Immunity
Review

Resistance Mechanisms to Immune-Checkpoint Blockade in Cancer: Tumor-Intrinsic and -Extrinsic Factors

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Inhibition of immune regulatory checkpoints, such as CTLA-4 and the PD-1-PD-L1 axis, is at the forefront of immunotherapy for cancers of various histological types. However, such immunotherapies fail to control neoplasia in a significant proportion of patients. Here, we review how a range of cancer-cell-autonomous cues, tumor-microenvironmental factors, and host-related influences might account for the heterogeneous responses and failures often encountered during therapies using immune-checkpoint blockade. Furthermore, we describe the emerging evidence of how the strong interrelationship between the immune system and the host microbiota can determine responses to cancer therapies, and we introduce a concept by which prior or concomitant modulation of the gut microbiome could optimize therapeutic outcomes upon immune-checkpoint blockade.

Introduction

There is now abundant evidence indicating that natural and/or therapy-induced immune responses against cancer antigens dictate better prognoses for patients across diverse histological types of neoplasia. Intratumoral infiltration with a range of immune cell populations correlates with favorable patient outcome (Bindea et al., 2013; Fridman et al., 2012; Galon et al., 2006; Kroemer et al., 2015) to the extent that analyses of the type, functional orientation, density, and spatial location of tumor-infiltrating lymphocytes (TILs) within distinct tumor regions have been developed into prognostic or predictive immune scoring systems for routine clinical practice (Galon et al., 2013). Such clinical observations, backed by extensive experimental evidence, underscore the concept of cancer immunosurveillance, where emerging tumors are generally eradicated by the immune system except under circumstances where cancer cells have evolved to escape immune detection. This can occur via the surplus sources of immunosuppression in the tumor microenvironment (TME) (Joyce and Fearon, 2015) or through immunoediting (Matsushita et al., 2012; Schreiber et al., 2011), in which the immune system itself participates in the selection of immune-resistant cancer cell clones.

The unprecedented rise and success of cancer immunotherapy over the past decade—a direct result of the concept of immunosurveillance—has revolutionized the clinical management of a wide array of malignancies that were previously endowed with dismal prognosis. At the forefront of immunotherapy development are immune-checkpoint blockers (ICBs), which have seen enormous and unparalleled success in cancer therapy as a result of their broad bioactivity across many histological tumor types, the durability of their responses, and cures observed even in metastatic and chemoresistant diseases. Among the checkpoint-blocking strategies, the two most prominent (in terms of their clinical success to date) are the targeting of cytotoxic-T-lymphocyte-associated protein 4 (CTLA-4) and the interaction between programmed cell death 1 (PD-1) and programmed cell death ligand 1 (PD-L1).

Early studies in the field of T cell regulation identified the expression of *Ctla4* (the gene encoding CTLA-4) upon T cell activation. CTLA-4 has very high structural homology to the costimulatory molecule CD28, and it could also bind B7 molecules present on antigen-presenting cells (APCs) with much higher affinity and avidity than CD28 (Schildberg et al., 2016; Sharma and Allison, 2015a). CTLA-4 was subsequently found to be a competitive antagonist of the CD28-B7 interaction in that it effectively blocks costimulation at the T-cell-APC interface and thus abrogates activation of T cell responses (Krummel and

Allison, 1995; Walunas et al., 1994). The knowledge of the function of CTLA-4 led to the hypothesis that blocking its action could allow T cell responses to persist, which had implications for developing an understanding of tumor immunology around that time. Several lines of preclinical evidence supported this concept, leading to the generation of ipilimumab, a monoclonal antibody (mAb) against human CTLA-4 for clinical trial (Leach et al., 1996; Sharma and Allison, 2015a, 2015b). In addition to having a recognized function in facilitating costimulation, therapy with anti-CTLA-4 mAb can also deplete regulatory T (Treg) cells from the TME, presumably because of the high expression of CTLA-4 on the surface of this immunosuppressive T cell population, resulting in released suppression of anti-tumor cytotoxic T lymphocyte (CTL) activity (Simpson et al., 2013). The preclinical and clinical successes of CTLA-4 blockade, accompanied by the discoveries of the many additional immune checkpoints, pioneered a new field of immune-checkpoint therapy, which brought forth the second significant targetable pathway in today's cancer armamentarium: the PD-1-PD-L1 axis.

The function of PD-1 as an immune checkpoint was established upon identification of one of its ligands, PD-L1 (Freeman et al., 2000). PD-1, like CTLA-4, is expressed on activated T cells, and its activation has been shown to downregulate signaling mediated on antigen recognition by the T cell receptor (Fourcade et al., 2010; Sakuishi et al., 2010; Sharma and Allison, 2015a; Woo et al., 2012; Zou et al., 2016). PD-1 has two ligands, PD-L1 and PD-L2. PD-L2 is predominantly expressed on APCs, whereas PD-L1 can be expressed on many cell types, including tumor cells, immune cells, epithelial cells, and endothelial cells (Sharma and Allison, 2015b; Zou et al., 2016). PD-L1 expression has been associated with exposure to interferon-γ (IFN-γ; e.g., after anti-tumor Th1 cell responses; Dong et al., 2002; Gao et al., 2009) and provides an efficient means for tumor cells to evade T cell immunosurveillance. The PD-1 pathway is further complicated by its known interactions with other ligands and receptors; for example, PD-L1 can bind CD80 molecules on the surface of T cells, and evidence suggests that PD-L2 is capable of binding T-cell-expressed RGMb (Xiao et al., 2014; Zou et al., 2016). The relationship among PD-L1, PD-L2, PD-1, CD80, and RGMb in terms of their interactions, expression regulation, cellular expression profile, and functional relevance must still be defined, yet it might yield new targets for checkpoint blockade (Zou et al., 2016).

To date, four ICBs have been approved by the FDA: (1) ipilimumab, a CTLA-4-blocking mAb approved for unresectable or metastatic melanoma; (2) pembrolizumab, a PD-1-blocking mAb licensed for individuals with unresectable metastatic melanoma or individuals with advanced metastatic non-small-cell lung carcinoma (NSCLC) whose tumors express PD-L1; (3) nivolumab, a PD-1-targeted mAb approved for unresectable or metastatic melanoma, advanced metastatic NSCLC progressing with or following platinum-based chemotherapy, and advanced (metastatic) renal cell carcinoma; and (4) atezolizumab, a PD-L1-targeted mAb recently approved for treatment of locally advanced or metastatic urothelial carcinoma not responding to platinum-based chemotherapy (Sharma and Allison, 2015b; Zou et al., 2016). These approved ICBs are likely to receive regulatory approval for treatment of additional cancers, and furthermore, other ICBs are likely to join this new class of therapeutics (Buqué et al., 2015). Notable clinically advanced examples include PD-L1-targeting ICBs that, similarly to the approved ICBs, have induced remarkable tumor regressions in melanoma, NSCLC, and renal cell carcinoma (Brahmer et al., 2012; Zou et al., 2016). Although head-to-head comparisons have not been performed, the levels of clinical response and toxicities following therapies with anti-PD-1 and anti-PD-L1 mAbs appear to be generally consistent.

Given the exceptional improvements in objective response rates, time to progression, and overall survival in some patients, it is an unfortunate fact that often the majority of patients fail to respond to ICBs or must stop their treatment (in some cases despite encouraging regressions) because of the development of immune-related adverse events (irAEs). For ICBs, although the metrics are unprecedentedly successful, the clinical data thus far indicate that the response rates for patients treated with ipilimumab are approximately 15% (Hodi et al., 2010; Robert et al., 2011); those following targeting of the PD-1-PD-L1 axis rarely exceed 40% (and are often considerably beneath this figure), and there are a large number of partial responders (Brahmer et al., 2012; Hamid et al., 2013; Topalian et al., 2012). Consequently, there are two overriding questions: (1) why is there a degree of heterogeneity in responses to ICBs, and (2) how can ICB coverage be extended to the majority of cancer patients who do not see control or regression of their cancer?

The answers to these questions will be revealed, to some extent, with further in-depth understanding and investigative targeting of immunoregulatory mechanisms within the TME. Many factors operating within the TME inhibit the therapeutic activities of ICBs (and immunotherapies in general), for example, through the contributions of Treg cells, myeloid-derived suppressor cells (MDSCs), and indole 2,3-dioxygenase (IDO) activity toward an immunosuppressive environment, as well as tumor-cell-autonomous factors including mutational load, oncogenic signaling pathways, expression of PD-L1, and downregulation of major histocompatibility complex (MHC) class I (Pardoll, 2012; Topalian et al., 2015). It is becoming clearer, however, that tumor-intrinsic influences are not the only factors affecting the outcome of immunotherapy (Figure 1). This is well demonstrated by the regular and intriguing observation that the same transplantable tumor cell line injected into haploidentical, syngeneic mice can result in highly variable tumor growth curves during immunotherapy (and some animals fail to respond at all; Rescigno, 2015; Sivan et al., 2015; and our own observations). Perhaps providing a reason for this heterogeneity, emerging evidence is revealing that cancer immunotherapy is additionally influenced by host-related and environmental factors affecting immune system function. We dedicate a large part of this review to describing these less-considered tumor-extrinsic factors that potentially dictate success or failure (and possibly even irAEs) after anti-cancer ICB immunotherapy, and we pay particular attention to the immunomodulatory potential of the intestinal microbiota in light of new and exciting findings showing how distinct commensal bacteria modulate immunotherapy-driven anti-cancer effects.

Resistance to Immune-Checkpoint Blockade within the Tumor Microenvironment

It is now increasingly accepted that, rather than working alone, cancer cells develop close interactions with the extracellular

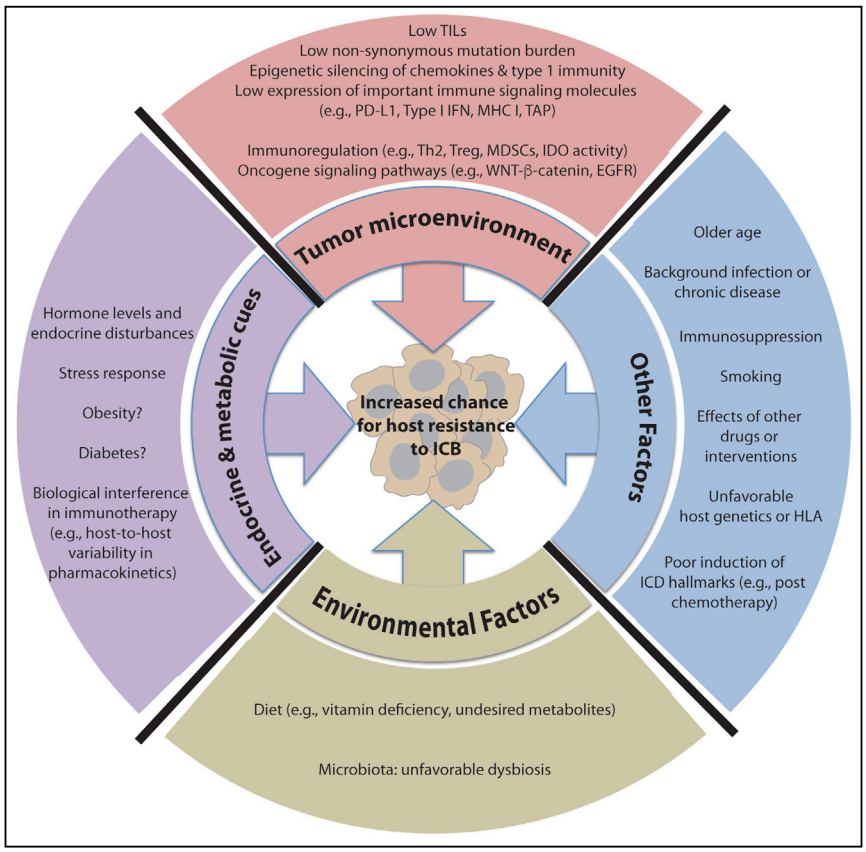

Figure 1. Major Factors Contributing to Primary Resistance to ICB Therapy

Many potential tumor-related, host-related, and environmental factors can explain the degree of heterogeneity seen with ICB immunotherapies. These can be categorized into influences from the tumor microenvironment, endocrine and metabolic factors, environmental factors, and other influences such as age and unfavorable host genetics.

matrix, stromal cells, and immune cells that together form the TME (Hanahan and Coussens, 2012). These constituents of the TME infrastructure facilitate a chronic inflammatory, immunosuppressive, and pro-angiogenic intratumoral environment in which cancer cells are able to adapt and grow with a lower likelihood of detection and eradication by host immunosurveillance.

Immunoregulatory Pathways Operating within the TME

T cells undertake the bulk of immunosurveillance, but to perform this role, they must (1) become properly activated by tumor-antigen-presenting dendritic cells (DCs) in peripheral lymph nodes; (2) home to the tumor, extravasate from tumor blood vessels, and infiltrate barriers such as stromal tissue to reach malignant cells; and (3) recognize and respond to their target (e.g., lytic granule release by CTLs kills the recognized malignant cell), which requires sufficient tumor cell exposure of peptide-MHC complexes and the overcoming of potential immunoregulatory mechanisms (Joyce and Fearon, 2015). Developing tumors often actively prevent one or more of the above requirements for T cell immunosurveillance in an effort to evade immune-mediated tumor control; thus, given that the efficacy of ICB therapy is driven by T cells, this effective immune evasion can ultimately determine failures in ICB treatment.

The upregulation of PD-L1 in the TME by tumor cells and APCs is one such strategy by which tumors evade immunosurveillance and is the premise behind the aforementioned therapies using PD-1-PD-L1 blockade (Zou et al., 2016). Tumor PD-L1 expression has been shown to reflect an immune-active TME (Dong et al., 2002; Gao et al., 2009) and is strongly associated with efficacious responses to anti-PD-1 (Taube et al., 2014; Tumeh et al., 2014) and anti-PD-L1 mAbs (Herbst et al., 2014). Comparison of anti-PD1-sensitive and anti-PD1-resistant tumors, which paradoxically both contain $CD8^+$ T cells, has suggested that intratumoral Treg cells might be responsible for limiting anti-PD1 mAb efficacy in cases where intratumoral T cell numbers appear sufficient (Ngiow et al., 2015).

The catabolism of tryptophan within the TME is increasingly recognized as a contributor to the suppression of anti-tumor immune responses. Tryptophan is catabolized by the rate-limiting enzyme IDO, expressed in myeloid cells and cancer cells, to yield immunosuppressive metabolites such as kynurenine. The actions of these metabolites, together with depletion of the essential amino acid tryptophan, inhibit the clonal expansion of T cells and can induce T cell anergy and apoptosis (Platten et al., 2012). Accordingly, the combination of IDO inhibitors and ICB therapy has been shown to increase TILs and their functional capacities in the TME and thus mediates rejection of both IDO-expressing and -nonexpressing poorly immunogenic tumors in an experimental setting (Holmgaard et al., 2013; Spranger et al., 2014). Clinical studies are currently assessing the safety and efficacy of IDO blockade combined with ICBs (IDO inhibitor plus ipilimumab [NCT: NCT02073123 and NCT01604889]; IDO inhibitor plus nivolumab [NCT: NCT02327078]).

The prominence of Treg cells, Th2 cells, and MDSCs that arise from and contribute to TME immunosuppression form a significant obstacle to ICB therapies via suppression of ICB-regenerated anti-tumor CTL and Th1 cell responses (Coussens et al., 2013). Depletion of such cell types, as might be possible for Treg cells (Ménétrier-Caux et al., 2012), could be used in combination with ICB therapies. It is interesting to note that PD-L1, IDO, and Treg cell immunoregulation operating within tumors might in fact follow, rather than precede, T cell infiltration (Spranger et al., 2013). This brings hope that such immunoregulatory mechanisms can be overcome or prevented with the right modulation of immune contexture for the reestablishment of an existing T cell tumoricidal capability.

Epigenetic Silencing of Th1 and Tc1 Chemokine Secretion

Stromal cells of the TME can exclude T cells from interacting directly with cancer cells. This can be achieved via TME-mediated

regulation of the local proliferation of T cells within tumors, control over T cell viability, regulation of T cell spatial distribution related to cancer cells, or control by the TME over the migration of T cells from the circulatory system into tumors (Joyce and Fearon, 2015). Epigenetic silencing of the genes encoding the chemokines Cxcl9 and Cxcl10 (potentially mediated by polycomb repressive complex 2 [PRC2]; Nagarsheth et al., 2016), which direct T cell trafficking to tumors, is an example of the latter (Peng et al., 2015). Indeed, treatment with epigenetic modulators has been shown to remove the repression over these Th1-cell-type chemokines and increase TILs and thus result in enhanced therapeutic efficacy of PD-L1 checkpoint blockade (Peng et al., 2015).

Importance of Type I IFN Signaling

TMEs with a significant lack of type-I-IFN-producing DCs will naturally result in limited anti-tumor T cell priming (Diamond et al., 2011; Fuertes et al., 2011) and thus a limited pool of useful T cells for ICB therapy to reactivate. In line with this, sufficient stimulation of innate sensory pathways (such as TLR3-IFNAR stimulation following anthracycline chemotherapy-induced immunogenic cell death [ICD] of tumor cells) can induce type I IFN that in turn stimulates secretion of the chemokine CXCL10 for TIL recruitment to tumor beds (Sistigu et al., 2014). Similarly, in vivo studies have demonstrated an important role for type I IFN production following activation of the stimulator of interferon genes (STING) pathway in the BATF3 lineage of DCs; this role is necessary for optimal T cell recruitment to tumors and spontaneous anti-tumor T cell responses (Corrales and Gajewski, 2015; Deng et al., 2014; Diamond et al., 2011; Fuertes et al., 2011; Woo et al., 2014) and suggests a potential application for STING agonists as cancer therapeutics (Corrales and Gajewski, 2015).

In vivo peritumoral injection of immunostimulatory RNA in immune-cell-poor melanomas has been observed to initiate cytotoxic inflammatory responses within the TME and inhibition of tumor growth driven by type I IFN responses by various immune cells. Moreover, this type I IFN activation allowed for prolonged survival when the PD-1-PD-L1 axis was subsequently targeted (Bald et al., 2014). In terms of clinical development, initial trials combining CTLA-4 blockade with IFNα-2b therapy have demonstrated clinical activity, which might be attributable to reduced MDSC populations (Tarhini et al., 2012a; Tarhini et al., 2012b). Several trials are currently investigating other ICB and IFNα-2b combinations (Buqué et al., 2015).

Cancer-Cell-Autonomous Mechanisms of Resistance to Immune-Checkpoint Blockade

Oncogenic Signaling

Cancer-cell-autonomous cues might be responsible for TIL-negative tumors, and cancer cell genetic evolution might affect the efficacy of immunotherapies. Activation of the oncogenic WNT-β-catenin signaling pathway in melanoma cells has been shown to correlate with the absence of T cell and CD103$^+$ DC infiltration into the TME. This is due to β-catenin-mediated suppression of the chemokine CCL4. A detrimental consequence of this is resistance to anti-PD-L1 and anti-CTLA-4 mAb-based therapies in experimental murine tumor models (Spranger et al., 2015). Similarly, loss of phosphatase and tensin homolog (PTEN) and activation of the PI3-kinase pathway in cancer cells can also promote resistance to ICBs (Peng et al., 2016) and has been linked to increased PD-L1 expression and immunoresistance in human glioma (Parsa et al., 2007). Other evidence has shown that alterations in chromosomal region 9p24.1 in Hodgkin's lymphoma can induce the expression of PD-1 ligands through Janus kinase (JAK)-signal transducer and activator of transcription (STAT) signaling (Ansell et al., 2015). Furthermore, Akbay et al. reported a correlation between activation of the epidermal growth factor receptor (EGFR) pathway and a signature of immunosuppression manifested by upregulation of PD-1, PD-L1, CTLA-4, and inflammatory cytokines (Akbay et al., 2013). This immunoablative fingerprint was associated with decreased CTLs and increased markers of T cell exhaustion in mice bearing EGFR-driven adenocarcinoma of the lungs, albeit PD1 blockade could restore effector T (Teff) cell functions and prolong survival in this model. Tumor cell downregulation of MHC class I molecules and TAP deficiencies is an additional common mechanism of tumor immune escape and renders any endogenous or therapeutic anti-tumor T cell responses ineffective (Haworth et al., 2015).

Mutational Status

The anti-tumor activity initiated by ICBs in tumors is most likely explained by the (re)activation of T cell responses against neo-antigens (i.e., T cell epitopes newly formed after tumor-specific mutations; reviewed in Schumacher and Schreiber, 2015). Therefore, the mutational status of the cancer will profoundly influence the success of ICB therapies. New lines of evidence support this theory. Genomic and bioinformatic approaches have identified tumor neo-antigens as a major class of T cell rejection antigens after anti-PD-1 and/or anti-CTLA-4 therapy in transplantable carcinogen-induced cancer models (Gubin et al., 2014). In this study, checkpoint blockade was also seen to alter both the quality (as monitored by gene-expression changes in isolated CD8$^+$ TILs) and the magnitude of the intratumoral T cell response against these neo-antigens. Evidence from clinical studies supports this through the findings that immune-checkpoint blockade enhances neo-antigen-specific T cell responses in melanoma (van Rooij et al., 2013) and NSCLC (Rizvi et al., 2015).

Building on these observations, it has been additionally proposed that higher mutational loads can predict sensitivity to immune-checkpoint blockade. Higher non-synonymous mutation burden or microsatellite-instability statuses have been correlated with durable clinical benefit to blockade of the PD-1-PD-L1 pathway (Le et al., 2015; Rizvi et al., 2015). A genetic basis for clinical benefit to CTLA-4 blockade, where high clonal neo-antigen load and low antigen intra-tumoral heterogeneity are associated with improved overall survival following treatment, has similarly been identified (Snyder et al., 2014; Van Allen et al., 2015).

Inflammation and Metabolic Cues

Contributions from metabolic and inflammatory processes within the TME could participate in quashing the desired impacts of ICBs. Tumor cell cyclooxygenase (COX) activity has been recently suggested as a driver of immune suppression via its production of prostaglandin E2, which suppresses immunity and fuels an inflammatory environment favoring tumor outgrowth (Zelenay et al., 2015). Similarly, glucose consumption by tumor cells can place a metabolic restriction on T cells in the TME, leading to suppressed mTOR activity, glycolytic capacity, and IFN-γ production within T cells and a consequential progression of

tumors. Interestingly, this was found to be reversed by the use of ICBs in an experimental setting; the authors predicted that ICB therapies would thus be most effective in patient tumors with higher glycolytic rates (Chang et al., 2015). Recent evidence has shown that modulation of cholesterol metabolism can also be used to potentiate the anti-tumor response of $CD8^+$ T cells. Inhibition of cholesterol esterification endowed $CD8^+$ T cells (but not $CD4^+$ T cells) with an improved control over melanoma growth and metastasis in mice, which could also be observed with avasimibe, an inhibitor of the key cholesterol esterification enzyme ACAT1. Combined therapy of avasimibe plus anti-PD-1 mAb showed higher efficacy than did monotherapies in controlling tumor progression, signifying that this has clinical potential (Yang et al., 2016).

Pre-mortem Cell Stress and Damage-Associated Molecular Patterns

Endoplasmic reticulum (ER) stress and autophagy dictate the immunogenicity of cell death in tumors (Galluzzi et al., 2015; Kroemer et al., 2013). These pre-mortem stresses condition the expression or release of damage-associated molecular patterns (DAMPs) as "come and eat me" signals to recruit professional APCs (e.g., DCs) that will recognize and engulf dying bodies; in doing so, they facilitate the processing and cross-presentation of tumor antigens to cognate T cells. Hence, TIL-negative oncogene-driven autochthonous lung carcinoma fails to respond to ICB therapy, non-immunogenic cytotoxicants (based on cisplatinum or taxane usage), and the combination of these therapeutics. Instead, the administration of appropriately selected chemotherapies ahead of ICB therapy, capable of transforming tumors into better immunological areas with significant T cell infiltration (i.e., restoring a high Teff-Treg ratio), improves ICB-mediated inhibition of tumor progression (Pfirschke et al., 2016). Recently, the clinical relevance of an immunogenic cell death in tumors has been demonstrated by several studies showing that tumor-related exposure of several DAMPs—notably surface membrane exposure of calreticulin, release of the TLR4 ligand HMGB1, and the abundance of LC3B puncta in the cytosol of tumor cells (as a surrogate marker of active autophagy machinery)—is associated with TIL infiltrates and favorable clinical outcome (Fucikova et al., 2016; Ladoire et al., 2015). This suggests that the form of cell death operating within tumors determines the success of ICBs and other cancer immunotherapeutics; the absence of an appropriate response to ER stress or of autophagy induction in tumor cells could, for example, predict resistance to ICB therapies in human cancers.

Host-Related Factors Contributing to Poor Immunotherapeutic Responses

The individual characteristics of the tumor-bearing host—including age, diet, hormones, human leukocyte antigen (HLA) type, genetic polymorphisms, and other individual factors such as smoking and secondary ongoing infections or diseases—can condition the success of immunotherapy.

Age

Aging of an individual is well known to correlate with functional limitation of immunity and has significant effects upon both innate and adaptive immune responses (Fulop et al., 2011). Because cancer incidence increases with age, such that over 60% of new cancers occur in subjects over 65 years of age (Fulop et al., 2011), cancer immunotherapy must mobilize the functionality of a senescent immune system. Hallmark age-related alterations to the immune system include (1) reduction in APCs and their functionality (e.g., reduced expression of TLR and costimulatory molecules); (2) diminished numbers of lymphocytes and therefore potentially reduced clonally heterogeneous responses to maintain immunosurveillance; (3) the appearance of T cells showing markers of terminal differentiation refractory to activation; (4) chronic inflammatory signaling, including increasing circulating levels of pro-inflammatory cytokines such as interleukin-1 (IL-1), IL-6, and tumor necrosis factor (TNF); and (5) a surge in suppressive immune cell populations, including MDSCs and Treg cells, within tumors (Goronzy and Weyand, 2013; Pawelec et al., 2010; Tomihara et al., 2013). Hence, MDSCs have been reported to be potential predictors of resistance to the anti-CTLA-4 mAb ipilimumab in patients with prostate cancer or melanoma (Martens et al., 2016; Meyer et al., 2014; Santegoets et al., 2014). A recent meta-analysis comparing the efficacy of ICBs between younger and older patients (age cutoff of 65–70 years) has, however, revealed that the overall survival benefit is significant in both groups: subgroup analyses based on the ICB used and tumor type showed consistent survival benefit in both younger and older cohorts in most cases. An exception was the subgroup of older patients treated in four trials of anti-PD-1 mAb (although this needs confirmation with greater statistical power); here, older patients did not see improved overall survival, whereas younger patients did (Nishijima et al., 2016). Nonetheless, advanced immunosenescence potentially forms a formidable barrier to ICB therapy; consequently, the immune function of older patients should ideally be investigated and factored into the choice of treatment.

HLA Type

Immunotherapies based on tumor-associated antigen (TAA) CTL responses (e.g., vaccination) are restricted by certain MHC class I haplotypes, which depend upon the individual patient's HLA type (Mitchell et al., 1992; Schadendorf et al., 2006). Understandably, HLA type is therefore a key eligibility criterion in clinical studies involving tumor peptide vaccination. HLA type can also determine the success or failure of full-length-protein anti-tumor vaccines, which might otherwise be expected to overcome inter-individual HLA type differences. This has been well illustrated in a clinical study of a recombinant NY-ESO-1 antigen vaccine where CTL responses were seen only in subjects expressing HLA-Cw3 and HLA-B35 alleles, although patients without these alleles were still able to mount $CD4^+$ T cell and antibody responses (Bioley et al., 2009). HLA-class-I-associated immunodominance for the induction of CTL responses thus makes an important factor in the choice of immunotherapy, and HLA association with responsiveness should be systematically included in future immunotherapy trials.

Genetics

Distinct germline polymorphisms in immune cell receptors have the potential to place limits on immunotherapies. Given consistent associations between certain FcγRIII receptor polymorphisms and the outcome of rituximab (anti-CD20) treatment in rheumatoid arthritis, FcγRIII polymorphism has been suggested to influence the efficacy of rituximab in follicular lymphoma, although data to the contrary signify that this remains

to be confirmed (Hargreaves et al., 2015). Differences in antibody-dependent cell-mediated cytotoxicity (ADCC) by natural killer (NK) cells or monocytes due to certain FcγRIII polymorphisms have also been associated with the outcome of trastuzumab treatment in HER2-positive breast cancer (Musolino et al., 2008) and similarly in cetuximab treatment of head and neck squamous cell carcinoma cell lines (López-Albaitero et al., 2009).

Release of HMGB1 by dying tumor cells is pivotal to the successful immunogenicity instigated by radiotherapy and certain chemotherapy regimens (Apetoh et al., 2007). A sequence polymorphism in *TLR4*, the gene coding for toll-like receptor 4, exists in approximately 12% of white individuals (Asp299Gly) and prevents binding of HMGB1 to TLR4 in a negative-dominant fashion (Arbour et al., 2000). A retrospective analysis of patients with breast cancer revealed that carriers of the loss-of-function Asp299Gly protein relapsed more quickly after immunogenic radiotherapy and chemotherapy than those carrying the normal TLR4 receptor (Apetoh et al., 2007). Similar negative correlations with treatment outcome have been documented with loss-of-function alleles of the genes coding for TLR3 (which senses RNA and DNA-RNA hybrids released from dying cells) (Chen et al., 2015) and formyl peptide receptor 1 (FPR1; which senses annexin A1 spilled from dead cells) (Vacchelli et al., 2015) in patients with breast cancer and also for loss-of-function *TLR4* and *FPR1* alleles in colorectal cancer (Tesniere et al., 2010; Vacchelli et al., 2015). It should be noted that these negative associations have been found in the context of immunogenic chemotherapies (anthracyclines and oxaliplatin for the treatment of mammary and colorectal carcinomas, respectively) yet have not been investigated in the context of ICBs. Irrespective of this consideration, they illustrate that the host immune system can be heterogeneous in its capacity to sense DAMPs that condition anticancer immunosurveillance.

Diet and Metabolism

Patients receiving immunotherapies are recommended to maintain a healthy diet with sufficient intake of vitamins and minerals to boost the functioning of the immune system. Sufficient intake of vitamin E, for example, might be of particular importance in some cancers, given evidence showing a role for this lipid-soluble antioxidant in boosting $CD4^+$ T cell number and Th1 cell functions in patients with colorectal cancer (Malmberg et al., 2002).

Beyond the metabolic restrictions that tumor cells can place on T cells within the TME (Chang et al., 2015), several energy-balance-related host factors are known to affect cancer treatments (Hursting and Berger, 2010). Immunotherapies are also likely to be among those affected, although limited dedicated research is currently available. Host insulin and insulin-like growth factor 1 signaling leads to downstream activation of the mammalian target of rapamycin (mTOR) complex, which lies at the heart of several metabolically important pathways (e.g., the PI3K pathway) and can determine drug resistance. Evidence indicates that activation of the PI3K pathway can mediate trastuzumab resistance in patients with breast cancer (Berns et al., 2007). Differing diet, metabolism, and hormonal cues might therefore also affect the course of ICB immunotherapy.

Background Chronic Infections

Beyond TME-associated inhibition, perturbations in immune signaling caused by background chronic viral infections can also affect tumor infiltration by T cells. Chronic infection with hepatitis C virus (HCV) is such an example. Chronic HCV infection is strongly associated with an N-terminally truncated form of CXCL10, which inhibits Teff cell and NK cell chemotaxis to virally infected cells and tumors by antagonizing CXCR3-mediated signaling by biologically active CXCL10 (Casrouge et al., 2011; Riva et al., 2014). Antagonistic CXCL10 is generated through dipeptidylpeptidase 4 (DPP4)-mediated truncation of CXCL10, and both DPP4 and N-terminally truncated CXCL10 correlate with increasing liver disease and treatment failure in HCV patients (Casrouge et al., 2011; Ragab et al., 2013; Riva et al., 2014). It has been recently shown that in vivo post-translational processing of chemokines by DPP4 inhibits T cell migration to tumors and that DPP4 inhibition improves adjuvant-based immunotherapy, adoptive T cell transfer, and checkpoint blockade with anti-CTLA-4 and anti-PD-1 mAbs (Barreira da Silva et al., 2015). DPP4 inhibition (which is already used in the clinic to protect the agonist forms of incretin hormones) might therefore be a useful adjunctive to immunotherapy in cancer patients with chronic HCV infection, given that this could boost intratumoral levels of bioactive CXCL10 (Barreira da Silva et al., 2015). It is highly likely that similar immune-signaling modulations resulting from other chronic infections will affect tumor immunotherapy.

Perhaps as a result of the novelty of the field, there is limited evidence so far for how these described host-related factors might affect cancer immunotherapy, and even so, potential inhibitory characteristics such as HLA type and host genetics are not amenable to manipulation. However, an interesting sideline here is the fact that many of these factors alter the gut microbiome, which in turn could determine the specifications of immune responses, including those elicited by immunotherapy.

The Unsuspected Input from Intestinal Microflora to Successful Cancer Immunotherapy

The Gut Microbiota and Immune "Fitness"

Recent estimates suggest that the human cells in our bodies are outnumbered by bacteria (Sender et al., 2016), the majority of which inhabit the gut. Through a significant degree of coevolution, gut microbiota thrive in a mutually beneficial, symbiotic relationship with the host. Gut commensals exert a battery of functions for the optimal health of an organism, including the processing of nutrients, degradation of xenobiotics, protection from pathogenic microbes, regulation of intestinal barrier homeostasis, and maturation of the immune and enteric nervous systems (Eberl, 2010; Kabouridis and Pachnis, 2015). In turn, the intestine provides a warm, nutrient-rich, and protective environment for the many species of gut-resident microbes. Although the composition of microbial communities is generally stable within each individual (Gophna, 2011), there exists a vast level of inter-individual heterogeneity (even between identical twins; Turnbaugh et al., 2009a) that is largely determined by the diet (Turnbaugh et al., 2009b; Wu et al., 2011) and environment (Lin et al., 2013; Yatsunenko et al., 2012) of the host but also by other factors such as medications (e.g., antibiotics; Ubeda and Pamer, 2012) and smoking (Biedermann et al., 2013). Thus, the gut microbiota communities represent a significant source of human genetic and metabolic diversity.

Given the massive bacterial content of the normal ("eubiotic") gut microbiota, a remarkable characteristic of the host immune

system is its ability to tolerate a vast number of commensals while still being able to perform immunosurveillance against invading pathogens. To form this agreeable balance, the gut microbiota and the host immune system have become closely intertwined through evolution to ensure mutual survival and are thus highly sensitive to changes in their partner (Belkaid and Hand, 2014). Therefore, the scale of inter-individual microbiome variability suggests that there is likely to be a similar degree of variability in the host immune system, potentially affecting its functionality. This is exemplified in cases where the microbiota-immune balance is perturbed.

A disequilibrium in the mutualistic symbiosis linking the host and intestinal bacteria—leading to a deviated (functional) repertoire of the normal gut microbiome (termed "dysbiosis")—can result in wide and diverse autoinflammatory and autoimmune pathologies, such as obesity (Cotillard et al., 2013; Le Chatelier et al., 2013), diabetes (Burrows et al., 2015), inflammatory bowel diseases (Dalal and Chang, 2014), and nonalcoholic fatty liver disease (Spencer et al., 2011) but also extraintestinal disorders including autoimmune encephalomyelitis (Lee et al., 2011), rheumatoid arthritis (Kamada et al., 2013), and cancer (Louis et al., 2014). Disorders involving the gut microbiota have been described as participating in colorectal carcinogenesis (Garrett, 2015; Wang et al., 2014), but they might also influence the development of extraintestinal cancers (e.g., breast cancer and hepatocellular carcinoma; Dapito et al., 2012; Velicer et al., 2004; Xuan et al., 2014; Yoshimoto et al., 2013). Corresponding with this, gut microbiota govern TLR5-dependent differences in the growth of extramucosal malignant progression by facilitating tumor-promoting inflammation (Rutkowski et al., 2015).

Several studies have shown how distinct bacteria, or bacterial products, can promote alterations in immune responses. The gastrointestinal tract is a privileged environment for the induction of $Foxp3^+$ Treg cells in response to oral antigens, which play a pivotal role in the maintenance of immune tolerance (Belkaid and Hand, 2014). This dedicated property of the gut results, at least in part, from specialized populations of gut-resident APCs such as $CD103^+CD11b^+$ DCs, which can induce Treg cells through their production of the cytokine TGF-β and the vitamin A metabolite retinoic acid (Coombes et al., 2007; Mucida et al., 2007; Sun et al., 2007). Components of the microbiota also facilitate Treg cell induction and function (e.g., IL-10 production)—this was first documented for polysaccharide A (PSA) produced by the prominent human symbiont *Bacteroides fragilis* (Mazmanian et al., 2008; Round et al., 2011). Later work identified that a variety of *Clostridia* strains also participate in Treg cell induction (partly through creating a TGF-β-rich environment; Atarashi et al., 2013; Atarashi et al., 2011) and that certain short-chain fatty acids (SCFAs), particularly butyrate, result from the bacterial metabolism of otherwise indigestible dietary components (Arpaia et al., 2013; Furusawa et al., 2013; Smith et al., 2013). Certain commensals promote the development of $CD4^+$ T helper responses. Perhaps in correspondence with this, most IFN-γ-producing (Th1) and IL-17-producing (Th17) T cells during steady state are located in the gastrointestinal tract and develop in response to microbial signals (Belkaid and Hand, 2014). For example, segmented filamentous bacteria (SFB) are potent inducers of Th17 and Th1 cells in the small intestine (Goto et al., 2014; Ivanov et al., 2009). However, mono-association of mice with SFB could induce auto-inflammatory arthritis (Wu et al., 2010) and multiple-sclerosis-like symptoms in an autoimmune encephalomyelitis (EAE) model (Lee et al., 2011), which suggests that certain individual components of the microbiota (termed "keystone species"; Belkaid and Hand, 2014) can drive antigen-independent, extraintestinal immune responses that ultimately trigger systemic autoimmunity. In line with this, SFB-induced Th17 cells have been visualized to migrate from the gut at the onset of autoinflammatory arthritis, and their accumulation in the spleen correlated with autoantibody titers in this model (Morton et al., 2014). Presumably, such keystone species must be kept in check by the immune system.

A direct consequence of the mutualistic symbiosis linking the host and intestinal bacteria is that the latter can determine the "immune fitness" of an individual, promoting and calibrating both innate and adaptive immunity. Besides a clear link with immune-mediated disease control and autoimmune disease, a less-appreciated aspect of dysbiosis is the way in which it affects and determines the outcome of therapies involving input from the immune system. A particularly prominent (and timely) illustration here is cancer immunotherapy.

Cytotoxicants and CpG Immunotherapy Require an Intact Intestinal Microflora

A number of studies have led to an understanding that gut microbiota and antineoplastic interventions profoundly affect each other. Chemotherapy and radiotherapy result in gastrointestinal mucositis in a significant proportion of cancer patients and can have direct or indirect (i.e., immune-mediated) cytotoxic effects on intestinal bacteria, thus culminating in dysbiosis (Touchefeu et al., 2014; Zitvogel et al., 2015). Alternatively, or in addition, the gut microbiota can influence both therapeutic and adverse effects of anti-cancer interventions either through pharmacodynamic (e.g., undesirable drug reactivation in the gut; Wallace et al., 2010) or immunological (Iida et al., 2013; Viaud et al., 2013) mechanisms.

A modulation of anti-cancer immune responses by gut microbiota has been documented for chemotherapy (platinum salts and cyclophosphamide [CTX]; Iida et al., 2013; Viaud et al., 2013). In this setting, the underlying mechanisms appear to be different depending on the type and actions of anti-cancer compounds. The alkylating agent CTX promotes the translocation of distinct Gram-positive bacteria (such as *Enterococcus hirae*) from the gut to secondary lymphoid organs, allowing cognate antibacterial effector "pathogenic Th17" (pTh17; Th17 possessing characteristics of Th1 cells, e.g., IFN-γ production) and memory Th1 cell immune responses to be elicited, which contribute to the anti-tumor efficacy of CTX (Viaud et al., 2013). Consistent with these data, a healthy gut microbiota has been shown to be necessary for oxaliplatin (an immunogenic platinum salt anti-neoplastic agent)-mediated tumor infiltration by myeloid cells, which promote tumor regression through the production of reactive oxygen species (Iida et al., 2013). In addition, this study demonstrated that a eubiotic gut microbiota similarly governs the therapeutic success of CpG oligonucleotide plus anti-IL-10R mAb immunotherapy. Here, tumoricidal activity was dependent on TNF-α release by myeloid cells, dictated by *Alistipes shahii* and TLR4 ligands (Iida et al., 2013).

These initial breakthroughs linking the gut microbiota to the immune-mediated efficacies of anti-cancer therapies highlighted

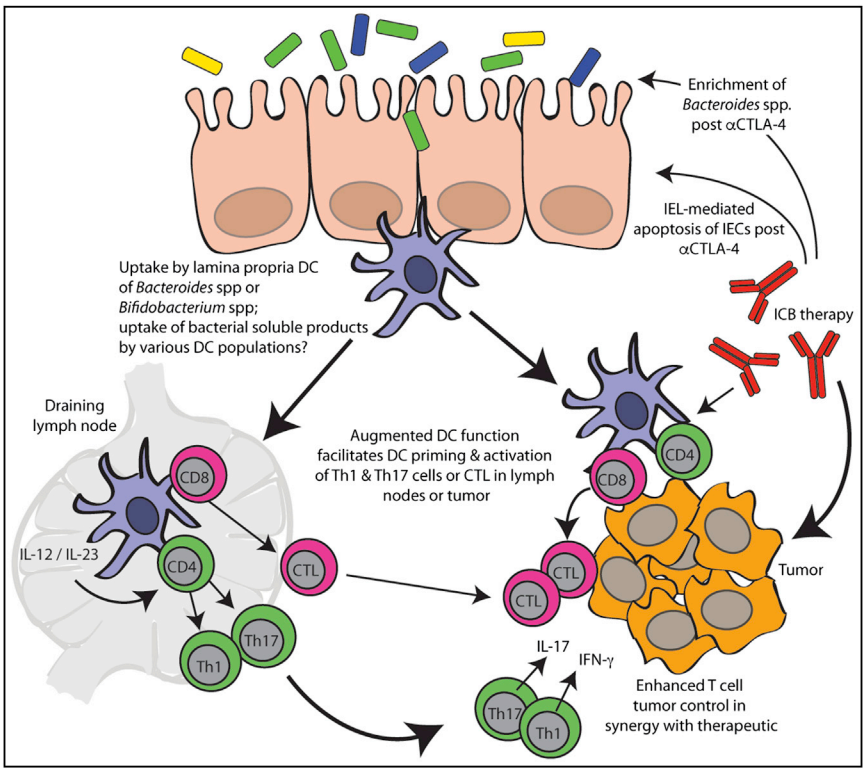

Figure 2. Immune-Checkpoint Blockade Mobilizes the Gut Microbiota to Promote Anti-tumor Immune Responses

Uptake of distinct bacterial species (e.g., *Bacteroides fragilis* and *Bifidobacteria*) or bacteria-derived products by DCs in the context of ICBs can significantly enhance DC antigen-processing and -presentation functions (i.e., upregulation of costimulatory molecules and antigen-presentation molecules such as CD40 and MHC class II) and ensure DC production of cytokines such as IL-12. Together, this DC activation increases the generation of anti-tumor T cells and increases intratumoral T cell numbers. Alterations in the composition of the gut microbiota can affect host anti-cancer immunity. Blockade of CTLA-4 has also been found to modulate the microbiota composition and the function and integrity of the intestinal mucosal barrier, which could facilitate the immune-mediated therapeutic efficacy of anti-CTLA-4 mAb immunotherapy. Abbreviations are as follows: ICB, immune-checkpoint blockade; CTL, cytotoxic T lymphocyte; Th, T helper; DC, dendritic cell; IEL, intestinal epithelial lymphocyte; IEC, intestinal epithelial cell; IFN, interferon; and IL, interleukin.

the importance of an intact commensal microbiota in cancer therapy and suggested the possibility of (re)establishing a favorable microbiota in patients with ineffective pre-existing enteric microbial microflora that might be associated with a poor prognosis.

Influence of Gut Microbiota on ICB Immunotherapy

CTLA-4 Blockade: Recent evidence indicates that CTLA-4 blockade, by compromising the homeostatic equilibrium between intraepithelial lymphocytes and intestinal epithelial cells, induces the accumulation of distinct *Bacteroides spp.* in the inner part of the mucus layer. At this interface, within reach of mucosal DCs, *Bacteroides fragilis* was observed to elicit potent and IL-12-dependent Th1 cell immune responses beneficial for the host against the tumor (Figure 2; Vétizou et al., 2015). The clinical relevance of these findings was inferred from analysis of the gut microbiome composition of patients with metastatic melanoma before and after one or two injections with the clinically approved anti-CTLA-4 mAb ipilimumab, which revealed three distinct microbiome clusters ("enterotypes") segregated by *Bacteroides* and *Prevotella* genera (*Alloprevotella* and *Prevotella* drove cluster A, and distinct *Bacteroides spp*. drove clusters B and C). Fecal microbial transplantation of feces harvested from each of these patient clusters into germ-free tumor-bearing mice highlighted that the microbial composition of cluster C, rich in immunogenic *Bacteroides spp.* (mainly contributing to the niching of *B. fragilis*), could restore anti-CTLA-4 mAb efficacy, whereas cluster B enriched with tolerogenic *Bacteroides* species mediated complete resistance to the mAb (Vétizou et al., 2015).

Bifidobacterium and TIL Infiltrates in the TME: A parallel study to that above has demonstrated a role for distinct components of the gut microbiota, especially *Bifidobacterium*, in promoting natural anti-tumor immune responses (Sivan et al., 2015). Here, Sivan et al. compared the anti-tumor CTL responses in genetically similar C57BL/6 tumor bearers derived from two different mouse facilities, the Jackson Laboratory (JAX) and Taconic Farms (TAC), to have differing commensal microbes. JAX and TAC mice exhibited significant differences in the growth kinetics of subcutaneously implanted melanomas; more aggressive tumors in TAC mice were attributable to lower tumor-specific T cell responses elicited in draining lymph nodes and poor intratumoral accumulation of tumor-antigen-specific $CD8^+$ T cells. The aggressive neoplastic growth in TAC mice could be reduced to the rates seen in JAX mice after either JAX fecal transplantation or cohousing between the mice. The 16*S* ribosomal RNA sequencing of feces gene amplicons identified *Bifidobacterium* as associated with the enhanced tumor control. Hence, oral feeding of TAC mice with *Bifidobacterium* or cohousing of TAC and JAX mice restored CTL responses and allowed the host to control tumor progression by activating the processing and presentation machinery of intratumoral DCs. Importantly, *Bifidobacterium*-induced TIL enrichment of the TME also allowed for enhanced anti-tumor effects mediated by anti-PD-L1 mAb immunotherapy (Figure 2; Sivan et al., 2015).

Interestingly, although both studies reveal the importance of the gut microbiota to optimal therapy with ICBs, they differ with respect to the specific bacteria identified as causing these effects. In addition, whereas the gut microbiota augmented the anti-tumor response to anti-PD-L1 mAb, it was absolutely required for that of anti-CTLA-4 mAb. Although this might reflect background microbiome differences between the C57BL/6 strains from distinct commercial suppliers, there is a complexity here that must be addressed in future studies. However, a similar observation between the two studies was that translocation of bacteria did not occur after immunotherapy (Sivan et al., 2015; Vétizou et al., 2015) and that

microbial-dependent DC activation (i.e., increased expression of MHC classes I and II and IL-12 production) appears to be indispensable for the efficacy of checkpoint blockade.

Uncoupling ICB Toxicity from Efficacy

Severe gastrointestinal toxicity, particularly colitis, frequently occurs in patients upon immunotherapy. Notably, immune responses following CTLA-4 blockade with ipilimumab not only execute their program systemically in secondary lymphoid organs contributing to reinstating cancer immunosurveillance but also are directed at sites where microbiota are abundant. Indeed, ipilimumab-treated patients develop anti-microbiota antibodies (Berman et al., 2010) that appear to be associated with irAEs such as rashes, hepatitis, and colitis (Beck et al., 2006; Berman et al., 2010; Weber et al., 2012). Gut microbiota might therefore be implicated in these phenomena, and the eagerly anticipated breakthrough of one day being able to uncouple efficacy from toxicity could stem from a better understanding of the disequilibrium between commensals and pathobionts.

Vétizou et al. (2015) characterized the effects of the microbiota on the irAEs mediated by anti-CTLA-4 mAb in preclinical mouse models. The anti-CTLA-4 mAb induced a subclinical colitis (defined by a histopathological score of microscopic lesions and release of the anti-microbial peptide lipocalin-2 in the caecum and feces), which was more prominent in mice kept in specific-pathogen-free (SPF) conditions than in germ-free animals, suggestive of a role for distinct commensals in this particular anti-CTLA-4-induced irAE. However, administration of the combination of *B. fragilis* and *Burkholderia cepacia*, mandatory for restoring the efficacy of CTLA-4 blockade in antibiotic-treated animals, failed to aggravate subclinical colitis and instead induced a protection against intestinal lesions. This protection was associated with the capacity of *B. fragilis* to promote the proliferation of $ICOS^+$ Treg cells in the lamina propria, possibly through mobilizing plasmacytoid DCs (this cell type has been observed to accumulate and mature in mesenteric lymph nodes after *B. fragilis* monocolonization of germ-free mice treated with anti-CTLA-4 mAb; Dasgupta et al., 2014; Vétizou et al., 2015). Supporting this assumption, the co-blockade of ICOS or IL-10 with CTLA-4 resulted in an overt and deadly colitis in tumor bearers reared in SPF conditions. This model of *B. fragilis* and *B. cepacia* bicolonization demonstrates that efficacy and toxicity of anti-CTLA-4 mAb can be dissociated and provides hope that the same could be accomplishable through the establishment of an attuned microbiota in patients treated with ipilimumab and/or other ICBs (Vétizou et al., 2015). Moreover, a recent report has similarly described a protective role offered by the *Bacteroidetes* phylum (namely *Barnesiellaceae unclassified* and *Rikenellaceae unclassified*; detected by 16S ribosomal RNA sequencing of feces) against the development of ipilimumab-associated colitis (Dubin et al., 2016). Ongoing studies are working to identify bacterial components that are predictive for colitis and other toxicities during CTLA-4 blockade in patients.

Novel Therapeutic Avenues for Improving the Coverage of Immune-Checkpoint Blockade

Using Existing Therapeutic Tools to Remove Hurdles to Immunotherapy

As highlighted here and in several excellent reviews (Buqué et al., 2015; Pardoll, 2012; Postow et al., 2015; Sharma and Allison, 2015a, 2015b; Zou et al., 2016), significant effort is currently in progress to improve the coverage of ICBs either by concomitantly stimulating immune functions or targeting other immunoregulatory mechanisms at play within the TME. Indeed, more than 50 combinatorial approaches involving ICBs with immunostimulatory cytokines or agonists (e.g., GM-CSF, IFNα-2b, and TLR agonists), inhibitors of IDO, peptide-based vaccines, targeted therapies, oncolytic virotherapy, chemotherapies, radiation therapy, or other ICBs (Buqué et al., 2015) are currently under clinical trial. Many of these combinations are likely to yield successes and improvements over ICB monotherapy; however, toxicities such as irAEs are also predicted to feature heavily and will determine the viability of these strategies. Additional inventive approaches for uncoupling the efficacy of ICBs from their toxicity involve local (as opposed to systemic) administration, which has been shown to produce abscopal effects (i.e., tumor-targeting immune-mediated regressions in distant, untreated lesions; Marabelle et al., 2013) and modulation of the gut microbiota, described below.

Envisioning a Greater Coverage of Immune-Checkpoint Blockade through Manipulating the Microbiome

The recent evidence that inter-individual differences in intestinal microflora are a source of the heterogeneity in immunotherapeutic efficacy and toxicity indicates that it might be possible to improve the therapeutic index of ICBs with "adjunctive oncomicrobiotics," i.e., live immunogenic commensals, derivatives of such commensals, and/or perhaps antibiotics that selectively target immunosuppressive microbes. As indicated by several studies (Iida et al., 2013; Sivan et al., 2015; Vétizou et al., 2015; Viaud et al., 2013), such improvements in efficacy correspond to directing increased numbers of TILs of the right functionality. Theoretically, TIL infiltration of tumor beds depends on the quality of the priming of naive T cells. This is determined by the presence of antigen-presenting DCs in the proximity of dying or apoptotic tumor cells (Broz et al., 2014; Galluzzi et al., 2015; Sandel et al., 2005), by existing neoantigens or high mutational load allowing high-avidity TCR engagement and proliferation (Tran et al., 2015), and by an appropriate "immunogenic milieu" in which DAMPs can be released (e.g., the mimicking of viral infection and consequent triggering of type I IFN receptor signaling pathways; Diamond et al., 2011; Fuertes et al., 2011; Sistigu et al., 2014). In addition to these aspects, another input to the immunogenic milieu responsible for quality TILs, or the reinstating of defective anti-cancer T cell responses, is derived from immune sensing of the bacterial species present at that particular time in the microflora.

Implementation of Adjunctive Oncomicrobiotics

The first consideration in the future use of microbiota-conditioning strategies is the fact that our knowledge of cancer-associated intestinal dysbiosis, and how this might influence the clinical benefits of immunotherapy for a given patient, remains fragmentary. Nonetheless, due progress is being made on this front. Recently, integrated catalogs of reference genes of the human gut microbiome have been reported from metagenomics and metatranscriptomics analyses from MetaHIT and the Human Microbiome Project (Li et al., 2014; Nielsen et al., 2014), and "culturomics" has been coupled with MALDI-TOF mass spectrometry for the identification of new commensals (Lagier et al., 2015). Between them, these catalogs reach a saturated

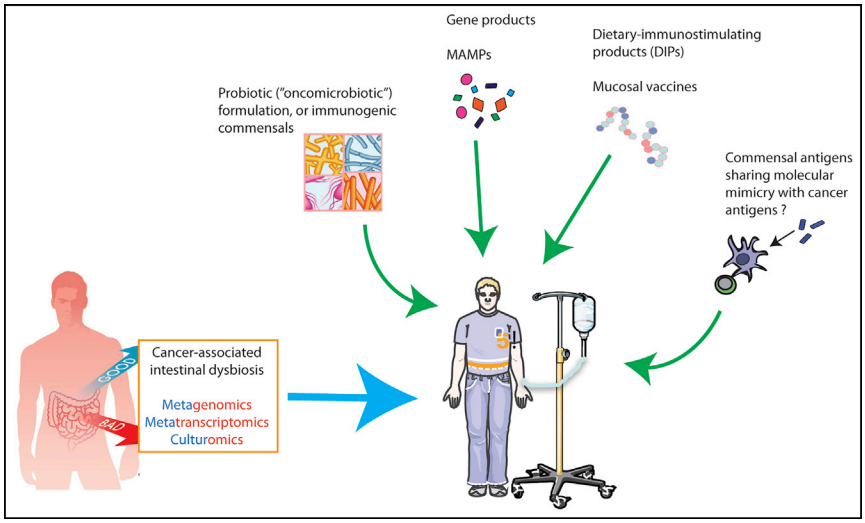

Figure 3. Mobilizing the Gut Microbiota to Circumvent Primary Resistance to ICB in Patients

Determining the precise microbiome of a given patient is becoming possible with the development of metagenomics, metatranscriptomics, and culturomics platforms. The gathered information from these techniques can be used for selecting an approach that will facilitate a particular cancer immunotherapy. Ancillary therapeutic approaches that can be selected to achieve this might include probiotic formulations, gene products, products containing MAMPs, DIPs, mucosal vaccines, or potentially commensal antigens with molecular mimicry to tumor antigens.

coverage of the core gene content and functions of the microbiome, enabling the possibility that oncologists can one day use these databanks to explore cancer-associated dysbiosis.

The second consideration involves the findings that mucosal (re)colonization with live (and not dead) immunogenic bacteria (capable of eliciting bacteria-specific Th1 cell immune responses), such as *E. hirae* or *B. fragilis* either alone or combined with *B. cepacia* or *Bifidobacterium*, is required for reinstating anti-cancer adaptive T cell responses (Sivan et al., 2015; Vétizou et al., 2015; Viaud et al., 2013). Hence, a specific probiotic formulation consisting of one or several of these immunogenic commensals (considering a choice of the most effective isolates of these species) would be necessary for testing in preclinical models and clinical trials to prevent or compensate for primary resistance to ICBs (Figure 3). Appropriate and standardized processes respecting good manufacturing procedures and rigorous quality control will guarantee bacterial identity, viability, and gastrointestinal survival throughout shelf life, as has been shown for the treatment of *C. difficile* (Auclair et al., 2015). Importantly, phase I trials will be necessary for dysbiotic, solid-tumor-bearing patients to ensure safety and satisfactory pharmacoimmunodynamics of the products (i.e., the capacity of such probiotic compositions to elicit Th1 immune responses against these commensals). Beyond the more tangible application of probiotics containing live immunogenic bacterial commensals, which could be called "oncomicrobiotics," future possibilities might take advantage of metatranscriptomic analyses to indicate novel gene products of immunogenic bacteria or products containing microbe-associated molecular patterns (MAMPs) that mediate their innate signaling within the host. Furthermore, the use of "prebiotics" (nondigestible compounds that stimulate the growth and/or functions of select gut microbiota), "postbiotics" (nonviable gut microbiota products that stimulate biological activities within the host, e.g., butyrate), or carefully selected antibiotics (i.e., that target an undesirable bacterial population) alongside ICBs could yield alternative combinatorial regimens of promise to cancer patients (Zitvogel et al., 2015). Confirmation of the particular bacterial species or families that impede ICB immunotherapy or promote immunotherapy-associated toxicity might warrant a more selective approach than is possible with existing antibiotics. Highly specific antimicrobials such as bacteriocins could be useful in such cases (Mathur et al., 2014).

The third consideration is that, besides providing a secondary costimulatory signal for T cell priming (or merely igniting innate signaling pathways involving TLR, NLR, or inflammasome platforms), immunogenic bacteria might provide the primary cognate MHC-peptide complex interaction with the TCR and hence elicite cognate immune responses that then cross-react with tumor antigens. Indeed, cross-reactivity of tumor-, self-, and pathogen-specific TCRs to microbiota-derived peptides or epitopes has been documented with relation to cancer (Dutoit et al., 2002; Rubio-Godoy et al., 2002; Snyder et al., 2014), autoimmunity, and infection, respectively (recently reviewed in Zitvogel et al., 2016). Moreover, among the CTL anti-tumor specificities responding to anti-CTLA-4 treatment, some are suggested to be due to cross-reactivity with bacterial epitopes (Snyder et al., 2014). This possibility warrants further investigation into whether commensals and cancer antigens share epitopes that could engage cross-reactive TCRs of high avidity.

Hence, dietary immunostimulating products (DIPs) or mucosal vaccines composed of effective tumor or bacterial epitopes plus bacterial adjuvants could be envisioned to prevent or compensate for primary resistance to ICBs or other immunotherapies (Figure 3). It remains to be established whether such mucosal DIPs are more effective than conventional parenteral vaccines at eliciting pTh17 immune responses against these commensals. Similarly, the transfer of high-avidity TCR-recognizing commensal antigens sharing high molecular mimicry with cancer antigens to the autologous T cell pool of cancer recipients could become another approach to restoring sensitivity to ICBs (Snyder et al., 2014; Zitvogel et al., 2016).

Conclusions

A plethora of tumor- and host-related factors combine in diverse ways to define the heterogeneity in clinical responses to ICB anti-cancer therapy. A variety of adjunctive therapeutics are being combined with ICBs to reduce this heterogeneity by improving the probability, duration, and potency of clinical activity; many of these have seen success, albeit often in parallel with increased toxicities. A new breakthrough in this

arena, which could be feasible beside multi- (or mono-)therapeutic strategies, is manipulation of the gut microbiota. Just as the microbiome is modified during cancer, possibly supporting oncogenesis, we have the opportunity to manipulate the microbiome toward a status that promotes immune-mediated tumor control. However, the exact microbial constitution of this status for a given ICB (or other immunotherapy) and the particular approaches clinicians should use to manipulate the microbiome remain to be determined. As a perspective, precise guidelines for the avoidance and preference of defined dietary compounds, the administration of probiotics or selective antibiotics, and the supplementation of specific bacterial strains ("oncomicrobiotics") or their products should be envisaged as a combined strategy for avoiding dysbiosis, restoring eubiosis, favoring homeostatic gut immunity, and stimulating effective anti-cancer immunosurveillance elicited by immunotherapy.

AUTHOR CONTRIBUTIONS

J.M.P., M.V., and L.Z. wrote and prepared figures for the manuscript. G.K., R.D., B.R., and M.P.R. edited and provided comments to improve the manuscript. M.V., R.D., M.P.R., B.R., T.Y., J.M.P., P.L., M.C., I.G.B., and L.Z. performed significant studies in the subject area of this manuscript.

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