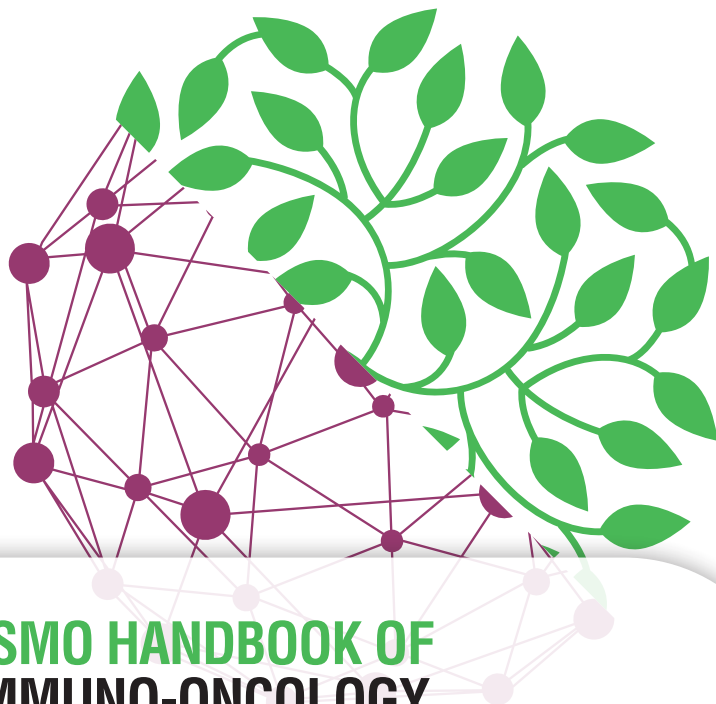




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ESMO HANDBOOK OF IMMUNO-ONCOLOGY

Edited by John B.A.G. Haanen · Raffaele Califano ·
Iwona Lugowska · Marina Chiara Garassino

ESMO Handbook Series



ESMO HANDBOOK OF **IMMUNO-ONCOLOGY**



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Edited by

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Abbreviations

2L	Second-line
5-FU	5-Fluorouracil
AACR	American Association of Cancer Research
Ab	Antibody
ABC	Anti-PD-1 Brain Collaboration
AC	Atypical carcinoid
ACT	Adoptive T cell therapy
ADC	Antibody–drug conjugate
ADCC	Antibody-dependent cellular cytotoxicity
ADCP	Antibody-dependent cellular phagocytosis
AE	Adverse event
AFP	Alpha-foetoprotein
AIDS	Acquired immunodeficiency syndrome
AIRE	Autoimmune regulator
ALCL	Anaplastic large cell lymphoma
ALK	Anaplastic lymphoma kinase
ANCA	Anti-neutrophil cytoplasmic antibody
APC	Antigen-presenting cell
APECED	Autoimmune polyendocrinopathy candidiasis ectodermal dystrophy
ASCO	American Society of Clinical Oncology
ASCT	Autologous stem cell transplantation
Atezo	Atezolizumab
B-ALL	B cell precursor acute lymphoblastic leukaemia
BCG	Bacillus Calmette-Guérin
BCLC	Barcelona Clinic Liver Cancer
bid	Twice daily
BITE	Bispecific T cell engager
BM	Brain metastasis
B-NHL	B cell non-Hodgkin lymphoma
BSC	Best supportive care

BV	Brentuximab vedotin
CAF	Cancer-associated fibroblast
CAR	Chimeric antigen receptor
ccRCC	Clear cell renal cell carcinoma
CEA	Carcinoembryonic antigen
CFU	Colony forming unit
cHL	Classical Hodgkin lymphoma
ChT	Chemotherapy
CI	Confidence interval
CIN	Chromosomal instability
CLL	Chronic lymphocytic leukaemia
CNS	Central nervous system
CPD	Confirmed progressive disease
CPK	Creatine phosphokinase
CPS	Combined positivity score
CR	Complete response
CRC	Colorectal cancer
CRS	Cytokine-release syndrome
CRT	Chemoradiotherapy
CTLA-4	Cytotoxic T-lymphocyte antigen 4
CV	Coxsackievirus
DCR	Disease control rate
DFS	Disease-free survival
DLBCL	Diffuse large B cell lymphoma
DLL3	Delta-like 3
DLT	Dose-limiting toxicity
dMMR	Mismatch repair deficiency
Doc	Docetaxel
DOR	Duration of response
DTIC	Dacarbazine
Durva	Durvalumab
EBV	Epstein-Barr virus
EC	Endometrial cancer
ECM	Extracellular matrix
ECOG	Eastern Cooperative Oncology Group
DFS	Event-free survival
EGFR	Epidermal growth factor receptor
EMA	European Medicines Agency

EMT	Epithelial–mesenchymal transition
EOC	Epithelial ovarian cancer
EORTC	European Organization for Research and Treatment of Cancer
ESMO	European Society for Medical Oncology
ETOP	European Thoracic Oncology Platform
EU	European Union
FcγR	Fcγ receptor
FDA	Food and Drug Administration
FGFR	Fibroblast growth factor receptor
FL	Follicular lymphoma
FU	Follow-up
GC	Gastric cancer
GCB	Germinal centre B cell-like
GEJ	Gastroesophageal junction
GGT	Gamma-glutamyl transferase
GI	Gastrointestinal
GITR	Glucocorticoid-induced tumour necrosis factor receptor
GM-CSF	Granulocyte–macrophage colony-stimulating factor
GMP	Good Manufacturing Practice
GOG	Gynecologic Oncology Group
gp100	Glycoprotein 100
GPC3	Glypican 3
Gr	Grade
GS	Genomically stable
GU	Genitourinary
HAART	Highly active antiretroviral therapy
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HDACi	Histone deacetylase inhibitor
HIF-1	Hypoxia-inducible factor 1
HIV	Human immunodeficiency virus
HL	Hodgkin lymphoma
HLA	Human leukocyte antigen
HNSCC	Head and neck squamous cell carcinoma
HPV	Human papillomavirus
HR	Hazard ratio
HRCT	High-resolution computed tomography

HRT	Hormone replacement treatment
i.m.	Intramuscular
i.v.	Intravenous
IC	Immune cell
ICB	Immune checkpoint blockade
ICI	Immune checkpoint inhibitor
ICOS	Inducible T cell co-stimulator
Id	Idiotypic
IDO	Indoleamine 2,3-dioxygenase
IFG-1R	Insulin-like growth factor-1 receptor
IFN	Interferon
IFNGR	IFN- γ receptor
IFN-α	Interferon-alpha
IFN-γ	Interferon-gamma
Ig	Immunoglobulin
IHC	Immunohistochemistry
IL	Interleukin
IL13Rα2	Interleukin-13 receptor α 2
IL-7R	Interleukin-7 receptor
IO	Immuno-oncology
Ipi	Ipilimumab
IPRES	Innate anti-PD-1 resistance
irAE	Immune-related adverse event
iRECIST	Response Evaluation Criteria In Solid Tumours in immunotherapy trials
IRF	Independent review facility
IRF1	Interferon regulatory factor 1
irRC	Immune-related response criteria
ITT	Intention to treat
JAK	Janus kinase
KIR	Killer cell immunoglobulin-like receptor
LA	Locally advanced
LAG-3	Lymphocyte-activation gene 3
LCNEC	Large cell neuroendocrine carcinoma
LDH	Lactate dehydrogenase
LN	Lymph node
mAb	Monoclonal antibody
MAGE-A	Melanoma-associated antigen genes-A

MAGE-A3	Melanoma-associated antigen-A3
MALT	Mucosa-associated lymphoid tissue
MCC	Merkel cell carcinoma
MCPyV	Merkel cell polyomavirus
MDSC	Myeloid-derived suppressor cell
MHC	Major histocompatibility complex
M1c	Visceral metastatic disease
MMR	Mismatch repair
mOS	Median overall survival
MPM	Malignant pleural mesothelioma
MPR	Major pathological response
MRI	Magnetic resonance imaging
mRNA	Messenger RNA
MSI	Microsatellite instability
MSI-H	Microsatellite instability-high
MSKCC	Memorial Sloan Kettering Cancer Center
MSLN	Mesothelin
MTD	Maximum tolerated dose
mTOR	Mammalian target of rapamycin
MUC	Mucin
N/A	Not applicable
NASH	Non-alcoholic steatohepatitis
NCCN	National Cancer Center Network
NCI	National Cancer Institute
NE	Not evaluated
NED	No evidence of disease
NGS	Next-generation sequencing
Nivo	Nivolumab
NK	Natural killer
NMIBC	Non-muscle invasive bladder urothelial carcinoma
NO	Nitric oxide
NR	Not reached
NSCLC	Non-small cell lung cancer
NSQ	Non-squamous
NY-ESO-1	New York-oesophageal squamous cell carcinoma-1
Obs	Observation
OPC	Oropharyngeal cancer
ORR	Objective response rate

OS	Overall survival
OSCC	Oesophageal squamous cell carcinoma
PAP	Prostatic acid phosphatase
PARP	Poly(adenosine diphosphate-ribose) polymerase
PBMC	Peripheral blood mononuclear cell
PCD	Programmed cell death
PCNSL	Primary central nervous system lymphoma
PD-1	Programmed cell death protein 1
PDGFR	Platelet-derived growth factor receptor
PD-L1	Programmed death-ligand 1
PE	Platinum/etoposide
Pembro	Pembrolizumab
PET	Positron emission tomography
PFS	Progression-free survival
PMBCL	Primary mediastinal large B cell lymphoma
PMED	Particle-mediated epidermal delivery
POLD	Polymerase D
POLE	Polymerase E
PR	Partial response
PRO	Patient-reported outcome
PS	Performance status
PTEN	Phosphatase and tensin homologue
PTL	Primary testicular lymphoma
PTLD	Post-transplant lymphoproliferative disorder
pts	Patients
qd	Once daily
QOL	Quality of life
qXw	Every X weeks
R/R	Relapsed/refractory
R0	No tumour at the margin
RCC	Renal cell carcinoma
RECIST	Response Evaluation Criteria In Solid Tumours
REP	Rapid expansion protocol
RFA	Radiofrequency ablation
RFS	Recurrence-free survival
RNAseq	RNA sequencing
RNS	Reactive nitrogen species
ROC	Receiver operating characteristic

Rova-T	Rovalpituzumab tesirine
RP2D	Recommended phase II dose
RR	Response rate
RS	Reed-Sternberg
RT	Radiotherapy
RTOG	Radiation Therapy Oncology Group
RT-PCR	Reverse transcription polymerase chain reaction
s.c.	Subcutaneous
SBRT	Stereotactic body radiotherapy
SCCC	Squamous cell carcinoma of cervix
scFv	Single chain fragment of variable region
SCLC	Small cell lung cancer
SD	Stable disease
SLP	Synthetic long peptide
SoC	Standard-of-care
SOM	Sum of measurement
SQ	Squamous
STAT	Signal transducer and activator of transcription
TAA	Tumour-associated antigen
TACE	Transcatheter arterial chemo-embolisation
TAM	Tumour-associated macrophage
TAP	Transporter associated with antigen processing
TC	Tumour cell
TCGA	The Cancer Genome Atlas
TCHRBCL	T cell-rich, histiocyte-rich large B cell lymphoma
TCR	T cell receptor
T_{eff}	Effector T cell
TERT	Telomerase reverse transcriptase
TGF	Transforming growth factor
TIGIT	T cell immunoreceptor with immunoglobulin and inhibitory motif
TIL	Tumour infiltrating lymphocyte
TIM-3	T cell immunoglobulin and mucin domain 3
TKI	Tyrosine kinase inhibitor
TLR	Toll-like receptor
TLS	Tumour lysis syndrome
TMA	Tissue micro-array
TMB	Tumour mutation burden

TME	Tumour microenvironment
TNF	Tumour necrosis factor
TNFSFR	TNF superfamily receptor
T-NHL	T cell non-Hodgkin lymphoma
TNM	Tumour Node Metastasis
TP	Timepoint
TPS	Tumour proportion score
trAE	Treatment-related adverse event
T_{reg}	T regulatory cell
TTP	Time to progression
T-VEC	Talimogene laherparepvec
UC	Urothelial carcinoma
UPD	Unconfirmed progressive disease
US	United States
UV	Ultraviolet
VEGF	Vascular endothelial growth factor
VEGFR	Vascular endothelial growth factor receptor
VISTA	V-domain immunoglobulin suppressor of T cell activation
WCLC	World Conference on Lung Cancer
WES	Whole-exome sequencing
WHO	World Health Organization

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Above all, I would like to thank you, dear reader, for your trust in us.

Professor John B.A.G. Haanen, on behalf of all the Editors

Preface



Immuno-oncology is a rapidly evolving field. Within just a few years, immunotherapy has been approved as an important treatment option for patients across many cancer types. It is likely that we are still at the beginning of this revolution, which is changing the lives of our patients. Never before has a new treatment paradigm had such an impact on survival – even for cancer types that seemed incurable, we now see long-term remission extending to the metastatic setting.

I see immunotherapy moving in several directions. First, combination therapies are being studied in several clinical trials over many disease types. In order to be successful, we need to understand the defects in the tumour microenvironment that need to be overcome by the therapy, requiring in-depth and large-scale biomarker research. Second, immunotherapy is moving from the palliative to the curative setting, resulting in more patients being treated.

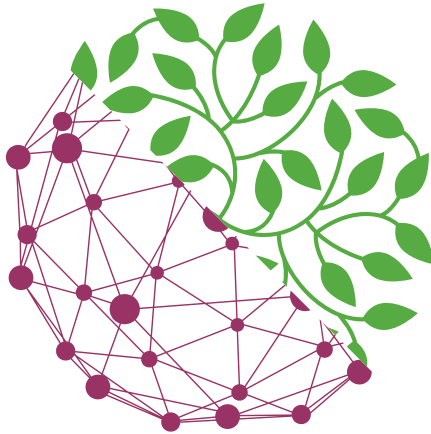
In this handbook, specialists in the field have covered many aspects of immuno-oncology, from basic immunology to the current state-of-the-art clinical immunotherapy in different disease types, including management of immune-related toxicities. This comprehensive overview provides a strong base to understand the theory, mechanisms of action, clinical developments and the future of immuno-oncology, and, therefore, is a ‘must read’ for any professional involved in the care of cancer patients.

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Section I:

Introduction to immunology and immunotherapy

1



I.I Immune Checkpoints

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Definition

Immune checkpoint molecules are key modulators of the anti-tumour T cell immune response. They are present on T cells, antigen-presenting cells (APCs) and tumour cells; their interaction activates either inhibitory or activating immune signalling pathways. Examples of inhibitory immune checkpoints shown to induce a negative signal to T cells are:

- Cytotoxic T-lymphocyte antigen 4 (CTLA-4, also known as CD152)
- Programmed cell death protein 1 (PD-1, also known as CD279)
- Lymphocyte-activation gene 3 (LAG-3)
- T cell immunoglobulin and mucin domain 3 (TIM-3)
- V-domain immunoglobulin suppressor of T cell activation (VISTA)

Inhibitory immune checkpoints play a vital role in maintaining immune self-tolerance. Indeed, negative co-stimulatory signals help to prevent T cells from showing autoimmune reactions.

On the other hand, co-stimulatory immune checkpoints have been shown to enhance T cell expansion and survival. Examples are:

- CD40
- OX40 (also known as CD134)

- 4-1BB (also known as CD137)
- Glucocorticoid-induced tumour necrosis factor (TNF) receptor (GITR)
- Inducible T cell co-stimulator (ICOS, also known as CD278)

Other intracellular metabolic pathways play a critical role in the activation of immune cells and could, by extension, be considered as immune checkpoints. For instance, in tumour cells and myeloid cells, indoleamine 2, 3-dioxygenase (IDO) and arginase are key enzymes which, by depleting amino acids, can inhibit the effector functions of T cells. However, we will focus here on immune checkpoint molecules: membrane-expressed receptors and ligands, which determine, at the level of the intercellular synapse, if an immune cell becomes activated or inhibited. We will also concentrate on immune checkpoints involved in the activation of T cells, as they are of current clinical interest. However, other immune checkpoints play a critical role for the modulation of other subsets of immune cells (e.g. CD40 for B cells).

Immune Synapse

Immune Recognition of Tumour Antigens by T Cells

During the priming phase of anti-tumour immunity, tumour antigens are presented to T cells via APCs, such as dendritic cells (DCs) or macrophages. The specificity of T cell activation against a tumour antigen relies on the cognate recognition of the antigen presented by the major histocompatibility complex (MHC) proteins on the surface of APCs and the T cell receptor (TCR). During the effector phase of the anti-tumour immune response, primed T cells will recognise the tumour antigens presented by MHC molecules expressed by the tumour cells. CD8⁺ and CD4⁺ T cells can recognise peptides presented by MHC-I and MHC-II molecules, respectively. This TCR/MHC interaction provides the first signal for T cell activation (signal 1).

Co-stimulatory Molecules

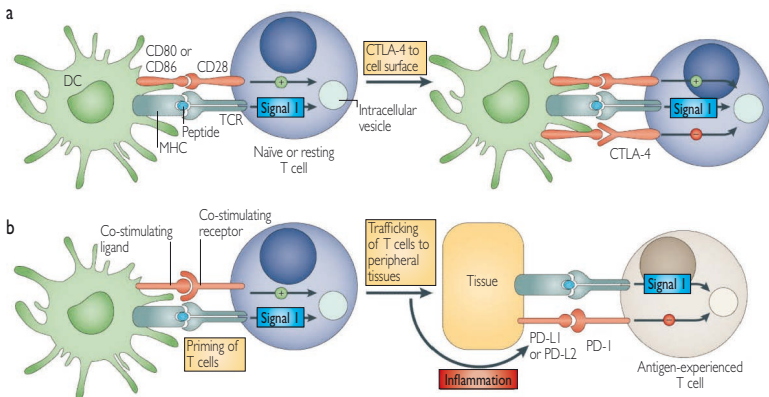
The activation of a T cell also requires a second signal, provided by co-stimulatory molecules. The first co-stimulatory molecules historically identified belong to the immunoglobulin B7 superfamily. CD80 (also known as B7.1) and CD86 (also known as B7.2) are expressed at the surface of either APCs or cancer cells, and act as activating ligands of the co-stimulatory receptor CD28 expressed on the surface of T cells (signal 2). More recently, other co-stimulatory immune checkpoints have been described, such as OX40 (CD134), 4-1BB (CD137) or GITR (CD357). These TCRs belong to the TNF superfamily receptors (TNFSFRs) and their activation enhances T cell survival and effector functions. From the same family, CD40 is expressed on APCs and amplifies T cell activation by increasing antigen presentations. Interestingly, co-stimulatory molecules are also highly expressed on immunosuppressive regulatory T cells (T_{regs}). The activation of T_{regs} favours immune self-tolerance. Defective T_{regs} have been associated with autoimmune disorders, while intratumoural T_{regs} have been associated with a worse prognosis in many cancers.

Co-inhibitory Receptors

Upon T cell activation, negative feedback loops can prevent overstimulation of self-reactivity. Like the CD28 receptor structure, but with opposite effects, the co-inhibitory receptor CTLA-4 has been shown to bind to CD80 and CD86 with a much higher affinity than CD28, delivering inhibitory signals to T cells and therefore blocking T cell activation. The membrane expression of CTLA-4 is mostly found on $CD4^+$ T cells, notably T_{regs} (Figure 1). Upon activation, the PD-1 receptor can be upregulated on T cells and can interact with two ligands: programmed death-ligand 1 (PD-L1) (also known as B7H1 or CD274) and PD-L2 (also known as B7DC or CD273). Once bound to its ligands, PD-1 confers a negative signal to effector T cells, thereby inhibiting their cytotoxic functions. CTLA-4 and PD-1 are usually highly expressed on intratumoural T cells and their stimulation is thought to contribute to the overall inhibition of anti-tumour T cells.

Figure 1 Evolution of CTLA-4 and PD-1 immune checkpoint expression in the immune response.

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- The CTLA-4-mediated immune checkpoint is induced in T cells at the time of their initial response to antigen. The level of CTLA-4 induction depends on the amplitude of the initial T cell receptor (TCR)-mediated signalling. High-affinity ligands induce higher levels of CTLA-4, which dampens the amplitude of the initial response. After the TCR is triggered by antigen encounter, CTLA-4 is transported to the cell surface. Therefore, CTLA-4 functions as a signal dampener to maintain a consistent level of T cell activation.
- By contrast, the major role of the PD-1 pathway is not at the initial T cell activation stage but rather to regulate inflammatory responses in tissues by effector T cells recognising antigen in peripheral tissues. Inflammatory signals in the tissues induce the expression of PD-1 ligands. IFN- γ is predominantly produced by T helper 1 (T_H1) cells. Excessive induction of PD-1 on T cells in the setting of chronic antigen exposure can induce an exhausted or anergic state in T cells.

Abbreviations: CTLA-4, cytotoxic T-lymphocyte antigen 4; DC, dendritic cell; IFN, interferon; MHC, major histocompatibility complex; PD-1, programmed cell death protein 1; PD-L1/2, programmed death-ligand 1/2; TCR, T cell receptor.

Immune Checkpoint-targeted Therapies

The scientific rationale that anti-tumour T cells could be blocked in their functions by co-inhibitory receptors led to the idea of designing antagonistic antibodies to dampen the CTLA-4/B7 and PD-1/PD-L1/2 interactions, and unleash the effector signals on T cells either at the priming or effector phases. This idea has been a major paradigm shift in the strategy to treat cancers, where instead of designing tumour-targeted therapies, we

would now design immune-targeted therapies in order to break the cancer immune tolerance, restoring T cell recognition against tumour cells.

Technical Procedures

As opposed to chemotherapies (ChTs) and tumour-targeted therapies, which aim to directly destroy cancer cells, immune checkpoint-directed therapies bind lymphocyte ligands or receptors to enhance the lymphocyte activation and allow a cytotoxic anti-tumour immune response. The first immune checkpoint-targeted therapies developed in the clinic were humanised or fully human monoclonal antibodies selected for their antagonistic properties against immune checkpoints such as CTLA-4, PD-1 and PD-L1. They have demonstrated promising clinical activity in more than 30 different cancer types in early phase trials. Patients with a tumour response share a common feature: their response is more durable than has been observed to date with ChTs and tumour-targeted therapies. This durability of tumour response has translated into significant overall survival (OS) benefits in several phase III clinical trials. Another characteristic of these drugs is their safety profile: they can trigger autoimmune and inflammatory toxicities in patients, so-called immune-related adverse events (irAEs).

Different isotypes have been used so far in the clinic (Table 1). These antibodies usually have a long half-life and are usually infused intravenously (i.v.) with varying intervals of administration. Anti-PD-1 and anti-PD-L1 antibodies were initially developed on weight-based dosing. However, results from several clinical trials have shown no correlation between dose, efficacy and toxicity for anti-PD-(L)1, and most compounds are now developed with a flat dose, sufficient to saturate the target. For the anti-CTLA-4 antibody ipilimumab, there was no dose-limiting toxicity (DLT) identified in early phase trials. However, a recent randomised study in patients with metastatic melanoma (MM) has shown that ipilimumab was more active and more toxic at 10 mg/kg than the approved dose of 3 mg/kg. This dose–efficacy relationship of ipilimumab currently raises questions about the mechanism of action of anti-CTLA-4 antibodies and the optimal dose to be used when combined with anti-PD-(L)1 antibodies.

Table 1 First Generation of Immune Checkpoint-targeted Monoclonal Antibodies

Target	Name	Isotype
Anti-CTLA-4	Ipilimumab	IgG1
	Tremelimumab	IgG2
Anti-PD-1	Nivolumab	IgG4
	Pembrolizumab	IgG4
	PDR001	IgG4
Anti-PD-L1	Atezolizumab	Fc mut* IgG1
	Durvalumab	Fc mut IgG1
	Avelumab	IgG1

*Fc mut: antibodies which have been mutated in their Fc part in order to avoid Fc receptor activation and antibody-dependent cellular cytotoxicity (ADCC).

Abbreviations: CTLA-4, cytotoxic T-lymphocyte antigen 4; Ig, immunoglobulin; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1.

New antibodies targeting inhibitory immune checkpoints such as LAG-3, T cell immunoreceptor with immunoglobulin and inhibitory motif (TIGIT), VISTA and TIM-3, and co-stimulatory checkpoints such as OX40, CD40, 4-1BB, GITR and ICOS are currently being evaluated.

Predictive and/or Prognostic Biomarkers of (Potential) Clinical Relevance

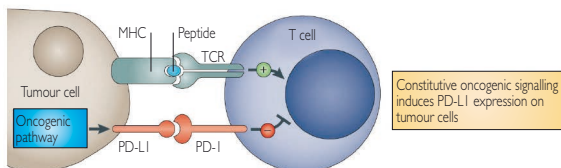
PD-L1 Staining

The tumoural expression of PD-L1, assessed by immunohistochemistry (IHC) staining, has been identified as a biomarker associated with a higher chance of tumour response in patients treated with anti-PD-L1 antibodies and a better OS in multiple tumour types. The PD-L1 status of a tumour relies both on the IHC staining kit and the scoring methods. Because of the heterogeneity of assays, there is no consensus on a cut-off defining a PD-L1-high tumour. PD-L1 can be expressed either constitutively via an oncogenic pathway or induced by inflammatory cytokines such as interferon-gamma (IFN- γ) (Figure 2). IFN- γ can also lead to the upregulation of PD-L1 at the surface of any cell in the tumour microenvironment, and activated T cells could be double-positive for PD-1 and PD-L1.

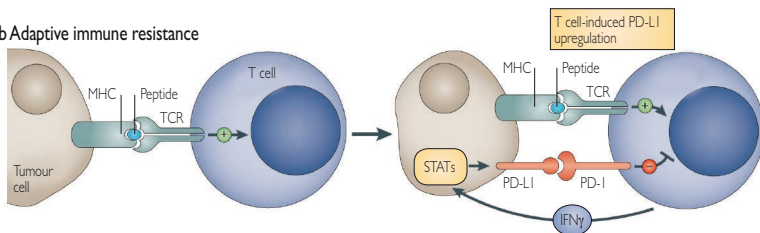
Figure 2 Expression of PD-L1 on tumour cells.

From Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer* 2012; 12:252–264. Reprinted with permission of Nature/Springer/Palgrave. Copyright ©2012.

a Innate immune resistance



b Adaptive immune resistance



- (a) In some tumours, constitutive oncogenic signalling can upregulate PD-L1 expression on all tumour cells, independently of inflammatory signals in the tumour microenvironment. Activation of the Akt and signal transducer and activator of transcription 3 (STAT3) pathways has been reported to drive PD-L1 expression.
- (b) In some tumours, PD-L1 is induced in response to inflammatory signals. Adaptive induction may be a common mechanism for the expression of multiple immune checkpoint molecules in tumours.

Abbreviations: IFN, interferon; MHC, major histocompatibility complex; PD-I, programmed cell death protein I; PD-L1, programmed death-ligand 1; STAT, signal transducer and activator of transcription; TCR, T cell receptor.

Pembrolizumab is, at present, the only anti-PD-1 antibody to be approved by the Food and Drug Administration (FDA) for a selected population of PD-L1-positive patients in non-small cell lung cancer (NSCLC) and gastric cancer.

Inflammatory Tumours and CD8⁺ T cells

In several tumour types, tumours with IFN- γ gene expression profile and a high level of tumour-infiltrative CD8⁺ T cells have better responses and survival following anti-PD-(L)1 therapy.

Mutational Load

Tumours with a high mutational load have been correlated with OS benefits following treatment with ipilimumab in MM, with pembrolizumab

in NSCLC and with atezolizumab in bladder cancers. It is currently believed that a high tumour mutation burden (TMB) yields numerous immunogenic cancer cell neo-epitopes, that may be recognised by T cells upon presentation by MHC molecules. However, the TMB seems to be a prognostic marker independent of the intratumoural inflammatory gene expression profile. The assessment of TMB is currently evaluated from either tumour samples or circulating tumour DNA.

Mismatch Repair Status

Tumours with DNA mismatch repair deficiency (dMMR)/microsatellite instability-high (MSI-H) have shown great sensitivity to anti-PD-(L)1 therapies. It is currently believed that tumours harbouring an erroneous MMR system will accumulate DNA mutations, which can lead to the presence of high levels of mutation-associated neoantigens, most recognised by immune cells. Tumours identified as having a dMMR/MSI-H status are eligible for treatment with pembrolizumab in the USA.

Blood Biomarkers

A high neutrophil to lymphocyte ratio has been associated with poor outcomes in patients treated with ipilimumab and anti-PD-(L)1 therapy across different tumour types. High levels of serum lactate dehydrogenase (LDH) or soluble CD25 have been associated with poor prognosis, although none is currently used in the clinic.

Microbiota

In preclinical models, the gut microbiota has been identified as a key modulator of the immune system by enhancing T cell activation and infiltration into tumours. The impact of the gut microbiome on anti-PD-(L)1 efficacy remains to be demonstrated in humans, but is currently under active investigation.

Clinical Results

Immune checkpoint-targeted therapies have received FDA approvals in ten tumour types or categories of cancer between 2011 and 2017: MM, NSCLC, renal cell carcinoma (RCC), urothelial cancers, head and neck

squamous cell carcinoma (HNSCC), Hodgkin lymphoma (HL), Merkel cell carcinoma (MCC), hepatocellular carcinoma, gastric cancer and a range of MSI-H cancers.

Anti-CTLA-4

The only anti-CTLA-4-blocking antibody that has received FDA approval is ipilimumab in MM patients, first as monotherapy in 2011, and in combination with nivolumab in 2015. Approval was based on the pivotal data of the CheckMate 067 trial, with an objective response rate (ORR) of 72.1% with nivolumab plus ipilimumab versus 21.3% with ipilimumab alone and statistically significant updated OS results for the combination versus ipilimumab (not reached [NR] versus 19.9 months in the ipilimumab group). Similarly, first-line combination therapy with nivolumab and ipilimumab has recently demonstrated clinical benefit in patients with previously untreated advanced or metastatic RCC. Results from the phase III CheckMate 214 trial showed significant improvement in OS (NR versus 26 months) and progression-free survival (PFS) (11.6 months versus 8.4 months) compared with sunitinib in intermediate- and poor-risk patients with metastatic RCC. In advanced NSCLC, the phase I CheckMate 012 trial showed significant clinical benefit for this combination, with an overall response in up to 47% of the patients; a phase III trial (CheckMate 227) is currently ongoing to confirm these results. This combination is also currently being evaluated in patients with unresectable pleural mesothelioma, in the CheckMate 743 study. In patients with advanced MM and patients with relapsed malignant mesothelioma, tremelimumab failed to demonstrate significant survival benefits compared with standard-of-care (SoC) ChT and placebo, respectively. Recently, the combination of durvalumab and tremelimumab did not reach the PFS outcome primary endpoint in the MYSTIC study in first-line treatment for patients with metastatic NSCLC, while the OS analysis is still pending.

Anti-PD-1

Nivolumab was first approved for patients with MM (CheckMate 066 and CheckMate 037) and for adjuvant therapy of resected stage III melanoma (CheckMate 238). Nivolumab is approved for patients with squamous (CheckMate 017) and non-squamous (CheckMate 057) NSCLC,

RCC (CheckMate 025), HNSCC (CheckMate 141), urothelial carcinoma (CheckMate 275), dMMR metastatic colorectal cancer (CheckMate 142) and classical HL after failure of first-line therapies. Treatment in patients with HL must follow relapse after autologous haematopoietic stem cell transplantation and post-transplantation brentuximab vedotin (CheckMate 205 and CheckMate 039). Most surprisingly, in NSCLC, nivolumab failed to demonstrate its superiority over ChT in the randomised phase III study CheckMate 026, in first-line treatment of patients with tumours with PD-L1 tumour expression $\geq 5\%$. Of note, there was imbalance in terms of TMB level between the two therapeutic arms, which could have contributed to this negative result. Indeed, patients with a high TMB showed a higher ORR and PFS when treated with nivolumab compared with ChT, and the inverse was shown in patients with low TMB. Interestingly, there was no correlation between the level of tumour PD-L1 expression and TMB.

Like nivolumab, pembrolizumab has been approved as second-line treatment of refractory/relapsing MM, NSCLC (with PD-L1 $>1\%$), HNSCC, classical HL, urothelial carcinoma and any solid tumour expressing MSI-H status. In previously untreated advanced or metastatic NSCLC, pembrolizumab has been approved for patients harbouring PD-L1 expression on at least 50% of tumour cells, with an ORR of 44.8% versus 27.8% in the ChT group, from the pivotal phase III KEYNOTE-024 trial. Also, the combination of pembrolizumab with carboplatin and pemetrexed is now a SoC for patients with metastatic NSCLC, irrespective of PD-L1 expression, based on the results of the KEYNOTE-021 study (ORR 55% versus 29%). Pembrolizumab has also been approved as first-line treatment of cisplatin-ineligible urothelial carcinoma patients, thanks to the results of KEYNOTE-052 (ORR of 29%).

Anti-PD-L1

Anti-PD-L1-blocking antibodies have also been approved in certain other tumour types, such as advanced bladder carcinoma for durvalumab and atezolizumab, based on the results of the phase III DANUBE and phase II IMvigor 210 trials, respectively. In patients with metastatic NSCLC, atezolizumab has been approved based on the results of the

phase II POPLAR and phase III OAK trials with OS of 12.6 months versus 8.9 months for second-line treatment. Recently, durvalumab has shown statistically significant improvement in PFS (16.8 months versus 5.6 months) after chemoradiotherapy in patients with locally advanced NSCLC (PACIFIC trial). A third anti-PD-L1 agent, avelumab, was approved in 2017 as both second-line treatment of metastatic urothelial carcinoma and first-line treatment of metastatic MCC.

Potential Future Developments

Because tumour-targeted therapies mostly confer improvements in PFS, and immune checkpoint-targeted therapies seem to provide greater OS benefits (at least for metastatic disease), the combination of the two categories of agents may significantly improve both survival and durable responses in many cancer types. Also, the combination of immunotherapies is currently investigated in many clinical trials in multiple tumour types. By boosting the efficacy of the immune system, co-stimulatory checkpoint agonists could also be of interest to enhance the anti-tumour response generated by immune checkpoint blockers. The modulation of innate immune cells with immune checkpoint antibodies, pattern recognition receptor agonists, or oncolytic viruses could also boost the adaptive immune system. Another class of antibodies targeting both tumour cells and T cells (so-called bispecific T cell engager antibodies) is currently being evaluated and could be of interest in combination with anti-PD-L1 antibodies.

Although immune checkpoint-targeted antibodies confer long-term durable responses, a greater understanding of primary and secondary resistance mechanisms to these agents is key for the future development of cancer immunotherapy and patient selection.

Declaration of Interest:

Dr Mahjoubi has reported no conflicts of interest.

Dr Rizvi is an investigator and co-investigator of industry-sponsored clinical trials using immune checkpoint-targeted drugs. He has provided consulting services and has participated in scientific advisory boards of companies developing immune checkpoint-targeted therapies. He has

served as a consultant to AstraZeneca, Lilly, Roche/Genentech, Pfizer, Novartis, Bristol-Myers Squibb and Merck Sharp & Dohme. He is a shareholder in Gritstone Oncology.

Dr Marabelle is an investigator and co-investigator of industry-sponsored clinical trials using immune checkpoint-targeted drugs. He has provided consulting services and has participated in scientific advisory boards of companies developing immune checkpoint-targeted therapies. He has received consultancy fees and honoraria from AstraZeneca, Merck Serono, Roche/Genentech, Pfizer, Novartis, Lytix Pharma, Bristol-Myers Squibb and Merck Sharp & Dohme. He has no stock ownership.

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I.2 Adoptive T Cell Therapy

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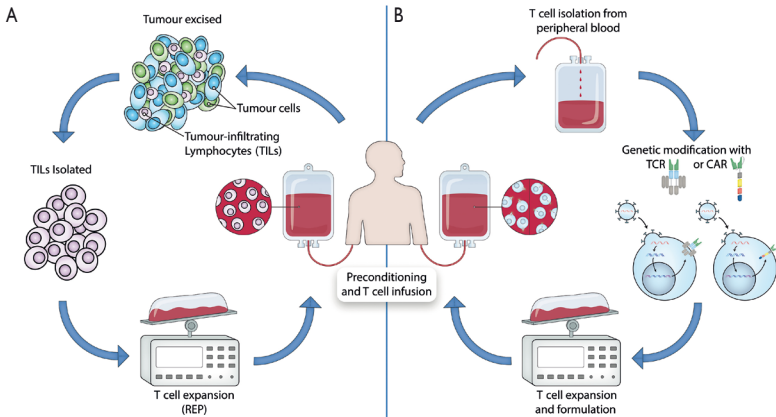
The goal of adoptive T cell therapy (ACT) is to generate a robust immune-mediated antitumour response through infusion of *ex vivo* manipulated T cells. ACT strategies with the aim of utilising T cells to destroy tumours can be divided into: (1) the isolation of naturally occurring tumour-specific T cells from existing tumour masses and (2) the genetic modification of blood-derived T cells to allow for specific recognition of tumour cells. In both settings, T cells are manipulated *ex vivo* followed by an expansion process and eventually infused into the lymphodepleted patient (Figure 1).

Naturally Occurring Tumour-specific T Cells

Traditional ACT involves the isolation of tumour infiltrating lymphocytes (TILs) from tumour tissue, followed by *ex vivo* massive expansion until enough T cells can be reinfused intravenously into the patient (Figure 1A). Since strategies based on isolation of tumour-specific T cells from the blood have so far achieved limited clinical success, this will not be discussed further here.

TILs constitute a heterogeneous population of cells, including mostly T cells and natural killer (NK) cells, which may be present in any solid tumour. Already in the 1980s, it was observed that TILs can recognise and kill cultured autologous tumour cells *in vitro*. Direct tumour killing is mostly mediated by CD8⁺ T cells, present within TILs. Thus, TILs are generally enriched for tumour-specific T cells, which have penetrated the

Figure 1 Different adoptive T cell transfer approaches to harness the immune system in cancer therapy.



- (A) Adoptive transfer of anti-tumour T cells isolated from within a patient's tumour. TILs are extracted from surgically resected tumour samples, then expanded *in vitro*, followed by re-infusion into the lymphodepleted patient.
- (B) T cells from patient peripheral blood are isolated and expanded in culture, and genetically modified to express either a TCR or a CAR that confers the ability to specifically recognise and destroy tumour cells when re-infused into the lymphodepleted patient.

Abbreviations: CAR, chimeric antigen receptor; REP, rapid expansion protocol; TCR, T cell receptor; TIL, tumour infiltrating lymphocyte.

tumour microenvironment. Most importantly, TILs can recognise several antigens that are uniquely expressed on the individual patient's tumour cells (including neoantigens arising from somatic genetic alterations, aberrantly expressed cancer/testis antigens, overexpressed self-antigens and some lineage antigens). Thus, TILs may orchestrate a highly specific multi-target attack directed towards the individual cancer.

The natural ability of TILs to kill autologous tumours can be exploited therapeutically with ACT. In its most widespread application, TIL-based ACT involves the isolation of TILs from tumour tissue of the patient, followed by massive expansion of unselected TILs and infusion back into the patient. Before infusion, lymphodepleting chemotherapy (ChT) is administered to create 'physical space' for the high number of TILs. Following infusion, the immune-stimulating cytokine interleukin-2 (IL-2)

is administered to the patient to support the survival and continued expansion of the TILs *in vivo*.

Briefly, resected tumour tissue is minced or digested and, following 1–3 weeks of *in vitro* culture in media containing high doses of IL-2, TILs are released and initially expanded. After the initial expansion, TILs are expanded massively without any further selection using the rapid expansion protocol (REP) for about 2 weeks, before being infused back to the patient; typically, around 100×10^9 cells are infused. Now, a very large population of expanded TILs is circulating in the peripheral blood of the patient. The natural capacity of TILs to reach tumour sites and kill autologous tumour cells is crucial to ensure tumour regression.

This TIL ACT approach has been found to mediate complete and durable responses in 10%–20% of patients with metastatic melanoma, and can also yield clinical responses in other selected types of solid tumours. In principle, any patient with a resectable tumour which contains tumour-reactive T cells can benefit from this approach, the constraint being the consistent generation and laborious *ex vivo* expansion of huge numbers of TILs. Furthermore, classical TIL ACT comprises intensive treatment regimens with high-dose conditioning ChT and high-dose IL-2, necessitating hospitalisation for around 3 weeks. All patients experience grade 3 and 4 toxicity and the treatment centre should be experienced in managing these potentially serious adverse events. In general, TIL ACT is only offered in one treatment cycle.

Active research is exploring how to improve the efficacy of TIL-based ACT in melanoma, to extend its efficacy to several other tumour types, as well as to increase its availability to reference cancer centres.

Genetically Modified T Cells

The approach of using genetically modified T cells is based on the premise that the antigen specificity of T cells can be manipulated by genetic engineering to target antigens expressed by tumours. This is especially valid in situations in which endogenous antitumour reactivity is lacking. This has been accomplished by transducing T cells with either tumour antigen-specific T cell receptors (TCRs) or with chimeric antigen receptors (CARs) (Figure 1B).

TCRs are naturally occurring surface receptors on T cells that can recognise peptide antigens presented on the surface of host cells by the major histocompatibility complex (MHC)/human leukocyte antigen (HLA) system.

Genetically modified TCR gene therapy has the purpose of altering T cell specificity through the expression of a new TCR alpha and beta chain pair that is specific for a tumour antigen (Figure 2A). For this purpose, TCRs from rare T cells have been identified that are able to recognise naturally processed and expressed tumour antigens, allowing them to specifically attack malignant tissue. However, as TCRs bind to peptide/MHC complexes on the surface of tumour cells, the tumour-specific TCRs can only be used in a patient population that has this specific MHC or HLA allele.

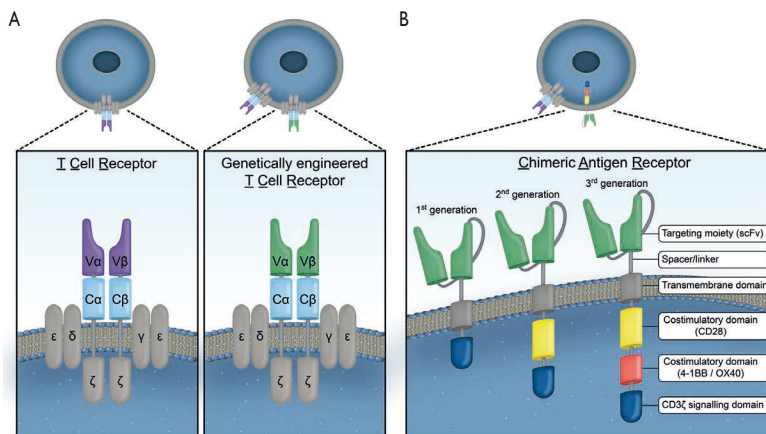
After the isolation and sequencing of these tumour-specific TCRs, they are cloned into retro- or lentiviral vectors, which can be used to transduce peripheral blood T cells from patients *ex vivo*, followed by expansion and re-infusion (Figure 1B).

In most cases, tumour antigen-specific T cells targeting self-antigens isolated from cancer patients are typically of low affinity, because of the impact of central tolerance to these antigens on the T cell repertoire. Attempts to overcome this issue have included the:

- Engineering of high-affinity TCRs by affinity maturation
- Generation of murine TCRs by immunising transgenic mice that express an HLA allele plus human tumour antigen, and
- Isolation of TCRs in an allogeneic setting, in which T cells are induced *in vitro* against a foreign HLA-peptide complex, as the repertoire is not limited by thymic selection

In the first proof-of-principle study using genetically modified TCRs, T cells from metastatic melanoma patients were transduced with a TCR directed against HLA-A*0201/MART-1 peptide, which was cloned from TIL isolated from a resected melanoma lesion of an HLA-A*0201 patient who had responded to TIL treatment. Sustained objective responses were demonstrated in a minor proportion of treated metastatic melanoma (MM) patients with no significant toxicity, and infused TCR-modified T cells persisted for more than a year. Other trials have subsequently dem-

Figure 2 Genetically modified T cells.



- (A) T cells recognise their target by the TCR complex, which is composed of the TCR α and β chain for recognition and the CD3 chains for signalling. T cells can be genetically engineered with defined specificity by expression of recombinant TCR $\alpha\beta$ chains of known specificity.
- (B) CARs are composed of an scFv derived from the antigen-binding domain of antibodies, fused to the CD3 ζ transmembrane and intracellular signalling domains from the TCR complex. Additional intracellular signalling domains are added for co-stimulatory signals, such as the CD28 and 4-1BB signalling domains, to yield second- and third-generation CARs.

Abbreviations: CAR, chimeric antigen receptor; scFv, single-chain fragment of variable region; TCR, T cell receptor.

onstrated significant and prolonged tumour regression in cancer patients using genetically modified TCRs against glycoprotein 100 (gp100), (melanoma), NY-ESO-1 (melanoma, synovial sarcoma), melanoma antigen A3 (MAGE-A3), (myeloma, melanoma) and carcinoembryonic antigen (CEA), (colorectal carcinoma).

Patients treated with high-dose conditioning ChT all experience toxicity including neutropaenia and risk of sepsis. In addition, potential safety risks are associated with the use of genetically modified T cell therapies, with the most critical related to:

- On-target off-tumour toxicity, when infused T cells recognise normal tissue due to expression of the same antigen (I)e gp100 and MART-1 which are expressed by both melanoma cells and normal melanocytes

- Off-target reactivity, when infused T cells can cross-react against peptides other than the targeted ones, and
- Cytokine-release syndrome (CRS), when infused T cells induce sudden and dramatic increase of inflammatory cytokines

The genetic modification of T cells with CARs combines antibody-like recognition with T cell-activating function (Figure 2B). The construction of a CAR relies on the identification of a suitable antibody to a cell surface molecule of interest, and, in contrast with the TCR modification approach, CAR recognition does not rely on peptide processing or presentation by MHC. Thus, all surface-expressed target molecules represent a potential CAR-triggering epitope.

The first-generation CARs were composed of an antigen-binding region (a single-chain antibody variable fragment [scFv]) derived from an antibody with the desired specificity, which was fused to the CD3 ζ T cell signalling domain, allowing T cell activation comparable to triggering of the native TCR (Figure 2B). These early CARs provided only activation signal 1 to T cells, and were shown to lead to CAR-T cell anergy upon repeated antigen stimulation. Second-generation CARs contain an additional co-stimulatory domain, such as CD28 and 4-1BB, which provides activation signal 2 upon scFv engaging the target antigen (Figure 2B). CAR-T cells carrying CD28 or 4-1BB signalling moieties have demonstrated potent antitumour activity in clinical trials and clinically meaningful response rates. Third-generation CARs, which incorporate an additional co-stimulatory domain (Figure 2B), are now in development to further potentiate persistence and activity of infused CAR-T cells.

A multitude of clinical trials have demonstrated robust efficacy and frequently durable responses using CAR-T cells targeting CD19, a B cell-lineage antigen expressed on the surface of both normal and malignant B cells. CAR-T cells specific for CD19 have been used effectively to treat patients with ChT-refractory B cell malignancies including marginal zone lymphoma, aggressive B cell lymphomas, chronic lymphocytic leukaemia, and adult and paediatric acute lymphoblastic leukaemia (ALL).

The collective experience from the treatment with CD19-specific CAR-T cells across different centres using different co-stimulatory domains and gene transfer methods can be summarised as:

- Patients should receive lymphodepleting ChT
- Patients with ALL, in particular, have very high response rates
- Off-tumour toxicity is primarily limited to B cell aplasia, a condition that can be clinically managed with prophylactic infusions of immunoglobulin
- Patients often develop severe CRS, and
- There is no clear dose–response relationship between the number of CAR-T cells infused and the likelihood of response

CAR-T cell therapy against solid tumours has yielded limited success thus far. Potential obstacles include:

- Inefficient T cell homing to the tumour site
- More difficult antigen selection due to high antigen heterogeneity across the same malignancy
- Physical barriers to tumour infiltration by T cells
- High risk of on-target, off-tumour toxicity because potential target antigens are more likely to be expressed in other essential organs, and
- Potent immunosuppressive factors that render T cells dysfunctional in the tumour microenvironment

Active preclinical research and clinical trials are attempting to overcome obstacles in the application of CAR-T cells to solid cancer types, by assessing novel CAR designs with additional receptors and ligands to ‘armour’ the CAR, gene transfer methods, treatment protocols and different targets, including CEA for colorectal cancers, disialoganglioside GD2 for neuroblastoma and sarcoma, prostate-specific membrane antigen (PSMA) for prostate cancer and melanoma, epidermal growth factor receptor variant III (EGFRvIII) and interleukin-13 receptor $\alpha 2$ (IL13R $\alpha 2$) for glioblastoma.

Concluding Remarks

TIL ACT can induce long-term remission in patients with otherwise treatment-resistant widespread MM. The use of TIL ACT is, however, still experimental and restricted to reference cancer centres with expertise in TIL production and clinical management.

The first two commercial gene-modified CD19-targeting CAR-T cell products have been approved by the Food and Drug Administration (FDA) as a standard therapy targeting CD19-positive B cell malignancies with significant clinical efficacy.

Indications of clinical effect in certain solid cancer types have been reported but a major clinical breakthrough for gene-modified TCR/CAR-T cells is still awaited.

Declaration of Interest:

Dr Svane has reported honoraria/consultation fees for Roche, Novartis, Merck, Merck Sharp & Dohme, Celgene, Incyte, Pfizer, Bristol-Myers Squibb, AstraZeneca, TILTh Bio and IO Biotech. Dr Svane is a co-founder of IO Biotech.

Dr Donia has reported no potential conflict of interest.

Dr Met has reported no potential conflict of interest.

Further Reading

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1.3 Vaccines (Dendritic Cell Vaccines, Peptide Vaccines, DNA Vaccines, RNA Vaccines, Oncolytic Viruses)

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Definition (Introduction to the Concept and Development of Cancer Vaccine Strategies)

Active immunotherapy with cancer vaccines aims to instruct the host immune system to recognise cancer as a foreign ‘non-self’ tissue and mount specific immune responses that eliminate malignant cells. Malignant diseases typically evolve by evading anti-tumour immunity, and cancer vaccines aim to (re-)establish immune responses against tumour-associated antigens (TAAs) and turn cold tumours (few or no spontaneous tumour infiltrating lymphocytes [TILs]) into hot tumours (many TILs). Cancer vaccines may induce *de novo* immune responses, by stimulating tumour-specific T cells from the naïve repertoire, and/or boost existing suboptimal responses by providing new/stronger antigenic stimuli.

In recent years, it has become evident that successful cancer vaccines need to meet a number of important criteria.

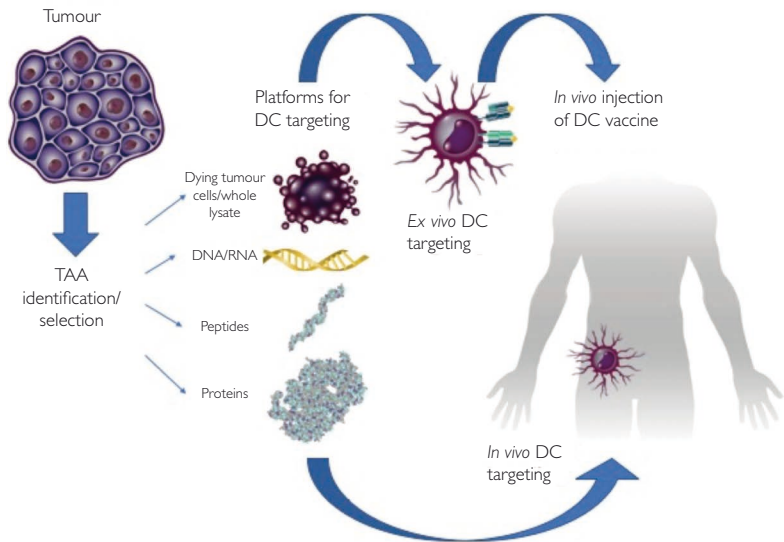
First, selection of TAAs, for which the T cell repertoire is not blunted by central immunological tolerance (i.e. thymic negative selection), is warranted (Figure 1). This restricts immunogenic TAAs for optimal vaccines to two main categories of ‘non-(or distant-from)-self’ antigens: oncogenic virus antigens and mutation-based neoantigens, for which cognate precursor T cells should exist in the human repertoire. Vaccination against such neoantigens was only recently successful in patients with melanoma and fits with the observation that neoantigen load in patients with different types of cancers (melanoma, lung and renal cell carcinoma, and microsatellite instable tumours) is strongly associated with the success of immune checkpoint blockade therapy (anti-cytotoxic T-lymphocyte antigen 4 [CTLA-4] and anti-programmed cell death protein 1 [PD-1]). These findings have added tumour-specific neoantigens to the list of candidate antigens to target in cancer vaccines and ushered in a new era for cancer vaccine design. We can now develop personalised vaccines targeting tumour-associated neo-epitopes, identified based on a genome-wide analysis of tumour-specific expressed mutanome.

Second, selection of the vaccine platform that delivers sufficiently concentrated antigens to vaccine-draining lymph nodes (LNs) for dendritic cell (DC) presentation to both CD4⁺ and CD8⁺ T cell precursors in the absence of antigenic competition with irrelevant sequences is also key (Figure 1). Successful vaccine platforms include DNA, RNA and synthetic long peptides (SLPs), consisting respectively of concentrated nucleic acids encoding TAAs or peptides harbouring the T cell epitopes themselves. These platforms can be used via direct injection or loaded onto DCs. Recombinant virus platforms can also be used, but suffer from induction of T cell responses against vector sequences, causing antigenic competition with the inserted TAAs. Recombinant protein vaccines have not been successful, mainly because of the inefficient nature of exogenous protein processing in DCs, resulting in the induction of weak CD4 T cell responses in the absence of CD8 T cell responses.

Third, vaccines need strong adjuvants. RNA and DNA vaccines have built-in adjuvants, whereas SLP vaccines need to be supplemented with an appropriate adjuvant, such as Montanide-ISA-51, poly I:C (toll-like receptor [TLR] 3 ligand), CpG (TLR9 ligand) and stimulator of type I interferon (IFN) pathway (e.g. STING agonists).

Fourth, experience with therapeutic cancer vaccines shows the advantage of their use in combination with immune-modulatory treatments that counteract the immune hostile cancer microenvironment, such as standard chemotherapy or checkpoint blockade (Massarelli et al, 2018).

Figure 1 Variables in cancer vaccine design. Selection of (1) TAAs to target, (2) platform/adjuvant for TAA delivery to use, (3) DC targeting strategy (ex vivo or in vivo) are among the most important aspects to consider in cancer vaccine design



Abbreviations: DC, dendritic cell; TAA, tumour-associated antigen.

Essential Processes Involved

As discussed above, optimal cancer vaccines must combine the most adequate antigens, vaccine platform, adjuvants and immunomodulatory treatment. TAAs should primarily target DCs and proper DC maturation must be induced so that they traffic to secondary lymphoid organs and activate TAA-specific T cells. Both CD4⁺ and CD8⁺ T cells should be stimulated, as CD4⁺ T cells are needed to programme and sustain CD8⁺ T cell responses through DC signalling and also generate specific T cell memory. Each step and vaccine component is critical in the design of effective cancer vaccines; for example, suboptimal DC loading and/or maturation or pulsing of non-professional antigen-presenting cells (APCs) with TAAs may promote tolerance rather than tumour rejection. Some cancer vaccination protocols have incorporated more than a single adjuvant with the aim to better induce the desired anti-tumour immune responses and therapeutic effects (e.g. poly I:C plus Montanide with anti-NY-ESO-1 SLP vaccine).

The route of administration of vaccines is also crucial to efficiently target antigens to DCs *in vivo* and/or activate the T cell pool that can be more easily redirected toward the tumour site. In general, subcutaneous (s.c.) administration or delivery into DC-rich LNs have been preferred for peptides/DC-based vaccines, and intramuscular (i.m.) injection for DNA-based vaccines. In the latter case, the efficiency of vaccination was crucially improved by electroporation. To deliver liposome-encapsulated RNA vaccines, intravenous (i.v.) administration has been advocated, because these liposomes can reach numerous LN-DCs following i.v. administration. Further studies are needed to understand how to select the administration route of cancer vaccines to more efficiently direct T cells toward cancer tissues.

Finally, based on the way TAAs are delivered into DCs, cancer vaccines can be divided into two major categories:

1. Vaccines targeting DCs with TAAs *in vivo*, through direct injection of TAAs (via one of the above-mentioned platforms) together with DC maturation stimuli for *in vivo* DC antigen uptake (Figure 1).
2. Vaccines targeting DCs with TAAs *ex vivo*, through *ex vivo* DC pulsing

with TAAs (provided in different formats), and manufacturing of a cellular product that is ready to stimulate T cells upon *in vivo* injection (Figure 1).

Direct *in vivo* DC loading with TAAs is clearly the way forward, because *ex vivo* loading of DCs is laborious and expensive and requires patient-specific DC preparation. Moreover, direct *in vivo* DC-targeting by current therapeutic vaccines has become very efficient. Treatment with *ex vivo* loaded DC can be more relevant to show mode of action and proof of concept. However, multiple strategies in both vaccine categories have been tested in cancer patients. Here we summarise the development and therapeutic activity of those that led to the most relevant clinical results.

DC Vaccines

Ex vivo TAA-loaded DCs have been extensively used as a vehicle to vaccinate against cancer *in vivo* because of the crucial role of properly activated DCs in the initiation of effective T cell responses. This strategy requires DC generation/isolation and *in vitro* pulsing with TAAs in presence of the proper activating/maturing stimuli and ensures complete control of the DC product. The procedure may be further optimised to generate properly activated antigen-loaded DCs ready to traffic to LNs and stimulate specific T cells *in vivo*. One of the problems has been that *ex vivo*-activated DCs often do not traffic efficiently to LNs upon intracutaneous, s.c. or i.m. injection (see below).

Technical Procedures Involved

Variables associated with the design of DC-based vaccines are numerous and require precise consideration to maximise the therapeutic efficacy. These include:

- DC source or lineage
- Antigen-engulfing strategy
- Levels of DC maturation and/or activation
- Route of vaccine administration

In initial studies with DC-based vaccines, DCs were generated from CD34⁺ haematopoietic progenitors or, more commonly, from peripheral

blood (PB)-derived monocytes following incubation with a cocktail of maturation cytokines, typically granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin (IL)-4. More recently, circulating natural DC subsets pulsed *ex vivo* with TAAs have been tested as a vaccine, achieving even better therapeutic effects.

The form of antigens used to load DCs is a crucial aspect to consider when designing anti-cancer vaccination strategies, since it affects major histocompatibility complex (MHC) antigen presentation and thereby the induction of cytotoxic CD8⁺ and/or helper CD4⁺ T cell responses. Class I- or II-MHC-restricted peptides have been largely exploited to pulse DCs *ex vivo*. Although peptides can be easily synthesised under Good Manufacturing Practice (GMP) requirements, this logistical advantage is offset by the need to pre-determine patient-specific MHC-restricted tumour-derived peptides and the limited stability of exogenous peptide-MHC complexes. Use of recombinant antigenic proteins to load DCs, which allows DC physiological processing of the peptides, besides being restricted by limited access to clinical grade proteins, has the drawback of preferentially channelling peptides into the endocytic compartment, thus predominantly stimulating CD4⁺ T cell responses. Use of SLPs encompassing multiple MHC-I and MHC-II peptide specificity can potentially overcome these problems. As an additional strategy, loading DCs with dying tumour cells, whole-tumour lysate or exosomes or transfecting DCs with tumour-derived complementary DNA (cDNA) or messenger RNA (mRNA) has been used. These options allow for natural processing and selection of multiple epitopes (known and unknown, including unique mutant TAAs that are expressed by the individual tumour) for MHC presentation, but antigen cross-presentation efficiency is uncertain. Transfection of tumour cDNA or mRNA into DCs via electroporation has the disadvantage of channelling TAAs primarily into the MHC-I presentation pathway, thus limiting effective CD4⁺ T cell responses. Pulsing DCs with dying tumour cells generates epitopes for both CD8⁺ and CD4⁺ T cell cross-presentation. In addition, killing tumour cells for cancer vaccine preparation with agents causing immunogenic death provides additional immunogenic and danger signals for DC activation and maturation (e.g. heat shock proteins, calreticulin or

TLR ligands). However, by widening the spectra of TAAs against which immune responses are elicited, whole-tumour-proteome-based vaccines have an increased risk of inducing autoimmune reactions to self-antigens shared with healthy tissues.

The level of DC maturation/activation to reach *ex vivo* is also a critical factor. In DC-based vaccines, the goal is to partially activate antigen-loaded DCs and differentiate them only to the point that they acquire LN migratory capacity and become responsive to full-activation (licensing) stimuli *in vivo*, once they reach the LNs, where they can then prime cognate T cells.

Finally, the administration route of antigen-loaded DCs has to be considered for the induction of effective T cell immune responses. Although intranodal injection is the most efficient way to deliver DCs into secondary lymphoid organs, it is still unknown whether overloading this tissue with large amounts of DCs really increases immune responses in humans. Thus far, s.c. and intradermal injections have been the most widely used modalities to administer DCs, because of the easy access of DCs to the regional LNs and more efficient induction of protective immunological memory compared with i.v. DC delivery.

Great interest and effort have been directed toward DC-based vaccines to immunise against cancer and results from these studies have been instrumental in clarifying the mechanisms underlying efficacy of active immunotherapy against established tumours.

Clinical Results

Numerous DC-based vaccines have been tested in clinical trials, with promising safety and efficacy profiles. DCs loaded with TAA cocktails achieved clinical benefit associated with tumour-specific immune responses in advanced melanoma patients and confirmed the relevance of co-activating CD4⁺ T helper cells to promote cytotoxic CD8⁺ T cell responses in patients. The most relevant example in this vaccine category is Sipuleucel-T for the treatment of hormone-refractory prostate cancer patients. This vaccine has been the first active cancer immunotherapy to gain regulatory approval from the Food and Drug Administration (FDA)

upon demonstration of a median survival improvement of 4.1 months and a death risk reduction of 22.5% in a randomised, double-blind phase III trial. Sipuleucel-T is generated by culturing autologous PB mononuclear cells (PBMCs) with the prostate cancer antigen prostatic acid phosphatase (PAP) fused to GM-CSF as an adjuvant for APC antigen uptake and activation. This strategy partially reduces the difficulties associated with DC-based vaccine production, as it does not require the generation of DCs and instead uses APCs naturally present in patients' PBMC samples. This procedure has been demonstrated to increase intra-tumour infiltration of PAP-specific T cells. Sipuleucel-T is currently under investigation as a monotherapy or in combination with hormone therapy in prostate cancer patients in phase II and III trials. A similar strategy was previously employed to immunise cancer patients against mutant *KRAS* and *p53*. In this case, SLPs encompassing patient-specific mutations in *KRAS* and *p53* were used to pulse patient-derived PBMCs, which were administered i.v. *in vivo* upon irradiation. This strategy, which somehow pioneered the current concept of vaccination against tumour-associated neoantigens, showed impressive clinical results in patients developing specific immune responses.

DC-based whole-tumour cell vaccines also demonstrated clinical efficacy in advanced cancer patients. As an example, autologous DCs pulsed with whole lysate from three allogeneic melanoma cell lines (TRIMEL) induced immune activation associated with increased survival and disease stabilisation in most of the treated metastatic melanoma (MM) patients. Interestingly, in a phase II trial with MM patients, DCs pulsed with autologous melanoma-derived antigens showed superior anti-tumour activity compared with the same irradiated whole-tumour cells, further substantiating the key role of proper DC activation for efficient development of anti-tumour immunity. In a pilot study with 18 relapsed indolent B cell lymphoma patients, it was shown that potent anti-tumour activity coupled with multifaceted immune activation upon vaccination with autologous DCs pulsed with apoptotic bodies generated by inducing immunogenic cell death in autologous tumour cells (Di Nicola et al, 2009). Importantly, this whole-tumour cell-based vaccine could activate T cell responses against the malignant B cell-associated immunoglobulin idiotype in responder patients.

The use of autologous DCs transfected to express TAAs and immunostimulatory molecules has also shown therapeutic immune responses in clinical trials. Administration of autologous DCs modified with a pox vector encoding the TAA's carcinoembryonic antigen (CEA) and mucin 1 (MUC1) (PANVAC™) reduced recurrence and prolonged survival in tumour-resected disease-free colorectal cancer patients. Co-electroporation of DCs with different mRNA molecules encoding one melanoma-associated antigen (tyrosinase, MAGE-A3, MAGE-C2 or gp100), CD40L, CD70 and a constitutively active isoform of TLR4 (TriMixDC-MEL) safely and effectively induced tumour-specific CD8⁺ T cell responses in advanced melanoma patients. A fully personalised vaccine generated with autologous DCs co-electroporated with amplified tumour RNA and synthetic CD40L RNA (AGS-003) was tested in metastatic renal cell carcinoma in combination with the tyrosine kinase inhibitor sunitinib. This strategy was well tolerated and achieved durable responses associated with CD8⁺ T cell activation. A phase II trial comparing AGS-003 + sunitinib versus sunitinib alone is ongoing (NCT01582672).

Peptide Vaccines

Peptides derived from TAAs have been widely tested in clinical trials to immunise against cancer. The understanding of the minimal rules that allow peptides to bind to MHC-I opened up the design of peptide-based vaccines. MHC-I binds short peptides (8–10 amino acids long), whose N- and C-terminal residues serve to anchor the peptide-binding groove through hydrophobic interactions in a way that can be computationally predicted. MHC-II binds peptides with different length variants (11–30 amino acids long) and the prediction of MHC-II-restricted peptides is less efficient. In cancer vaccine manufacturing, both short peptides with high MHC-I binding affinity or long peptides encompassing multiple epitopes for both MHC-I and MHC-II binding have been exploited. The administration route of peptide vaccines is particularly relevant, as the peptides need to reach DC-rich sites for proper immunisation.

Vaccination with short peptide sequences, predicted to bind patient-specific MHC-I, has been tested in many clinical trials with poor success, for the following reasons:

1. Short peptides bind MHC-I exogenously on all nucleated cells, which causes peptides to bind to a large majority of cells that lack co-stimulatory molecules and may therefore induce antigen-specific tolerance.
2. Short peptides rapidly distribute throughout the body and may therefore lead to antigen presentation outside vaccine-draining LNs in the absence of an adjuvant.
3. MHC-I-binding peptides stimulate only CD8⁺ T cells, leading to suboptimal and short-lived CD8⁺ T cell activation in the absence of CD4⁺ T cell help.

In contrast, vaccination with SLPs has shown much more robust CD4⁺ and CD8⁺ T cell response induction against both cancer-testis antigens (such as NY-ESO-1) and viral antigens (such as human papillomavirus [HPV]16 E6/E7). SLP vaccination relies on SLP processing by professional APCs for antigen presentation in both MHC-I and MHC-II molecules. In the case of HPV16 E6/E7, the need for MHC characterisation/selection has been avoided by incorporating a set of 13 long peptides, representing the complete sequence of the oncogenic HPV16 proteins E6 and E7, ensuring that all possible CD4⁺ and CD8⁺ T cell epitopes in this sequence of 256 amino acids are processed by DCs in vaccinated patients. Indeed, all patients have been shown to respond to this SLP vaccine, so the use of SLPs does not necessarily require the prediction of patient-specific MHC-restricted epitopes and allows for incorporation of powerful adjuvants, such as TLR ligands, thus coupling DC targeting with simultaneous DC activation. However, potential competition for MHC binding among the different peptides incorporated into polyvalent vaccines must be considered. This may be avoided by injection of no more than six or seven SLPs in a single i.m. or s.c. site (Kenter et al, 2008).

Technical Procedures Involved

GMP production of SLPs followed by freeze drying and cryopreservation is needed for vaccination with SLPs. Shortly before vaccination, the

lyophilised peptides are dissolved in an appropriate solvent, mixed or emulsified with adjuvant and injected i.m. or s.c.

Clinical Results

A global analysis of all the studies with peptide vaccines carried out in MM patients at the National Cancer Institute (NCI) showed very limited therapeutic effects, with an overall objective response rate of 2.9%. Most, if not all, of these studies were unfortunately conducted with short peptides representing exact MHC-I-binding sequences, a suboptimal vaccine platform (see above). Moreover, most of these trials were performed with sequences derived from differentiation antigens or cancer-testis antigens, against which the T cell repertoire may be blunted by central tolerance. Also, in these studies no treatment with immunomodulators was applied, further reducing the chances of clinical benefit as argued at the beginning of this chapter. Peptide vaccines have continued to be assessed in patients, with some vaccines reaching evaluation in phase III clinical trials.

In MM patients, vaccines based on gp100 peptides and the water-in-oil adjuvant Montanide have been tested in phase III trials in combination with high-dose IL-2 or the anti-CTLA-4 antibody (Ab) ipilimumab. Not surprisingly, based on the use of short peptides and a possibly tolerant T cell repertoire, these vaccines did not show significant clinical improvement and the therapeutic effects were not associated with the development of gp100-specific immunity, suggesting that gp100 in that formulation is not an effective vaccine. Tecemotide, an anti-MUC1 lipopeptide-based vaccine, has been studied in advanced non-small cell lung cancer (NSCLC) patients. Results from a phase III study indicated improved clinical benefit in patients who had previously received concurrent chemoradiotherapy. However, evaluation of tecemotide in further phase III trials failed to confirm these results (NCT01015443, NCT02049151). GV1001, a vaccine based on a 16-amino-acid-long peptide encompassing the active site of human telomerase reverse transcriptase and GM-CSF, has achieved promising results in early phase studies with advanced NSCLC and pancreatic cancer patients. However, further development in phase III trials produced unconvincing results (NCT01579188). Again, telomerase is a self-molecule and specific

T cells may be thymically deleted and GM-CSF, although capable of attracting immature DCs, may not be an ideal adjuvant in this setting.

Immunisation against mutant oncoproteins or viral antigens with multi-epitope vaccines (either peptide mixtures or long peptides) seems to represent a more effective strategy based on results from early phase trials. Vaccination with mutant KRAS peptides and GM-CSF followed by surgical resection was effective in inducing specific immune responses and prolonging survival in pancreatic cancer patients. A vaccine based on a cocktail of HPV16 E6 and E7 synthetic peptides induced frequent regression in patients with premalignant high-grade vulvar intraepithelial neoplastic lesions in association with the induction of specific immune responses. The same vaccine proved ineffective and significantly less immunogenic in patients with recurrent cervical cancer, in agreement with the profound immunosuppression in the setting of established cancer. However, when this vaccine was combined with timed standard-of-care chemotherapy consisting of carboplatin and paclitaxel, excellent immunogenicity was observed in patients with recurrent or metastatic cervical cancer. The mechanism was shown to be depletion of immunosuppressive myeloid cells and synergy of the platinum compound with tumour necrosis factor alpha (TNF- α) released from tumour-specific T cells (van der Sluis et al, 2015; Welters et al, 2016). Recent data indicate that this combination therapy induces strong T cell responses against the vaccine associated with prolonged overall survival (Melief et al, in preparation). The same vaccine also doubled the overall response rate in patients with incurable HPV16⁺ oropharyngeal cancer when combined with the anti-PD-1 nivolumab (Marsarelli et al, 2018). Recently, a vaccine based on SLPs harbouring mutant sequences predicted to bind to MHC-I showed strong immunogenicity and reduced disease recurrences in melanoma patients with high recurrence risk following surgery. Interestingly, patients who relapsed responded to a subsequent anti-PD-1 treatment (Ott et al, 2017).

DNA Vaccines

DNA-based vaccines are a straightforward approach to immunise against TAAs. However, they are generally poorly immunogenic, and DNA encoding xenogenic antigens or antigens fused with adjuvant molecules

have been exploited to more efficiently break tolerance against TAAs. Accordingly, therapeutic DNA vaccines have achieved the greatest success in the treatment of virus-driven cancers, such as cervical intraepithelial neoplasia lesions caused by high-risk HPV16 or HPV18.

Technical Procedures Involved

Because DNA has to be transcribed to RNA and then translated into protein, a highly efficient procedure must be used to ensure that enough DNA reaches cells in which these processes occur. Electroporation and particle-mediated epidermal delivery (PMED) appear to serve this purpose. DNA vaccines use plasmids of bacterial origin and, as such, have the built-in adjuvant CpG, a TLR9 ligand.

Clinical Results

DNA vaccines delivered by electroporation have induced robust T cell responses to the E6 and E7 proteins of high-risk HPV16 and 18. Moreover, in a randomised phase II trial, more high-grade cervical epithelial neoplasia lesions regressed following HPV DNA vaccination than spontaneously (Trimble et al, 2015). Vaccination with xenogenic and human gp100-encoding plasmid DNA by means of either i.m. injection or PMED has also been demonstrated to be safe and capable of inducing specific T cell responses in melanoma patients (Ginsberg et al, 2010; Yuan et al, 2013).

RNA vaccines

RNA vaccines encoding TAAs are currently being developed in several laboratories. One particularly efficient RNA delivery platform is encapsulation in DC-targeting liposomes.

Technical Procedures Involved

Naked RNA is generally injected i.m. or into LNs, whereas RNA-encapsulated liposomes are injected into LNs or i.v. to achieve optimal loading and processing by DCs for T cell cross-presentation of the antigens. In addition, antigen-encoding RNA can be codon-optimised to increase protein production. RNA vaccines have built-in adjuvants such as TLR3 ligand and TLR7/8 ligand.

Clinical Results

A self-adjuvanted RNA-based vaccine (CV9103) encoding the antigens PSA, PSCA, PSMA and STEAP1 was well tolerated and induced immune responses against multiple epitopes in a phase I/IIa study with advanced prostate cancer patients (Kübler et al, 2015). Very recently, personalised neo-epitope-containing liposomal RNA vaccines generated specific immune responses in advanced melanoma patients (Sahin et al, 2017), similar to SLP vaccines (Ott et al, 2017).

Oncolytic Viruses

Oncolytic virotherapy, based on the use of different viruses, such as modified herpes viruses, as self-expanding bio-therapeutics that selectively infect and kill cancer cells while sparing normal tissues, represents a promising immunotherapeutic approach (Lichty et al, 2014). Killing of tumour cells upon virus infection generates a local inflammatory environment that results in tumour antigen release and recruitment of immune cells, which in turn contribute to the amplification of anti-tumour immunity.

Technical Procedures Involved

This approach couples the direct anti-tumour cytotoxic effects of viruses replicating within malignant cells and the induction of an anti-viral immune response, which is expected to drive immune effector functions toward the tumour site, thus increasing the probability of a reaction against the released TAAs. To improve the immunological activity of oncolytic viruses, they can be engineered to express pro-inflammatory molecules as immune adjuvant.

Clinical Results

T-VEC (talimogene laherparepvec), a genetically engineered herpes simplex virus to express GM-CSF, was the first oncolytic virus therapy approved by the FDA for the treatment of unresectable MM, based on results of the OPTIM trial that compared T-VEC with GM-CSF in patients with advanced unresectable melanoma.

Coxsackievirus A21 (CVA21), a picornavirus with oncolytic potential, has been tested for the treatment of different solid cancers and has shown both local and distant clinical responses (Andtbacka et al, 2015), further demonstrating the therapeutic potential of *in situ* vaccination with oncolytic viruses.

Predictive and/or Prognostic Biomarkers of (Potential) Clinical Relevance

Thus far, the following parameters have been found to correlate with clinical activity of anti-cancer vaccines:

- **Tumour burden:** patients with less-advanced diseases are more likely to benefit from active immunotherapies (Hale et al, 2012)
- **Immune responses, in particular T cell responses:** clinical trials with therapeutic cancer vaccines have shown that development of anti-tumour immune responses correlates with improved clinical outcomes (Constantino et al, 2017)
- **Immune gene signatures (e.g. IFN- γ response pathway),** as a measure of immune activation that takes into account the complex molecular network of the tumour immune microenvironment
- **Baseline expression level of vaccine-targeted antigen and MHC molecules in tumour cells:** target antigens must be expressed and properly presented on MHC in tumour cells for efficient T cell recognition and killing of malignant cells

Potential Future Developments

Clinical results from phase III trials with cancer vaccines have, overall, shown limited clinical benefit. The flaws in vaccine design and lack of co-treatment signalled above are likely to have contributed to this.

Apart from these previously discussed flaws in cancer vaccine design and application, efficacy of appropriate cancer vaccination/immunomodulation can be thwarted by additional tumour immune evasion mechanisms:

- Down-regulation or loss of TAAs and MHC molecules in tumour cells

- Immunosuppression (indoleamine 2,3-dioxygenase [IDO], myeloid-derived suppressor cells [MDSCs], M2 macrophages and regulatory T cells [T_{regs}])
- Poor intra-tumour T cell infiltration
- Tumour-specific T cell anergy/exhaustion
- Up-regulation of immune checkpoints and specific ligands in the tumour microenvironment (CTLA-4, PD-1, programmed death-ligand 1 [PD-L1], T cell immunoglobulin and mucin domain 3 [TIM-3], lymphocyte-activation gene 3 [LAG-3], etc.)

Nevertheless, the recent clinical successes with immune checkpoint blockade therapy have formally demonstrated that immunotherapy can cure patients with advanced cancer. These results may be attributed to concurrent inhibition of key molecules regulating immunosuppression (CTLA-4 and PD-1 pathway) and activation of multiple immune responses against non-self-mutant tumour-associated neoantigens. This information has provided renewed interest in pursuing similar or even better results with active immunotherapy. Based on the novel insights obtained from a variety of research lines, including treatment with checkpoint blockade, chemo-immunotherapy, IDO inhibition, T_{reg} depletion and use of beneficial immunomodulators, we can now rationally improve cancer vaccine design toward increased anti-tumour efficacy based on the following directions:

- 1. Personalised vaccines against tumour-associated neoantigens (to avoid central tolerance-mediated elimination of specific high-affinity T cells).**

Immune peptidome analysis of MHC-bound peptides, tumour whole-exome and RNA sequencing coupled with computational prediction of immunogenic peptides binding to patient-specific MHC haplotypes can be used to identify new non-self tumour-specific antigens for improved cancer vaccine design. Initial clinical results are promising, and this personalised immunotherapy approach might soon become an affordable reality in clinical practice (Ott et al, 2017; Sahin et al, 2017).

- 2. Combination with immunomodulatory agents: develop rational combinations with strategies that reduce immunosuppression/activate tumour immunity in patients with established cancers.**

If neoantigen-based vaccines can bypass central tolerance, they still have to work against peripheral tolerance and tumour-immune escape mechanisms (exhaustion/anergy and immunosuppression). Therefore, it is very likely that, even when generated with neoantigens, cancer vaccines will not be able to achieve major clinical results as monotherapy. Strategies that inactivate the most important immunosuppressive mechanisms and/or stimulate cytotoxic response may be optimally combined with cancer vaccines. The increased availability of clinically approved agents with immunomodulatory functions offers new opportunities for optimal combination strategies to potentiate the efficacy of active immunotherapy. In addition, the progressive understanding of homeostatic mechanisms that control strength and duration of immune responses together with the renewed interest in assessing immune functions of conventional anti-cancer agents have led to the identification of many precision drugs that can favour immune activation, such as:

- **Immunogenic chemotherapy:** thalidomide, cisplatin; carboplatin + paclitaxel (van der Sluis et al, 2015; Welters et al, 2016).
- **Radiotherapy** (Finkelstein et al, 2012; Formenti and Demaria, 2009; Hasumi et al, 2011).
- **Checkpoint blockade:** preclinical evidence (Curran et al, 2010; Fu et al, 2014; Hurwitz et al, 1998; Mkrtichyan et al, 2013; Wada et al, 2013); clinical evidence (Hodi et al, 2003; Yuan et al, 2011).
- **Immunostimulatory Abs targeting TNFR family members:** CD40, OX40, GITR, 4-1BB (Tacken et al, 2007).
- **Abs against immunosuppressive cytokines or cytokine receptors.**
- **Pro-inflammatory cytokines** (γ C cytokines: IL-2, IL-7, IL-15).

Despite the availability of a myriad of new agents, there is a renewed interest in the use of vaccination as a first step to boost a robust anti-cancer immune response. *‘Proper integration of immunotherapeutic and anti-neoplastic approaches may thus be key to overcome these limitations and improve cancer control’* (Zappasodi et al, 2018).

Declaration of Interest:

Dr Melief is Chief Scientific Officer of ISA Pharmaceuticals, a biotech company developing synthetic long peptide vaccines. As such he receives a salary and participates in a management participation plan.

Dr Zappasodi has reported no potential conflicts of interest.

Dr Garassino has declared grants/supports from Merck Sharp & Dohme and Eli Lilly; advisory board/consultancy for: Inivata, Merck Sharp & Dohme, Boehringer Ingelheim, Bristol-Myers Squibb, Eli Lilly, Roche, AstraZeneca, Novartis, Celgene, MedImmune, Pfizer, Takeda and Ignyta.

Dr Di Nicola has reported no potential conflicts of interest.

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I.4 Biomarkers for Immunotherapy

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Introduction

Biomarkers are biological characteristics which are associated with behavioural properties of disease processes. For malignant diseases, biomarkers are either prognostic of disease outcome, independent of treatment or predictive of treatment response. In the context of immunoncology (IO), predictive biomarkers are the main focus but there are also relevant prognostic issues.

Biological Background for Biomarkers in Immunotherapy

There are multiple mechanisms and parameters that appear to determine the outcome of tumour–immune interactions. Some of these overlap, some appear distinct and there is considerable variation between different tumours and patients. An approach to rationalise the best way of determining how best to treat a patient based on current and plausible future biomarkers has been explored in a ‘cancer immunogram’, which proposes a dynamic approach in adding biomarkers as more data become available. Within this proposed approach there are seven broad categories where biomarkers are potentially found. These are summarised as follows:

- (1) The ‘foreignness’ of the tumour
- (2) Tumour inflammation
- (3) The presence or absence of immune checkpoints
- (4) Soluble immune inhibitors
- (5) Inhibitory tumour metabolism
- (6) The general immune status of the patient, and
- (7) The question of whether or not the tumour cells (TCs) are susceptible to a re-invigorated immune response

This is an excellent overview of what are a relatively limited number of trial-proven biomarkers currently in use, as well as a larger number of potential biomarkers which have still to find their clinical utility. This chapter focusses on biomarkers for the use of immune checkpoint inhibitors (ICIs) in solid tumours and the discussion will concentrate on those biomarkers which are already in widespread clinical use or for which there are emerging trial data. Most available data concern non-small cell lung cancer (NSCLC) and melanoma.

The immune system has the ability to recognise foreign, non-self antigens (epitopes) and mount a specific immune response through antibody-directed humoral and T cell-driven cellular immunity. Less antigen-specific responses also occur involving natural killer (NK) cells and macrophages, among other cell types. T cell- and macrophage-driven responses are very important in anti-tumour immunity. The most important source of neoantigens is probably through gene mutations, leading to the production of abnormal, new proteins. It is possible, however, that other aberrant metabolic events in TCs could lead to structurally abnormal proteins which may appear ‘non-self’ to the immune system. Many solid tumours have a high mutational burden, especially lung cancers and malignant melanoma, related to tobacco and ultraviolet light carcinogenesis. A high tumour mutation burden (TMB) infers, but does not guarantee, a high neoantigen load. High antigenicity infers the possibility of high immunogenicity, but this is also not guaranteed. These steps may fail in malignant cells for various reasons. There is evidence that when neoantigens are clonal rather than subclonal, they are more likely

to be associated with better outcome from immune checkpoint blockade (ICB); this infers more efficient antigenicity. Immunogenicity infers a specific immune response generated against these neoantigens but this may not occur.

Even if the immune response occurs, and the specific T cell immunity is available and present in the tumour, it may not be effective. A range of regulatory mechanisms (cellular or molecular) may control and negate the immune response at the point of efficacy in the tumour microenvironment (TME) – several of these are incorporated into the ‘cancer immunogram’, including checkpoints, soluble immune inhibitors and inhibitory tumour metabolism leading to an immune-suppressive microenvironment. Importantly, among these mechanisms are negative immune-regulatory checkpoints, ligand-receptor moieties which, when bound, switch off specific immune cell activity.

That clinically apparent tumours can evolve, while bearing a high cellular mutational load, highlights the existence of mechanisms which allow TCs to avoid antigen-specific T cell killing. As described, the reasons for this escape in any one tumour are potentially numerous. This creates the potential for several concurrent biomarker approaches, not only addressing why the tumour may escape an immune response, but also providing data on the likelihood of an immune response actually being available. Among these immune inhibitory mechanisms, the interactions of cytotoxic T-lymphocyte antigen 4 (CTLA-4)/CD80/86 and programmed cell death protein 1 (PD-1)/programmed death-ligand 1 (PD-L1) as negative immune checkpoints are important in a wide range of human solid tumours and are now also important targets for ICIs.

This simplified description of the tumour immune response and its regulation encapsulates its three essential components. These are:

- TC immunogenicity rendering the TCs visible to the immune system
- The successful generation of a specific cellular response to those immunogenic TCs, and
- Active mechanisms to escape, avoid or negate that immune response, either within the TME or lymphoid tissues

These three components also provide the rationale for biomarker approaches which have been most pursued in IO: TMB, tumour inflammation and immune checkpoint ligand expression, specifically the cellular expression of PD-L1. The other factors alluded to in the Immunogram could be considered as environmental factors. This does not mean to say that they are less important, but there is less trial-based evidence, so far, to support their use. Serum lactate dehydrogenase (LDH) levels are a surrogate for an immune suppressive tumour metabolic environment and may prove to be a valuable measure, especially in melanoma and response to anti-CTLA-4; measures of general immune status could be helpful but there is conflicting, and limited, evidence to support their use now. The use of immunohistochemistry (IHC) to determine PD-L1 expression within tumours is by far the most widely developed biomarker for ICI therapy and this will be considered first.

PD-L1 Immunohistochemistry

The only biomarker currently in widespread use for selecting patients for anti-PD-1 axis agents in the treatment of NSCLC is the expression of PD-L1 on the surface of TCs and the immune cells that infiltrate it. The current status of various drugs with their trial-validated assays is given in Table 1. These assays have also been used with variable benefit with various drugs in different indications in melanoma, head and neck squamous cell carcinoma, and urothelial and renal cell carcinoma.

These differences in the requirement for testing and of ‘cut-offs’ derive from trial data showing a variable relationship between level of PD-L1 expression and sensitivity to IO drugs. There is, nevertheless, in most trials a direct relationship between the level of PD-L1 expression, which is a biological continuous variable, and the likelihood of the tumour responding to an IO agent, although this is by no means a constant correlation and, as a result, no absolute consensus on the use of PD-L1 expression as a biomarker across tumour types and anti PD-1/PD-L1 agents has been achieved. The choice of cut-off is driven by a number of factors and will not create an absolute distinction between ‘responders’ and ‘non-responders’. Whether used as a companion or a complementary test, the outcome does provide clinically useful information.

Table 1 Approved and Investigational PD-L1 Diagnostic Assays in NSCLC

	Bioassay ^a	Cut-offs in clinical trials	PD-L1 level for 1 st line	PD-L1 level for 2 nd line	Diagnostic status	Approved <i>in vitro</i> diagnostic PD-L1 expression levels
Nivolumab	pharmDx 28-8	TCs ≥ 1%, ≥5% ≥ 10%	N/A	None	Complementary; testing not required 28-8: US/EU: NSQ NSCLC SP263: EU: NSQ NSCLC	US/EU: all patients eligible EU: all patients eligible
	Ventana SP263					
Pembrolizumab	pharmDx 22C3 Ventana SP263	TCs ≥ 1%, ≥50%	TCs ≥ 50%	TCs ≥ 1%	Companion; testing required US/EU: SQ and NSQ NSCLC	US/EU: ≥50% (previously untreated); ≥1% (previously treated)
Atezolizumab	Ventana SPI42	TCs + ICs – various ^{**}	N/A	None	Complementary; testing not required US: SQ and NSQ NSCLC	US: all patients eligible
Durvalumab	Ventana SP263	TCs ≥ 25%	N/A	N/A	Not approved for durvalumab in any setting	Not available
Avelumab	pharmDx 73-10	TCs ≥ 1%	N/A	N/A	Not approved for avelumab in any setting	Not available

* All assays score cells at any intensity.

Ventana SPI42 scored with the following cut-offs: **TCs: TC0 <1%, TC1 ≥1%–<5%, TC2 ≥5%–<50%, TC3 ≥50%; **ICs:** IC0 <1%, IC1 ≥1%–<5%, IC2 ≥5%–<10%, IC3 ≥10%.

Abbreviations: EU, European Union; IC, immune cell; N/A, not applicable; NSCLC, non-small cell lung cancer; NSQ, non-squamous; PD-L1, programmed death-ligand 1; SQ, squamous; TC, tumour cell; US, United States.

Implementing PD-L1 testing presents a number of challenges, which can be framed as four questions:

- Which diagnostic test should be used?
- How can accurate assessment be assured?
- Which types of specimen can be used?
- How should the results be reported?

Which diagnostic test should be used?

Each of the drugs currently available for treating NSCLC was developed with its own diagnostic test. It is impractical for laboratories to carry multiple testing platforms for essentially the same biomarker. Concerted efforts in harmonising these tests has led to some welcome rationalisation (Table 1).

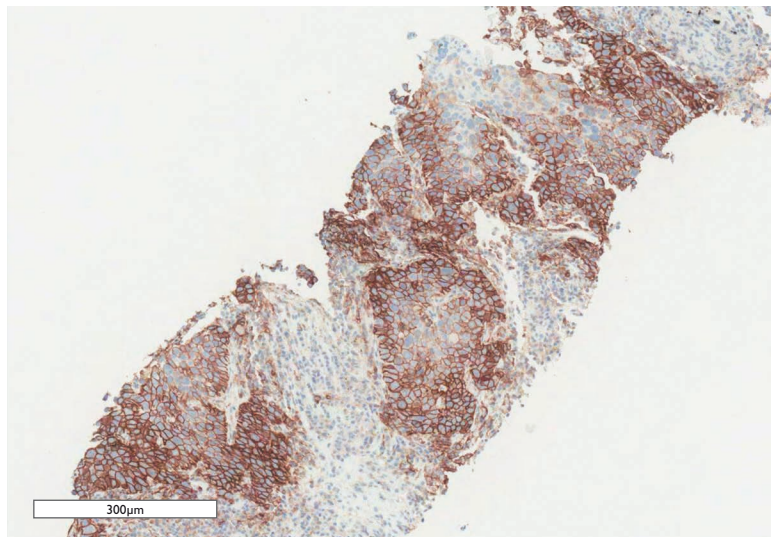
Studies reveal good correlation between the pharmDx 22C3, pharmDx 28-8 and Ventana SP263 assays, assessing expression on TCs. In contrast, the Ventana SP142 assay reveals a higher threshold for PD-L1 expression by TCs, but a higher sensitivity for immune cells. This assay can be positive through tumour or immune cell expression (Table 1). Comparative data for the pharmDx 73-10 assay are not yet available. The Ventana SP263 assay is applicable to guiding the use of nivolumab and pembrolizumab as well as of durvalumab, the drug for which it was originally developed.

How can accurate assessment be assured?

Assessing PD-L1 expression differs from most other IHC in its subtlety, heterogeneous expression and confounding staining patterns in many tumours. Relevant expression is the sometimes delicate delineation of the cell membrane (Figure 1). Cytoplasmic expression is ignored. For all assays except SP142, when used in NSCLC, only TCs are assessed – the tumour proportion score (TPS) is expressed as a percentage. The Ventana SP142 assay is more complex, since TPS and the area of tumour infiltrated by PD-L1-expressing immune cells are both assessed (Table 1).

Experience in reading PD-L1 tests in one tumour type will not necessarily be transferrable to other tumours, as the characteristics of both tumours and PD-L1 expression may differ significantly.

Figure 1 PD-L1 immunohistochemistry. In this example, where the tumour proportion score is well over 50%, some variation in intensity of membrane staining can be seen in tumour cells. In the surrounding stroma there are some PD-L1-expressing immune cells. Courtesy: JR Gosney.



Abbreviation: PD-L1, programmed death-ligand 1.

Which types of specimen can be used?

A major shortcoming in the development of tests for assessing PD-L1 expression in clinical trials was the exclusion of ‘cytology’ specimens. This led to the belief that only tissue biopsies can be used. There are anecdotal reports but little published evidence that alcohol fixation, commonly used in many cytology laboratories, is deleterious to PD-L1 epitopes. More data are required. Cytology is so integral to NSCLC diagnostics that it will be difficult not to use such samples. Cytology samples do not permit application of the immune cell staining rules for the SP142 assay.

Heterogeneity of PD-L1 expression within and between tumour deposits and the possibility of sampling error, leading to unrepresentative PD-L1 scoring, is well appreciated. It is usually impossible to avoid this potential problem in practice; generous or multiple biopsy specimens reduce its influence, but obtaining these is not always practicable or even possible.

Can pre-first-line chemotherapy (ChT) archived material be relied upon to provide a result accurate enough to guide second-line therapy? Clearly, the ideal specimen is the most recent, but re-biopsy is often not practical or ethical and, in clinical trials where this question was addressed, there was no significant difference between ‘fresh’ and ‘archive’ material use. As a general guide, there is evidence to suggest that archived specimens less than 3 years old are suitable for analysis if no more recent material is available.

How should the results be reported?

Reporting of PD-L1 expression should include objective data with its interpretation in context. It is important to state which diagnostic test assay was used and whether at least 100 TCs were assessed – some assays mandate a 50-cell minimum. An actual TPS is best reported although sometimes, if the sample is challenging, an indication of which range of TPS the sample falls into can be given.

Tumour Mutation Burden

The biological rationale for TMB or mutational load as a predictor of response has been discussed already. It is a surrogate for tumour immunogenicity as it reflects neoantigen load.

There are several ways of assessing tumour mutational load. It may be measured directly, through whole-exome sequencing, and expressed as mutations per Mbase in DNA. It may be inferred by the number of mutations found after large, targeted panel next-generation sequencing, an approach which correlates with actual ‘whole’ mutational burden. Specific factors associated with a higher likelihood of high mutational prevalence, such as loss of function mutations in mismatch repair (MMR) genes, microsatellite instability-high (MSI-H) and DNA polymerase D1 (*POLD1*) and E (*POLE*) mutations, are interesting candidate biomarkers, and have shown very effective enrichment for response to ICIs in some tumour types such as colorectal and endometrial carcinomas. Common mutations in NSCLC such as in *KRAS* and *p53* seem to be associated with more highly mutated disease. These, in turn, are associated with, in lung cancer, a smoking history, and both this and evidence of tobacco carcinogen-associated transversions are also biomarkers of interest.

TMB can predict response to the CTLA-4 inhibitor ipilimumab in melanoma, the PD-1 inhibitors pembrolizumab and nivolumab in NSCLC and the PD-L1 inhibitor atezolizumab in NSCLC and urothelial carcinoma. MMR and MSI-H can select patients for benefit from PD-1 axis inhibitors in a range of tumours including gastric and colorectal cancers, while MSI-H has been approved for the use of pembrolizumab by the Food and Drug Administration (FDA) as a biomarker independent of tumour type. *KRAS* mutation and a smoking history are associated with benefit from second-line nivolumab versus docetaxel in lung adenocarcinoma, while epidermal growth factor receptor (*EGFR*) mutations, which are associated with a low TMB and a never smoking history, favour docetaxel.

There is no consensus about what defines a ‘high’ TMB. It has been defined in a variety of different ways in different trials. The more genome that has to be sequenced to estimate TMB, the less practical the biomarker appears, at least with current technology, in terms of turnaround time, cost and possible feasibility. The prospect of a small number of targeted mutations to predict high TMB is very attractive, but this surrogate for actual TMB is likely to be different depending on tumour type. MSI-H is relatively frequent and an established biomarker in colorectal carcinoma but is extremely uncommon in NSCLC, for example. *p53* and *KRAS* mutations are common in lung adenocarcinomas but not so in melanoma. TMB or a surrogate has the potential to select patients who might benefit from a range of ICIs which might be used currently or in the future. It does not, however, inform about the actual inhibitory mechanism(s) active in an individual tumour, which might be targeted by appropriate therapy.

There is also another potential aspect to the mutational landscape in tumours with respect to immunotherapy. While a high mutational burden may present a relatively poor prognostic factor in many tumour types, there is also emerging evidence that genomic alterations can predict the possibility of hyper-progression of disease during PD-1/PD-L1 blockade. Data are few but *MDM2/MDM4* amplification and some *EGFR* alterations have been associated with risk of hyperprogression on immunotherapy. The molecular mechanisms and predictors of this phenomenon require further investigation.

Tumour Inflammation

Immunotherapy using ICB will not work if there is no immune response available to be released from an inhibited state. It is therefore completely intuitive that evidence of this immune response could be a useful biomarker to predict response to such drugs. It is important that good prognostic effects do not confound any predictive effect that a tumour inflammation biomarker might have in the context of ICB. Tumour inflammation has been assessed in many ways, from simple assessments of tumour infiltrating immune cells (TIICs – mostly lymphocytes and macrophages), microanatomical TIIC localisation in the tumour, and detailed characterisation of these cells – CD3⁺ and CD8⁺ T cells, macrophages (CD68⁺), myeloid-derived suppressor cells (MDSCs), inhibitory FoxP3⁺ T cells and Langerhans antigen-presenting cells (S100⁺). The ‘immunoscore’ assessing CD3⁺ and CD8⁺ T cells in the tumour core and invasive margin has bettered Tumour Node Metastasis (TNM) stage as a prognostic factor, considered as a predictive biomarker in melanoma, gastric and breast cancer among others.

There is already evidence in melanoma that the effects of nivolumab and ipilimumab are greater in inflamed tumours. Similar studies are ongoing in breast, colorectal, urothelial and other cancers including NSCLC. In melanoma and NSCLC, immune-related gene expression signatures using mRNA profiling on tumour samples have been used with some success as a way of enriching for ‘inflamed tumours’. Data have been published on a number of mRNA-based expression signatures of genes related to the activity and regulation of the immune response; the interferon-gamma (*IFN-γ*) gene has been a consistent member of these panels. These assessments have shown predictive value for response to pembrolizumab, nivolumab and atezolizumab in different settings. In the IMpower150 trial (first-line ChT plus bevacizumab with or without atezolizumab in NSCLC), however, direct comparison of a three-gene signature (*PD-L1*, *IFN-γ* and *CXCL9*) and PD-L1 IHC showed there was no difference in terms of predictive power for outcome between these two biomarker approaches. In other tumour types, the immune cells are the focus of attention and more data are awaited. Another important factor, in terms of actual adoption in the clinic, will be the practicality of

implementation. If complex and expensive biomarker approaches are no better than simple ones, it will be hard to justify their use.

Future IO Biomarker Strategies

Despite the interest in combining CTLA-4 or PD-1/PD-L1 inhibitors with other immunotherapies or with ChT to improve efficacy and patient inclusivity, it seems likely that biomarkers will continue to be required to select patients in at least some indications. The CheckMate 026 trial is the first indication, at least in NSCLC, that combining biomarkers may improve patient selection. This makes sense and combined biomarker approaches could be used, provided there is clinical validation and the assays are practical.

New checkpoint targets such as T cell immunoglobulin and mucin domain 3 (TIM-3) and lymphocyte-activation gene 3 (LAG-3) and other regulators such as indoleamine 2,3-dioxygenase (IDO) are both potential targets and biomarkers for therapy, alone or in combination. These and established target biomarkers such as PD-L1 may be considered primary IO biomarkers, since they represent an immune inhibitory mechanism which is being targeted. If the biomarker is truly absent and not active in the tumour, these therapies are very unlikely to work. Biomarkers such as TMB and inflammation are secondary IO biomarkers whose predictive power is likely independent of the drug/target being considered. They only predict a probability of an available immune response that might be activated by blockade of inhibitory mechanisms. Emerging biomarkers such as the gut microbiome, general immune status and an inhibitory tumour metabolic environment could be seen as over-arching conditional factors which may offset an effect of treatment, despite more specific biomarkers suggesting checkpoint inhibition should work. More data are required to allow us to understand how these additional factors should be incorporated into any decision-making algorithm.

Conclusion

The need for biomarkers for IO will continue. Due to the multifaceted nature of the tumour immune response, a single biomarker in this arena is unlikely to satisfy clinical requirements for high selective performance.

Dissatisfaction with biomarkers to date (mainly PD-L1 IHC) is somewhat unjustified and reflects unrealistic expectations, but has driven attempts to find alternatives. For different tumour types and different drugs, these biomarkers are also likely to be variable. Although not perfect, the most commonly used IO biomarker is PD-L1 IHC. This is relatively easy to measure and building on this moderately performing biomarker should be the way forward.

Declaration of Interest:

Dr Gosney is a paid advisor to and speaker for Abbvie, Agilent, AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, Diaceutics, Lilly & Co, Merck Sharp & Dohme, Novartis, Pfizer and Roche.

Dr Haragan has reported no potential conflicts of interest.

Dr Laing has reported no potential conflicts of interest.

Dr Kerr is a paid advisor to and speaker for Abbvie, AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, Lilly & Co, Merck Sharp & Dohme, Merck Serono, Novartis, Pfizer, Roche and Roche Diagnostics.

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I.5 Resistance to Immunotherapy

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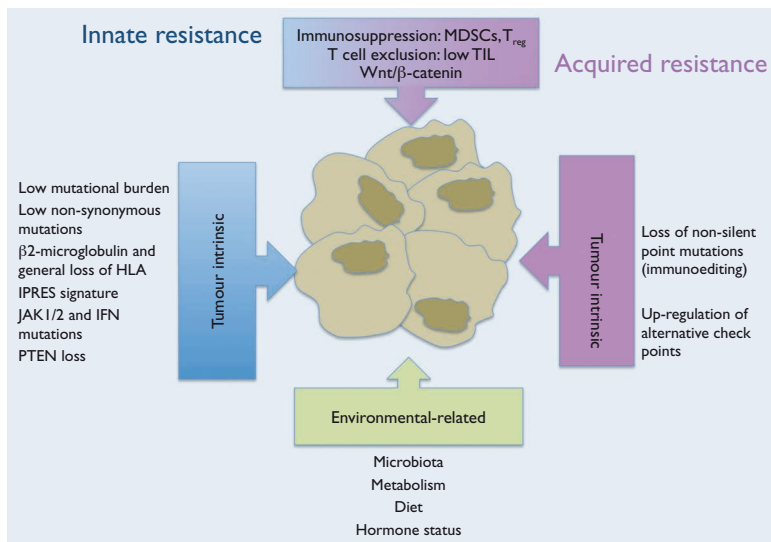
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Introduction

Although the immune system is efficiently armed to eliminate tumours viewed as 'altered self', immune responses to developing tumours are often modulated or suppressed. The main goal of immunotherapy is to induce and boost the ability of immune cells to destroy cancer. This can be achieved using different strategies such as cancer vaccines, adoptive transfer of tumour infiltrating lymphocytes (TILs), chimeric antigen receptor (CAR)-T cells and immune checkpoint inhibitors (ICIs).

ICIs have demonstrated efficacy in many tumour types including metastatic melanoma and advanced non-small cell lung cancer (NSCLC), also showing durable clinical response. However, the majority of patients show resistance to ICIs. Such resistance may be either innate, if they never responded, or acquired, if it follows an objective tumour response (Figure 1). This resistance can be further subdivided into intrinsic, if elicited by the tumour itself, or extrinsic, when resulting from the interaction with normal stromal cells in the tumour microenvironment (TME). Environmental factors such as microbiota, diet, hormone levels and metabolism can further contribute in establishing ICI resistance.

Figure 1 Mechanisms of resistance to ICI immunotherapy.



Abbreviations; HLA, human leukocyte antigen; ICI, immune checkpoint inhibitor; IFN, interferon; IPRES, innate anti-programmed cell death protein 1 resistance; JAK, Janus kinase; MDSC, myeloid-derived suppressor cell; PTEN, phosphatase and tensin homologue; TIL, tumour infiltrating lymphocyte; T_{reg} , regulatory T cell.

Tumour-intrinsic Mechanisms of ICI Resistance

Innate Mechanisms

The impact of the mutational status on ICI resistance

The tumour mutation burden (TMB) is a quantitative measure of the total number of mutations per coding area of the tumour genome, and is considered a new clinical marker able to predict response to immunotherapy. In this context, recent advances in sequencing technology have led to the identification of thousands of somatic mutations in single cancer samples, and the definition of hyper-mutated (melanoma and NSCLC) versus low-mutated (breast, pancreas) tumour types. In essence, tumours with high TMB are those caused by exposure to mutagens (such as ultraviolet radiation for melanoma or cigarette smoke for lung cancer),

or characterised by defects in DNA damage repair machinery. The idea is that tumours with the highest number of somatic mutations are likely to be enriched in neoantigens, and therefore potentially more immunogenic.

According to this hypothesis, the best response rate to ICIs is observed in tumours with high frequencies of somatic mutations, such as melanoma and lung cancer. In melanoma, the clinical benefit of cytotoxic T-lymphocyte antigen 4 (CTLA-4) blocking has been associated with the neoantigen repertoire. Indeed, Snyder et al (2014) identified a neoantigen signature that was specifically present in patients who strongly responded to CTLA-4 blockade. The identification of immunogenic peptides and evaluation of the corresponding antigen-specific T cell response is complex and unlikely to be routinely applied, due to the high costs, high technology skills and facilities required. In brief, the tumour is analysed for the presence of non-synonymous somatic mutations in expressed genes by exome sequencing. An algorithm is then used to predict proteasome processing and human leukocyte antigen (HLA)-class binding, allowing the identification of epitopes likely to be recognised by CD8⁺ T cells. High affinity predicted peptides are synthesised, and TILs or peripheral blood CD8⁺ T cells from the patient are tested for reactivity through a tetramer-based staining strategy. This approach allows monitoring of antigen-specific T cell activity at different time points: before, during or after treatment.

As for anti-CTLA-4 immunotherapy, the mutational load also influences the sensitivity to programmed cell death protein 1 (PD-1) blockade. Available clinical data indicate a positive correlation between somatic mutation frequency and the clinical benefit of PD-1/programmed death-ligand 1 (PD-L1) inhibitors, and tumours with the highest somatic mutation rate, such as mismatch repair (MMR)-deficient cancers (melanoma and NSCLC) showed the highest overall response rate. Whole-exome sequencing of NSCLC treated with pembrolizumab, nivolumab and atezolizumab showed, in independent cohorts, higher non-synonymous mutation burden associated with improved objective response, durable clinical benefit and progression-free survival (PFS). Results for the phase III clinical trial CheckMate 227, presented at the American Association of Cancer Research (AACR) meeting in April 2018, showed that in NSCLC patients with high TMB, nivolumab plus ipilimumab

provided improved benefit compared with chemotherapy (ChT) or anti-PD-1 monotherapy and yielded durable responses, sparing the use of ChT in the first-line setting.

Overall, studies on melanoma and NSCLC have shown that high mutational load and presence of antigen-specific CD8⁺ T cells are associated with improved overall survival (OS) in patients treated with ICIs. However, despite this favourable genomic landscape, most patients do not respond to immunotherapy, suggesting that the mutational load could be only one factor leading to effective patient response. Possible explanation stems from the fact that CD8⁺ T cell-dependent killing of cancer cells requires efficient presentation of tumour antigens by HLA-I molecules. Chowell and colleagues (2018) investigated whether germline HLA-I genotype influences T cell recognition of tumour peptides and response to ICI immunotherapies. In a large advanced cancer patient cohort (>1500) they found that heterozygosity at HLA-I loci was associated with better survival than homozygosity for one or more HLA-I genes. Maximal heterozygosity at all HLA-I loci ('A,' 'B' and 'C') improved OS after ICIs, compared with patients who were homozygous for at least one HLA locus.

Data showing that tumours characterised by relatively low mutational burden (such as renal cell carcinoma) can respond positively to immune checkpoint blockade (ICB) further support the idea that additional factors are likely to play an important role in modulating response to ICIs.

Somatic mutations in antigen presentation machinery genes

To avoid recognition and elimination by CD8⁺ T cells, cancer cells often harbour mutations in genes associated to major histocompatibility complex (MHC) class I presentation, such as β 2-microglobulin or transporter associated with antigen processing (TAP). Alternatively, the lack of MHC molecules can be associated with the partial loss of chromosome 6 that harbours MHC class I and class II genes. HLA loss variants can appear at different phases of tumour progression. Indeed, cell lines established from the same melanoma patient at metastatic stage IV of disease exhibited partial or complete HLA loss, not detectable in cell lines established earlier at stage III. Consistently, Sucker et al (2014) showed that earlier

lesions were infiltrated by higher numbers of T cells, and corresponding cell lines showed higher T cell stimulatory capacity compared with those derived from stage IV metastases. Such HLA loss may result in escape variants no longer recognised by specific CD8⁺ T cells, and represents the main obstacle in overcoming immunotherapy resistance. Additionally, Chowell et al (2018) showed dependency of ICB responsiveness on HLA heterozygosity with diminished response in case of loss of HLA heterozygosity.

Presence of specific transcriptome signatures

Hugo and colleagues (2016) demonstrated that although the TMB is associated with an improved OS in melanoma patients treated with anti-PD-1 immunotherapy, the mutational status was not able to predict the response to anti-PD-1 therapy, suggesting that other genomic or non-genomic features contribute to ICI response. A transcriptional signature known as IPRES (innate anti-PD-1 resistance) was found to characterise innate resistant tumours. Notably, this signature was under-represented in melanoma patients resistant to anti-CTLA-4 treatment, indicating that innate mechanisms of resistance to anti-PD-1 or anti-CTLA-4 immunotherapy are likely different. Nevertheless, the IPRES signature was found to be enriched in other anti-PD-1-resistant solid tumours, including pancreatic adenocarcinoma, clear cell renal cell carcinoma and lung adenocarcinoma. This signature comprises genes of the epithelial-mesenchymal transition (EMT), immunosuppressive genes, monocyte and macrophage chemotactic genes, and genes related to wound healing and neo-angiogenesis. The mechanisms responsible for EMT-induced immunosuppression are not clear but may be related to deregulated expression of immune checkpoints and alterations in cytokine and chemokine production/activities. A different composition of extracellular matrix (ECM) molecules is also able to influence the recruitment of immune-suppressive cells and/or to exclude T cell infiltration, and could be considered a possible mechanism for ICI resistance. Indeed, high-grade breast cancers with high expression of ECM molecules show a suppressive environment characterised by the enrichment in myeloid-derived suppressor cells (MDSCs) and the lack of T cells, together with EMT features.

Mutations in JAK1/2 and interferon signalling

Another intrinsic mechanism of ICI resistance, found in both melanoma and colon cancer, is the presence of homozygous loss-of-function mutations in the Janus kinases, JAK1 and JAK2, tyrosine kinases that are essential for interferon (IFN) intracellular signalling. In addition to JAK1/2 mutations, alteration in IFN signalling pathways, leading to anti PD-1 or CTLA-4 resistance, could also be linked to somatic mutations in other genes related to the IFN- γ pathway, such as the IFN- γ receptor (IFNGR)1 and IFNGR2 and interferon regulatory factor 1 (IRF1). The potential benefit of IFN- γ produced by T cells reaching the tumour site (i.e. direct tumour apoptosis, as well as increased antigen presentation by up-regulation of MHC class I molecules, and production of chemokines that attract T cells) would be lost by defective IFN- γ signalling. JAK1/2 mutations have also been found in other tumour histotypes, such as prostate and breast cancers. Defective JAK1/2 pathways, besides being caused by tumour pre-existing genetic mutations, may also derive from epigenetic silencing and are therefore considered an acquired mechanism of ICI resistance.

Although IFNs are important in the generation of an anti-tumour immune response, recent evidence indicates that in conditions of prolonged IFN signalling and antigen exposure, they may have immunosuppressive roles. For example, they are responsible for the up-regulation of PD-L1 and of other inhibitory pathways that may all contribute to ICI resistance. Indeed, it has recently been shown that persistent type II IFN signalling induces signal transducer and activator of transcription 1 (STAT1)-related epigenomic changes in tumours, and enhances expression of ligands for different T cell inhibitory receptors, contributing to the establishment of a resistance programme.

Phosphatase and tensin homologue loss

Phosphatase and tensin homologue (PTEN) loss commonly occurs in several cancers, including in approximately 30% of melanomas where it correlates with resistance to ICI immunotherapy. Knock-down of PTEN decreases the ability of T cells to kill tumour cells expressing the melanoma tumour antigen gp100. Moreover, silencing of PTEN reduces the ability of adoptively transferred T cells to kill melanoma tumours *in vivo*

when compared with tumours expressing PTEN. Melanoma patients with tumours that express PTEN generally achieved greater reduction of tumour size upon ICI treatment in comparison with patients with tumours not expressing PTEN.

Wnt/ β -catenin pathway activation

Aberrant regulation of the Wnt/ β -catenin pathway has been linked to cancer development and progression, more aggressive behaviour and worse prognosis in different types of cancers. For example, active Wnt/ β -catenin signalling has been reported in one-third of melanoma tumours; recently the laboratory of Thomas Gajewski (Department of Pathology, University of Chicago, IL, USA) provided an elegant demonstration of activation of Wnt/ β -catenin signalling contributing to the lack of T cell infiltration in melanoma. Using spontaneous mouse melanoma models, he identified the mechanism by which active β -catenin signalling in the tumour cells results in T cell exclusion and, consequently, resistance to anti-PD-1 or anti-CTLA-4 therapy.

Tumour-intrinsic active β -catenin pathway may therefore contribute to the ‘non-T cell inflamed’ tumour phenotype, alternatively referred to as ‘cold’, in contrast to ‘hot’ tumours, characterised by T cell infiltration.

The latter phenotype shows this positive prognostic value for different types of early-stage cancer. In the metastatic setting, it is generally associated with better response to different immunotherapies, including ICB, cancer vaccines and adoptive T cell transfer.

Acquired Mechanisms

Loss of non-silent point mutations

Although neoantigens represent attractive therapeutic targets, they also contribute to cancer immunoediting. This process involves T cell-selective pressure on cancer cells, which results in the selection of less immunogenic tumour cell clones, which are spared from T cell killing. As an example, the analysis of matched pre-treatment and resistant tumours from NSCLC patients identified genomic changes that resulted in loss of mutation-associated neoantigens in the resistant clones.

Tumour-extrinsic Mechanisms of ICI Resistance

Immunosuppression

Among extrinsic factors that can negatively impact on ICI-based immunotherapy, the generation of an immune-suppressive TME is one of the most relevant. Tumour immune suppression depends on the recruitment of a variety of immune cells, including MDSCs, dendritic cells (DCs), tumour-associated macrophages (TAMs) and regulatory T cells (T_{regs}), that through common or cell type-specific mechanisms suppress T cell recruitment and responses. Several pieces of evidence, mostly obtained from melanoma patients, suggest that high tumour infiltration by MDSCs is associated with poor prognosis and resistance to ICI therapy.

MDSCs are mostly characterised by the production of nitric oxide (NO) that, reacting with O_2 in the TME, allows the generation of different reactive nitrogen species (RNS). RNS modify, post-translationally, chemokines involved in T cell recruitment at the tumour site, including CCL2. Of note, nitrosylated CCL2 maintains its capacity to recruit immunosuppressive myeloid cells but fails to sustain T cell recruitment. MDSCs can also release the metabolic enzyme indoleamine 2,3-dioxygenase (IDO) that inhibits T cell expansion and promotes the conversion of naïve T cells into T_{regs} . On the same line, TAMs can also promote the immunosuppressive environment by acting on T_{regs} via immunosuppressive cytokines, such as interleukin-10 (IL-10) and transforming growth factor β (TGF β). Notably, the number of intratumoural T_{regs} could be responsible for resistance to anti-PD-1 therapy in tumours that would have been expected to positively respond to immunotherapy because they are enriched in infiltrating CD8⁺ T cells.

Emerging evidence also points to an indirect mechanism through which TAMs and myeloid cells can mediate ICI resistance. These cells can capture anti-PD-1 monoclonal antibody (mAb) from the surface of T cells with a mechanism involving Fc γ receptors (Fc γ R), dampening the efficacy of the antibody. On this line, Arlauckas et al (2017) demonstrated in mice that *in vivo* blockade of Fc γ R before treatment with anti-PD-1 mAb enhanced immunotherapy-induced tumour regression.

Generation of a Lymphocyte-excluded State

Effective immunotherapy relies on cancer cells being killed by cytotoxic T cells. Therefore, a relevant step in this process is the physical interaction between antigen-specific T cells, generated within the draining lymph nodes, and tumour cells. Different factors can impair the recruitment of T cells in the TME. Cancer cells and cancer-associated fibroblasts can also contribute to T cell exclusion through ECM fibre production and cross-linking. Tumours whose genetic programme includes ECM molecules showed a different enrichment in immune-related genes. ECM-rich high-grade breast tumours are impaired in the expression of genes related to natural killer (NK), T and B cells, which otherwise are enriched in their non-ECM rich, less aggressive counterparts. Conversely, tumours with a high collagen density are enriched in myeloid cells localised in close contact with tumour cells. It has been shown that T cells easily migrate in a loose collagen matrix, while, on the contrary, a dense collagen matrix hampers T cell migration. This suggests that the interaction between the ECM signature with immune and stromal signatures that are independently prognostic, *per se*, might be informative for patient selection for the most appropriate immune-based therapeutic approach.

Up-regulation of Alternative Checkpoint Pathways

Immunotherapy may affect the TME up-regulating alternative checkpoint pathways and therefore contribute to acquiring resistance to ICIs. This has been demonstrated for T cell immunoglobulin and mucin domain 3 (TIM-3), both in NSCLC patients and corresponding mice models. Similarly, T cells can up-regulate lymphocyte-activation gene 3 (LAG-3) and CTLA-4 after anti-PD-1 treatment.

Environmental Host Factors

Tumour Metabolism

Accumulating evidence suggests that the metabolic interplay between cancer and immune cells can play an important role in the regulation of the immune response and, consequently, in regulating response to immunotherapy. Such cross-talk is based on effector T cell and tumour cell use of the same metabolic pathway and their competition to obtain energy.

In the TME, the oncogenic mutations, hypoxic condition and/or the low uptake of blood-borne nutrient shift the metabolism from a mitochondrial oxidative phosphorylation towards an aerobic glycolysis (Warburg effect). A similar shift also occurs in lymphocytes upon activation. During activation, T cells reprogramme themselves from a mitochondrial to a glycolytic metabolism. As a result, in the TME, highly proliferating tumour cells would deprive T cells from environmental nutrients, leading to T cell energy/inactivation. Interestingly, a direct loop has been identified between PD-L1 and the change in metabolic pathway in tumour cells. Chang and collaborators (2015) showed that PD-L1 can directly trigger glycolysis and Akt activation in tumour cells while suppressing mammalian target of rapamycin (mTOR) activation in T cells through glucose deprivation. Checkpoint blockade with anti-PD-L1 antibodies reverted this condition by promoting T cell activation. It is not completely clear how blocking PD-L1 is sufficient to revert the metabolic pathway in the different cancer types, but a possible phosphorylation of the short cytoplasmic PD-L1 tail has been suggested. Like glucose metabolism, amino acid metabolism can impact T cell activation. For example, tumour cells can produce the enzyme IDO, which depletes the amino acid tryptophan in the TME, resulting in T cell inhibition. Finally, cancer metabolism can also be changed by DNA damage repair. DNA damage response has been shown to regulate metabolic pathways and MMR-deficient patients showed higher objective clinical responses to anti-PD-1 therapy compared with MMR-proficient patients. Overall, these data suggest considering dietary and pharmacological approaches targeting tumour metabolism for combination treatment in association to immunotherapy.

The Microbiota

Microbiota defines commensal bacteria with homeostatic functions that are present at mucosal sites. The microbiota can affect different aspects of tumour biology, including transformation and response to immunotherapy. Alterations in the microbiota can result from exposure to environmental factors (i.e. diet, toxins, drugs) and pathogens. Enteric pathogens have the greatest potential to induce microbial dysbiosis and to trigger local and systemic auto-inflammatory conditions that, in turn, can promote cancer development in the gut, but also in other extra-intestinal

sites (i.e. hepatocellular carcinoma and breast cancer). The mutual relationship between the gut microbiota and the immune system suggests the potential relevance of the gut microbiota in modulating host response to immunotherapy. Pioneering studies in murine models showed that antibiotic-mediated disruption of the microbiota impaired the effectiveness of CpG-based immunotherapy. In humans, some evidence suggests that the efficacy of CTLA-4 and PD-1 blockade depends on distinct *Bacteroides* species of the gut microbiota. Specific microbiota can also prevent the development of colitis, a common side effect of ICI immunotherapy.

Metagenomics, metatranscriptomics and culturomic platforms now provide the opportunity to determine the microbiome of a patient, opening the possibility of using probiotics, prebiotics and/or carefully selected antibiotics in preparation for subsequent ChT or immunotherapy alone or in combination.

Unmet Needs

Two of the most important unmet needs are the identification of predictive biomarkers of responsiveness, and the development of suitable response criteria. The need for biomarkers of response to immunotherapeutic agents relies on the great variability of responses to ICIs and, therefore, the difficulties of patient selection for appropriateness of care. One of the most widely used biomarkers to predict response to anti-PD-1 is the expression of PD-L1 on tumour cells detected by immunohistochemistry (IHC). However, contradictory results have been obtained regarding the role of PD-L1 expression as a marker of response, as in some cases tumours that were negative for PD-L1 did respond, whereas PD-L1-expressing tumours did not. Although the different criteria (cytoplasmic versus surface expression) and antibodies used to evaluate PD-L1 expression partially explain such unexpected results, the biological mechanisms behind this complex picture are still to be identified, but a match with the co-presence of PD-1-positive CD8 cells should probably identify the responding PD-L1-positive tumours.

Another unmet need is the definition of ICI response criteria. Considering their mechanisms of action, patient response to these treatments

cannot be measured with conventional criteria such as World Health Organization (WHO) or Response Evaluation Criteria In Solid Tumours (RECIST). In some cases, the initial response is characterised by an apparent increase in tumour size that, however, is not due to enhanced tumour cell proliferation but rather to increased T cell infiltration. This phenomenon, referred to as ‘pseudoprogression’, prompted the proposal of new response criteria in 2009, the immune-related response criteria (irRC). Although proven useful in some instances, several issues related to irRC such as tumour measurement (bidimensional versus unidimensional) and timing of response assessment (4-week window or longer timeframe) remain to be addressed. The reported cases of hyperprogression quickly following PD-1 or PD-L1 ICI treatment are cause for concern. The remarkable clinical and imaging worsening may affect roughly 20% of treated patients with epidermal growth factor receptor (*EGFR*) alteration and 4% of those with MDM2 family amplification, but other undefined mechanisms might be involved.

Finally, the many possibilities of combination therapies (with radio/ChT or immune co-stimulation) that could be given together or that require proper sequence should be carefully assessed using the adaptive trial design.

Declaration of Interest:

Dr Sangaletti has reported no potential conflicts of interest.

Dr Chiodoni has reported no potential conflicts of interest.

Dr Colombo has reported no potential conflicts of interest.

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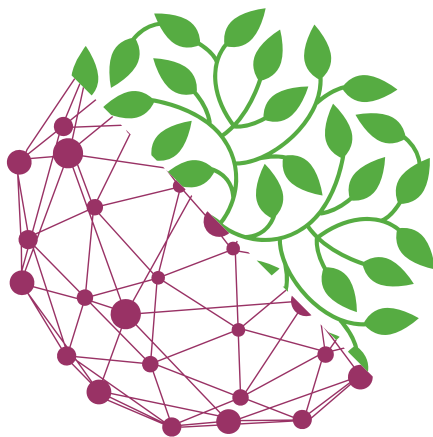
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Section 2:

Immunotherapies in
specific disease groups –
State of the art



2.1 Melanoma

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Overview of Immunotherapy in Melanoma

Recent advances in cancer treatment have ushered in a novel era of immunotherapy, providing new treatment options. Melanoma has always been described as an immunogenic tumour, and several immunomodulatory strategies have been tested. Nevertheless, chemotherapy (ChT) remained the standard-of-care (SoC) for metastatic melanoma (MM) until 2011, with response rates (RRs) of 15%–20%, albeit with no overall survival (OS) benefit. Before immune checkpoint inhibitors (ICIs), the only approved immunotherapeutic agent for MM was high-dose interleukin-2 (IL-2). With an overall response rate (ORR) of 16% and a median response time of 8.9 months, the main benefit of this treatment was the possibility of a sustained response, with 28% of patients showing no signs of progression after 5 years. IL-2 was, however, susceptible to induce severe treatment-related toxicities, and could therefore only be considered a treatment option for fit patients in specialised centres.

ICIs revolutionised the treatment of metastatic/unresectable melanoma, with several randomised clinical trials showing survival benefits, finally leading to the approval of the first immune checkpoints by regulatory authorities, FDA (Food and Drug Administration) and EMA (European Medicines Agency): the anti-CTLA-4 (cytotoxic T-lymphocyte antigen 4) antibody ipilimumab and subsequently the anti-PD-1 (programmed cell death protein 1) antibodies nivolumab and pembrolizumab.

Immunotherapy in Metastatic/Unresectable Melanoma

Anti-CTLA-4 Antibodies

Two pivotal phase III trials (CA 184-002 and CA 184-024) led to the approval of ipilimumab. In previously treated patients, ipilimumab 3 mg/kg was associated with an improvement in median survival of 3 months compared with gp100 vaccine therapy. More importantly, there was a tail on the survival curve, with some patients gaining a sustained benefit. A phase III trial compared ipilimumab 10 mg/kg in combination with dacarbazine (DTIC) versus DTIC alone in treatment-naïve patients. Median OS was significantly better in the combination group (11.2 versus 9.1 months), but, as the combination was associated with unexpectedly high liver toxicity, it was not submitted for approval. Ipilimumab treatment is associated with a substantial risk of immune-related adverse events (irAEs). In clinical trials, >80% of patients treated with ipilimumab reported adverse events (AEs): 10%–26% experienced grade ≥ 3 irAEs (enterocolitis in 34 [6.7%] patients, hepatotoxicity in 8 [1.6%], dermatitis in 13 [2.5%] and endocrinopathies in 9 [1.8%] patients). A pooled analysis of 1861 patients treated with ipilimumab with a maximum follow-up (FU) of 10 years revealed a 3-year OS of 21% and a *plateau* on the OS curve, representing the long-term responders subgroup. Based on these results, ipilimumab became an SoC in the first and subsequent lines settings.

The superiority shown by anti-PD-1 agents in clinical trials made them the drugs of choice as first-line immunotherapy, leaving ipilimumab (when not used in combination with an anti-PD-1) with an uncertain role in the treatment algorithm of melanoma.

An important question is the activity of ipilimumab after another first-line single-agent PD-1 therapy. A retrospective study of the KEYNOTE-006 trial (see below) showed an RR of 16% and 1-year OS of 68% in patients progressing on pembrolizumab and treated in second line with ipilimumab. Similarly, in the CheckMate 067 study (below), 26% of patients progressing on nivolumab received ipilimumab, contributing to the 3-year OS of 52% for the nivolumab arm.

Anti-PD-1 Antibodies

The clinical development of anti-PD-1 antibodies was a milestone in the treatment of advanced melanoma. Pembrolizumab and nivolumab are currently approved in this indication. Phase I and II trials showed significant clinical activity associated with these agents, with a favourable toxicity profile. In the phase III CheckMate 037 trial, nivolumab (3 mg/kg every 2 weeks [q2w]) was compared with investigator's choice ChT in previously treated patients who progressed on ipilimumab or BRAF inhibitors. ORR, the primary endpoint, was higher in the nivolumab group: 31.7% versus 10.6%. In first-line setting, nivolumab was compared with DTIC in *BRAF* wild-type melanoma patients in the CheckMate 066 trial. The primary endpoint was met, with a median OS not reached in the nivolumab group compared with 10.8 months in the DTIC group. Nivolumab was associated with a better progression-free survival (PFS) (5.1 versus 2.2 months) and ORR (40.0% versus 13.9%).

The phase III KEYNOTE-006 trial was designed to compare pembrolizumab with ipilimumab in checkpoint inhibitor-naïve patients. OS and PFS were co-primary endpoints of this study, and patients were randomised 1:1:1 to receive one of two schedules of pembrolizumab (10 mg/kg, q2w or q3w) or ipilimumab 3 mg/kg q3w. Results showed a median OS not yet reached in the pooled pembrolizumab arms versus 16 months in the ipilimumab arm, and a 2 year-OS also superior for the anti-PD-1 (55% versus 43% with ipilimumab). The co-primary endpoint of PFS was met, with a median of 5.6 and 4.1 versus 2.8 months for each pembrolizumab arm and ipilimumab, respectively, and ORR of 36% and 37% versus 13%, also favouring pembrolizumab.

Pembrolizumab and nivolumab have shown a significantly better safety profile compared with ipilimumab. The incidence of grade 3–4 treatment-related adverse events (trAEs) with PD-1 inhibitors ranges from 10%–16%, compared with 19%–27% with ipilimumab. The most common anti-PD-1-associated toxicities are fatigue, cutaneous toxicity (rash and pruritus), diarrhoea and endocrinopathies.

One of the important benefits of immunotherapy is the possibility of achieving sustained responses, due to activation of the immune system

leading to memory being established. This potential for prolonged clinical response also raises the question of how long to continue treatment in responding patients. The first glimpse of an answer came from the long-term results of the KEYNOTE-001 and -006 trials. In the latter, treatment was halted after 2 years of pembrolizumab. Results from the 104 patients who completed therapy and had a median FU of 9.7 months revealed that 98% were alive. The estimated PFS at this timepoint was 91% for the overall population, 95% for patients who achieved a complete response (CR), 91% for patients with partial response (PR) and 83% for patients with stable disease (SD). Trials are ongoing or planned in Canada and the UK to address this question directly.

Combination of Checkpoint Inhibitors

The exciting results achieved with single immune checkpoint agents led to the investigation of combinations of ICIs with different mechanisms of action (anti-PD-1 and anti-CTLA-4).

This approach was initially investigated in the phase II CheckMate 069 and in the phase III CheckMate 067 trials. The latter compared ipilimumab 3 mg/kg plus nivolumab 1 mg/kg or placebo for 4 cycles, followed by nivolumab 3 mg/kg q2w or placebo, versus single-agent nivolumab or ipilimumab 3 mg/kg. The trial was designed to compare the nivolumab arms with ipilimumab, but not to compare the nivolumab arms directly. ORR and median PFS were significantly higher for the combination arm (Table 1). At 2 years, the survival curves had separated, with an OS rate of 64% for the combination versus 59% and 45% for the single agents nivolumab and ipilimumab, respectively. A recent update showed a 3-year OS rate of 58% for the combination and 52% for the nivolumab arm. Subgroup analysis showed that the benefit for combination therapy was higher in younger patients, those with *BRAF*-mutated tumours and those with low programmed death-ligand 1 (PD-L1) expression. These results were, however, at the expense of high toxicity, with grade 3–4 AEs in 58% of patients treated with the combination. Based on these results, the combination regimen of ipilimumab plus nivolumab was approved by the regulatory authorities.

Table 1 Immunotherapy in Melanoma Clinical Trials

	Treatment naïve	ORR (%)	mPFS (months)	mOS (months)	1-year OS (months)	2-year OS (months)	3-year OS (months)	Grade 3/4 AEs (%)
Ipilimumab (10 mg/kg; q3w) + DTIC vs DTIC (850 mg/m ² ; q3w) (CA184-024; NCT00324155)	Yes	15.2	3	11.2	47.3	28.5	20.8	56.3
	No	10.3	3	9.1	36.3	17.9	12.2	27.5
Pembrolizumab (2 mg/kg or 10 mg/kg; q3w) vs ChT (KEYNOTE-002; NCT01704287)	Yes	21–25	2.9	13.4–14.7	–	36–38	–	11–14
	No	4	2.7	11	–	30	–	26
Pembrolizumab (10 mg/kg; q2w or q3w) vs Ipilimumab (3 mg/kg; q3w) (KEYNOTE-006; NCT01866319)	Yes	36–37	4.6–5.1	–	68–74	55	–	17
	No	13	2.8	16.0	59	43	–	20
Nivolumab (3 mg/kg; q2w) vs ChT (CheckMate 037; NCT01721746)	Yes	27.0	3.1	15.7	–	–	–	14
	No	10.0	3.7	14.4	–	–	–	34
Nivolumab (3 mg/kg; q2w) vs DTIC (1000 mg/m ² ; q3w) (CheckMate 066; NCT01721772)	Yes	40.0	5.1	–	72.9	57.7	–	11.7
	No	13.9	2.2	10.8	42.1	26.7	–	17.6
Nivolumab + Ipilimumab (1 mg/kg + 3 mg/kg; q3w x 4 → Nivolumab 3 mg/kg; q2w) vs Nivolumab (3 mg/kg; q2w) or Ipilimumab (3 mg/kg; q3w) (CheckMate 067; NCT01844505)	Yes	58	11.5	–	73	64	58	59
	No	44	6.9	37.6	74	59	52	21
	No	19	2.9	19.9	67	45	34	28

Abbreviations: AE, adverse event; ChT, chemotherapy; DTIC, dacarbazine; mOS, median overall survival; mPFS, median progression-free survival; ORR, overall response rate; OS, overall survival; qXw every X weeks.

Assuming that much of the toxicity seen with combination therapy was due to the use of full-dose ipilimumab 3 mg/kg, the combination of pembrolizumab and reduced-dose ipilimumab was investigated in the phase Ia-Ib KEYNOTE-029 study. Ipilimumab dose was reduced to 1 mg/kg and combined with 4 doses of pembrolizumab 3 mg/kg q3w, followed by pembrolizumab alone. This combination showed an incidence of grade 3–4 AEs of 42% and an ORR of 57% in the exploratory analysis. The results of a phase III study are expected soon.

Brain Metastasis

Metastatic disease in the central nervous system is present at diagnosis in approximately 10% of MM patients, leading to significant morbidity. Surgery and stereotactic radiotherapy are the main treatment for patients with small-volume metastases, with *BRAF*-directed therapy active in eligible patients. Recently, combined checkpoint inhibition has emerged as a new option for these patients. Compelling evidence from two phase II studies, CheckMate 204 and Anti-PD-1 Brain Collaboration (ABC), was recently presented and supports the combined use of nivolumab and ipilimumab in this subgroup, leading to favourable RRs with concomitant systemic control. Of note, both trials only included asymptomatic patients with brain metastasis.

The CheckMate 204 trial included steroid-free patients with at least one brain lesion. The primary endpoint was intracranial clinical benefit – a composite endpoint including CR, PR and SD for more than six months. The intracranial ORR was 56%, with 19% of patients achieving CR. Extra-cranial responses were largely concordant with intracranial responses and the six-month PFS rate exceeded 65%. Similarly, in the combination cohort of the Australian ABC trial, an intracranial benefit of 42% and a 15% CR rate were achieved.

Talimogene Laherparepvec

Talimogene laherparepvec (T-VEC) is an oncolytic herpes virus genetically modified to express granulocyte–macrophage colony-stimulating factor (GM-CSF). Intra-lesional injection results in both tumour destruction and recruitment of dendritic cells, leading to immune activation and

a distant effect. T-VEC was licensed based on the OPTIM study, which showed a durable response at 6 months compared with GM-CSF in patients with inoperable stage IIIc and IVM1a disease. Promising results have also been seen in combination with ICIs. The phase IB trial Masterkey-265 reported a confirmed ORR of 57.1% and an unconfirmed CR rate of 28.8% for T-VEC combined with pembrolizumab.

Adjuvant Immunotherapy in Melanoma

Until recently, the adjuvant setting presented a major gap in melanoma treatment. The treatment of choice in this setting used to be high-dose interferon-alpha (IFN- α) for stage IIB/III melanomas. Several trials (Eastern Cooperative Oncology Group [ECOG] E1684, E1690, E1694) showed a benefit in recurrence-free survival (RFS), but inconsistent results in terms of survival benefit. A meta-analysis of IFN trials showed a very modest impact on OS, and no clear impact of dose or duration of treatment. The FDA approved pegylated-IFN (PEG-IFN) for stage III melanoma patients, based on the European Organisation for Research and Treatment of Cancer (EORTC) 18991 trial in 1256 patients, which reported significant improvement in RFS in favour of PEG-IFN- α 2b. Updated results with a median FU of 7.6 years showed that the greatest benefit was seen in patients with microscopic nodal disease who had ulcerated primary tumours, and no benefit was seen in non-ulcerated tumours.

Ipilimumab was investigated in the adjuvant setting in two randomised phase III trials comparing it with placebo (EORTC 18071) and high-dose INF- α (ECOG 1609). In the EORTC trial, ipilimumab (4 doses of 10 mg/kg q3w, and then every 3 months for 3 years) showed favourable outcomes in RFS, distant metastasis-free survival (DMFS) and OS. Results evidenced a 5-year RFS of 40.8% versus 30.3%, and a 5-year OS rate of 65.4% versus 54.4% in favour of ipilimumab. Ipilimumab was associated with a high rate of toxicity, with 41.6% of grade 3–4 irAEs and five deaths. This trial led to the approval of adjuvant ipilimumab (10 mg/kg) by the FDA, but ipilimumab was not submitted to the EMA for approval.

More recently, the results of CheckMate 238 were reported (Table 2). This trial tested nivolumab versus ipilimumab for 1 year and included

Table 2 Adjuvant Immunotherapy in Melanoma Clinical Trials

	Duration of treatment	Minimum follow-up (months)	1-year RFS (%)	HR for relapse	3-year OS	HR for OS
Nivolumab (3 mg/kg q2w) vs ipilimumab (10 mg/kg q3w x 4 → q12w) (CheckMate 238; NCT02388906)	1 year	18	70.5 60.8	0.65	–	–
Pembrolizumab (200 mg q3w) vs placebo (KEYNOTE-054; NCT02362594)	1 year	–	–	0.57	–	–
Dabrafenib + Trametinib (150 mg bid + 2 mg qd) vs placebo (Combi-AD; NCT01682083)	1 year	30	88 56	0.47	86 77	0.57
Vemurafenib (960 mg bid)	1 year	–				
Cohort I (stage IIC, IIIA, IIIB) Vemurafenib vs placebo			84.3 66.2	0.54	–	–
Cohort II (stage IIC) Vemurafenib vs placebo (Brim-8; NCT01667419)			78.9 58	0.80	–	–

Abbreviations: bid, twice daily; HR, hazard ratio; OS, overall survival; qd, once daily; qXw, every X weeks; RFS, recurrence-free survival.

stage IIIB, IIIC or resected stage IV patients. The primary endpoint was RFS. Nivolumab showed a higher 12-month RFS rate (70.5% versus 60.8% with ipilimumab), which was significantly longer. The toxicity profile was also better with nivolumab, with 14.4% of AEs grade 3–4 versus 45.9% with ipilimumab. Nivolumab has recently been approved as an adjuvant treatment by the FDA. OS data are pending.

Another ongoing phase III clinical trial is evaluating adjuvant pembrolizumab versus placebo in stage III melanoma patients (EORTC 1325/KEYNOTE-054 trial). In April 2018, a press release reported that the study had met the primary endpoint with a hazard ratio of 0.57 for RFS with pembrolizumab (98.4% confidence interval: 0.43–0.74; $p < 0.0001$).

Immunotherapy Versus Targeted Therapy

For patients with a *BRAF*-driver mutation, the combination of *BRAF* with *MEK* inhibitors is currently an SoC. Targeted treatment is associated with higher responses and shorter time-to-response compared with immunotherapy, with a distinct toxicity profile. Both treatment approaches have the potential for sustained durable disease control in patients with favourable prognostic factors. It is unclear how to optimally sequence treatments for patients with *BRAF* mutations. Several clinical trials are ongoing to address this question. There is evidence that targeting the *MAPK* pathways has a direct impact on the immune system, including increased melanoma antigen expression, decreased immunosuppressive cytokine production, increased CD8⁺ T cell infiltration, increased T cell clonality, increased PD-L1 expression and Class I major histocompatibility complex (MHC) upregulation. This benefit is lost in tumours becoming resistant to targeted therapy. Rather than treat to progression, one strategy under evaluation is to switch to immunotherapy after a short induction treatment with targeted therapy, and to switch back if the patient subsequently progresses on immunotherapy, so that the melanoma would still be sensitive to targeted therapy.

Another approach is a triplet combination of *BRAF/MEK* and anti-PD-1 inhibitors, now under evaluation in clinical trials. In previously untreated *BRAF*-mutant melanoma patients, COMB-I (NCT02967692),

a phase III study of dabrafenib + trametinib \pm PDR001 (a PD-L1 inhibitor) was developed to explore the efficacy and toxicity of combinations. TRILOGY (NCT02908672), a phase III study with atezolizumab (anti-PD-L1 antibody), vemurafenib and cobimetinib, is also addressing this question. Management of overlapping toxicities poses a challenge, e.g. drug-induced versus immune hepatitis (NCT02130466, NCT02967692, NCT02908672).

In the absence of randomised, prospective data to help guiding current treatment decisions, it is crucial to consider both patient and disease characteristics (performance status, tumour-related symptoms, co-morbidities, tumour burden, growth rate) and patient wishes, to select the best treatment approach.

The Endless Search for a Biomarker

A significant number of patients do not benefit from ICIs, with approximately 30%–40% of patients refractory to single anti-PD-1 treatment. Identifying a predictive biomarker has been a major focus over the last few years.

Given the mechanism of action of anti-PD-1 agents, expression of the target on tumour cells would be a logical biomarker to study. However, PD-L1 expression is not a very reliable biomarker for many reasons, including results being dependent on the platform used, expression being inducible, etc. There is evidence of an increased RR with increased expression, though tumours with low expression can still respond to PD-1 inhibitors. PD-L1 expression can help to identify patients more likely to benefit from combination immunotherapy. In the CheckMate 067 study, patients with low levels of PD-L1 expression responded better to combination ipilimumab plus nivolumab versus single-agent nivolumab (PFS of 11.2 versus 5.3 months), the PFS for patients with $>5\%$ expression of PD-L1 being similar for both treatment arms at 14 months.

High rates of somatic mutations are believed to translate into increased neo-epitope formation, contributing to tumour immunogenicity. However, mutational load itself is not enough to account for response to ICIs, and neoantigen expression (particularly clonal neoantigens) is responsi-

ble for T cell activation. The presence of key signalling pathways is also required to allow spontaneous T cell response. Understanding these key factors on an individual patient basis allows treatment strategies to be tailored, but remains an experimental approach.

Recent data suggest a link between toxicity and response. A systematic review of patients treated with immunotherapy showed that those who developed vitiligo-like depigmentation had a two- to four-fold lower risk of disease progression and death, respectively. Furthermore, there was no evidence that patients receiving immunosuppression in the form of high-dose corticosteroids or infliximab had a lower RR than untreated patients. Recent subgroup analysis of the CheckMate 067 study has shown that patients discontinuing treatment because of toxicity had a higher RR than those not experiencing significant toxicity.

Future Perspectives

The landscape of melanoma treatment has changed dramatically over the last 7 years, transforming the outcome for patients. There is no evidence that the pace of progress is slowing. New, rational combinations are being tested, focusing on increasing the immune response, reducing toxicity and personalising treatment for individual patients. The outcomes for rare subtype of melanomas (e.g. mucosal, uveal), which are biologically distinct from cutaneous melanoma, remain poor and the development of more effective systemic therapy is mandatory.

While ICIs result in durable responses, this is not the case for all patients. Many different strategies are being studied to improve outcomes for patients: strategies to increase T cell infiltration of tumours, antigen release and recognition, to modulate the tumour microenvironment (TME), evaluation of new checkpoint inhibitors and combination with targeted therapy. Combination strategies are being evaluated in many clinical trials. Some examples are given below, but this is not a comprehensive list and the reader is directed to clinicaltrials.gov for more information.

IDO-1 (indoleamine 2,3-dioxygenase 1) is overexpressed in several cancers, including melanoma, resulting in suppression of T cell function

within the TME. While IDO-1 inhibitors have no activity as single agents, an RR of 53% was observed in a phase II study when indoximod, an IDO inhibitor, was given in combination with pembrolizumab. Similar results were observed with the epacadostat/pembrolizumab combination.

Histone deacetylase inhibitors (HDACis) were also combined with ICIs. The ENCORE 601 phase II trial tested pembrolizumab in combination with entinostat and demonstrated favourable results that warrant phase III confirmation.

Another appealing concept is the concurrent engagement of the target cell antigen and CD3 receptor BITE (bispecific T cell engager), leading to activation of polyclonal cytotoxic T cells and resulting in target lysis. Several new BITEs have been developed (carcinoembryonic antigen, human epidermal growth factor receptor 2, prostate-specific antigen), and are currently being tested both in monotherapy and in combination with anti-PD-1 agents.

Adoptive cell therapy can result in a durable benefit in selected patients, particularly the 20% that achieve a CR. Ongoing studies are comparing tumour infiltrating lymphocyte (TIL) therapy with ICIs and evaluating the role of low-dose IL-2 to reduce the significant toxicity of this treatment, making it an option for more patients. A major focus is on identifying biomarkers of response, either at the start of or on treatment. These will allow more rational use of these effective and expensive treatments. The exciting results from adjuvant trials will undoubtedly translate into approvals in this setting, but how this will impact on the treatment of advanced disease is unknown. Immunotherapy in melanoma sets a precedent for advances in many other cancer types, and will continue to do for the foreseeable future.

Declaration of Interest:

Dr Teixeira de Sousa has been a consultant/advisor/paid speaker for Roche, Merck Sharp & Dohme, Novartis and Bristol-Myers Squibb.

Dr Mansinho has reported no potential conflicts of interest.

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2.2 Merkel Cell Carcinoma

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Overview

Merkel cell carcinoma (MCC) is an aggressive skin malignancy with a high disease-associated mortality rate. The carcinogenesis of MCC in the northern hemisphere is predominantly associated with the Merkel cell polyomavirus (MCPyV) in around 80% of tumours and a high mutational burden characterised by an ultraviolet (UV) signature in the remainder. Both viral- and UV-associated carcinogenesis result in persistent expression of immunogenic antigens (viral proteins or neoantigens), which provides a strong rationale for testing immunotherapy in this disease (Becker et al, 2017). Indeed, immune checkpoint inhibitors (ICIs) for treatment of metastatic MCC are extremely effective and have led to a remarkable improvement in patient outcomes. In this chapter, we review the recent advances in MCC treatment with a special focus on ICIs. We also discuss the currently unmet treatment needs of MCC patients and the future directions for MCC research.

Introduction

MCC is an aggressive neuroendocrine skin cancer with a disease-associated case fatality rate three times that of malignant melanoma (46% versus 15%). MCC is an uncommon cancer with an estimated 2500 cases in the United States in 2013. The number of cases in Europe

is less clear, since most of the cancer registries are restricted to individual countries; however, it is assumed that the incidence is comparable to that of the Caucasian population in the United States (Stang et al, 2018). The reported incidence is constantly increasing since the initial description by Toker in 1972. This increase is in part due to heightened awareness and improved detection, but is also likely due to the higher prevalence of known risk factors for MCC (immune suppression; Caucasian >50 years of age with extensive prior sun exposure).

MCC is an aggressive cancer with prognosis dependent on the stage at presentation. Stages I and II represent low-risk and high-risk primary disease, respectively, while stages III and IV include the presence of nodal and distant metastases, respectively. The reported 5-year relative survival (an estimate for disease-specific survival) for patients with local, nodal and metastatic disease is 64%, 39% and 18%, respectively. Although surgery and/or radiotherapy (RT) may be curative for patients with loco-regional MCC without distant metastases, relapse is common and often difficult to treat. There is no established adjuvant systemic therapy after definitive management. For patients with distant metastatic disease not amenable to surgery or RT, systemic chemotherapy (ChT) was, until recently, the only treatment option beside best supportive care. The reported objective response rate (ORR) with either mono- or poly-ChT regimens is high, in some reports up to 60%. However, the clinical benefit is usually short-lived with a median progression-free survival (PFS) of only 3 months, and the impact on survival is unclear and thought to be modest at best.

Fortunately, rapid strides have recently been made in our understanding of the biology of MCC providing a strong rationale for the investigation of immunotherapies in this aggressive disease. These initial investigations have been extremely successful, leading to remarkable advances in therapies for metastatic MCC in a relatively short period of time.

Immunology of MCC

Epidemiological data had long suggested a strong link between MCC and the immune system. Individuals with T cell dysfunction (solid organ transplant recipients, human immunodeficiency virus [HIV]-infected

patients with acquired immunodeficiency syndrome [AIDS] or chronic lymphocytic leukaemia patients) have a 5- to 15-fold increased risk of developing MCC (Stang et al, 2018). MCC tumours sometimes regress following improvement in immune function. Additionally, there are several reported cases (Pang et al, 2015) of complete spontaneous regression (a far greater number than expected for its rarity). These epidemiological data had raised the possibility of an infectious aetiology for MCC. Indeed, the discovery of the MCPyV in 2008 provided the missing link between MCC and its strong association with the immune system. This strong association was independently confirmed in an unbiased gene expression analysis of MCC tumours, which revealed overexpression of immune response genes in tumours with favourable prognoses (Paulson et al, 2011). Intra-tumoural infiltration of CD8⁺ lymphocytes was found to be an independent predictor of improved survival among MCC patients in a cohort of 156 MCC cases. It should be noted, however, that a substantial number of these cases did not express the MCPyV-derived oncoproteins on a messenger RNA (mRNA) level. Indeed, approximately 20% of MCC cases in the United States and Europe, and up to 70% of cases in Australia, lack detectable tumour-associated MCPyV DNA or oncoproteins. Strikingly, the mutational burden of virus-negative MCC is even higher than that of melanoma, and has a signature suggestive of UV-induced mutations. It is likely that these genetic changes lead to generation and expression of novel epitopes and subsequently, neoantigen-directed immune responses. Thus, these observations readily explain the important role of cellular immune responses in the natural history of both MCPyV- and UV-associated MCC.

Since the discovery of the prognostic impact of CD8⁺ tumour infiltrating lymphocytes (TILs), our understanding of the host–virus immune interactions in MCC pathogenesis has increased rapidly with new insights into both humoural and cellular immunity in MCC patients. In patients with MCPyV-positive (MCPyV⁺) tumours, there is now ample evidence for ongoing expression of viral proteins in tumour cells and their recognition by the adaptive (humoural as well as cellular) arm of the immune system. Levels of MCPyV T antigen-specific antibodies correlate with tumour burden in MCC patients, and this observation has led to the development of a clinically validated assay (*AMERK*) for surveillance

of high-risk patients with MCPyV⁺ MCC tumours. MCPyV-specific T cells have been isolated from the peripheral blood or tumours of affected patients and are even being investigated for therapy after *ex vivo* expansion and adoptive transfer.

Despite this persistent expression of immunogenic proteins, MCCs that become clinically evident are able to evade host immune responses. Our understanding of the immune evasion mechanisms employed by MCC tumours continues to evolve rapidly. The progression from the immune equilibrium phase to the immune escape phase may occur due to changes in tumour cell population that may acquire new immune-evasive characteristics, or due to changes in the host immune system that may get suppressed either generally or more selectively toward the tumour cells. Both of these broad mechanistic categories appear relevant to MCC. The tumour cell characteristics include mechanisms such as down-regulation of antigen presentation, resulting in major histocompatibility complex (MHC)-I loss to become ‘less visible’ to the adaptive immune system, or decreased susceptibility to immune control mechanisms to become ‘more resistant’ to the effects of the cytotoxic immune cells. The host immune features include systemic immune suppression, either therapeutically or due to co-morbid immune suppressive diseases, or more commonly due to immune senescence, an erosion of the immune response with ageing. MCC tumour cells also establish a local immune suppressive tumour microenvironment (TME) via production of immunosuppressive cytokines, or via recruitment of immunosuppressive cells, such as CD4⁺CD25⁺ regulatory T cells (T_{regs}) or myeloid-derived suppressor cells. In response to chronic antigen exposure, antigen-specific CD8⁺ T cells in the MCC TME often develop an exhausted phenotype with poor effector function, sustained expression of inhibitory receptors (such as programmed cell death protein 1 [PD-1], T cell immunoglobulin and mucin domain 3 [TIM-3]), and a transcriptional state distinct from that of functional effector or memory T cells.

Immunotherapy of MCC

The above-mentioned data have provided the rationale for immunomodulation to treat MCC. These immunotherapy efforts have focused on a

multitude of approaches aiming to render cancer cells more visible to the immune cells, reinvigorate existing immune responses, generate new ones or simply use the viral targets for selective delivery of cancer therapeutics to tumours. Several early phase immunotherapy trials, including intra-tumoural interleukin-12 (IL-12) injection, intra-tumoural injection of the toll-like receptor 4 (TLR4) agonist G100 and adoptive T cell therapy, have all provided preliminary evidence of the potential efficacy of a variety of immune-based approaches in MCC. However, the most remarkable successes have occurred with the ICIs, which are discussed in detail below.

ICIs

The discovery of programmed death-ligand 1 (PD-L1) expression on tumour and immune cells in both MCPyV⁺ and MCPyV-negative (MCPyV⁻) MCC tumours provided a rationale for investigating checkpoint inhibitors targeting PD-1 or PD-L1 in MCC. The presence of PD-1 and PD-L1 in the MCC TME reflects the result of chronic antigen presentation of processed viral proteins and UV-induced neoantigens. Consequently, anti-PD-1 and anti-PD-L1 antibodies have been investigated as first-line and as second-line or later therapy in patients with advanced-stage MCC.

Pembrolizumab is a humanised immunoglobulin (Ig)G4 anti-PD-1 monoclonal antibody (mAb) and is being investigated for first-line systemic treatment of immunocompetent patients with advanced MCC in a phase II clinical trial (NCT02267603). The first report of this trial included 26 patients with unresectable stage IIIB or stage IV MCC, of whom 16% had a complete response (CR) and 40% a partial response (PR), resulting in an ORR of 56% (Response Evaluation Criteria In Solid Tumours [RECIST] v1.1). While the ORR is not strikingly different from what would have been expected from front-line ChT, the responses and PFS are remarkably more durable than those expected from ChT (Figure 1). Twelve of the 14 confirmed responses (86%) were ongoing at last follow-up, with the median follow-up being close to 8 months. Response to pembrolizumab did not correlate significantly with PD-L1 expression, a biomarker that has been evaluated extensively in several trials of PD-1

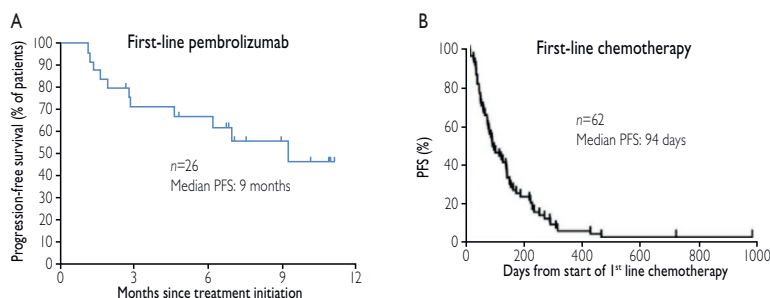
pathway blockers. Importantly, responses were seen in both MCPyV⁺ and MCPyV⁻ tumours, consistent with immunogenicity of both subtypes. Twenty-six patients were included in the safety analysis and treatment was generally well tolerated, with 77% of patients reporting an adverse event (AE) of any grade, of which 15% were grade 3 or 4. AEs were consistent with prior reports in other cancer types and were managed well through the discontinuation of pembrolizumab and, if necessary, glucocorticoid treatment. The results also led to the listing of pembrolizumab as a therapeutic option in the 2017 National Cancer Center Network (NCCN) guidelines.

Concurrently with the above-mentioned study, another phase II study (NCT02155647) was investigating avelumab in immunocompetent patients with metastatic MCC who had previously received one or more lines of cytotoxic ChT. Avelumab is a human IgG1 anti-PD-L1 mAb with a wild-type IgG1 fragment crystallisable (Fc) region that may, in addition to blocking PD-1/PD-L1 interactions, activate natural killer (NK) antibody-dependent cellular cytotoxicity (ADCC). In this much larger pivotal phase II trial, 88 patients with ChT-refractory distant metastatic (stage IV) disease were treated, of whom 9% had a CR and 23% a PR, resulting in an ORR of 32%. Responses were impressively durable, with the proportion of responses of ≥ 6 months being 92%. Similar to the pembrolizumab study, responses to avelumab occurred quickly (generally at the time of the first scan at 6 weeks) and occurred irrespective of PD-L1 expression or MCPyV status of the MCC tumours. Avelumab was well tolerated, with 70% of patients reporting an AE, but only 5% of grade 3 and no grade 4 events. Only fatigue (24%) and infusion-related reaction (17%) occurred in more than 10% of patients. Based on the impressive results from this phase II study, avelumab received approval by the United States Food and Drug Administration (FDA), SwissMedic and the European Medicines Agency (EMA) in 2017 for the treatment of metastatic MCC, regardless of prior ChT administration. This trial was expanded to include MCC patients who are treatment-naïve to systemic therapy in the metastatic setting. Preliminary results of the first 39 patients enrolled in part B were presented at the 2017 European Society for Medical Oncology (ESMO) congress. At the time of the data cut-off, the ORR with first-line avelumab was 62%, with 14% of patients experi-

Figure 1 Comparison of PFS and other outcomes in patients with advanced MCC treated with pembrolizumab (panel A) in the first-line setting in a clinical trial (Nghiem et al, 2016) with patients treated with first-line cytotoxic chemotherapy (panel B) in a retrospective single-institution cohort study (Iyer et al, 2016).

Note: These data do not come from a randomised controlled trial, but are derived from two separate studies and hence, are fraught with limitations of comparing across studies.

Panel A from Nghiem PT, Bhatia S, Lipson EJ, et al. PD-1 blockade with pembrolizumab in advanced Merkel-cell carcinoma. *N Engl J Med* 2016; 374:2542–2552. Reprinted with permission of Massachusetts Medical Society. Copyright ©2016. Panel B from Iyer JG, Blom A, Doumani R, et al. Response rates and durability of chemotherapy among 62 patients with metastatic Merkel cell carcinoma. *Cancer Med* 2016; 5:2294–2301.



Treatment and outcomes	First-line Pembrolizumab (N=26)	First-line Chemotherapy (N=62)
ORR (per RECIST v1.1)	56%	55%
Median DOR	NR (>8+ months)	2.9 months
Median PFS	9 months	3 months
PFS rate at 6 months	67%	26%

Abbreviations: DOR, duration of response; MCC, Merkel cell carcinoma; NR, not reached; ORR, objective response rate; PFS, progression-free survival; RECIST, Response Evaluation Criteria In Solid Tumours.

encing a CR and 48% of patients experiencing a PR. Sixty-seven per cent of patients had a PFS rate of 3 months.

Yet another ongoing study (NCT02488759) is investigating nivolumab, an anti-PD-1 antibody, in patients with virus-associated cancers including MCC. Patients with metastatic MCC are enrolled regardless of MCPyV status or prior ChT. Preliminary results were presented at the

2017 American Association of Cancer Research (AACR) congress. ORR in 22 patients was an impressive 64%. The majority (75%) of the responses occurred by ~Week 8. Responses were durable, with 75% of the responses ongoing at a median follow-up time of ~12 months. As in the studies mentioned above, responses were noted regardless of PD-L1 expression or MCPyV status. The trial is ongoing and has added another cohort investigating the combination of nivolumab plus low-dose ipilimumab (1 mg/kg) in metastatic MCC patients. This trial is also investigating the neo-adjuvant use of nivolumab (two doses total) in loco-regional MCC prior to surgery (\pm RT).

The impressive and concordant results from the above-mentioned trials using three different drugs blocking the PD-1/PD-L1 interaction have offered powerful new options to clinicians for managing advanced MCC. All of these ICIs have been remarkably well tolerated with low rates of \geq grade 3 treatment-related AEs and no treatment-related deaths. The response rates appear to be higher in treatment-naïve patients and lower in patients with prior ChT exposure. The responses occur quickly and at a frequency similar to that expected with front-line ChT, but are much more durable and will likely lead to a meaningful improvement in overall survival, with reasonably good quality of life (QoL). Additionally, these studies suggest that in both MCPyV⁺ and MCPyV⁻ tumours, a large proportion of patients have MCC-specific T cells that can be reactivated to provide clinically beneficial anti-tumour activity. Taken together, these data suggest that PD-1/PD-L1-based immunotherapy should be considered as the new standard-of-care for treatment of patients with metastatic MCC, regardless of MCPyV status. This is reflected in the recent listing of avelumab, pembrolizumab and nivolumab as the preferred treatment options for metastatic MCC in the 2018 NCCN guidelines, although avelumab is currently the only FDA- and EMA-approved therapy for metastatic MCC.

Unmet Needs and Future Directions

The durable responses to PD-1/PD-L1-blocking antibodies confirm the importance of immune mechanisms in MCC pathogenesis. However, not all patients respond to immunotherapy and some develop secondary resistance. Thus, a key question remains as to what tumour or host char-

acteristics might be used to predict response and/or resistance. In addition to finding predictive biomarkers, there is a direct unmet need for finding effective therapies in ~50% of immunocompetent patients who do not respond to PD-1/PD-L1 blockade. Mechanistic studies to understand both intrinsic and acquired mechanisms of resistance are critical to uncovering new rational therapies to overcome these. Given the heterogeneity of MCC tumours and individual variations in host immune systems, it is unlikely that one single approach will be effective in all patients. Rather, a combination of various strategies and personalisation to the unique biological characteristics of MCC tumours in individual patients will be required.

Facilitated by the ongoing excitement surrounding cancer immunotherapy, several trials of novel immunotherapeutic approaches (both innate and adaptive) are already ongoing in patients with advanced MCC. One innate immunotherapy approach is using allogeneic irradiated activated natural killer (NK)-92 cells (a NK cell line derived from a patient with large granulocytic leukaemia) in combination with an IL-15 agonist in MCC patients who may have received prior PD-1/PD-L1 blockade (NCT02465957). Another innate immunotherapy approach is studying intra-tumoural administration of TTI-621, a recombinant fusion protein targeting CD47, that regulates phagocytosis in patients with injectable MCC lesions (NCT02890368). Trials are underway to evaluate the oncolytic virus talimogene laherparepvec (T-VEC) administered intra-tumourally, both as a monotherapy or in combination with RT (NCT02819843), or with anti-PD-1 (nivolumab) treatment in patients with advanced MCC (NCT02978625). The profound success with checkpoint inhibitors has also raised interest in clinical studies using combinations of other therapies with ICIs. A triple-combination study of tremelimumab (an anti-cytotoxic T-lymphocyte antigen 4 [CTLA-4] antibody), durvalumab (an anti-PD-L1 antibody), and TLR3 agonist poly-ICLC in advanced MCC (NCT02643303) is testing the hypothesis that the TLR3 agonist will influence the TME and potentiate the activity of the ICIs. A study to investigate localised upregulation of antigen expression (using RT or interferon) plus adoptive immunotherapy (MCPyV T antigen-specific T cells) with avelumab is also ongoing (NCT02584829). Efforts are also underway to test the safety and efficacy of several ICIs (ipilimumab, nivolumab and avelumab) in the adjuvant set-

ting in patients with loco-regional MCC amenable to definitive therapy with surgery \pm RT (NCT02196961 and NCT03271372). It is indeed an exciting time for investigation of novel targeted and/or immune therapies in this fascinating malignancy.

Conclusion

An improved understanding of the biology and immunology of MCC has revolutionised the therapeutic possibilities in advanced MCC. The immune system appears to be playing a major role in MCC biology, irrespective of their virus- or UV-associated carcinogenesis. A new era in the systemic therapy of metastatic MCC has begun with the recent successes of immune checkpoint blockade. Promising new immunotherapy- and molecularly-targeted therapy approaches are in development. An improved understanding of tumour immunology and immune escape mechanisms operative in MCC will facilitate the rational development of new treatment strategies to overcome primary and secondary resistance of MCC to immune-modulating therapies.

Declaration of Interest:

Dr Bhatia has received advisory board honoraria from Genentech and EMD-Serono; his institution (University of Washington) has received research funding from EMD-Serono, Merck, Bristol-Myers Squibb, Oncosec and Immune Design.

Professor Becker has received speaker honoraria from Amgen, Merck Serono and Pfizer; he has received advisory board honoraria from Amgen, CureVac, eTheRNA, Lytix, Merck Serono, Novartis, Rigontec and Takeda; and he has received research funding from Boehringer Ingelheim, Bristol-Myers Squibb and Merck Serono. Professor Becker's activities with Bristol-Myers Squibb, Merck Serono and Pfizer are related to the submitted report (therapy of advanced MCC).

Further Reading

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2.3 Thoracic Malignancies

2.3.1 Non-small Cell Lung Cancer

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Current Scope of Immunotherapy of NSCLC

Lung cancer, of which non-small cell lung cancer (NSCLC) represents 85%, accounts for 13% of all cancer diagnoses worldwide, and remains the leading cause of cancer deaths worldwide. Mortality rates in Europe and North America are now declining in men, and in some countries in women, reflecting the evolution of the tobacco epidemic. The aetiological association with exposure to tobacco carcinogens is of particular relevance for cancer immunotherapy, as current approaches targeting immune checkpoints exhibit increased responses in tumours with a high number of somatic mutations, such as smoking-induced NSCLC. Conversely, immune checkpoint blockade (ICB) has been significantly less successful in never-smokers, including a majority of anaplastic lymphoma kinase (*ALK*)-rearranged or epidermal growth factor receptor (*EGFR*)-mutated NSCLC. Overall, an estimated 25% of NSCLCs are not directly attributable to smoking, representing 15% in men and 53% in women, globally.

Blockade of the programmed cell death protein 1 (PD-1)–programmed death-ligand 1 (PD-L1) receptor–ligand pair, a dominant mediator of immune resistance in the tumour microenvironment (TME), represents the mainstay of current immunotherapy of NSCLC. Anti-PD-1 monoclonal antibodies (mAbs) nivolumab and pembrolizumab, and anti-PD-L1 mAb atezolizumab have all demonstrated an improvement in overall survival (OS) compared with chemotherapy (ChT) in the second line and

later lines of therapy of advanced NSCLC. Pembrolizumab has demonstrated an OS benefit over cisplatin-based doublet ChT in NSCLC patients with high PD-L1 expression ($\geq 50\%$ on tumour cells [TCs]) in the front-line setting. Furthermore, consolidation therapy with durvalumab delays tumour progression or death after chemoradiotherapy (CRT) of locally advanced (LA) NSCLC. Studies of adjuvant therapy of early disease with the same compounds are ongoing. Beyond blockade of the PD-1/PD-L1 axis, combination approaches directed at other immune checkpoints including cytotoxic T-lymphocyte antigen 4 (CTLA-4), immuno-oncology (IO)–IO combinations, IO–ChT combinations, as well as cellular immunotherapy using tumour infiltrating lymphocytes (TILs), dendritic cell vaccines and more, are being actively investigated, but have not yet been established in phase III studies and/or received regulatory approval. Individual trial results will be further developed in this chapter.

Predictive Biomarkers of Immunotherapy

Response of Clinical Relevance

Predictive biomarkers that can direct the rational use of PD-1/PD-L1 checkpoint inhibitors are warranted by the low response rates (RRs) in the range of 20% observed in an unselected pre-treated NSCLC population. Currently, PD-L1 expression level by immunohistochemistry (IHC) on TCs and possibly on tumour infiltrating immune cells is the only biomarker of immunotherapy response of clinical relevance, and the only biomarker having been prospectively validated in at least one randomised trial. PD-L1 expression can be used to prioritise treatment sequencing (i.e. first-line therapy for high expressors), but it cannot be defined as an absolute selection criterion, as well demonstrated by the existence of responses and clinical benefit over standard therapy in pre-treated patients despite PD-L1 negativity. Due to parallel development of immune checkpoint inhibitors (ICIs), a number of antibodies (28-8, 22C3, SP142, SP263) using various platforms (Dako, Ventana, Leica), methodologies, tumour material, scoring methods and PD-L1 thresholds have been developed. A robust consensus cannot currently be reached, and harmonisation as well as quality assessment efforts are urgently needed.

The Blueprint PD-L1 IHC Assay Comparison Project, an industry–academic collaboration, compared the four assays used in clinical trials. Despite the observation of an identical performance of three assays out of four on TCs (28-8, 22C3, SP263), it also showed that interchanging the testing assay can lead to patient misclassification. In addition, biological limitations of these assays should be highlighted, including temporal and treatment-related fluctuations, as well as a significant intratumoural heterogeneity. Of current clinical use is the companion diagnostic test 22C3 required for the use of pembrolizumab in pre-treated patients, for whom $\geq 1\%$ positivity is required, and for the use of pembrolizumab in the front-line setting, where $\geq 50\%$ positivity is required. Testing is either not required (28-8 or SP263 for nivolumab, SP142 for atezolizumab) or not approved in all other clinical situations, outside clinical trials to date. Other possibly non-correlated immune biomarkers are under active investigation, including:

- Tumour mutational load derived from whole-exome sequencing or gene-panel testing
- Immune gene signatures using microarray or RNA sequencing (RNAseq)
- T cell receptor (TCR) clonality
- TILs phenotype by multiparametric IHC

First-line Therapy

Anti-PD-1/PD-L1 antibodies have become a standard-of-care (SoC) in the first-line setting for most NSCLC patient subgroups, either as monotherapy, or as part of a ChT-IO or IO-IO combination.

In patients with PD-L1 expression on $\geq 50\%$ of TCs (as assessed by the 22C3 assay in the trial) and no *EGFR*-sensitising mutation or *ALK* gene rearrangement, pembrolizumab was associated with significantly longer progression-free survival (PFS) and OS, with fewer adverse events (AEs) than platinum-based doublet ChT. Median PFS (mPFS), the primary endpoint, reached 10.3 months versus 6 months (hazard ratio [HR] for progression or death 0.50), and median OS (mOS) 30 versus 14.2 months (HR for death 0.63), with a 24-month OS rate of 51.5% versus

34.5% for pembrolizumab and ChT, respectively. Also improved were RR (44.8% versus 27.8%), median duration of response and grade 3 or higher treatment-related AEs (trAEs): 26.6% versus 53.3%. These efficacy and tolerability results establish pembrolizumab monotherapy as the standard first-line therapy in this patient population.

Several randomised phase III studies have demonstrated an OS benefit for the addition of anti-PD-1/PD-L1 antibodies to platinum-based doublet ChT. In non-squamous NSCLC patients, the addition of pembrolizumab to platinum drugs and pemetrexed improved 12-month survival from 49.4% to 69.2%, and RRs from 18.9% to 47.6%; the addition of atezolizumab to carboplatin, paclitaxel and bevacizumab improved OS (HR for death 0.78). In squamous NSCLC, the addition of pembrolizumab to carboplatin and paclitaxel/nab-paclitaxel improved median OS from 11.3 to 15.9 months (HR for death 0.64). In all these trials, the OS benefit was seen across all relevant patient subgroups, including those with low or absent tumour and/or immune cell PD-L1 expression. Furthermore, the rate of grade 3 or higher AEs, in the range of 58 to 69%, was not increased by the addition of the checkpoint inhibitors.

Adding further complexity to first-line immunotherapy, a high tumour mutational burden (TMB), defined as ≥ 10 mutations (somatic, coding base substitutions and short indels) per megabase of genome by the FoundationOne CDx assay, defines a population that derives a significant PFS benefit from an ipilimumab and nivolumab combination as compared with platinum-based doublet ChT (HR for disease progression or death 0.58), with 43% versus 13% of patients being progression-free at 1 year. TMB was independent of PD-L1 expression level. While OS data remain immature at the time of writing, these data support consideration of both PD-L1 expression levels and TMB as predictive biomarkers for clinical decision-making in the first-line setting.

Second and Later Lines of Therapy

Nivolumab, pembrolizumab and atezolizumab have all been compared to SoC docetaxel in NSCLC patients pre-treated with platinum-based ChT, all showing an improvement in OS. Nivolumab improved OS in non-squamous NSCLC patients from 9.4 months to 12.2 months (HR

for death 0.78), and in squamous NSCLC patients from 6 to 9.2 months (HR for death 0.59). Pembrolizumab improved OS in PD-L1-positive NSCLC patients pre-treated with one line of platinum-based ChT from 8.2 to 14.9 months (HR for death 0.54). Atezolizumab improved OS in NSCLC patients pre-treated with one or two lines of ChT from 9.6 to 13.8 months (HR for death 0.73). In the latter trial, OS benefit was consistent across subgroups including PD-L1-negative NSCLC, with the noteworthy exception of *EGFR*-mutated NSCLC, where RRs were as low as 5% and HR for death ranged from 1.18 to 1.24. Never-smokers derived less benefit, with an HR of 0.71–1.02 compared with current or former smokers.

PFS is generally not consistently improved in an unselected population. To date, OS is considered as a better endpoint to evaluate immunotherapy for the treatment of NSCLC. This specificity might be attributed to the non-proportional HR observed for the benefit of IO versus ChT, requiring a sufficient observation time, as well as to a combination of factors including some cases of pseudoprogression and the probable existence of antitumour immune activity beyond progression and along subsequent lines of therapies. Duration of responses is consistently longer with ICB than with docetaxel, in the range of 16–17.2 months. Patient-reported outcomes (PROs) and AEs strongly favour ICB, with patients' health status improving from baseline during the first year of treatment, while docetaxel patients' health status remained stable relative to baseline during their shorter time on treatment. The incidence of grade ≥ 3 trAEs is 15% versus 40%–50% for ICB and docetaxel, respectively. Exploratory analyses of a randomised trial suggest that treatment should be administered at least until progression or unacceptable toxicity, rather than for a fixed duration. Whether this also applies specifically to patients in complete response is unknown and further data regarding treatment duration are greatly needed.

The efficacy and tolerability results of these four trials establish ICB with anti-PD-1/PD-L1 mAbs in monotherapy as an SoC after platinum-based ChT, with the current exception of *EGFR*-mutant and *ALK*-rearranged NSCLC, where tyrosine kinase inhibitors (TKIs) and docetaxel should be considered first outside clinical trials, and the benefit of immunotherapy is reported to be lower although still existing in some of these patients.

Immunotherapy in Particular Subsets of NSCLC

The role of ICB has been less well studied in patients with untreated central nervous system (CNS) metastases, with common actionable genetic driver alterations, in patients with pre-existing autoimmune disorders, in elderly patients and/or patients with poor PS.

Patients with brain metastases (BMs) have generally been either excluded from clinical trials or included only after prior local therapy (radiotherapy or surgery). Limited evidence on the safety and activity of ICB in patients with stable, asymptomatic untreated BMs does not allow firm conclusions on safety and activity to be drawn. In patients with treated BMs, ICB is not associated with a higher incidence of AEs. Subgroup analyses from one phase III trial showed a significant OS benefit of atezolizumab in patients with baseline BMs compared with docetaxel (HR for death 0.54, versus 0.75 in the cohort without BMs). Furthermore, the time to development of new BMs was significantly prolonged (HR 0.42). These data establish ICB as the SoC in the second line, provided BMs are supratentorial, asymptomatic and have been successfully treated. The safety and efficacy in patients with spinal metastases or leptomeningeal disease remains unknown.

ICB in *EGFR*-mutated or *ALK*-rearranged NSCLC has only been evaluated in subgroup analyses of phase III trials. A meta-analysis of relevant trials showed no OS benefit over docetaxel for *EGFR*-mutated NSCLC in the second-line setting. Overall, RRs in the *EGFR*-mutated subgroup have been significantly lower than in the wild-type population, with higher PD-L1 expression being associated with higher RRs. The subset of *ALK*-positive (*ALK*⁺) NSCLC has consistently been too small to reach meaningful conclusions. The only prospective trial performed in heavily pre-treated patients nonetheless suggested an encouraging OS, leaving ICB as an attractive option after platinum-based ChT and at least one TKI. An ongoing prospective randomised trial is evaluating the role of ICB after first-line *EGFR* TKI in T790M- NSCLC patients (CheckMate 722, NCT02864251).

ICB in elderly or poor PS patients relies on limited evidence, but represents an attractive option due to the manageable safety profile. In the large

CheckMate 171 trial, the safety of nivolumab was consistent with prior studies of nivolumab in previously treated squamous NSCLC, with no new safety signals. Tolerability in patients aged ≥ 70 years (n=279) or with Eastern Cooperative Oncology Group (ECOG) PS 2 (n=98) was comparable with the overall population (Popat et al, 2017).

ICB in patients with pre-existing autoimmune disorders is developed in Chapter 3.3 (Immunotherapy in Special Populations)

Immunotherapy of Locally Advanced NSCLC

About one-third of NSCLC patients present with locally advanced (LA) disease, which carries a poor prognosis, with an OS rate of 15% at 5 years, mainly due to distant relapse. Consolidation therapy with the anti-PD-L1 mAb durvalumab after CRT of unresectable stage III NSCLC increased mPFS from 5.6 to 16.8 months compared with placebo (HR for progression or death 0.52), with a consistently observed benefit across subgroups. Also improved was time to death or distant metastasis. OS data were immature at the time of presentation. The rate of irAEs was 24% in the durvalumab group versus 8% with placebo, with no significant increase in the rate of severe (grade ≥ 3) pneumonitis (3.4% with durvalumab versus 2.6% with placebo); treatment discontinuation rate due to pneumonitis was 6.3% versus 4.3%. Pending the presentation of OS data, improvement of PFS of almost 17 months is unprecedented in stage III NSCLC, and this clinically meaningful difference merits consideration of durvalumab as a new SoC consolidation therapy after CRT; durvalumab is being further evaluated in resectable NSCLC in randomised trials (CheckMate 816 [NCT02998528]), as well as in unresectable LA-NSCLC after CRT (CheckMate 816 [NCT02998528]) (Table 1).

Immunotherapy for Early-stage NSCLC

Adjuvant ICB after surgical resection is being investigated in several randomised trials: KEYNOTE-091/PEARLS (NCT02504372), IMpower10 (NCT02486718), BR 31 (NCT02273375) and ANVIL (NCT02595944); see Table 1. Due to the nature of the disease-free survival (DFS) endpoint, results are not expected before 2021.

Table 1 Ongoing Randomised Phase II and Phase III Trials in Early and Locally Advanced NSCLC

Strategy	Trial (NCT reference)	Clinical Phase	Treatment	Patient target	Primary endpoint
Neoadjuvant IB-IIIa	CheckMate 816 (NCT02998528)	III	Nivo+ipi vs ChT	326	MPR
Neoadjuvant IIIa	(NCT03081689)	II	Nivo+ChT	46	PFS
Neoadjuvant IA-IIIa (I station)	(NCT03158129)	II	Nivo or Nivo+ipi	66	MPR
Neoadjuvant IB (>3 cm)-IIIa	TOP 1501 (NCT02818920)	II	Pembro	32	Surgical feasibility
Neoadjuvant IB (>4 cm)-IIIa non N2	PRINCEPS (NCT02994576)	II	Atezo	60	Toxicities leading to surgical delay
Neoadjuvant IB-IIIa	MAC (NCT02716038)	II	Atezo + ChT	30	MPR
Neoadjuvant IB-IIIa	(NCT02927301)	II	Atezo	180	MPR
Neoadjuvant IIIa	SAKK 16/14 (NCT02572843)	II	Durva + ChT	68	EFS
Neoadjuvant I (>2 cm)-IIIa	(NCT02904954)	II	Durva or Durva +SBRT	60	DFS
Neoadjuvant IB-II	IONESCO (NCT03030131)	II	Durva	81	R0 resection
Adjuvant IB (>4 cm)-IIIa	ANVIL (NCT02595944)	III	Nivo vs obs	714	DFS OS
Adjuvant IB (>4 cm)-IIIa	PEARLS (NCT02504372)	III	Pembro vs placebo	1380	DFS
Adjuvant IB (>4 cm)-IIIa	IMpower010 (NCT02486718)	III	Atezo vs BSC	1127	DFS
Adjuvant IB (>4 cm)-IIIa	BR-31 (NCT02273375)	III	Durva vs placebo	1100	DFS
Unresectable IIIa/B	RTOG 3505 (NCT02768558)	III	CRT → Nivo vs placebo	660	OS PFS
Unresectable IIIa/B	ETOP Nicolas (NCT02434081)	II	Nivo + CRT	78	Grade ≥3 pneumonitis
Unresectable IIIa/B	(NCT03102242)	II	Atezo → CRT	63	DCR
Unresectable I	(NCT03050554)	(I) / II	Avelumab + SBRT	56	RFS

Abbreviations: Atezo, atezolizumab; BSC, best supportive care; ChT, chemotherapy; CRT, chemoradiotherapy; DCR, disease-control rate; DFS, disease-free survival; Durva, durvalumab; EFS, event-free survival; ETOP, European Thoracic Oncology Platform; ipi, ipilimumab; MPR, major pathological response; NCT, clinicaltrials.gov identifier; Nivo, nivolumab; NSCLC, non-small cell lung cancer; obs, observation; OS, overall survival; Pembro, pembrolizumab; PFS, progression-free survival; R0, no tumour at the margin; RFS, recurrence-free survival; RTOG, Radiation Therapy Oncology Group; SBRT, stereotactic body radiotherapy.

Potential Future Developments

Securing better outcomes for NSCLC patients requires sharper patient selection through efficient immune profiling, the rational use of drug combinations and better study endpoints to accelerate development. The multiple methods currently available to monitor the mechanisms of immune escape and the effects of therapeutic measures (*in situ* immunophenotyping, genomic and transcriptomic analyses, among others) will be integrated into a bio-informatic routine able to condense the information into the essential features needed to guide clinical decision-making. Combinations tailored to the immune microenvironment will maximise efficacy by tackling the mechanisms of immune escape. Agents currently in clinical and preclinical investigation in NSCLC will lead to combinations that will enlarge the proportion of patients exhibiting a tumour response, including:

- Immune checkpoint modulators (anti-PD-1/PD-L1, CTLA-4, T cell immunoglobulin and mucin domain 3 [TIM-3], lymphocyte-activation gene 3 [LAG-3], KIR, OX40, NKG2A, V-domain immunoglobulin suppressor of T cell activation [VISTA], etc.)
- Agents enhancing cellular effector function (CD122-biased interleukin-2 [IL-2] variants, IL-15 superagonists, IL-2-based fusion immunocytokines, transforming growth factor-beta [TGF- β]-traps, etc.)
- Adjuvants (stimulator of interferon genes [STING] agonists)
- Metabolic immunotherapy agents (indoleamine 2,3-dioxygenase [IDO] inhibitors, A2a receptor inhibitors)
- Adjuvants promoting antigen-presenting cell function (toll-like receptor agonists)

Table 2 Phase III Trials of Anti-PD-1/PD-L1 Checkpoint Inhibitors in Advanced Pre-treated NSCLC, Median OS Results (Continued)

Trial Study drug	CheckMate 017 Nivolumab 3 mg/kg q2w	CheckMate 057 Nivolumab 3 mg/kg q2w	OAK Atezolizumab 1200 mg q3w	KEYNOTE-010 • Pembrolizumab 2 mg/kg q3w • Pembrolizumab 10 mg/kg q3w
>5% (TC23 or IC23) [#]				
Cross histology			16.3	0.67 (0.49–0.90)
Squamous	10 [†] 6.4 [‡] (0.31–0.89)		10.4 ^{††} 9.7 ^{††}	0.76 ^{††}
Non-squamous		18.1 [†] 8.1 [†]	18.7 ^{††} 11.3 ^{††}	0.61 ^{††}
≥10% (N/A) [#]				
Cross histology				
Squamous	11 [†] 7.1 [†] (0.28–0.89)			
Non-squamous		19.4 [†] 8 [†] 0.40		
≥50% (TC3 or IC3) [#]				
Cross histology			20.5	8.9
			17.5 ^{††} 11.6 ^{††}	0.57 ^{††}
Squamous			22.5 ^{††} 8.7 ^{††}	0.35 ^{††}
Non-squamous				
			20.5	8.2
			14.9	17.3
			0.41 (0.27–0.64)	2 mg/kg; 0.54 (0.38–0.77) 10 mg/kg; 0.50 (0.36–0.70) ^{††}

[†]HR 96.85% CI; ^{††}HR 95.92% CI; [‡]ASCO 2015; ^{†††}WCLC 2016.

[#]BMS PD-L1 assay measures PD-L1 expression on tumour cells; Roche PD-L1 assay measures PD-L1 expression on tumour and immune cells.

Abbreviations: 2L, second-line; ASCO, American Society of Clinical Oncology; Atezo, atezolizumab; CI, confidence interval; Doc, docetaxel; HR, hazard ratio; IC, immune cell; N/A, not applicable; Nivo, nivolumab; NSCLC, non-small cell lung cancer; NSQ, non-squamous; OS, overall survival; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; PFS, progression-free survival; qXw, every X weeks; SQ, squamous; TC, tumour cell; TPS, tumour proportion score; WCLC, World Conference on Lung Cancer.

Table 3 Phase III Trials of Anti-PD-1/PD-L1 Checkpoint Inhibitors in Advanced Pre-treated NSCLC, Landmark OS Results and Further Endpoints

Trial	CheckMate 017	CheckMate 057	OAK	KEYNOTE-010
Study drug	Nivolumab 3 mg/kg q2w	Nivolumab 3 mg/kg q2w	Atezolizumab 1200 mg q3w	• Pembrolizumab 2 mg/kg q3w • Pembrolizumab 10 mg/kg q3w
N	272	582	850	1033
Patient population	2L SQ Stage IIIB/IV NSCLC	2L+ NSQ Stage IIIB/IV NSCLC	2L+ Stage IIIB/IV or recurrent NSCLC	2L+ Metastatic NSCLC (PD-L1 TPS ≥ 1%)
Primary endpoint	OS	OS	OS	OS and PFS
Efficacy	Nivo N=135	Doc N=137	Doc N=425	Doc N=343
1-year OS, %	42.1	23.7	55	41
18-months OS, %	28 [†]	13 [†]	40	27
2-year OS, %	23 [‡]	8 [‡]	31	21
Median PFS, months	3.48	2.83	2.8	3.9
ORR, %	20	9	13.6	18
mDOR, months	25.2	8.4	16.3	6.2
Safety	58 [†]	86 [†]	64	64 [†]
Gr 3-4 trAEs, %	7 [‡]	57 [‡]	15	13 ^{***}
			43	17 ^{***}
				36 ^{***}

[†]ASCO 2015; [‡]ESMO 2015; ^{***}WCLC 2015; ^{††}ESMO 2016, ^{***}Grade 3-5 trAEs

Abbreviations: 2L, second-line; ASCO, American Society of Clinical Oncology; Atezo, atezolizumab; CI, confidence interval; Doc, docetaxel; ESMO, European Society for Medical Oncology; HR, hazard ratio; mDOR, median duration of response; Nivo, nivolumab; NR, not recorded; NSCLC, non-small cell lung cancer; NSQ, non-squamous; ORR, objective response rate; OS, overall survival; PD-1, programmed cell death protein 1; PD-L1, programmed cell death ligand 1; PFS, progression-free survival; qXw, every X weeks; SQ, squamous; TPS, tumour proportion score; trAE, treatment-related adverse event; WCLC, World Conference on Lung Cancer.

Declaration of Interest:

Professor Peters has reported no conflicts of interest.

Dr Zimmermann has reported no conflicts of interest.

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2.3 Thoracic Malignancies

2.3.2 Small Cell Lung Cancer

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Introduction

Small cell lung cancer (SCLC) accounts for approximately 15% of all bronchogenic carcinomas, occurring almost exclusively in current or former smokers. SCLC has distinct clinical and pathological characteristics defined by rapid growth, propensity for early metastasis, initial response to chemotherapy (ChT) and radiotherapy (RT) with a high rate of relapse with treatment-resistant disease.

Initial treatment with concurrent platinum/etoposide and thoracic RT is the standard-of-care for limited-stage SCLC, yielding response rates of up to 80%–90%. Data from the phase III CONVERT study confirmed that there was no significant difference between a once-daily (66 Gy in 33 fractions over 6.5 weeks) and twice-daily (45 Gy in 30 twice-daily fractions over 3 weeks) RT schedule. Patients with stable disease or response after ChT should be considered for prophylactic cranial irradiation.

Four to six cycles of platinum/etoposide ChT is the standard first-line treatment for extensive-stage SCLC. No statistically significant difference in overall survival (OS) or progression-free survival (PFS) has been

found between cisplatin- and carboplatin-based combinations. Consolidation thoracic RT has been found to improve 2-year OS and PFS in patients with residual thoracic disease following initial ChT. The survival benefit of thoracic RT does not extend to patients who obtain a complete response to initial systemic treatment.

Choice of regimen for relapsed disease is dependent on length of response to initial treatment. Patients with platinum-sensitive disease (relapse >90 days after completion of ChT) may be re-challenged with platinum/etoposide. Second-line topotecan or combination therapy (cyclophosphamide/doxorubicin/vincristine) are acceptable options in resistant (relapse within 90 days) or refractory (no response to initial systemic treatment) disease, but have limited efficacy.

Despite good initial response to ChT and availability of second-line options, the prognosis of SCLC is exceptionally poor. Median OS for limited disease is 15–20 months, diminishing to 8–13 months for extensive disease, hence the need for novel treatments to improve patient outcomes.

Aside from immunotherapeutic strategies, efforts to identify molecular targets in SCLC are ongoing. Agents showing potential activity include the Aurora A kinase inhibitor alisertib and poly(adenosine diphosphate-ribose) polymerase (PARP) inhibitors. Molecular targeted agents that have failed to demonstrate sufficient activity in SCLC include the mammalian target of rapamycin (mTOR) inhibitor temsirolimus, hedgehog inhibitor vismodegib and agents targeting insulin-like growth factor 1 receptor (IFG-1R) cixutumumab and linsitinib.

Antibody–drug conjugate rovalpituzumab tesirine (Rova-T) targets gene delta-like 3 (*DLL3*). *DLL3* is highly expressed in neuroendocrine tumours, including 80% of SCLC. In a phase I study (NCT01901653), objective response was observed in 60 patients with progressive SCLC receiving Rova-T. In 26 patients with high *DLL3* expression (>50%), the objective response rate (ORR) was 39%. On the basis of promising phase I data, Rova-T is currently under investigation for treatment-naïve and relapsed/refractory SCLC in a number of ongoing trials.

Rationale for Immunotherapy in SCLC and Predictors of Response

SCLC is associated with over 200 non-synchronous mutations, of which 95% are single-based substitutions, consistent with exposure to polycyclic aromatic hydrocarbons present in tobacco smoke. These somatic mutations are thought to be potential targets for T cells activated by immune checkpoint inhibitors (ICIs); however, the individual mutations predictive for response to checkpoint blockade are yet to be identified. In certain disease groups associated with a high non-synchronous mutation load, improved efficacy of ICIs has been observed, leading to a strong rationale for development of immunotherapy trials in SCLC.

The development of predictive biomarkers is an area of great interest. Use of tumour programmed death-ligand 1 (PD-L1) expression as a predictive biomarker has been studied, predominately in the NSCLC population. The methodology for detection and definition of 'PD-L1 positivity' is not uniform between trials. Literature suggests that while PD-L1 positivity may enrich for a population that derives the greatest clinical benefit from anti-programmed cell death protein 1 (PD-1)/PD-L1 therapy, a low or negative PD-L1 expression does not exclude achieving a clinical benefit. Use of PD-L1 expression as a predictive biomarker is also limited by tumour heterogeneity, fluidity of PD-L1 expression within the tumour microenvironment and lack of standardisation of use of PD-L1 assays. Further work is required to determine the predictive impact of PD-L1 expression in SCLC. There is also a fundamental need to explore additional predictive markers for immunotherapy in SCLC.

Whole-exome sequencing (WES) techniques have been employed to characterise the mutational landscape in SCLC tumour samples. Exploratory data from CheckMate 032 (NCT01928394) found a correlation between high tumour mutation burden (TMB) and improved response rate to combination checkpoint blockade. Further prospective data are warranted to confirm the predictive nature of TMB.

Early preclinical work in defining the tumour microenvironment in SCLC associated the presence of tumour infiltrating lymphocytes (TILs)

expressing checkpoint molecules with improved OS. A larger patient population is required to confirm the predictive and prognostic role of TILs in SCLC.

Clinical Results

Prior to the era of immune checkpoint blockade (ICB), vaccines and immunomodulating agents were investigated in SCLC with limited success. The anti-idiotypic antibody Bec2 mimics cell membrane ganglioside GD3, which is overexpressed in up to 60% of SCLCs. Unfortunately, a randomised phase III study of Bec2/BCG (Bacillus Calmette-Guérin) vaccination failed to show survival benefit in limited-disease SCLC. Maintenance interferon-alpha (IFN- α) following induction ChT also failed to demonstrate improved survival.

Contemporary immunotherapy trials in SCLC have largely focused on ICB. Results from clinical trials investigating immunotherapy in SCLC are summarised in Table 1.

Table 1 Results from Immunotherapy Trials in SCLC

Drug (Trial identifier)	Target	Phase	N	Regimen	OS	PFS	ORR
Extensive stage – First-line							
a/ Ipilimumab (NCT01450761)	CTLA-4	III	1132	PE + Ipi 10 mg/kg q3w from cycle 3, followed by Ipi 10 mg/kg q12w vs PE + placebo	11m vs 10.9m HR 0.94, $p=0.3775$	4.6m vs 4.4m HR 0.85, $p=0.0161$	62% vs 62%
Extensive stage – Second-line and beyond							
b/ Pembrolizumab (NCT02054806)	PD-1	I	24	Pembro 10 mg/kg q2w	9.7m	1.9m	37.5
c/ Ipilimumab/Nivolumab (NCT01928394)	CTLA-4 / PD-1	I/II	159	Ipi 3 mg/kg + Nivo 1 mg/kg q3w $\times 4$, followed by Nivo 3 mg/kg q2w vs Nivo 3 mg/kg q2w	7.9m vs 4.1m	not reported	25 vs 11

Abbreviations: CTLA-4, cytotoxic T-lymphocyte antigen 4; HR, hazard ratio; Ipi, ipilimumab; Nivo, nivolumab; ORR, overall response rate; OS, overall survival; PD-1, programmed cell death protein 1; PE, platinum/etoposide; Pembro, pembrolizumab; PFS, progression-free survival; qXw, every X weeks; SCLC, small cell lung cancer.

Anti-cytotoxic T-Lymphocyte Antigen 4 (CTLA-4) Blockade

No OS benefit was observed with the addition of ipilimumab to standard platinum/etoposide for treatment-naïve extensive-stage SCLC in a randomised double-blind phase III trial (NCT01450761, see Table 1, a). In this study, 1132 patients were assigned in a 1:1 ratio to receive cisplatin or carboplatin plus etoposide for 4–6 cycles, plus ipilimumab 10 mg/kg or placebo every 3 weeks beginning with cycle 3. Following induction, maintenance ipilimumab or placebo was administered every 12 weeks. OS was evaluated in the 954 patients who received at least one dose of blinded study therapy. Median OS was 11 versus 10.9 months (hazard ratio [HR]: 0.94; 95% confidence interval [CI]: 0.81–1.09; $p=0.3775$) and median PFS was 4.6 versus 4.4 months (HR: 0.85; 95% CI: 0.75–0.97) for the ChT/ipilimumab and ChT/placebo arms, respectively. No subgroups demonstrated greater benefit with the addition of ipilimumab versus ChT alone. Rates of grade 3–4 adverse events (AEs) and treatment discontinuation were higher (18% versus 2%) in the ChT/ipilimumab arm. Lack of corresponding T cell activation within the tumour microenvironment or ChT-induced immunosuppression limiting T cell activation and proliferation have been hypothesised as contributing factors to the lack of benefit from additional CTLA-4 blockade.

PD-1 and PD-L1 Blockade

Pembrolizumab

KEYNOTE-028 (NCT02054806, see Table 1, b) was a non-randomised, multicohort phase Ib study which evaluated the safety and efficacy of pembrolizumab in 24 patients with extensive-stage SCLC who had progressed after platinum-based ChT. All patients had a PD-L1 expression $\geq 1\%$ as evaluated by immunohistochemistry (IHC). Pembrolizumab 10 mg/kg was given every 2 weeks for up to 2 years, until progressive disease or intolerable toxicity. Sixteen out of 24 (66.7%) patients experienced treatment-related AEs (trAEs) with grade 3–5 reported in two patients (8.3%). ORR was 37.5% (95% CI: 18.8–59.4) with one complete response and eight partial responses. Durable responses were observed with a 9-month median duration of response. Median PFS and median OS were 1.9 months (95% CI: 1.7–5.9 months) and 9.7 months (95% CI: 4.1 months–not reached), respectively.

Nivolumab

In the SCLC cohort of the phase I/II open-label CheckMate 032 (NCT01928394, see Table 1, c), unselected patients progressing after at least one previous platinum-containing regimen received either nivolumab 3 mg/kg every 2 weeks or nivolumab + ipilimumab (1 mg/kg + 1 mg/kg, 1 mg/kg + 3 mg/kg or 3 mg/kg + 1 mg/kg, intravenously) every 3 weeks for 4 cycles, followed by nivolumab 3 mg/kg every 2 weeks. Anti-tumour response and tolerability of nivolumab ± ipilimumab reported at interim analysis led to the addition of a randomised expansion cohort (3:2) assigned to nivolumab monotherapy (n=147), or nivolumab + ipilimumab (1 mg/kg + 3 mg/kg) followed by nivolumab 3 mg/kg every 2 weeks (n=95).

Updated analysis from the non-randomised cohort showed a higher ORR (25% versus 11%) in the combination arm (nivolumab 1 mg/kg + ipilimumab 3 mg/kg, n=61) compared with nivolumab alone (n=98). Combination therapy also improved median OS compared with nivolumab monotherapy (7.9 versus 4.1 months). Durable responses were observed in both treatment arms: nivolumab versus combination 1-year OS was 27% versus 40%, and 2-year OS 14% versus 26%. The incidence of any grade (73% versus 55%) and grade 3–4 toxicity (37% versus 12%) was higher in the combination arm. These are the first reported data demonstrating durable tumour response and manageable safety profile in SCLC patients for combination checkpoint inhibition in the second-line setting.

Results from the randomised expansion cohort confirm higher ORR (21% versus 12%) in the combination arm (n=95) versus monotherapy (n=147). Mature survival data are awaited.

Within the pooled non-randomised and randomised cohorts (n=401), exploratory biomarker analysis utilising TMB was undertaken in 211 (53%) evaluable patients and stratified into high, medium and low TMB subgroups. In patients receiving combination therapy, high TMB was associated with higher ORR (46%) compared with the medium or low TMB subgroups (ORR 16% and 22%, respectively). Improved 1-year survival was observed in the high TMB subgroup (62%) compared with the medium and low TMB subgroups (20%, 23%).

In patients receiving nivolumab monotherapy, a higher ORR was also observed in the high TMB subgroup (21%) versus the medium and low TMB subgroups (7%, 5%), although not to the same magnitude as in the combination arm. Prospective investigation of TMB is warranted in future immunotherapy SCLC trials.

A phase I/II study (NCT02247349), evaluating BMS-986012 (fully human monoclonal antibody targeting fucosyl-GM1) plus nivolumab after platinum-based first-line therapy, has reported a partial response in four out of 16 evaluable patients. Updated efficacy and biomarker analysis are awaited.

Future Developments

Several clinical trials investigating ICB in treatment-naïve and pre-treated SCLC are currently in progress (Table 2).

Limited Stage

A phase I trial is investigating the combination of pembrolizumab and concurrent RT either with ChT (cisplatin/carboplatin and etoposide) or alone (NCT02402920, see Table 2, a). The primary endpoint is to determine the maximum tolerated pembrolizumab dose given in combination with RT \pm ChT. Secondary endpoints are PFS (using immune-related response criteria [irRC]) and safety. The study plans to enrol 80 patients. The estimated primary completion date is July 2023.

A phase II trial is assessing the efficacy of pembrolizumab added to concurrent ChT \pm RT (NCT02934503, see Table 2, b). The primary endpoint is to evaluate the change in PD-L1 expression status as determined by IHC in pre-treatment and archival samples. Secondary endpoints are PFS, OS, ORR and safety. The study plans to enrol 60 patients. The estimated primary completion date is October 2019.

The use of combination nivolumab and ipilimumab as consolidation therapy after standard chemoradiotherapy (CRT) for limited-stage SCLC is currently under investigation in the randomised open-label phase II trial STIMULI (NCT02046733, see Table 2, c). Following CRT, unselected patients will be randomised to an induction phase of

nivolumab 1 mg/kg + ipilimumab 3 mg/kg every 3 weeks for 4 cycles, followed by a maintenance phase (nivolumab 240 mg every 2 weeks for 12 months) or observation. Co-primary endpoints are OS and PFS.

Extensive Stage

First-line

Part E of the phase I study KEYNOTE-011 (NCT01840579, see Table 2, d) is enrolling extensive-stage SCLC patients to receive pembrolizumab in combination with either cisplatin or carboplatin plus etoposide to assess the safety and tolerability of the combination therapy. The primary outcome measure is the number of patients experiencing dose-limiting toxicities. The study plans to enrol a total of 75 patients over all five parts. Recruitment has closed, with an estimated study completion date in April 2020.

The European Organisation for Research and Treatment of Cancer (EORTC)-led phase II study REACTION (NCT02580994) will evaluate platinum/etoposide \pm pembrolizumab in the first-line setting for extensive-stage SCLC patients. The estimated primary completion date is August 2020.

The phase III study KEYNOTE-604 (NCT03066778, see Table 2, f) plans to enrol 430 patients to receive platinum/etoposide \pm pembrolizumab with PFS and OS as co-primary endpoints. Secondary endpoints are ORR and safety. The estimated primary completion date is January 2019.

The phase III study IMpower133 (NCT02763579, see Table 2, g) is investigating carboplatin/etoposide plus atezolizumab or placebo. Co-primary endpoints are PFS and OS. Secondary endpoints include ORR and safety. The study plans to enrol 500 patients. The estimated primary completion date is August 2019.

The phase III study CASPIAN (NCT03043872, see Table 2, i) plans to randomise 795 patients to receive platinum/etoposide versus platinum/etoposide plus durvalumab versus platinum/etoposide plus durvalumab and tremelimumab. The primary endpoint is OS and secondary endpoints are PFS, ORR and safety. The estimated primary completion date is March 2019.

Table 2 Ongoing Clinical Trials Investigating Immunotherapy in SCLC

Trial identifier	Drug / Target	Phase	Regimen	N	Primary endpoint	Status (estimated primary completion date)
Limited stage						
a/ NCT02402920	Pembro / PD-1	I	MK-3475 + PE + Thoracic RT	80	MTD	Recruiting (Jul 2023)
b/ NCT02934503	Pembro / PD-1	II	Pembro + PE + Thoracic RT	60	Change in PD-L1 expression status	Recruiting (Oct 2019)
c/ NCT02046733	Ipi + Nivo / CTLA-4 PD-1	II	Ipi + Nivo followed by maintenance Nivo vs Observation	260	OS, PFS	Recruiting (Oct 2019)
Extensive stage – First-line						
d/ NCT01840579	Pembro / PD-1	I	In Part E, Pembro + Cisplatin + Etoposide	75 (overall 5 parts of the study)	Number of participants experiencing DLT	Ongoing but not recruiting participants (May 2018)
e/ NCT02934503	Pembro / PD-1	II	PE + Pembro and RT	60	PFS	Recruiting (Oct 2019)
f/ NCT03066778	Pembro / PD-1	III	Pembro + PE vs Placebo + PE	430	PFS	Recruiting (Jan 2019)
g/ NCT02763579	Atezo / PD-L1	III	Atezo + Carboplatin + Etoposide vs Placebo + Carboplatin + Etoposide	500	PFS, OS	Recruiting (Aug 2019)
h/ NCT02537418	Tremelimumab Durva / CTLA-4 PD-L1	Ib	Durva + Tremelimumab vs Tremelimumab	175	RP2D	Ongoing but not recruiting participants (Dec 2018)
i/ NCT03043872	Tremelimumab Durva / CTLA-4 PD-L1	III	Durva + Tremelimumab + PE vs Durvalumab + PE vs PE	795	OS, PFS	Recruiting (Mar 2019)

Table 2 Ongoing Clinical Trials Investigating Immunotherapy in SCLC (Continued)

Trial identifier	Drug / Target	Phase	Regimen	N	Primary endpoint	Status (estimated primary completion date)
Extensive stage – Maintenance						
j/ NCT03043599	Ipi Nivo / CTLA-4 PD-1	I/II	Consolidative Ipi + Nivo and maintenance Nivo with Thoracic RT after platinum-based ChT	52	RP2D	Ongoing but not recruiting participants (Apr 2021)
k/ NCT02538666	Ipi Nivo / CTLA-4 PD-1	III	Nivo vs Ipi + Nivo vs Placebo	810	PFS, OS	Recruiting (Sep 2018)
Extensive stage – Second-line and beyond						
l/ NCT03026166	Ipi Nivo / CTLA-4 PD-1	I/II	Rova-T and Nivo or Rova-T and Nivo plus Ipi 1 mg/kg or Rova-T and Nivo plus Ipi 3 mg/kg	90	Number of participants experiencing DLT	Recruiting (Feb 2020)
m/ NCT02963090	Pembro / PD-1	II	Pembro vs Topotecan	98	PFS	Ongoing but not recruiting participants (May 2019)
n/ NCT02701400	Tremelimumab Durva / CTLA-4 PD-L1	II	Tremelimumab + Durva vs Tremelimumab + Durva + SBRT	20	PFS, ORR	Recruiting (Aug 2019)
o/ NCT02481830	Nivo / PD-1	III	Nivo vs Topotecan or Atracuricin	480	OS	Ongoing but not recruiting participants (Aug 2018)

Abbreviations: Atez, atezolizumab; ChT, chemotherapy; CTLA-4, cytotoxic T-lymphocyte antigen 4; DLT, dose-limiting toxicity; Durva, durvalumab; Ipi, ipilimumab; MTD, maximum tolerated dose; Nivo, nivolumab; ORR, objective response rate; OS, overall survival; PD-1, programmed cell death protein-1; PD-L1, programmed death-ligand 1; PE, platinum/etoposide; Pembro, pembrolizumab; PFS, progression-free survival; Rova-T, Trovafipituzumab tesine; RP2D, recommended phase II dose; RT, radiotherapy; SBRT, stereotactic body radiotherapy; SCLC, small cell lung cancer.

An open label, randomised phase Ib study of durvalumab (MEDI4736) ± tremelimumab with or without standard ChT for solid malignancies will include a cohort of previously untreated patients with SCLC (NCT02537418, see Table 2, h). Final data collection is expected in December 2018.

Maintenance treatment

A phase I/II study (NCT03043599, see Table 2, j) is evaluating, in the safety run-in phase I portion, the recommended dose for the following phase II study of ipilimumab and nivolumab with combined thoracic RT (30 Gy in 10 fractions) and nivolumab/ipilimumab following standard treatment with 4–6 cycles of platinum-based ChT. The phase II portion of this study aims to estimate the 6-month PFS rate. In both parts, this trial plans to enrol a total of 52 patients. The estimated primary completion date is April 2021.

The phase III study CheckMate 451 (NCT02538666, see Table 2, k) is investigating immunotherapy as maintenance treatment after completion of first-line platinum/etoposide. A total of 810 patients will be randomised to receive nivolumab versus nivolumab/ipilimumab versus placebo. The co-primary endpoints are PFS and OS with secondary endpoints being ORR and safety. The estimated primary completion date is September 2019.

Second-line and beyond

The CheckMate 331 phase III trial (NCT02481830, see Table 2, o) randomised 480 pre-treated patients to receive nivolumab versus topotecan or amrubicin. The primary endpoint is OS of nivolumab versus ChT. Secondary endpoints are PFS, ORR and safety. The trial has completed accrual and will report in 2019.

A multicentre, randomised, open-label phase II study of pembrolizumab versus topotecan (NCT02963090, see Table 2, m) is enrolling 98 patients with recurrent SCLC in a 2:1 fashion. PD-L1 expression will be determined at baseline although subjects will be enrolled regardless of PD-L1 status. In the topotecan arm, progressing patients will be allowed to

crossover to the pembrolizumab arm. The primary endpoint is PFS. The study has completed recruitment with an estimated primary completion date in May 2019.

A randomised phase II trial (NCT02701400, see Table 2, n) is enrolling 20 patients with recurrent SCLC to receive tremelimumab plus durvalumab \pm RT. The co-primary endpoints are PFS and ORR. The estimated primary completion date is August 2019.

A phase I/II study (NCT03026166, see Table 2, l) is investigating the safety of Rova-T administered in combination with nivolumab or nivolumab plus ipilimumab in 90 patients with recurrent SCLC. The primary endpoint is to evaluate the number of patients experiencing dose-limiting toxicities. Secondary endpoints are ORR, PFS and OS. The estimated primary completion date is February 2020.

Declaration of Interest:

Dr Tay has reported no potential conflicts of interest.

Dr Rossi has reported no potential conflicts of interest.

Dr Califano has reported no potential conflicts of interest.

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2.3 Thoracic Malignancies

2.3.3 Mesothelioma

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Definition

Malignant pleural mesothelioma (MPM) has been known for its resistance to a variety of therapies, and has therefore been the focus of new treatment approaches such as immuno-oncology treatment. Although mesothelioma is not a typical immunogenic tumour, in the past it has been observed that some patients with MPM responded well on instillation of BCG (*Bacillus Calmette-Guérin*) or after the development of an empyema (Webster et al, 1982). In the 20th century, some groups observed that immune infiltration in biopsies predicted for better survival. Mesothelioma is also infiltrated by immune effector cells, cytokines and regulatory T cells (Hegmans et al, 2006; Anraku et al, 2008). This led to the idea that the immune system could play an important role in the biology of MPM.

Predictive and/or Prognostic Biomarkers of Clinical Relevance

Mesothelioma has a moderate expression of programmed death-ligand 1 (PD-L1); 20%–40% of patients have an expression of >1%. The non-epithelioid histological subtype has a significantly higher number of PD-L1-positive (PD-L1⁺) patients. PD-L1-negative (PD-L1⁻) patients have a significantly better prognosis than PD-L1⁺ patients, with a median survival of 16.3 months versus 4.8 months, respectively. The effect of PD-L1 status on prognosis does not depend on the histology (Cedr s et al, 2015; Mansfield et al, 2014).

Mesotheliomas have a low protein-altering mutation rate. Compared with other cancers, it is in the lowest third of the tumour mutational burden landscape (Chalmers et al, 2017). There is no significant difference in mutational burden between histological subtypes of mesothelioma (Bueno et al, 2016). Despite this low mutational burden, in a subgroup of patients with mesothelioma, immunotherapy is beneficial, possibly due to the presence of immune cells in the tumour microenvironment.

The prognostic significance of immune cells infiltrating the tumour has been investigated in several studies. With more CD4-expressing cells or CD8⁺ lymphocytes in the mesothelioma, there is a tendency for longer survival. High levels of interleukin-7 receptor (IL-7R) are associated with an increased risk of death. CD163⁺ cells and their ratio to tumour infiltrating lymphocytes (TILs) (CD8⁺ T cells and CD20⁺ B cells) are an independent marker of prognosis in mesothelioma (Ujii et al, 2015).

Clinical Results

Unlike the turbulent development in melanoma and lung cancer, the number of studies in MPM has developed at a slow pace. The studies reported in peer-reviewed journals or presented at major meetings are listed in Table 1. Most of these studies focus on the anti-programmed cell death protein 1 (PD-1) monoclonal antibodies nivolumab and pembrolizumab.

Data emerging from these studies indicate that the objective response rate (ORR) is comparable with the results obtained in lung cancer and other tumours, but there seems to be no clear correlation between PD-L1 expression level and response. In general, the primary endpoint of the second-line studies is the disease control rate (DCR) at 12 weeks. Long-term survivors have not yet been reported due to the recent initiation of these studies.

PD-1 Blockade

One phase Ib study, KEYNOTE-028, examined pembrolizumab in a variety of tumour types. This is the only study that included patients who expressed PD-L1 (defined as >1%), including a subset of 25 patients with MPM. The ORR for mesothelioma was 20% and the DCR was 72%.

Table 1 Completed Studies of Immuno-oncology Therapy for Mesothelioma Patients

Study	Drug(s) ^a	Phase	# Pts	Outcome
Determine (Maio et al, 2017)	Tremelimumab vs placebo 2:1	IIb	571	DCR: 28% vs 22% OS: 7.7 vs 7.3 months
NivoMes (Quispel-Janssen et al, 2017)	Nivolumab	II	33	DCR: 50% ORR: 15%
JAVELIN Solid Tumor (Hassan et al, 2016)	Avelumab	IB	53	DCR: 57% ORR: 9.4% mPFS: 17 weeks
KEYNOTE-028 (Alley et al, 2017)	Pembrolizumab 10 mg/kg q2w For PD-L1 >1%	IB	25	DCR: 72% ORR: 20% mPFS: 5.4 months mOS: 18 months
Pembrolizumab (Kindler et al, 2017) NCT02399371	Pembrolizumab	II	34	DCR 76% ORR 21% mPFS: 6.2 months mOS: not reached
MAPS 2 (Scherpereel et al, 2017)	Nivolumab vs Nivolumab + Ipilimumab (1:1)	II	125	DCR: 43% vs 52% ORR: 17% vs 26%
INITIATE NCT03048474	Ipilimumab + Nivolumab	II	38	DCR: 72% ORR: 28%
DC vaccine (Cornelissen et al, 2016)	DC-based immunotherapy + Cyclophosphamide	I	10	DCR: 80% Reduces regulatory T cells Safe
Antimesothelin immunotoxin (Hassan et al, 2014)	Cisplatin + Pemetrexed + SSIP	I	24	Safe Well tolerated PR: 77%

^aStandard dosages of therapy, unless otherwise specified

The number between brackets stands for bibliographic references listed at the end of this chapter.

Abbreviations: DC, dendritic cell; DCR, disease control rate; mOS, median overall survival; mPFS, median progression-free survival; ORR, objective response rate; OS, overall survival; PD-L1, programmed death-ligand 1; PR, partial response; Pts, patients; qXw, every X weeks.

The clinical benefit (complete response [CR] + partial response [PR] + stable disease [SD]) at 6 months was 40%. Median overall survival was 18 months. Historical data on median overall survival with second-line therapy ranges from 5.7 to 10.9 months.

Five patients (20%) presented treatment-related adverse events (trAEs) of grade ≥ 3 , including thrombocytopaenia, dyspnoea, increase in alanine aminotransferase, neutropaenia, decrease in appetite and pyrexia (Alley et al, 2017).

An interim analysis of a phase II study with single-agent pembrolizumab confirmed the DCR and limited toxicity profile (Kindler et al, 2017). In Switzerland, data collected from patients who received pembrolizumab for relapsed MPM were reviewed retrospectively. Response rates and survival outcomes were promising in the unselected population, and comparable with clinical trials for patients with Eastern Cooperative Oncology Group (ECOG) performance status 0–1 and second-line treatment (as were the inclusion criteria for KEYNOTE-028).

Comparable results were reported when nivolumab was used (Quispel-Janssen et al, 2017).

PD-L1 Blockade

Limited studies have been performed with PD-L1 blockers. The JAVELIN Solid Tumor study, a phase Ib trial, tested the use of avelumab in 53 patients. ORR was 9.4% and DCR 57%. Median progression-free survival was 17 weeks. The toxicity profile was acceptable; four patients (7.5%) had trAEs of grade ≥ 3 (colitis, lymphopaenia, increased gamma-glutamyl transferase [GGT] or creatine phosphokinase [CPK]) (Hassan et al, 2016).

Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4) Blockade

One of the largest studies performed in MPM is the use of tremelimumab in second and third line. A total of 571 patients were randomised to receive tremelimumab or placebo (2:1). The preliminary safety profile of tremelimumab was acceptable. This was a negative study, since no difference in the primary endpoint (overall survival) was noted (Maio et al, 2017).

Combination Checkpoint Inhibitors

In the MAPS2 trial, 125 patients were included and received either nivolumab or nivolumab plus ipilimumab. Interim analysis for the first 108 patients showed a DCR of 43% at 12 weeks with nivolumab and 52% with nivolumab plus ipilimumab. ORR was 17% with nivolumab alone and 26% with nivolumab plus ipilimumab (Scherpereel et al, 2017).

An interim analysis of 26 patients in the Dutch INITIATE trial

(NCT03048474), a phase II trial in which patients receive nivolumab plus ipilimumab, showed comparable results with a DCR of 69% and ORR of 27% at 12 weeks. Toxicity was relatively low.

Potential Future Developments

In Table 2, ongoing studies are reported. For checkpoint inhibitors, two trials explore the toxicity and changes in immunological microenvironment with immunotherapy as neoadjuvant treatment for surgery. One study investigates the toxicity of pembrolizumab when given after radiotherapy.

A few studies investigate the difference in efficacy for chemotherapy (ChT) versus immunotherapy, some in first line and some in further lines.

Adoptive Cell Therapy

A few phase I studies are investigating the safety and feasibility of intrapleural or intravenously administered human chimeric antigen receptor (CAR)-modified T cells in patients with mesothelin (MSLN)-expressing cancers. No results have been published for mesothelioma.

Anticancer Vaccines

Dendritic cells (DCs) have been used in tumour cell vaccinations for mesothelioma. Cornelissen et al described 10 patients in whom DC vaccination was given after immune modulation of the body with cyclophosphamide. This resulted in radiographic disease control in 8 out of 10 patients. Seven of these 10 patients survived 24 months or more, and two patients were alive at 50 and 66 months after treatment (Cornelissen et al, 2016).

This approach is now being investigated in two other trials (see Table 2). The European DENIM phase III trial will test DC-based immunotherapy with allogeneic tumour lysate as maintenance treatment after ChT.

Immunotoxin Immunotherapy

MSLN is overexpressed in mesothelioma. SS1P is an immunotoxin consisting of an anti-MSLN antibody fragment fused to pseudomonas exotoxin. Hassan et al showed that SS1P can be administered safely and had an impres-

sive tumour response in mesothelioma. Thirteen out of 24 patients received the maximum tolerated dose, and 77% demonstrated a partial response with SS1P in combination with ChT (Hassan et al, 2014).

Another MSLN-targeted immunotoxin currently being investigated is

Table 2 Ongoing Studies of Immuno-oncology Therapy for Mesothelioma Patients

Study	Drug(s)	Phase	# Pts	Primary endpoint	Remarks
Neoadjuvant pembrolizumab NCT02707666	Pembrolizumab before surgery	I	15	Toxicity γ gene expression	University of Chicago
Adjuvant pembrolizumab NCT02959463	RT + adjuvant Pembro (± surgery or ChT)	I	24	Toxicity	MD Anderson
Durvalumab tremelimumab + surgery NCT02592551	Durva + surgery Durva + tremelimumab + surgery Control arm + surgery	II Window of opportunity study	8 8 4	CD8/T _{reg} ratio and ICOS	Single centre Houston
Pembro vs ChT NCT02784171	Cisplatin + pemetrexed Cisplatin + pemetrexed + Pembro Pembro alone	II	126	PFS	Canada
PROMISE NCT02991482	Pembro vs standard of care	III	142	PFS	ETOP study
Durvalumab and tremelimumab NCT03075527	Durva q4w + tremelimumab q4w	II	40	ORR	Dana-Farber Institute
PrE0505 NCT02899195	Durva q4w + ChT	II 1 st line	55	OS	ECOG study
CheckMate 743 NCT02899299	Nivo + ipi vs platinum+ pemetrexed	III 1 st line	600	OS and PFS	Multinational
NIBIT-MESO-1 NCT02588131	Durva + tremelimumab	II 1 st and 2 nd line	40	ORR	Italian study
KEYNOTE-158 Pembrolizumab NCT02628067	Pembro	II	1350	ORR	Multinational
MesoDec NCT02649829	Autologous DC vaccination	I/II	20	Feasibility and safety	Single centre Antwerp
MesoCancerVac NCT02395679	DCs loaded with allogeneous cell lysate	I	9	Tolerability	Single centre Rotterdam
Oncolytic virus NCT02714374	Neoadjuvant GL-ONC1 vaccinia ± eculizumab	IB	36	trAE	Single centre San Diego
NCT01503177	Intrapleural measles virus	I	36	AE	Mayo Clinic

Abbreviations: AE, adverse event; ChT, chemotherapy; DC, dendritic cell; Durva, durvalumab; ECOG, Eastern Cooperative Oncology Group; ETOP, European Thoracic Oncology Platform; ICOS, inducible T cell co-stimulator cells; Ipi, ipilimumab; OS, overall survival; ORR, objective response rate; Nivo, nivolumab; Pembro, pembrolizumab; PFS, progression-free survival; Pts, patients; qXw, every X weeks; RT, radiotherapy; trAE, treatment-related adverse event; T_{reg}, regulatory T cell.

LMB-100.

Oncolytic Viral Therapy

For vaccinia immunotherapy, there is still only preclinical research. Two phase I studies are investigating the toxicity of oncolytic viral therapy for mesothelioma (see Table 2, NCT02714374 and NCT01503177).

Declaration of Interest:

Dr Baas has received research grants from or has served as a consultant for Merck and Bristol-Myers Squibb.

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Further Reading

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2.3 Thoracic Malignancies

2.3.4 Other Thoracic Malignancies: Thymic Malignancies and Others

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Thymic Malignancies

Definition

Thymic malignancies represent a heterogeneous group of rare thoracic cancers, which may be aggressive and difficult to treat. Thymic epithelial tumours are classified according to the World Health Organization (WHO) system, which distinguishes thymomas from thymic carcinomas. Thymomas combine epithelial tumour cells with lymphocytes and are further subdivided into different types (so-called A, AB, B1, B2 and B3), based on the relative proportion of non-tumoural lymphocytic component and resemblance to normal thymic architecture. Thymic carcinomas are similar to their extra-thymic counterpart, the most frequent subtype being squamous cell carcinoma – which may be differentiated from primary lung squamous cell carcinoma as thymic squamous cell carcinoma shows expression of CD5 and CD117. While thymomas are usually slow-growing tumours with a tendency toward local and regional invasion, thymic carcinomas are more aggressive tumours with frequent metastatic spread to lymph nodes and distant sites.

Key Points to Consider for Immunotherapy

Autoimmunity in the thymus and in thymomas

The physiological role of the thymus is to induce central tolerance to self-antigens, through the control of the differentiation and the subsequent positive and negative selection of immature T cells. This process is deregulated along with thymic carcinogenesis:

- Immature thymoma-derived lymphocytes may escape the disorganised tumour environment without passing through the thymic medulla where self-tolerance is induced.
- Medullary thymic epithelial cells present with defects with regard to their unique capability to express tissue-related antigens, related to a loss of expression of the transcription factor AIRE (autoimmune regulator), similar to that described in APECED (autoimmune poly-endocrinopathy candidiasis ectodermal dystrophy). This leads to self-reactive thymocytes not driven to programmed cell death, and thereby not deleted from the immune repertoire.
- Thymic carcinogenesis may be associated with genetic changes that impair the development of T cells and generate an increased number of self-reactive lymphocytes. The finding that patients can develop an autoimmune disease after thymectomy has been challenging these concepts and is currently not fully understood.

In the non-neoplastic thymus, programmed cell death protein 1 (PD-1)/programmed death-ligand 1 (PD-L1) interaction negatively regulates beta selection. However, it also modulates positive selection, as PD-1 deficiency may lead to a significant alteration of the mature T cell repertoire. PD-1 is also involved in CD8⁺ T cell tolerance through peripheral intrinsic mechanisms, such as deletion or functional inactivation. PD-1 also facilitates the peripheral differentiation of CD4⁺ T cells into regulatory T cells (T_{regs}).

Autoimmune disorders are a clinical hallmark of thymomas

One-third of patients diagnosed with thymoma present at the time of diagnosis with autoimmune disorders, the most frequent being myasthenia gravis. Other frequent disorders include pure red cell aplasia (5% of

cases) and hypogammaglobulinaemia (5% of cases). Those disorders are therefore not paraneoplastic syndromes which would recover after resection of the tumour. As they are more related to self-reacting lymphocytes than escaped from negative selection in the thymus, those autoimmune diseases usually have a course that is independent from the evolution of the tumour.

Systematic immunological check-up is recommended when a diagnosis of thymic epithelial tumour is suspected; autoimmune disorders, even if latent at the time of diagnosis, may significantly impact any therapeutic intervention, including surgery, radiotherapy (RT), chemotherapy (ChT) and especially immunotherapy, with a risk of acute exacerbation. Autoimmune disorders are not observed in thymic carcinomas, which may still be associated with true paraneoplastic syndromes related to the direct secretion of cytokines or hormones by tumour cells.

PD-L1 Expression in the Thymus and in Thymic Epithelial Tumours

PD-L1 expression, while observed in >90% of epithelial cells of the normal thymus with a medullar tropism respecting Hassall's corpuscles, was also identified in thymomas and thymic carcinomas using various immunohistochemistry (IHC) protocols (Table 1) (Arbour et al, 2017; Cho et al, 2017; Giaccone et al, 2017; Padda et al, 2015; Weissferdt et al, 2017; Yokoyama et al, 2016a; Yokoyama et al, 2016b). The significance of this finding as a rationale for the assessment of immune checkpoint inhibitors (ICIs) targeting PD-1 or PD-L1 remains debatable, given:

- The high frequency of PD-1 and PD-L1 expression in the non-neoplastic thymus
- The fact that in thymomas, the presence of immature and mature T cells surrounding tumour cells is part of the prototypic architecture, and not a marker of actual antitumour response
- The potential immune modulation induced by ChT or targeted agents (such as reported with sunitinib), which are part of the standard treatment strategy in advanced disease and may lead to modulation of PD-L1 expression in immune cell populations including T_{regs}

Table 1 Selected Studies Assessing PD-L1 Expression in Thymoma and Thymic Carcinoma

	Techniques	Thymoma		Thymic carcinoma	
	Antibody	n	PD-L1 positive (n, %)	n	PD-L1 positive (n, %)
National Cancer Center Tokyo	TMA, clone E1L3 (H-score, 1% cut-off)	101	22 (23%)	38	26 (70%)
Stanford University	TMA, clone 5H1 (intensity high)	65	44 (68%)	4	3 (75%)
Memorial Sloan Kettering Cancer Center	Slides, clone E1L3 (25% of tumour cells cut-off)	12	11 (94%)	12	4 (34%)
Kurume University	EPRI 161 (H-score, 20% cut-off)	82	44 (54%)	25	20 (80%)
MD Anderson Cancer Center	Slides, clone E1L3 (5% of tumour cells cut-off)	74	47 (64%)	26	14 (54%)
Cedars-Sinai Medical Center	Clone SP142 (1% of tumour cells cut-off)	38	35 (92%)	8	4 (50%)

Abbreviations: PD-L1, programmed death-ligand 1; TMA, tissue micro-array.

Clinical Results with PD-1 and PD-L1 ICIs

About 20%–30% of thymomas and 70%–80% of thymic carcinomas may present with unresectable, recurrent and/or metastatic disease. In this setting, current options include cytotoxic combination regimens, combining platin with anthracyclines, etoposide or taxanes, and targeted agents such as sunitinib; response and survival rates are usually limited, ranging from 20%–30%, and 6–7 months, respectively.

To fulfil the unmet need for prolonged survival in this setting, several phase II trials were initiated using ICIs targeting PD-1 or PD-L1, after several case reports were published. Concerns include both safety, with the potential risk of worsening latent autoimmune disorders, and efficacy, in the setting of a low tumour mutation burden potentially limiting immunogenicity.

One phase II trial was conducted with pembrolizumab, a fully humanised immunoglobulin (Ig)G4 antibody that targets the PD-1 receptor, in patients with advanced refractory thymic carcinomas (NCT02364076). In this study, any history of autoimmune disease or other malignancy requiring treatment were exclusion criteria. Pembrolizumab was given at

200 mg every 3 weeks. Out of 41 patients, six (15%) patients developed serious autoimmune disorders: two cases of polymyositis and myocarditis, with complete recovery with steroids but requiring a pacemaker for complete auriculo-ventricular block; one case of pancreatitis, hepatitis and diabetes mellitus type 1; one case of bullous pemphigoid, recovering with steroids; one case of polymyositis and hepatitis; and one case of transaminitis; three patients had to discontinue treatment after one of these adverse events (AEs). Response rate was 23% including one complete response (CR), eight partial responses (PRs) and 21 (53%) patients with stable disease (SD); median duration of response was 23 months. Median progression-free and overall survival (PFS and OS) were 4.2 and 24.9 months, respectively. PD-L1 expression (using IHC with Dako 22C3 antibody) was observed in $\geq 50\%$ of tumour cells for 10 patients, six of whom had response to pembrolizumab; only three patients out of the 27 with PD-L1 expression by tumour cells $< 50\%$ had any response. Correlation between response to pembrolizumab and T cell inflammation signature was also reported in 32 patients.

A similar trial was conducted in Korea (NCT02607631). This phase II trial enrolled 26 patients with thymic carcinoma and seven patients with thymoma, and results were presented at the ASCO 2017 Annual Meeting. Response and SD rates were 24% and 55% respectively, with PFS 6.1 and 9 months, respectively. Treatment-related AEs (trAEs) \geq grade 3 associated with immune-related AEs (irAEs) included hepatitis (four cases), myocarditis (three cases), myasthenia gravis (two cases), thyroiditis (one case), anti-neutrophil cytoplasmic antibody (ANCA)-associated rapidly progressive glomerulonephritis (one case), colitis (one case) and subacute myoclonus (one case). Despite management with high-dose corticosteroids and other immunosuppressive agents, eight patients discontinued study treatment.

Finally, a phase I trial is ongoing with avelumab, a fully human, IgG1 anti-PD-L1 antibody under clinical development (NCT03076554). In this trial, eight patients (seven with thymoma [2 type B3, 1 type B2/B3, 3 type B2, and 1 type B1] and one with thymic carcinoma) were treated; two patients with thymoma had confirmed PR, two had unconfirmed responses, three

(including the patient with thymic carcinoma) had SD, and one had progressive disease. Interestingly, three patients experienced tumour response after a single dose of avelumab. In five patients, trAEs were also irAEs, including myositis, precluding continuation of avelumab. Following treatment with oral steroids, these events resolved completely in three cases and incompletely in one case. Correlative studies suggested that avelumab induces infiltration of the tumour by macrophages, natural killer (NK) cells and activated T lymphocytes, suggesting real induction of antitumour responses, not only lymphocytic depletion with the treatment.

Overall, the conclusions of those studies are the following:

- Immunotherapy with ICIs targeting PD-1 or PD-L1 shows promising efficacy in thymic malignancies, with response rates and duration of response in line with reported studies in other solid tumours
- Toxicity is a major concern, despite systematic baseline workup for autoimmunity, with frequent occurrence of severe autoimmune AEs, mostly consisting of myocarditis, myositis and hepatitis, possibly favoured by previous treatments with anthracyclines and RT
- Immunotherapy is therefore not a standard-of-care in thymic carcinoma and should not be delivered in an off-label setting, especially if the patient is eligible for ongoing clinical trials

Potential Future Developments

In Europe, the European Organization for Research and Treatment of Cancer (EORTC) and the European Thoracic Oncology Platform (ETOP) are now starting a single-arm, multicentre, phase II study – the NIVOTHYM trial – to assess the efficacy of nivolumab alone or combined with ipilimumab in patients with advanced, refractory type B3 thymomas and thymic carcinomas. A strict autoimmune workup is planned (NCT03134118). A phase I/II trial with pembrolizumab in thymic carcinoma and thymoma is also being initiated (NCT03295227).

Other Rare Thoracic Malignancies

Rare thoracic tumours are defined as lung, pleural, mediastinal or cardiac tumours with unusual histology. Overall, these tumours account for <1%

Table 2 *Reported Results of Clinical Trials with Anti-PD-1/PD-L1 in Thymic Malignancies*

	Thymoma				Thymic carcinoma				Grade ≥3 adverse events	
	n	Response	Stable disease	Outcome	n	Response	Stable disease	Outcome	Rate	Events
	n (%)	n (%)	n (%)		n (%)	n (%)	n (%)		n (%)	
Giaccone et al (2017)					41	9 (23%)	21 (53%)	mPFS: 4.2 months mOS: 24.9 months	6 (15%)	Myositis, myocarditis, pancreatitis, hepatitis, pemphigoid
Cho et al (2017)	7	2 (29%)	5 (72%)	mPFS: 6.1 months	26	6 (23%)	13 (50%)	mPFS: 6.1 months	9 (27%)	Myositis, myocarditis, myasthenia, hepatitis
Rajan et al (2017)	7	4 (57%)	2 (28%)	NR	1	0 (0%)	1 (100%)	NR	5 (71%)	Myositis

Abbreviations: mOS, median overall survival; mPFS, median progression-free survival; NR, not reached; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1.

of all primary thoracic tumours, while they correspond to more than 100 different histological, clinical, radiological and prognostic entities. Some rare histological subtypes are specific to the lung or the pleura, whereas others, rarely occurring within the thorax, correspond to tumours more frequent in other organs. The most frequent rare pulmonary tumours are, in decreasing order of frequency: neuroendocrine tumours (carcinoids and large cell carcinomas), inflammatory myofibroblastic tumours, mucosa-associated lymphoid tissue (MALT) lymphoma and sarcomatoid carcinomas. Limited data about immune checkpoint expression and immunotherapy have been reported so far in rare pulmonary tumours, with the exception of large cell neuroendocrine carcinomas.

Definition

Neuroendocrine tumours of the lung are a distinct family of tumours, sharing morphological, immunohistochemical, molecular, clinical and outcome characteristics. The major categories of neuroendocrine tumours of the lung include small cell lung cancer (SCLC) and large cell neuroendocrine carcinoma (LCNEC), both of which are high-grade neuroendocrine tumours, while typical carcinoid and atypical carcinoid are

considered as low- and intermediate-grade malignant tumours, respectively. The diagnosis of LCNEC requires IHC for neuroendocrine markers, including NCAM/CD56, chromogranin A and synaptophysin.

PD-L1 Expression in LCNEC

PD-L1 expression was observed in 10% of LCNEC cases in a series of 106 surgical cases from Japan, using the E1L3N antibody and a 1% cut-off using the H-score method.

Clinical Results with PD-1 and PD-L1 ICIs

A retrospective cohort of 18 patients treated with nivolumab (nine cases) or pembrolizumab (one patient) was reported at the 2016 World Conference on Lung Cancer; response was observed in one patient, SD in eight patients, and progression in eight patients. Median PFS was 57 weeks.

Declaration of Interest:

Dr Girard has reported consultancy from AstraZeneca, Bristol-Myers Squibb, Hoffman-La Roche, Merck Sharp & Dohme and Pfizer.

Dr Merveilleux du Vignaux has reported no potential conflicts of interest.

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2.4 Genitourinary Malignancies

2.4.1 Renal Cancer

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Introduction

Clear cell renal cell carcinoma (ccRCC) has long been known as an appropriate tumour model for the development of immune agents. The rationale beyond this assumption relied on:

- The high lymphocytic infiltration observed in this disease
- Clinical observations of spontaneous systemic disease regression after resection of the primary tumour, and
- The long-term remissions observed in some patients with cytokines such as high-dose interleukin-2 (IL-2)

Therefore, investigators have rapidly integrated new immune-modulatory treatment regimens for advanced RCC patients into their clinical trials. Over the past 3 years, checkpoint inhibitors have transformed the standard-of-care (SoC) not only in second line but now also in first line, creating a real paradigm shift after a decade of anti-angiogenic supremacy in this disease.

The objective of this chapter is to present the clinical results of immune-modulating agents in RCC and to frame the future developments in this disease.

Clinical Results

Until 2015, the treatment of metastatic RCC was built on two classes of drugs: the vascular endothelial growth factor (VEGF)/VEGF receptor (VEGFR) axis inhibitors, and the mammalian target of rapamycin (mTOR) inhibitors.

Nivolumab Single Agent in Second and/or Third Line

Nivolumab, an immunoglobulin (Ig)G1 monoclonal antibody directed against programmed cell death protein 1 (PD-1), was initially tested in 33 patients with refractory ccRCCs in a phase I trial (Topalian et al, 2012) demonstrating strong antitumour activity at every dose level, ranging from 0.1 mg/kg to 10 mg/kg, with an objective response reported in nine out of 33 patients (27%). Responses were durable, ongoing after one year of treatment for five patients. Eighteen patients had disease control, including stable disease (SD) and partial response (PR), with a 54% progression-free survival (PFS) rate at 24 weeks. Subsequently, a large phase II study with 168 patients randomised to three dose levels of nivolumab (0.3 mg/kg, 2 mg/kg, 10 mg/kg) reported a response rate (RR) in the range of 20%–22% in previously treated patients, and a median overall survival (OS) of 18.2 months (80% confidence interval [CI]: 15.3–26.0), with 11% of grade 3 or 4 adverse events (AEs) (Motzer et al, 2015a).

These findings prompted the CheckMate 025 phase III trial of nivolumab versus everolimus in advanced ccRCC following one or two lines of prior anti-angiogenic therapy (Motzer et al, 2015b). OS was improved by nivolumab, with a median OS of 25 months (95% CI: 21.8–not evaluated [NE]), versus 19.6 months (95% CI: 17.6–23.1) with everolimus (hazard ratio [HR] 0.73; 98.5% CI: 0.57–0.93, $p=0.0018$). Among patients treated with nivolumab ($n=410$), the objective response rate (ORR) was 25% versus 5% with everolimus (odds ratio: 5.98; 95% CI: 3.68–9.72). No difference in PFS was observed: median PFS with nivolumab was 4.6 (3.7–5.4) months versus 4.4 (3.7–5.5) months with everolimus, HR 0.88 (95% CI: 0.75–1.03). Consistent benefit of nivolumab over everolimus was seen across all pre-specified subgroups (Escudier et al, 2017a), including across the Memorial Sloan Kettering Cancer Center

(MSKCC) risk classification groups, irrespective of the number of prior anti-VEGFR therapies (one versus two). Interestingly, treatment benefit beyond progression was reported with a 14% RR after progression (Escudier et al, 2017b). It is noteworthy that the use of programmed death-ligand 1 (PD-L1) staining did not allow patient selection, with similar magnitude of benefit between PD-L1-negative (PD-L1⁻) patients (HR 0.79) and PD-L1-positive (PD-L1⁺) patients (HR 0.77). However, as in previous reports, the PD-L1⁺ patients carried a dismal prognosis compared with PD-L1⁻ patients. Safety analyses reported that 76 out of 410 patients (19%) treated with nivolumab experienced grade 3 or 4 treatment-related AEs (trAEs). The most frequent AEs were fatigue (33%), pruritus (14%) and nausea (14%). Immune-related AEs (irAEs) encompassed pneumonia, rash or endocrinopathies. Most of them were reversible following treatment with corticosteroids.

Based on these results, nivolumab was granted approval by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for the treatment of ccRCC following prior anti-angiogenic therapy. Therefore, nivolumab has been integrated into the guidelines for second and later lines of treatment, and is widely used depending on national reimbursement policies (Escudier et al, 2016; Powles et al, 2016; Choueiri and Motzer, 2017).

Nivolumab + Ipilimumab Combination in First Line

Combination of two checkpoint inhibitors, namely nivolumab and ipilimumab (a cytotoxic T-lymphocyte antigen 4 [CTLA-4]) inhibitor, have demonstrated encouraging results in the phase I setting of CheckMate 016 (Hammers et al, 2017). Two different regimens of this combination have been evaluated, with similar efficacy but better tolerability for the nivolumab 3 mg + ipilimumab 1 mg regimen. Furthermore, CheckMate 214 was a global randomised phase III trial, testing the combination of two immune checkpoint inhibitors (ICIs), ipilimumab and nivolumab (3 mg/kg nivolumab intravenously [i.v.] + 1 mg/kg ipilimumab i.v. every 3 weeks for 4 doses, then 3 mg/kg nivolumab i.v. every 2 weeks), compared with sunitinib (50 mg sunitinib orally once daily for 4 weeks out of each 6-week cycle) (Motzer et al, 2018a). The patient population con-

sisted of treatment-naïve advanced or metastatic ccRCC patients. The trial had three co-primary endpoints of RR, PFS and OS in intermediate- and poor-risk groups as defined by the International Metastatic Renal Cell Carcinoma Database Consortium (IMDC). Outcomes in the overall population (intention to treat [ITT]) were a secondary endpoint.

Overall, 1096 patients were randomised in the ITT population, including 847 with intermediate- or poor-risk disease. Respectively 23%, 61% and 17% of patients were in good-, intermediate- and poor-risk groups; 24% of the ITT population and 28% of the intermediate-/poor-risk population were PD-L1+ (>1% of tumour cell staining with 288 antibody). With a median follow-up of 25.2 months, the study was positive on two of the co-primary endpoints. The ORR was 42% for the patients treated with the combination versus 27% for patients treated with sunitinib ($p<0.0001$). Median OS was not reached for the immuno-oncology (IO) combination (95% CI: 28.2–not reached [NR]) versus 26 months for sunitinib (95% CI: 22–NR) (HR 0.63; 99.8% CI: 0.44–0.89). The third endpoint, PFS, did not reach statistical significance: median PFS for nivolumab + ipilimumab was 11.6 (8.5–15.5) months versus 8.4 (7–10.8) months for sunitinib (HR 0.82; 99.1% CI: 0.64–1.05). Furthermore, a higher rate of complete responses (CRs), 9%, as well as durable response, were reported in the nivolumab + ipilimumab arm. Taken altogether, these results will establish nivolumab + ipilimumab as a new SoC in first line for intermediate- and poor-risk patients. Secondary endpoints included investigating outcomes in the ITT population. Results showed nivolumab + ipilimumab was associated with a significant advantage for both ORR (39% versus 32%) and OS (HR 0.68; 99.8% CI: 0.49–0.95).

Notably, in the nivolumab + ipilimumab arm, 79% of patients received all four doses of ipilimumab. Treatment discontinuation due to AEs was 15% and 7% for nivolumab + ipilimumab and sunitinib, respectively. Grade 3–5 AEs were more common with sunitinib than with the IO combination (63% versus 46%, respectively). The most common grade 3–4 AEs with nivolumab + ipilimumab were fatigue (37%), pruritus (28%) and diarrhoea (27%), compared with diarrhoea (52%), fatigue (49%) and palmar-plantar erythema (43%) for sunitinib.

Exploratory endpoints investigated outcomes in favourable-risk patients and according to tumour PD-L1 expression level. Results in the favourable-risk population showed RRs of 29% (95% CI: 21–38) versus 52% (95% CI: 43–61), and median PFS of 15.3 months (95% CI: 9.7–20.3) versus 25 months (95% CI: 20.9–NE) for nivolumab + ipilimumab and sunitinib, respectively (HR 2.18; 95% CI: 1.29–3.68). These results support the use of sunitinib over the combination in the favourable-risk population. Exploratory analysis of the biomarker identified that PD-L1⁺ tumours were associated with better outcomes for both RR and PFS with the doublet IO than with sunitinib (HR 0.48; 95% CI: 0.28–0.82). In contrast, in the PD-L1⁻ tumours, PFS was similar (HR 1.0; 95% CI: 0.74–1.36), suggesting that in this study this biomarker appears potentially predictive for PFS.

Atezolizumab + Bevacizumab Combination

Improving long-term survival of patients might rely on ICI combinations associated with anti-angiogenic therapies; this hypothesis is under investigation in a large number of combination trials.

The first results have included phase I combination as well as randomised phase II for the doublet atezolizumab + bevacizumab. The phase I study initially highlighted the biological rationale behind the combination, thanks to sequential tumour biopsies providing preliminary evidence of enhanced anti-tumour immune responses following treatment with bevacizumab and atezolizumab + bevacizumab (Wallin et al, 2016). The randomised phase II IMmotion 150 study compared atezolizumab single agent (1200 mg i.v. every 3 weeks) versus atezolizumab + bevacizumab (15 mg/kg every 3 weeks) versus sunitinib (50 mg daily 4-weeks on/2-weeks off) as first-line treatment for metastatic ccRCC. It is noteworthy that, at progression, a cross-over was allowed from the two single-agent arms to the combination arm.

Overall, 305 patients have been enrolled and stratified based on PD-L1 expression (on tumour cells and immune cells) as well as on MSKCC prognostic groups. The primary endpoint was OS in the ITT population and in the PD-L1⁺ population. With a median follow-up of 20.7 months, there was no difference in primary endpoint (HR 1.00; 95% CI: 0.69–1.45) in the atezolizumab + bevacizumab arm versus the sunitinib arm, nor in the atezolizumab single-agent arm (HR 1.19; 95% CI: 0.82–

1.71) compared with the sunitinib arm. However, a signal was reported in the biomarker-selected population (PD-L1⁺), as median PFS in this population was 14.7 (8.2–25.1) months versus 7.8 (3.8–10.8) months for sunitinib (stratified HR 0.64; 95% CI: 0.38–1.08). Median PFS for atezolizumab as single agent was 5.5 months (stratified HR 1.03; 95% CI: 0.63–1.67) compared with the sunitinib arm. Additionally, the RR was higher (36% in the combination arm versus 28% for sunitinib in the PD-L1⁺ population). On the safety analysis, the most common AEs were similar to the ones known for each agent, and grade 3 or 4 AEs were reported in 40%, 16% and 57% of patients for the atezolizumab + bevacizumab, bevacizumab and sunitinib arms, respectively. Interestingly, efficacy of the combined arm was further reported in the monotherapy arms (atezolizumab or sunitinib) after crossover upon progression (Atkins et al, 2017). Overall, 26% of patients achieved objective response after crossover, with a median PFS of 8.8 (5.6–13.7) months.

More recently, the randomised phase III IMmotion 151 trial compared atezolizumab + bevacizumab (15 mg/kg every 3 weeks) versus sunitinib (50 mg daily 4-weeks on/2-weeks off) as first-line treatment for metastatic ccRCC (Motzer et al, 2018b). Overall, 915 patients have been enrolled, including 362 patients with PD-L1⁺ tumours. The primary endpoint was PFS in the PD-L1⁺ population. With a median follow-up of 15 months, there was a benefit on PFS (HR 0.74; 95% CI: 0.57–0.96) in the atezolizumab + bevacizumab arm with a median PFS of 11.2 (8.9–15) months, compared with the sunitinib arm with a median PFS of 7.7 (6.8–9.7) months. The same benefit was reported in the ITT population: median PFS was 11.2 (9.6–13.3) months for the atezolizumab + bevacizumab arm versus 8.4 (7.5–9.7) months for the sunitinib arm (HR 0.83; 95% CI: 0.7–0.97). Furthermore, the RR was higher (43% in the combination arm versus 35% for sunitinib in the PD-L1⁺ population). At time of reporting, data for OS analysis were not mature, given that only 30% of patients had an OS event at data cut-off, with a median OS NR for the atezolizumab + bevacizumab arm versus 23.3 (21.3–NR) months for sunitinib (HR 0.68; 95% CI: 0.46–1.00). On the safety analysis, grade 3 or 4 AEs were reported in 40% and 54% of patients for the atezolizumab + bevacizumab and sunitinib arms; AEs leading to treatment discontinuation were reported in 5% and 8% of patients, respectively.

Other Combinations

Many other combinations have been investigated in phase I/II. Table 1 presents the studies with available results focusing on RCC populations. This representation is not meant to encourage cross-trial activity comparison, but to highlight the ORRs seen in treatment-naïve populations ranging from 45% to 73%. These results have prompted several phase III trials, summarised below.

Table 1 Combination Phase I/II Studies of VEGFR TKI with PD-1/PD-L1 Inhibitors in Patients with Metastatic RCC: Activity

Combination	Nivolumab + Sunitinib	Nivolumab + Pazopanib	Pembrolizumab + Axitinib	Avelumab + Axitinib	Pembrolizumab + Lenvatinib	Nivolumab + Tivozanib
Reference	Amin et al (2014)	Amin et al (2014)	Atkins et al (2018)	Choueiri et al (2018)	Lee et al (2017)	Escudier et al (2018)
N	33	20	52	55	30	18
ORR	52	45	73	58	63	64
CR	NE	NE	7.7	5.5	0	0
PR	NE	NE	65.4	53	63	64

Abbreviations: CR, complete response; NE, not evaluated; ORR, objective response rate; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; PR, partial response; RCC, renal cell carcinoma; TKI, tyrosine kinase inhibitor; VEGFR, vascular endothelial growth factor receptor.

Potential Future Developments

Ongoing Phase III Combination Trials

Table 2 presents the ongoing phase III trials of VEGF/VEGFR-targeted therapy with IO agents in the first-line setting. All these trials have been designed to compare the combination therapy against SoC single-agent VEGF therapy with sunitinib. However, given the recent results of the CheckMate 214 pivotal study (nivolumab + ipilimumab versus sunitinib), a new standard is being set and head-to-head comparison of the different doublet approaches would have been of interest both for the community and the regulatory agencies.

Table 2 Ongoing Randomised Phase III Combination Checkpoint Inhibitors in the First-line Setting for Metastatic RCC

Study	IMmotion I51	MK-3475-426/ KEYNOTE-426	JAVELIN Renal 101	Study 307	CheckMate 9ER
Investigational arm(s)	Atezolizumab + Bevacizumab	Pembrolizumab + Axitinib	Avelumab + Axitinib	Lenvatinib + Everolimus or Lenvatinib + Pembrolizumab	Cabozantinib + Nivolumab
Control arm	Sunitinib	Sunitinib	Sunitinib	Sunitinib	Sunitinib
NCT reference	NCT02420821	NCT02853331	NCT02684006	NCT02811861	NCT03141177
N	830	840	830	735	630
Primary objective	<ul style="list-style-type: none"> • PFS in PD-L1+ population • OS 	<ul style="list-style-type: none"> • PFS • OS 	<ul style="list-style-type: none"> • PFS in PD-L1+ population • OS in PD-L1+ population 	PFS	PFS in intermediate-/poor-risk randomised participants

Abbreviations: NCT, clinicaltrials.gov identifier; OS, overall survival; PD-L1+, programmed death-ligand 1-positive; PFS, progression-free survival; RCC, renal cell carcinoma.

Open Questions with IO Use

Sequence trials

None of the previously discussed trials addresses the question of combination versus sequence trials. Therefore, it is unknown if a sequential strategy of priming the immune system with a VEGF/VEGFR-targeting agent first is superior to an upfront combination approach. Ongoing trials of sequencing single agents may help to define the optimal strategy, such as the SUAVE Trial (NCT03035630), a randomised phase II study planned to randomise patients in a 1:1 ratio to one of two first-line medication treatment arms (sunitinib or avelumab); once disease progression has been documented, subjects will receive second-line medication (either avelumab or axitinib).

Adjuvant use

The role of PD-1/PD-L1 axis inhibition in the perioperative setting for localised RCC is unknown. Several small phase I/II studies are investigating the neoadjuvant approach, while large phase III trials have been launched in the post-nephrectomy population. Interestingly, disease-free survival (DFS) as primary endpoint, commonly accepted by the FDA and EMA, may be discussed with this class of agents. Once

again, the question of the comparator arm is being discussed, given the heterogeneous results of the role of sunitinib in the subset of high-risk patients. To date, five phases III studies have been initiated: nivolumab pre- and post-nephrectomy is investigated against surveillance (PROSPER, NCT03055013), atezolizumab against placebo (NCT03024996), nivolumab + ipilimumab against placebo (NCT03138512), durvalumab ± tremelimumab versus observation (RAMPART, NCT03288532), and finally pembrolizumab against placebo (NCT03142334), with further trials in development. These adjuvant trials are summarised in Table 3. Until now, only clinico-pathological features are being used for patient selection for these trials. No integration of biological recurrence score is currently used as selection criteria.

Table 3 *Adjuvant Trials for Metastatic RCC*

Trial	PROSPER NCT03055013	IMmotion010 NCT03024996	CheckMate 914 NCT03138512	KEYNOTE-564 NCT03142334	RAMPART NCT03288532
Agent	Nivolumab	Atezolizumab	Nivolumab + Ipilimumab	Pembrolizumab	Arm B: durvalumab Arm C: durvalumab + tremelimumab
Control	Observation	Placebo	Placebo	Placebo	Observation
Duration	1 month in neoadjuvant + 9 months adjuvant	12 months	6 months	12 months	12 months
N	766	664	800	950	1750
Endpoint	DFS	IRF-assessed DFS	DFS	DFS	DFS
Specificity	Neoadjuvant and adjuvant	Cohort of metastasectomy patients		Cohort of metastasectomy patients	Multi-arm multi-stage design
Study Population	RCC of any histology ≥T2NxM0 or T any N+ M0 disease for which radical or partial nephrectomy is planned	ccRCC • T2 Gr4 • T3a Gr3–4 • T3b/c any Gr • T4 any Gr • N+ any T, Gr • or metastasectomy of lung, soft tissue, LN >12 months	ccRCC • pT2a, Gr3 or Gr4, N0 • pT2b, any Gr, N0 • pT3, any Gr, N0 • pT4, any Gr, N0 • pT any, any Gr, N1 M0	ccRCC Intermediate-/high-risk RCC: • pT2, Gr 4 N0, M0 • pT3, any Gr, N0, M0 High-risk RCC: • pT4, any Gr N0, M0 • pT, any stage, any Gr, N+ • M0 M1 NED	RCC of any histology Leibovich score 3–11

Abbreviations: ccRCC: clear cell renal cell carcinoma; DFS, disease-free survival; Gr, grade; IRF, independent review facility; LN, lymph node; NED: no evidence of disease; N, node; M, metastasis; RCC, renal cell carcinoma; T, tumour.

Biomarkers

As for many other tumour types, the quest for predictive biomarkers for IO raises more questions than answers. The pitfalls of these investigations are discussed in Chapter 1.4 ('Biomarkers of Response to Immunotherapy'). Specifically, in RCC, PD-L1 biomarker expression carries an unfavourable prognostic value. Regarding the predictive value of PD-L1 immunohistochemistry staining for nivolumab, or the combination of nivolumab and ipilimumab, no selection based on PD-L1 biomarkers should be recommended. With regards to the staining performed in the IMmotion 150 and the IMmotion 151 studies (atezolizumab + bevacizumab versus sunitinib), the potential interest of biomarkers is still a matter of debate, and may be related to the potential immune-modulating role of VEGF inhibition by bevacizumab.

Other unsolved questions

While nivolumab has been integrated into all guidelines for systemic therapies for RCC, and nivolumab + ipilimumab combination is likely to become the new SoC in the first-line setting for intermediate- and poor-prognosis patients, many unanswered questions, more class-related, are being identified. These include the question of treatment duration for long responders, the question of treatment beyond progression in patients with unconventional response and the potential role of rechallenge after treatment discontinuation in responding patients. Some academic trials are being developed to address some of these questions. As an example, the TITAN RCC (NCT02917772) study investigated the role of the addition of ipilimumab to single-agent nivolumab as a rescue strategy in resistant disease to single-agent nivolumab, either upfront or as acquired resistance.

Conclusion

Over the past 2 years, single-agent nivolumab has integrated the treatment landscape of metastatic RCC with demonstration of OS benefit over active agents in second line and, more recently, nivolumab combined with ipilimumab has shown superiority over sunitinib in the first-line setting. These major changes are shifting the paradigm of VEGF inhibition as the cornerstone of RCC systemic therapy strategy and opening the way for combination strategies.

Further research is ongoing, especially combination of PD-1/PD-L1 inhibitors with VEGF/VEGFR axis inhibition, as well as combination with other ICIs. The appropriate timing for IO use in RCC is under investigation, including clinical trials in the perioperative setting (adjuvant or neoadjuvant). A major limitation with this class of agents is related to the lack of biomarkers for patient selection.

Declaration of Interest:

Dr Albiges has declared advisory / consulting roles for Novartis, Pfizer, Sanofi, Amgen, Bristol-Myers Squibb, Ipsen, Roche and Astellas.

Dr Strijbos has declared advisory / consulting roles for Novartis, Bristol-Myers Squibb, Sanofi and Janssen Pharmaceuticals; research grants from Janssen Pharmaceuticals.

Dr Rodriguez-Vida has declared advisory / consulting roles for Novartis, Pfizer, Sanofi, Bristol-Myers Squibb, Roche, Astellas, Janssen and Bayer.

Dr Escudier has declared advisory / consulting roles for Bayer, Novartis, Pfizer, Exelixis, Bristol-Myers Squibb, Ipsen and EUSA pharma; honoraria from Pfizer, Novartis, Bayer, Bristol-Myers Squibb, Ipsen, Exelixis and Roche/Genentech.

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2.4 Genitourinary Malignancies

2.4.2 Bladder Cancer

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Introduction and Context

Despite improvements in the clinical, surgical and medical management of locally advanced or metastatic urothelial carcinoma (UC), little progress has been made over the past three decades and the outcomes of patients with advanced disease remain poor (Necchi et al, 2017). In general, UC is considered to be a chemosensitive disease, and systemic platinum-based combination chemotherapy (ChT) is the standard-of-care (SoC) for neoadjuvant, adjuvant and first-line therapy. The objective response rate (ORR) to cisplatin-based first-line ChT is approximately 50%, with some differences according to baseline prognostic factors. However, most patients ultimately progress on first-line ChT, and no ChTs have yet demonstrated a survival benefit over active comparators in these patients. Commonly used second-line agents include taxanes, pemetrexed and vinflunine, all of which have demonstrated only limited activity and an ORR of 5%–20%. The ‘median’ overall survival (OS) was 6.9 months in the most up-to-date trial-level meta-analysis (Raggi et al, 2016).

Of note, standard ChT for UC often causes significant haematological and non-haematological adverse events (AEs), usually seen after either first-line cisplatin- or non-cisplatin-based ChT. Another limitation in

ChT administration is frequent ineligibility for cisplatin due to the standard criteria adopted in UC (for example, patients being required to have a creatinine clearance greater than 60 mL/minute). As a result, the overall rates of ChT administration in UC are rather low, particularly in the early stage perioperative setting. Thus, there is a need for new drugs at all stages of the disease, with comparable or better ORRs and favourable toxicity profiles when compared with standard ChT.

The treatment of various solid cancers has been revolutionised by the advent of various immunotherapeutic strategies, particularly those targeting T cell inhibitory pathways such as the immune checkpoint inhibitors (ICIs). In 2014 Powles et al reported the results of a phase I basket study describing the clinical activity of atezolizumab in metastatic UC. This anti-programmed death-ligand 1 (PD-L1) antibody rapidly received breakthrough designation status from the United States Food and Drug Administration (FDA) in June 2014, and was approved by the FDA in May 2016 for the treatment of patients with platinum-refractory metastatic UC. The use of systemic immunotherapy is not completely novel, since UC is known to be immunogenic. Immunotherapy for UC has already been pursued with the establishment of *Bacillus Calmette-Guérin* (BCG) intra-vesical therapy as the SoC for high-grade non-muscle invasive bladder UC (NMIBC), which was the first immunotherapy approved by the FDA for malignancy.

Basic research over the last few decades has provided vital insight into the molecular pathogenesis and characteristics of UC, as documented by the updated findings from The Cancer Genome Atlas (TCGA) project (Robertson et al, 2017). Epitopes induced by random mutations in tumour cells play an important role in the immunogenicity of tumours and the generation of an adaptive immune response, and UC is considered to be one of the most immunogenic cancers among all malignancies, due to its high mutational frequency, rating first in the retrospective analysis from the Memorial Sloan Kettering Cancer Center (Zehir et al, 2017). In this chapter, we present the latest evidence on the role, efficacy and safety of immunotherapy in patients with advanced metastatic UC.

Clinical Results

Use of ICIs as Front-line Therapy for Metastatic UC

To date, there is no established standard first-line ChT in UC patients unable to receive cisplatin-based ChT, and ineligibility to cisplatin affects approximately 50% of patients diagnosed with metastatic UC (according to internationally recognised criteria). Although cisplatin can be safely replaced by carboplatin in unfit patients, carboplatin-based ChT is less effective, with an inferior ORR (averaging 30%–40%) and shorter median OS (8–10 months according to baseline prognostic factors) (Necchi et al, 2017). Therefore, more effective and less toxic treatments are greatly needed, and immunotherapy targeting the programmed cell death protein 1(PD-1)/PD-L1 axis offers a unique opportunity for these patients.

In this context, atezolizumab has received accelerated approval from the FDA and the European Medicines Agency (EMA) for the treatment of patients with advanced UC ineligible for cisplatin-containing ChT. This approval was based on the results of Cohort 1 from the open-label, single-arm, phase II IMvigor 210 study (Balar et al, 2017a). In this cohort, 119 patients received 1200 mg of atezolizumab intravenously (i.v.) every 21 days, until unacceptable toxicity occurred or there was evidence of progressive disease (PD). The ORR in the intention-to-treat (ITT) population was 23% (95% confidence interval [CI]: 16–31), including a complete response (CR) in 9% of patients. Disappointingly, there was no improved response in patients with demonstrated immune cell (IC) PD-L1 expression, as the ORR was 28% (95% CI: 14–47) in IC2/3-positive (IC2/3+), and 21% in IC-negative patients. Similar trends were seen regarding OS, as median OS was 12.3 months (95% CI: 6–not estimable) for IC2/3+ patients and 19.1 months (95% CI: 9.8–not estimable) for IC0/1+ patients. Overall, the incidence of treatment-related adverse events (trAEs) of any grade was 66%, and fatigue, diarrhoea and pruritus occurred in more than 10% of patients.

In May 2017, the FDA also granted pembrolizumab conditional approval for the same patient population. This approval was based on data from the open-label, single-arm, phase II KEYNOTE-052 study (Balar et al, 2017b).

The trial evaluated pembrolizumab in 370 patients, in a design very similar to that of IMvigor 210. Patients received 200 mg of pembrolizumab every 3 weeks until PD or the development of unacceptable toxicity. The ORR was 24% in the total population (95% CI: 24–34), including 5% CR. PD-L1 expression was assessed in this study using the Dako antibody clone 22C3, and the combined positivity score (CPS) was developed. This score evaluates the number of PD-L1-staining cells (tumour cells and ICs) out of the total number of viable tumour cells. A CPS cut-off of 10% was determined to be the optimal enrichment cut-off for predicting response using receiver operating characteristic (ROC) curve analysis along with the ORR and biomarker prevalence profile. At the time of the most recent update, the ORR in all patients was 29% (95% CI: 25–34), and a trend toward improved responses was observed in PD-L1-positive (PD-L1⁺) patients (39% in patients with CPS \geq 10%). Mature data from KEYNOTE-052 were presented at the American Society of Clinical Oncology (ASCO) 2018 Annual Meeting by Vuky et al (2018). The median duration of response was not reached (95% CI: 21.4 months–NR), including 82% who had a response of \geq 6 months and 68% who had a response of \geq 12 months. The median OS was 11.5 months (95% CI: 10.0–13.3) and the 6- and 12-month OS rates were 67% and 48%, respectively. The median PFS was 2.3 months (95% CI: 2.1–3.4) and the 6- and 12-month PFS rates were 34% and 22%, respectively. The safety profile was consistent with previous trials using pembrolizumab.

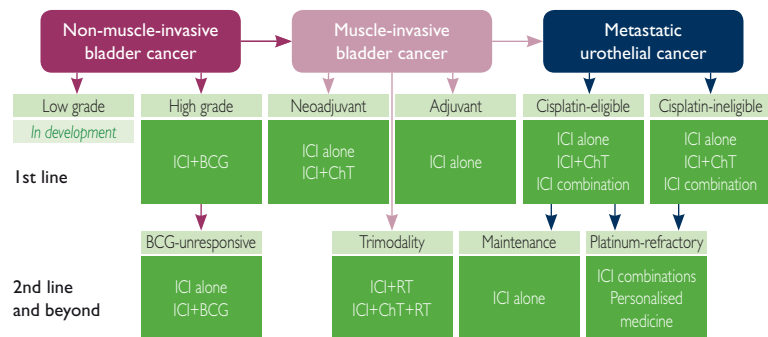
Additional data are awaited from multiple ongoing, randomised, phase III studies (NCT02853305, NCT02807636, NCT02516241, NCT03036098). These studies compare single-agent anti-PD-1/PD-L1 immunotherapy combined with anti-cytotoxic T-lymphocyte antigen 4 (CTLA-4) antibodies with standard ChT, or a chemo-immunotherapy combination with the SoC. Of note, all patients may be included in these studies, regardless of cisplatin eligibility. There are also clinical trials dedicated to cisplatin-ineligible patients. In particular, KEYNOTE-672/ECHO-307 (NCT03361865) is a randomised phase III trial comparing the combination of pembrolizumab with an anti-indoleamine 2,3-dioxygenase (IDO)1 compound, epacadostat, versus a combination of pembrolizumab and placebo. The reason for combining pembrolizumab with epacadostat is based on the promising results obtained in salvage therapy for metastatic UC, which will be presented later.

To date, there are no data to support the use of ICIs as first-line treatment in patients eligible for cisplatin-based therapy outside of clinical trials, as well as no evidence supporting the use of the PD-L1 biomarker for selecting patients for immunotherapy in ChT-naïve patients. However, new developments in immunotherapy treatments may result in substantial changes in the entire therapeutic paradigm of UC over the next couple of years.

Immunotherapy as Salvage Therapy for Platinum-treated, Advanced UC

An explosion of clinical trials has greatly changed the therapeutic paradigm of platinum-treated advanced UC, as illustrated in Figure 1 and Table 1. Multiple studies with single-agent ICIs targeting the PD-1/PD-L1 axis have shown promise in patients with metastatic UC treated with ChT. Several agents are currently FDA-approved in UC, and some of them (nivolumab, pembrolizumab and atezolizumab) are also EMA-approved (Necchi et al, 2018).

Figure 1 Future development of PD-L1/PD-1 inhibitors in urothelial cancer.



Abbreviations: BCG, Bacillus Calmette-Guérin; ChT, chemotherapy; ICI, immune checkpoint inhibitor; PD-1, programmed cell death protein-1; PD-L1, programmed death-ligand 1; RT, radiotherapy.

Atezolizumab

Atezolizumab was the first ICI approved in ChT-treated, metastatic UC. Accelerated FDA approval was granted on the results of a single-arm, phase II study (IMvigor 210, Cohort 2), which included 310 patients with locally advanced or metastatic UC previously treated with platinum-based therapy (Rosenberg et al, 2016). The primary outcome of

ORR was obtained in 15% (45/310) of patients, with 5% experiencing CR. Patients with a higher expression of PD-L1 (i.e. IC score 2 or 3 using the Ventana antibody SP142) in the infiltrating ICs in the tumour microenvironment had better ORR (26%) than patients with no or weak expression (IC 0-1). With a median follow-up duration of 21 months at the time of the most recent update, the median duration of response (DOR) had not yet been reached in all patients and a few delayed CRs (from previous partial response [PR]) or PRs (from previous stable disease [SD]) had occurred. The safety profile was consistent with the data from Cohort 1. However, substantial controversy has been raised from the results of the IMvigor 211 trial. This study was an open-label, randomised, phase III trial comparing atezolizumab versus standard ChT as second- or third-line therapy after the failure of platinum-based therapy. In a hierarchical statistical approach, the study failed to meet the primary endpoint of improved OS in PD-L1⁺ patients (hazard ratio [HR]: 0.87, 95% CI: 0.63–1.21, $p=0.41$). However, atezolizumab showed clinical OS benefit versus ChT in the ITT population (median follow-up: 17.3 months), durable responses were demonstrated in the ITT population and ongoing response was achieved in the majority of responders with a favourable safety profile when compared with ChT (Necchi et al, 2018).

Table 1 Immunotherapy in Second-line Treatment of Urothelial Carcinoma: A Summary of Efficacy and Toxicity

Trial	Efficacy			Grade 3–4 Adverse Events	
	ORR	PFS	OS	Total	Immune-mediated
Atezolizumab ^a	15% (95% CI: 11–19)	2.7 months (95% CI: 2.1–3.9)	7.9 months (95% CI: 6.6–9.3)	16%	5%
Pembrolizumab ^b	21.1% (95% CI: 16.4–26.5)	2.1 months (95% CI: 2.0–2.2)	10.3 months (95% CI: 8.0–11.8)	15%	4.5%
Nivolumab ^c	19.6% (95% CI: 15.0–24.9)	2.0 months (95% CI: 1.87–2.63)	8.74 months (95% CI: 6.05–NR)	18%	7%
Durvalumab ^d	17.8% (95% CI: 12.7–24.0)	1.5 months (95% CI: 1.4–1.9)	18.2 months (95% CI: 8.1–NR)	6.8%	2.1%
Avelumab ^e	17% (95% CI: 12–24)	1.5 months (95% CI: 1.4–2.6)	6.5 months (95% CI: 4.8–9.5)	8%	8%

Abbreviations: CI, confidence interval; NR, not reached; ORR, objective response rate; OS, overall survival; PFS, progression-free survival.

^aIMvigor 210 (Necchi et al, 2018; Rosenberg et al, 2016)

^bKEYNOTE-045 (Necchi et al, 2018)

^cCheckMate 275 (Necchi et al, 2018)

^dA phase I/II study to evaluate MEDI4736 (Necchi et al, 2018)

^ePhase I JAVELIN Solid Tumor trial (Necchi et al, 2018)

A 1-year OS of 39% in the ITT population of IMvigor 211 was consistent with the efficacy seen in previously reported studies, including a 1-year OS of 37% in Cohort 2 of IMvigor 210.

Pembrolizumab

Pembrolizumab was first investigated in the expanded cohort of the phase I KEYNOTE-012 trial. Patients with locally advanced or metastatic UC, with no limit on previous therapies, were treated with pembrolizumab 10 mg/kg every two weeks until CR, progression or unacceptable toxicity. Thirty-three patients were enrolled, of whom 33% had received ≥ 3 prior therapies and 66% had presented with visceral or bone metastases. The ORR was 25%, with 3 (11%) CRs and 4 (14%) PRs. After a median follow-up duration of 13 months, median DOR had not been reached and the 12-month PFS rate was 19%. ORR in patients with PD-L1 expression was 38%, and safety outcomes were comparable with those reported with atezolizumab (Necchi et al, 2018).

Based on these results, the phase III KEYNOTE-045 trial was initiated with the aim of comparing pembrolizumab with standard ChT according to physicians' choice as a salvage therapy for UC (Bellmunt et al, 2017). In this study, 542 patients were randomised to receive pembrolizumab 200 mg i.v. every 3 weeks for a maximum of 2 years, versus 3-weekly docetaxel, paclitaxel or vinflunine. The OS analysis of patients with CPS $\geq 10\%$ showed a 43% reduction in the risk of death in patients treated with pembrolizumab compared with standard ChT (HR: 0.57; 95% CI: 0.37–0.88; $p=0.0048$). The median OS was 8 months (95% CI: 5.0–12.3) with pembrolizumab, versus 5.2 months (95% CI: 4.0–7.4) with ChT, and more sustained responses were seen with pembrolizumab compared with ChT. Fewer trAEs were seen with pembrolizumab compared with ChT for any grade (60.9% versus 90.2%), and especially for grade 3–5 AEs (15.0% versus 49.4%). Findings from this landmark trial represent the first immunotherapy agent to demonstrate an OS benefit over an active comparator in locally advanced or metastatic UC, with level I evidence.

Durvalumab

Additional results from other phase II trials using checkpoint inhibitors have now also been presented, leading to overlapping results (Necchi et al, 2018). Durvalumab 10 mg/kg given every 2 weeks was evaluated in 20 patients unselected for PD-L1 expression in the expanded cohort of a phase I trial in UC. Patients were then selected based on tumour cell PD-L1 expression, with a cut-off of 5% (using Ventana SP263 antibody). Sixty-one patients were enrolled, with an ORR of 31%, 46.4% in the PD-L1⁺ subgroup (with a 25% cut-off in both tumour cells and ICs) and 0% in the PD-L1-negative subgroup. The median DOR was not reached after a median follow-up of 6.5 months. According to the most recent study update (n=191), disease response was observed regardless of PD-L1 expression (ORR 27.6% [n=27; 95% CI: 19.0–37.5] and 5.1% [n=4; 95% CI: 1.4–12.5] in patients with high and low or negative expression of PD-L1, respectively).

Avelumab

Avelumab was also administered at 10 mg/kg every 2 weeks in a total of 249 patients enrolled in the expansion cohort of a phase I study; and the confirmed ORR was 17% (95% CI: 12–24), regardless of PD-L1 expression. Nivolumab results presented from the CheckMate 275 phase II study included 275 patients who had progressed on a platinum-based regimen. The ORR was 19.6% in all patients, and a trend toward enriched responses was found in PD-L1⁺ patients (5% cut-off on tumour cells only; Dako antibody, clone 28-8). The ORR was 28.4% in patients expressing PD-L1 \geq 5% and 15.8% in patients expressing PD-L1 <5%. Similar trends were observed for OS.

Potential Future Developments

Although salvage combination immunotherapy with the use of a double regimen (i.e. anti-PD-1/PD-L1 plus anti-CTLA-4) or even a triple regimen (by adding cabazitaxel) may result in improved ORR and enhanced survival benefit compared with single-agent ICIs, the toxicity and sustainability of such combinations will likely limit their overall use (Necchi et al, 2018). The combination of pembrolizumab and epacadostat appears to be among the combinations demonstrating the most promis-

ing trade-off between efficacy and safety. Epacadostat, an anti-IDO1 agent, was combined with pembrolizumab in the phase I-II ECHO-202/KEYNOTE-037 trial. The study enrolled 40 patients with UC previously treated with ChT and reported an ORR of 35% (Smith et al, 2017). However, the development of epacadostat in UC is currently on hold based on the negative results achieved in the phase III KEYNOTE-252/ECHO-301 trial (NCT02752074) in unresectable or metastatic melanoma (Long et al, 2018).

Several uncertainties remain regarding the optimal use of these therapies. One issue is that the optimal duration of treatment for responders, as well as the optimal interruption of treatment for patients who progress, is still under debate. In a post-hoc analysis of the IMvigor 210 study, approximately 5% of delayed responses were observed after the first evidence of PD, denoting a disease-modifying activity induced by immunotherapy that is still largely unaccounted for. Response to ChT after ICIs will represent another future challenge, and the optimal sequence of chemo-immunotherapy will require further information from ongoing phase III trials.

Retrospective studies have aimed to improve clinical prognostic factors in the salvage setting, and to apply new prognostic models to the context of salvage immunotherapy. In particular, a new validated 6-factor prognostic model for OS (including Eastern Cooperative Oncology Group performance status [ECOG-PS], liver metastases, platelet count, neutrophil-lymphocyte ratio, lactate dehydrogenase [LDH] and anaemia) was proposed in the setting of salvage atezolizumab for advanced UC. The applicability of this model to other PD-1/PD-L1 inhibitors and PD-L1 immunohistochemistry assays warrants additional investigation (Pond et al, 2018).

The search for the optimal biomarker approach will require further new research. For example, two key factors were associated with a response to atezolizumab in the IMvigor 210 trial: the T_{eff} gene signature and the TCGA luminal II subtype ($p=0.0072$). Luminal-I tumours displayed low T_{eff} expression, and may be regarded as an ‘immune desert’ in their microenvironment according to Rosenberg et al (2016). Interestingly, fibroblast growth factor receptor 3 (*FGFR3*) mutations appear to be more common in luminal-I UC, which has a low expression of markers associated with an immune response, including CD8-T-effector gene

expression levels. This suggests that *FGFR3* mutations occur within a group of tumours less likely to benefit from immune checkpoint inhibition. In fact, the reported ORR to previous anti-PD-1/PD-L1 treatment and time-to-progression for patients with specific *FGFR* alterations are lower than those reported in studies of a population unselected for *FGFR* alterations. Of note, the outcomes of patients with *FGFR2/3* mutations or gene fusions, previously treated with PD-1/PD-L1 inhibitors and included in the BLC2001 study (erdafitinib in patients with advanced UC), have been presented: the ORR to anti-PD-1/PD-L1 agents was only 3.6% (95% CI: 0.1–18.3), and the median time-to-progression was 3.4 months (Siefker-Radtke et al, 2018). A combination of ICIs and pan-FGFR inhibitors might therefore be particularly beneficial for molecularly-selected patients.

Tumour mutation burden (TMB), evaluated by quartile split using the FoundationOne test, was significantly associated with improved response and survival to atezolizumab (Rosenberg et al, 2016). Similar findings were reported for patients treated with nivolumab from translational analyses of the CheckMate 275 study, as well as in patients treated with avelumab.

Future investigations in this field will include efforts to develop clinical trials with ICIs in patients with non-metastatic disease, and such clinical trials in early-stage disease may help to reinvigorate the collaboration between urologists and medical oncologists. Three phase III adjuvant immunotherapy trials comparing ICIs with placebo or observation alone (NCT02450331, NCT02632409 and NCT03244384) are now recruiting patients. These trials focus on the population of patients with pathological evidence of high-risk disease at radical cystectomy who have already received neoadjuvant ChT or cannot receive cisplatin-based adjuvant ChT. Additionally, preoperative (neoadjuvant) trials are evaluating ICIs, especially pembrolizumab and atezolizumab (NCT02736266, NCT02662309). Pending the initiation of large randomised trials of chemo-immunotherapy compared with standard neoadjuvant ChT, these studies are harnessing the window-of-opportunity approach by providing a short treatment course preceding radical cystectomy, and the pathological response to treatment represents their primary endpoint.

Most noteworthy, multiple phase II trials have now entered the NMIUC field, particularly for patients who develop BCG-refractory disease and for whom no standard conservative options exist. Interestingly, there is no need for randomised control arms in these studies, as the objective is to improve outcomes while preserving the quality of life of these patients. The study furthest ahead in recruitment is the KEYNOTE-057 study (NCT02625961), which provides up to 2 years of pembrolizumab therapy in a single-arm design, and the results are highly anticipated. It is hoped that the results of these ongoing and upcoming trials will greatly expand our knowledge of the optimal timing and combinations of immunotherapy, and will lead to the widespread use of chemo-immunotherapy as the first-line SoC in urothelial cancer.

Declaration of Interest:

Dr Necchi has reported no potential conflicts of interest.

Dr Crolley has reported no potential conflicts of interest.

Dr Powles has reported no potential conflicts of interest.

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2.5 Gastrointestinal Malignancies

2.5.1 Microsatellite Instability-high

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Cancer is the second leading cause of death globally. Gastrointestinal (GI) cancers of the colon, rectum and stomach accounted for 1 518 000 of the 8.8 million cancer-related deaths documented in 2015. Early diagnosis, access to evidence-based approaches and quality healthcare services are critical for improved prognosis and survival. Historically, GI cancers have been treated with cytotoxics and biological therapies. Clinicians have traditionally used clinical parameters, such as laboratory tests, imaging and pathological results as key drivers for therapeutic

tic decision-making. However, the molecular profiles of tumours are becoming more and more important for optimal treatment. The field of oncology is increasingly shifting toward novel targets detected in the genome as predictors of therapeutic benefit. One of these novel targets is mismatch repair deficiency (dMMR). dMMR tumours are also microsatellite instability-high (MSI-H). A new era in cancer care is emerging and allows for tissue/site-agnostic therapeutic methods to be a critical part of standard-of-care therapeutic decision-making, as evidenced by the recent registration of pembrolizumab for dMMR tumours by the Food and Drug Administration (FDA), and nivolumab for MSI-H metastatic colorectal cancers (CRCs).

The aim of this chapter is to describe the differential pathogenesis of MSI-H tumours, methods of detection for MSI status and clinical implications for therapy.

Pathogenesis of MSI

Short tandem repeats found in DNA are repeats of the same base or sequence of bases and are found in microsatellite regions. Microsatellite regions are error-prone due to the greater likelihood of slippage caused by DNA polymerases mistakenly inserting or deleting bases. Mismatch repair (MMR)-proficient cells recognise and correct these different base–base mispairs introduced into microsatellites during DNA synthesis. In the case of dMMR tumours, these errors are not corrected. Nucleotide mutation rates can depend on the intrinsic stability of nucleotides, their sensitivity to mutagens and the fidelity of DNA replication and repair. MSI is a function of broken MMR proteins and is used as a marker for dMMR. MMR machinery defects are caused by a deficiency in one of the four MMR proteins (MSH2, MSH6, MLH1 and/or PMS2,), which leads to defects in the core heterodimers MLH1/PMS2 and MSH2/MSH6, responsible for correcting mismatches as they are encountered.

Pathogenesis of MSI is dependent on the biallelic inactivation of MMR genes caused by either (1) germline mutations, i.e. Lynch syndrome (LS), (2) sporadic mutations or (3) epigenetic silencing. LS, also known as hereditary non-polyposis colorectal cancer (HNPCC), is an autosomal

dominant disease caused by a germline mutation found in one or more of the MMR genes. Biallelic inactivation of the remaining wild-type allele can be caused by either loss of heterozygosity, somatic mutations or hypermethylation of MSH2 due to a germline deletion of the epithelial cell adhesion molecule.

Screening for HNPCC is critical because individuals are at a higher risk of developing cancer of the colon, endometrium, ovaries, kidneys, bladder, stomach, small bowel, bile ducts and brain; thus screening has implications for family members. The highest increase of risk is for development of endometrial and colorectal cancers. On the other hand, epigenetic silencing of the *MLH1* promoter gene seems to be the primary cause of sporadic MSI, and is associated with a global increase of aberrant DNA methylation of CpG islands. Additionally, sporadic MSI is often associated with a somatic *BRAF* V600E mutation, which is less common in Lynch-associated cancers. Although rare, sporadic MSI can arise from biallelic somatic inactivation of the genes encoding for a component of the MMR heterodimer complex. Lastly, accumulating evidence indicates a new subtype of MSI-H CRC caused by a mutated microRNA (miRNA) pathway which may be correlated with prognosis.

Who Does This Apply To?

A broad range of tumour types have at least a small percentage of MSI-H cancers, with the highest incidence in colon, oesophageal, rectal, stomach, small intestine and endometrial cancers. Furthermore, dMMR occurs in cancers of the biliary tract, pancreas, ovary and prostate. Estimated percentages for dMMR are the following: approximately 7% of oesophageal cancer, 15% of colon cancers (2.5% germline mutations, 12.5% sporadic mutations), 22%–33% of endometrial cancers, 8% of cervical cancer, 22% of sporadic gastric cancer and 0%–2% of skin and breast cancers. More studies are underway to discover the percentage of dMMR tumours in other cancer types, as seen recently with Warth and colleagues (2016), testing for MSI status in pulmonary carcinomas and finding approximately four MSI-H cases of the 480 tested.

Clinical, Pathological and Genetic Criteria Involved in Detection of dMMR

Detection of MMR status is necessary, due to the globally recognised importance in identifying patients with LS. For detection of LS status, the Amsterdam II criteria and revised Bethesda guidelines are currently used. However, Bethesda guidelines seem to have both a higher sensitivity and specificity. Both criteria use information regarding family history of cancer, age at diagnosis and tumour histology. These criteria may not be applicable in some populations. Yan et al (2016) questioned the use of these criteria in Chinese populations, where the large number of small families (attributable to the historical one-child policy) makes it harder to meet all the specified criteria.

In addition to clinical criteria, MMR testing is required and can be done in multiple ways. Clinicians commonly use immunohistochemistry (IHC) staining and polymerase chain reaction (PCR)-based MSI testing, with a sensitivity upwards of 90% for phenotypic detection of loss of dMMR protein expression and MSI status. Both are acceptable methods to screen for dMMR and, most importantly, LS. Currently, the National Comprehensive Cancer Network (NCCN) and the European Society for Medical Oncology (ESMO) recommend universal testing for MSI-H status in all patients with CRC. MSI testing is performed on fresh, frozen or paraffin-embedded tumour tissue using the approved National Cancer Institute (NCI) panel. Initially, the NCI created a panel with five microsatellite markers to determine MSI status, including two mononucleotide (BAT25/26) and three dinucleotide (D2S123, D5346 and D17S249) repeats. This has been modified and an alternative molecular method for detection has been established, which has proven to be more specific and sensitive than the original NCI panel, and is based exclusively on mononucleotide markers.

IHC is an additional method for detecting MSI status, and is globally available. IHC can identify the affected gene by detecting loss of its protein product. Loss of expression of a protein can help direct testing for germline mutations associated with LS. If loss of MLH1 protein expression is evident in a CRC sample, it is also recommended to test for a

mutation in the *BRAF* oncogene. This is the most cost-effective approach to confirm a sporadic case and exclude LS as a clinical diagnosis. In the absence of a mutation in *BRAF*, germline testing for a mutation in *MLH1* should be completed. IHC testing has a sensitivity of 83%, regardless of the MMR gene involved, with a specificity of 89%. It is convenient, inexpensive and may detect loss of MSH6 protein that can be missed with PCR-based MSI testing. However, the most common issue with IHC testing is the variability in fixation of the tumour tissue, which can affect the quality of staining detected. Biopsies may be a possible solution to circumvent variability, as they show complete and uniform rather than variable fixation.

MSI and IHC testing have shown to be highly concordant with a sensitivity of >90% and specificity of 100%. A recent study demonstrated the equivalence of MSI testing and MMR IHC in a cohort of patients with endometrial carcinomas, supporting previously reported concordance rates of over 92%.

A newer technology available for dMMR detection is next-generation sequencing (NGS), which enables detection of MSI as well as genetic mutations, allowing for massive parallel sequencing. NGS allows for high-yield output of dozens to hundreds of genes which can be simultaneously sequenced, as well as determination of epigenetic methylation patterns. NGS- and MSI PCR-based testing present a high degree of correlation in the fraction of unstable loci detected (R-squared values of 0.86 and 0.94, respectively). When compared with PCR-based testing, NGS:

- Might eliminate the need for separate testing for MSI status (of note, using tumour tissue to test for biomarkers can give rise to a few challenges, which include insufficient samples and inadequate tumour cellularity, so that repeated testing as in the case of IHC and subsequent PCR-based MSI testing might not be feasible)
- Allows for a far greater number of microsatellite markers to be examined
- Has the capacity to quantify the degree of MSI displayed by a tumour in terms of number and distribution of novel microsatellite repeat-length polymorphisms

One major limitation of using NGS for solely MSI screening is the utilisation of expensive targeted gene-capture sequencing. However, technologies such as MiSeq allow for a less costly platform using smaller NGS machinery and a streamlined data processing pipeline which is easier to operate, does not require a large computational infrastructure and requires less time. Results from MiSeq when compared with PCR-based testing showed high concordance (100% concordance with a few exceptions). BAT25 and BAT26 were 100% accurate when compared with traditional MSI testing, yielding 100% sensitivity (95% confidence interval [CI]: 83.2–100) and 100% specificity (95% CI: 88.4–100). Furthermore, technologies such as MANTIS (Microsatellite Analysis for Normal Tissue InStability) allow for the rapid detection of pan-cancer MSI and have shown highest overall sensitivity of 99.68% compared with MSI Sensor (96.48%) and MSINGS (76.06%). However, NGS is not the gold standard testing method to detect MSI, as inadequate sample cellularity and cost remain the major concerns.

Historical Prognostic and Predictive Value of MSI

Before the advent of immuno-oncology, testing for MSI has been restricted to localised CRC, where MSI status has been shown to have both prognostic and predictive value.

Popat et al (2005) stratified survival in CRC patients with MSI status and confirmed the relationship between MSI-H and improved survival rates, with a combined hazard ratio (HR) for overall survival (OS) of 0.65 (95% CI: 0.59–0.71). When it comes to different pathological stages, the excellent prognosis for stage II CRC patients with dMMR supports observation in this group. However, Mohan and colleagues (2016) suggested that MSI-H status was associated with worse outcomes in stage III versus stage I/II CRC, thus supporting the use of adjuvant chemotherapy (ChT) in patients with positive lymph nodes. In stage IV patients, MSI status seems to confer a worse prognosis.

Regarding the predictive value of MSI status to ChT in CRC, studies have examined the relationship between MSI status and response to 5-fluorouracil (5-FU)-based adjuvant ChT. Studies demonstrated the lack of benefit

of 5-FU single-agent therapy for MSI-H patients, a situation that could be reverted by adding oxaliplatin to the ChT backbone, which is clearly recommended for stage III patients. However, it is unclear whether MSI can be used as a predictive biomarker for 5-FU-based therapy in the metastatic setting, as researchers suggest that the molecular and epigenetic heterogeneity of MSI-H tumours may prevent the use of MSI status alone as a biomarker to guide ChT decisions in metastatic CRC.

In line with the observations made in CRC, the MAGIC trial for gastric cancer, which tested perioperative ChT, found that patients with dMMR and MSI-H may be better served by a surgery-only approach. However, this finding has to be prospectively validated.

Clinical Rationale of Using MSI as a Predictive Biomarker for Programmed Cell Death Protein 1 Antibody Response

Certain histopathological and genetic parameters related to disease prognosis in MSI-H tumours support the immunogenicity of these tumours: (1) a hypermutated phenotype, (2) the degree of T cell infiltrate and (3) the level of programmed death-ligand 1 (PD-L1) expression. Tumours that exhibit a high mutation rate due to a defective MMR system are especially sensitive to recognition by an endogenous anti-tumour immune response. This is seen either in LS-associated cancers and sporadic GI and endometrial cancers, causing accumulation of anywhere from hundreds to thousands of somatic mutations because of the dMMR system. Clinical trials have used this rationale to test immune checkpoint inhibition in tumours known to have both a hypermutable nature and large number of mutations. Based on this rationale, Le and colleagues (2017) clinically confirmed in a phase II study that a large proportion of mutation-associated neoantigens (MANAs) in dMMR tumours do in fact make MSI-H patients good candidates for programmed cell death protein 1 (PD-1) axis inhibition.

Furthermore, MSI-H tumours have a high number of tumour infiltrating lymphocytes (TILs) in dMMR CRCs. MSI-H cancers with marked T cell infiltration may have a more favourable prognosis, which would warrant using this marker for clinical decisions. It is evident that MSI-H

cancers have a strong cytotoxic immune response present; so why do patients still progress? The answer lies in the well-researched PD-1/PD-L1 pathway. PD-L1 (CD 274) is a ligand to PD-1 and is expressed on the cell surface of tumour cells with some exceptions (CRCs often express PD-L1 proteins on TILs and/or myeloid cells rather than the tumour itself). PD-L1 expression down-modulates infiltrating T cells in the tumour microenvironment through upregulation of PD-L1 protein, enabling tumours to survive and harbour immune resistance. Blocking the binding of PD-1 to PD-L1 may lead to the reactivation of cytotoxic T-lymphocytes, therefore helping the immune system to recognise and attack the cancer. However, the expression of PD-L1 is not a definitive marker of response to anti-PD-1 blockade. Studies using PD-1 inhibitors (nivolumab and pembrolizumab) in advanced MSI-H CRCs have shown durable responses regardless of PD-L1 expression.

Clinical Implications

Clinical trials have demonstrated the utility of MSI status as a biomarker of response to PD-1 blockade. In 2015, data from a PD-1 blockade clinical trial testing response to pembrolizumab in patients with dMMR tumours showed that MMR status predicted clinical benefit from immune checkpoint blockade for patients with advanced metastatic CRC and non-CRCs. MSI status was a significant predictor of both the immune-related objective response rate (ORR; 40% in dMMR CRC, 71% in dMMR non-CRC, 0% in MMR-proficient CRC) and immune-related progression-free survival (PFS) rate at 20 weeks (78%, 67% and 11%, respectively). Follow-up data on a larger cohort showed an objective radiographic response in 46 of 86 patients (53%; 95% CI: 42–64) with 21% (n=19) achieving a complete radiographic response. Disease control was achieved in 66 of 86 patients (77%; 95% CI: 66–85). It is important to note that ORR was similar between CRC and other cancer subtypes, providing further evidence that MSI-H status may be considered a strong predictive biomarker across tumour types.

Data from KEYNOTE-016, -164, -012, -028 and -158 included 148 patients and resulted in accelerated approval of pembrolizumab by the FDA for adult and paediatric patients with unresectable or metastatic MSI-H or

dMMR refractory solid tumours, for whom there are no alternative treatment options, as well as for patients with MSI-H or dMMR CRC who are resistant to fluoropyrimidine, oxaliplatin and irinotecan. KEYNOTE-158 and KEYNOTE-164 provided early evidence that MSI-H metastatic advanced refractory CRC and non-CRC patients benefit tremendously from immunotherapy. Patients enrolled in these trials had a median follow-up of 7.4 months for MSI-H CRC and 4.5 months for MSI-H non-CRC; ORR for both cancer types was 26.2% (95% CI: 15.8–39.1) and 42.9% (95% CI: 21.8–66), respectively.

Similarly, nivolumab, also a PD-1 inhibitor, showed efficacy in treating MSI-H/dMMR colorectal tumours, and was granted approval by the FDA for treatment of patients aged 12 years and older with dMMR and MSI-H metastatic CRC that had progressed following treatment with fluoropyrimidine, oxaliplatin and irinotecan. CheckMate 142 enrolled patients with MSI-H metastatic refractory CRC to evaluate the clinical benefit of immune checkpoint inhibition therapy for patients with a faulty MMR system. Results from this trial showed that nivolumab was well tolerated and provided durable response and disease control. Furthermore, these results were achieved in dMMR/MSI-H metastatic CRC across all subgroups tested for PD-L1 expression, *BRAF* and/or *KRAS* mutations, as well as LS. This study also tested the combination therapy of ipilimumab plus nivolumab in patients with dMMR metastatic CRC, and has shown promising preliminary efficacy. CheckMate 032 tested nivolumab alone or in combination with ipilimumab and demonstrated clinical activity in both MSI-H and non-MSI-H ChT-refractory metastatic oesophagogastric cancer. Of note, MSI-H patients had an ORR of 29% in the three nivolumab groups versus non-MSI-H patients who showed an 11% ORR. This study also showed clinical benefit independent of PD-L1 status across all groups. Furthermore, both CheckMate 142 and 032 found that most adverse events were manageable, and consistent with the reported safety profile for ipilimumab and nivolumab in other solid tumours.

Molecular characterisation of cancers has ushered in the age where it is increasingly important to identify predictive and prognostic markers before making any treatment decisions. Analysis of molecular hetero-

geneity and subsequent incorporation of these new insights into clinical practice has led to a substantial shift in the way clinicians think about treatment for cancer. MSI is present at a low but definite rate across multiple solid tumour histologies. Given the durable clinical benefit of PD-1 inhibitors in patients with advanced, treatment-refractory MSI-H disease, it is becoming increasingly important for clinicians to identify these patients. Planned studies will investigate if this treatment strategy could be moved to earlier lines of therapy as well, potentially changing the therapeutic landscape for these patients. Further efforts are needed to identify the resistance mechanisms to immune activation, which would increase the degree of benefit from immunotherapies in this population.

Declaration of Interest:

Dr Aulakh has reported no potential conflicts of interest.

Dr Le has declared research funding and advisory board honoraria for Bristol-Myers Squibb, and research funding, advisory board and speaking honoraria for Merck.

Dr Karamouzis has reported no potential conflicts of interest.

Dr Argilés has reported no potential conflicts of interest.

Dr Tabernero has declared participation in advisory boards of Bayer, Boehringer Ingelheim, Genentech/Roche, Lilly, Merck Sharp & Dohme, Merck Serono, Novartis, Roche, Sanofi, Symphogen and Taiho.

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2.5 Gastrointestinal Malignancies

2.5.2 Gastric and Oesophageal Malignancies

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Introduction

Gastric and oesophageal cancer are diagnosed in more than 1 million patients annually. For patients with advanced or metastatic gastric or oesophageal cancer, limited treatment options are available. Standard cytotoxic chemotherapy (ChT) in first- and second-line settings results in a median overall survival (OS) of generally <18 months, and, except for trastuzumab in human epidermal growth factor receptor 2 (HER-2)-positive cancers and the anti-angiogenic monoclonal antibody (mAb) ramucirumab, development of targeted therapies has fallen short of expectations in gastro-oesophageal adenocarcinoma. For squamous cell oesophageal cancer, there are no targeted therapies available.

The Immune Environment in Gastro-oesophageal Cancer

Molecular Subtypes and the Immune Response

Epstein-Barr virus (EBV) and microsatellite instability (MSI) subtypes of gastric cancer have characteristics associated with enhanced rates of response to immuno-oncology therapies. EBV tumours demonstrate frequent amplification of the chromosome 9p, which encodes programmed death-ligand 1 (PD-L1) and PD-L2; and PD-L1 expression is high in EBV-positive

(EBV⁺) gastric tumours. Mismatch repair-deficient (dMMR) gastric cancers display a hypermutated genome and are also associated with high levels of PD-L1 expression and tumour infiltrating lymphocytes. EBV and MSI gastric tumours also demonstrate a high interferon (IFN)- γ response gene signature compared with chromosomal instable (CIN) and genomic stable (GS) tumours; this signature has been associated with increased sensitivity to anti-programmed cell death protein 1 (PD-1) therapy. However, immunogenic MSI and EBV⁺ tumours are less commonly found in patients with metastatic gastric cancer than in patients with operable disease.

There are less data regarding the implications of the CIN and GS subgroups and the immune response; however, gene expression data suggest that inflammatory cytokines such as interleukin (IL)-1 β , IL-2, IL-3, IL-21, IL-27 and INF- γ , which are highly expressed in EBV⁺ tumours, are suppressed in CIN cancers. Emerging data suggest that chromosomal instability and resulting aneuploidy and copy number alterations may act as a mechanism of immune evasion; therefore, CIN cancers, which are characterised by genomic instability, may be intrinsically immunologically evasive.

PD-L1 Expression and Cytotoxic T Cells in Gastro-oesophageal Cancer

The proportion of gastric and gastro-oesophageal cancers which are PD-L1-positive is sensitive to the methodology used to determine PD-L1 status; tumour cells are less frequently PD-L1-positive (PD-L1⁺) than immune infiltrating cells, and the infiltrating edge of the tumour is more likely to contain PD-L1⁺ cells than the centre. In gastric cancer, CD8⁺ T cell infiltration and PD-L1 expression are both linked to OS in multiple datasets.

Immune Checkpoint Blockade in Gastro-oesophageal Cancer

Single-agent Immune Checkpoint Blockade (ICB)

Nivolumab

ONO-4538-12. Nivolumab is a humanised, immunoglobulin G4 mAb against PD-1 which is licensed to treat melanoma, non-small cell lung cancer (NSCLC) and renal cell carcinoma. In the ONO-4538-12

(ATTRACTION-2) trial, which was a phase III randomised study, patients with unresectable advanced or recurrent gastro-oesophageal cancer who were refractory or intolerant to ≥ 2 standard ChT regimens were randomised to either nivolumab 3 mg/kg every 2 weeks or placebo. The primary end-point of the trial was OS. Recruitment was limited to patients from Asian countries (Japan, Korea and Taiwan). A total of 493 patients were recruited and randomised in a 2:1 ratio to nivolumab or placebo. Nivolumab significantly improved median OS from 4.1 to 5.3 months (hazard ratio [HR] 0.63; 95% confidence interval [CI]: 0.50–0.78, $p < 0.0001$). Additionally, survival at 12 months was almost doubled for nivolumab-treated patients; this was 26.6% for nivolumab and 10.9% for placebo. RECIST (Response Evaluation Criteria in Solid Tumours) responses were observed in 12% of nivolumab-treated patients; however, some reduction in tumour size was observed in 40% of patients. Results according to PD-L1 status were available for 192 patients (39%). PD-L1 expression on $\geq 1\%$ tumour cells was observed in 13.5% of patients. In ATTRACTION-2, a survival benefit for nivolumab treatment was observed for patients with and without PD-L1 expression. Therefore, nivolumab is effective in chemorefractory gastric cancer, regardless of PD-L1 status.

In ATTRACTION-2, treatment with nivolumab was well tolerated; only 11% of patients experienced a grade 3 or 4 adverse event (AE) and only 1% had an AE which led to treatment discontinuation. The most common AEs associated with nivolumab treatment included pruritus (9%), diarrhoea (7%), rash (6%), fatigue, decreased appetite and nausea (5% each), elevation of aspartate aminotransferase (AST), hypothyroidism and pyrexia (3% each).

CheckMate 032 (single-agent nivolumab data). CheckMate 032 is a phase I/II non-randomised study of nivolumab (anti-PD-1) versus combination nivolumab + ipilimumab (anti-cytotoxic T-lymphocyte antigen 4 [CTLA-4]) at two different dose schedules in non-Asian patients with previously treated gastro-oesophageal cancer, unselected for PD-L1 status. The primary endpoint of the study was objective response rate (ORR). In CheckMate 032, 59 patients were treated with nivolumab 3 mg/kg every 2 weeks. The ORR associated with nivolumab therapy was 12%, median duration of response 7.1 months (95% CI: 3.0–13.2). In CheckMate 032, 42/59 patients treated with nivolumab single agent

were evaluable for PD-L1 status; of these, 38% were PD-L1⁺ in $\geq 1\%$ of tumour cells. Radiological responses were seen in patients who were PD-L1⁺ and PD-L1-negative (PD-L1⁻). For all nivolumab-treated patients, median progression-free survival (PFS) was 1.4 months (95% CI: 1.2–1.5), and median OS 6.2 months (95% CI: 3.4–12.4); 12-month OS was 39%, and 18-month OS 25%. In CheckMate 032, treatment with single-agent nivolumab was associated with a similar spectrum of toxicity to that observed in ATTRACTION-2, implying no difference in toxicity in Asian and non-Asian populations.

Table 1 Gastric and Gastro-oesophageal Cancer Patients Treated with Nivolumab and Pembrolizumab and Combination Nivolumab/Ipilimumab in ATTRACTION-2, KEYNOTE-059 and CheckMate 032 Trials

	ATTRACTION-2	KEYNOTE-059	CheckMate 032		
	Nivolumab	Pembrolizumab	Nivolumab only	Nivo 1 + IPI 3	Nivo 3 + IPI 1
N	330	259	59	49	52
Geographic region	Japan, Korea Taiwan	Mixed Asian and non-Asian	Finland, Germany, Italy, Spain, UK, USA	Finland, Germany, Italy, Spain, UK, USA	Finland, Germany, Italy, Spain, UK, USA
Age median (range)	62 (20–83)	62 (24–89)	60 (29–80)	53 (27–77)	58 (19–81)
Tumour site:					
• Gastric	272 (82%)	124 (48%)	19 (32%)	22 (45%)	18 (35%)
• GEJ/oesophageal	58 (18%)	134 (52%)	40 (68%)	27 (55%)	34 (65%)
Number of prior regimens:					
• 0–1	0	0	10 (17%)	7 (14%)	16 (31%)
• 2	69 (21%)	134 (52%)	20 (34%)	19 (39%)	16 (31%)
• 3	137 (42%)	75 (29%)	19 (32%)	11 (22%)	12 (25%)
• >3	124 (37%)	50 (19%)	10 (17%)	12 (24%)	7 (13%)
ORR	30 (11.2%)	30 (12%)	7 (12%)	12 (24%)	4 (8%)
PFS (months)	1.61 (1.5–2.3)	2.0 (2.0–2.1)	1.4 (1.2–1.5)	1.4 (1.2–3.8)	1.6 (1.4–2.6)
6-month PFS	20.2%	14.6%	17%	24%	12%
12-month PFS	7.6%	NR	8%	17%	10%
OS (median, months)	5.3 (4.63–6.41)	5.5 (4.2–6.5)	6.2 (3.4–12.4)	6.9 (3.7–11.5)	4.8 (3.0–8.4)
12-month OS	27%	NR	39%	39%	24%
Toxicity					
All Grade	141 (43%)	159 (61%)	69 (41%)	84 (41%)	75 (39%)
Grade 3–5	34 (10%)	46 (18%)	17 (10%)	47 (23%)	27 (14%)

Abbreviations: GEJ, gastro-oesophageal junction; Ipi, ipilimumab; Nivo, nivolumab; NR, not reported; ORR, objective response rate; OS, overall survival; PFS, progression-free survival.

Comparing the patients treated with nivolumab in ATTRACTION-2 and CheckMate 032 (Table 1), it appears that the cohorts were very similar in profile and nivolumab response rates were almost identical. It might be possible to argue from the similarity of these data that the survival benefit associated with nivolumab in PD-L1 unselected chemorefractory patients in the ATTRACTION-2 study might also be applicable to non-Asian patients.

Pembrolizumab

KEYNOTE-012 (chemorefractory gastric cancer, PD-L1 selected). Pembrolizumab is a humanised immunoglobulin G4 mAb targeting PD-1, which is licensed to treat melanoma, NSCLC and MSI cancers of any tumour site, and has now received Food and Drug Administration (FDA) approval for chemorefractory PD-L1⁺ gastric cancer. In the preliminary KEYNOTE-012 trial, patients were screened for PD-L1 expression prior to study entry, using a $\geq 1\%$ positive criterion on tumour cells or contiguous immune infiltrate; using this cut-off, 65 (40%) of the patients were PD-L1⁺ and 39 patients were treated on study. An objective response rate (ORR) of 22% was reported in the 36 patients evaluable for response. On repeated assessment of PD-L1 status using biopsy on trial, 8/32 evaluable patients (25%) were PD-L1⁻, highlighting the complexity of PD-L1 assessment in gastric cancer patients.

KEYNOTE-059 Cohort 1 (chemorefractory gastro-oesophageal cancer, PD-L1 unselected). In KEYNOTE-059 Cohort 1, 259 patients who had previously been treated with two or more lines of ChT were treated with pembrolizumab 200 mg every 3 weeks. Using the combined positivity score (CPS) for PD-L1 expression (where CPS is the number of PD-L1⁺ cells of any type [tumour, macrophage, lymphocyte] divided by the total number of tumour cells $\times 100$), 57% of tumours were PD-L1⁺. In KEYNOTE-059 Cohort 1, radiological responses were observed in 12% of all patients (16% of PD-L1⁺ patients and 6% of PD-L1⁻ patients); however, 42% of patients observed a change in lesion size. These results are remarkably consistent with those observed for single-agent nivolumab. Importantly, response rates and disease control rates (DCRs) were higher in patients who were treated with pembrolizumab in the third- versus the fourth-line setting (16% versus 7% and 31% versus 23% for ORR and

DCR, respectively). This could indicate that ICB is more effective in the earlier stages of cancer therapy. In KEYNOTE-059 Cohort 1, median PFS for all patients was 2 months (95% CI: 2.0–2.1) and median OS 5.5 months (4.2–6.5), comparable with single-agent nivolumab in ATTRACTION-2 and CheckMate 032. In KEYNOTE-059 Cohort 1, grade 3–5 AEs occurred in 18% of patients, leading to treatment discontinuation in 3% of patients. Immune-related AEs of all grades occurred in 19% of patients, the most frequent of these being hypothyroidism (9%) and hyperthyroidism (3.5%); colitis, infusion-related reactions and pneumonitis occurred in 2% of patients. Notably, 4% of patients were MSI-high (MSI-H); of these, 57.1% had a radiological response, of which 14.3% were complete radiological responses. This confirms the immunogenicity of MSI-H tumours and, in the future, may provide a treatment option for MSI-H gastric cancer patients not benefiting from preoperative or adjuvant ChT in retrospective analysis of several randomised clinical trials.

Regarding outcomes in patients who have been treated with only one line of ChT, at the time of writing, initial results of the KEYNOTE-059 trial (pembrolizumab versus paclitaxel) have been released; this trial was negative for its primary endpoint of an improvement of OS in PD-L1⁺ patients.

KEYNOTE-059 Cohort 3 (treatment-naïve). Preliminary efficacy results have been reported from a small cohort (n=31) of PD-L1⁺ treatment-naïve gastro-oesophageal cancers treated with pembrolizumab 200 mg every 3 weeks. In this group of patients, ORR was 26% (95% CI: 12%–45%), of which 7% were complete responses (CRs). This ORR is higher than that observed in later lines of therapy for pembrolizumab. Seventy-seven per cent of patients had a reduction in target lesion size, and median OS was an impressive 20.7 months (95% CI: 9.2–20.7).

Anti-PD-1 therapy in the second-line setting

Pembrolizumab was compared with paclitaxel ChT in the second-line setting in the KEYNOTE 061 trial. The trial initially recruited PD-L1⁻ and PD-L1⁺ patients; however, the primary endpoint was in PD-L1⁺ patients (CPS score ≥ 1). Pembrolizumab failed to improve OS compared with paclitaxel in the PD-L1⁺ patient group (n=395); median OS was 9.1 months in pembrolizumab-treated patients and 8.3 months for paclitaxel

(HR 0.82; 95% CI 0.66-1.03; one-sided $p=0.0421$). Notably, the survival curves crossed in the trial, and standard statistical assessments of outcome may be less valid. In subgroup analysis, pembrolizumab appeared more effective in patients who had high levels of PD-L1 expression (CPS ≥ 10) and in patients with excellent performance status (Eastern Cooperative Oncology Group performance status [ECOG PS]=0).

Avelumab

Avelumab, a fully humanised anti-PD-L1 antibody licensed for the treatment of Merkel cell carcinoma, has been evaluated in gastric and gastro-oesophageal cancer patients. In the phase IB JAVELIN Solid Tumor study, patients were treated with avelumab 10 mg/kg every 2 weeks. Eighty-nine patients were treated with maintenance avelumab, of whom 93% had been treated with only one line of prior ChT. The median duration of maintenance treatment was 12 weeks (range 2–62 weeks). Of patients treated with maintenance avelumab with PD-L1 status available, 38.5% were PD-L1⁺ at the 1% level on tumour cells. In the switch maintenance group, ORR was 9.0%. ORR was higher in PD-L1⁺ patients at 1% (10% versus 3%) and 5% (9% versus 5%) tumour cell levels; however, these analyses contain relatively few patients ($n=52$) in total. For all patients treated with maintenance avelumab, the median PFS was 12 weeks; median OS not reported. PFS appeared to be improved for PD-L1⁺ patients treated with avelumab; it was 17.6 versus 11.6 weeks for PD-L1⁻ and PD-L1⁺ patients, respectively.

In the second-line JAVELIN trial group, 62 patients were treated with avelumab, of whom 9.7% had a partial response. Few patients ($n=22$) in the second-line cohort had tissue available for PD-L1 expression; however, ORR appeared higher in PD-L1⁺ at 1% (18.2% versus 9.1%) and 5% PD-L1 expression levels (28.6% versus 6.7%). Median PFS was 6 weeks in the second group. Toxicity outcomes are similar for avelumab to other ICB drugs, except for infusion-related reactions, which occurred in 12.6% of the entire cohort. At the time of going to press, a trial of avelumab versus ChT in chemorefractory patients (JAVELIN Gastric 300) was initially reported as negative for an OS benefit.

Combination Immunotherapy

Combination anti-PD-1 plus anti-CTLA-4

Nivolumab plus ipilimumab (CheckMate 032). As single-agent anti-PD-1 therapy is associated with modest benefits in gastro-oesophageal cancer (ORR 10%–15%), the value of combination ICB in gastro-oesophageal cancer is also being explored.

In two of the phase I/II non-randomised CheckMate 032 study arms, nivolumab and ipilimumab were assessed at two dose levels: nivolumab 3 mg/kg plus ipilimumab 1 mg/kg every 3 weeks (N3 + I1) or nivolumab 1 mg/kg plus ipilimumab 3 mg/kg every 3 weeks (N1 + I3). Objective radiological response rates were higher in patients treated with the N3 + I1 regimen (24% versus 8%), as well as 12-month survival (35% versus 24%). Survival at 18 months was 28% for N1 + I3 patients and 13% for N3 + I1 patients (and 25% for N3 patients). For the subsequent randomised trial, the N1 + I3 regimen has been chosen for further investigation. ORRs appeared to be incremented by PD-L1⁺ status in both the N3 + I1 and N1 + I3 groups, and were as high as 40% in the N1 + I3 arm. However, as the number of patients from which this analysis is drawn is very small (n=10), this estimate is imprecise.

In CheckMate 032, patients who were treated with the combination of nivolumab and ipilimumab had higher rates of AEs compared with patients treated with single-agent nivolumab. In the N1 + I3 arm of CheckMate 032, 35% of patients had a grade 3 or 4 serious AE (versus 17% in the N3 + I1 arm and 5% in the nivolumab single-agent arm). Similarly, 20% of patients treated with N1 + I3 discontinued treatment due to AEs, compared with 10% of N3 + I1 and 5% of nivolumab-alone treated patients.

Therefore, although combination ICB for patients with gastro-oesophageal cancer is associated with increased response rates and encouraging survival, this comes at a cost of increased toxicity. This may have contributed to the early closure to recruitment of the N1+ I3 arm of the CheckMate 649 phase III randomised trial. However, response and survival results are awaited.

Combination Immunotherapy and Anti-angiogenic Therapy

Pembrolizumab plus ramucirumab

Vascular endothelial growth factor (VEGF) signalling and angiogenesis may act as negative regulators of the immune response. Inhibition of angiogenesis with ramucirumab is associated with survival benefits in gastro-oesophageal cancer, both as a single agent and in combination with paclitaxel ChT. In preclinical models, combining anti-angiogenic therapy with ICB led to enhanced anti-tumour efficacy. The JVDF trial combined the anti-VEGF receptor 2 (VEGFR2) mAb ramucirumab with the anti-PD-1 antibody pembrolizumab in a phase Ia/b trial in two cohorts (treatment-naïve and previously treated) of gastro-oesophageal cancer patients. PD-L1 assessment was based on CPS using Agilent antibody 22C3, and 54% of patients were PD-L1⁺. Partial responses were seen in 7% of patients, and stable disease (SD) in 44%. In the JVDF study, the median PFS and OS in the previously treated cohort were 2.6 months (95% CI: 1.5–4.2) and 6.4 months (95% CI: 4.2–12.6), respectively, whereas 6-month PFS and 12-month OS were respectively 26% and 35%. These preliminary data, although encouraging for patients with previously treated gastro-oesophageal cancer, are not significantly different from those observed for single-agent anti-PD-1 or anti-PD-L1 therapy in the same setting.

In the first-line setting (Cohort A2), 28 chemo-naïve patients were treated with ramucirumab and pembrolizumab. Twenty-five per cent of partial responses were observed, with 68% disease control. Median PFS was 5.3 months and 6-month PFS 35%; however, median OS was not reached.

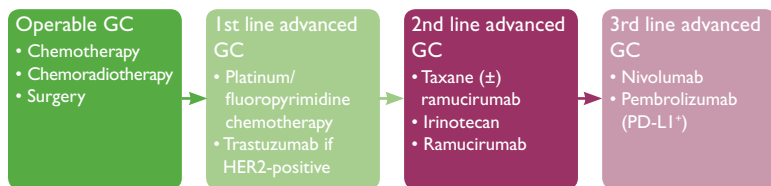
Toxicity for the combination of anti-angiogenic and immune therapy were expected. Hypertension occurred in 12% of the second- and further-line cohort and 18% of the first-line cohort, with grade 3 hypertension in 7% and 11%, respectively. Grade 3 colitis occurred in 7% of patients in the second-line cohort, which appears to be slightly more common than with anti-PD-1 or PD-L1 therapy alone.

Combination Anti-PD-1 Plus ChT

The KEYNOTE-059 study (Cohort 2) evaluated the effect of pembrolizumab and cisplatin–fluoropyrimidine ChT in HER2-negative treatment-

naïve gastro-oesophageal and gastric cancer patients. The primary end-point of the study was safety and tolerability; 25 patients were treated (17 Asian patients [68%] and 8 non-Asian patients [32%]). Using the CPS score previously described, 64% of patients were PD-L1⁺. The ORR associated with combination ChT plus pembrolizumab therapy was 60% (95% CI: 39–69), which is higher than that expected with combination ChT (normally 30%–48%). In KEYNOTE-059 Cohort 2, different rates of response were observed in PD-L1⁺ and PD-L1⁻ patients (69% versus 38%, respectively). Median PFS was 6.6 months, and median OS 13.8 months in all patients. These data are encouraging; however, they must be treated cautiously in view of the small numbers of patients enrolled in the trial. A majority (76%) of patients experienced grade 3–4 toxicity; however, the most common toxicities observed were consistent with ChT: neutropaenia (64%), stomatitis (20%) and poor appetite/anaemia and fatigue (8%).

Figure 1 Algorithm for management of gastro-oesophageal cancer with use of immunotherapy.



Abbreviations: GC, gastric cancer; PD-L1, programmed death-ligand 1.

Squamous Oesophageal Cancer

There are few data reported relating to the utility of ICB in oesophageal squamous cell carcinoma (OSCC). In the KEYNOTE-012 study, patients with both adenocarcinoma and OSCC were recruited. Of 17 patients with OSCC, the objective radiological response rate was 29%. In a phase II non-randomised study of nivolumab in chemorefractory OSCC, 65 patients were treated, with a centrally reviewed objective radiological response rate of 17%. Notably, median OS was 10.8 months, which is encouraging for a patient group which was refractory to both platinum and taxane ChT and had a history of significant alcohol and tobacco use. No results according to PD-L1 status were presented.

Conclusions and Future Directions

The ATTRACTION-2 study has provided level I evidence of the efficacy of ICB in gastric and gastro-oesophageal cancer. Despite the limitations that the results have been obtained in Asian patients only and may warrant further confirmation in Caucasian patients, nivolumab is likely to become a standard-of-care for patients with chemorefractory disease. Additionally, pembrolizumab has received a license in the United States for PD-L1⁺ gastric cancer patients who have been treated with two or more lines of ChT. Unfortunately, two second-line trials are negative. Ongoing trials will help to delineate the role of ICB in the first-line setting, which may be as single agent, in combination with other immune inhibitory molecules or with ChT. At the time of writing, PD-L1 is not an accurate predictive biomarker for efficacy of anti-PD-1 therapy in gastric and gastro-oesophageal cancer; standardisation of PD-L1 assessment may provide further refinement, but, as a benefit from ICB has been clearly observed in PD-L1⁺ tumours, it may be considered preferable as a biomarker to enrich for response rather than exclude patients from treatment. To date, the relatively immunogenic gastric cancer subtype MSI has provided the low-hanging fruit for anti-PD-1 therapy, but further research on the interaction between CIN and GS subtypes and responses to immunotherapy are required. Work on genomic and transcriptomic biomarkers such as tumour mutation burden and the IFN- γ response signature will be informed by progress in other disease types. However, it is crucial to recognise that, as yet, a minority of patients appear to benefit from ICB, and understanding the mechanisms of immune evasion for the majority of patients will be necessary in order to design effective strategies for combination therapy in the future.

Declaration of Interest:

Dr Arnold has reported consulting and advisory services, speaking or writing engagements, and public presentations for Roche, Merck Serono, Bayer Healthcare, Servier, BTG, Terumo, Sanofi Oncology and Eli Lilly. Dr Smyth has received honoraria for an advisory role from Five Prime Therapeutics, Bristol-Myers Squibb, Gritstone Oncology and Servier.

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2.5 Gastrointestinal Malignancies

2.5.3 Hepatocellular Carcinoma

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Introduction

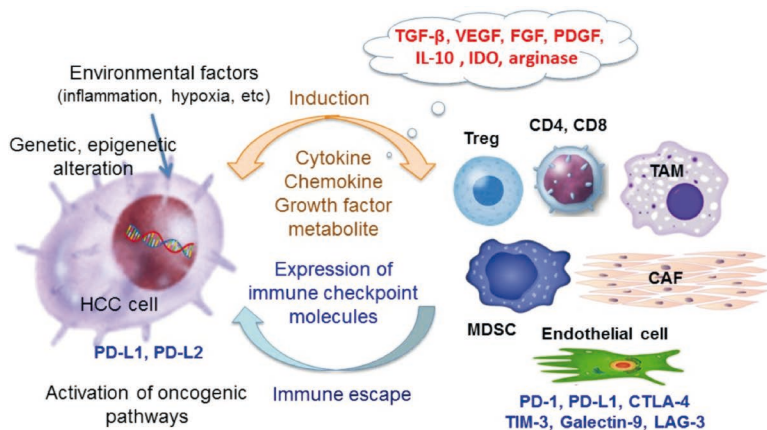
Hepatocellular carcinoma (HCC) originates from chronic fibro-inflammatory disorders of the liver such as chronic viral hepatitis, alcohol-induced hepatitis and non-alcoholic steatohepatitis. In most cases of hepatocarcinogenesis, HCC develops as a result of sustained viral infection or chronic inflammation, leading to the accumulation of genetic mutations and selection of hepatocytes with potent growth ability. In addition to genetic alterations in hepatocytes, recent studies highlight the importance of immune responses that impair cancer immunosurveillance by tissue immune cells. It is now generally accepted that a wide variety of immunosuppressive mechanisms operating in HCC tissue play a critical role in the generation of cancer microenvironment, and interference with these immunological pathways leads to therapeutic benefit in advanced HCC patients. Herein, we will focus on the current understanding of HCC immunobiology and the state of the art in HCC immunotherapies.

HCC Immunobiology

An immunosuppressive tumour milieu is created by cross-talk between tumour cells and immune cells. Cancer cells express tumour-associated antigens (TAAs), such as alpha-fetoprotein (AFP), New York-oesophageal squamous cell carcinoma-1 (NY-ESO-1), telomerase reverse transcriptase (TERT) and melanoma-associated antigen genes A (MAGE-A). In addition to these typical TAAs, HCCs also express neoantigens specific to individual tumours. HCCs evade the immune system even though classical TAAs and neoantigens can be detected by immune cells. Several mechanisms have been proposed for the explanation of such immune escape (Figure 1).

First, failure to process TAAs into antigenic peptide results in ineffective presentation of TAAs in HCCs. Second, HCC cells and immune cells in the tumour microenvironment (TME) produce a large amount of immunosuppressive soluble factors such as transforming growth factor (TGF)- β , interleukin (IL)-10, indoleamine 2,3-dioxygenase (IDO) and arginase. These immunosuppressive mediators inhibit activation of both innate and adaptive immunity cells. Third, HCC cells and immune cells in the TME express immune checkpoint molecules. Immune checkpoint molecules are fail-safe mechanisms to prevent excessive activation of T cells and are reviewed in Chapter 1.1. Immune checkpoint molecules include cytotoxic T-lymphocyte antigen 4 (CTLA-4), programmed cell death protein 1 (PD-1), T cell immunoglobulin and mucin domain-3 (TIM-3) and lymphocyte-activation gene 3 (LAG-3). Programmed death-ligand 1 (PD-L1) and PD-1 are preferentially expressed on HCC cells and T cells, respectively. The interaction between PD-L1 and PD-1 results in T cell exhaustion, and then, as a result, HCC development is accelerated by escape from T cell-mediated cancer immune surveillance. In preclinical models, blockade of these immune checkpoint pathways leads to HCC eradication and, in clinical samples, markers of T cell exhaustion in tumoural tissue often portend worse disease-specific survival outcomes. Finally, alterations in the cellular compartment of the TME, such as the presence of myeloid-derived suppressor cells (MDSCs), tumour-associated macrophages (TAMs) and T regulatory cells (T_{regs}), serve to further suppress an effective immune response in HCC. Interaction between effector T cells and these immunosuppressive cells contributes to the generation of tolerogenic immune responses in HCC.

Figure 1 Establishment of immunosuppressive environment in HCC.



Abbreviations: CAF, cancer-associated fibroblast; CTLA-4, cytotoxic T-lymphocyte antigen 4; FGF, fibroblast growth factor; HCC, hepatocellular carcinoma; IDO, indoleamine 2,3-dioxygenase; IL-10, interleukin-10; LAG-3, lymphocyte-activation gene 3; MDSC, myeloid-derived suppressor cell; PD-1, programmed cell death protein 1; PD-L1/2, programmed death-ligand 1/2; PDGF, platelet-derived growth factor; TAM, tumour-associated macrophage; TGF-β, transforming growth factor-β; TIM-3, T cell immunoglobulin and mucin domain-3; T_{reg}, T regulatory cell; VEGF, vascular endothelial growth factor.

Current Landscape of Immune Checkpoint Inhibition in HCC

CTLA-4 Blockade

Tremelimumab, a fully human immunoglobulin (Ig)G2 monoclonal antibody (mAb), is an antagonist of CTLA-4 on activated T cells and was recently evaluated in 20 patients with hepatitis C virus (HCV)-related HCC (Table 1). The study population included patients who failed prior treatment with sorafenib and had a large burden of disease with impaired liver function (57% Barcelona Clinic Liver Cancer [BCLC] C, 43% Child Pugh B, 29% portal vein invasion, 29% AFP >400 UI/mL). Notably, three of 17 (17.6%) evaluable patients attained a confirmed partial response (PR). These data are provocative and suggest that a patient subset might attain durable disease control with this treatment modality. The relatively high proportion of grade 3 and 4 transaminitis (45%) observed with CTLA-4 blockade was concerning, although impairments in liver func-

tion were reversible and did not progress to liver failure. Another critical observation from this study was that CTLA-4 blockade did not worsen hepatitis C virus (HCV) viraemia. Three patients achieved a transient complete virological response, and a patient subset had a transient decrease in HCV viral load. Given these results, continued development of CTLA-4 blockade in HCC is warranted and, currently, areas of exploration include CTLA-4 blockade in combination with other immune checkpoint blockers (namely with anti-PD-1/PD-L1 mAbs), pairing with regional therapy and, importantly, attempts to mitigate CTLA-4-based toxicity.

Table 1 Reported Results from Completed and Ongoing Clinical Trials Using Immune Checkpoint Blockade in Patients with Advanced HCC Who Failed, Declined or Were Intolerant to Prior Sorafenib

Clinical trial number	Agent	Design and size	ORR % (95% CI)	mDOR months (95% CI)	mOS months (95% CI)
NCT01008358	Tremelimumab	II (N=20)	17.6 (not reported)	not reported	8.2 (4.6–21.3)
NCT01853618	Tremelimumab + TACE/RFA	I (N=32)	26.3 (9.1–51.2)	not reported	12.3 (9.3–15.4)
NCT01658878	Nivolumab	I (N=48) II (N=214)	Phase I: 15 (6–28) Phase II: 20 (15–26)	17 (6–24) 9.9 (8.3–NE)	15 (9.6–20) not reported
NCT02702414	Pembrolizumab	II (N=104)	16.3 (9.8–24.9)	8.2 (2.3–8.3)	not reported
NCT01693562	Durvalumab	II (N=40)	10.3 (2.9–24.2)	not reported	13.2 (6.3–21.1)
NCT02519348	Durvalumab + tremelimumab	I (N=40)	15 (not reported)	not reported	not reported

Abbreviations: CI, confidence interval; HCC, hepatocellular carcinoma; mDOR, median duration of response; mOS, median overall survival; NE, non-evaluable; ORR, overall response rate; TACE/RFA, trans-arterial chemoembolisation/radiofrequency ablation.

PD-1 and PD-L1 Blockade

Several mAbs blocking PD-1 and PD-L1 are in development as monotherapy for HCC (Tables 1 and 2). Nivolumab, a human IgG4 mAb to PD-1, has been extensively tested in HCC. Based on the results from a large phase I/II study, nivolumab received expedited Food and Drug Administration (FDA) approval in 2017 for advanced HCC after failure or intolerance to sorafenib. In the study, 262 patients with advanced HCC and intact hepatic function were treated with nivolumab every 2 weeks in a dose escalation (n=48, 0.1 mg to 10 mg/kg) and a dose expansion (n=214, 3 mg/kg). Importantly, the agent was well tolerated

– 25% of patients experienced grade 3/4 toxicity in dose escalation; the most common events of any grade included transaminitis (31%), increases in amylase (15%) and lipase (15%), rash (31%) as well as pruritus (23%). Low frequencies of immune-related adverse events (irAEs) typical of this class of compound were also observed, such as hepatitis, adrenal insufficiency and diarrhoea. Hepatitis B virus (HBV) reactivation or seroconversion was not reported, and some patients had a transient decrease in HCV viraemia. The objective response rate (ORR) was 15% in the dose-escalation arm with a median duration of response (DOR) of 17 months and median overall survival (OS) of 15 months. The dose expansion confirmed these findings – safety was comparable and the ORR was 20% (95% confidence interval [CI]: 15–26). Responses were seen across HCC aetiologies and in patients who were treatment-naïve or heavily pretreated. As a condition of accelerated approval, further trials will be required to verify the clinical benefit of nivolumab.

Table 2 Status of Late-stage Clinical Trials of Immune Checkpoint Inhibitors in Patients with HCC

Clinical trial number	Agent	Target	Design	Endpoint	Status
Advanced HCC: Not surgical or transplant candidates; ineligible or failed prior embolisation					
NCT02576509	Nivolumab vs sorafenib	PD-I	Phase III	TTP/OS	Accrual complete
NCT02702401	Pembrolizumab vs BSC	PD-I	Phase III	PFS/OS	Accrual complete
NCT03062358	Pembrolizumab vs BSC-Asia	PD-I	Phase III	OS	Active accrual
NCT03298451	Durvalumab ± tremelimumab vs sorafenib	PD-L1 CTLA-4	Phase III	OS	Active accrual
NCT03434379	Atezolizumab + bevacizumab vs sorafenib	PD-L1 VEGF	Phase III	ORR/OS	Active accrual
Early-stage HCC: candidates for surgery					
NCT03383458	Nivolumab vs observation	PD-I	Phase III	RFS	Active accrual

Abbreviations: BSC, best supportive care; CTLA-4, cytotoxic T-lymphocyte antigen 4; HCC, hepatocellular carcinoma; ORR, objective response rate; OS, overall survival; PD-I, programmed cell death protein 1; PD-L1, programmed death-ligand 1; PFS, progression-free survival; RFS, recurrence-free survival; TTP, time to progression; VEGF, vascular endothelial growth factor.

An open-label, multicentre, randomised phase III study of nivolumab versus sorafenib in patients with advanced HCC has completed recruitment, and results are currently pending (NCT02576509). Patients with unresectable or metastatic HCC who were treatment-naïve and with Child Pugh A liver function were randomised 1:1 to receive nivolumab at 240 mg intravenously every 2 weeks or sorafenib at 400 mg orally twice a day. Stratification factors included HCC aetiology, vascular invasion, extrahepatic spread and geography. The primary endpoint of the study is OS. With an expected sample size of 726 patients, the study has a 90% power to detect a hazard ratio (HR) of 0.74 with a two-sided error of 0.04 for OS.

Durvalumab, a human IgG1κ mAb to PD-L1, has also been tested in a small phase I/II study of advanced HCC patients who failed prior treatment with sorafenib (93% of patients). Of 39 evaluable patients, four attained a confirmed PR (ORR 10.3%; 95% CI: 2.9–24.2). The median OS was 13.2 months with 56.4% of patients alive at one year. The anti-PD-1 mAb pembrolizumab is currently being evaluated in sorafenib-pretreated patients in a single-arm, phase II trial and in two randomised phase III studies against placebo in the second-line setting (NCT02702414, NCT02702401 and NCT03062358).

Finally, other agents targeting PD-1/PD-L1, such as PDR001, are studied in HCC-specific trials (NCT02795429), while several other agents are being evaluated for safety and efficacy in basket studies.

Combination Immune Checkpoint Blockade

Although these data are promising, it is important to acknowledge that the majority of HCC patients will progress or will not attain durable disease control with either CTLA-4 or PD-1/PD-L1 monotherapy, due to innate and acquired resistance to each of these treatments. To improve efficacy, biomarker and patient strategies will be of critical importance and are reviewed later in this chapter under ‘Future directions and issues specific to HCC’. Alternatively, combination immune checkpoint therapies may improve anti-tumour efficacy (Tables 1 and 2).

The most relevant example is combination blockade with CTLA-4 and PD-1/PD-L1 mAbs. The scientific rationale is that immune checkpoint

molecules function at different times in the lifecycle of effector T cells – CTLA-4 regulates naïve T cell priming and anergy, while PD-1/PD-L1 functions to blunt the immune response of effector T cells in the periphery. Thus, blocking these two pathways is proposed to stimulate T cell activation further, leading to enhanced tumour eradication. Preclinical data indicate that dual blockade is synergistic and, in the clinic, combination therapy results in statistically higher response rates and improved outcomes over monotherapy in a number of solid tumours. In HCC, both durvalumab and tremelimumab (NCT02519348), and nivolumab and ipilimumab (NCT01658878, NCT03222076), are being evaluated in phase I/II clinical trials, and planning for pivotal phase III studies is expected or ongoing. For example, a randomised, open-label, multicentre phase III study of durvalumab with or without tremelimumab versus sorafenib in advanced HCC patients opened to accrual late 2017 (NCT03298451). This 4-arm study will enrol about 1200 patients and explore two dose schedules of durvalumab and tremelimumab combination therapy. The primary endpoint is OS.

A critical question, of course, is whether the added toxicity of combination therapy will be offset by improved efficacy and outcomes. Another important question is whether sequential or combination checkpoint blockade might prevent resistance. For example, a recent clinical report indicates that TIM-3, a checkpoint protein, may mediate resistance to PD-1 monotherapy, and an antagonist of TIM-3 in combination or in sequence with PD-1 therapy *in vivo* improves anti-tumour efficacy. As more data become available, it is expected that multiple immune checkpoint inhibitor (ICI) doublets, and perhaps triplets, will be assessed in HCC. Presently, anti-PD-1/PD-L1 therapy is being paired with agents targeting TIM-3 (NCT03099109), LAG-3 (NCT03005782, NCT01968109) and KIR (NCT01714739) in HCC patients.

Combination Tyrosine Kinase Inhibitors and Immune Checkpoint Blockade

Hepatomas are sensitive to tyrosine kinase inhibition, and clinically several multitargeted tyrosine kinase inhibitors (TKIs) have demonstrated a survival benefit in advanced HCC, including sorafenib, lenvatinib,

regorafenib and cabozantinib. These agents collectively block vascular endothelial growth factor receptor (VEGFR) and platelet-derived growth factor receptor (PDGFR) with varying potency and each agent preferentially blocks distinct signalling cascades (i.e. cabozantinib/MET and lenvatinib/fibroblast growth factor receptor [FGFR] 1-4). From a purely empirical and practical standpoint, pairing each of these agents with anti-PD-1 therapy might be warranted, although emerging biological data indicate that TKIs clearly affect immune effectors, antigen presentation, the TME and the vasculature, and may serve to blunt or augment the immune response to cancer. In HCC, several preclinical studies have suggested that certain TKIs might act synergistically with anti-PD-1 therapy and, as such, several early phase studies are underway exploring the safety and tolerability of anti-PD-1 therapy with many agents, not limited to sorafenib (NCT03211416, NCT01658878, NCT02988440), lenvatinib (NCT03006926), cabozantinib (NCT03299946, NCT01658878) and axitinib (NCT03289533). In addition, selective inhibitors of VEGF, MET and FGFR4 are currently being evaluated with studies using bevacizumab (NCT03434379), capmatinib (NCT02795429) and FGF401 (NCT02325739), respectively.

Alternative Immunotherapeutic Strategies

Oncolytic Viruses

Oncolytic viruses function by two major mechanisms: direct viral replication within tumour cells leading to tumoural lysis, and activation of cell-mediated tumour specific immunity. Pexa-Vec (pexastimogene devacirepvec), an oncolytic virus derived from vaccinia, has been evaluated in a randomised phase II study of high-dose versus low-dose intratumoural injection in advanced HCC patients (n=30). OS was significantly longer in the high-dose arm compared with the low-dose arm (median: 14.1 months versus 6.7 months, *p*-value 0.02). In contrast, a phase IIb clinical trial of 129 HCC patients who failed sorafenib therapy did not achieve the primary endpoint of prolonging OS in Pexa-Vec-treated patients compared with best supportive care. Importantly, the study population included patients with impaired hepatic function (Child Pugh-B7), large vessel involvement and allowed a heavy burden of disease. A phase III

study of Pexa-Vec in combination with sorafenib versus sorafenib alone (NCT02562755) and a phase I/II study of Pexa-Vec in combination with nivolumab (NCT03071094) in first-line HCC patients are ongoing at the time of writing, with more refined inclusion and exclusion criteria.

Adoptive Cellular Therapy

Adoptive cellular therapies use tumour infiltrating lymphocytes (TILs), modified T cell receptors (TCRs) and chimeric antigen receptors (CARs), and these approaches are all being investigated in HCC. However, all three modalities call for extensive clinical infrastructure since TCRs and CARs require genetic modification to target specific tumour antigens, the former in a major histocompatibility complex (MHC)-restricted manner and the latter in a non-MHC restricted manner. Common antigens studied to date include AFP and glypican 3 (GPC3), as both are abundantly expressed in HCC with limited expression in normal tissues. Several studies are underway to evaluate GPC3-CAR in patients with advanced and refractory HCC (NCT03146234, NCT02715362, NCT03130712, NCT03198546), as well as AFP-specific TCRs (NCT03132792). Future success of these approaches will depend on safe antigen selection, common MHC haplotypes for TCR-based research (especially given the wide ethnic variability observed with HCC) and careful patient selection given the need for cytoreductive treatments prior to adoptive transfer.

Future Directions and Issues Specific to HCC

Application of Immunotherapy to Earlier Stages of Disease

Clinical trials of immunotherapy have mostly enrolled patients with advanced-stage HCCs that are refractory to conventional treatments, such as radiofrequency ablation (RFA) and trans-arterial chemoembolisation (TACE). However, these procedures clearly affect the immune response and may release TAAs and/or contribute to the induction of an anti-tumour immune reaction. Intriguingly, tremelimumab in combination with RFA or TACE in advanced HCC (BCLC-C) patients was found to be safe and led to a 26.3% ORR. Median OS was favourable at 12.3 months for a heavily pretreated population. However, the effect of immune checkpoint blockade at earlier stages of HCC has not been

confirmed, and several pilot studies are ongoing to assess safety and efficacy (NCT03033446, NCT03143270, NCT03099564, NCT02821754). Furthermore, ICIs are also being tested in the surgical setting, and several adjuvant and neoadjuvant studies are in planning or ongoing (NCT03222076, NCT03383458).

Biomarkers and Patient Selection

Specific biomarkers that predict the effect of immunotherapy, including ICIs, have not been clarified for HCC. The largest clinical effort to date has been the analysis of tumoural PD-L1 expression in the phase I/II study of nivolumab – no correlation between response and PD-L1 level was observed. Several other tumoural markers are currently being evaluated, including viral markers, TILs, immune effector composition, cytolytic score by proteomic analysis and tumoural genomics. Several studies indicated that mutational load and inactivation of mismatch repair genes are strongly associated with efficacy of PD-1/PD-L1 inhibitors. However, mutations in mismatch repair genes, microsatellite instability and hypermutation are rare in HCC. HCC as a field has also been at the forefront of imaging, and investigation here will no doubt continue to include novel magnetic resonance imaging (MRI) modalities, texture and vasculature analyses, as well as functional T cell imaging (e.g. 89Zr-Df-IAB22M2C, NCT03107663).

Issues Specific to HCC

As nearly 90% of HCC patients have cirrhosis and most patients have viral hepatitis, several issues specific to HCC and immune-based therapy must be addressed. First, transaminitis related to checkpoint blockade must be evaluated carefully to include an assessment of viral reactivation, cross-sectional imaging to rule out progression and hepatic/portal vein involvement, and history to exclude other potential confounders such as toxin-induced injury (i.e. alcohol). In the event of immune-mediated hepatic dysfunction, prompt treatment with corticosteroids or other immunosuppressive medications may be required to prevent liver failure. Second, a subset of HCC patients progress after prior liver allograft; in these cases immune-based therapy is contraindicated and case reports

have shown acute rejection following anti-PD-1 therapy. Third, procurement of fresh tumour tissue for biomarker analysis may be inappropriate, specifically in cases of poor hepatic function and/or severe hepatic dysfunction. Thus, functional imaging, as noted above, must be explored in HCC. Finally, specifically to HCV-related HCC, it is unclear if treatment of the virus will affect immune-based treatment.

Conclusions

The field of HCC treatment is rapidly evolving and, at present, nivolumab is the first immunotherapy approved for the treatment of advanced HCC. The immediate and critical questions for this field are whether this new modality improves OS in the metastatic setting or not, and at what point during the course of the disease should anti-PD-1 therapy be applied (i.e. first or second line). Several groups are actively evaluating combination therapies as well as enrichment strategies to improve anti-PD-1 therapy. Immune checkpoint blockade is rapidly moving to earlier stages of disease and is being evaluated in the context of a variety of regional therapies. Novel treatments, such as oncolytic viruses and cellular therapy, are under active investigation. These collaborative and global efforts will undoubtedly lead to progress and to a new armamentarium of treatments for patients with this devastating disease.

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2.6 Immunotherapy in Head and Neck Tumours (HPV⁺ and HPV⁻)

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Immunotherapy in Head and Neck Cancer – the Rationale

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common malignancy worldwide. Tobacco and alcohol use account for the majority of HNSCC, while a substantial proportion of oropharyngeal cancers (OPCs) appear to be associated with high-risk human papilloma-viruses (HPVs). Despite advances in multimodality treatment, the 5-year progression-free survival (PFS) rates of patients with locally advanced disease do not exceed 40%–50%, and survival in recurrent or metastatic settings remains dismal. In-depth elucidation of cancer immunology indicated that immunotherapy represents the future in HNSCC treatment. The effector arm of immunotherapy is regulated by positive and negative co-signalling checkpoint pathways.

Complete activation of T cells depends on the regulation of a ‘dual-signal’ system. The first signal is derived from specific binding between

T cell receptor and a major histocompatibility complex class. The second signal is mediated by the interaction between antigen-presenting cell (APC)-expressed co-stimulatory molecules and the corresponding receptor or ligand on the T cell surface. In addition, to ensure that T cells are not overstimulated, negative co-stimulatory molecules regulate T cells, mainly cytotoxic T-lymphocyte antigen 4 (CTLA-4)-B7 and programmed cell death protein 1 (PD-1)/programmed death-ligand 1 (PD-L1) signalling pathways. After PD-1 and PD-L1 bind with each other in activated T cells, tyrosine in the structural domain of PD-1 undergoes phosphorylation, causing dephosphorylation of the downstream protein kinases SYK and PI3K. This leads to inhibition of downstream proteins, such as Akt and ERK. Finally, inhibition of the transcription and translation of genes and cytokines required by T cell activation leads to the regulation of T cell activity. After invasion by tumour cells, these signal channels are used to inhibit T cell activation and evade attack by the immune system.

PD-1 is a member of the CD28/CTLA-4/inducible T cell co-stimulator (ICOS) co-stimulatory receptor family. PD-L1 and PD-L2 are PD-1 ligands belonging to the B7 superfamily. The structure of PD-L1 and PD-L2 comprise an extracellular region consisting of immunoglobulin (Ig)V-like structures in series and Ig constant region-like structures, a hydrophobic transmembrane region and an intracellular region with a short cytoplasmic tail. The affinity of PD-L1 extracellular region with PD-1 is lower than that of PD-L2. However, the PD-L1 extracellular region can also bind with the B7-A (CD80) extracellular region. Only a few studies have focused on PD-L2 since it is expressed in activated macrophages and dendritic cells (DCs). PD-1 is mainly expressed on activated T cells after induction by T cell antigen receptor and cytokine receptor. PD-L1 is expressed constitutively at low levels on APCs and a wide variety of non-haematopoietic cell types. Inflammatory cytokines, such as type I and type II interferons (IFNs) as well as tumour necrosis factor alpha (TNF- α) and vascular endothelial growth factor (VEGF), can induce PD-L1 expression.

HNSCC cells upregulate PD-L1 expression through several mechanisms. The first is the activation of the epidermal growth factor recep-

tor (EGFR) and its associated downstream effector pathways. Increased expression of STAT3 and hypoxia-inducible factor 1 (HIF-1) transcription factors can upregulate PD-L1 expression. The second mechanism is the amplification of genes coding PD-L1 (*9p24.1*). The third is the induction of Epstein–Barr virus (EBV): EBV-positive HNSCCs, although not frequent, can result in high expression of PD-L1 even without amplification of the *9p24.1*. Despite reports that HPV-positive (HPV+) HNSCCs overexpress PD-L1, it has recently been shown that increased expression of PD-1/PD-L1 in the microenvironment of HNSCC is independent of HPV status. Additionally, in the tumour microenvironment (TME), the stimulatory effects of inflammatory factors can also induce PD-L1 expression, IFN- γ being the most important.

At present, immune checkpoint inhibitors (ICIs) have been studied in HNSCC patients, and the most extensively used are CTLA-4, PD-1 and PD-L1 monoclonal antibodies (mAbs). The anti-tumour effect consists of inhibition of the activity of immune checkpoints, blockade of immunosuppression in the TME and reactivation of the immune response of T cells to the tumour.

Immunotherapy for Locally Advanced HNSCC

The current therapeutic standard for locally advanced (LA) HNSCC consists of multimodality treatment. In this setting, immunotherapy may be complementary to chemotherapy (ChT), radiotherapy (RT) and surgery.

Immunotherapy and Surgery

In some LA, potentially resectable, HNSCC, ChT can be administered before surgery, with a double intent: downstaging primary disease to improve the success of radical surgery and reducing the risk of distant metastases. Since induction immunotherapy could play a similar role, several ICIs are currently under investigation in this setting.

The CheckMate 358 trial is exploring the safety and activity of pre-surgical nivolumab in malignancies with viral aetiology (NCT02488759). Preliminary data report that neither major toxicities nor delays in surgery have been observed after two courses of anti-PD-1 therapy. In this vein,

Ferris et al (2016) reported presurgery radiological tumour reduction in 11 of 23 (48%) evaluable patients. A further trial is studying the role of induction nivolumab and RT, followed by surgery and 6 cycles of adjuvant nivolumab (NCT03247712).

Within another ongoing trial (NCT02296684), no severe immune-related adverse events (irAEs) nor delays in surgery were observed after a single course of pre-surgical pembrolizumab, resumed after postoperative (chemo)RT. In this study, a pathological treatment effect in $\geq 70\%$ of resected tumour or lymph nodes was observed in 29% of cases. MEDI6469 is a mAb directed against OX40, a molecule belonging to the TNF receptor superfamily. Safety and activity of this drug in the neo-adjuvant setting are being tested in an ongoing study (NCT02274155).

Immunotherapy and RT

RT-induced cell damage is mediated by both physical and biological pathways. RT leads to host immune system activation through different mechanisms: inducing an inflammatory TME, increasing tumour antigen presentation and enhancing anti-tumour cytotoxicity of CD8⁺ T cells. Bystander effect is a radiation-induced injury to anatomical regions not included in the radiation volume but close to it. On the contrary, the abscopal effect consists of shrinkage of a non-irradiated tumour site distant from the RT site. While the former is mostly physically mediated, the latter also involves biological processes, notably immunological ones. In preclinical models, radiation-mediated cell death induces the expression of tumour-associated antigens. The activation of APCs leads to T cell stimulation and selection of clones able to react against tumour cells, even if distant from the RT target. This represents a rational basis for associating ICIs with RT. In murine models, anti-CTLA-4 antibodies induce this abscopal effect with fractionated but not single-dose radiation. Anti-PD-L1 agents concomitant with RT reduce tumour infiltration of myeloid-derived suppressor cells (MDSCs) and activate CD8⁺ T cells through TNF- α . An ongoing clinical trial is exploring the association of pembrolizumab with curative intensity-modulated RT (IMRT) in patients ineligible to receive cisplatin (NCT02609503).

Immunotherapy and Concurrent ChT/Biological Therapy plus RT

Cancers not responding to preoperative anti-EGFR agents have an immune suppressive microenvironment. Indeed, tumour infiltration of FoxP3⁺ T_{reg} cells is more evident and CTLA-4 expression is higher in patients not responding to induction cetuximab. Two trials are ongoing to explore the safety of ipilimumab in association with cetuximab and RT (NCT01860430 and NCT01935921). In turn, cetuximab itself induces tumour cell death through antibody-dependent cellular cytotoxicity (ADCC), complements mediated activity and enhances activity of T CD8⁺ cells. Preliminary data revealed a dose-limiting skin toxicity, different from typical cetuximab-induced acne-like rash.

Results of a phase I trial evaluating pembrolizumab with concomitant chemoradiotherapy (CRT) (NCT02586207) were reported in 2017 at the American Society of Clinical Oncology (ASCO) Annual Meeting. Treatment discontinuations due to irAEs were observed in 11% of patients and, in 85% of subjects, total cumulative cisplatin dose was higher than 200 mg/m².

Nivolumab is currently under evaluation with IMRT and weekly cisplatin (NCT02764593). An ongoing phase III trial (NCT03349710) is exploring the safety and activity of nivolumab with cisplatin (or without, in ChT-ineligible patients) combined with RT in patients with LA HNSCC. In this study, nivolumab is delivered every 3 weeks during RT, then monthly for 6 months. Another phase III study (NCT03040999) is evaluating the role of combining pembrolizumab with CRT. A priming pembrolizumab dose or placebo is given one week before CRT, followed by two doses during CRT, and an additional 14 doses after CRT, totalling 17 pembrolizumab or placebo infusions.

Further trials are studying the safety and activity of avelumab with CRT (NCT02952586) or with RT plus cetuximab (NCT02999087). Durvalumab is also being investigated with RT and cetuximab in the DUCRO-HN trial (NCT03051906).

Intra- or Peri-tumour Immunotherapy

In addition to systemic treatments, some immune-modulating agents can be administered topically. In 2002, an Italian study (De Stefani et al)

showed that peri-lymphatic injections of interleukin-2 (IL-2) in patients with resectable carcinoma of the oral cavity and oropharynx could delay disease recurrences.

Peri-tumourally-delivered immunotherapies are promising approaches in the management of HNSCC. Nevertheless, LA tumours are often close to vital structures, notably neck vessels, therefore an accurate risk/benefit ratio should be balanced in every patient when considering such treatments.

Immunotherapy in Recurrent/Metastatic Head and Neck Cancer – Evidence and Future Directions

The main PD-1-targeting agents approved in HNSCC so far are pembrolizumab and nivolumab, both being humanised PD-1-inhibiting IgG4 mAbs with high specificity. They have been approved (nivolumab in the USA and Europe, pembrolizumab only in the USA) for the treatment of patients with recurrent/metastatic HNSCC with disease progression on or after platinum-containing ChT.

Pembrolizumab

A single-arm phase II study evaluated fixed-dose pembrolizumab (200 mg every 3 weeks for 24 months) in 171 patients with recurrent/metastatic HNSCC after failure of platinum-based treatment and cetuximab. The objective response rate (ORR) was 16%, with median duration of response 8 months. Response rates were similar in all HPV and PD-L1 subgroups. Median PFS was 2.1 months and median overall survival (OS) 8 months. At the time of analysis, 109 patients (64%) experienced a treatment-related AE (trAE), with 26 of them (15%) experiencing a grade ≥ 3 event. KEYNOTE-040 is an open-label, phase III study of pembrolizumab versus standard-of-care treatment in patients who had recurrence or progression after a platinum-containing regimen. Randomisation was stratified by Eastern Cooperative Oncology Group (ECOG) performance status (PS), HPV status and PD-L1 tumour proportion score (TPS). After median follow-up of 7.3 months, pembrolizumab prolonged median OS in the intention-to-treat (ITT) population

(8.4 versus 7.1 months), but the difference did not achieve statistical significance (hazard ratio [HR] 0.81, one-sided $p=0.0204$), while there was no difference in PFS. It is noteworthy that pembrolizumab achieved better OS (11.6 versus 7.9 months; HR 0.58) in patients with PD-L1-expressing tumours (TPS $\geq 50\%$).

Nivolumab

Nivolumab was directly evaluated in a randomised phase III trial, in which 361 patients with recurrent/metastatic HNSCC that had progressed on or within 6 months of platinum-based therapy were given nivolumab (3 mg/kg every 2 weeks; 240 cases) or standard therapy (121 cases). The primary endpoint was OS. The ORR of the nivolumab group was 13.3%, versus 5.8% in the standard therapy cohort. Up to 18 December 2015, the median OS of the nivolumab group was 7.7 months and 22% patients had reached 18-month PFS, whereas in the standard-treatment arm the median OS was 5.1 months and only 8.3% patients had reached 18-month PFS. The survival advantage of nivolumab was irrespective of p16 status and PD-L1 positivity, while in an exploratory analysis it was shown that the benefit was greater in patients without prior exposure to cetuximab. Regarding toxicity, 139 patients in the nivolumab group experienced trAEs (58.9%), mainly fatigue, nausea and rash; in addition, 31 patients (13.1%) experienced grade ≥ 3 trAEs. Eighty-six patients in the standard-therapy group experienced trAEs (77.5%), mainly nausea, fatigue and anaemia; among them, 39 patients (35.1%) experienced grade ≥ 3 trAEs. Therefore, it seems that the curative effect and survival benefit of nivolumab were more than doubled and longer than with a traditional regimen, while the number of serious adverse reactions was about one-third of that of the traditional regimen. Additionally, it was shown that nivolumab resulted in improvement of functional and symptom domains, whereas investigator's choice led to clinically meaningful deterioration.

Other PD-I/PD-L1-targeted Agents

Many PD-L1-targeted agents are currently under evaluation for HNSCC treatment. Among them is durvalumab, a human IgG1 mAb that blocks PD-L1 from binding to its receptors and is clinically tested in patients

with recurrent/metastatic HNSCC. Early-phase clinical trials have evaluated durvalumab (10 mg/kg every 2 weeks) in such patients, also showing high PD-L1 expression ($\geq 25\%$ staining of tumour cells). This agent showed an ORR of 16.2%, with a toxicity similar to that of PD-1 inhibitors. In the overall cohort, median PFS was 2.1 months and median OS 7.1 months.

As single agents, anti-PD-1/PD-L1 molecules have durable response rates of 14%–32% in the second-line setting in recurrent/metastatic HNSCC. However, only a minority of patients derives benefit from single-agent immunotherapies, with some patients not responding to treatment at all, and others attaining a limited response followed by tumour progression. Ongoing clinical trials use T cell checkpoint inhibitors in combination with other therapeutic approaches to enhance immunotherapy benefit in head and neck cancer patients. These combinations use other co-inhibitory checkpoints (e.g. anti-CTLA-4 agents), co-stimulatory checkpoints (e.g. anti-OX40 antibodies) and other molecules in the TME (e.g. IDO inhibitors).

Immuno-oncology in Head and Neck Cancer: Predictive Biomarkers?

The benefit of immunotherapy in HNSCC is not accessible to all patients. Based on the literature, ORR of immunotherapy in HNSCC ranges from 13.6% (Ferris et al, 2016) to 22% (Bahleda et al, 2017). In HNSCC, ORR is crucial, considering that specific disease-related issues, such as infections, bleeding or pain, may improve thanks to tumour reduction. Furthermore, a better selection for immunotherapy could avoid toxicities. Therefore, the identification of clinical and/or preclinical predictive factors of immunotherapy response is eagerly advocated.

To date, no factor warrants the decision whether or not to prescribe immunotherapy. Despite this, quite a considerable amount of data is available.

PD-L1 Expression

One important marker is PD-L1 expression on tumour and/or immune cells. PD-L1 is the direct (durvalumab, atezolizumab) or indirect (nivolumab, pembrolizumab) target of all immunotherapeutic agents.

In this scenario of the immune response prediction, PD-L1 expression is emerging as the most promising factor, but there are still limitations to confirm its predictive use. First, there is no consensus about the percentage of PD-L1 positive cells (cut-off) to define a tumour as PD-L1-positive (PD-L1⁺). Second, the type of cells (tumour and/or stromal) to be considered for PD-L1 expression has not been established yet. Table 1 summarises these discrepancies for anti PD-1/PD-L1 agents studied in HNSCC.

As shown in Table 1, another limitation is the type of assay used to measure PD-L1. Rimm et al (2017) compared these assays in detecting PD-L1 on samples of non-small cell lung cancer (NSCLC). They found that three out of four assays identified similar PD-L1-positivity, but one of them (Ventana SP142) proved to be a low-performance test, with half of PD-L1⁺ patients detected. The same concordance has already been demonstrated in HNSCC.

Considering these limitations, in the nivolumab study (Ferris R et al, 2016) a relationship between PD-L1 expression and outcome was observed. In fact, PD-L1 expression >1% was notably related to better OS when compared with tumours expressing PD-L1 <1%.

In KEYNOTE-012 (phase Ib trial with pembrolizumab), with PD-L1 measured only on tumour cells as TPS, ORR was similar among PD-L1⁺ and PD-L1-negative (PD-L1⁻) patients (19% in PD-L1⁺ versus 18% in PD-L1⁻, $p=0.461$). When PD-L1 was detected on tumour and stromal cells in a combined positivity score (CPS), ORR was significantly different (21% in PD-L1⁺ versus 6% in PD-L1⁻, $p=0.023$). By CPS use, PFS and OS were also significantly increased in PD-L1⁺ patients treated with pembrolizumab.

Table 1 PD-L1 Detection in Studies Testing Immunotherapy for HNSCC

	Nivolumab	Pembrolizumab	Atezolizumab	Durvalumab
Type of assay	Dako/Agilent 28-8	Dako/Agilent 22c3	Ventana SP142	Ventana SP263
Type of cells	Tumour cells	Tumour cells Stromal cells	Tumour cells Stromal cells	Tumour cells
Cut-off	≥1%, ≥5%, ≥10%	≥50% tumour cells ≥1% stromal cells	≥1%, ≥5%, ≥10%	≥25%

Abbreviations: HNSCC, head and neck squamous cell carcinoma; PD-L1, programmed death-ligand 1.

In KEYNOTE-055 (phase II trial with pembrolizumab), ORR was higher in PD-L1⁺ (on tumour and immune cells) than in PD-L1⁻ patients: 18% versus 12%, respectively.

In the KEYNOTE-040 study (phase III trial with pembrolizumab), ORR was progressively higher in the overall population and in subgroups identified as PD-L1⁺ by CPS >1% and TPS ≥50% (ORR: 14.6%, 17.3% and 26.6%, respectively).

These data strengthen the concept that a higher expression of PD-L1 (regardless of cut-off or type of cells considered for PD-L1 detection) corresponds to a higher response rate to anti-PD1 agents. This was confirmed in two other studies with atezolizumab and durvalumab (Zandberg et al, 2017), both being anti-PD-L1 agents. In the atezolizumab study, seven patients with low PD-L1 expression (<5%, detected on tumour and stromal cells) had significantly lower ORR than 25 patients with high PD-L1 expression (>5%): 14% versus 24%, respectively.

In the durvalumab study, the overall population was already selected as ‘high PD-L1⁺’, as only patients with PD-L1 >25% (on tumour cells) were included.

Human Papillomavirus

HPV-positivity is not predictive, as responses to immunotherapy were also observed in HPV-negative patients. A trend to higher ORR in HPV⁺ patients was observed but not confirmed in the atezolizumab and KEYNOTE-040 studies. Moreover, in the nivolumab phase III study (Ferris et al, 2016), in HPV⁺ patients, ORR was not significantly different according to PD-L1 expression (32.9% in HPV⁺ PD-L1⁺ >1% versus 26.1% in HPV⁺ PD-L1⁺ <1%).

PD-L2 Expression

Other molecules, such as PD-L2, are involved in the PD-1/PD-L1 axis and could explain why PD-L1⁻ patients also occasionally respond to anti-PD-1/PD-L1 drugs.

Yearley et al (2017) analysed PD-L2 expression on tumour, stromal and endothelial cells in seven cancer subtypes: gastric, melanoma,

kidney, bladder, lung, breast cancer (triple-negative) and HNSCC. Among these tumours, results for HNSCC showed higher PD-L2 expression on tumour cells. PD-L2 was intermediate on stromal cells for all considered cancers, and low on endothelial cells. In HNSCC, PD-L2 was predictive of response, regardless of PD-L1. OS and PFS were better in PD-L2⁺ than in PD-L2⁻ patients. ORR was higher with concomitant PD-L1⁻ and PD-L2⁺.

Other Possible Predictive Factors

Another potentially predictive factor is IFN- γ , which seems to play a role in blocking the anti-tumour immune response (Abiko et al, 2015). Regularly, IFN- γ induces higher PD-L1 expression on immune, stromal and tumour cells, thus enabling the interaction between PD-L1 and PD-1 on the intra-tumour T lymphocytes. Therefore, tumours expressing the IFN- γ gene signature, highly producing IFN- γ (inflamed gene expression profile [GEP]), have a higher probability to respond to anti-PD-1/PD-L1 drugs and to improve OS and PFS.

Another determinant of response is the tumour mutation burden (TMB). In advanced tumours, response to immunotherapy was higher if TMB was higher (Le et al, 2015). This is due to a higher exposition of neo-antigens in highly-mutated tumours. In this way, these neo-generated epitopes are more easily recognisable by the immune system when amplified by immunotherapy.

The combination of different predictors may represent the best strategy to select patients for immunotherapy. In NSCLC patients treated with durvalumab, the concomitant lack of PD-L1 and IFN- γ signature reached a negative predictive value of 97% (Higgs et al, 2016).

The last factor is the gut microbiome. The influence of different composition of gut microbiome on response to anti-PD-1 agents was recently reported in other tumours, such as kidney and NSCLC (Routy et al, 2018). In HNSCC, the only available data come from the nivolumab study, where there was no difference in saliva microbiome composition between responders and non-responders. Further studies are needed to clarify the predictive role of microbiome.

Conclusion

Almost 10 years after the approval of cetuximab, immunotherapy is beginning to enrich the therapeutic armamentarium for recurrent/metastatic HNSCC. Moreover, immune agents offer medical oncologists a great opportunity to possibly change the management of this cancer, also in earlier disease stages. Unfortunately, current evidence confirms that only a minority of HNSCC patients may benefit from immunotherapy. Therefore, research efforts are focused on identifying the main characteristics of immune responders.

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2.7 Immunotherapies in Lymphoma

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Introduction

Lymphomas were one of the first cancers to show sensitivity to manipulations of the immune system; in particular, indolent lymphomas responded to interferon and vaccination in studies performed in the 1980s. Lymphomas are a big and rather heterogeneous group of tumours arising from the immune system and dissecting the value of different types of immunotherapy on each single lymphoma entity goes beyond the purpose of this chapter. We will therefore address the most relevant ones in the context of each different type of immunotherapy.

Monoclonal Antibodies

Monoclonal antibodies (mAbs) are used in lymphoma treatment either in their ‘naked’ form (no other molecule attached to them) or as vectors of cytotoxic substances (such as the anti-CD30 brentuximab vedotin [BV], active in Hodgkin lymphoma) or of radioactive isotopes (such as ⁹⁰Y-ibritumomab tiuxetan, active in follicular lymphoma [FL]). As the latter is finally a technique to deliver targeted chemotherapy (ChT) and radiotherapy (RT) rather than pure immunotherapy, it will not be addressed in this chapter.

The anti-CD20 mAb rituximab revolutionised the management of patients with FL, diffuse large B cell lymphoma (DLBCL) and other CD20-positive

B cell non-Hodgkin lymphomas (B-NHLs). Rituximab is particularly active, with little toxicity, due to its ability to kill normal and cancer B cells (bearing CD20 on their surface) while totally sparing T cells and other body tissues (all CD20-negative). The introduction of this drug (usually given in association with ChT) has improved the median survival of patients with B cell lymphomas by 20%–30% in the last 20 years.

The mechanisms of action of rituximab are classically believed to be based on so-called ‘passive immunotherapy effects’, including complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP) and programmed cell death (PCD). Nevertheless, some elements suggest that rituximab could also elicit an active immune response with expansion of specific cytotoxic T lymphocytes.

Facts Suggesting a ‘Vaccinal Effect’ of Rituximab

In vitro experiments suggest that rituximab treatment causes inflammatory death of lymphoma cells, releasing antigens from apoptotic cells and promoting the uptake, processing and cross-presentation of lymphoma cell-derived antigens by antigen-presenting cells (APCs) to T cells, thus inducing expansion and activation of specific cytotoxic T lymphocytes. Findings from a clinical trial of patients with FL treated with rituximab alone provide evidence that the ‘passive’ immunotherapy with rituximab can elicit an active T cell response. To increase the ‘vaccinal effect’ of rituximab, phase II clinical trials combining immunomodulatory cytokines with rituximab in patients with indolent B-NHL were performed: combining rituximab with interferon (IFN)- α or macrophage colony-stimulating factor seems to improve both the quality and duration of clinical response. Also, the onset of maximal response several months after the last infusion of rituximab and the long-lasting complete remissions in responding FL patients, even when treated for a short time, suggest that, in addition to passive immunotherapy, an active ‘vaccinal effect’ could be contributing to the activity. Finally, patients with Waldenström’s macroglobulinaemia treated with rituximab alone show a gradual reduction of serum immunoglobulin (Ig)M over several months after the last infusion, which supports this hypothesis.

To improve antitumour activity and Fc binding affinity, several new generations of anti-CD20 mAbs have been engineered. The second-generation mAbs (ocrelizumab, veltuzumab, ofatumumab) are designed as humanised or fully human with unmodified Fc domain, with the aim of reducing immunogenicity compared with the first-generation chimeric mAb rituximab. The third-generation mAbs are humanised and modified in the Fc region. The modification of Fc domain may improve antitumour activity in patients with low-affinity versions of the Fc receptor expressed on their tumour cells, due to increased binding affinity and increased cell death through ADCC mechanisms (obinutuzumab).

Other mAbs active in lymphomas are:

- Epratuzumab: anti-CD22
- Lumiliximab: anti-CD23
- Otlertuzumab: anti-CD37
- Milatuzumab: anti-CD74
- Polatuzumab: anti-CD79b
- Galiximab: anti-CD80.

Interferons

IFNs are small proteins and glycoproteins produced by cells in response to viral infections. The antiproliferative activity of IFN consists of direct and indirect effects. Direct effect is mediated through cancer cell growth inhibition by cell cycle arrest, apoptosis or differentiation. Indirect effect occurs via expansion and activation of specific cytotoxic T lymphocytes and natural killers (NKs), inhibition of angiogenesis and induction of cytokines.

A meta-analysis evaluating the role of INF- α -2b in FL showed prolongation of survival and remission duration when IFN was given in the context of relatively intensive initial ChT at a dose of ≥ 5 million units. However, the use of systematically administered INF- α -2b in treating B cell lymphoma was limited by its short half-life and rather high toxicity.

Treatment with IFN plays a key role in a group of rare hepatitis C virus (HCV)-associated indolent B-NHLs. The association between HCV and

B-NHL is now widely accepted. The most accepted pathogenetic models rely on chronic antigenic stimulation of lymphocyte receptors by viral antigens eliciting B cell proliferation.

Vaccination

Despite significant progress in therapy, indolent lymphomas remain incurable. Using vaccines to induce tumour-specific immune response is one possible strategy to eradicate the residual lymphoma cells.

Idiotypic (Id) is the unique and tumour-specific variable component of the surface Ig present on each B lymphoma cell. Most clinical trials of Id vaccines have been performed on patients with FL, because the indolent nature of FL allows the necessary time for the individual vaccine to be prepared and for the patient's immune system to recover after immunosuppressive ChT. Early studies have shown that the treatment with Id vaccines can elicit a tumour-specific response and favourably affect disease-free and overall survival (DFS and OS) in patients responding to vaccination. The promising results of Id vaccines from small single-arm studies was unfortunately not confirmed in randomised phase III studies. Out of three randomised clinical trials, only one showed prolonged DFS in a subgroup of vaccinated patients with FL. The significant differences in trial design and vaccine production are most likely responsible for the failures and conflicting results of the trials. However, the trials do provide several valuable insights with respect to clinical use of Id vaccine approaches.

What have we learnt from vaccination clinical trials?

- Factors correlating with improved OS after vaccination:
 - Achieving a durable complete response (CR)/CR unconfirmed after induction ChT
 - Induction of specific anti-Id antibody via vaccination
- Clinical setting, in which the vaccine is likely to be most effective:
 - Vaccination as a consolidation therapy to eradicate residual lymphoma cells after induction therapy
- Type of lymphoma:
 - Indolent B cell lymphomas respond well to the induction therapy

- Vaccination timing:
 - 2–6 months after completion of ChT in patients with sustained remission
- Tolerability and toxicity of vaccination:
 - Non-immunosuppressive, non-myelotoxic, well tolerated, mostly injection site reactions or flu-like symptoms
- Manufacturing procedures:
 - In the studies showing survival benefit, the vaccine was generated from hybridoma
- Possible differences in immunogenicity of Id vaccine according to the isotype of the Fc region:
 - Vaccination with IgM-Id, but not with IgG-Id, significantly prolonged DFS compared with matched controls

Vaccination in lymphoma should not be abandoned, but several open theoretical and practical questions need to be answered before a new generation of trials can be performed.

Immune Checkpoint Inhibitors

Like solid tumours, lymphoid malignancies have developed different mechanisms to evade the immune system, allowing lymphoma cells to escape immune surveillance. Cumulating data support the theory that functions of inhibitory molecules, such as programmed cell death protein 1 (PD-1)/programmed death-ligand 1 (PD-L1), cytotoxic T-lymphocyte antigen 4 (CTLA-4), lymphocyte-activation gene 3 (LAG-3) or T cell immunoglobulin and mucin domain 3 (TIM-3), are dysregulated in lymphoid malignancies.

The expression of PD-L1 varies considerably across different types of lymphoma, probably due to the diverse mechanisms responsible for the expression of PD-1 and co-inhibitory molecules. Moreover, while in solid tumours PD-L1 is highly expressed on cancer cells but minimally expressed in surrounding normal tissue, in several types of lymphoma a major component of PD-L1 expression is derived from tumour infiltrating

macrophages, histiocytes or stromal cells in the tumour microenvironment (TME). For example, FL cells do not express PD-L1, but histiocytes in the TME strongly express PD-L1. In T cell-rich, histiocyte-rich large B cell lymphoma (TCHRBCL), the predominant histiocytes adjacent to scattered malignant B cells show a very strong PD-L1 expression. Similarly, Reed-Sternberg (RS) cells comprise only a small proportion of the overall tumour cellularity and PD-L1-positive cells in classical Hodgkin lymphoma (cHL); the predominant tissue macrophages show a strong PD-L1 expression. This suggests that both tumour cells and background inflammatory cells in the TME can provide immune escape signals.

There are various mechanisms leading to PD-L1 overexpression in lymphomas. In principle PD-L1 expression can be induced and upregulated by extrinsic and/or intrinsic signals.

Extrinsic signals: INF- γ secreted by tumour infiltrating lymphocytes (TILs) leading to upregulation of PD-L1 in the TME. This mechanism may be responsible for overexpression of PD-L1 on tumour infiltrating macrophages in lymphomas with cytokine-rich inflammatory TME (cHL, TCHRBCL).

Intrinsic signals: So far, four mechanisms have been identified:

1. **Genetically-driven** PD-L1 and PD-L2 expression. Copy number alterations (amplifications or gains) and/or translocations involving *9p24.1*/PD-L1/PD-L2 are associated with PD-L1 overexpression in RS cells in cHL, primary mediastinal large B cell lymphoma (PMBCL), Epstein-Barr virus (EBV)-negative primary central nervous system lymphoma (PCNSL), primary testicular lymphoma (PTL) and in a subset of DLBCL. The amplification of *9p24.1* also induces JAK2 amplification, which further stimulates PD-L1 expression via the JAK/STAT pathway.
2. **EBV infection-driven** overexpression of PD-L1 via the JAK/STAT pathway in cHL, EBV-positive (EBV⁺) DLBCL, EBV⁺ immunodeficiency-related DLBCL, EBV⁺ post-transplant lymphoproliferative disorder, plasmablastic lymphoma, primary effusion lymphoma and extranodal NK/T cell lymphoma. Chronic viral infections are known to be able to abuse the PD-1/PD-L1 pathway to induce immune tolerance (EBV, hepatitis B, C or human immunodeficiency virus [HIV]).

3. **PD-L1 3'-untranslated region disruption**-induced PD-L1 expression
4. **Dysregulated JAK/STAT signalling pathway** is responsible for PD-L1 expression in activated B cell-like (ABC) DLBCL, which more commonly expresses PD-L1 compared with germinal centre B cell-like (GCB) DLBCL and in anaplastic lymphoma kinase (ALK)-positive anaplastic large cell lymphoma (ALCL).

Results from clinical trials demonstrated that PD-1 blockade is an attractive way to reconstitute the host's immune function in lymphomas. The responses to PD-1 blockade vary significantly between different types of lymphoma, probably due to the various mechanisms responsible for the expression of PD-1, which highlights the striking biological differences between different lymphomas. The most promising results have been reported in patients with relapsed/refractory (R/R) cHL. Based on the positive results from two studies, cHL is the first haematological malignancy in which an anti-PD-1 antibody, nivolumab, has been approved as a salvage therapy in patients with failure after prior autologous stem cell transplantation (ASCT) and brentuximab vedotin (BV). Less striking responses have been observed in patients with DLBCL, FL and some T cell NHL (T-NHL). Monotherapy with anti-PD-1 inhibitors was disappointing in patients with chronic lymphocytic leukaemia (CLL), highlighting the need for combinations with other synergistic drugs (Table 1).

It is of great importance to explore biomarkers able to predict responders to anti-PD-1/PD-L1 inhibitors. Methods such as immunohistochemistry for PD-L1/PD-L2 protein tissue expression, chromosome analysis or fluorescent *in situ* hybridisation (FISH) to detect aberrations on *9p24.1*/PD-L1/PD-L2 locus or reverse transcription polymerase chain reaction (RT-PCR) to detect gene rearrangements involving PD-L1 and PD-L2 could help to discover the best biomarker.

Open questions about ICIs in lymphoma:

- How to identify patients who are candidates for the treatment (biomarkers)?
- What is the best agent to combine with anti-PD-1/PD-L1 in individual types of lymphoma?

- Optimal timing of PD-1 blockade when combined with ChT – prior to, concomitantly or after the cytotoxic treatment?
- Long-term toxicity? (when used earlier with curative intention)

Table 1 Checkpoint Clinical Efficacy – Results from Selected Clinical Trials

Study	Condition	ORR	CR	SD	PFS
Nivolumab (CheckMate 039) Phase I	23 R/R cHL 31 R/R B-NHL 11 DLBCL 10 FL 23 R/RT-NHL	87% 26% 36% 40% 17%	17% 10% 18% 10% 0%	13% 52% 27% 60% 43%	86% (6 months)
Nivolumab (CheckMate 205) Phase II	143 R/R cHL (arms A, B)	68%	8%–22%	NA	77% (6 months)
Pembrolizumab (KEYNOTE-013) Phase I	31 R/R cHL 19 R/R PMBCL	65% 41%	16% 13%	23% 35%	64% (6 months)
Pembrolizumab (KEYNOTE-087) Phase II	210 R/R cHL (arms A, B, C)	65%–74%	22%–29%	12%–17%	NA
Nivolumab + BV Phase I/II	6 R/R cHL	100%	50%	NA	NA
Nivolumab + ipilimumab + BV Phase I	8 R/R cHL	100%	62%	NA	NA
Nivolumab + ibrutinib Phase II	12 R/R or high-risk CLL	66%	0%	NA	NA
Pidilizumab + rituximab Phase II	29 R/R FL	66%	52%	NA	18.8 months (median)
Atezolizumab + obinutuzumab Phase I	23 R/R FL 20 R/R DLBCL	56% 12%	26% 6%		10.2 months

Abbreviations: BV, brentuximab vedotin; B-NHL, B cell non-Hodgkin lymphoma; cHL, classical Hodgkin lymphoma; CLL, chronic lymphocytic leukaemia; CR, complete remission; DLBCL, diffuse large B cell lymphoma; FL, follicular lymphoma; NA, not available; ORR, objective response rate; PFS, progression-free survival; PMBCL, primary mediastinal large B cell lymphoma; R/R, relapsed/refractory; SD, stable disease; T-NHL, T cell non-Hodgkin lymphoma.

Chimeric Antigen Receptor-T cell Therapy

The adoptive transfer of chimeric antigen receptor (CAR)-T cells demonstrated remarkable success in patients with B cell malignancies, most notably in patients with R/R B cell precursor acute lymphoblastic leukaemia (B-ALL), with up to 90% complete remission rate using CD19 CAR-T cells. Positive results are also increasingly described in patients with R/R B-NHL and CLL.

CAR-T cell therapy constitutes a unique personalised autologous ‘living cell therapy’ capable of expanding and persisting during the life of the patient.

CD19 CAR-T cells (Figure 1) are directed against the surface protein CD19, for which expression is restricted to B cells and B cell precursors. Its consistent expression on the surface of most B cell malignancies and missing expression on pluripotent stem cells is of great advantage. CD19 (like CD20) is an example of an ideal tumour-associated antigen (TAA) – highly and homogeneously expressed on tumour cells, but not (or only weakly) expressed on vital normal tissues. Significant antitumour efficacy of CD19 CAR-T cells in B cell malignancies can serve as a proof of principle for adoptive T cell therapy (Table 2).

Table 2 CD19 CAR-T Cells Clinical Efficacy – Results from Selected Clinical Trials

Study	Condition	ORR	CR	Comments	Neurotoxicity grade ≥3	CRS grade ≥3
AXI-CEL; KTE-C19 ZUMA-1 Phase II	101 R/R aggressive B-NHL	82% 41% at 6 months	54% 36% at 6 months	<ul style="list-style-type: none"> Median DOR 8.2 months, not reached for patients in CR Median OS not reached 	28%	13% 43% received tocilizumab, 27% received steroids
CTL019JULIET Phase II	141 R/R DLBCL enrolled, 85 infused	59% 45% at 3 months	43% 37% at 3 months	Median DOR not reached	13%	26% 16% received tocilizumab, 11% received steroids

Abbreviations: B-NHL, B cell non-Hodgkin lymphoma; CAR, chimeric antigen receptor; CR, complete remission; CRS, cytokine-release syndrome; DLBCL, diffuse large B cell lymphoma; DOR, duration of response; ORR, objective response rate; OS, overall survival; R/R, relapsed/refractory.

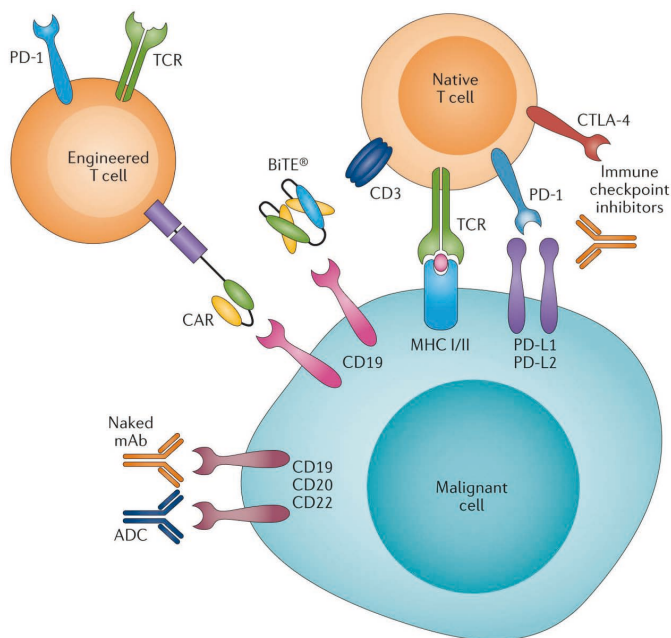
The encouraging results with CD19-directed CAR-T cells led to investigation of CAR-T cells directed to other haematological antigens such as CD20, CD22, CD30 and CD5.

Factors favouring the efficacy of CAR-T cells in haematological malignancies compared with solid tumours include:

- Fewer ‘physical barriers’ to efficiently infiltrate the tumour
- Fewer immunosuppressive factors and barriers preventing CAR-T cells to reach tumour cells

Figure 1 Mechanisms of action of immunotherapy modalities. Native T cells can recognise tumour-specific antigens in an MHC-dependent manner. The T cells also require co-stimulation for activation. Upon antigen recognition, without co-stimulatory signal, or with the stimulation of inhibitory molecules, such as through the PD-1/ PD-L1 axis, the T cells can be induced to anergy or become exhausted. Immune checkpoint inhibitors can block the inhibitory signal of T cells to avert T cells from anergy. BiTE® antibodies bring T cells and malignant cells into close proximity through dual antigen binding, and can induce T cell activation without co-stimulatory signals. T cells can also be engineered to express CARs to recognise cell-surface molecules independent of MHC. Later-generation CARs have both TCR and co-stimulatory signalling components, thereby activating the T cells without additional co-stimulatory signal.

From Batlevi CL, Matsuki E, Brentjens RJ, Younes A. Novel immunotherapies in lymphoid malignancies. *Nat Rev Clin Oncol* 2016; 13:25-40. Reprinted with permission of Massachusetts Medical Society. Copyright © 2016.



Abbreviations: ADC, antibody–drug conjugate; BiTE®, bispecific T cell engager antibody; CAR, chimeric antigen receptor; CTLA-4, cytotoxic T-lymphocyte antigen 4; mAb, monoclonal antibody; MHC, major histocompatibility complex; PD-1, programmed cell death protein 1; PD-L1, programmed cell death-ligand 1; TCR, T cell receptor.

- Homogeneous expression of target antigen within the tumour, even in disseminated disease
- Manageable and not life-threatening on-target, off-tumour toxicity

The major, potentially fatal toxicities of CAR-T cell treatment are a severe ‘cytokine storm’ associated with the rapid T cell proliferation and severe neurotoxicity. After the infusion, the CAR-T cells interact with tumour cells and the patient’s immune system, resulting in a widespread, toxic release of pro-inflammatory cytokines, thus leading to tumour lysis syndrome (TLS) and cytokine-release syndrome (CRS). It has been shown that the severity of TLS and CRS correlated with the tumour burden before CAR-T cell infusion. The pathogenesis of neurological toxicity remains unclear; however, immunological mechanisms are assumed. Symptoms, which were mostly self-limiting and reversible, lasting 2–3 days, included aphasia, confusion, delirium, somnolence or even seizures. A specific long term on-target, off-tumour toxicity induced by CD19 CAR-T cells is B cell aplasia, leading to severe hypogammaglobulinaemia. Sustained B cell aplasia is a marker of continued CD19 CAR-T cell persistence and is observed in long-term responders. In all trials, the anti-tumour effect correlated with the persistence and proliferation of CAR-T cells in the peripheral blood of patients.

Tumour antigen escape, resulting in CD19-negative relapse, has emerged as a major challenge for long-term disease control after CD19 CAR-T cell therapy. This phenomenon was most commonly observed in approximately 14% of B-ALL responders and may be overcome by dual-targeted CAR-T cells.

Bispecific Antibodies

Conventional mAbs do not directly recruit T cells into the tumour. In contrast, synthetic bispecific antibodies are designed to recruit polyclonal T cells into the tumour and harness their ability to kill tumour cells. Blinatumomab is a first-in-class T cell-engaging bispecific antibody, known as a bispecific T cell engager. It has two linked single-chain variable segments, one targeting CD3 on T cells and the other directed against CD19 (Figure 1). This bispecific CD19/CD3 construct is designed to

redirect previously unstimulated polyclonal T cells toward malignant CD19-expressing cells and to induce their destruction.

Blinatumomab demonstrated impressive single-agent activity in R/R B-ALL, for which it was approved. Current data suggest that the responses are generally short-lived and should ideally be followed by allogeneic transplantation. Phase I and II trials with blinatumomab in patients with R/R B-NHL have demonstrated activity. The objective response rate (ORR) across all subtypes was 69% with 37% complete remissions. The highest activity was observed in patients with FL with ORR 80% and 40% complete remissions. In addition to haematological toxicity, two-thirds of patients suffered from neurological side effects similar to CAR-T cell-associated neurotoxicity. However, patients with R/R DLBCL have poor outcome, and in responding patients blinatumomab may represent a bridging therapy to a potentially curative autologous or allogeneic transplantation.

In the future, combination with immunomodulatory drugs may theoretically increase the T cell activation and proliferation induced by blinatumomab. Combination with PD-1/PD-L1 inhibitors may be the way to restore the function of antigen-exhausted TILs and increase the blinatumomab-induced mobilisation and activation of T cells.

Declaration of Interest:

Dr Ballova has reported no potential conflicts of interest.

Professor Ghielmini has reported receiving honoraria from Roche, Celgene, Mundipharma, Janssen, Abbvie, Bayer and Gilead.

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2.8 Gynaecological Malignancies

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The Role of Immunotherapy in Ovarian Cancer

There are approximately 65 000 new cases of ovarian cancer per year in Europe, with 43 000 deaths, making ovarian cancer the fourth highest cause of cancer death in women. The majority of patients are diagnosed at advanced stages. In this setting, there is a great unmet need as the 5-year survival rate for women diagnosed with stage III and IV disease remains 25% despite treatment strategies that involve both surgery and chemotherapy (ChT). Immunotherapy therefore offers a novel potential treatment strategy, either alone or in combination with other systemic anti-cancer therapy.

The Immune Microenvironment in Ovarian Cancer

The importance of an anti-tumour immune response in epithelial ovarian cancer (EOC) was highlighted when Zhang et al (2003) identified that the presence of tumour infiltrating lymphocytes (TILs) was associated with a significant improvement in progression-free survival (PFS) and overall survival (OS). Here, CD3⁺ TILs were identified within tumour islets in 54.8% of ovarian cancer specimens, and their presence was associated with an increase in the 5-year survival rate from 4.5% to 38.0%. However, despite the survival advantage associated with TILs, their role within the tumour microenvironment remains a subject of investigation. While tumour infiltrating CD8⁺ lymphocytes have emerged as an independ-

ent prognostic factor in EOC, with their presence being associated with increased OS and PFS, lymphocytes located in the stroma do not appear to impact survival. Clinical outcome is also associated with the ratio of effector T cells (T_{eff}) to regulatory T cells (T_{regs}) within EOC tumours, and the presence and composition of immune infiltrates vary between histological subtypes. Immune infiltrates are most commonly seen in high-grade serous ovarian cancer versus other EOC histological subtypes (e.g. endometrioid, clear cell), and the infiltrates seen in the former are more likely to be associated with a favourable prognosis. It has therefore been proposed that high-grade serous ovarian cancer has a more accessible immunophenotype when compared with the other subtypes of EOC.

As in other tumour types, programmed death-ligand 1 (PD-L1) expression on tumour cells has been investigated as both a prognostic and predictive biomarker in ovarian cancer. Levels of expression vary between EOC histological subtypes, with high levels most commonly being found in high-grade serous ovarian cancer. PD-L1 appears to be expressed predominantly on tumour-associated macrophages rather than on the tumour cells themselves, although the exact role that these cells play is unclear. High levels of PD-L1 expression are associated with a less favourable prognosis, with a median OS of 6.48 years (± 0.62) versus 9.56 years (± 0.82) for tumours exhibiting low levels of expression. This association is independent of other poor prognostic factors such as distant metastases and residual tumour after surgery. Furthermore, PD-L1 levels are inversely correlated with the presence of tumour infiltrating CD8⁺ T lymphocytes, suggesting that aberrant signalling through this pathway may dampen the anti-tumour immune response in ovarian cancer. PD-L1 blockade in murine models of ovarian cancer also appears to inhibit the growth of tumour xenografts, further raising the prospect that anti-programmed cell death protein 1 (PD-1)/PD-L1 therapies may offer a potential treatment strategy in ovarian cancer.

Immunotherapy Trials

Anti-PD-1-directed therapy

Both nivolumab and pembrolizumab are monoclonal antibodies (mAbs) targeted against PD-1. The activity of nivolumab in ovarian cancer was

tested in a phase II trial of 20 Japanese women with platinum-resistant disease. Patients were randomised to receive nivolumab at a dose of 1 mg/kg or 3 mg/kg every 2 weeks for 6 cycles (4 doses per cycle) or until progression. The best overall response observed was 15% with a median PFS of 3 months (95% confidence interval [CI]: 0.7–3.9) and median OS of 20 months (95% CI: 7.0–not reached). Two patients within the 3 mg/kg group experienced a durable complete radiological response, one of whom had clear cell carcinoma of the ovary.

KEYNOTE-028 (NCT02054806) was a non-randomised, phase Ib trial of pembrolizumab in advanced solid tumours. The ovarian cancer cohort consisted of 26 patients who had progressed following prior treatment, with either PD-L1 expression >1% in the tumour cells or PD-L1-positive (PD-L1⁺) staining in the stroma. Patients received pembrolizumab 10 mg/kg every 2 weeks for 2 years or until disease progression. The objective response rate (ORR) was 11.5%, with one patient experiencing a complete response and two further patients a partial response (PR).

Microsatellite instability-high (MSI-H) has been reported in approximately 10% of unselected EOC cases, but, as with many other ‘Lynch syndrome’ malignancies, most of these present at an early stage and are cured by surgery. So, although pembrolizumab received Food and Drug Administration (FDA) approval for the treatment of MSI-H tumours that have progressed following prior treatment with no satisfactory alternative treatment options, it is unlikely that many ovarian cancer patients will be able to access immunotherapy through this route.

Anti-cytotoxic T lymphocyte antigen 4-directed therapy

Ipilimumab is a mAb which targets cytotoxic T-lymphocyte antigen 4 (CTLA-4) and has been tested in a phase II trial of 40 women with platinum-sensitive ovarian cancer. Here, subjects received an induction phase consisting of ipilimumab 10 mg/kg every 3 weeks for 4 doses, followed by a maintenance phase of ipilimumab 10 mg/kg every 12 weeks. Treatment continued until toxicity or disease progression. Although an ORR of 10.3% was reported, grade ≥ 3 toxicity was experienced by 50% of participants, and only two patients completed the induction phase.

Since then, it has been demonstrated that lower doses have sufficient efficacy in other tumour types and are associated with less toxicity. Future planned studies at different doses will help define the role of anti-CTLA-4 therapies in ovarian cancer.

Anti-PD-L1-directed therapy

The mAbs avelumab and durvalumab bind PD-L1, thereby preventing its interaction with PD-1. Avelumab 10 mg/kg was administered every 2 weeks to 124 patients with refractory or recurrent ovarian cancer until progression, unacceptable toxicity or withdrawal of consent. A PR was observed in 12 patients and stable disease was seen in five, with an ORR of 9.7% (95% CI: 5.1–16.3) for the entire cohort. When patients were stratified using a threshold of $\geq 1\%$ PD-L1 expression, the ORR was 12.3% (95% CI: 5.1–23.7) in the PD-L1⁺ cohort and 5.9% (95% CI: 0.1–28.7) in the PD-L1-negative (PD-L1⁻) group.

In summary, the ORR seen in the nivolumab, pembrolizumab and avelumab trials reported in ovarian cancer are similar, ranging from 10% to 15%. Of the studies discussed, only the nivolumab and avelumab studies have reported results by histological subtypes. So far, durable responses have been noted in patients with clear cell histology, a subtype which has limited effective treatment options.

Future Developments for Immune Checkpoint Inhibitors

The combination of the immune checkpoint inhibitors (ICIs) ipilimumab and nivolumab has proven to be more effective compared with monotherapy in patients with untreated malignant melanoma, albeit with an increased incidence of grade 3/4 toxicity. The NRG oncology study GY003 (NCT02498600) is currently investigating the potential benefit of combining these two ICIs in patients with recurrent ovarian cancer, but using ipilimumab at the lowest dose of 1 mg/kg every 21 days, due to the high incidence of \geq grade 3 toxicity observed when higher doses were used.

ICIs have also entered clinical trials in combination with ChT. For example, JAVELIN Ovarian 200 (NCT02580058) is a randomised, phase III clinical trial that evaluates the activity of the addition of avelumab to pegylated

liposomal doxorubicin in platinum-resistant EOC. Pembrolizumab in combination with weekly paclitaxel is being explored in a phase II study of recurrent EOC (NCT02440425). The ATALANTE study (NCT02891824) is a randomised placebo-controlled phase III trial that investigates the potential benefit of adding the PD-L1 inhibitor atezolizumab to carboplatin and bevacizumab in the context of platinum-sensitive relapse. ICIs have also reached first-line ovarian cancer trials in combination with carboplatin and paclitaxel as well as maintenance therapy, e.g. avelumab (JAVELIN Ovarian 100, NCT02718417) and pembrolizumab (NCT02520154).

The principal rationale for combining targeted agents with ICIs includes enhanced neoantigen presentation following targeted treatment. This has the potential to augment the anti-tumour response. Studies include durvalumab in combination with the vascular endothelial growth factor (VEGF) inhibitor cediranib and the poly(adenosine diphosphate ribose) polymerase (PARP) inhibitor olaparib (NCT02734004), and nivolumab ± ipilimumab with rucaparib versus rucaparib alone for patients whose tumours are loss of heterozygosity-high (CeNuRION, ISRCTN10490346). Several phase III trials of ICIs combined with PARP inhibitors and bevacizumab in the first-line treatment of ovarian cancer are also planned.

Beyond ICIs

While most existing trial data regarding the role of immunotherapy in the treatment of ovarian cancer pertain to ICIs, other strategies have also been tested, including vaccines and the adoptive transfer of T cells expressing chimeric antigen receptors (CARs). Both abagovomab and oregovomab are cancer vaccines. Abagovomab consists of a variable epitope which resembles cancer antigen 125 (CA125) and oregovomab is a murine mAb with an affinity for CA125. Both have been the subject of randomised placebo-controlled phase III trials as maintenance therapy following primary ChT; abagovomab was not associated with improved relapse-free survival (RFS) or OS, and oregovomab did not increase time to relapse (TTR). The results of the TRIOC (NCT01556841) trial, a placebo-controlled trial of TroVax, a vaccine directed against the 5T4 tumour-associated antigen, in women with asymptomatic

CA125 relapsed ovarian cancer, are awaited. CAR therapy is a promising treatment modality in multiple tumour types and is currently at an early stage of development in ovarian cancer. Early phase trials of this approach include targeting NY-ESO-1 (NCT01567891) and mesothelin (NCT02159716; NCT01583686).

The Role of Immunotherapy in Cervical Cancer

Squamous cell carcinoma of the cervix (SCCC) is the second most commonly diagnosed cancer among women, with more than 58 000 new cases per year, and the third leading cause of cancer death in less developed countries. Worldwide spread of Pap (Papanicolaou) smear screening has increased the diagnosis rate of precancerous lesions and early-stage tumours, which has dramatically reduced the incidence of advanced cervical cancer presentations in western countries, where there has been significant uptake among the population. It is hoped that the introduction of specific vaccines will decrease the incidence of any human papillomavirus (HPV) 16/18-associated cervical cancers in the future. It is, however, important to understand that the HPV vaccines do not cover all strains of HPV, just the current most common.

Immune Response and SCCC

It is estimated that 75%–80% of women are infected by HPV during their lifetime, but most women respond with an appropriate immune response. However, a process known as immuno-editing allows HPV-infected cells to overcome immune-surveillance in some women, permitting selection of cancer clones with increased resistance to immune detection and elimination and resulting in tumour growth. In SCCC, immunotherapy could play an important role, since restoring the immune response against cancer cells could stop tumour growth and inhibit progression of precancerous alterations and initial tumours.

The activation of the PD-1/PD-L1 pathway is possibly involved in SCCC and other HPV-related squamous cell cancers. Many authors have demonstrated expression of PD-1/PD-L1 in about 54%–67% of SCCC, which is correlated with progression of precancerous lesions to invasive cancer, tumour grade and prognosis.

Vaccines

The existing vaccines against HPV cannot eliminate pre-existing HPV infections, but are effective in preventing infection by types 16, 18, 6 and 11. There is also a suggestion that they can be used to boost existing weak immune responses. There is a strong interest in discovering further therapeutic vaccines with the aim of stimulating the immune system, eliminating infections and stopping transformation processes; they consist of viral vectors, but also peptides, nucleic acids or dendritic cells (DCs).

ADXS11-001 is a biologically engineered, live-attenuated *Listeria monocytogenes* (LM) vaccine. These vectors are internalised by antigen-presenting cells, and once inside they escape the lysosome, releasing in the cytosol a highly immunogenic fusion protein, which promotes the differentiation of cytotoxic T-lymphocytes and tumour infiltration.

GOG/NRG-0265 is an ongoing study in patients with metastatic or locally advanced HPV-related SCCC. Twenty-six patients were treated with three doses of ADXS11-001, 1×10^9 colony forming units (CFUs), monthly: 91% of patients reported grade 1–2 toxicities (nausea, vomiting, chills, fatigue and fever). Mean PFS was 3.1 months, mean survival was 7.7 months and 12-month survival was 38.5%. An Indian phase II trial, involving 110 patients with recurrent or persistent SCCC randomised to receive three or four doses of ADXS11-001 with cisplatin-based ChT, showed a response rate of 11% and a disease control rate of 41%, with a manageable safety profile. A phase III study, testing ADXS11-001 plus chemoradiotherapy (CRT) in locally advanced high-risk SCCC, is currently ongoing (NCT02853604).

The results of a trial which included late-stage HPV16⁺ SCCC was presented at the American Society of Clinical Oncology (ASCO) 2017 meeting. Three HPV16 vaccine doses were given 2 weeks after the second, third and fourth cycle of ChT. Robust T cell responses were observed and remained sustained until at least 30 days after the sixth cycle. A significant positive correlation was observed between the strength of the vaccine-induced immune response and OS (NCT02128126).

Anti-PD-1/PD-L1-directed Therapy

The KEYNOTE-028 SCCC cohort demonstrated a 6-month PFS rate of 13%, 6-month OS rate of 66.7% and ORR of 12.5% in 24 advanced SCCC patients treated with pembrolizumab 10 mg/kg every 2 weeks. No grade 4–5 adverse events (AEs) occurred and only two patients discontinued pembrolizumab due to a grade 3 AE (colitis and Guillain-Barré syndrome).

Preliminary results of the phase II study KEYNOTE-158 were presented at ASCO 2017. Patients with recurrent/advanced SCCC were treated with pembrolizumab at a flat dose of 200 mg every 3 weeks. Among the first 47 patients, ORR was 17%. Among the 15 patients who had ≥ 27 weeks of follow-up, ORR was 27%.

A randomised phase II open-label multicentre study, evaluating standard CRT plus pembrolizumab, is ongoing. Primary outcomes are safety and efficacy (NCT02635360).

Hollebecque et al presented the results of a phase I/II study evaluating nivolumab 240 mg every 2 weeks in HPV-related squamous vulvar, vaginal and cervical tumours at ASCO 2017. SCCC patients experienced a good toxicity profile and demonstrated promising clinical activity (ORR: 26.3%; PFS: 5.5 months). Nivolumab is currently under investigation in a phase II trial involving metastatic, recurrent or persistent SCCC patients (NCT02257528) in combination with lirilumab (anti-killer cell immunoglobulin-like receptor [KIR] antibody) and ipilimumab (anti-CTLA-4 antibody) in patients with advanced solid tumours (NCT01714739).

Anti-CTLA-4-directed Therapy

Mayadev et al reported the safety, tolerability and efficacy of a Gynecologic Oncology Group (GOG) phase I study evaluating escalating ipilimumab doses ranging from 3 to 10 mg/kg in combination with definitive CRT in node-positive SCCC at ASCO 2017. The data suggest that immunotherapy has potential activity (the secondary endpoint of 1-year disease-free survival [DFS] was 74%) and a good toxicity profile: the most common AEs were grade 1–2 diarrhoea, rash and endocrinopathies. The incidence of acute grade 3 toxicity was 16%, which resolved without

consequences. With a median follow-up of 12 months, there were no major late toxicities reported (NCT01693783). A phase II study aiming to evaluate the safety and efficacy of single-agent ipilimumab in patients with metastatic or recurrent SCCC is ongoing. At present no studies evaluating ipilimumab in combination with other therapies are ongoing.

Adoptive TILs Therapy

In 2015, Stevanović et al published their experience in nine metastatic patients with SCCC, previously treated with platinum-based ChT and CRT, and reported a 33% ORR after a single infusion of TILs and interleukin-2 (IL-2).

The Role of Immunotherapy in Endometrial Cancer

Endometrial cancer (EC) is the most common gynaecological cancer in developed countries. In Europe, the number of new cases was around 100 000 in 2012. The immunological landscape in endometrial tissue is characterised by a balance between the task of defending against infections and the need to welcome an allogeneic foetus. Sex hormones directly guide this state with cyclic modifications: macrophages, neutrophils and natural killer (NK) cells are numerous during menstruation, while B and T cells increase during the follicular phase and lose their cytotoxicity during the luteal phase to facilitate implantation.

Immune Microenvironment and EC

Data regarding the prognostic value of TILs are controversial: some authors showed that elevated tumour infiltration is associated with low-grade endometrial lesions, while others support the opposite. A study focusing on the ratio of $CD8^+ T_{eff} / CD4^+ T_{reg}$ cells confirmed that a high ratio is associated with more favourable outcomes in type I ECs.

Data on tumour-associated macrophages are more consistent: their presence was associated with aggressive features (angiogenic profile, lymphovascular and myometrial invasion and node metastasis) in all reported studies.

The expression of PD-L1 in EC has been estimated between 67% and

100% on tumour cells. Recently, a new molecular classification of EC identified four genomic groups:

1. *Polymerase E (POLE)* ultramutated
2. MSI hypermutated
3. Copy number low
4. Copy number high

Howitt et al demonstrated that ultramutated *POLE* and hypermutated MSI are characterised by high levels of neoantigens and TILs, which is counterbalanced by over-expression of PD-1/PD-L1. These subgroups are potential candidates for immunotherapy.

Anti-PD-1/PD-L1-directed Therapy

In the KEYNOTE-028 study, 24 locally advanced or metastatic EC patients progressing after platinum-based ChT received pembrolizumab 10 mg/kg every 2 weeks. The majority of patients had endometrioid adenocarcinoma (n=17; 70.8%), while high-grade serous carcinoma, carcino-sarcoma and mixed other histotypes were 12.5%, 8.3% and 4.2%, respectively. Nineteen patients had tumours evaluable for MSI status; of these, one patient (5.3%) had MSI-high status and 18 patients (94.7%) had non-MSI-high status. The single patient classified as MSI-high had progressive disease as best response and, among the patients achieving PRs, one patient had non-MSI-high status but was found to present a *POLE* mutation.

After 69.9 weeks of median follow-up, ORR was 13%, and 6-month PFS and OS rates were 19.9% and 68.8%, respectively. The authors concluded that pembrolizumab had a good preliminary tumour activity and an acceptable toxicity profile: AEs occurred in 54.2% of patients (pruritus, asthenia, fatigue, pyrexia and decreased appetite); only three patients experienced grade 3 AEs and no patients died or discontinued pembrolizumab due to an AE.

At ASCO 2017, Makker et al presented the results of a multicentre phase Ib-II trial: 23 patients with metastatic EC were enrolled and received oral lenvatinib (a multi-targeted tyrosine kinase inhibitor) 20 mg/day plus

pembrolizumab 200 mg intravenously every 3 weeks. The reported ORR was 48% and toxicities were generally manageable, with no grade 3–5 toxicities reported. The most common AEs were hypertension, fatigue, arthralgia, diarrhoea and nausea. The results of a phase II study with pembrolizumab in tumours with mismatch repair deficiency (dMMR) have recently been reported. In the cohort of nine patients with solid tumours other than colorectal cancer (two were EC), the ORR and the PFS rate were 71% and 67%, respectively.

Currently, both pembrolizumab and avelumab are under investigation in patients with EC:

- In the metastatic setting, given in combination with carboplatin-paclitaxel (NCT02549209; EudraCT: 2016–004403–31)
- In the first-line treatment of patients with potentially resectable advanced disease (NCT02630823)
- In recurrent/persistent disease in selected EC populations (*POLE* mutation/hypermutation or dMMR) (NCT02899793; NCT02912572)

A multicentre phase II trial is ongoing in advanced/recurrent EC with single-agent durvalumab 1500 mg monthly (ACTRN12617000106336).

At ASCO 2017, Fleming et al reported the results of a trial with atezolizumab in advanced/recurrent EC: the ORR was 13% and the safety profile was acceptable; clinical benefit appeared to be related to higher PD-L1 expression. Atezolizumab is currently being tested in an ongoing phase Ib study with advanced tumours, including EC, in combination with carboplatin–cyclophosphamide (NCT02914470).

Finally, a phase I trial evaluating the combination of nivolumab with ipilimumab (anti-CTLA-4) in different cohorts of rare tumours, including EC, is ongoing (NCT02834013).

Declaration of Interest:

Dr Stewart has reported no potential conflicts of interest.

Dr Banerjee has reported no potential conflicts of interest; she has participated as an investigator in immunotherapy trials (Merck Sharp & Dohme, Roche, Tesaro and Pfizer).

Dr Lorusso has reported no potential conflicts of interest.
Dr Tripodi has reported no potential conflicts of interest.

Further Reading

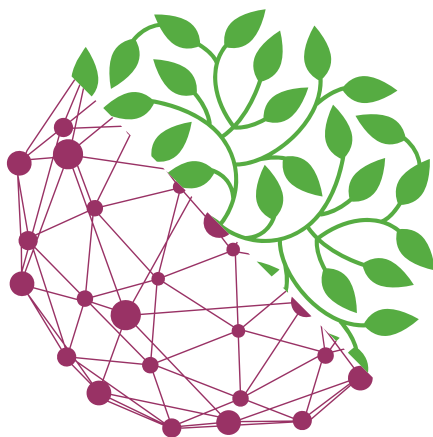
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Section 3:

Implications for clinical practice

3



3.1 Management of Adverse Events Related to Immune Checkpoint Blockade

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Definition

The blockade of cytotoxic T-lymphocyte antigen 4 (CTLA-4) and programmed cell death protein 1 (PD-1)/programmed death-ligand 1 (PD-L1) is associated with a unique spectrum of immune-related adverse events (irAEs) related to T cell activation (Haanen et al, 2017). CTLA-4 and PD-1 regulate immune responses at different levels (Boutros et al, 2016). Their blockade may affect any organ or tissue, leading sometimes to severe, and even fatal, issues. The focus of this review is to describe the adverse event (AE) profile of immune checkpoint inhibitors (ICIs) based on their mechanisms of action, and to provide guidelines for physicians to safely implement the use of these agents in routine practice.

Processes Involved

CTLA-4 Pathway

CTLA-4 is expressed on T cells, both on CD4 and CD8 effector T cells, as well as on CD4 regulatory T cells (T_{regs}). It regulates T cell activation at

early stages in the lymph nodes. There is accumulating evidence that the currently available anti-CTLA-4 antibodies also function by depleting T_{regs} from the tumour microenvironment (TME). CTLA-4 plays a key role in the development of immune tolerance at early stages. Blockade of CTLA-4 using the antibody ipilimumab results in a large and non-specific activation of the immune system, leading to a wide spectrum of irAEs. IrAEs may occur weeks or even months after the initiation of ipilimumab.

PD-1 Pathway

PD-1 regulates T cell activity in the peripheral tissues and the TME (Boutros et al, 2016). The blockade of PD-1 in the TME and the tissues, using pembrolizumab or nivolumab, induces a spectrum of irAEs which is somewhat distinct from that observed with CTLA-4 blocking, and with a lower rate of grade 3–4 irAEs (around 15% versus 25%).

Global Management

Education and communication are of paramount importance to successfully manage patients treated with ICIs, because this new treatment approach has a mechanism of action different from that of chemotherapies or targeted therapies. Referral to an expert and/or specialist (gastroenterologist, hepatologist, endocrinologist, neurologist or dermatologist) for management of specific AEs should also be considered in collaboration with the treating physician.

There are several published algorithms on the treatment of irAEs (Haanen et al, 2017). Differential diagnoses such as infection should be ruled out, particularly in patients with enterocolitis, hepatitis and pneumonitis. Grade 1 irAEs do not usually require ICI interruption, and symptomatic treatment should be prescribed. For grade 2 AEs, the treatment is withheld until symptoms return to grade 0 or 1, and oral systemic therapy is required. If grade 2 AEs persist, or for grade 3 irAEs, high-dose (intravenous) steroids are prescribed and, in most cases, the treatment with ICIs is permanently stopped. If irAEs are refractory to high-dose steroids, alternative immune-suppressive treatments should be used. These vary according to the target organ and the hypothesised underlying mechanisms: anti-tumour necrosis factor (TNF) for severe enterocolitis, mycophenolate mofetil for severe

hepatitis and intravenous immunoglobulin for antibody-mediated autoimmune diseases. In life-threatening conditions, anti-thymocyte globulin may be used; however, there are no evidence-based data in this setting and, therefore, no standardised management.

Clinical Results

IrAEs attributed to CTLA-4 and/or PD-1 blockade may affect various organs or tissues, with higher prevalence for the skin, gastrointestinal tract and endocrine system.

IrAEs attributed to CTLA-4 blockade occur in 60%–85% of patients treated with ipilimumab 3 mg/kg (Haanen et al, 2017; Boutros et al, 2016), being of grade 3–4 in 23% of patients. Treatment-related deaths have been reported in approximately 2% of patients, due to perforation of the colon, liver failure, septic shock with multiorgan dysfunction and capillary leak syndrome. However, many of these deaths were reported in the early years of ipilimumab development, at a time when awareness and management of toxicity was less well established than it is now.

The anti-PD-1 antibodies appear to cause a distinct spectrum of irAEs. Pembrolizumab 10 mg/kg every 2 or 3 weeks was compared with ipilimumab 3 mg/kg in advanced melanoma in a phase III study (KEY-NOTE-006) (Robert et al, 2015). AEs of any grade occurred in 72%–79% of patients treated with pembrolizumab, and in 73% of patients treated with ipilimumab. The rates of AEs of grade 3–4 were lower with pembrolizumab (10%–13%) than with ipilimumab (20%). Permanent treatment discontinuation related to the study drug was lower with pembrolizumab (4%–7%) than with ipilimumab (9%).

Similarly, nivolumab showed a favourable safety profile. In a pooled analysis of four studies, 576 patients with advanced melanoma received nivolumab 3 mg/kg (Weber et al, 2017). Treatment-related AEs of any grade occurred in 71% of patients, among which 10% were grade 3–4. No drug-related death was reported.

The combination of anti-CTLA-4 and anti-PD-1 antibodies is associated with a higher incidence and a larger spectrum of AEs, due to the mecha-

nisms of action of these two drugs that act at different levels of immune activation and on distinct lymphocyte subtypes. Most of the AEs occurred within the induction phase (the first 3 months of treatment). In the phase III study CheckMate 067, the incidence of AEs was higher in the combination group compared with monotherapy (Wolchok et al, 2017). AEs of any grade occurred in 86%, 96% and 86% of patients in the nivolumab, nivolumab plus ipilimumab and ipilimumab groups, respectively. Grade 3 or 4 AEs were highest in the combination group (59% versus 21% and 28% with monotherapies). However, grade 3 or 4 AEs generally resolved with immuno-modulatory treatments (Wolchok et al, 2017).

Skin Toxicity

Skin irAEs occur in 43%–45% of patients treated with ipilimumab, and in 34% of patients treated with nivolumab or pembrolizumab (Haanen et al, 2017; Boutros et al, 2016; Hua et al, 2016). The most frequent skin irAEs are primarily of grade 1 or 2 and include pruritus, rash, erythema and vitiligo (Haanen et al, 2017; Boutros et al, 2016). Rash primarily affects the trunk and extremities and may be predominant around naevi, suggesting an inflammatory reaction against melanocytes (Boutros et al, 2016; Wolchok et al, 2017). Histologically, a perivascular lymphocytic (CD4⁺ and CD8⁺ T cells) and eosinophilic infiltrate extending into the superficial dermis and up into the epidermis may be observed (Hua et al, 2016). Skin irAEs are generally managed symptomatically with emollients, antihistamines and topical glucocorticosteroids. They generally do not require dose skipping or treatment discontinuation. Grade 3 rashes require dose skipping until improvement to grade 1 and treatment with oral prednisone 0.5 to 1 mg/kg/day. Vitiligo is more frequently described in patients with melanoma (where it seems to be associated with clinical responses) than with other cancers, suggesting that there is recognition of normal melanocyte-associated antigens when the immune system reacts against malignant melanocytes (Hua et al, 2016).

Rarely, life-threatening Stevens-Johnson syndrome and toxic epidermal necrolysis may occur with histologically severe leukocytoclastic vasculitis and necrosis of keratinocytes (Lacouture et al, 2014), requiring permanent treatment discontinuation, urgent hospitalisation in a derma-

tology unit and initiation of intravenous methylprednisolone 1–2 mg/kg/day. Tapering should be over no less than 4 weeks.

Rare skin and mucosa toxicities, generally of grade 1 or 2, have been documented with ICIs: alopecia, dry mouth and skin, eczema, lichenoid skin reactions, hair repigmentation, mucosal inflammation and photosensitivity (Boutros et al, 2016).

Gastrointestinal Toxicity

Diarrhoea and colitis are more frequent in patients treated with ipilimumab than in those receiving nivolumab or pembrolizumab. When combined, ipilimumab and nivolumab induce the highest incidence of toxicities (Boutros et al, 2016).

Enterocolitis due to ipilimumab

Diarrhoea occurs in 33% and colitis in 8%–22% of patients treated with ipilimumab (Haanen et al, 2017; Boutros et al, 2016). Diarrhoea is generally watery. Severe abdominal pain, vomiting, haematochezia, weight loss and fever are less frequent. Extra-intestinal manifestations including arthralgia, skin disorders, endocrine disorders, hepatitis, nephritis, pericarditis and pancreatitis are commonly associated. Bowel perforation is rare (Haanen et al, 2017; Boutros et al, 2016; Robert et al, 2015).

Lower gastrointestinal endoscopy shows erythema, mucosal friability, erosion or ulceration, primarily in the distal colon. The sigmoid colon and the rectum are frequently affected. Sigmoidoscopy is generally sufficient to make the diagnosis. Histological features include neutrophilic inflammation (46%), lymphocytic infiltration (15%) or both (38%) (Haanen et al, 2017; Boutros et al, 2016; Marthey et al, 2016). Neutrophilic inflammation is predominantly associated with cryptitis and crypt abscesses, whereas lymphocytic inflammation is characterised by increased CD8⁺ T lymphocytes within the crypt epithelium and elevated CD4⁺ lymphocytes in the lamina propria (Haanen et al, 2017; Boutros et al, 2016; Lacouture et al, 2014). Granulomas and chronic inflammation are rare. Endoscopic and/or microscopic inflammation of the oesophagus, stomach, duodenum and ileum may be associated.

Initial workup of diarrhoea in patients treated with ipilimumab includes serum electrolytes, urea, creatinine, C-reactive protein and stool search for enteropathogens and *Clostridium difficile* toxins. Grade 0 and 1 diarrhoea can be managed with antidiarrhoeal agents and oral hydration, but many progress to higher grade colitis. Grade 3, 4 and persistent or complicated (dehydration, fever, tachycardia or haematochezia) grade 2 diarrhoea require a sigmoidoscopy or colonoscopy. ICIs should be discontinued and oral or intravenous corticosteroids should be initiated at 1 mg/kg/day. Patients with grade 3 or 4 diarrhoea or colitis (≥ 7 stools per day) should be hospitalised and empirical treatment commenced. Intravenous hydration and systemic methylprednisolone 1–2 mg/kg/day should be prescribed. In patients with significant response to methylprednisolone, a switch to the oral form should be made within 3–5 days and tapering during the following 8–12 weeks is recommended. Patients with severe colitis who do not respond to 3–7 days of intravenous corticosteroids should be prescribed infliximab at a dose of 5 mg/kg of body weight.

Enterocolitis due to anti-PD-1 antibodies

Different patterns of gastrointestinal irAEs induced by anti-PD-1 antibodies have been reported: acute colitis similar to that induced by anti-CTLA-4 antibodies, microscopic colitis, upper gastrointestinal involvement and pseudo-obstruction (Collins et al, 2017). Most of these respond to corticosteroids.

Hepatotoxicity

Hepatitis occurs in 5%–10% (grade 3 in 1%–2%) of patients treated with ipilimumab, nivolumab and pembrolizumab and in 25%–30% (grade 3 in 15%) of patients treated with the combination of ipilimumab and nivolumab (Haanen et al, 2017; Boutros et al, 2016). Grade 3 hepatitis is more frequently associated with nivolumab or pembrolizumab (15%) than with ipilimumab monotherapy (1%–2%). Initial workup includes serum transaminases, bilirubin and prothrombin time. Viral hepatitis, liver metastasis, alcohol and other drug-specific toxic reactions should be eliminated.

Liver biopsy may be recommended in severe hepatitis to rule out other causes (Haanen et al, 2017). Histological features include lobular

hepatitis and, in some cases, sinusoidal histiocytosis and central vein endotheliitis (Haanen et al, 2017; Gupta et al, 2015; Kim et al, 2013); rarely, they include portal tract inflammation and cholangitis, similar to changes observed in non-alcoholic steatohepatitis (NASH).

For grade 1 hepatitis, treatment with ICIs may be continued. In the case of grade 2 hepatitis (as gauged by the elevation of transaminases or total bilirubin), ICIs should be withheld, and liver function tests monitored twice weekly. Oral high-dose prednisone at 1 mg/kg or methylprednisolone at 0.5 to 1 mg/kg/day should be initiated if transaminases and total bilirubin do not improve within 1–2 weeks. ICIs may be resumed after corticosteroid tapering, once serum transaminases and total bilirubin improve to grade 1 hepatitis. If transaminases and total bilirubin do not decrease despite initiation of corticosteroids, methylprednisolone at a dose of 2 mg/kg/day should be used and ICIs permanently discontinued. For grade 3 or 4 transaminase or total bilirubin elevation, ICIs should be permanently discontinued, and corticosteroids (methylprednisolone) initiated at 1–2 mg/kg/day. If transaminases and bilirubin do not decrease within 2–3 days after corticosteroid initiation, oral mycophenolate mofetil at 1000 mg twice daily should be considered as well as anti-thymocyte globulins (Chmiel et al, 2011). Patients should be referred to a hepatologist and liver biopsy considered in corticosteroid-refractory cases (Chmiel et al, 2011).

Endocrine Toxicity

ICIs may affect the endocrine system. Endocrine disorders do not generally require interruption or cessation of ICIs. Hypothyroidism and hyperthyroidism are more frequent with anti-PD-1 therapy, whereas hypophysitis is more frequent with anti-CTLA-4 treatment.

Hypophysitis

Ipilimumab induces endocrinopathies in 6%–8% of patients, primarily the pituitary gland (Haanen et al, 2017; Boutros et al, 2016). Hypophysitis occurs in 1%–6% of patients treated with ipilimumab and in 8% of patients treated with the combination of ipilimumab and nivolumab (Haanen et al, 2017; Boutros et al, 2016). It is very rare in patients

treated with nivolumab or pembrolizumab monotherapy. Hypophysitis may be subtle, but often manifests as headache, vertigo, nausea, diplopia, weakness or hypotension. Hypophysitis must be differentiated from brain metastases. When an immune-related endocrinopathy is suspected, a complete workup is necessary to determine pituitary, thyroid, adrenal and gonadal status. Brain magnetic resonance imaging (MRI) with pituitary images may be considered to exclude brain metastasis and potentially identify enlargement of the pituitary gland.

When hypophysitis is suspected, hormone replacement treatment (HRT) should be initiated without awaiting a confirmed diagnosis if there is no other apparent cause. Symptoms generally improve with adapted HRT, and ICIs may usually be continued or resumed in patients on stable doses of HRT. High-dose corticosteroids are not necessary, except if neurological problems or headaches are present. In that case, according to the 2017 European Society for Medical Oncology (ESMO) Clinical Practice Guidelines, an initial dose of systemic (methyl)prednisolone at 0.5–1 mg/kg should be administered, followed by tapering depending on resolution of symptoms over up to 4 weeks and long-term adapted HRT (Haanen et al, 2017).

Thyroid disorders

Thyroid disorders are more frequent with pembrolizumab and nivolumab or the combination of ipilimumab and nivolumab, whereas hypophysitis is more frequent with ipilimumab (Haanen et al, 2017; Boutros et al, 2016). Thyroid disorders occur in 1%–5% of patients treated with ipilimumab, 5%–10% of patients treated with pembrolizumab or nivolumab and 20% of patients treated with the combination of ipilimumab and nivolumab (Haanen et al, 2017; Boutros et al, 2016). Thyroid disorders related to anti-PD-1 antibodies present with either a transient thyrotoxicosis followed by hypothyroidism (or euthyroidism) or hypothyroidism without an initial thyrotoxicosis. Symptomatic thyrotoxicosis may require short-term treatment with beta-blockers and, in severe cases, steroids and anti-thyroid drugs (e.g. propylthiouracil). It is generally reversible, but hypothyroidism usually requires long-term HRT.

Adrenal insufficiency

Adrenal insufficiency results in low levels of cortisol (8am cortisol level below 275 nmol/L) and normal to high levels of adrenocorticotrophic hormone (ACTH) (Haanen et al, 2017; Boutros et al, 2016). With adrenal insufficiency, ICIs may be continued, but immediate HRT with oral hydrocortisone at 20–10–10 mg/day is required to avoid adrenal crisis. Symptoms of adrenal crisis include severe dehydration, electrolyte disturbance, hypotension or shock, and require urgent hospitalisation to initiate methylprednisolone intravenously. Sepsis or infection should be ruled out in adrenal crises.

Pneumonitis

Pneumonitis is the most common pulmonary irAE and occurs more frequently with nivolumab or pembrolizumab monotherapy or the combination of ipilimumab and nivolumab than with ipilimumab alone. Other lung conditions such as asthma-like, sarcoid-like reactions and myocarditis can also occur and should be excluded in a patient with dyspnoea. Lung complications are less frequent with ipilimumab alone (Haanen et al, 2017). Recently, pneumonitis has been better characterised in a large retrospective analysis of patients receiving anti-PD-1/PD-L1 monotherapy or in combination with anti-CTLA-4 therapy (Naidoo et al, 2017). Among the 915 patients treated, pneumonitis occurred in 4.6%. The incidence of pneumonitis was higher with the combination immunotherapy versus monotherapy (10% versus 3%). Seventy-two per cent of the pneumonitis cases were grade 1–2, and 86% improved with drug withholding and immunosuppression. When pneumonitis is suspected, a high-resolution computed tomography (HRCT) scan is required. Radiological features are heterogeneous. They include ground-glass opacities (in 10%), interstitial infiltrates (in 6%), cryptogenic organising pneumonia-like patterns (in 5%) and hypersensitivity (in 3%) (Kim et al, 2013). In 4% of cases, pneumonitis does not fit a specified subtype classification.

The management of pneumonitis depends on the severity of symptoms. In grade 1 pneumonitis (asymptomatic patients with radiological changes only), ICIs may be continued with close monitoring. In grade 2 pneumonitis (mild dyspnoea), treatment should be withheld and bron-

choscopy, bronchoalveolar lavage and biopsy considered if infection or disease progression are also suspected. Antibiotics should be initiated if an infection is suspected. Otherwise, oral prednisolone 1–2 mg/kg/day should be initiated with tapering over no less than 4 weeks when symptoms improve. ICIs may be resumed if symptoms improve to grade ≤ 1 within 12 weeks and prednisolone reduced to ≤ 10 mg/day. In a grade 3 or 4 pneumonitis (severe dyspnoea, hypoxia, acute respiratory distress symptoms), ICIs should be definitively discontinued and the patient hospitalised, with respiratory and intensive care specialists consulted. Bronchoscopy, bronchoalveolar lavage and biopsy should be considered and methylprednisolone 2–4 mg/kg/day immediately initiated. When symptoms improve to grade ≤ 1 , oral prednisolone may be initiated at 1–2 mg/kg/day with tapering over no less than 4 weeks. If symptoms do not improve within 48–72 hours after corticosteroid initiation, infliximab (5 mg/kg) or anti-thymocyte globulin should be administered.

Rare Immune-related Toxicities

Rarely (in $\leq 1\%$), unexpected irAEs may occur and require urgent consultation with a specialist to avoid a serious outcome (Haanen et al, 2017; Boutros et al, 2016). Several studies have reported neurological, cardiac, ocular and haematological toxicities.

Neuropathies

Neurological toxicities include myasthenia gravis, Guillain-Barré syndrome, meningo-radiculo-neuritis, granulomatous central nervous system inflammation and aseptic meningitis. In a retrospective analysis of 59 trials involving 9208 patients treated with ICIs, immune-related neuropathies occurred in 3.8%, 6.1% and 12% of patients treated with anti-CTLA-4, anti-PD-1 and combined anti-CTLA-4 and anti-PD-1 drugs, respectively (Haanen et al, 2017). Therefore, patients should be monitored for signs or symptoms of motor, sensory or autonomic neuropathy (Gu et al, 2017). In patients with grade 1 neurological symptoms, ICIs may be stopped or continued with very careful monitoring. Unless an alternative aetiology has been identified, signs and symptoms of neuropathy should be considered immune-mediated. In patients with

durable grade 1 or 2 symptoms (not interfering with daily activities), ICIs should be withheld and prednisolone at 0.5–1 mg/kg/day should be initiated. In grade 3–4 symptoms (interfering with daily activities or life-threatening), ICIs should be permanently discontinued and high-dose prednisolone at 1–2 mg/kg/day should be initiated, with a tapering over at least 30 days. Plasmapheresis, systemic immunoglobulin or other immunosuppressants (e.g. mycophenolate) may be required.

Cardiac toxicities

Rare cases of cardiac toxicities have been reported with ICIs. Recent cases of myocarditis and myositis have been reported with combined ICIs (Johnson et al, 2016). Among 20 594 patients treated with nivolumab, ipilimumab or combined ipilimumab and nivolumab, 18 cases of drug-related severe myocarditis were reported (0.09%). Patients who received the combination immunotherapy tended to have more frequent and severe myocarditis than those who received nivolumab alone (0.27% versus 0.06%; $p < 0.001$; 5 fatal events versus 1). Severe myositis (grade 3–4) also appeared more frequently with the combination immunotherapy (0.24% versus 0.15%). Therefore, patients should be monitored for cardiac symptoms when they are treated with ICIs, and an immediate consultation with a cardiologist is recommended. High-dose systemic methylprednisolone at 1–2 mg/kg/day should be initiated. Other immunosuppressive drugs such as infliximab, mycophenolate mofetil, tacrolimus and anti-thymocyte globulin may be considered if symptoms do not improve with corticosteroids.

Ocular toxicities

Rare cases of ocular toxicities may occur with ICIs. They include blepharitis, conjunctivitis, episcleritis, iritis, scleritis and uveitis. The incidence per 1000 person-months of all grade uveitis was 3 (0; 80) (Boutros et al, 2016). Several studies have shown that patients treated with anti-CTLA-4 agents who were affected by diarrhoea or colitis were also likely to be affected by uveitis or episcleritis (Haanen et al, 2017; Boutros et al, 2016). In grade 1 or 2 AEs, topical steroids such as 1% prednisolone acetate suspension and iridocyclitics should be initiated. In grade 3 or 4 AEs, ICIs

should be permanently discontinued and systemic high-dose prednisolone at 1–2 mg/kg/day initiated with a tapering over at least 30 days.

Haematological toxicities

ICIs may be associated with the development of immune-mediated cytopenias, autoimmune haemolytic anaemia, pure red blood cell aplasia and aplastic anaemia (Cooling et al, 2017). A case of fatal aplastic anaemia was recently reported with the combination of ipilimumab and nivolumab (Helgadottir et al, 2017). In the summary of product characteristics, grade 3 and 4 anaemia, neutropaenia and thrombocytopaenia occurred in 2.8%, 0.7% and 1.2% of patients treated with the combination immunotherapy (n=448). Haematological toxicities tend to occur early during ICI treatment, often concomitant with irAEs of other organs (Helgadottir et al, 2017). The management of these irAEs is not well defined. Patients should be referred to an expert team to avoid a fatal issue.

Declaration of Interest:

Dr Boutros has declared consultancy for Bristol-Myers Squibb.

Dr Caronnel has declared consultancy for Abbvie (lecture), Bristol-Myers Squibb (lecture and board), Enterome (board), Janssen (board), Mayoly Spindler (research grant and lecture), Merck Sharp & Dohme (lecture and board), Pfizer (lecture and board) and Takeda (lecture). Additional disclosures more than 2 years old: consultancy for Falk, Ferring (travel grant and board), Genentech (board), Hospira (lecture), Otsuka (board) and Vifor (board).

Caroline Robert has declared consultancy for Roche, Novartis, Bristol-Myers Squibb, Merck Sharp & Dohme, Merck and Pierre Fabre.

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3.2 Immune Checkpoint Blockade: Response Patterns and Assessment of Response

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The Problem

Immune-based therapies are a major advancement in patient care and have caused a paradigm shift in the landscape of cancer treatment. Treatment of patients with immune checkpoint blockade (ICB) can result in unconventional tumour ‘response’ patterns in comparison with those seen using classic chemotherapy (ChT) drugs. Concerns about using the existing tools to evaluate tumour response to immune-based treatments have led to the development of dedicated systems of response evaluation for immunotherapeutic drugs.

Response Patterns in Immunotherapy

Response to ICB requires T cell activation and this mechanism of action is postulated to result in unusual patterns of tumour ‘response’ that can resemble tumour flare. This type of response appears to be more pronounced and more frequent than previously described for classic ChT drugs. From the time of the first melanoma trials, some patients whose disease met the criteria for disease progression based on traditional response criteria went on to have late but deep and durable responses. A decrease in tumour burden after an initial apparent increase, or after

the development of ‘new’ lesions, has been termed ‘pseudoprogression’. This apparent tumour size increase detected on imaging is thought to be caused by T cell infiltration as a result of immune activation, rather than by tumour cell proliferation. Thus, while imaging may detect an increase in size of lesions (which may result in previously undetectable lesions becoming visible), it does not represent true disease progression. This has occasionally been confirmed by biopsy as inflammatory cell infiltrates or necrosis. The most commonly reported immune-related response pattern is a decrease in size of target lesions in combination with development of new lesions, although initial tumour enlargement with subsequent slow steady decrease in tumour burden has also been reported. Although initially reported as an early event, more recent observations indicate that pseudoprogression can also occur later during the course of therapy after an initial period of stable disease (SD) or even partial response (PR).

Although pseudoprogression has been well described and the phenomenon has attracted much attention, it is important to recognise that the true incidence of pseudoprogression in cancer patients treated with immune checkpoint inhibitors (ICIs) is low. In studies of patients with melanoma receiving cytotoxic T-lymphocyte antigen 4 (CTLA-4) and programmed cell death protein 1 (PD-1) inhibitors, the reported incidence rates of pseudoprogression are below 10%. An incidence of 5% was reported in patients with advanced stage non-small cell lung cancer (NSCLC) treated with nivolumab, and the incidence might be even lower in other cancer types such as bladder cancer and renal cell carcinoma. Detailed, objective data on potential immune responses are available for less than one-third of studies performed, and comparisons of the true incidence between studies are hampered by the lack of a gold-standard method for immune response assessment.

The degree of tumour regression after an initial size increase that has been observed in patients who display pseudoprogression is variable and dependent on the method of assessment. In melanoma patients treated with pembrolizumab, increases in tumour burden of <20% from baseline were associated with longer overall survival (OS) compared with patients with a $\geq 20\%$ increase. However, in this study the frequency of patients with an unconventional response pattern was <5%, and patients with

tumour shrinkage at all time points were not compared with the group with 0%–20% increase in burden. For patients treated with ipilimumab, survival in patients with unconventional or delayed responses was similar compared with patients with responses captured by traditional criteria. Prospective analysis is required of the impact of unconventional response patterns, measured in a standardised fashion, on patient survival.

Other types of unusual ‘response’ patterns have also been reported, including hyperprogression, reported to occur in up to 9% of patients and more commonly in elderly patients. Hyperprogressive disease is defined as progression at 12 weeks with a doubling of tumour growth rate after the start of therapy compared with before treatment. The abscopal effect refers to the rare observation that anti-tumour effects of radiotherapy (RT) can be observed outside the radiation field. This is likely due to antigen-presenting cell (APC) migration and T cell activation in draining lymph nodes following local RT. However, RT can also recruit immunosuppressive cells to tumours and, for this reason, the combination of immunotherapy and RT requires further study. An abscopal effect after local RT has been described in numerous case reports of patients treated with ICB, but is yet to be confirmed in a prospective clinical trial.

When unusual response patterns occur during therapy, they pose dilemmas in clinical decision-making in terms of risk versus benefit of continuing therapy. There is frequently the hope that the apparent increase in tumour burden is an early indicator of response rather than true, and perhaps aggressive, progression. In immunotherapy trials, patients may be allowed to continue therapy beyond progression when their treating physician considers them to be deriving continued therapeutic benefit. Similarly, in routine clinical practice, decisions regarding continuation of immunotherapy beyond progression are made subjectively based on the overall assessments of clinical improvement and treatment tolerance. However, since pseudoprogression appears to be a relatively uncommon event, and indeed perhaps less frequent than hyperprogression, an apparent increase of tumour burden is more likely to reflect true progression than pseudoprogression. In case of true progression, continued treatment may delay the start of effective salvage therapy for many weeks. Therefore, the establishment, validation and implementation of a standardised

strategy to evaluate immune-related responses in patients receiving ICIs, both within clinical trials and in routine practice, is essential.

Criteria for Response

Cancer patients on active treatment undergo scheduled restaging scans and radiographic measurements of tumour lesions to determine the extent of change in tumour size. A standardised methodology to define tumour response was initially developed by the World Health Organization (WHO) in 1992 and later simplified in 2000 by the Response Evaluation Criteria In Solid Tumours (RECIST) working group after validation in a large data warehouse. In 2009, RECIST was refined to RECIST version 1.1. These criteria are the preferred platform for defining response to therapy in clinical trials, and frequently guide routine practice. When using RECIST criteria, a significant one-dimensional increase in the size of tumour lesions, the development of new lesions and/or, in exceptional circumstances, an unequivocal increase in non-target disease are considered disease progression. In contrast to when there is partial or complete tumour response, a confirmatory scan for progression is not required.

The majority of immunotherapy trials have used RECIST v1.1 to define the primary response-based endpoints. This is due to the lack of validated alternatives and because regulatory agencies continue to base the approvals of novel agents on RECIST-defined outcomes. The recognition that pseudoprogression followed by delayed responses may result in an inaccurate estimation of the true data of progression prompted the development and use of immune-related response criteria (irRC). The first consensus-based irRC guidelines were published in 2009 and were based on the WHO criteria using bi-dimensional measures. In these guidelines, new lesions did not define progressive disease, but their measurements were included in the sum of the measures of target lesions. Four years later, the guidelines were revised to incorporate uni-dimensional rather than bi-dimensional measurements and have been referred to as irRECIST. Unfortunately, only a few clinical trials have used irRC or irRECIST as the primary criteria to define their endpoints, although others have included irRC, irRECIST or similar modified criteria as secondary response criteria. However, these criteria are not always consistently

applied, hampering validation and leading to concerns about the comparability of data across trials. This is worrisome, as changes in tumour burden are often used as surrogates of survival or quality of life.

These concerns prompted the RECIST working group, together with representatives from academia, pharmaceutical companies and regulatory agencies, to develop a guideline for the use of a modified RECIST (named iRECIST) to ensure consistent trial design and data collection. The iRECIST guideline describes a standard approach to solid tumour measurement and definitions for objective change in tumour size for use in immunotherapy clinical trials. Initial response is measured according to RECIST v1.1. If the RECIST v1.1 criteria for tumour progression are met, this is defined as unconfirmed progressive disease (iUPD) for which confirmation is required. In the absence of clinical deterioration, patients continue treatment until the confirmation scan 4–8 weeks later. If there is worsening of disease bulk on the next scan, this is considered to be confirmed progressive disease (iCPD). Importantly, providing progression is not confirmed during the next scan, then complete response (CR), PR or SD (using RECIST v1.1 criteria and compared with baseline) can still be assigned at a subsequent timepoint, despite a previous iUPD. New lesions are managed using RECIST v1.1 principles (definitions of measurable, target and non-target disease) but are not added to the sum of target lesions. iRECIST recommends that the confirmatory scan after an unconfirmed progression is performed with an interval of at least 4 weeks but no more than 8 weeks after iUPD, to ensure that patients remain fit for salvage therapy. In the absence of salvage therapy or predictable tumour kinetics, a longer interval may be considered appropriate, providing it is well described in the trial protocol.

The similarities and changes in iRECIST compared with RECIST v1.1 are summarised in Table 1 and illustrated in Figure 1. An explanatory video, training slides and an example of a case record form can be found on the RECIST website (<http://recist.eortc.org/irecist/>; 15 May 2018, date last accessed). The iRECIST guideline was published in early 2017 and iRECIST will, once enough data have been accumulated, be evaluated as a potential novel standard for response measurement in immunotherapy trials.

Table 1 Summary of Similarities and Differences between RECIST v1.1 and iRECIST

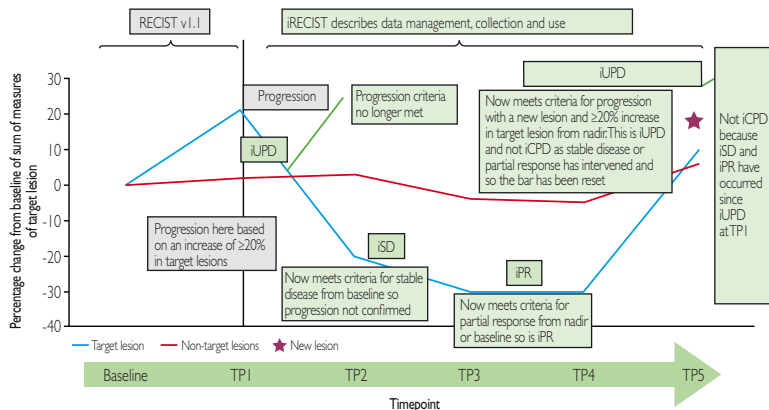
	RECIST v1.1	iRECIST
Measurable disease		
-Non-lymph node	≥10 mm (longest diameter)	≥10 mm (longest diameter)
-Lymph node	≥15 mm (shortest diameter)	≥15 mm (shortest diameter)
Target lesions	Maximum 5 Maximum 2 per organ site	Maximum 5 Maximum 2 per organ site
Non-target lesions	Other (non-)measurable lesions	Other (non-)measurable lesions
Calculation of SOM	Total diameter of target lesions	Total diameter of target lesions
Complete response	Disappearance of target lesions All lymph nodes <10 mm Confirmation required	Disappearance of target lesions All lymph nodes <10 mm Confirmation required
Stable disease	<30% decrease SOM, <20% increase SOM	<30% decrease SOM, <20% increase SOM
Partial response	≥30% decrease SOM Confirmation required	≥30% decrease SOM Confirmation required
Progressive disease	≥20% increase SOM and ≥5 mm increase lesion or New lesion or Non-target PD No confirmation required	≥20% increase SOM and ≥5 mm increase lesion or Non-target PD resulting in iUPD Confirmation required and in the meantime continuation of therapy when clinical deterioration is absent
New lesions	Define PD	Result in iUPD. Maximum 5, 2 per organ site, recorded as new target lesion (in separate iSOM). iCPD is only assigned if at next assessment additional new lesions appear or an increase in size of new lesions is seen (≥5 mm for sum of new lesion target or any increase in new lesion non-target); the appearance of new lesions when none have previously been recorded can also confirm iCPD
Confirmation of progressive disease	Not required	Required at the next assessment (4–8 weeks later) If not performed, reasons must be recorded including reason for treatment discontinuation
Clinical status	Not recorded	Record performance status, disease-related symptoms and intensification of symptom management

Best overall response and time-point of best overall response must be recorded for both RECIST v1.1 and iRECIST.

Abbreviations: iCPD, confirmed progressive disease; iUPD, unconfirmed progressive disease; PD, progressive disease; RECIST, Response Evaluation Criteria In Solid Tumours; SOM, sum of measurement.

Figure 1 RECIST v1.1 and iRECIST: an example of assessment.

From Seymour L, Bogaerts J, Perrone A, et al. iRECIST: guidelines for response criteria for use in trials testing immunotherapeutics. *Lancet Oncol* 2017;18:e143–e152. Reprinted with permission of Macmillan Publishers Ltd. Copyright © 2017.



Prefix 'i' indicates immune responses assigned using iRECIST; others without 'i' are confirmed by RECIST v1.1.

Abbreviations: iCPD, complete progressive disease; iPR, partial response; iSD, stable disease; iUPD, unconfirmed progressive disease; RECIST, Response Evaluation Criteria in Solid Tumours; TP, timepoint.

Potential Future Developments

Clinical trial data collected according to RECIST v1.1 and iRECIST (recorded prospectively or with retrospective measurements derived from central imaging review) will be collected to create an immunotherapy data warehouse with the goal of validating iRECIST or suggesting modifications. Although tumour kinetics can be assessed from studies that have incorporated iRECIST, the collection of additional data from imaging performed prior to enrolment is also recommended and should be incorporated into protocols and informed consent documents, to allow the evaluation of hyperprogression as well as pseudoprogression. For trials combining RT and ICIs, the collection of data on timing of RT and tumour lesions irradiated should be considered.

Future validation of the warehouse will clarify outstanding issues regarding timing and frequency of scans. Scans are currently recommended every 6–12 weeks, with scans for progression 4–8 weeks after iUPD in

addition to the standard scan schedule. Early response evaluation at 4–6 weeks after start of therapy has been suggested to facilitate identification of rapid progressors. An intensive scanning schedule provides detailed information to guide clinical decisions and a wealth of information for validation of iRECIST, but these advantages must be weighed against burden to patients, radiation exposure and costs.

It is important to recognise that iRECIST is a guideline for the management of data collection and has not yet been formally validated as new criteria for response-based endpoints for clinical trials of immune-based therapies. This approach was adopted because it was clear in the rapidly moving environment of immune-based cancer therapies that multiple different criteria were being developed that would limit the ability to create a warehouse. It is also the reason why iRECIST recommends the use of RECIST v1.1 to define primary response-based endpoints, with iRECIST used as exploratory secondary endpoints, at least for pivotal trials. Universal adoption of iRECIST may additionally aid decision-making in routine practice, although the guidelines are currently not designed for use outside the trial setting.

Simultaneously, several other ongoing initiatives are searching for a potential role for other imaging techniques. Functional positron emission tomography (PET) imaging, visualising expression of checkpoints or markers of immune activation, is of interest as well as detailed analysis of tumour characteristics using regular imaging modalities, known as radiomics. Furthermore, combination of imaging results with clinical, blood- or tumour-derived biomarkers may further refine predictions of tumour response to ICIs.

Declaration of Interest:

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3.3 Immunotherapy in Special Populations

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Introduction

Clinicians are often faced with complex decision-making around the administration of immune checkpoint inhibitors (ICIs) in groups of patients who appear to be at greater risk of an immune-related adverse event (irAE). These so-called ‘special populations’ include patients with a pre-existing autoimmune condition, the elderly and those with a poor performance status (PS).

There are several treatment and patient factors to consider in this situation. Single-agent anti-programmed cell death protein 1 (PD-1) and anti-programmed death-ligand 1 (PD-L1) antibodies do not induce the same frequency of severe irAEs as anti-cytotoxic T-lymphocyte antigen 4 (CTLA-4) monotherapy and combinations of anti-PD-1/PD-L1 with anti-CTLA-4, and may be preferable in many of the groups discussed below. The risk/benefit ratio for patients being considered for adjuvant therapy is also different to those with metastatic disease.

This chapter provides guidance for clinicians faced with treating patients who belong to a special population. The vast majority of these patients would not have been eligible for large randomised trials. Therefore, the majority of the literature on special populations is retrospective and anecdotal. The care of these individuals is optimised in a large centre

with clinicians experienced in the management of complex and rare irAEs. Liaison with physicians in associated specialties is important to help anticipate and mitigate potential toxicity. Table 1 summarises the key points discussed in this chapter.

Table 1 *Key Points for the Management of Immunotherapy in Special Populations*

Prior ICI toxicity	Based on limited retrospective data, administration of anti-PD-1 agents after ipilimumab toxicity is generally safe and does not usually reproduce the same toxicity. Similarly, resumption of anti-PD-1 treatment after combination ICI therapy may be considered, particularly in those with toxicities more attributable to ipilimumab (e.g. colitis)
Pre-existing autoimmunity	Ipilimumab and anti-PD-1 antibodies are generally safe, although flares can occur; and maintenance immunosuppression may reduce efficacy of ICIs
Transplant recipients	Allograft rejection may occur; however, this does not necessarily compromise the potential for tumour response
Chronic infections	ICI treatment appears to be safe in patients with controlled hepatitis B and C infection, as well as HIV
Older adults	Age alone should not be a limiting factor for ICI therapy; however, a more comprehensive functional assessment should be considered
Poor performance status	In patients compromised by disease with a high chance of response, ICI treatment can be administered with close monitoring
Organ dysfunction	The metabolism of ICIs does not rely upon renal or hepatic function; haemodialysis is not a contraindication
Pregnancy & fertility	ICIs theoretically pose a risk to the foetus; endocrine toxicities may negatively impact fertility
General comments	Randomised, prospective evidence is lacking to inform management across these patient groups Liaison with multidisciplinary teams and experienced academic centres is recommended for complex cases

Abbreviations: HIV, human immunodeficiency virus; ICI, immune checkpoint inhibitor; PD-1, programmed cell death protein 1.

Clinical Results/Patient Groups

Prior ICI Toxicity

Retrospective, multicentre studies in advanced melanoma have looked at cohorts of patients treated with nivolumab or pembrolizumab who experienced prior significant toxicity with single-agent ipilimumab and also combination ICI therapy. In a series by Menzies et al (2017), of the 67 patients receiving anti-PD-1 therapy who required immunosuppression for prior ipilimumab-related toxicity (including patients who had

received infliximab and anti-thymocyte globulin for colitis and hepatitis, respectively), 21% developed a grade 3–4 irAE and 12% had to discontinue their anti-PD-1 therapy. Only two patients experienced a recurrence of their ipilimumab toxicity, and these were grade 1–2 in severity. In this group, characterised by predominantly visceral metastatic disease (M1c), the response rate was 40%.

Gutzmer et al (2017) reported outcomes on a series of 22 patients treated with anti-PD-1 therapy after experiencing an ipilimumab-related toxicity (10 of whom only had grade 2 irAEs). Two of these patients, both of whom experienced colitis, were still on treatment for their irAE at the time of PD-1 initiation. Only one patient experienced a recurrence of his ipilimumab-related toxicity and five patients developed a new irAE. These were all grade 2 in severity, manageable with medical treatment and did not require discontinuation. The response rate in this group was 46%.

The resumption of anti-PD-1 treatment subsequent to toxicity with combination anti-PD-1/anti-CTLA-4 therapy was examined in 2018 in a series of 80 patients by Pollack et al. The median number of combination ICI cycles prior to cessation was two, with 69% stopping due to grade 3 and 4 adverse events (AEs). The median time to resuming anti-PD-1 treatment was 58 days (range 14–395), and this was for ongoing maintenance in 81% and for progression in 16% (two cases unknown). Upon resumption of anti-PD-1 therapy, 50% experienced an AE of any grade, with 18% experiencing grade 3–5 AEs. One patient died due to Stevens-Johnson syndrome/toxic epidermal necrolysis. It was uncommon for colitis to recur (6%), whereas 17% had a recurrence of hepatitis. Patients still receiving steroids for or having symptoms of the AE at the time of anti-PD-1 resumption had higher rates of toxicity (55% versus 31% and 30% versus 17%, respectively). Of the 16% resuming treatment for progressive disease, 31% had a partial response.

These series provide some reassurance that treatment with anti-PD-1 agents after significant toxicity with ipilimumab-containing regimens may be safe and effective. Nonetheless, patients should be informed that they are at risk of further irAEs that may be severe and, in rare circumstances, fatal. Whether repeated administration of combination

anti-CTLA-4/anti-PD-1 or PD-L1 is appropriate in this setting is unclear and remains at the discretion of the treating physician. In one series by Spain et al (2017), of three consecutive melanoma cases rechallenged with combination ipilimumab and nivolumab on the background of prior grade 3 toxicity with ipilimumab + nivolumab, recurrent toxicity was seen in one patient and a different grade 3 toxicity in another. All irAEs were manageable and clinical benefit was observed; however, more data are required to draw any conclusions about the safety of combination therapy in this context, and it cannot therefore be recommended outside major immunotherapy centres.

Pre-existing Autoimmunity

Patients with pre-existing autoimmune diseases (ADs) such as rheumatoid arthritis, multiple sclerosis and inflammatory bowel disease are theoretically at risk of a flare in their condition with the augmented immune response facilitated by ICIs.

Two multicentre series have reported outcomes for patients with a variety of pre-existing autoimmune disorders, including rheumatological and endocrine conditions, myositis and neurological disorders, who were treated with anti-PD-1 agents. In the series by Menzies et al (2017), of the 52 patients identified, 29% had 'active' symptoms of their AD and 38% were on immunosuppressants at treatment commencement. Thirty-eight per cent of patients experienced a flare in their AD and this was more common in the group with active symptoms. Proportionately, the majority occurred in those with a background rheumatological condition (52%). Flares were mostly mild, but in two patients anti-PD-1 therapy was ceased. Notably, the response rate was lower for patients on background immunosuppression (15% versus 44%) but did not differ between those with and without a flare (35% and 31%, respectively). In the series by Gutzmer et al (2017) (n=19; including 5 cases of autoimmune thyroiditis which would not have excluded participation in trials and not thought to be significant), 42% had a flare but no one discontinued treatment. At the start of therapy, 33% were on immunosuppression, 32% on hormone replacement and 35% on no additional therapy for their AD. The reported response rate was 32% to anti-PD-1 therapy.

Patients treated with ipilimumab for advanced melanoma who had a history of AD were evaluated by Johnson et al (2016). In this series of 30 patients, 20% remained on steroids and 23% on other immunomodulatory therapies when ipilimumab was commenced. Exacerbations of the autoimmune condition requiring treatment occurred in eight patients (27%). These consisted of worsening of existing symptoms, and treatment with low-dose corticosteroids (up to 30 mg prednisolone) was effective in six of the eight patients. The other two required prednisolone at 1 mg/kg. Overall in this cohort the tumour response rate was 20%.

In summary, selected patients with pre-existing ADs, be that ‘active’ (i.e. ongoing symptoms) or ‘inactive’ (i.e. no current manifestations), may be safely treated with ICI monotherapy. In the ‘active’ group, flares of AD are common but appear to be manageable and rarely result in treatment cessation. The decision to treat such patients must be made with multidisciplinary support, with the likelihood of response and ramifications of a flare of the underlying disorder discussed. Background immunosuppression should be weaned to the lowest dose possible to optimise the chance of response. These patients warrant more frequent clinical review. In patients with active symptoms or in those who require ongoing immunomodulatory treatment, we would not recommend combination ICI therapy based on a lack of safety data at present.

Transplant Recipients

Administration of ICIs in patient with solid organ transplants carries a risk of graft rejection; however, control of metastatic spread is often a greater priority. The implications of a renal allograft rejection are very different to those of a liver or heart transplant. Factors such as the age of the graft, the extent of donor–recipient human leukocyte antigen (HLA) matching, prior rejection episodes and the level of maintenance immunosuppression may all impact on the risk of rejection.

There have been no systematic studies to date on the risk of graft rejection with ICI treatment. Many case reports describe rejection with anti-PD-1 therapy, whereas only two describe rejection with anti-CTLA-4 therapy. There are several reported cases where the allograft has not been affected and patients have derived clinical benefit from anti-CTLA-4

treatment; however, in the one case reported where anti-PD-1 therapy did not lead to rejection, no response was reported. There is a stronger scientific rationale for why PD-1 blockade may lead to rejection, and this may explain the bias in the anecdotal literature toward anti-PD-1-mediated rejection. Nonetheless, tumour shrinkage may still occur even after graft rejection and responses may be durable.

Where possible in this cohort, the use of targeted therapy or chemotherapy is advisable; however, administration of ICIs is not absolutely contraindicated. Involvement of the transplant physician is crucial. Ideally, maintenance immunosuppression should be minimised prior to ICI administration, and the use of mammalian target of rapamycin (mTOR) inhibitors instead of calcineurin inhibitors may be considered. Although reduction of maintenance immunosuppression may in theory result in tumour shrinkage due to restoration of the immune response, prospective evidence of this is lacking, and it appears more relevant to post-transplant lymphoproliferative disorders (PTLDs) than solid organ malignancies. Treating oncologists need to monitor for the symptoms of graft rejection, which can be non-specific and may occur within the first cycle of ICI initiation or later in the course of therapy. Graft salvage with high-dose steroids has only been described in one case.

Chronic Infections

Anti-PD-1 and anti-CTLA-4 agents have been safely administered to patients with metastatic cancer despite concurrent human immunodeficiency virus (HIV) and hepatitis C infection. In the published case reports of patients with HIV who have been treated with ICIs, all were on highly active antiretroviral therapy (HAART) with an undetectable viral load. ICIs may actually assist in reducing viral load and improving CD4 count in HIV infection, likely due to their ability to reverse T cell exhaustion. This has been described in a case report by Guihot et al (2018), and a multicentre series presented at the European Society for Medical Oncology (ESMO) 2017 annual congress by Rai et al.

Nivolumab was evaluated in a phase I/II trial of patients with hepatocellular carcinoma, many of whom had hepatitis B and C infection. No new safety signals were observed in this study. Patients with hepatitis B were

required to be on anti-viral therapy with a suppressed viral load, but concurrent treatment for hepatitis C was not mandated. Safe and successful treatment in the presence of an untreated hepatitis C infection with a detectable viral load has been reported.

Overall, ICI treatment appears to be safe in patients with chronic viruses controlled on therapy or who have undetectable viral loads. Although not reported, theoretically there is a risk of immune reconstitution syndrome in these patients. Consultation with the patient's managing specialist should be conducted. Quantification of viral load, as well as CD4 counts in the case of HIV, should be performed at the beginning of ICI therapy and periodically thereafter.

In patients at high risk of exposure, it is worth taking a thorough history and testing for latent bacterial infections such as *Mycobacterium tuberculosis*. Such patients should be discussed with an infectious diseases physician as to whether they warrant prophylaxis. As with chronic viral infections, preclinical work suggests PD-1 blockade may also be helpful as an adjunct to antibiotic treatment in tuberculosis. Consequent immune reconstitution may lead to an inflammatory response.

Older Adults

Retrospective studies have reviewed the use of ICIs in patients >70 years old. A systematic review and meta-analysis of several phase III ipilimumab studies failed to find different outcomes for patients aged 65–75 and >75 years old (Nishijima et al, 2016). This same analysis looked at anti-PD-1 treatment in these two age brackets but did not find a survival benefit over control treatment for those >75 years old. This is most likely attributable to small numbers. Consistent with the results from ICI monotherapy studies, a subgroup analysis from the CheckMate 067 trial showed that patients >75 years old did not have a higher rate of grade 3–4 irAEs across treatments. Combination ipilimumab + nivolumab was still significantly better for progression-free survival (PFS) and objective response rate (ORR) than ipilimumab monotherapy.

Although these studies do not raise concerns for patients >70 years old being treated with either monotherapy or combination regimens, the vast

majority of patients were Eastern Cooperative Oncology Group (ECOG) performance status (PS) 0 or 1, significantly limiting the ability to generalise these results. Age on its own should not be a contraindication for treatment; however, elderly patients in the clinic may have multiple co-morbidities and less physiological reserve to cope with toxicity. Geriatric evaluations may provide a more comprehensive perspective on functional status. If there is concern over tolerance of ICIs, single-agent anti-PD-1/PD-L1 agents are the safest choice in this group.

Poor PS

Clinical trials and most expanded access programmes of ICIs require an ECOG PS of 0 or 1 for treatment eligibility. As such, there is little published literature on outcomes with ICIs in patients with a PS >2. In the phase II study of atezolizumab for cisplatin-ineligible patients with urothelial cancer, 27% of patients were ECOG PS 2 (Balar et al, 2017). There was no apparent difference in response rate to treatment, although the trial was not powered for this comparison. In a patient whose PS is compromised by symptoms of cancer, and with a good chance of response, single-agent PD-1 or PD-L1 is a reasonable treatment choice. In an otherwise fit patient compromised by metastatic disease, a combination anti-CTLA-4/PD-1 or PD-L1 regimen may be appropriate.

Organ Dysfunction and Haemodialysis

ICIs are immunoglobulin (Ig)G antibodies, eliminated by intracellular catabolism. This makes their clearance independent of renal and hepatic function. Nonetheless, the evidence for their safety in patients with reduced organ function is limited. In one retrospective series of 27 patients with chronic kidney disease, congestive cardiac failure and hepatic impairment, worsening of baseline organ dysfunction was not attributed to anti-PD-1 treatment and was reversible with supportive care. Grade 3–4 irAEs were experienced in three patients (11%) and 59% derived clinical benefit from anti-PD-1 therapy (i.e. stable disease or response).

Reduced organ function may complicate the recovery from irAEs and this should be considered in the toxicity management strategy. For example, patients with chronic kidney disease and diarrhoea may warrant

admission for hydration and fluid balance. Immunomodulatory medications may require dose adjustment in this setting too.

Patients on haemodialysis may be treated with ICIs. The size of the antibodies is such that they are not removed by standard haemodialysis membranes. No AEs were noted in three patients on dialysis in the series by Kanz et al (2016).

Pregnancy and Fertility Implications

In patients who are pregnant, the use of ICIs is not deemed safe. The pregnant uterus is considered an immune-privileged site, and both the CTLA-4 and the PD-1/PD-L1 pathways play important roles in the protection of the foetus from maternal T cells that may recognise foreign antigens.

In patients of reproductive age who are considering future pregnancy, there is a potential impact on fertility, for example as a consequence of hypophysitis. In many of these patients, pregnancy may not be advisable due to their limited prognosis from the underlying malignancy. However, with durable remissions now possible for metastatic disease, this has become a grey area. The survival benefit associated with adjuvant ICIs in melanoma will see a greater proportion of patients undergo ICI therapy for whom loss of fertility may have a large impact.

Potential Future Developments

The potential for efficacy with ICIs across numerous tumour types means that, for many patients with metastatic disease, the benefit of treatment outweighs the potential risks. In some tumour types where response rates are more modest, however, this may not be the case. Patients not represented in clinical trial populations require careful consideration, consent and monitoring for complications of therapy, to optimise their outcomes.

Declaration of Interest:

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3.4 Cancer Immunotherapy Trials: Challenges and Opportunities

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Introduction

Drug development to treat advanced cancer has, for the most part, followed a similar sequence. Agents that showed promise in preclinical testing were evaluated in first-in-man studies, which historically established the toxicity profile and identified a recommended dose and schedule for evaluation in larger scale efficacy-signalling studies. Once an efficacy signal was established in a single-arm phase II trial, the agent was tested against currently available standard-of-care in a randomised study to determine whether it represented an advance in therapeutic options.

Efficacy endpoints in these studies reflect the mechanism of action of chemotherapeutics and both their expected benefits and limitations in treating advanced disease. Most chemotherapy (ChT) drugs are not curative in the palliative (advanced disease) setting. Clinical benefit is obtained from a period during which tumour growth has been controlled. There is usually a cytoreductive effect with cytotoxic ChT if the cancer is sensitive to treatment. Internationally accepted response criteria (RECIST, Response Evaluation Criteria In Solid Tumours) were therefore developed to provide a robust and standardised way of assessing whether disease was responding to treatment or progressing. Both initial response rates and duration of response are assessed with RECIST. In randomised studies, differences in progression-free survival (PFS) are generally described as the mean – i.e. a single point on the PFS curve. The difference between the whole length of the curves rather than a single point is best described by the hazard ratio (HR). Overall survival (OS), when able to be reported, is again frequently described as mean and HR.

Because most palliative ChT aims to gain clinically worthwhile periods of disease control before the inevitable onset of resistance to therapy, these relatively short-term measurements of efficacy are reasonable. Response rates give an early readout of efficacy and the mean PFS is (a) a simple measure that enables cross-trial comparisons, despite the obvious flaws with the confounders that are present and (b) can be relevant where two survival curves separate early. Because ultimately all patients treated with palliative ChT progress, there tends not to be an extended tail on either the PFS or OS survival curves. However, this methodology may not be optimal for immunotherapeutic agents. Chapter 3.2 ('Immune Checkpoint Blockade: Response Patterns and Assessment of Response') describes new approaches to response measurement with modern immunotherapy.

The advent of immunotherapy has presented new challenges regarding clinical trial design. Immunotherapy is given with the hope of obtaining long-term durable disease control. Cancers that respond to immunotherapy appear to find it harder to escape control by an active immune system that also demonstrates plasticity in response to tumour evolution. Immunotherapy is, however, rarely given in an attempt to gain immediate palliation of highly symptomatic patients. Tumour cytoreduction is generally required to acutely palliate patients and, although response rates with modern immunotherapy are far greater than previously seen, there are many situations where alternative treatment modalities confer higher immediate response rates and therefore offer superior immediate palliation of symptoms.

Immunotherapy has the potential to induce an immune response that changes the natural history of metastatic disease. Patients who have RECIST-defined stable disease induced by immunotherapy may go on to have durable benefit despite the presence of measurable disease. Radiological imaging reports the presence of a mass and whether the size of the mass changes over time, but does not report the proportion of live cancer cells versus the size of immune infiltrate and whether the balance of these two cellular populations changes over time. It may therefore be possible to have a response to immunotherapy that has a highly significant impact upon OS not reflected in RECIST response rate. Equally, because of the possibility that ongoing generation of effective immunity takes time, PFS endpoints may not be reflective of OS endpoints. At one

end of the scale, this may manifest itself as pseudoprogression, where tumours grow before a delayed response. This is well described but relatively rare with most active immunotherapies. At the other end of the scale, it may be possible for durable benefit to be obtained with improvement in OS, but without this benefit being reflected with a significant improvement in PFS endpoints.

Trial design for immunotherapy therefore needs to take account of these issues.

Lessons from Pre-checkpoint Inhibitor Immunotherapy

The relatively recent development and introduction of immune checkpoint inhibitors (ICIs) into clinical practice followed many years of negative clinical trials with agents that were far less clinically active. There are, however, some important lessons that can be learnt from these studies, which may have implications for contemporary studies. Failed development was not just because agents were minimally active.

Inferior Experimental Arm Outcome

Many randomised large phase III vaccine studies proved to be negative, with a non-statistically significant inferiority of the experimental arm. The implication from these studies is that suboptimal attempts to induce immunity can induce a poorer clinical outcome. This is not a surprise from preclinical studies in which induction of anergic T lymphocytes is well described. However, the implication for clinical development of vaccines is that an adequately strong efficacy signal needs to be seen in earlier phase studies before testing in the phase III setting. This is not always straightforward – single-arm phase II clinical trials can often perform well against historical controls, and because the relevant clinical endpoint is durable benefit, response rates may not be instructive. There is an additional ethical dimension to these trials that potentially has wider implications. Despite patient information sheets becoming ever longer and more detailed, the possibility that the experimental treatment may be inferior to standard treatment is rarely mentioned forcefully.

Lack of Predictive Biomarkers

Interpretation of efficacy signals in phase II clinical trials was compounded by the lack of predictive biomarkers of clinically relevant immune responses. Investigators were lulled into the flawed belief that agents were likely to be clinically active because a number of immunological assays showed significant changes. Immunological assays were frequently performed on samples from peripheral blood and used non-tumour-specific measures of immune function, for example Elispot assays detecting T cell activation by release of interferon (IFN)- γ . This is an assay of peripheral T cell activation, but it is not a predictive marker of clinically significant anti-tumour immunity. In the absence of a proven predictive marker of clinical benefit, immunotherapies which may result in a prolonged benefit but no increase in response rate (some vaccines may fall into this category) are difficult to assess in a phase II setting and may therefore have a higher risk of failing in phase III trials.

Assumptions Regarding Efficacy of Immunotherapy with High Tumour Burden

In the pre-checkpoint inhibitor immunotherapy era, assumptions were sometimes made regarding the ability of a vaccine to stimulate effective anti-tumour immunity in a patient with a significant tumour burden and a degree of immunoparesis due to the presence of advanced disease. Immunotherapy was therefore felt to be a special case in comparison with conventional treatments. Rather than proving efficacy in the advanced disease setting before entering into larger scale adjuvant studies, a number of vaccines were tested in the adjuvant setting with inadequate evidence of activity in the metastatic setting. Limited evidence of efficacy was explained away with the thought that agents should be tested in the adjuvant setting where they were perceived to have a better chance of demonstrating efficacy. In the present era of more highly active immunotherapeutics, which demonstrate activity in both the metastatic and adjuvant settings, this flawed thinking should not be repeated.

Trial Design

There is an understandable pressure in drug development to make early decisions regarding the potential utility of a new agent. This means that

early readouts of efficacy such as response rate and median PFS can be given untoward emphasis. This is particularly inappropriate where a drug confers significant long-lasting benefit in a minority of patients. If survival curves in a randomised study separate after the mean, potentially useful agents may fail in development. Longer follow-up would allow time for improved HR, but this obviously takes precious time. The cytotoxic T-lymphocyte antigen 4 (CTLA-4) inhibitor tremelimumab was perceived as being inactive in a small randomised phase II trial due to these issues, whereas ipilimumab succeeded in development following a larger study with longer follow-up. The tremelimumab development pathway has been long and tortuous as a result.

Optimising Contemporary Immunotherapy Clinical Trials

The availability of active drugs with measurable efficacy has resulted in a sharp rise in confidence in the immunotherapy field. There are now multiple potential targets demonstrating promise in preclinical studies that may have clinical utility. Combinations of immunotherapies and combinations of immunotherapeutics with other modalities are in active assessment. To facilitate identification of the most promising developments, progress in the following areas would be very helpful.

Biomarker Development

Development of robust biomarkers that are predictive of durable benefit would facilitate selection of agents in what is an increasingly crowded field. Early phase clinical trials should have high-quality stringently applied translational programmes that aim to identify predictive potential biomarkers able to be validated in larger scale studies. The limited number of centres that usually contribute to early phase studies should enable high-quality sample collection. Examination of potential markers should not be limited to those easier assessments in the periphery but should also include mandated biopsy material from the tumour environment. Assessment of the tumour microenvironment using immunohistochemistry, gene expression profiling and other methodologies has the potential to identify markers predictive of response to treatment. Care

should be taken to distinguish between assays that demonstrate a robust predictive signal of benefit and those that simply reflect that the patient has been exposed to drug but are not predictive of significant anti-tumour efficacy. For example, reporting peripheral changes in T cell subsets confirms that some changes in immune parameters are induced by an agent but not more than that. It is the immunological equivalent of diarrhoea induced by a tyrosine kinase inhibitor – it demonstrates that the patient has taken the drug but not whether there is clinical benefit.

Predictive biomarkers allow appropriate selection of patients who are highly likely to gain durable benefit from treatment, thereby not exposing patients who will not benefit from an inactive and potentially toxic treatment. They also likely improve the cost effectiveness assessments performed by some reimbursement authorities. However, the required robustness of a biomarker that is used in decision-making to withhold a treatment from a patient is far greater than the requirements of one used to identify an enriched patient population with a higher chance of experiencing clinical benefit. These issues need to be considered if the risk of adopting biomarkers that exclude treatment from a group of patients who would otherwise benefit is to be avoided.

The Challenge of Combination Therapies

It is likely that immune-based future therapies will involve combinations of agents that result in increased efficacy. Recent breakthroughs have been with antibodies directed against immune checkpoint molecules. As multiple checkpoints are identified that have not yet been fully clinically tested as targets, it is likely that they will be tested in combination with clinically proven checkpoint inhibitors in so-called immuno-oncology (IO)–IO combinations. There is also justification to assess immunotherapies with non-immune conventional treatments. In melanoma and renal cancer IO-targeted therapy, combinations are in late-phase clinical trials and there are hypotheses supported by preclinical evidence that such combinations may prove advantageous. However, the attraction of a combination treatment is that it results in synergistic rather than additive benefit, and most studies are not designed to distinguish between these two outcomes. This issue is directly related to the biomarker issue described above. Identification of which patients are likely to

benefit from combination treatment as opposed to sequential treatment from single agents is an important issue that needs to be resolved. The danger in not doing so is that the speciality acquires combination therapies as a standard-of-care that are not advantageous but come at greater cost and toxicity.

Duration of Treatment and Toxicity Issues

Significant evidence has now accumulated that durable responses can persist after the withdrawal of treatment either due to toxicity, the demands of a trial protocol or investigator/patient preference. Clinical trial design regarding duration of treatment has historically been conservative. With relatively well tolerated agents, treatment duration has generally been until disease progression, dose-limiting toxicity or patient preference. If, however, immunotherapy has the potential to induce life-long disease control, the issue of treatment duration becomes more pressing. There is a natural inclination to not want to undertreat patients, which is understandable. There may, however, also be a commercial attraction to have long periods of treatment exposure which, from a clinical perspective, should be resisted. Trial design should explore duration of treatment at an earlier stage in drug development. Currently this issue is relegated to exploration by academic groups following licensing and it can therefore be many years before data emerge that confirm a limited duration of treatment is all that is necessary.

ICIs are associated with significant immune-mediated organ-specific toxicity which can, on occasion, be fatal. Current toxicity management algorithms using high-dose steroid-based regimens safely gain control of immune-mediated adverse events in the majority of patients. However, there is a group of patients who have refractory toxicities, and a larger group that have toxicity controlled at the expense of chronic high-dose steroid exposure and the associated side effects. There is inadequate investigation of immune-related toxicity management. Identification of alternative strategies is important in maintaining quality of life in patients exposed to these agents.

Response Assessment

Immunotherapy-induced responses can be more complex than those seen with ChT. There may be mixed responses, with some lesions progress-

ing and others regressing. Pseudoprogression has also been described with lesions getting larger before having a delayed response, and finally new lesions can appear before a delayed response. Concern has therefore been raised whether RECIST adequately identifies patients who are benefiting from immunotherapy. Modified RECIST criteria, iRECIST, have been developed to take account of this variability in quality of response. iRECIST appeared most useful with ipilimumab, a CTLA-4 inhibitor with low response rates but durable benefit in a minority of patients. More active immunotherapies have shown that RECIST response rates are adequate in defining the proportion of patients who have a cytoreductive immune-mediated tumour response. Investigators need to be aware that post-progression treatment should be allowed if there is evidence of any lesion responding. RECIST v1.1 is currently the standard methodology for response evaluation. iRECIST is still exploratory. If iRECIST proves to be clinically useful with further evaluation, it may become a new standard.

Summary

Immunotherapy represents a new challenge in trial design. The goal of treatment is to generate long-term durable responses; however, many conventional trial endpoints are relatively short term. A focus on short-term trial endpoints such as response rate and median PFS may risk missing active agents that could confer long-term benefit to patients. Landmark PFS analyses are now frequently described and are likely to be more representative of the ability of an agent to induce a durable response. Investigators should consider the value of landmark PFS analyses as a primary outcome measurement. Such considerations may need to be discussed in advance with licensing authorities depending on the phase of study being performed.

Because immunotherapies which are now proven to positively impact upon long-term survival are commercially available, many patients whose disease progresses following exposure to an investigational agent may have a subsequent active treatment and some will derive long-term benefit from exposure. Overall survival endpoints, while frequently being mandated by licensing and reimbursement authorities, risk increasingly

being confounded by such post-trial crossover. Landmark PFS analysis is likely to be the best measure of durable benefit from a specific agent, but greater work needs to be done to describe the relationship between landmark PFS and OS in patients receiving immunotherapy.

Finally, high-quality translational biomarker development work in early phase immunotherapy trials, which can be validated in larger scale later phase studies, is of the utmost importance and is likely to be the most useful advance that allows investigators to discriminate between the multitude of potential new agents and combinations of agents currently in development.

Declaration of Interest:

Dr Nathan has participated in advisory boards for AstraZeneca, Bristol-Myers Squibb, Ipsen, Merck, Merck Sharp & Dohme, Novartis and Pfizer.

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3.5 Future Perspectives

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Introduction

Only a few short years ago, a prediction that checkpoint inhibition would have made a lasting impact on cancer therapy, with approvals in the United States for ten different cancers, and surely more to come, would have been met with great scepticism, even derision. Currently, there are hundreds of combination immunotherapy trials in progress with checkpoint inhibitor antibodies as the backbone treatment, encompassing five approved programmed cell death protein 1 (PD-1) or programmed death-ligand 1 (PD-L1) antibodies and numerous other antibodies blocking the PD-1 pathway that are still investigational.

Progress in the field of immunotherapy for cancer has been breathtaking since the approval of ipilimumab for melanoma in 2011, yet benefit in most cancers has been incremental. For many of the common tumour types such as non-MSI-H (microsatellite instability-high) colon cancer, breast cancer that is not triple negative and prostate cancer, there is little evidence of benefit for checkpoint inhibition or immunotherapy in general. Since response rates for checkpoint inhibition vary from 15%–45%, a minority of patients benefit from this treatment.

In the future, attention will be paid to expanding the repertoire of tumour types that are amenable to immunotherapy, and many different refinements will improve the proportion of patients who benefit from this treatment. Virtually all patients will receive combination immunotherapy or targeted/immunotherapy in which the choice and sequencing of agents will be driven by biomarkers, both derived from the tumour and in the periphery. Outlined below are some of the pathways by which we will hopefully make progress in the next 5 years, which will increase the

proportion of patients with tumour types who already benefit from immunotherapy, prolong their duration of response and expand the population of patients that benefit from immunotherapy to include those with any type of invasive malignancy.

Precision Immunotherapy

The only current Food and Drug Administration (FDA)-approved biomarker for the use of checkpoint inhibition is PD-L1 staining, which, for all its shortcomings, may have relevance in choosing combination versus single-agent therapy in melanoma and has shown some utility in other cancers. Additional tumour-related and peripheral blood biomarkers, as discussed in Chapter 1.4 ('Biomarkers of Response to Immunotherapy'), will come into more common use. An amalgamated marker that includes PD-L1 staining, mutational load and T cell tumour infiltrate will likely be better than any individual marker in predicting outcome with checkpoint inhibition, and will be used to choose patients for therapy.

In the future, there will be many new agents developed so that all patients will be treated with combination immunotherapy. Lymphocyte activation gene 3 (LAG-3) levels on tumour infiltrating T cells has already been shown to be potentially useful in choosing patients who may respond to combination LAG-3/PD-1 antibody therapy and is a biomarker that is easily measured in tumours by using established immunohistochemistry or flow cytometry techniques.

In the near future, newer immunotherapies and combinations will be tested in immune 'basket' trials, in which patients will be characterised by their tumour amalgamated biomarker profile including a T cell:T regulatory cell (T_{reg}) ratio, PD-L1 staining and mutational burden, with the addition of a tumour expression signature.

The presence of a high tumour inflammation score will suggest monotherapy or combination PD-1 checkpoint inhibition. A peripheral blood T cell profile by LAG-3 and expression of other checkpoints will match patients to new antibodies like anti-LAG-3, anti-TIM-3 (T cell immunoglobulin and mucin domain 3) or anti-VISTA with or without PD-1 blockade.

Absence of the criteria for an inflamed tumour and no peripheral blood T cell markers would likely direct patients to novel investigational combinations. For example, if a patient is PD-L1-negative and has a high mutational load, he/she may do well with PD-1 blockade combined with ipilimumab and a third drug. PD-L1-positive tumour with the same mutational load may do just as well with PD-1 blockade with metabolic approaches such as targeting amino acid pathways of glutamine and L-arginine or fatty acid pathways, or taking advantage of differential effects of hypoxia on T cell subsets to promote tumour inflammation.

Peripheral blood and microbiome analyses will also reveal the likelihood of developing severe immune-related adverse events (irAEs), which may suggest that nivolumab and ipilimumab might not be a good choice, and would indicate that an alternative combination should be chosen. Understanding of, and manipulation of, the microbiome may improve the efficacy of current and future immunotherapies.

The use of circulating tumour DNA assays will also allow an assessment of early treatment failure and facilitate a switch to alternative immunotherapies, or may be used to verify that ‘pseudoprogression’ has occurred and that immunotherapy should actually continue, a phenomenon that, while not very common, may be seen 5%–10% of the time with checkpoint inhibitors, and can be difficult to diagnose.

Adoptive Cell Therapy

Adoptive cell therapy will soon be developed to the point where tumour infiltrating lymphocyte (TIL) therapy can be tested in a phase III multi-institutional trial in melanoma and other tumours, such as human papillomavirus (HPV)-positive head and neck cancers with centralised tumour processing and cell expansion. If the results of those trials are positive, it will encourage other trials in solid tumours using TIL or chimeric antigen receptor (CAR)-T cell approaches.

There is a significant effort to assess the toxicity and utility of CAR-T cells in solid tumours using folate receptor, mesothelin and other cell surface targets, and, if the cytokine release and other off-target toxicity can be ameliorated while maintaining clinical benefit, adoptive cell

therapy will be combined with checkpoint inhibition as another element in the immunotherapy repertoire.

CAR-T cells can be modified to enable them to be ‘armoured CARs’, by which they are modified to secrete interleukin (IL)-12 and engineered to make an anti-PD-1 antibody or other substances, which can overcome the immune suppression that is prevalent in many solid tumours and has impeded their successful use in those cancers thus far.

The use of artificial antigen-presenting cells (APCs) to expand human T cells can result in a significant and high degree of expansion over a period of 14–21 days, that is superior to the use of endogenous dendritic or other natural APCs and may allow tumour antigen-specific T cells to be grown to high numbers from the peripheral blood, in which they are infrequent but of course much more accessible than in a tumour. The development of rapid techniques to clone antigen-specific T cell receptors will facilitate new trials of T cell receptor gene transfer therapy, in which the T cell receptors may recognise either tumour-associated molecules, or neoantigens, which are discussed below.

Neoantigens

Neoantigens are tumour-specific molecules that arise in mutated proteins, which encode novel epitopes that can be recognised by T cells in the context of class I and II molecules. They are likely to comprise true tumour rejection antigens, since they are associated with a high likelihood of response to PD-1 blockade, and TILs that are neoantigen-specific are highly likely to induce tumour regression after adoptive transfer in gastrointestinal (GI) and lung cancer.

Many clinical research protocols at different centres around the world have employed neoantigen sequences derived by whole-exome sequencing of tumours in vaccine strategies, and small numbers of patients have been treated using complex and lengthy protocols to generate personalised neoantigen-specific vaccines, such as long peptides or within viral and plasmid vector constructs.

In the near term, either tumours or circulating tumour cells will be sequenced rapidly to define neoantigens that can be incorporated into either adoptive

cell transfer or vaccine trials using novel vaccine platforms. The amount of time required to generate a patient-specific vaccine is still a limitation, and the ability to distinguish a passenger neoantigen from a true tumour rejection antigen is still poorly developed. Because of these limitations, this strategy may remain appropriate as an adjuvant therapy for the time being, until sequencing technology and more immunogenic vaccine generation platforms allow a rapid generation of a potent personalised vaccine which could be used in a patient with metastatic cancer. An important advance would be the development of a vaccine that could be repeatedly administered and result in high levels of neoantigen-specific T cells, but that has not yet been achieved. The combination of newer, more potent vaccines and checkpoint inhibitors remains an appealing clinical immuno-oncology approach.

Epigenetic Treatment

Epigenetic treatment takes advantage of the concept that gene regulation which occurs in mammalian cells may be independent of the primary DNA sequence, but rather functions at the level of histone methylation or acetylation and DNA methylation. A number of drugs that are histone deacetylase inhibitors have already been approved for haematological cancers such as T cell lymphoma or multiple myeloma, but recent studies have suggested that drugs that impact on acetylation of specific histone families may have important immune-stimulating effects and result in down-modulation of suppressive T_{regs}.

These drugs are being tested in combination with checkpoint inhibitors, and early reports are that selective histone acetylase inhibitors can be safely and effectively combined with PD-1 blockade or combination PD-1 blockade with ipilimumab. These drugs, and histone methylase inhibitors, may be effective immune adjuvants, although their effects are rather broad and not tumour-specific.

EZH2 is a histone methylase that has been implicated in the function of T_{regs} and in the control of the expression of important regulatory molecules like FoxP3, and its inhibition has been shown to potentially suppress T regulatory numbers and function. EZH2 inhibitors are being tested in haematological malignancies and will enter trials as an adjunct to checkpoint inhibition soon.

Novel Antibodies and Cytokines

Novel antibodies and cytokines that are genetically modified and other antibodies that are repurposed from prior indications will come into common use in oncology. As an example, ‘masked’ antibodies are being developed that are active only at the tumour site because they have a peptide linker blocking the antigen-combining region of the immunoglobulin that is cleaved by tumour-specific proteases. These molecules will target the tumour microenvironment and have less systemic toxicity than current antibodies, due to their lower systemic exposure.

Innovative constructs that combine an engineered cytokine and a receptor as a fusion molecule can replicate the physiological binding of cytokines to a specific receptor, and may be more potent than a recombinant cytokine, as in the case of an IL-15/IL-15 receptor alpha molecule that has been shown to have activity in melanoma and bladder cancer.

IL-2 is a cytokine that was approved in 1998 for melanoma and has been used for metastatic renal cell cancer, but has a modest response rate and is very toxic, requiring inpatient treatment. Newer IL-2 variants have been genetically or chemically modified by polyethylene glycolation to decrease their binding to the high-affinity IL-2 alpha receptor, which has the advantage of diminishing suppressive T_{regs} function and proliferation while maintaining its effector T cell activity. These variants will likely enter general use in the near term and will be combined with checkpoint inhibition or replace IL-2 as a standalone therapeutic.

A variety of monoclonal antibodies have been approved for various non-cancer indications in the cardiac or inflammatory bowel disease fields. These antibodies have potential to decrease the chronic inflammatory changes that have been associated with metastatic cancer, and that can either down-modulate the immune benefit of checkpoint inhibition or have been associated with the toxicity of PD-1/PD-L1 and CTLA-4 blocking antibodies.

Infliximab, the tumour necrosis factor (TNF)-blocking antibody used in patients suffering from ulcerative colitis, has a clear track record of treating colitis associated with checkpoint inhibition. Vedolizumab, an alpha4-beta7 integrin-blocking antibody, has also been tested for its ability to suppress the colitis associated with combination checkpoint

blockade, and may be repurposed for that indication.

Canakinumab, an antibody targeting the cytokine IL-1 β , was tested in a large phase III trial to reduce C-reactive protein (CRP) and mortality from coronary artery disease. In that study, a reduction in mortality from lung cancer was seen to be associated with the CRP reduction. CRP is also associated with a poor outcome with many other cancers, and was shown to be associated with short survival in melanoma patients treated with PD-1 blockade. Canakinumab will be an excellent choice to be repurposed in combination with PD-1 blockade to overcome resistance to that treatment, associated with elevation in chronic inflammatory intermediates and acute phase reactants.

Adjuvant Immunotherapy

Adjuvant immunotherapy with checkpoint inhibition has been tested successfully in three completed randomised phase III trials in melanoma. In the EORTC 18071 trial, patients with high-risk resected stage III melanoma were randomly allocated to receive either ipilimumab at 10 mg/kg or placebo, and, after 5 years of follow-up, an advantage for ipilimumab was seen in both relapse-free and overall survival. The rate of grade 3 or 4 immune-related toxicity was over 40% in that trial, suggesting that ipilimumab was effective but quite toxic. When that regimen of adjuvant therapy was compared with the anti-PD-1 agent nivolumab as adjuvant therapy for high-risk stage IIIB/C and IV resected melanoma in the CheckMate 238 study, superior relapse-free survival and toxicity was shown for the PD-1 antibody compared with the active control arm with ipilimumab. In the recently published KEYNOTE-054 study, the PD-1 antibody pembrolizumab was superior in relapse-free survival to placebo in patients with resected stage III melanoma. The success of PD-1 blockade in melanoma as adjuvant therapy in two trials will spawn adjuvant trials in multiple other cancers like lung, bladder, renal cell, Merkel cell and head and neck cancer.

In the future, the biology of metastatic disease in most cancers will be altered because the majority of relapsed patients will likely have received adjuvant immunotherapy. This will change the landscape of cancer treatment, since treatment for immunotherapy-experienced patients may differ

considerably from that for immunotherapy-naïve patients. Patients will then be divided into two categories: those who relapse during or early after immunotherapy, and those who relapse late. The latter patients may benefit from additional immunotherapy of the same type; the first will probably need to switch to a new therapy. As novel combination immunotherapies enter the repertoire for metastatic disease, they will be incorporated into newer adjuvant regimens, which hopefully will reduce the proportion of patients who ever relapse and increase the number of patients who are cured of disease.

The vast majority of clinical trials in immuno-oncology excluded patients with underlying autoimmune diseases, those who required chronic steroids and those who had undergone organ allograft transplant. We know very little about the safety and efficacy of immunotherapy in these patients and answering those questions will become an increasingly important area of investigation.

Conclusions

The future of immunotherapy is bright, and the opportunities for new, curative treatments are manifold. Building on the base of potent checkpoint inhibitors, new combinations of immunotherapies will improve outcomes for patients and overcome innate and acquired resistance to immune treatment. As the complexities of the human immune system are revealed at the molecular level, our ability to manipulate it for patient benefit will grow until, in time, we will be able to overcome that disease we call cancer.

Declaration of Interest:

Dr Weber has stock or other ownership in Altor BioScience, Celldex and CytomX Therapeutics; received honoraria from Bristol-Myers Squibb, Merck, Genentech, AbbVie, AstraZeneca, Daiichi Sankyo, GlaxoSmith-Kline, Eisai, Altor BioScience, Lion Biotechnologies, Amgen, Roche, Ichor Medical Systems, Celldex, cCam Biotherapeutics, Pieris Pharmaceuticals, CytomX Therapeutics, Nektar, Novartis and Medivation; has had a consulting or advisory role with Celldex, Ichor Medical Systems, cCam Biotherapeutics, Lion Biotechnologies, Pieris Pharmaceuticals,

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Note:

Abbreviations used in the index are listed on pages xxv-xxxii.

Cross-references in italics refer either to terms within the same main entry (e.g. 'see above', or 'see below') or to generic entries (e.g. see *individual tumours*).

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Over the past decade, the field of immuno-oncology has truly come of age. Starting with the modest but important improvement in overall survival of metastatic melanoma patients with ipilimumab, blockade of the PD-1/PD-L1 axis has revolutionised management for a growing number of tumour types. There are already five anti-PD-1/PD-L1 drugs in the clinic and many more are being studied in clinical development programmes. These agents can be used in combination with old or new drugs, which may synergise with PD-1/PD-L1 blockade in order to improve response rates and survival by overcoming primary or adaptive resistance mechanisms. Biomarkers for selecting which patients are most likely to benefit from these drugs are in development, but the landscape is highly complex.

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