CURRENT ONCOLOGY
A Canadian Cancer Research Journal

REVIEW ARTICLE

A review of cancer immunotherapy: from the past, to the present, to the future

K. Esfahani MD MSc,* L. Roudaia MD,* N. Buhlaiga MD,* S.V. Del Rincon PhD,† N. Papneja MD,* and W.H. Miller Jr MD PhD*

ABSTRACT

Compared with previous standards of care (including chemotherapy, radiotherapy, and surgery), cancer immunotherapy has brought significant improvements for patients in terms of survival and quality of life. Immunotherapy has now firmly established itself as a novel pillar of cancer care, from the metastatic stage to the adjuvant and neoadjuvant settings in numerous cancer types. In this review article, we highlight how the history of cancer immunotherapy paved the way for discoveries that are now part of the standard of care. We also highlight the current pitfalls and limitations of cancer checkpoint immunotherapy and how novel research in the fields of personalized cancer vaccines, autoimmunity, the microbiome, the tumour microenvironment, and metabolomics is aiming to solve those challenges.

Key Words Immune checkpoint inhibitors, personalized cancer vaccines, immune-related adverse events, microbiome studies, metabolomics

Curr Oncol. 2020 April:27(S2)87–97 www.current-oncology.com

INTRODUCTION

The field of immuno-oncology has been transformational in the care of cancer patients. William B. Coley, now widely accepted as the father of immunotherapy, first attempted to harness the power of the immune system for treating cancer in the late 19th century. As an orthopedic surgeon who operated on patients with bone sarcomas, he noticed that some patients with significant postoperative wound infections—a common occurrence when aseptic technique had not yet been optimized—would undergo spontaneous regression of their unresected tumours. Beginning in 1891, Coley injected more than a thousand patients with mixtures of live and inactivated bacteria such as *Streptococcus pyogenes* and *Serratia marcescens* with the hope of inducing sepsis and strong immune and antitumour responses. His cocktail of bacteria became widely known as "Coley's toxin" and represents the first documented active cancer immunotherapy intervention[1]. Coley achieved durable complete remissions in several types of malignancies, including sarcoma, lymphoma, and testicular carcinoma. However, the lack of a known mechanism of action for Coley's toxin and the risks of deliberately infecting cancer patients with pathogenic bacteria caused oncologists to adopt surgery and radiotherapy as alternative standard treatments early in the 20th century[2].

It would take more than half a century before a better understanding of the key mediators of sepsis would shed some light on the mechanisms of action of Coley's toxin. Those mediators constitute a cytokine family including interleukins, interferons, and chemokines[3]. Once again, the race was on to apply those novel discoveries to cancer therapy[4]. Physicians and researchers achieved modest success with this novel approach, occasionally inducing clinical remissions with high-dose interleukin 2 (IL-2) in metastatic renal cell carcinoma[5] and debatable responses with interferon in stages III and IV melanoma[6]. Those modest successes were often counterbalanced with significant adverse events. Although novel methods of delivery such as pegylation would abate some of the toxicities, the sporadic and unpredictable immune responses seen with those therapies meant that only a small, carefully selected subgroup of cancer patients would benefit.

The next revolutionary wave in cancer immunotherapy came with the better understanding of the process of immune surveillance, by which innate immune cells eliminate cancer cells. The recent discovery of T cell immune checkpoints, such as CTLA-4 and PD-1, propelled the field of immuno-oncology into its current era and saw the awarding of the 2018 Nobel prize in Physiology or Medicine to Drs. Allison and Honjo. Those hardwired signals have the crucial task of maintaining a fine balance between immune

Correspondence to: Wilson H. Miller Jr, E710–755 Côte-Ste-Catherine Road, Montreal, Quebec H3T 1E2.
E-mail: wmiller@ldi.jgh.mcgill.ca ■ **DOI:** https://doi.org/10.3747/co.27.5223

surveillance against foreign pathogens or abnormal cells and autoimmunity. Blocking those T cell surface receptors results in enhanced autoimmunity that induces an immune response against tumours, but can also increase the chance of autoimmune reactions.

In this review article, we highlight the current standards of care in cancer immunotherapy, with a strong focus on immune checkpoint inhibitors (ICIs), their limitations and pitfalls, and promising novel approaches.

REVIEW

Overview of Checkpoint Inhibitors

Cancer immuno-editing is the process by which various immune system components protect the host against primary tumour development or enhance tumour escape, or both, either by sculpting tumour immunogenicity or attenuating antitumour immune responses[7]. The process is tightly regulated by immune checkpoints, which are immune-cell surface receptors controlling either the activation or the inhibition of immune responses. Activation of the immune system is, on the one hand, the desired outcome to achieve tumour control, but on the other hand, responsible for autoimmunity. The discovery and development of monoclonal antibodies against the inhibitory immune checkpoints CTLA-4 and PD-1 have resulted in dramatic antitumour responses by the upregulation of immune activation at various stages of the immune cycle.

Immune checkpoint inhibitor therapies are now widely indicated in numerous cancer types (Table I). Furthermore, numerous ongoing clinical trials are assessing the potential of other agonistic or inhibitory checkpoints to affect tumour-related outcomes (Table II). The checkpoints are not equal in their potential. For example, the agonistic OX40 antibody has modest clinical activity, but the CD28 antibody—even at very subtherapeutic doses—resulted in massive cytokine syndrome and the intensive-care hospitalization of the first 6 healthy volunteers treated[8]. In that light, finding the right combination of ICI therapy to induce the optimal amount of immune activation remains an active area of clinical research.

Modulating and Predicting Immune Toxicity for Better Efficacy

Immunotherapies are often limited by their immune-related adverse events (irAEs), an immune activation and inflammatory response against the host's healthy tissues. Immune activation against the host's tumour is the desired outcome, but irAEs are challenging to predict, diagnose, and treat. In the setting of metastatic melanoma, the addition of a CTLA-4 antibody to PD-1 blockade is associated with only an incremental increase in survival, but at the cost of more than double the rate of serious irAEs[9]. A recent meta-analysis reported a fatality rate of up to 1 patient in every 77 treated using an ICI combination[10]. For specific irAEs, such as immune-related myocarditis, the mortality rate is as high as 50% in treated patients[11]. Numerous predictors of irAEs have been proposed (baseline lymphopenia and eosinophilia, B cell changes, T cell repertoire, circulating IL-17, and gut microbiota changes[12–17]), but few have been prospectively validated.

TABLE I Indications for immune checkpoint inhibitors in advanced-stage cancers, as currently approved by Health Canada[a]

Agent	Melanoma	NSCLC	RCC	SCHNN	Bladder	Merkel cell carcinoma	Hepato-cellular carcinoma	Hodgkin lymphoma
CTLA-4 inhibitor								
Ipilimumab	All lines of Tx							
PD-1 inhibitors								
Pembrolizumab	All lines of Tx	All lines of Tx			2nd line Tx			After ASCT
Nivolumab	All lines of Tx	2nd line Tx	2nd line Tx	2nd line Tx			2nd line Tx	After ASCT
PD-L1 inhibitors								
Atezolizumab		2nd line Tx			2nd line Tx			
Avelumab						2nd line Tx		
Durvalumab		After CTxRT in stage III disease			2nd line Tx			
Combination CTLA-4 and PD-1 inhibition								
Ipilimumab–nivolumab	1st line Tx		1st line Tx					

[a] Obtained 25 May 2019 from Health Canada's Drug Product Database (https://www.canada.ca/en/health-canada/services/drugs-health-products/drug-products/drug-product-database.html).

NSCLC = non-small-cell lung cancer; RCC = renal cell carcinoma (clear cell); SCCHN = squamous-cell carcinoma of head and neck; Tx = treatment; ASCT = autologous stem-cell transplantation; CTxRT = chemoradiotherapy.

TABLE II Agonistic and antagonistic immune checkpoint modulators currently under investigation

Target	Drug	Company	Clinical phase
Costimulatory or agonist antibodies			
4-1BB (CD137)	Utomilumab	Pfizer Canada, Kirkland, QC	I
	Urelumab	Bristol–Myers Squibb, New York, NY, U.S.A.	I/II
	INBRX-105	Inhibrx, San Diego, CA, U.S.A.	I
ICOS (CD278)	GSK3359609	GlaxoSmithKline, Mississauga, ON	I/II
	JTX-2011	Jounce Therapeutics, Cambridge, MA, U.S.A.	I/II
GITR (CD357)	TRX 518-001	Leap Therapeutics, Cambridge, MA, U.S.A.	I/II
	MK-4166	Merck, Kenilworth, NJ, U.S.A.	I
	BMS-986156	Bristol–Myers Squibb, New York, NY, U.S.A.	I/II
	INCAGN01876	Incyte Biosciences International, Wilmington, DE, U.S.A.	I/II
CD70	ARGX-110 (cusatuzumab)	Argenx, Breda, Netherlands	I/II
CD27	CDX-1127 (varlilumab)	Celldex Therapeutics, Hampton, NJ, U.S.A.	I/II
OX40 (CD134)	PF-0451860	Pfizer Canada, Kirkland, QC	I/II
	MEDI0562/6469/6383	AstraZeneca Canada, Mississauga, ON	I
	GSK3174998	GlaxoSmithKline, Mississauga, ON	I
	BMS-986178	Bristol–Myers Squibb, New York, NY, U.S.A.	I/II
CD40	CP870893	Pfizer Canada, Kirkland, QC	I
	APX005M	Bristol–Myers Squibb, New York, NY, U.S.A.	I/II
Co-inhibitory or antagonist antibodies			
VISTA (B7-H5)	CA-170	Curis, Lexington, MA, U.S.A.	I
CCR4 (CD194)	Mogamulizumab	Kyowa Kirin, Tokyo, Japan	I/II
B7-H3 (CD276)	MGD009	Novartis Pharmaceutical, Ottawa, ON	I
	8H9	Y-mAbs Therapeutics, New York, NY, U.S.A.	I
TIM-3	TSR-022	Tesaro, Waltham, MA, U.S.A.	I
	MBG453	Novartis Pharmaceutical, Ottawa, ON	I/II
	Sym023	Symphogen A/S, Ballerup, Denmark	I
	MEDI9447 (oleclumab)	AstraZeneca Canada, Mississauga, ON	I
LAG-3 (CD223)	BMS-986016 (relatlimab)	Bristol–Myers Squibb, New York, NY, U.S.A.	I/II
	IMP321 (eftilagimod alpha)	Prima BioMed, Sydney, Australia	II
	LAG525	Novartis Pharmaceutical, Ottawa, ON	I/II
KIR (2DL1–3)	Lirilumab	Bristol–Myers Squibb, New York, NY, U.S.A.	I/II
IDO-1,2	Indoximod	NewLink Genetics, Ames, IA, U.S.A.	II
	Epacadostat	Incyte Biosciences International, Wilmington, DE, U.S.A.	II
TIGIT	Tislelizumab	BeiGene, Beijing, P.R.C.	I/II/III
	BMS-986207	Bristol–Myers Squibb, New York, NY, U.S.A.	I/II
	MTIG7192A	Genentech, San Francisco, CA, U.S.A.	II/III
	AB154	Arcus Biosciences, Hayward, CA, U.S.A.	I/II
A2aR	Ciforadenant	Corvus Pharmaceuticals, Burlingame, CA, U.S.A.	I
Transforming growth factor β	M7824	EMD Serono, Rockland, MA, U.S.A.	I/II
	Galunisertib	Eli Lilly and Company, Indianapolis, IN, U.S.A.	II
CD47	TTI-621	Trillium Therapeutics, Mississauga, ON	I
CD73	MEDI9447 (oleclumab)	AstraZeneca Canada, Mississauga, ON	I

TABLE II Continued

Target	Drug	Company	Clinical phase
Other pathways			
Toll-like receptors	Poly-ICIC	Ludwig Institute for Cancer Research, New York, NY, U.S.A.	I
	MGN1703 (lefitolimod)	Mologen, Berlin, Germany	I
	SD-101	Dynavax Technologies Corporation, Emeryville, CA, U.S.A.	I/II
	DSP-0509	Boston Biomedical, Cambridge, MA, U.S.A.	I/II
	Rintatolimod	Hemispherx Biopharma, Philadelphia, PA, U.S.A.	II
	CMP-001	Checkmate Pharmaceuticals, Cambridge, MA, U.S.A.	II
Interleukin 2 receptor	NKTR-214	Nektar Therapeutics, San Francisco, CA, U.S.A.	I/II/III
	RO6874281	Hoffmann–La Roche, Basel, Switzerland	I/II
	THOR-707	Synthorx, La Jolla, CA, U.S.A.	I/II
Arginase inhibitors	CB-1158	Incyte Corporation, Wilmington, DE, U.S.A.	I/II
Oncolytic peptides	LTX-315	Lytix Biopharma, Oslo, Norway	II
Interleukin 10	AM0010 (pegilodecakin)	Eli Lilly and Company, Indianapolis, IN, U.S.A.	I/II

Poly-ICLC = polyinosinic-polycytidylic acid–poly–L-lysine carboxymethylcellulose.

For serious irAEs, guidelines recommend broad immunosuppression consisting of corticosteroids, followed by one or more biologics (tumour necrosis factor inhibitors) or T cell suppressants (such as mycophenolate mofetil)[18–20]. Very little prospective knowledge has been developed about the consequences of those therapies for cancer-related outcomes. An analysis of the baseline use of corticosteroids in patients with lung cancer reported an association with worse survival outcomes[21]. Similarly, the use of high-dose steroids in the setting of immune-related hypophysitis in patients with metastatic melanoma was also associated with worse survival[22]. On the other hand, the use of corticosteroids in other clinical settings in which patients experience irAEs was not associated with a reduced response to ICI therapy or with survival[23]. More studies are needed to assess the optimal immunosuppression regimen to be used with ICIs to avoid impairing their efficacy. The use of mTOR (mechanistic target of rapamycin) inhibitors shows promise to abate toxicities without impairing ICI efficacy in the specific setting of organ transplantation[24–26].

Modulating cytokines in the setting of an ICI is a dual-edged sword. Many of those soluble factors, such as tumour necrosis factor α and IL-17, are called "pleiotropic cytokines" for the dual roles they play in immunity: on the one hand, they promote tumour surveillance; on the other hand, they can be key mediators of autoimmune reactions. As discussed earlier, circulating IL-17 is a biomarker for the prediction of ICI-induced colitis[16]. The addition of the IL-17 monoclonal antibody secukinumab for the treatment of immune-related colitis and psoriasis, while effective at abating immune toxicities, has been reported to induce tumour escape[27]. The same effect has not yet been reported for tumour necrosis factor α or IL-6. Tumour necrosis factor blockade seems not only to be safe for the treatment of ICI-related colitis, but in animal models of melanoma, also adds synergistic antitumour efficacy to PD-1 inhibition[28,29]. Those early observations collectively highlight the importance of further study of the role of various cytokines and immune cells in the pathogenesis of irAEs.

A New Era for Tumour-Specific Vaccines in Combination with ICIs

Despite promising results with ICIs, single-agent PD-1 inhibitor has an objective response rate that varies from almost nonexistent in pancreatic cancer and microsatellite-stable colonic adenocarcinoma, to an average of 15%–30% in most other tumour types, but 50%–80% in melanoma, Hodgkin lymphoma, squamous-cell carcinoma of the skin, and Merkel cell carcinoma. The addition of an anti–CTLA-4 agent increases the response rate, but comes with a significantly higher toxicity rate. A rational approach to achieving a higher response rate without increasing autoimmunity has been to combine an ICI with a therapy that can sensitize the host's immune system to the tumour in advance. Recent studies have shown that personalized neoantigen-based tumour-specific vaccines hold considerable promise.

Unlike hematologic malignancies, in which a common antigen is uniformly expressed on the surface of all malignant cells, making them amenable to targeted therapies such as therapy with chimeric antigen receptor T cells, solid tumours either lack such an antigen or undergo mutations under natural selection when exposed to therapeutic interventions such as monoclonal antibodies. Traditional cancer vaccines have failed for a number of potential reasons, including improper selection of a target antigen, lack of immunogenicity, or inadequate patient selection. In the new era of cancer vaccines, efficacy relies on computational pipelines geared to identify personal candidate neoantigens in real time. Comprehensive mutation analysis is performed by whole-exome sequencing, and based on affinity predictions, neo-epitopes encoded by somatic mutations in the tumour are selected given their probability of being presented by the individual's major histocompatibility class molecules[30–34]. One of the

most commonly used prediction algorithms for major histocompatibility class I binding, NetMHCpan (DTU Health Tech, Technical University of Denmark, Kongens Lyngby, Denmark), relies on state-of-the-art neural networks, putting the spotlight on the current power of bioinformatics for guiding precision immuno-oncology[35]. The concept has been translated to multiple phase I clinical trials evaluating neoantigen-based vaccines[36–38]. Other clinical trials are testing this novel vaccination strategy in combination with ipilimumab (NCT02950766 at https://ClinicalTrials.gov/) or nivolumab (NCT02897765), or as a personalized messenger RNA mutatome vaccine in combination with the PD-L1 inhibitor atezolizumab (NCT03289962).

The Crucial Role of the Tumour Microenvironment

An important advance in the field of immuno-oncology came from the increased understanding of the crucial role of the tumour microenvironment in the modulation of anticancer immune responses. In colorectal cancers, immune cell infiltration into the tumour microenvironment has been correlated with a strong immune response to treatment with ICIs, with even better correlation than for microsatellite instability[39,40]. Based on those findings, the concept of "immune contexture" has been proposed and validated, with tumours classified into four proposed categories (hot, excluded, immunosuppressed, and cold)[41,42]. Apart from the presence of tumour-infiltrating lymphocytes, additional features such as the expression of anti–PD-L1 on tumour-associated immune cells, genomic instability, and the presence of a pre-existing antitumour immune response have been described as characteristics of "hot" tumours, which are associated with a good response to ICIs[43]. Conversely, apart from being poorly infiltrated, "cold" tumours have also been described to be immunologically "ignorant" (scarcely expressing PD-L1) and characterized by high proliferation with a low mutational burden (low expression of neoantigens) and by low expression of antigen presentation machinery markers such as major histocompatibility class I[43]. Transforming "cold" tumours into fertile "hot" tumours responsive to ICIs is an active area of investigation.

Radiotherapy and chemotherapy have both been used in combination with ICIs to increase the antigenicity and priming potential of tumours, which in turn could be applied to turn "cold" tumours into "hot" ones. Ionizing radiation–induced immunogenic cell death and antigen release could potentially turn tumour cells into an *in situ* vaccine[44]. The outcome of that approach is not only local tumour control, but possibly a response at distant tumour sites through the abscopal effect[45]. On the other hand, chemotherapy can induce mutations, leading to the generation of neo-epitopes and therefore increasing the antigenicity of tumours[46]. Other approaches with proven benefit have been the local injection of oncolytic viruses into tumour beds. These native or genetically modified viruses selectively infect and replicate within tumour cells, eventually leading to tumour cell lysis and antigen release[47]. Again, that process results in local priming of the immune system, with responses seen both locally and systemically. The effect is accentuated when those therapies are combined with ICIs[48].

Another key targetable characteristic of "cold" tumours is strong expression of mesenchymal and collagen barrier molecules that prevent the migration of tumour-infiltrating lymphocytes to the tumour bed[49,50]. As an example, inhibition of transforming growth factor β, a key player in the formation of the mesenchymal barrier, when combined with an ICI resulted in a strong antitumour response in mouse models[51]. That approach is now being tested in clinical trials.

Finally, another strategy that can convert a "cold" to a "hot" tumour microenvironment uses inhibitors of oncogenic kinases (reviewed in Guo *et al.*[52]). The PI3K/AKT pathway, glycogen synthase kinase 3α/β, and Mnk1 and Mnk2 are often aberrantly activated in cancer, and appreciation for their tumour-extrinsic effects in the cells of the tumour microenvironment to promote immune suppression is growing. For example, Mnk1 and Mnk2, which are critical regulators of messenger RNA translation, have important immunomodulatory antitumor effects. Inhibitors of Mnk1 and Mnk2 can block the expression of secreted factors such as Nodal and Angptl4[53,54], inhibiting the survival of neutrophils[55] and suppressing the expression of PD-L1[56]. The Mnk1 and Mnk2 inhibitors are actively being pursued in the clinic (see NCT04261218, NCT03616834, and NCT03258398 at https://ClinicalTrials.gov/).

Targeting Tumour Metabolism in the Tumour Microenvironment

There is growing evidence that the tumour microenvironment supports inappropriate metabolic reprogramming, negatively affecting T cell function and resulting in attenuated antitumour immune responses[57,58]. In that context, targeting both tumour and T cell metabolism can beneficially enhance immunity in an inhospitable microenvironment and markedly improve the success of immunotherapies. As discussed earlier, TILs in the tumour microenvironment have significant prognostic and predictive significance. Their function is limited not only by immune checkpoints, but also by increasingly recognized "metabolic checkpoints"[59].

Rapidly dividing tumour cells show complex and dynamic metabolic reprogramming and high glycolytic activity, a phenomenon called the "Warburg effect," which is recognized as one of the hallmarks of carcinogenesis[60]. Thus, tumour cells impede the access of T cells to nutrients necessary for their activation and generate high levels of lactate. The resulting scarcity of nutrients and accumulation of metabolic waste products in the tumour microenvironment lead to a TIL metabolic switch that impairs optimal proliferation and function[61].

Recent evidence suggests that ICIs might directly sculpt the metabolic landscape in the tumour microenvironment, thus affecting the functioning of effector T cells. On the one hand, CTLA-4 and PD-1 binding to their respective ligands impairs the metabolic TIL phenotype by inhibiting glycolysis[62], thus causing reduced cytokine secretion and leading to an exhausted effector T cell phenotype[63]. On the other hand, ICIs also have the opposite effect on metabolic reprogramming of cancer cells. Ligation of PD-L1 directly upregulates glycolysis in cancer cells by promoting glucose uptake and production of lactate, thus promoting tumour

growth and metastasis[64,65]. Many therapeutic strategies have been proposed to tackle that imbalance.

The PI3K/AKT/mTOR pathway is well known to play a critical role in integrating the metabolism signals of cancer and immune cells. Recent preclinical evidence suggests that rapamycin, in combination with ICIs, augments cytotoxic and memory T cell function[24,66]. Another promising therapeutic is metformin in combination with ICIs. Metformin is known to target the mitochondrial respiratory complex I and to activate AMPK pathway signal transduction, a key pathway in T cell regulatory and metabolic functioning[67,68]. In a cohort of patients with metastatic melanoma who received metformin in combination with ICIs, favourable treatment-related outcomes (objective response rate, disease control rate, median progression-free survival, and median overall survival) were observed[69]. Those findings await further validation in larger randomized studies.

Besides glycolysis, another key element of metabolism dictating immune function in the tumour microenvironment is amino-acid catabolism. It is well established that L-arginine, tryptophan (Trp), and glutamine play fundamental roles in tumour progression and immunity[70]. Targeting those amino acids and their metabolic pathways in cancer therapy therefore becomes a promising strategy for the development of novel therapeutic agents. As one example, the depletion of tryptophan and the increase in kynurenine (Kyn) exert an important immunosuppressive function by activating T regulatory cells and suppressing the functioning of effector T cells[71]. The catabolic IDO1 enzyme in the Trp–Kyn–AhR metabolic pathway thus became an interesting therapeutic target. Despite promising results in early-phase clinical trials in a range of tumour types, a phase III study of the IDO1-selective inhibitor epacadostat in combination with pembrolizumab in metastatic melanoma showed no difference between the epacadostat–pembrolizumab group and the placebo–pembrolizumab group[72]. That resulted in a diminution of interest in IDO1 inhibitors; however, other approaches to inhibiting that pathway continue to be considered. Novel Trp–Kyn–AhR pathway inhibitors such as Kyn-degrading enzymes, direct AhR antagonists, and Trp mimetics are advancing in early-stage or preclinical development[73]. Despite the uncertainty surrounding IDO1 inhibition, ample preclinical evidence supports the continued development of Trp–Kyn–AhR pathway inhibitors to enhance ICI efficacy.

The Microbiome As a Master Regulator of Both ICI Efficacy and Toxicity

The host microbiome plays an important role in the efficacy of vaccine immune responses[74], the promotion of carcinogenesis[75–81], and the efficacy and toxicity of anticancer treatments[82–84], including ICIs[82,85]. A foundational study in mice showed that manipulation of the baseline flora of the gut microbiome affects melanoma growth kinetics and can enhance ICI efficacy[86]. Other preclinical studies showed that the efficacy of anti–CTLA-4 therapy can be compromised by antibiotic-induced dysbiosis or use of germ-free mice[87]. The efficacy of the ICI could be restored in antibiotic-treated mice after gavage with *Bacteroides fragilis* or *Bacteroides thetaiotaomicron* (order Burkholderiales), or both, through an enhanced IL-12–dependent type 1 T helper immune response[87]. Furthermore, analysis of stool from patients with ipilimumab-treated melanoma demonstrated selective enrichment of *B. fragilis* in clinical responders, possibly suggesting the presence of a CTLA-4 blockade–induced gut dysbiosis.

Other key studies have focused on identifying human microbiota signatures predictive of clinical ICI responses[88–92]. Patients with metastatic melanoma who were responders to anti–PD-1 therapy were shown to have enrichment of *Bifidobacterium longum, Collinsella aerofaciens,* and *Enterococcus faecium* in pre-treatment stool samples[89]. Subsequent fecal microbiota transplantation (FMT) of "responder" gut flora into germ-free mice was associated with improved melanoma tumour control through a CD8+ T cell immune response[89]. Furthermore, the ratio of "beneficial" to "non-beneficial" bacteria species in patients was the best predictor of an antitumour clinical response[89]. Another group identified enrichment of *Akkermansia muciniphila* in the microbiota of responders to anti–PD-1 or PD-L1 ICIs in 3 cancer subtypes. They further demonstrated that oral supplementation with *A. muciniphila, Alistipes indistinctus,* or *Enterococcus hirae* could restore ICI antitumour efficacy in germ-free mice colonized with bacterial species from non-responder FMT[88]. A third fundamental study showed that patients with melanoma responding to ICI had greater alpha diversity in their gut flora, with selective enrichment in order Clostridiales family *Ruminococcaceae,* especially genus *Faecalibacterium*[90]. On the other hand, the microbiomes in ICI non-responders showed a shift toward order Bacteroidales. Taken together, those studies demonstrate that the antitumour immune response depends on the composition of the gut microbiome and that antibiotic-induced dysbiosis is associated with reduced immune priming and primary resistance to immunotherapy. The deleterious effect of antibiotics given close to the time of ICI treatment was confirmed in retrospective analyses of ICI-treated patients with various cancers (renal cancer, non-small-cell lung cancer, urothelial cancer, and melanoma)[88,93–95]. Thus far, a modest overlap in key microbiome mediators of response to anti–PD-1 or PD-L1 therapies has been observed across cohorts, with an apparent common responder signature enriched in *A. muciniphila,* Clostridiales, *E. faecium, Eubacterium* species, the Firmicutes, and *Ruminococcus* species[96]. Although poor overlap between study cohorts might be a result of differences in technique or tumour type, additional heterogeneity of the gut microbiome linked to genetics, geography, lifestyle, or prior antibiotic and other drug exposure must be considered. Ultimately, "responder" gut profiles will likely reflect combinations of taxonomic orders and families rather than the presence or absence of one or a few particular species.

Besides priming the immune response to ICIs, the microbiome also modulates irAEs[17,91]. For instance, metagenomic sequencing found members of the Bacteroidetes phylum (families *Bacteroidaceae, Rikenellaceae,* and *Barnesiellaceae)* to be more abundant in stools obtained before treatment from patients with melanoma who did not develop ipilimumab-induced colitis, implying a protective effect of those microorganisms[17]. Importantly, specific microbial metabolic pathways (polyamine transport and vitamin B synthesis) were found to be predictive of resist-

ance to CTLA-4 blockade–induced colitis[17]. Another group corroborated the protective effect of a Bacteroidetes-rich phylotype against CTLA-4 blockade–induced colitis in patients with melanoma. They further demonstrated that favourable clinical responses and susceptibility to colitis were both correlated with baseline microbiota enrichment in phylum Firmicutes (unclassified *Ruminococcaceae, Clostridium* cluster XIVa, and *Blautia)* and, in particular, *Faecalibacterium,* which is known to exert an anti-inflammatory role in the gut[91]. Further, the resolution of refractory ICI-associated colitis in 2 patients with cancer was achieved by FMT from a healthy donor[97]. Thus, a critical goal of microbiome manipulation is to disentangle the modulation of toxicity from the preservation or enhancement of ICI efficacy[98].

There is a strong push to translate those newly acquired basic science findings about the microbiota into therapeutic clinical tools. Several trials are evaluating safety, efficacy, and immune profile changes in patients with ICI-resistant cancer treated with "complete responder" FMT (see NCT03353402, NCT03341143, and NCT03637803 at https://ClinicalTrials.gov/). The safety of FMT is particularly important and under scrutiny, given that FMT or bacterial colonization experiments in mice have revealed a potential for transfer of chronic diseases[99,100] or increased risk of tumorigenesis[76,101]. Probiotics—loosely defined as health-promoting live organisms or fermented foods—are so far being assessed mostly in clinical trials aiming to reduce anticancer treatment toxicities; only one registered trial is testing their efficacy in the context of ICIs (NCT03829111). The optimal probiotic cocktail for immunotherapy remains to be determined and might vary with the ICI or the tumour type. Additionally, reliable preparation of probiotics will be essential, likely requiring changes to their regulation. Ultimately, clinical studies of the effects of the microbiome on ICI efficacy or toxicity will have to consider other confounding factors known to affect the commensal microbiome, such as concomitant radiation therapy[102], exposure to antibiotics or other drugs (proton-pump inhibitors, antipsychotics, antimetabolites)[103], and diet (including method and composition).

SUMMARY

Cancer immunotherapy has dramatically changed survival and quality of life for patients. However, not all cancers are equal, and very few predictors of response and toxicity currently exist. Despite the rapid advances made in the field, immuno-oncology is still in its relative infancy, with numerous challenges and hurdles yet to be overcome. Over time, a realization grew that the standard tools used to assess choice of treatments in the era of chemotherapy and targeted therapies might not be valid for the new immunotherapies. As an example, the Response Evaluation Criteria in Solid Tumors (RECIST) used to assess response to treatments were modified to create iRECIST, which accounts for the novel patterns of response seen during immunotherapy, including tumour pseudoprogression[104]. In the same way that TNM staging has been crucial in guiding treatments in the era of chemotherapy, novel tools are required in the era of cancer immunotherapy. The Immunoscore has already been validated as adding important prognostic information to TNM staging in colon cancer[39]. The fact that T cells are currently widely recognized as the key mediators of antitumour efficacy with ICI treatment suggests that use of the Immunoscore is an attractive option to help guide treatment selection in other cancer types as well. Still, that option does not exclude the possible use of additional parameters that might provide further insights into the specifics of each case.

It is becoming more challenging to increase the efficacy of combination therapies already established in clinical practice. In metastatic melanoma, combined CTLA-4 and PD-1 blockade has achieved an unprecedented five-year overall survival above 50%[105]. In metastatic renal cell carcinoma, the same combination has been associated with an overall survival rate exceeding 60% at 3 years in the intention-to-treat population[106,107]. In the large landscape of ongoing early-phase clinical trials, few novel combinations have achieved a level of efficacy rivalling those new standards of care. What certainly remains to be improved is their safety profiles.

The approved induction and regimen dose of combination ICIs (ipilimumab 3 mg/kg and nivolumab 1 mg/kg every 3 weeks) in the setting of melanoma is associated with a 59% rate of grades 3–4 toxicities[108]. Preliminary results from CheckMate 511, which used alternative dosing (ipilimumab 1 mg/kg and nivolumab 3 mg/kg every 3 weeks), showed a significant improvement in toxicity without loss of efficacy[109]. Given that irAEs can sometimes be associated with mortality and significant lifelong morbidity (for example, *de novo* insulin-dependent diabetes, persistent pituitary dysfunction, or immune-related inflammatory arthropathies), predictors and novel strategies to abate those toxicities are urgently needed.

Another area of urgent need is to find novel treatments both for patients who are primary non-responders to ICIs and for those who develop secondary resistance to those therapies. Beyond ICI failure, very few treatments have been studied, and physicians often rely on previously validated standards of care for each specific cancer. Early observational data suggest that exposure to ICIs might modulate the response to standard treatments received after progression. For instance, exceptionally high response rates to chemotherapy have occasionally been documented after ICI failure[110,111]. Those observations might be secondary to immunotherapy having removed the inhibition initially exerted by tumour cells or other immune cells, followed by cytotoxic chemotherapy–mediated killing of tumour cells. On the other hand, progression-free survival and the adverse event profiles associated with exposure to targeted therapies (such as BRAF inhibition in melanoma) might be adversely affected by first-line exposure to ICIs[112].

To summarize, the future of cancer immunotherapy could rely on combination therapies using checkpoint inhibitors not with other novel checkpoint inhibitors, but rather with personalized cancer vaccines and novel targeted therapies directed at the tumour microenvironment, tumour glycosylation, and the host microbiome, as outlined in the present review. Advances in those fields will allow movement away from the current broad "shotgun" approach, which exposes all comers within the approved

indications to ICIs, to treatments tailored to the factors that make each cancer and host a unique pairing.

CONFLICT OF INTEREST DISCLOSURES

We have read and understood *Current Oncology*'s policy on disclosing conflicts of interest, and we declare the following interests: WHM reports personal fees from Bristol–Myers Squibb, Merck, Roche, Novartis, and Amgen outside the submitted work; and KE reports personal fees from Bristol–Myers Squibb outside the submitted work. LR and NB have no conflicts of interest to disclose.

AUTHOR AFFILIATIONS

*Departments of Medicine and Oncology, Segal Cancer Centre, Sir Mortimer B. Davis Jewish General Hospital, Rossy Cancer Network, McGill University, and †Department of Oncology, Lady Davis Institute, Sir Mortimer B. Davis Jewish General Hospital, McGill University, Montreal, QC.

REFERENCES

1. McCarthy EF. The toxins of William B. Coley and the treatment of bone and soft-tissue sarcomas. *Iowa Orthop J* 2006; 26:154–8.
2. Decker WK, Safdar A. Bioimmunoadjuvants for the treatment of neoplastic and infectious disease: Coley's legacy revisited. *Cytokine Growth Factor Rev* 2009;20:271–81.
3. Dinarello CA. Historical insights into cytokines. *Eur J Immunol* 2007;37(suppl 1):S34–45.
4. Lee S, Margolin K. Cytokines in cancer immunotherapy. *Cancers (Basel)* 2011;3:3856–93.
5. Rosenberg SA. Interleukin 2 for patients with renal cancer. *Nat Clin Pract Oncol* 2007;4:497. [Comment on: Twardowski P, Figlin RA. What are the indications for sorafenib treatment in patients with renal cell carcinoma? *Nat Clin Pract Oncol* 2007;4:456–7; and Stadler WM, Szmulewitz RZ. Sunitinib—a new standard of care for metastatic renal cell carcinoma. *Nat Clin Pract Oncol* 2007;4:458–9]
6. Di Trolio R, Simeone E, Di Lorenzo G, Buonerba C, Ascierto PA. The use of interferon in melanoma patients: a systematic review. *Cytokine Growth Factor Rev* 2015;26:203–12.
7. O'Donnell JS, Teng MWL, Smyth MJ. Cancer immunoediting and resistance to T cell-based immunotherapy. *Nat Rev Clin Oncol* 2019;16:151–67.
8. Suntharalingam G, Perry MR, Ward S, *et al.* Cytokine storm in a phase 1 trial of the anti-CD28 monoclonal antibody TGN1412. *N Engl J Med* 2006;355:1018–28.
9. Hodi FS, Chiarion-Sileni V, Gonzalez R, *et al.* Nivolumab plus ipilimumab or nivolumab alone versus ipilimumab alone in advanced melanoma (CheckMate 067): 4-year outcomes of a multicentre, randomised, phase 3 trial. *Lancet Oncol* 2018;19:1480–92.
10. Wang DY, Salem JE, Cohen JV, *et al.* Fatal toxic effects associated with immune checkpoint inhibitors: a systematic review and meta-analysis. *JAMA Oncol* 2018;4:1721–8.
11. Moslehi JJ, Salem JE, Sosman JA, Lebrun-Vignes B, Johnson DB. Increased reporting of fatal immune checkpoint inhibitor–associated myocarditis. *Lancet* 2018;391:933.
12. Schindler K, Harmankaya K, Kuk D, *et al.* Correlation of absolute and relative eosinophil counts with immune-related adverse events in melanoma patients treated with ipilimumab [abstract 9096]. *J Clin Oncol* 2014;32:. [Available online at: https://ascopubs.org/doi/abs/10.1200/jco.2014.32.15_suppl.9096; cited 9 September 2019]
13. Liudahl SM, Coussens LM. B Cells as biomarkers: predicting immune checkpoint therapy adverse events. *J Clin Invest* 2018;128:577–9.
14. Oh DY, Cham J, Zhang L, *et al.* Immune toxicities elicited by CTLA-4 blockade in cancer patients are associated with early diversification of the T-cell repertoire. *Cancer Res* 2017; 77:1322–30.
15. Diehl A, Yarchoan M, Hopkins A, Jaffee E, Grossman SA. Relationships between lymphocyte counts and treatment-related toxicities and clinical responses in patients with solid tumors treated with PD-1 checkpoint inhibitors. *Oncotarget* 2017;8:114268–80.
16. Callahan MK, Yang A, Tandon S, *et al.* Evaluation of serum IL-17 levels during ipilimumab therapy: correlation with colitis [abstract 2505]. *J Clin Oncol* 2011;29:. [Available online at: https://ascopubs.org/doi/10.1200/jco.2011.29.15_suppl.2505; cited 9 September 2019]
17. Dubin K, Callahan MK, Ren B, *et al.* Intestinal microbiome analyses identify melanoma patients at risk for checkpoint-blockade–induced colitis. *Nat Commun* 2016;7:10391.
18. Brahmer JR, Lacchetti C, Schneider BJ, *et al.* on behalf of the National Comprehensive Cancer Network. Management of immune-related adverse events in patients treated with immune checkpoint inhibitor therapy: American Society of Clinical Oncology clinical practice guideline. *J Clin Oncol* 2018;36:1714–68.
19. Haanen JBAG, Carbonnel F, Robert C, *et al.* on behalf of the ESMO Guidelines Committee. Management of toxicities from immunotherapy: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2018; 29(suppl 4):iv264–6.
20. Puzanov I, Diab A, Abdallah K, *et al.* on behalf of the Society for Immunotherapy of Cancer Toxicity Management Working Group. Managing toxicities associated with immune checkpoint inhibitors: consensus recommendations from the Society for Immunotherapy of Cancer (SITC) Toxicity Management Working Group. *J Immunother Cancer* 2017;5:95.
21. Arbour KC, Mezquita L, Long N, *et al.* Impact of baseline steroids on efficacy of programmed cell death–1 and programmed death–ligand 1 blockade in patients with non-small-cell lung cancer. *J Clin Oncol* 2018;36:2872–8.
22. Faje AT, Lawrence D, Flaherty K, *et al.* High-dose glucocorticoids for the treatment of ipilimumab-induced hypophysitis is associated with reduced survival in patients with melanoma. *Cancer* 2018;124:3706–14.
23. Petrelli F, Signorelli D, Ghidini M, *et al.* Association of steroids use with survival in patients treated with immune checkpoint inhibitors: a systematic review and meta-analysis. *Cancers (Basel)* 2020;12:pii:E546.
24. Esfahani K, Al-Aubodah TA, Thebault P, *et al.* Targeting the mTOR pathway uncouples the efficacy and toxicity of PD-1 blockade in renal transplantation. *Nat Commun* 2019; 10:4712.
25. Barnett R, Barta VS, Jhaveri KD. Preserved renal-allograft function and the PD-1 pathway inhibitor nivolumab. *N Engl J Med* 2017;376:191–2.
26. Sadaat M, Jang S. Complete tumor response to pembrolizumab and allograft preservation in renal allograft recipient on immunosuppressive therapy. *J Oncol Pract* 2018;14:198–9.
27. Esfahani K, Miller WH Jr. Reversal of autoimmune toxicity and loss of tumor response by interleukin-17 blockade. *N Engl J Med* 2017;376:1989–91.
28. Johnson DH, Zobniw CM, Trinh VA, *et al.* Infliximab associated with faster symptom resolution compared with corticosteroids alone for the management of immune-related enterocolitis. *J Immunother Cancer* 2018;6:103. [Erratum in: *J Immunother Cancer* 2019;7:107]
29. Bertrand F, Montfort A, Marcheteau E, *et al.* TNFα blockade overcomes resistance to anti–PD-1 in experimental melanoma. *Nat Commun* 2017;8:2256.
30. van Rooij N, van Buuren MM, Philips D, *et al.* Tumor exome analysis reveals neoantigen-specific T-cell reactivity in

an ipilimumab-responsive melanoma. *J Clin Oncol* 2013; 31:e439–42.
31. Robbins PF, Lu YC, El-Gamil M, *et al.* Mining exomic sequencing data to identify mutated antigens recognized by adoptively transferred tumor-reactive T cells. *Nat Med* 2013;19:747–52.
32. Matsushita H, Vesely MD, Koboldt DC, *et al.* Cancer exome analysis reveals a T-cell–dependent mechanism of cancer immunoediting. *Nature* 2012;482:400–4.
33. Castle JC, Kreiter S, Diekmann J, *et al.* Exploiting the mutanome for tumor vaccination. *Cancer Res* 2012;72:1081–91.
34. Hu Z, Ott PA, Wu CJ. Towards personalized, tumour-specific, therapeutic vaccines for cancer. *Nat Rev Immunol* 2018; 18:168–82.
35. Nielsen M, Andreatta M. NetMHCpan-3.0; improved prediction of binding to MHC class I molecules integrating information from multiple receptor and peptide length datasets. *Genome Med* 2016;8:33.
36. Sahin U, Derhovanessian E, Miller M, *et al.* Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer. *Nature* 2017;547:222–6.
37. Carreno BM, Magrini V, Becker-Hapak M, *et al.* Cancer immunotherapy. A dendritic cell vaccine increases the breadth and diversity of melanoma neoantigen–specific T cells. *Science* 2015;348:803–8.
38. Ott PA, Hu Z, Keskin DB, *et al.* An immunogenic personal neoantigen vaccine for patients with melanoma. *Nature* 2017;547:217–21.
39. Pages F, Mlecnik B, Marliot F, *et al.* International validation of the consensus Immunoscore for the classification of colon cancer: a prognostic and accuracy study. *Lancet* 2018;391:2128–39.
40. Camus M, Tosolini M, Mlecnik B, *et al.* Coordination of intratumoral immune reaction and human colorectal cancer recurrence. *Cancer Res* 2009;69:2685–93.
41. Gajewski TF, Corrales L, Williams J, Horton B, Sivan A, Spranger S. Cancer immunotherapy targets based on understanding the T cell–inflamed versus non–T cell–inflamed tumor microenvironment. *Adv Exp Med Biol* 2017;1036:19–31.
42. Fridman WH, Zitvogel L, Sautès-Fridman C, Kroemer G. The immune contexture in cancer prognosis and treatment. *Nat Rev Clin Oncol* 2017;14:717–34.
43. Hegde PS, Karanikas V, Evers S. The where, the when, and the how of immune monitoring for cancer immunotherapies in the era of checkpoint inhibition. *Clin Cancer Res* 2016;22:1865–74.
44. Golden EB, Apetoh L. Radiotherapy and immunogenic cell death. *Semin Radiat Oncol* 2015;25:11–17.
45. Liu Y, Dong Y, Kong L, Shi F, Zhu H, Yu J. Abscopal effect of radiotherapy combined with immune checkpoint inhibitors. *J Hematol Oncol* 2018;11:104.
46. McGranahan N, Furness AJ, Rosenthal R, *et al.* Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade. *Science* 2016;351:1463–9.
47. Kaufman HL, Kohlhapp FJ, Zloza A. Oncolytic viruses: a new class of immunotherapy drugs. *Nat Rev Drug Discov* 2015;14:642–62. [Erratum in: *Nat Rev Drug Discov* 2016;15:143]
48. Conry RM, Westbrook B, McKee S, Norwood TG. Talimogene laherparepvec: first in class oncolytic virotherapy. *Hum Vaccin Immunother* 2018;14:839–46.
49. Spranger S, Bao R, Gajewski TF. Melanoma-intrinsic beta-catenin signalling prevents anti-tumour immunity. *Nature* 2015;523:231–5.
50. Ros XR, Vermeulen L. Turning cold tumors hot by blocking TGF-β. *Trends Cancer* 2018;4:335–7.
51. Ganesh K, Massague J. TGF-β inhibition and immunotherapy: checkmate. *Immunity* 2018;48:626–8. [Comment on: Mariathasan S, Turley SJ, Nickles D, *et al.* TGFβ attenuates tumour response to PD-L1 blockade by contributing to exclusion of T cells. *Nature* 2018;554:544–8]
52. Guo Q, Huang F, Goncalves C, Del Rincón SV, Miller WH Jr. Translation of cancer immunotherapy from the bench to the bedside. *Adv Cancer Res* 2019;143:1–62.
53. Guo Q, Li VZ, Nichol JN, *et al.* Mnk1/Nodal signaling promotes invasive progression of breast ductal carcinoma *in situ*. *Cancer Res* 2019;79:1646–57.
54. Yang W, Khoury E, Guo Q, *et al.* Mnk1 signaling induces an Angptl4-mediated gene signature to drive melanoma progression. *Oncogene* 2020;:[Epub ahead of print].
55. Robichaud N, Hsu BE, Istomine R, *et al.* Translational control in the tumor microenvironment promotes lung metastasis: phosphorylation of eIF4E in neutrophils. *Proc Natl Acad Sci U S A* 2018;115:E2202–9.
56. Xu Y, Poggio M, Jin HY, *et al.* Translation control of the immune checkpoint in cancer and its therapeutic targeting. *Nat Med* 2019;25:301–11.
57. Martinez-Outschoorn UE, Peiris-Pagés M, Pestell RG, Sotgia F, Lisanti MP. Cancer metabolism: a therapeutic perspective. *Nat Rev Clin Oncol* 2017;14:11–31. [Erratum in: Martinez-Outschoorn UE, Peiris-Pagés M, Pestell RG, Sotgia F, Lisanti MP. Cancer metabolism: a therapeutic perspective. *Nat Rev Clin Oncol* 2017;14:113]
58. Kouidhi S, Elgaaied AB, Chouaib S. Impact of metabolism on T-cell differentiation and function and cross talk with tumor microenvironment. *Front Immunol* 2017;8:270.
59. Scharping NE, Delgoffe GM. Tumor microenvironment metabolism: a new checkpoint for anti-tumor immunity. *Vaccines (Basel)* 2016;4:pii:E46.
60. Kareva I, Hahnfeldt P. The emerging "hallmarks" of metabolic reprogramming and immune evasion: distinct or linked? *Cancer Res* 2013;73:2737–42.
61. Qorraj M, Böttcher M, Mougiakakos D. PD-L1/PD-1: new kid on the "immune metabolic" block [editorial]. *Oncotarget* 2017;8:73364–5.
62. Patsoukis N, Bardhan K, Chatterjee P, *et al.* PD-1 alters T-cell metabolic reprogramming by inhibiting glycolysis and promoting lipolysis and fatty acid oxidation. *Nat Commun* 2015;6:6692.
63. Saunders PA, Hendrycks VR, Lidinsky WA, Woods ML. PD-L2:PD-1 involvement in T cell proliferation, cytokine production, and integrin-mediated adhesion. *Eur J Immunol* 2005;35:3561–9.
64. Wangpaichitr M, Kandemir H, Li YY, *et al.* Relationship of metabolic alterations and PD-L1 expression in cisplatin resistant lung cancer. *Cell Dev Biol* 2017;6:pii:183.
65. Chang CH, Qiu J, O'Sullivan D, *et al.* Metabolic competition in the tumor microenvironment is a driver of cancer progression. *Cell* 2015;162:1229–41.
66. Mineharu Y, Kamran N, Lowenstein PR, Castro MG. Blockade of mTOR signaling via rapamycin combined with immunotherapy augments antiglioma cytotoxic and memory T-cell functions. *Mol Cancer Ther* 2014;13:3024–36.
67. Rena G, Pearson ER, Sakamoto K. Molecular mechanism of action of metformin: old or new insights? *Diabetologia* 2013;56:1898–906.
68. Ma EH, Poffenberger MC, Wong AH, Jones RG. The role of AMPK in T cell metabolism and function. *Curr Opin Immunol*. 2017;46:45–52.
69. Afzal MZ, Mercado RR, Shirai K. Efficacy of metformin in combination with immune checkpoint inhibitors (anti–PD-1/anti–CTLA-4) in metastatic malignant melanoma. *J Immunother Cancer* 2018;6:64.
70. Lukey MJ, Katt WP, Cerione RA. Targeting amino acid metabolism for cancer therapy. *Drug Discov Today* 2017;22:796–804.

71. Liu M, Wang X, Wang L, *et al.* Targeting the IDO1 pathway in cancer: from bench to bedside. *J Hematol Oncol* 2018;11:100.
72. Long GV, Dummer R, Hamid O, *et al.* Epacadostat plus pembrolizumab versus placebo plus pembrolizumab in patients with unresectable or metastatic melanoma (ECHO-301/KEYNOTE-252): a phase 3, randomised, double-blind study. *Lancet Oncol* 2019;20:1083–97.
73. Labadie BW, Bao R, Luke JJ. Reimagining IDO pathway inhibition in cancer immunotherapy via downstream focus on the tryptophan–kynurenine–aryl hydrocarbon axis. *Clin Cancer Res* 2019;25:1462–71.
74. Zimmermann P, Curtis N. The influence of the intestinal microbiome on vaccine responses. *Vaccine* 2018;36:4433–9.
75. Yoshimoto S, Loo TM, Atarashi K, *et al.* Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. *Nature* 2013;499:97–101.
76. Dejea CM, Fathi P, Craig JM, *et al.* Patients with familial adenomatous polyposis harbor colonic biofilms containing tumorigenic bacteria. *Science* 2018;359:592–7.
77. Di Domenico EG, Cavallo I, Pontone M, Toma L, Ensoli F. Biofilm producing *Salmonella* typhi: chronic colonization and development of gallbladder cancer. *Int J Mol Sci* 2017;18:pii:E1887.
78. Wang F, Meng W, Wang B, Qiao L. *Helicobacter pylori*–induced gastric inflammation and gastric cancer. *Cancer Lett* 2014;345:196–202.
79. Wu S, Rhee KJ, Albesiano E, *et al.* A human colonic commensal promotes colon tumorigenesis via activation of T helper type 17 T cell responses. *Nat Med* 2009;15:1016–22.
80. Rubinstein MR, Baik JE, Lagana SM, *et al. Fusobacterium nucleatum* promotes colorectal cancer by inducing Wnt/beta-catenin modulator annexin A1. *EMBO Rep* 2019;20:pii:e47638.
81. Kostic AD, Chun E, Robertson L, *et al. Fusobacterium nucleatum* potentiates intestinal tumorigenesis and modulates the tumor-immune microenvironment. *Cell Host Microbe* 2013;14:207–15.
82. Iida N, Dzutsev A, Stewart CA, *et al.* Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment. *Science* 2013;342:967–70.
83. Viaud S, Saccheri F, Mignot G, *et al.* The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide. *Science* 2013;342:971–6.
84. Geller LT, Barzily-Rokni M, Danino T, *et al.* Potential role of intratumor bacteria in mediating tumor resistance to the chemotherapeutic drug gemcitabine. *Science* 2017;357:1156–60.
85. McQuade JL, Daniel CR, Helmink BA, Wargo JA. Modulating the microbiome to improve therapeutic response in cancer. *Lancet Oncol* 2019;20:e77–91.
86. Sivan A, Corrales L, Hubert N, *et al.* Commensal *Bifidobacterium* promotes antitumour immunity and facilitates anti–PD-L1 efficacy. *Science* 2015;350:1084–9.
87. Vetizou M, Pitt JM, Daillere R, *et al.* Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. *Science* 2015;350:1079–84.
88. Routy B, Le Chatelier E, Derosa L, *et al.* Gut microbiome influences efficacy of PD-1–based immunotherapy against epithelial tumors. *Science* 2018;359:91–7.
89. Matson V, Fessler J, Bao R, *et al.* The commensal microbiome is associated with anti–PD-1 efficacy in metastatic melanoma patients. *Science* 2018;359:104–8.
90. Gopalakrishnan V, Spencer CN, Nezi L, *et al.* Gut microbiome modulates response to anti–PD-1 immunotherapy in melanoma patients. *Science* 2018;359:97–103.
91. Chaput N, Lepage P, Coutzac C, *et al.* Baseline gut microbiota predicts clinical response and colitis in metastatic melanoma patients treated with ipilimumab. *Ann Oncol* 2017;28:1368–79.
92. Frankel AE, Coughlin LA, Kim J, *et al.* Metagenomic shotgun sequencing and unbiased metabolomic profiling identify specific human gut microbiota and metabolites associated with immune checkpoint therapy efficacy in melanoma patients. *Neoplasia* 2017;19:848–55.
93. Derosa L, Hellmann MD, Spaziano M, *et al.* Negative association of antibiotics on clinical activity of immune checkpoint inhibitors in patients with advanced renal cell and non-small-cell lung cancer. *Ann Oncol* 2018;29:1437–44.
94. Hakozaki T, Okuma Y, Omori M, Hosomi Y. Impact of prior antibiotic use on the efficacy of nivolumab for non–small cell lung cancer. *Oncol Lett* 2019;17:2946–52.
95. Elkrief A, El Raichani L, Richard C, *et al.* Antibiotics are associated with decreased progression-free survival of advanced melanoma patients treated with immune checkpoint inhibitors. *Oncoimmunology* 2019;8:e1568812.
96. Derosa L, Routy B, Kroemer G, Zitvogel L. The intestinal microbiota determines the clinical efficacy of immune checkpoint blockers targeting PD-1/PD-L1. *Oncoimmunology* 2018;7:e1434468.
97. Wang Y, Wiesnoski DH, Helmink BA, *et al.* Fecal microbiota transplantation for refractory immune checkpoint inhibitor–associated colitis. *Nat Med* 2018;24:1804–8. [Erratum in: *Nat Med* 2019;25:188]
98. Wang F, Yin Q, Chen L, Davis MM. Bifidobacterium can mitigate intestinal immunopathology in the context of CTLA-4 blockade. *Proc Natl Acad Sci U S A* 2018;115:157–61.
99. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 2006;444:1027–31.
100. Ridaura VK, Faith JJ, Rey FE, *et al.* Gut microbiota from twins discordant for obesity modulate metabolism in mice. *Science* 2013;341:1241214.
101. Wong SH, Zhao L, Zhang X, *et al.* Gavage of fecal samples from patients with colorectal cancer promotes intestinal carcinogenesis in germ-free and conventional mice. *Gastroenterology* 2017;153:1621–33.
102. Gerassy-Vainberg S, Blatt A, Danin-Poleg Y, *et al.* Radiation induces proinflammatory dysbiosis: transmission of inflammatory susceptibility by host cytokine induction. *Gut* 2018;67:97–107.
103. Maier L, Pruteanu M, Kuhn M, *et al.* Extensive impact of non-antibiotic drugs on human gut bacteria. *Nature* 2018;555:623–8.
104. Seymour L, Bogaerts J, Perrone A, *et al.* on behalf of the RECIST working group. iRECIST: guidelines for response criteria for use in trials testing immunotherapeutics. *Lancet Oncol* 2017;18:e143–52.
105. Larkin J, Chiarion-Sileni V, Gonzalez R, *et al.* Five-year survival with combined nivolumab and ipilimumab in advanced melanoma. *N Engl J Med* 2019;381:1535–46.
106. Motzer RJ, Rini BI, McDermott DF, *et al.* on behalf of the CheckMate 214 investigators. Nivolumab plus ipilimumab versus sunitinib in first-line treatment for advanced renal cell carcinoma: extended follow-up of efficacy and safety results from a randomised, controlled, phase 3 trial. *Lancet Oncol* 2019;20:1370–85. [Erratum in: Correction to *Lancet Oncol* 2019;20:1370–85. *Lancet Oncol* 2019;20:e559]
107. Motzer RJ, Tannir NM, McDermott DF, *et al.* on behalf of the CheckMate 214 investigators. Nivolumab plus ipilimumab versus sunitinib in advanced renal-cell carcinoma. *N Engl J Med* 2018;378:1277–90.
108. Wolchok JD, Chiarion-Sileni V, Gonzalez R, *et al.* Overall survival with combined nivolumab and ipilimumab in advanced melanoma. *N Engl J Med* 2017;377:1345–56. [Erratum in: Neoadjuvant PD-1 blockade in resectable lung cancer; Nivolumab and ipilimumab in advanced melanoma; Overall

survival with combined nivolumab and ipilimumab in advanced melanoma; Prolonged survival in stage III melanoma with ipilimumab adjuvant therapy; Combined nivolumab and ipilimumab or monotherapy in untreated melanoma; Combined nivolumab and ipilimumab or monotherapy in untreated melanoma; Nivolumab and ipilimumab versus ipilimumab in untreated melanoma; Rapid eradication of a bulky melanoma mass with one dose of immunotherapy; Genetic basis for clinical response to CTLA-4 blockade; Genetic basis for clinical response to CTLA-4 blockade in melanoma; Nivolumab plus ipilimumab in advanced melanoma; Safety and tumor responses with lambrolizumab (anti–PD-1) in melanoma; Hepatotoxicity with combination of vemurafenib and ipilimumab. *N Engl J Med* 2018;379:2185]

109. Lebbé C, Meyer N, Mortier L, *et al.* Evaluation of two dosing regimens for nivolumab in combination with ipilimumab in patients with advanced melanoma: results from the phase IIIB/IV CheckMate 511 trial. *J Clin Oncol* 2019;37:867–75.
110. Dwary AD, Master S, Patel A, *et al.* Excellent response to chemotherapy post immunotherapy. *Oncotarget* 2017;8:91795–802.
111. Aguilera JV, Paludo J, Bangalore A, *et al.* Chemoimmunotherapy combination after PD-1 inhibitor failure to improve clinical outcomes in metastatic melanoma patients [abstract 9558]. *J Clin Oncol* 2018;36:. [Available online at: https://ascopubs.org/doi/abs/10.1200/JCO.2018.36.15_suppl.9558; cited 9 September 2019]
112. Xia CY, Wang DY, Mason R, *et al.* Activity of targeted therapy after failure of first-line immunotherapy in *BRAF*-mutant metastatic melanoma [abstract 9532]. *J Clin Oncol* 2018;36:. [Available online at: https://ascopubs.org/doi/abs/10.1200/JCO.2018.36.15_suppl.9532; cited 9 September 2019]