




# The Great Debate at 'Immunotherapy Bridge', Naples, December 5, 2019

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## ABSTRACT

As part of the 2019 Immunotherapy Bridge congress (December 4–5, Naples, Italy), the Great Debate session featured counterpoint views from leading experts on six topical issues in immunotherapy today. These were the use of chimeric antigen receptor T cell therapy in solid tumors, whether the Immunoscore should be more widely used in clinical practice, whether antibody-dependent cellular cytotoxicity is important in the mode of action of anticytotoxic T-lymphocyte-associated protein 4 antibodies, whether the brain is immunologically unique or just another organ, the role of microbiome versus nutrition in affecting responses to immunotherapy, and whether chemotherapy is immunostimulatory or immunosuppressive. Discussion of these important topics are summarized in this report.

## INTRODUCTION

Over recent years, extensive research has improved our understanding of tumor immunology and enabled the development of novel treatments that can harness the patient's immune system and prevent immune escape. Through numerous clinical trials and real-world experience, a large body of evidence of the potential for long-term survival with immunotherapy agents has been accumulated across various types of malignancies, starting in melanoma and extending to other tumors. Organized by the Fondazione Melanoma Onlus, Naples, Italy, the Immunotherapy Bridge Congress was first held in 2015 to provide a forum for international experts to discuss new approaches and strategies in the field of immunotherapy. As part of the fifth Immunotherapy Bridge congress (December 4–5, 2019, Naples, Italy), the Great Debate session featured counterpoint views from invited leading experts on six topical issues in immunotherapy today. These were the use of chimeric antigen receptor (CAR) T cell therapy in solid tumors, whether the Immunoscore in colon cancer should be more widely used in clinical practice, whether antibody-dependent cellular cytotoxicity

(ADCC) is important in the mode of action of anticytotoxic T-lymphocyte-associated protein 4 (anti-CTLA-4) antibodies, whether the brain is immunologically unique or just another organ, the role of microbiome versus nutrition in affecting responses to immunotherapy and whether chemotherapy is immunostimulatory or immunosuppressive. Discussion of these important topics are summarized in this report.

## USE OF CAR T IN SOLID TUMORS: YES OR NO Kunle Odunsi: yes

The cancer immunity cycle involves several stages that starts with release of cancer cell antigens, through priming, activation, trafficking and infiltration of T cells into tumors and finally tumor disruption. The use of CAR T cell or transgenic T cell receptor (TCR) therapy bypasses many of these steps and the success of this approach has revolutionized the treatment of several hematological malignancies. Now, there is increasing focus on the potential role of CAR T therapy for solid tumors.

The first question over the use of CAR T in solid tumors is what are we asking T cells to achieve? In liquid tumors, the tumor microenvironment (TME) is peripheral blood so it is reasonably straightforward for T cells to mediate their effects. However, in solid tumors T cells have to successfully traffic from the blood into solid tumors despite potential T cell chemokine receptor umor-derived chemokine mismatches. T cells then have to infiltrate the stromal elements of solid tumors in order to elicit tumor-associated antigen (TAA)-specific cytotoxicity. Even after successful trafficking and infiltration, T cells become rapidly dysfunctional owing to a TME that is hostile because of multiple metabolic, inhibitory and



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immunosuppressive factors, along with the issue of TAA loss or heterogeneity.

The ideal TAA for CAR T therapy in solid tumors should be selectively expressed on the surface of tumor cells at high levels, but not at all or at very low levels on the surface of normal tissues. Expression should be homogeneous and ideally across all tumor cells. The degree of specificity is also critical for safety, with the most feared complication of CAR therapy catastrophic and rapid on target-off tumor events than have the potential to be fatal. To date, a wide range of different target antigens have been used in clinical trials. None so far is ideal but we are learning how to refine strategies to better identify suitable targets.

Varying clinical outcomes have been reported across solid tumor antigen targets. CAR T cells specific to GD2 resulted in 3 of 11 patients with neuroblastoma having complete remissions, HER2 CARs for sarcoma resulted in 4 of 17 patients showing stable disease and 2 of 11 patients with lung cancer had partial responses with HER1 CARs.<sup>1</sup> These results suggest at least a hint that CAR T therapy may have potentially use in solid tumors.

The safety of CAR T therapy is a key issue and can only really be established in careful clinical trials. Possible solutions for improving safety include the use of 'self-limited' CAR cells which employ mRNA electroporation rather than lentivirus to transiently express the CAR receptor, or the insertion of suicide genes that can be activated in case of adverse events, for example, HSV-TK gene or inducible caspase 9 gene. It is also possible to increase the specificity of CARs by requiring two antigens to be recognized to promote activity or to use combinatorial recognition circuits (eg, synthetic Notch receptors). All these approaches can help to mitigate the risk of fatal outcomes.

TAA heterogeneity can be addressed by targeting multiple antigens at once, as shown by CARs targeting both CD19 and CD20 in B cell leukemia, which provides better 'killing coverage' and may prevent the development of resistance. Another approach is to use multi-functional CARs which can encode by-products, such as cytokines, to augment tumor killing.

CAR antigens have to be expressed on the cell surface but most solid tumor antigens are intracellular, so lessons can be learned from the use of TCR transgenic T cells. The first report of autologous T cells transduced with a TCR directed against the NY-ESO-1 antigen showed remarkable tumor regression in patients with synovial cell sarcoma or metastatic melanoma.<sup>2</sup> Patients who demonstrated responses to therapy were those with T cell persistence. Poor persistence of transferred CD8+ cells and insufficient CD4+ T cell support are two key challenges of adoptive T cell therapy. In a currently ongoing trial (NCT03691376), autologous engineered hematopoietic stem cells with tumor-recognizing CD4 TCRs are being used for long-term support of NY-ESO-1 CD8 TCR transduced T cells in recurrent or treatment-refractory ovarian cancer, with the aim of promoting long-term

persistence of TCR T cells. Relevance of this to CAR T is that persistence of CAR T cells may be improved by harnessing the differentiation stage in vivo. One strategy to address intracellular antigens is to generate TCR-mimic cells. For example, T cells modified to express the TCR-mimic CAR, WT1-28z, directed against the peptide portion of the intracellular onco-protein Wilms tumor 1 (WT1), specifically targeted and lysed HLA-A\*02:01+, WT1+ tumors and enhanced survival of mice engrafted with HLA-A\*02:01+, WT1+ tumors.<sup>3</sup>

With regard to T cell trafficking, it may be possible to harness our understanding of immune cell chemotaxis and migration in novel ways in order to generate T cell detection and homing circuits. This may involve using CARs that co-express chemokine receptors resulting in increased intratumoral migration of CAR T cells and tumor eradication. Oncolytic viruses that encode tumor targets and chemokines may also be used. In addition, the local injection of CARs into tumors is being explored in clinical trials in liver, head and neck and other cancers.

Various strategies can be used to help CARs survive a hostile solid TME.<sup>4</sup> Physical and metabolic barriers can be overcome by CARs that deplete fibroblast cells or degrade the extracellular matrix. Tumor-derived soluble factors and cytokines may be overcome by CARs that interrupt inhibitory adenosine and PGE2 signaling, or that express dominant negative transforming growth factor (TGF)- $\beta$ . Simultaneous depletion of immunosuppressive immune cells may be achieved by the use of alternative homeostatic cytokines, such as interleukin (IL)-7 and IL-21, to boost CAR efficacy without stimulating regulatory T cells (Tregs). Intrinsic regulatory mechanisms of T cells may be addressed by combining CAR therapy and programmed death-1 (PD-1) blockade, the use of PD-1 switch receptors to neutralize inhibitory PD-1 signaling, blocking CTLA-4 enhanced adoptive transfer, or engineering CAR T cells lacking inhibitory molecules (eg, diacylglycerol kinase).

Toxicities may be controlled by user-controlled regulatory strategies that potentially allow a physician to modulate the survival of T cells, as well as the timing, strength and location of their activity. In addition, it may be possible to insert feedback control systems into therapeutic T cells which allow them to autonomously monitor when adverse outcomes reach a critical stage. Various other immunoenvironmental advances are also being developed to further improve CAR T efficacy and safety.<sup>5</sup>

The ultimate goal of engineered immune cells (CAR T, TCR T) is to provide a reliable, safe and effective platform for treating cancer. Individual cancer types may present different challenges, and the types of engineered T cells that they need may be different. However, advances in synthetic biology and genome engineering are providing powerful tools to address the engineering needs of T cell therapy.

#### Chrystal M Paulos: no

CAR T cell therapies have been successful in hematologic malignancies but have been less effective to date in

## Use of CAR T therapy in hematological malignancies and solid tumors

	Solid tumors	Liquid tumors
<b>Heterogeneity</b>	Do not express same mix of surface Ag so, CAR T cells may not "see" solid tumors. TILs, CD4 help or multiple cars instead?	CAR T cells do not always kill all blood cancer cells.
<b>Microenvironment</b>	Some solid tumors express PD-L1, an immune-suppressive molecule.	T cells can circulate in the blood. Thus, CAR T cells can expand well in circulation and swiftly kill blood-derived malignancies.
<b>Delivery</b>	A solid mass of tumor cells thousands of layers thick are difficult for T cells to penetrate. Administration may be key?	FDA-approved CAR T cells are infused into blood, where they can easily target and lysis malignant B cells.
<b>Perfect antigen</b>	Ag on solid tumors are also expressed on healthy tissue. Thus, neoantigens shall be key targets.	Patients can live without normal B cells. Yet B cells deficiencies in patients might impair protection against viruses.

**Figure 1** Use of chimeric antigen receptor (CAR) T therapy in hematological malignancies and solid tumors. PD-L1, programmed death ligand-1; TIL, tumor-infiltrating lymphocyte.

treating patients with solid tumors (figure 1). Anti-PD-1, anti-CTLA-4 therapies as well as the transfer or tumor-infiltrating lymphocytes (TIL) have been successful across many solid tumors and strategies involving these in combination with one another and other immunotherapies may be a more worthwhile and feasible approach than pursuing the use of CAR T cells. This is not to say CAR T cells may not have a place in the treatment of solid tumors, but rather that other options may be more viable. This may be especially for those patients who do not have easy access to the expertise increasingly concentrated at major cancer centers.

Theoretically, CAR T therapies could have a role in the treatment of solid tumors but this will be an uphill challenge. There is a difficulty in designing a CAR against an antigen expressed only in the tumor and not in normal tissue. To date, clinical trials with CAR T cells in solid tumors have often demonstrated severe off-tumor toxicities since the targeted antigens are not completely foreign to the host, and even low expression in normal tissue can result in serious adverse effects.<sup>5</sup> Some TAAs have been identified that result in more limited off-tumor effects, but many of these targets for CAR T cells have shown poor clinical efficacy in patients, with disease stabilization the best response in many cases. In addition, solid tumors treated with CAR T cells may undergo antigen escape due to selection pressure that favors tumor cells lacking the targeted antigen. This highlights the major problem of tumor heterogeneity for solid tumor CAR treatments.

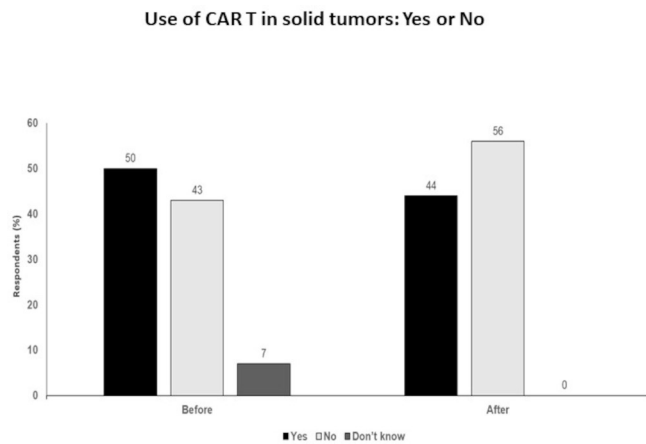
Even if the ideal solid tumor antigen could be identified and targeted, CAR T cell therapies face further obstacles including poor trafficking to the tumor site, difficulties in penetrating and infiltrating the tumor and limited persistence and proliferation within the host. Moreover, CAR T cells that can infiltrate the tumor can be functionally suppressed within a hostile TME, which is rich in suppressor cytokines, such as TGF- $\beta$  and IL-4, and inhibitory molecules including programmed death ligand-1 (PD-L1). Activated CAR T cells within the TME express high concentrations of exhaustion markers

such as PD-1, Tim-3, Lag3 and 2B4, indicative of the challenging TME.

It should also be noted that, although the peripheral blood is a much more welcoming environment for CAR T cells, the use of CAR therapies in hematological malignancies has not always succeeded. However, there has been a tendency for these failures to be less widely discussed compared with successes, with the possible result that, even in liquid tumors, the potential of CAR T therapies may be overstated. In solid tumors, CAR T therapy has many significant challenges to overcome. Although various bioengineering approaches are being tested to overcome these obstacles, it is not yet known whether these may be effective. It may be that TIL-based adoptive cell transfer together with cancer vaccines and anti-PD-1s represent more feasible therapeutic approaches. However, it is important to recognize that CAR T cell development for patients with solid tumors is still at an early stage. A danger is that CAR T cell therapy for solid tumors will be dismissed after a few failed trials, even though it may still represent a promising platform.

### Key points

- ▶ CAR T cell therapy has revolutionized the treatment of several hematological malignancies and there is increasing focus on its potential role in solid tumors.
- ▶ For CAR T therapy to be effective in solid tumors, T cells have to traffic into the tumor, infiltrate the stroma and overcome a hostile TME with multiple metabolic, inhibitory and immunosuppressive effects.
- ▶ TAAs in solid tumors should be selectively and homogeneously expressed on tumor cells at high levels but not at all or at very low levels on the surface of normal tissues.
- ▶ To date, clinical trials with CAR T cells in solid tumors have often demonstrated severe off-tumor toxicities since the targeted antigens are not completely foreign to the host, and low expression in normal tissue can result in serious adverse effects.



**Figure 2** Use of chimeric antigen receptor (CAR) T in solid tumors: yes or no. Audience response before and after debate.

- ▶ CAR T therapy has many significant challenges to overcome in solid tumors. Although various bioengineering approaches are being tested to overcome these obstacles, it is not yet known whether these will be effective (figure 2).

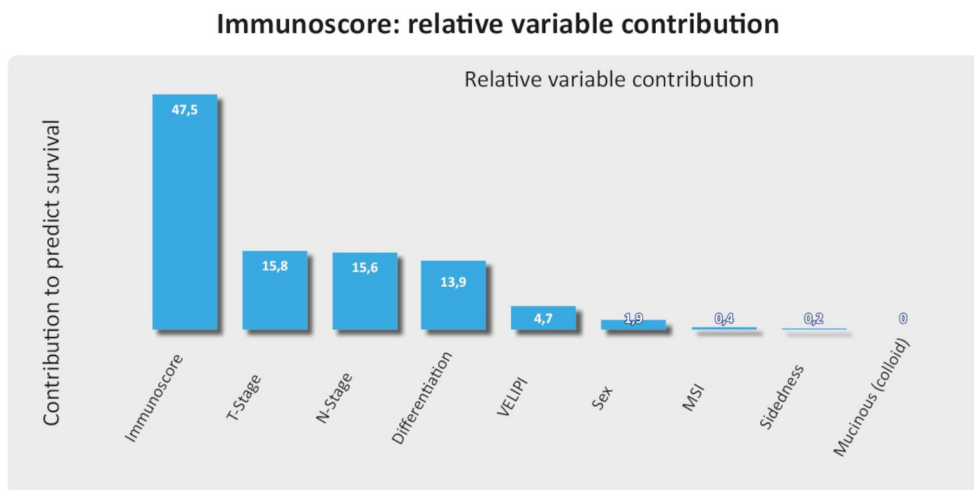
### IMMUNOSCORE IN COLON CANCER CLINICAL PRACTICE: YES OR NO

**Jerome Galon: yes**

The importance of lymphocyte infiltration was shown as early as 1921 with a report that lymphocytic infiltration influenced postoperative longevity in gastric carcinoma. However, the significance of this was largely ignored until the impact of tumor immune infiltrate on patient survival in colorectal cancer was shown.<sup>6</sup> This study was the first to show that the Immunoscore was highly significantly associated with time to disease recurrence in multivariate analysis, independent of age, sex, T stage, N stage, microsatellite instability and existing prognostic factors.<sup>6</sup> Immunoscore was also the only independent parameter associated with overall survival (OS). The lack of a

consensus definition was addressed by a worldwide effort that showed the Immunoscore assay to be an accurate and powerful prognostic factor.<sup>7</sup> In a multivariate regression model for OS stratified by center that combined Immunoscore with T stage, N stage, sex, Venous Emboli, Lymphatic Invasion, Perineural Invasion (VELIPI), histological grade, mucinous-colloid type, sidedness and microsatellite instability (MSI), Immunoscore was the most significant parameter.<sup>7</sup> Immunoscore also showed the highest contribution to predict survival (47.5%), in comparison to T stage (15.8%), N stage (15.6%), differentiation (13.9%), VELIPI (4.7%), sex (1.9%), MSI (0.4%), sidedness (0.2%) and mucinous colloid type (<0.01%) (figure 3).

The Immunoscore has clinical utility in colon cancer at all disease stages. In patients with stage I disease, the lower the Immunoscore the higher the risk of disease relapse, with the Immunoscore the only predictor of risk in multivariate analysis. Given this, patients with a low Immunoscore may require more careful follow-up after surgery. In stage II disease, the Immunoscore (low/intermediate or high) was prognostic for time to recurrence.<sup>7</sup> In these patients, chemotherapy may be considered in high-risk patients and the Immunoscore may be a more accurate way to identify these patients. Moreover, in stage II patients traditionally considered as high-risk (T4N0), a high Immunoscore predicted low-risk of recurrence. Immunoscore also showed the highest contribution to predict relapse (76.2%). Thus, patients considered high-risk based on staging may actually be low-risk based on Immunoscore and so could avoid unnecessary chemotherapy. In patients with locally advanced stage III cancer, stratification into five risk categories based on Immunoscore was predictive of time to recurrence. No patients in the highest Immunoscore category had disease recurrence or died over 8 years of follow-up. Thus, Immunoscore can be used to identify patients with stage III disease at high-risk and also those with minimal risk, who may not require chemotherapy.



**Figure 3** Immunoscore: relative variable contribution Immunoscore: contribution to predict survival, in comparison to T stage, N stage, differentiation, VELIPI, sex, microsatellite instability (MSI), sidedness and mucinous colloid type.

In a phase III randomized study of patients with stage III colon cancer, a comparison of 3 vs 6 months of chemotherapy was inconclusive, with non-inferiority of 3 months of therapy not confirmed.<sup>8</sup> However, Immunoscore was predictive of disease-free survival (DFS) over 6 years, with no recurrence or deaths in the highest Immunoscore group.<sup>9</sup> In patients treated with 6 months FOLFOX chemotherapy (Folinic acid, Fluorouracil and Oxaliplatin), a high Immunoscore significantly predicted response in all patients with stage III colon cancer as well as in low-risk (T1-3 and N1) and high-risk (T4 or N2) patients.<sup>10</sup> In patients with stage IV disease, Immunoscore applied to metastases should be added to pathological score and molecular status for routine clinical assessment. Of these three parameters, only Immunoscore had a significant contribution to the risk of time to recurrence and OS. Immunoscore also has the highest contribution to the risk of death in metastatic disease.

Immune markers comparable to Immunoscore have also been shown to be predictive of response to treatment in other cancers. For example, in metastatic melanoma, CD8+ density in the invasive margin was a better predictive marker of response to anti-PD-1 therapy than PD-L1 or other biomarkers.<sup>11</sup>

The Immunoscore stratifies patients into low-risk and high-risk and is the strongest prognostic parameter in univariate and multivariate analyses. It has been shown to be predictive of response to chemotherapy at all disease stages in colon cancer and is likely also predictive of response to immunotherapy. There is a strong argument for introducing a 'I' for immune into the TNM classification of cancer. Immunoscore is an approved in vitro diagnostic for clinical use in colon cancer in Europe and there are certified laboratories in the USA and China. Currently, non-inclusion in treatment guidelines and reimbursement issues are two of the major obstacles to more widespread adoption.

#### **Carlo Bifulco: no**

The demonstration in 2006, that the type, density and location of immune cells within tumors, as quantified by the Immunoscore, was a better predictor of patient survival than histopathological methods used to stage colorectal cancer,<sup>6</sup> was a major and unexpected paradigm change, since at the time, cancer immunotherapy was not a mainstream accepted therapeutic option and only limited pre-existing evidence was available that supported the role of immune infiltrates in influencing patient outcomes. The study clearly showed that the assessment of immune infiltrates is a powerful prognostic factor, potentially more predictive of outcomes than traditional TNM staging, highlighting the need for a fundamental change in assessing patients with cancer that includes the host immune response and demonstrating that relying solely on tumor characteristics is insufficient to fully capture the biology of cancer.

Multiple subsequent publications have assessed and validated the Immunoscore in larger cohorts. In 2018, an

international task force consortium assessed the Immunoscore assay in patients with TNM stage I–III colon cancer and showed that it provided a robust and reproducible estimate of the risk of disease recurrence in patients.<sup>7</sup> These and other similar findings, strongly support the clinical and analytical validity of the Immunoscore, and argue for the incorporation of the Immunoscore as a new component of an improved TNM-Immune (TNM-I) cancer classification.

A critical aspect of the Immunoscore that has however so far prevented a wide adoption in the routine clinical practice is the lack of a definitive demonstration of its clinical utility. Although interpretations of what clinical utility actually is can vary, an undeniable key aspect with regard to the utility of biomarkers is whether they can predict treatment responses and thereby drive treatment decision-making. This ability to predict treatment response is a key driver in the uptake of diagnostic assays, as shown by a plethora of widely adopted companion diagnostics that are now approved and included in the clinical treatment guidelines for many cancer types.

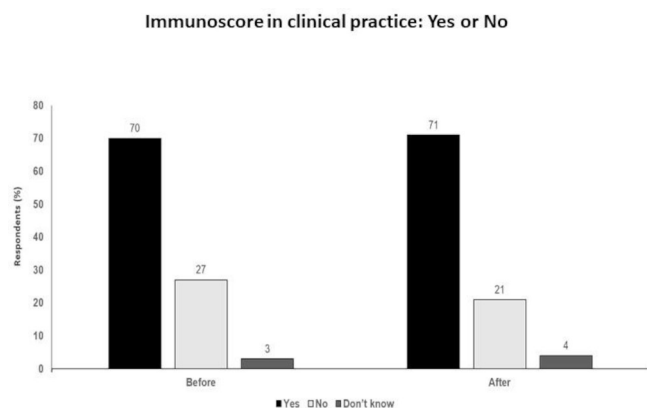
A possible explanation for the lack of a more extensive exploration of the possible predictive value of the Immunoscore is that the dominance of the framework of targeted therapies, based on a relatively simple biomarker approach, that is, the presence or absence of the targetable mutation in the patients cancer, has brought to a very narrow interpretation of the concept of companion diagnostics and has discouraged the exploration of more complex biomarkers. A second contributing factor may be incidental, and possibly related to the Immunoscore being initially developed and best established in colon cancer. Colon cancer is also the cancer type where high MSI (MSI-H) is most frequently positive, and the Food and Drug Administration (FDA) accelerated approval of pembrolizumab for patients with MSI-H or mismatch repair-deficient solid tumors, granted in 2017, provided one of the strongest biomarkers predictive of responsiveness to immunotherapies that is currently available. Of note, this strength of MSI-H as a biomarker does not preclude the future possibly of incorporating the Immunoscore into a refined IO responsiveness assessment that further stratifies MSI-H patients. Another future open opportunity for Immunoscore is the neoadjuvant setting. As an example, a recent trial of neoadjuvant atezolizumab in urothelial carcinoma reported that the presence of activated CD8+ GZMB+ T cells correlated with outcome, whereas other emerging biomarkers, such as tumor mutational burden (TMB), did not.<sup>12</sup>

A broader consideration is that Immunoscore only provides a snapshot of the state of the overall adaptive immune response to the tumor. Although this information is extremely powerful, a deeper insight into the mechanisms that modulate the TME and ultimately enable the tumor immune evasion will be required for the development and rational deployment of novel therapeutic strategies. This deeper understanding of the biology of the TME is urgently needed, as there is

now a wide appreciation that PD-L1 and TMB status are by themselves not sufficient to guide immune-oncology therapies. As a consequence, multiple multiplexed multiparametric immunohistochemistry/immunofluorescence platforms and strategies are currently actively being developed to address these questions. These novel platforms can enable the acquisition of a large numbers of phenotypic biomarkers in a spatial context, and could significantly impact the field, similarly to what the transition from Sanger to next-generation sequencing (NGS) recently did for genomics. Ultimately, porting the analytical strengths of the Immunoscore to these 'next-generation' platforms tools will likely be needed to overcome the limitations of the current generation of immune-oncology biomarkers.

### Key points

- ▶ The Immunoscore was first shown to be the only independent parameter associated with overall OS in colon cancer and a consensus definition of the Immunoscore was addressed by a worldwide effort that showed the assay to be an accurate and powerful prognostic factor.
- ▶ Various studies support the clinical and analytical validity of the Immunoscore and its incorporation as a new component of an improved TNM-I cancer classification.
- ▶ The Immunoscore has clinical utility in colon cancer at all disease stages and has been shown to be predictive of response to chemotherapy and is likely also predictive of response to immunotherapy.
- ▶ However, the lack of a specific clinically relevant predictive indication for the Immunoscore may have limited its more widespread adoption.
- ▶ Immunoscore is an approved *in vitro* diagnostic for clinical use in colon cancer in Europe and there are certified laboratories in the USA and China.
- ▶ Currently, non-inclusion in treatment guidelines and reimbursement issues are two of the major obstacles to more widespread adoption (figure 4).



**Figure 4** Immunoscore in clinical practice: yes or no. Audience response before and after debate.

## IS ADCC IMPORTANT FOR ANTI-CTLA-4 MODE OF ACTION? YES OR NO

**Sergio A Quezada: yes**

Anti-CTLA-4 antibodies alter the intratumoral balance between effector (Teff) and Treg cells and increase the Teff:Treg ratio. In an *in vivo* melanoma model, anti-CTLA-4 blocked the inhibitory activity of CTLA-4 antibodies on Teff and Treg cells, with high levels of surface CTLA-4 on Treg cells relative to Teff cells promoting preferential depletion of Treg cells at the tumor site.<sup>13</sup> This resulted in enhanced antitumor Teff cell activity capable of inducing tumor regression.

The effect of anti-CTLA-4 on Tregs is dependent on the presence of Fc $\gamma$  receptor (Fc $\gamma$ R)-expressing macrophages within the TME and is natural killer (NK) and complement-independent.<sup>14</sup> Fc $\gamma$ Rs are key drivers of ADCC. In mice, ADCC is regulated predominantly by the low-affinity receptors Fc $\gamma$ RIII and Fc $\gamma$ RIV, as well as the high-affinity Fc $\gamma$ RI. Treg depletion by FcIV macrophages is key to the antitumor activity of anti-CTLA-4, as shown by the high complete response rate to anti-CTLA in wild-type mice, which was absent if FcIV knockout mice were used.

Anti-CTLA-4 has a dual Treg depletion and Teff blocking effect in mice, meaning ADCC is important for anti-CTLA-4 activity. However, while this is true for mice, there is no consensus of the role of ADCC in anti-CTLA-4 activity in humans. The rules of engagement for mouse FcRs binding to rat or hamster IgG are different than those for human FcRs binding to human IgG.

In human cancers, CTLA-4 is expressed preferentially on tumor-infiltrating Tregs and this is shown in different cancer types, including melanoma, non-small-cell lung cancer and renal cell carcinoma. Using chimeric antimurine CTLA-4 antibodies with human IgG1 to model ipilimumab, IgG1 wild-type antibody demonstrated superior ADCC activity to an IgG1 mutant isotype with no binding to Fc $\gamma$ Rs, while IgG1 mutant with optimized Fc $\gamma$ R-binding promoted enhanced ADCC activity. The IgG1 isotype with enhanced ADCC promoted the depletion of tumor-infiltrating Tregs. Human anti-CTLA-4 IgG1 mutant promoted superior antitumor activity *in vivo* in a mouse model expressing human FcRs. Tumor growth was equivalent in untreated mice and those treated with the Fc-silent IgG1 mutant, demonstrating that CTLA-4 blockade alone is insufficient to promote tumor rejection in the context of human Fc $\gamma$ R-IgG interactions.

In humans, evidence for a role of Fc $\gamma$ R-mediated effector function in tumor-targeting antibody-based cancer therapies (eg, rituximab) derives from studies demonstrating an association between clinical responses and activating Fc $\gamma$ Rs that confer higher binding affinity to IgG1. If part of anti CTLA-4's activity is based on Fc $\gamma$ R engagement and Treg depletion, the presence of FcR polymorphisms that increase affinity for IgGs should correlate with enhanced antitumor activity. In patients with melanoma treated with ipilimumab, FcR polymorphism was associated with an improved response to anti-CTLA-4 among those

with a high mutational burden (ie, more immunogenic, inflamed tumor) but not those with a low mutational burden. More evidence for a role of ADCC in humans is provided by IPEX syndrome, which in effect represents a human model of FOXP3 knockout and the symptoms of which are similar to the immune-related side effects of anti-CTLA-4 treatment.

In conclusion, FcR engagement and Treg depletion is a key component of the activity of anti-CTLA-4 antibodies in mice. In humans, the presence of FcR polymorphism and high TMB correlates with increased responses to anti-CTLA-4. If ipilimumab is a partial Treg depleter then this may explain the need for high-affinity FcR polymorphism and, if so, then most of its toxicity is driven by blocking Treg activity. These data may point toward the need for a high-ADCC anti-CTLA-4.

#### John Timmerman: no

The critical question being debated is not whether Treg depletion during anti-CTLA-4 treatment is important but whether ADCC elicited by anti-CTLA-4 antibodies is clinically relevant.

In the study by Laurent *et al*,<sup>15</sup> CTLA-4 expression and ipilimumab reactivity were analyzed in melanoma models. However, most results were from cell lines with very heterogenous expression of CTLA-4, which is mostly intracellular and not accessible to therapeutic antibodies. Significant cell surface CTLA-4 expression was infrequent and only seen in 14% of cell lines. Moreover, cell lines can begin to aberrantly express antigens in vitro after prolonged culture. In an in vivo xenograft model, the co-engraftment of ipilimumab-treated melanoma cells with human allogeneic NK cells delayed tumor growth, as compared with mice receiving control xenografts. However, this delay was only modest and limited to the cell line with highest CTLA-4 expression. No data were reported from clinically relevant studies showing, for instance, correlation between response to ipilimumab and cell surface CTLA-4 expression.

In a study of 29 patients with advanced cutaneous melanoma, ipilimumab engaged ex vivo with FcγRIIIA (CD16)-expressing monocytes resulting in ADCC-mediated Treg depletion.<sup>16</sup> However, clinical studies have shown that FOXP3+ Tregs are not depleted from peripheral blood during ipilimumab treatment.<sup>17</sup> The study also reported that patients responding to ipilimumab had higher peripheral counts of non-classical monocytes at baseline, but this is only indirect evidence of ADCC involvement in an antitumor immune response.

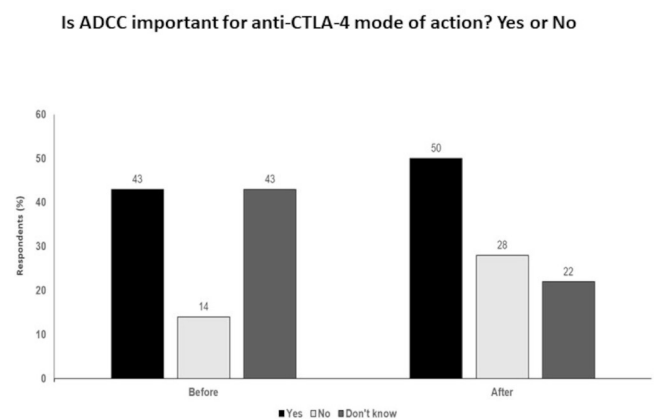
In a mouse model expressing FcγRs, antibodies with higher FcγR binding drove superior anti-tumor responses and survival. However, these studies only measured FcR binding and not ADCC. FcR binding could simply allow, after binding to an NK or monocyte cell surface, greater avidity ligation and blocking of CTLA-4 on the surface of T cells, irrespective of actual ADCC. Response to ipilimumab in humans also correlated with CD16a-V158F polymorphism; however, as previously, this is only a measure of

binding to the effector cell surface, and not actual ADCC or tumor cell killing in vivo. Thus, there are convincing data that FcR binding is important but no direct evidence that ADCC is involved.

Finally, even if ADCC is active in anti-CTLA-4 therapy, trying to engineer novel more potent anti-CTLA-4 antibodies for enhanced ADCC seems unlikely to improve outcomes, given that 20 years of effort to improve ADCC in anti-CD20 antibodies in lymphoma has failed to yield an antibody with clearly improved efficacy over rituximab, an IgG1 similar to ipilimumab.

#### Key points

- ▶ FcγRs are key drivers of ADCC and FcR engagement and Treg depletion is a key component of the activity of anti-CTLA-4 antibodies in mice.
- ▶ In a mouse model expressing FcγRs, antibodies with higher FcγR binding drove superior anti-tumor responses and survival. However, these studies only measured FcR binding and not ADCC.
- ▶ If part of anti CTLA-4's activity is based on FcγR engagement and Treg depletion, the presence of FcR polymorphisms that increase affinity for IgGs should correlate with enhanced antitumor activity. In humans, the presence of FcR polymorphism and high TMB correlates with increased responses to anti-CTLA-4. However, this may show that FcR binding is important but is not direct evidence that ADCC is involved.
- ▶ If ipilimumab is a partial Treg depleter then this may explain the need for high-affinity FcR polymorphism and, if so, then most of its toxicity is driven by blocking Treg activity.
- ▶ These data may point toward the need for a high-ADCC anti-CTLA-4. However, efforts to improve ADCC in anti-CD20 antibodies in lymphoma has failed to yield an antibody with clearly improved efficacy over rituximab (figure 5).



**Figure 5** Is antibody-dependent cellular cytotoxicity important for anticytotoxic T-lymphocyte-associated protein 4 mode of action? Yes or no. Audience response before and after debate.

Pivotal stage IV melanoma trials  
VS  
melanoma brain metastases-specific trials

Pivotal stage IV melanoma trials vs MBM-Specific

Trial	Number of patients
Ipi+gp100	676
Ipi+DTIC	502
BRIM-3	645
BREAK-3	250
COMBI-V	704
COMBI-D	423
coBRIM	495
KEYNOTE-002	540
KEYNOTE-006	834
CheckMate-037	631
CheckMate-067	945
COLLUMBUS	577
<b>TOTAL</b>	<b>6711</b>

Trial	Number of patients
Break MB	172
Vemurafenib	146
Ipi-CVG	72
NIBIT-M1	20
Pembrolizumab	18
COMBI-MB	125
CheckMate-204	119
ABC Collaboration	76
<b>TOTAL</b>	<b>746</b>

**Figure 6** Pivotal stage IV melanoma trials versus melanoma brain metastases (MBM)-specific trials.

### THE BRAIN IS JUST ANOTHER ORGAN? YES OR NO

Hussein A Tawbi: yes

Patients with brain metastases greatly outnumber those with primary brain tumors, with up to 30% of patients with metastatic cancer having brain metastases at some point. Brain metastases are also a strong prognostic indicator of very poor outcomes, with median survival of typically only 3–6 months.

In metastatic melanoma, brain metastases are an everyday problem in the clinic with 30%–40% of patients having brain metastases at the time of diagnosis, increasing to up to 60% during treatment and up to 80% at time of death. Historically, median survival of patients with melanoma has been approximately 8 months, with the presence of brain metastases reducing this to around 4 months. Recent advances in targeted therapy and immunotherapy have resulted in greatly improved outcomes, with over 50% survival at 5 years. Pivotal phase III studies of these new agents have included almost 7000 patients with melanoma. However, patients with brain metastases were excluded from all these trials (figure 6). Reasons for this include their expected worse prognosis with immediate neurological deterioration, the belief that drugs are unable to penetrate the brain, and the assumption that that brain is not immunologically responsive. There is also the underlying supposition that brain metastases may be better treated with surgery and/or radiation.

The brain is one of most protected parts of body, with many difficult to penetrate layers that makes access from the blood is limited, including an active efflux through the blood-brain barrier. However, many drugs can overcome the blood-brain barrier, even though they may have been specifically designed not to enter the brain, for example, to reduce toxicity.

In a phase I trial of dabrafenib in 10 patients with melanoma and untreated brain metastases, changes in intracranial and extracranial tumor size were similar, with 9 patients having an intracranial response.<sup>18</sup> In the BREAK-MB (melanoma with brain metastases) trial,

which was the first to specifically enroll patients with melanoma with brain metastases, intracranial responses of 39% in patients with no previous brain treatment and 31% in patients with prior brain treatment were achieved.<sup>19</sup> Several other trials have also since shown positive intracranial responses. In the COMBI-MB (melanoma with brain metastases) trial, dabrafenib plus trametinib was active with a 60% intracranial response rate and a manageable safety profile in patients with BRAF V600-mutant melanoma with brain metastases, although the median duration of response was relatively short.<sup>20</sup> Median progression-free survival (PFS) was also shorter than observed in patients without brain metastases. The combination of encorafenib plus binimetinib may be a more effective targeted combination for brain metastases and is being investigated using higher doses in the ongoing POLARIS trial.

Immunotherapy is also effective in the brain. In a phase II trial, ipilimumab showed activity in patients with advanced melanoma and brain metastases, in particular those who were asymptomatic and not receiving corticosteroids.<sup>21</sup> Overall response rate (ORR) and PFS were similar with ipilimumab in patients with or without brain metastases. PD-1 monotherapy does appear less effective against brain metastases, with an intracranial response of 22% with pembrolizumab in patients with melanoma with brain metastases not requiring steroids, compared with the 30%–40% responses rates usually seen in patients without brain metastases.<sup>22</sup> However, this does not mean the brain should be treated differently to other organs, since similar response rates have been seen in liver metastases with pembrolizumab. Combination immunotherapy may be more effective than single-agent therapy. In the CheckMate-204 study of nivolumab plus ipilimumab in patients with unirradiated brain metastases, intracranial and extracranial benefit was similar (57% vs 56%).<sup>23</sup> However, complete responses were higher in the brain. Responses were also durable, with intracranial progression prevented for over 6 months in 64% of patients.



Symptomatic patients on steroids had lower response rates, which may relate to steroid use rather than the presence of brain metastases. Triplet combinations of a BRAF inhibitor plus MEK inhibitor plus anti-PD-1/PD-L1 have also shown promise in patients with brain metastases.<sup>24</sup> Beneficial effects have also been observed with other treatments in patients with breast or lung cancers.

The brain is truly just another organ and, moreover, offers the potential for novel combinations. Checkpoint inhibitor therapy combined with stereotactic radiosurgery (SRS) was associated with decreased distant and local intracranial failure compared with SRS alone in a retrospective study of patients with melanoma<sup>25</sup> and several prospective trials are ongoing to further assess this potential synergy. One positive consequence of this may be to encourage greater multidisciplinary efforts, with radiation oncologists working more closely with medical as well as neuro-oncologists in brain metastases-focused clinics.

#### Hideho Okada: no

Although checkpoint inhibition has resulted in response rates of over 50% in patients with melanoma with brain metastases,<sup>23</sup> outcomes in patients with primary brain tumors have been less positive. In preliminary analysis of the CheckMate-143 trial, nivolumab failed to prolong OS of patients with recurrent glioblastoma (GBM), and this arm of the trial was prematurely closed.<sup>26</sup> This poses the question of why there is a difference in response between metastatic and primary brain tumors.

GBM is more infiltrative and has been shown to disseminate throughout the brain and central nervous system (CNS), with cells found in the brain stem even when not originally detectable. Because of this, GBM is more protected by the blood-brain barrier than brain metastases. Penetration of IgG antibodies through the intact blood-brain barrier is only around 4%. There may also be more profound lymphopenia in recurrent GBM, especially after immunosuppressive standard of care chemoradiation. In addition, melanoma is much more immunogenic than GBM, with a higher TMB. However, there are some common challenges in primary and secondary brain tumors, including heterogeneity and primary/acquired resistance and common mechanisms of immune escape, such as heterogeneity, major histocompatibility complex downregulation and immunosuppressive molecules.

The brain is not just another organ. The idea of the brain as immunologically privileged dates to the first observation a century ago that rat sarcoma grew well when transplanted into the mouse brain parenchyma, but not when implanted into subcutaneous or muscle tissue. It was subsequently shown that if recipient spleen was co-transplanted with the foreign tumor in the brain parenchyma, tumor growth was inhibited. This has been attributed to the blood-brain barrier but, although a contributory factor, this is not the primary explanation. This immunoprivilege is relative and not absolute, is confined to the

CNS parenchyma, applicable to both adaptive and innate immunity, and mostly a result of defects in the afferent arm in adaptive immunity.

It has been shown that cerebral spinal fluid (CSF) can contain T cells and CAR T cells, which is used as evidence that these can cross the blood-brain barrier. However, CSF differs from the fluid in the brain parenchyma, the interstitial fluid (ISF), and there is a barrier between the two compartments between which cells are not freely exchanged. Antigen-presenting cells (APCs) in the CSF can drain into lymph nodes, but there is no clear route for those in the ISF. Thus, a major part of the brain's immuno-privilege is a defect in antigen presentation.<sup>27</sup>

With regard to the afferent arm in adaptive immunity, some trafficking of T cell can occur. In an experimental model, ovalbumin CD4+ T helper peptide vaccine promoted protective immunity via induction of CD8+ cytotoxic T lymphocytes (CTLs) against subcutaneous but not intracranial melanomas.<sup>27</sup> Subcutaneous tumors responded to either class I or class II peptides, while intracranial tumors did not respond to class II peptide. IHC revealed extensive infiltration of CD11c+ dendritic cells (DCs) in subcutaneous but not intracranial tumors.

There are also metabolic differences between extracranial and brain metastases. Analysis of melanoma brain metastases and patient-matched extracranial metastases identified significant immunosuppression in brain metastases, with reduced T cell, DC and macrophage infiltration and enrichment of oxidative phosphorylation (OXPHOS).<sup>28</sup> OXPHOS inhibition improved survival of mice bearing MAPK inhibitor-resistant intracranial melanoma xenografts and inhibited melanoma brain metastases formation.

Brain damage may also be associated with systemic immunosuppression. In GBM as well as other cancers with intracranial metastases, T cells appear to be sequestered to the bone marrow.<sup>29</sup> Thus, lesions in the brain may suppress the systemic immune response.

Finally, the brain may be susceptible to a systemic immune response. Impaired antigen presentation may lead to lack of tolerance meaning the brain is more prone to autoimmunity. The brain is the only organ in which autoimmunity can be induced, as shown by a mouse model of multiple sclerosis, experimental autoimmune encephalomyelitis. Also, in paraneoplastic cerebellar degeneration, peripheral activation of cdr2-specific CTLs is likely to contribute to the subsequent development of the autoimmune neuronal degeneration.<sup>30</sup> Both cancer and cerebellar cells express a common antigen, cdr2, which triggers a systemic CTL response, which can be responsible for ataxia in patients with undiagnosed cancers (eg, ovarian cancer). Severe neurotoxicity events associated with adoptive T cell therapy also suggest an immune effect on the brain. TCR targeting MAGE-A3 caused severe damage to brain gray matter by recognizing MAGE-A12 expression in the brain, resulting in two deaths.<sup>31</sup> In addition, high IL-6,

IL-2, granulocyte macrophage colony-stimulating factor and vascular endothelial growth factor levels have been observed in CSF during neurotoxicity, with both CD20 CAR and non-CAR T cells accumulating in the CSF and in the brain parenchyma.<sup>32</sup>

In summary, immunological privilege of the brain may be primarily characterized by impaired antigen presentation. However, there are also other issues, such as differential metabolism and T cell sequestration to the bone marrow. Nonetheless, the brain (and cancer in the brain) can be susceptible to the systemic immune response once the response is primed against brain-associated antigens.

### Key points

- ▶ Patients with brain metastases are often excluded from immunotherapy trials due to their expected worse prognosis, the belief that drugs are unable to penetrate the brain and the assumption that that brain is not immunologically responsive.
- ▶ Immunotherapy is effective in the brain and combination immunotherapy may be more effective than single-agent therapy. Nivolumab plus ipilimumab resulted in durable intracranial benefit in patients with melanoma with unirradiated brain metastases.
- ▶ Checkpoint inhibitor therapy combined with SRS has also been associated with decreased distant and local intracranial failure compared with SRS alone and several prospective trials are ongoing to further assess this potential synergy.
- ▶ However, checkpoint inhibition has been less effective in patients with primary brain tumors, which may be more infiltrative and less immunogenic than melanoma brain metastases.
- ▶ Immunological privilege of the brain may be primarily characterized by impaired antigen presentation but there are also other factors, such as differential metabolism and T cell sequestration to the bone marrow.
- ▶ Nonetheless, the brain can be susceptible to the systemic immune response once the response is primed against brain-associated antigens (figure 7).

## IS THE MICROBIOME OR NUTRITION MORE IMPORTANT FOR RESPONSE TO IMMUNOTHERAPY?

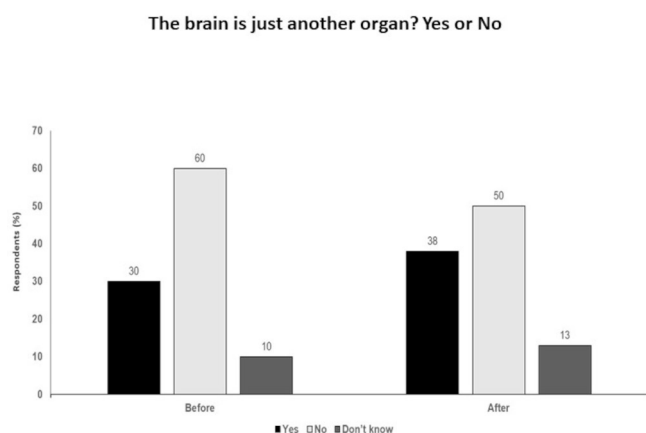
### Giorgio Trinchieri: microbiome

It is now widely accepted that the composition of the gut microbiome may contribute to the efficacy of cancer therapy, primarily by modulating the antitumor immune response through training infiltrating myeloid and APCs in distant tumors. This has shown in a series of studies, involving CpG-oligodeoxynucleotides, oxaliplatin, cyclophosphamide, ACT and immune checkpoint inhibitors. Until recently, most data were from mouse models but several clinical studies have now suggested a role of microbiota composition in response to treatment, including anti-PD-1 therapy.<sup>33–35</sup>

However, even though there is increasing evidence that the microbiome has a role in response to therapy, we do not yet know what constitutes a favorable or unfavorable microbiome. A critical question is whether we can somehow target the microbiome to improve therapeutic response. NGS and improved culture technology means it is now possible to better characterize the microbiome, with regard to identifying bacterial species that are present. However, modifying the microbiome is more difficult and various approaches have been suggested, including antibiotics, probiotics, prebiotics, diet and fecal microbial transplant (FMT).

Although several studies have indicated that microbiome composition influences response to anti-PD-1 therapy, no single bacterial species has been identified as positively correlated with response across different studies. Because it is not currently known which bacteria should be targeted, a possible strategy is to transplant fecal microbiota from a patient who responds to treatment to a non-responding patient. FMT is being assessed in ongoing trials in patients with melanoma who are refractory to anti-PD-1 treatment. In an ongoing study at the University of Pittsburgh, patients who are non-responders to pembrolizumab at 12 weeks are receiving FMT from a responder, with promising preliminary results. Fecal samples are also being transplanted into germ-free mice to assess whether responses correlate with those seen in patients.

Although there is as yet no clear indication of which bacteria are important, another approach is the use of a bacterial consortium. A consortium of 11 bacterial strains from healthy human donor feces that induces interferon (IFN)- $\gamma$ -producing CD8 T cells in the intestine was shown to enhance the therapeutic efficacy of immune checkpoint inhibitors in syngeneic tumor models.<sup>36</sup> This approach has the benefit of using a well characterized product. However, the microbiome represents a balanced ecological system and it is unclear how this might be affected by the introduction of such a consortium. Another potential strategy is the introduction of a single bacterial species that has been shown to correlate with response. Commensal *Bifidobacterium* have been shown to promote antitumor immunity and facilitate anti-PD-L1 efficacy in mice<sup>37</sup> and a trial is now ongoing in an attempt



**Figure 7** The brain is just another organ? Yes or no. Audience response before and after debate.

to identify a single species that may improve response to therapy in melanoma.

Targeting the microbiome represents a promising approach. Future goals include the discovery of reliable microbiome-related biomarkers for prediction of response and stratification of patients and the identification of favorable microbiomes for fecal transfer from responder patients or healthy donors. Identification of consortia of commensal bacteria that favor a clinical response and identification of perturbations (eg, through diet, prebiotics) able to induce or maintain a favorable microbiome composition will also be necessary.

#### Jennifer McQuade: nutrition

There is now an impressive body of evidence linking gut microbiota with response to immunotherapy. In contrast, there is very little data on the possible role of diet and nutrition on immunotherapy response. However, despite several studies identifying bacteria associated with improved response to anti-PD-1 therapy, there appears to be very limited overlap in terms of proresponse bacterial species across different cohorts. This could in part be attributed to differences in processing and sequencing of cultures. Other factors include geographic region and environmental influences, in particular diet. It may be that the real importance is not the particular bacterial species that are present, but rather their function.

Very little is known about the mechanisms by which gut microbiota influences antitumor immunity. Although the determinants of the gut microbiome has been well studied in other settings, what factors determine the microbiome in patients receiving immunotherapy has not been well studied to date. Gut microbiota are inherently modifiable, with <10% being genetically determined. Modifiable factors that influence the microbiome include body mass index, psychological factors, medications (eg, antibiotics, probiotics) and diet. Habitual diet is a key determinant of the gut microbiota, with a plant-based diet resulting in a microbiome with different characteristics to that seen with a meat-based diet.<sup>38</sup> Gut microbiome composition can also be rapidly changed by switching from a high-fat, low-fiber diet to a low-fat, high-fiber diet or vice versa. In a controlled feeding study, switching to a high-fiber, low-fat African-style diet from a high-fat, low-fiber western-style diet was associated with increased saccharolytic fermentation and butyrogenesis and suppressed secondary bile acid synthesis in an African-American cohort.<sup>39</sup>

Although there is relatively little overlap in bacteria identified as being proresponse to immunotherapy, many of the putative proresponse bacteria have strong dietary correlations from prior population-based studies. For example, many proresponse bacteria are fiber-fermenting bacteria which are typically present at higher abundance in people with higher intake of fiber-rich plant foods, leading to downstream production of short-chain fatty acids (eg, butyrate, propionate) in the gut. The interaction between the production of short-chain fatty acids by the microbiome and the development of the gut mucosal

layer and mucosal immunity is quite well studied but how this relates to antitumor immunity is less well known and is an area of active investigation.

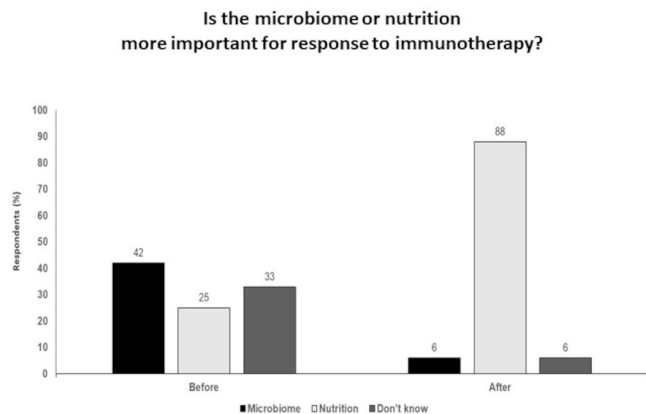
The microbiota is a key mediator of the effects of a plant-based diet on human health, but is not the only one. For instance, the diet may exert a direct positive effect on the immune system for example, antineoplastic effects exerted by polyphenols. A plant-based high-fiber diet is consistent with current dietary recommendations in cancer, although these recommendations are primarily based on data on cancer prevention. Future studies should prospectively investigate the effects of dietary fiber on the gut microbiome and immune response in the setting of immune checkpoint inhibitors.

Diet should be assessed in all observational microbiome cohorts and both habitual diet and baseline microbiota may influence response to microbiome modulation interventions including FMT and probiotic use.

In conclusion, the gut microbiota and its metabolites may mediate the effect of diet. The function of the microbiome is more important than its composition and this is shaped by diet. Moreover, diet may have other beneficial effects not mediated by the microbiome. Dietary intervention based on promoting a wholefood, plant-based high-fiber diet may be more cost-effective, acceptable and provide wider health benefits than approaches solely based on microbiome modulation.

#### Key points

- ▶ Several clinical studies have now suggested a role of gut microbiota composition in response to treatment, including anti-PD-1 therapy.
- ▶ Various approaches to render the microbiome more responsive to therapy have been suggested, including the use of antibiotics, probiotics, prebiotics, diet and FMT.
- ▶ Future goals include the discovery of reliable microbiome-related biomarkers for prediction of response and stratification of patients and the identification of favorable microbiomes.
- ▶ Although the microbiome appears to influence response to immunotherapy, the factors that determine the microbiome and how patients can achieve a proresponse microbiome are less well known.
- ▶ There appears to be very limited overlap in terms of proresponse bacterial species across different cohorts, which could in part be attributed to differences in environmental influences and in particular diet. It may be that the real importance is not the particular bacterial species that are present, but rather their function.
- ▶ Habitual diet is a key determinant of the gut microbiota, and gut microbiome composition can be rapidly changed by altering diet.
- ▶ The microbiota is a key mediator of the effects of diet on health, but is not the only one. For instance, the diet may exert a direct positive effect on the immune



**Figure 8** Is the microbiome or nutrition more important for response to immunotherapy? Audience response before and after debate.

system, for example, antineoplastic effects exerted by polyphenols (figure 8).

### IS CHEMOTHERAPY IMMUNOSTIMULATORY OR IMMUNOSUPPRESSIVE?

#### Claus Garbe: immunostimulatory

It is well known that chemotherapy can be immunosuppressive but, conversely, under certain circumstances, it may have an immunostimulatory effect. Nearly all patients receiving cytotoxic agents broad-based chemotherapy will experience immunosuppression since, by design, chemotherapy targets rapidly dividing cells. Since immune system cells divide quickly, they are among the many targets of chemotherapy. Major clinical sequelae of immunosuppressive chemotherapy can include bacterial, viral or fungal infection.

The immunosuppressive effects of chemotherapy were shown by a study in patients with metastatic melanoma, in which adjuvant treatment with IFN- $\alpha$  resulted in a significant DFS and OS benefit over observation after complete lymph node dissection.<sup>40</sup> However, the addition of dacarbazine to IFN- $\alpha$  reversed the beneficial effect, that is, the immunostimulatory effect of IFN- $\alpha$  was negated by the immunosuppressive effect of chemotherapy.

However, in certain situations, chemotherapy may have a positive immunostimulatory effect when used with immunotherapy. Responses to adoptive cell transfer of autologous TILs with IL-2 is improved by pretreatment with lymphodepleting myeloablative cyclophosphamide and fludarabine chemotherapy.<sup>41</sup> Agents such as paclitaxel or gemcitabine may also promote the elimination or inactivation of immunosuppressive Tregs or myeloid-derived suppressor cells (MDSCs), resulting in enhanced antitumor immunity.<sup>42</sup> Treg elimination or inactivation by low-dose cyclophosphamide (metronomic regimen) or paclitaxel, which is a mitotic inhibitor that selectively reduces Treg number and function while sparing effector T lymphocytes, can promote antitumor immunity and enhance the efficacy of immunotherapy. MDSC elimination, inactivation and/or differentiation into proinflammatory cells, for example, with gemcitabine

or fluorouracil, also enhances antitumor immunity and fosters the response to immunotherapy. Cytotoxic therapy may also promote the release of tumor antigens and thereby stimulate the immune response.<sup>43</sup> Use of cytotoxic drugs can destroy cancer cells and thereby release antigens with the subsequent immune response leading to tumor eradication. In the absence of a response to checkpoint blockade, chemotherapy, for example, paclitaxel and carboplatin in melanoma, can be used to promote tumor antigen release in order to stimulate the immune response.

There are only limited data on optimal regimens of chemotherapy in these instances and we are essentially at the start of learning how cytotoxic agents can be combined with immunotherapy. For example, the ideal duration of chemotherapy in these circumstances is unknown.

In summary, long-term use of chemotherapy is always immunosuppressive. However, chemotherapy can be used with adoptive T cell transfer and other immunotherapies for complete and incomplete myeloablation. Short-term administration of chemotherapy can also be used for the targeted removal of Tregs and MDSCs and to promote the immune response by antigen release.

#### Samir N Khleif: immunosuppressive

All chemotherapy acts on the cell cycle to damage rapidly dividing cells. Chemotherapeutic drugs are selected based on their direct cytotoxic effects on highly proliferating cells and cells are more sensitive to chemotherapeutic agents when in active phases of replication. This leads to an association with significant toxicities, which occur secondary to the effect of chemotherapy on highly proliferative normal cells. Cytotoxic agents, even administered short-term at standard doses, can lead to severe lymphodepletion, with greater reductions in CD4+ than CD8+ T cells. As an example of this issue, in 88 patients with primary breast cancer, lymphodepletion occurs after chemotherapy in the majority of patients. The levels of B and T cells were significantly reduced 2 weeks after chemotherapy and some of these cells remained significantly depleted even 9 months after chemotherapy.<sup>44</sup> Chemotherapy can also affect NK cells. These kind of changes occur regardless of the type of regimen and was long-lasting after just four cycles of chemotherapy. For example, docetaxel has a pronounced immunosuppressive effect on NK function.<sup>45</sup> Taxanes have cell-subset dependent effects, suppressing T, B and NK cells while having a stimulatory effect on macrophages.<sup>46</sup> Overall, standard doses of chemotherapy, even when given short-term, has a long-lasting effect on almost the whole repertoire of the immune system.

Chemotherapy also increases the immune-suppressive environment inhibiting effector cells and increasing negative regulators of the immune system. Bleomycin stimulates TGF- $\beta$ -dependent expansion of Tregs and doxorubicin-cyclophosphamide chemotherapy increases levels of circulating MDSCs.<sup>47 48</sup> Increased levels of circulating MDSCs correlate with decreased T cell responses.

Increased MDSCs with cyclophosphamide can attenuate antitumor CD4+ T cell response through the PD-1/PD-L1 axis,<sup>49</sup> and cyclophosphamide therapy-induced monocytes possess immunosuppressive activities.

Chemotherapy can also induce intrinsic immune resistance in cancer cells. Treatment of human or murine triple-negative breast cancer cells with cytotoxic agents promoted an immune evasion phenotype, with a marked increase in breast cancer cells expressing CD47+C-D73+PDL1+.<sup>50</sup> Etoposide has been shown to increase expression of PD-L1 on retinoblastoma cells and can cause T cell apoptosis by inducing T cell unresponsiveness, stimulating secretion of cytokines such as IL-10 and suppressing immune response.<sup>51</sup> Chemotherapy also induces nuclear factor kappa B (NF- $\kappa$ B) activation in cancer cells, which promotes the expression of cytokines and chemokines that modulate the interaction between tumor cells and other cellular components of the TME.<sup>52</sup> NF- $\kappa$ B activation is linked to IL-34 production which enhances immunosuppression of tumor-associated macrophages and mediates the survival of chemoresistant lung cancer cells.

To conclude, chemotherapy is clearly immunosuppressive since it inhibits the number and function of effector immune cells, increased the immune suppressive environment and induces intrinsic immune resistance in cancer cells.

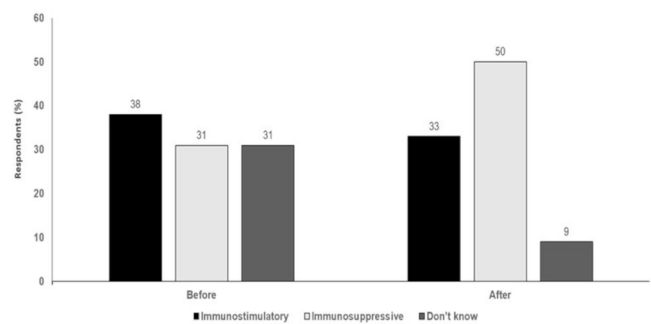
### Key points

- ▶ Chemotherapy is immunosuppressive since it inhibits the number and function of effector immune cells, increased the immune suppressive environment and induces intrinsic immune resistance in cancer cells.
- ▶ Overall, standard doses of chemotherapy, even when given short-term, have a long-lasting effect on almost the whole repertoire of the immune system.
- ▶ However, although chemotherapy is generally immunosuppressive it can, under certain circumstances, have an immunostimulatory effect.
- ▶ Chemotherapy can be used with adoptive T cell transfer and other immunotherapies for complete and incomplete myeloablation.
- ▶ Short-term administration of chemotherapy can also be used for the targeted removal of immunosuppressive Tregs and MDSCs and to promote the immune response by antigen release (figure 9).

### CONCLUSIONS

Counterpoint views from leading experts on four controversial clinical issues in immunotherapy today were presented during these Great Debate sessions. Given the constraints of the format and the intended nature of the session, each presentation was not intended as a rigorous assessment of the field but rather provided an opportunity to highlight some important areas of debate within immunotherapy. It may be that there are no clear right or wrong answers to these questions; however, it is hoped that these discussions can help focus attention on these

### Is chemotherapy immunostimulatory or immunosuppressive?



**Figure 9** Is chemotherapy immunostimulatory or immunosuppressive? Audience response before and after debate.

issues, stimulating further debate and encouraging the research needed to improve our understanding of immunotherapy and thereby further improve outcomes for patients.

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