OPTIMISING CANCER IMMUNOTHERAPY: CHALLENGES AND OPPORTUNITIES

Summary of Presentations from a prIME Oncology Scientific Exchange on 21st May 2016 in Amsterdam, Netherlands

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MEETING SUMMARY

Cancer immunotherapy has moved to the forefront in the treatment of patients with cancer, providing a unique opportunity to achieve dramatic and lasting anti-tumour responses in a variety of tumour types. When it comes to patient selection and development of novel immunotherapeutic agents and combinations, so far we have merely scratched the surface of this therapeutic approach. Leading experts in the field of cancer immunotherapy gathered in Amsterdam, Netherlands, on 21st May 2016 for a Scientific Exchange to discuss the current status of immunotherapy within the field of oncology and explore the future of this evolving therapeutic strategy. Current challenges and limitations regarding the use of immunotherapy were addressed for tumour types such as melanoma, lung cancer, bladder cancer, and renal cell carcinoma (RCC). Recent advances and future directions in the areas of immunotherapy biomarkers and mechanisms of resistance were also examined. Current evidence for combination strategies with immunotherapy was highlighted, including combinations with other immunotherapies or with radiotherapy. Below is a summary of the key points discussed during this scientific exchange.

Current Immunotherapy Strategies in the Management of Solid Tumours

Doctor Mario Colombo

The field of cancer immunotherapy is expanding at an extremely rapid pace. After being named the breakthrough of the year in 2013 by *Science*,[1] cancer immunotherapy has continued to revolutionise patient care across an ever-increasing range of malignancies. Immunotherapy seeks to initiate an anti-tumour immune response or augment any existing immune response, ultimately resulting in tumour regression and disease control. This requires cancer antigen presentation, priming and activation of T cells, infiltration of activated T cells into the tumour, and finally recognition and killing of cancer cells.[2] However, the inherent genetic heterogeneity of tumours and the propensity for tumour cells to escape or avoid immune targeting continues to encourage investigation of novel approaches and combination strategies. Several immunotherapies are now available and ongoing clinical trials are exploring a myriad of novel immunotherapeutic approaches, including vaccine therapies, adoptive transfer of tumour-infiltrating lymphocytes (TILs), antibody therapies targeting either immune checkpoints or activation pathways, and chimeric antigen receptor T cells.

Perhaps the most exciting achievement thus far in the use of cancer immunotherapies is the rapid and durable responses observed in a number of tumour types, albeit only in a minority of patients treated.[3] T cell activation, differentiation, and function are controlled by complex interactions between co-stimulatory and co-inhibitory molecules, with overlapping signalling pathways driving an ever-changing tide of immune response.[4] T cell signalling is carefully regulated both transcriptionally and post-transcriptionally. Cell surface expression of co-signalling molecules is continuously modulated within the tumour microenvironment and can differ greatly depending on the milieu of receptors and ligands present on the surface of surrounding cells, including antigen presenting cells (APCs). In addition, several co-signalling receptors can interact with more than one ligand, adding another level of regulation based on the differential expression of specific receptor–ligand pairs that act as either stimulatory or inhibitory signals.

The existence of unique tumour-specific antigens is one of the key premises behind cancer immunotherapy, allowing selective targeting of tumour cells without killing normal host cells. Recent advances in next-generation sequencing and algorithms for epitope prediction have opened the door for rapid identification of tumour neo-antigens.[5] Whole-exome sequencing and *in silico* tools to predict major histocompatibility complex (MHC) Class I molecule presentation can now quickly identify a neoepitope that can then be validated through T cell epitope screening to

assess T cell reactivity.[6] This type of information can be used to identify predictive biomarkers or new targets for immunotherapy, as well as develop neo-antigen vaccines or adoptive cell transfer therapies.

Ongoing research is aimed at expanding and refining the use of immunotherapeutic approaches in the treatment of patients with cancer. While immunotherapy is associated with rapid and durable responses, some patients never respond or develop resistance to these approaches. Improved patient selection is needed, fuelling continued investigation of biomarkers to predict response. Our understanding of resistance mechanisms is also increasing, providing the rationale for novel immunotherapeutic agents and combinatorial strategies.

Experience with Immunotherapy in Clinical Practice: Identifying Limitations and Challenges

Doctor Alexander M. M. Eggermont, Doctor Joachim G. Aerts, Doctor Thomas Powles

Melanoma

Immunotherapy has long been an important part of the treatment landscape for melanoma.[7] Cytotoxic T lymphocyte-associated-protein 4 (CTLA-4) and programmed cell death-protein 1 (PD-1) are negative regulators of T cell activation and can contribute to immune evasion by tumour cells.[8] Monoclonal antibody inhibitors of these checkpoint pathways enhance T cell proliferation and function, perpetuating T cell activation and reawakening the silenced anti-tumour immune response. CTLA-4 is thought to primarily limit early phases of T cell activation, while PD-1 inhibits T cell activity in the effector phase within tissues and tumours.

The CTLA-4-targeted monoclonal antibody ipilimumab became the first immune checkpoint inhibitor approved for the management of previously-treated melanoma and was later approved as first-line treatment, where it has replaced chemotherapy.[7] Long-term survival data with 10 years of follow-up from a pooled analysis of Phase II and Phase III ipilimumab trials demonstrated a prolonged survival benefit for ipilimumab in patients with untreated or pretreated melanoma, and a plateau in the survival curve starting at approximately 3 years with around 20% of patients achieving long-term disease control.[9] A non-randomised subset analysis also showed no significant difference in survival between the 3 mg/kg and 10 mg/kg dosing regimens.

Ipilimumab was also investigated as an adjuvant therapy in the Phase III EORTC 18071 trial.[10] Ipilimumab significantly prolonged median recurrence-free survival compared with placebo, regardless of extent of nodal disease or ulcerative status. Ipilimumab is limited by its adverse event profile, including specific immune-related adverse events (irAEs) such as pruritis, rash, diarrhoea, colitis, hypophysitis, and increases in compounds detected by liver function tests. In the EORTC 18071 trial, most of the Grade 2–5 irAEs had occurred by the fourth or fifth dose of ipilimumab and approximately half of patients receiving ipilimumab had to discontinue therapy due to an adverse event. This implies that the majority of benefit from adjuvant ipilimumab was gained from those first four or five doses.

An important question this study did not address is whether the same magnitude of benefit could be achieved by simply treating patients with ipilimumab at the time of disease progression rather than immediately following surgery. Within the field of melanoma, the clinical value of adjuvant therapy versus salvage therapy at progression continues to be an area of debate. This will be addressed in the Phase III EORTC 1325 trial evaluating adjuvant therapy with the PD-1 inhibitor pembrolizumab in high-risk, Stage III melanoma, as patients will be unblinded at the time of disease relapse and those previously allocated to placebo treatment will be offered pembrolizumab.[11]

The PD-1 checkpoint inhibitors pembrolizumab and nivolumab have demonstrated significant efficacy in patients with ipilimumab-naïve and ipilimumab-pretreated melanoma and were both approved in 2015 for the treatment of patients with unresectable or metastatic disease.[7] Both of these agents demonstrated superiority to chemotherapy in ipilimumab-refractory patients and nivolumab improved overall survival (OS) compared with dacarbazine in treatment-naïve patients.[7,12] The durability of response to PD-1 blockade is similar to that seen with ipilimumab, although the actual response rates tend to be higher with PD-1 inhibitors.[7] In Phase III randomised trials, both pembrolizumab and nivolumab demonstrated superior efficacy to ipilimumab with regards

to overall response rate (ORR) and median progression-free survival (PFS) in patients with advanced melanoma. Pembrolizumab also demonstrated an OS advantage over ipilimumab. In addition, the safety profile of PD-1 inhibition appears to be more favourable than CTLA-4 blockade, further supporting the use of these agents. Based on these data, PD-1 inhibition should be strongly considered as first-line therapy for most patients with advanced melanoma. If a patient has *BRAF*-mutated metastatic disease with a very high tumour load and rapid disease progression, a BRAF inhibitor may be preferred as first-line therapy, with immunotherapy offered later in the disease course.

The combination of ipilimumab and nivolumab was also recently approved for patients with unresectable or metastatic melanoma based on significant improvements in ORR and median PFS over ipilimumab alone in the CheckMate 067 and 069 trials.[13,14] Interestingly, in the CheckMate 067 trial, patients expressing programmed cell death-ligand 1 (PD-L1) showed a similar median PFS with either nivolumab alone or the combination of nivolumab and ipilimumab.[13] This suggests the combination may not be necessary in those expressing PD-L1. In the CheckMate 069 trial, the OS curves for nivolumab plus ipilimumab and ipilimumab alone continue to converge over time, with only a 10% OS benefit for the combination over ipilimumab alone at 24 months.[14] The combination of nivolumab and ipilimumab is also associated with a higher rate of Grade 3 or 4 adverse events (56.5%) compared with nivolumab alone (20%) or ipilimumab alone (27%) and over one-third of patients in the CheckMate 067 trial had to discontinue therapy due to an adverse event.[13] The potential for efficacy and toxicity should be carefully discussed when considering this combinatorial treatment option.

The oncolytic intralesional therapy talimogene laherparepvec (T-VEC) was also recently approved for the treatment of patients with unresectable melanoma. T-VEC is an attenuated oncolytic virus that expresses human granulocyte macrophage-colony stimulation factor (GM-CSF), which is involved in recruitment and activation of APCs and stimulation of tumour-specific T cell response.[15] In the Phase III OPTiM study, intralesional T-VEC was associated with a higher rate of durable and objective responses compared with subcutaneous GM-CSF and prolonged median OS by 4.4 months (p=0.051). Responses to T-VEC were seen in both injected and uninjected lesions and T-VEC was well-tolerated. Ongoing clinical trials are investigating T-VEC in combination with other immunotherapies, including checkpoint inhibitors.[16,17]

Lung Cancer

The vast majority of lung cancers exist in a highly immunosuppressive microenvironment, leading to immune evasion and tumour progression.[18] This has resulted in investigation of several immunotherapy strategies, including vaccines and immune checkpoint inhibitors. PD-1 and PD-L1 inhibitors have demonstrated exciting efficacy and safety in patients with metastatic lung cancer. In a randomised Phase III trial, nivolumab significantly improved ORR, median PFS, and median OS compared with docetaxel in patients with advanced squamous non-small cell lung cancer (NSCLC) with disease progression during or after first-line chemotherapy, reducing the risk of death by 41%.[19] Nivolumab also significantly improved ORR and median OS compared to docetaxel in a Phase III trial of non-squamous NSCLC with progression during or after platinum doublet chemotherapy.[20] This efficacy was particularly evident in patients with higher levels of PD-L1 expression. In both trials, nivolumab was well-tolerated compared with docetaxel, with Grade 3 or 4 adverse events in ≤10% of patients in the nivolumab arms compared to approximately 55% in the docetaxel arms of each trial.[19,20]

The PD-1 inhibitor pembrolizumab and the PD-L1 inhibitor atezolizumab have also demonstrated superiority over docetaxel in patients with previously treated advanced NSCLC expressing PD-L1 in Phase II/III studies.[21,22] Median PFS and OS benefit was most evident in patients with high PD-L1 expression (≥50% of tumour cells expressing PD-L1). Both checkpoint inhibitors were well-tolerated compared with chemotherapy. Updated data from the KEYNOTE-001 trial of pembrolizumab and the POPLAR trial of atezolizumab versus chemotherapy showed favourable long-term OS benefit in patients with PD-L1 plus advanced NSCLC.[23,24]

Close examination of the OS curve for nivolumab in the non-squamous NSCLC trial shows a slight superiority for chemotherapy in the first 6 months after treatment initiation, with slightly more patients in the nivolumab arm dying early in the treatment course,[20] and the survival benefit of nivolumab only appearing after 6 months, by which time one-third of patients had already died.

This calls into question the approach of one-size-fits-all therapy in which every patient receives second-line checkpoint inhibitor therapy. There may be a specific subset of patients who display an unusual immunogenic response, are insensitive to immunotherapy, and may progress too quickly to receive any potential benefits from chemotherapy. The lack of benefit could be related to levels of PD-L1 expression, although further studies are needed. Unfortunately, identification of these patients who may not respond to immunotherapy is difficult given the complex immunosuppressive environment of lung cancer and the limited predictive biomarkers for response to immunotherapy.

Interestingly, there is a further decline in the probability of survival approximately 18 months following initiation of nivolumab for squamous NSCLC, suggesting that many patients that initially responded develop resistance to immunotherapy at around this timepoint.[19] This represents another patient subset needing a biomarker to identify, as these patients may benefit from sequential therapy to boost the anti-tumour response and prolong survival. A good initial tumour response is needed for patients to become long-term survivors, so those with only stable disease at 3 months may need to be considered for combination or sequential therapy. These strategies are currently under investigation, but will need to be carefully evaluated for both efficacy and safety.

Important questions remain, including which patients with lung cancer should be treated with immunotherapy and the optimum duration of treatment. Immunotherapy for all patients with NSCLC may not be ideal, particularly if there is a subset displaying an unusual immunogenic response that is detrimental to their long-term survival. Further studies to identify reliable biomarkers for response and resistance are needed to improve patient selection. Quality of life is also an extremely important factor in the decision to use immunotherapy, as these agents are typically better tolerated than chemotherapy and are often preferred by patients.

Bladder Cancer

The treatment of advanced bladder cancer had seen very little improvement in the last three decades, with limited treatment options and poor patient outcomes until the advent of immunotherapy.[25] Urothelial bladder cancers have high rates of somatic mutations and often express high levels of PD-L1, providing a rationale for the investigation of PD-1 and PD-L1 inhibitors. A Phase II study evaluated first-line atezolizumab in patients ineligible for cisplatin and demonstrated durable response rates and promising effects on survival.[26] This study also showed durable responses (ORR 16%) in a cohort of 310 patients who had progressed following platinum-based chemotherapy.[27] While responses were observed in all PD-L1 patient subgroups, PD-L1 expression on ≥5% of tumour infiltrating immune cells appeared to be an important biomarker for both response and prolonged survival. Exploratory analyses showed that The Cancer Genome Atlas subtypes of bladder cancer were associated with different levels of PD-L1 expression and a corresponding correlation with response to atezolizumab.[28] Mutational load was also predictive of response to this checkpoint inhibitor. Atezolizumab is now approved in the USA for patients with locally advanced or metastatic urothelial carcinoma with progression during or following platinum-based chemotherapy.[29]

Pembrolizumab and the novel PD-L1 inhibitors avelumab and durvalumab have also demonstrated efficacy as monotherapy in Phase I/II trials in advanced urothelial cancer, with response rates of 28–50% in patients with PD-L1-positive tumours.[30-32] Nivolumab elicited responses in 24% of patients with advanced urothelial cancer and promising effects on survival that appeared to be unrelated to PD-L1 status.[33] Ongoing randomised trials are further exploring checkpoint inhibition in bladder cancer, including a trial directly comparing atezolizumab with second-line chemotherapy in patients with metastatic transitional cell carcinoma (TCC) who failed platinum-based chemotherapy.[34] A second randomised study is comparing the PD-L1 inhibitor durvalumab alone versus durvalumab plus the CTLA-4 inhibitor tremelimumab versus chemotherapy for untreated metastatic TCC.[35] Patients will be crossed over at disease progression, providing some insight into the efficacy of sequential therapy using chemotherapy and immunotherapeutic agents. Phase III trials of avelumab maintenance therapy following first-line chemotherapy[36] and of adjuvant atezolizumab or nivolumab post-cystectomy are also ongoing.[37,38]

Renal Cell Carcinoma

In RCC, nivolumab demonstrated superiority to everolimus in patients with advanced disease following one or two prior regimens of

antiangiogenic therapy.[39] Nivolumab significantly improved ORR and prolonged median OS, although unlike in other cancers no plateau was observed in the survival curve for nivolumab, with continuing attrition over time with both treatment approaches. PD-L1 status was prognostic but not predictive of OS benefit. Median PFS was not significantly different between the two treatment arms and further follow-up will be needed to fully understand the survival benefit associated with nivolumab in RCC. The combination of nivolumab and ipilimumab is also under investigation in advanced RCC and has demonstrated preliminary efficacy in a Phase I trial.[40,41]

Tumour angiogenesis is an important driver of RCC progression and antiangiogenic therapy is a key component of the treatment landscape. Antiangiogenic therapies such as bevacizumab have an immunogenic effect and ongoing clinical trials are now evaluating combinations of immunotherapy with antiangiogenic therapy in RCC, including atezolizumab plus bevacizumab.[42] This combination demonstrated promising efficacy in a Phase I trial in advanced RCC, leading to an ongoing Phase II study comparing atezolizumab alone versus atezolizumab plus bevacizumab versus sunitinib in untreated advanced RCC and a Phase III study comparing the combination with sunitinib.[42-44] The anti-PD-L1 therapy avelumab is also being evaluated in combination with axitinib in patients with advanced RCC.[45]

Tumour-associated macrophages promote angiogenesis, invasion, and immunosuppression in a variety of tumour types and may play a particularly important role in RCC.[46] This suggests that second-generation immunotherapy combinations and novel agents targeting macrophages may be needed to see more dramatic efficacy in this tumour type. Inhibitors of macrophage colony stimulating factor 1 (CSF-1) and its receptor (CSF-1R), as well as toll-like receptor and CD40 agonists are under investigation as potential strategies to target tumour-associated macrophages.[2,47] Manipulation of tumour-associated macrophages is complex, as macrophage depletion may inhibit the ability to elicit a strong T cell-mediated response while activation of macrophages can lead to significant production of interleukin-10. Because the immune system is constantly seeking equilibrium, stimulation of one pathway often leads to upregulation of an opposing pathway and can counteract the desired immunogenic effect of the therapy. This complex interplay needs to be carefully considered when designing novel immunotherapies and combinatorial strategies.

Predictive Biomarkers for Cancer Immunotherapy

Doctor Mario Colombo

Despite the success of cancer immunotherapy, not all patients respond to therapy and those who do respond often experience toxicities that can negatively impact on quality of life. Thus the ability to select patients who will most likely benefit from immunotherapy and determine which immunotherapy would work best for an individual, given the expanding number of available agents, is an important objective of current research. High-throughput technologies provide potential tools for immune monitoring and biomarker discovery.[3] One strategy is assessment of TILs, as a lack of T cells within the tumour negatively impacts on the capacity for an immune response. In patients with colorectal cancer, *in situ* immunohistochemical staining and gene expression profiling to evaluate the type, density, and location of immune cells within a tumour sample provided valuable prognostic information.[48] This 'immunoscore' examines the distribution and functional orientation of CD3+ lymphocytes, CD8+ cytotoxic T cells, and memory T cells in the tumour core and invasive margin and has demonstrated superior prognostic utility to the TNM classification system.

PD-L1 expression is an important biomarker for response to PD-1 and PD-L1 inhibitors, although its precise role in specific tumour types is still under investigation.[49] PD-L1 may function primarily as an indicator of immune recognition, reflecting the presence of T cells at the tumour site and sensitivity to interferon gamma. PD-L1 expression patterns differ considerably among the different tumour types, including whether expression is primarily on tumour cells, immune cells, or both. In addition, some tumours display focal PD-L1 expression or show heterogeneous expression across multiple metastases with variation over time. This potentially impacts the utility of PD-L1 as a biomarker for immunotherapy. Increased PD-L1 expression can result from oncogenic signalling or gene mutations within the tumour cell itself (innate resistance) or from activated T cells stimulating surrounding tumour and immune cells to express PD-L1 (acquired resistance).[50]

PD-L1 testing has several technical challenges and can be complicated by the dynamic nature of the immune system.[49] Tumour tissue quantity and quality is important to ensure accurate testing. The percentage cut-offs for PD-L1 positivity are low, sometimes requiring distinction between 1%, 3%, and 5% positive cells within a tumour section. From a biological viewpoint, it is challenging to understand how such small differences in expression can influence responsiveness to immunotherapy. Presumably, PD-1 blockade can create a chain reaction of immune activation from just a small initial population of PD-L1-positive cells that results in clinically meaningful tumour regression. The available diagnostic assays for PD-1/PD-L1 immunohistochemistry differ somewhat with regards to their sensitivity and specificity.[51] While several of the companion diagnostic assays have recently shown comparable PD-L1 staining on the same tissues, suggesting relatively good concordance, careful interpretation, and improved understanding of the inherent differences in these assays is needed.

Mutational load has also been put forth as a potential biomarker for immunotherapy, as increased mutational heterogeneity can create tumour-specific neo-antigens that would potentially elicit an immune response.[52] Tumours with mismatch repair (MMR) deficiencies such as microsatellite instability have particularly high mutational loads, which appears to confer sensitivity to immunotherapy.[53] In support of this, a recent study of the checkpoint inhibitor pembrolizumab demonstrated a response rate of 62% and 60% in MMR-deficient colorectal and non-colorectal tumours, respectively. In contrast, no MMR-proficient colorectal tumours responded to pembrolizumab in this study. However, not all tumour mutations will result in an immunogenic neo-antigen and the challenge lies in being able to accurately identify the appropriate immunologic target.

There are several metabolic enzymes and pathways involved in the control of immune function that represent potential targets for immunotherapy. Tumour cells and nearby immune cells are in continuous competition for nutrients. Studies suggest tumour cells can restrict the availability of glucose to surrounding T cells via PD-L1 signalling, resulting in immunosuppression.[54] Another example is indoleamine 2,3-dioxygenase (IDO), an immune-inhibitory molecule expressed by tumour cells and infiltrating myeloid cells.[55]

IDO mediates the kynurenine pathway of tryptophan degradation, resulting in depletion of tryptophan needed for T cell function. This leads to suppression of effector T cells, enhancement of regulatory T cells, and subsequent immune escape. Lastly, in response to signals from activated T lymphocytes, myeloid suppressor cells can block T cell proliferation through manipulation of arginine metabolism by inducing the two enzymes nitric oxide synthase 2 and arginase 1.[56] Induction of either enzyme alone leads to reversible blockade of T cell proliferation, while induction of both enzymes simultaneously results in T cell apoptosis.

There remains a clear unmet need for immunotherapy biomarkers, as it is not realistic to treat every patient with these agents. Ultimately, biomarkers for immunotherapy need to distinguish 'hot' tumours from 'cold' tumours. This might be accomplished with an expanded Immunoscore-type algorithm that takes into account variables such as PD-L1 expression, interferon gamma signatures, MHC Class I and II expression, CD8+ T cell density, mutational load, and other parameters. This readout could provide an 'immune temperature' for each individual tumour to identify responders from non-responders.

Resistance to Immune Checkpoint Blockade

Doctor George Coukos

The exciting efficacy produced by immune checkpoint inhibitors across multiple tumour types is, in most situations, unfortunately coupled with development of resistance to these agents. The precise mechanisms of resistance to immune checkpoint inhibitors are poorly understood, in part due to failure to consistently biopsy and evaluate tumours at progression on immunotherapy. Depending on the tumour type and location, serial biopsies can be very challenging. Liquid biopsies of systemic peripheral blood are much more convenient, can easily be repeated, and may enable prediction of tumour response and/or development of resistance. While peripheral blood samples are unlikely to fully represent the exact immune composition at the tumour site, they could provide important insight into the overall immune environment. A recently published study showed that isolated CD8+, PD-1+ T cells in the peripheral blood of four

patients with melanoma were representative of the TILs recognising specific tumour neo-antigens.[57] Although this was a very small study, it presents the possibility of using peripheral blood as a mirror for the tumour-immune microenvironment. It is important to remember the potential for background noise in peripheral blood samples, as the circulating markers will reflect not only the tumour-specific immune response, but also immune responses to infections, inflammation, and other events. Further study will be required to determine how liquid biopsies and tissue biopsies can best be utilised to fully understand the complex resistance strategies tumours employ to evade immunotherapy.

One of the major mechanisms of resistance to immune checkpoint blockade is absence of tumour-infiltrating T cells. T cells must already be embedded at the tumour site in order to achieve a good anti-tumour immune response. In a retrospective study of ovarian cancer, 5-year OS was significantly higher in patients whose tumours contained infiltrating T cells compared with those without T cell infiltration (74% versus 12%).[58] A meta-analysis performed in 2012 of studies from multiple tumour types also identified the presence of T cells within the tumour as a good prognostic indicator.[59] Mouse models of ovarian cancer and retrospective analysis of melanoma tumour samples clearly showed that the absence of TILs predicted failure of PD-L1 blockade.[60,61] While some tumours have spontaneous infiltration of T cells, 50–70% of solid tumours lack tumour-infiltrating T cells and would not be expected to respond to T cell activation. This potentially explains why only 30–50% of tumours respond to immune checkpoint inhibitor monotherapy. Based on T cell infiltration, tumours can be divided into immunogenic and non-immunogenic. The non-immunogenic group however comprises both tumours that have no T cells at all (immune ignorant or immune desert) and those that only have T cells at the invasive margin (immune exclusion).

Immunogenicity is also related to mutational load and the presence of neo-antigens, as discussed previously, with mutational load predicting for response to pembrolizumab in NSCLC and MMR-deficient tumours.[53,62] Importantly, a linear relationship does not exist between the number of neo-antigens in a tumour and the level of response to PD-1 blockade, indicating that additional factors likely influence tumour immunogenicity and response to therapy. Adding to this complexity, McGranahan et al.[63] recently published a study showing that clonal neo-antigens enhance sensitivity to immune checkpoint inhibitors, while subclonal neo-antigens were associated with a poor response to these agents.[63] Interestingly, administration of chemotherapy appeared only to add subclonal neo-antigens and did not improve response to immunotherapy. Immune targeting of clonal, dominant neo-antigens that are shared by all the branches of the tumour could potentially eliminate all of the tumour clones. In contrast, significant subcloning will create neo-antigens that are not shared universally within the tumour and allow subclones to evade the immune response. These data suggest that administration of immunotherapy earlier in the disease course before subcloning occurs may be more beneficial, although many tumours exhibit significant heterogeneity at diagnosis.

Strategies to improve response to PD-1/PD-L1 blockade in tumours that are already immunogenic include pushing TILs harder to increase the immune response, eliminating more inhibitory signals, or expanding the pool of tumour-reactive T cells. Activating immune receptors such as OX40, GITR, CD137, CD27, and HVEM are potential targets for agonistic antibodies that could be combined with existing immunotherapy to augment immune response (Figure 1).[4] Increased activation of the immune system will undoubtedly result in emergence of further regulatory counterpoints, or 'breaks' to achieve equilibrium. Novel inhibitory receptors beyond CTLA-4 and PD-1, including TIM-3, VISTA, and LAG-3, represent targets for blocking antibodies to eliminate additional inhibitory signals.

As mentioned previously, an apparently important negative regulator of immune response is IDO, which catabolises tryptophan and shuts down T cells. Studies of immune checkpoint blockade in IDO knockout mice showed that upregulation of IDO is a potential mechanism of resistance to agents inhibiting CTLA-4, PD-1, PD-L1, and GITR.[64] Combined blockade of IDO and immune checkpoint signalling appeared to be synergistic in this mouse model, suggesting a rationale for combination therapy strategies that could prevent or overcome resistance to checkpoint blockade monotherapy.
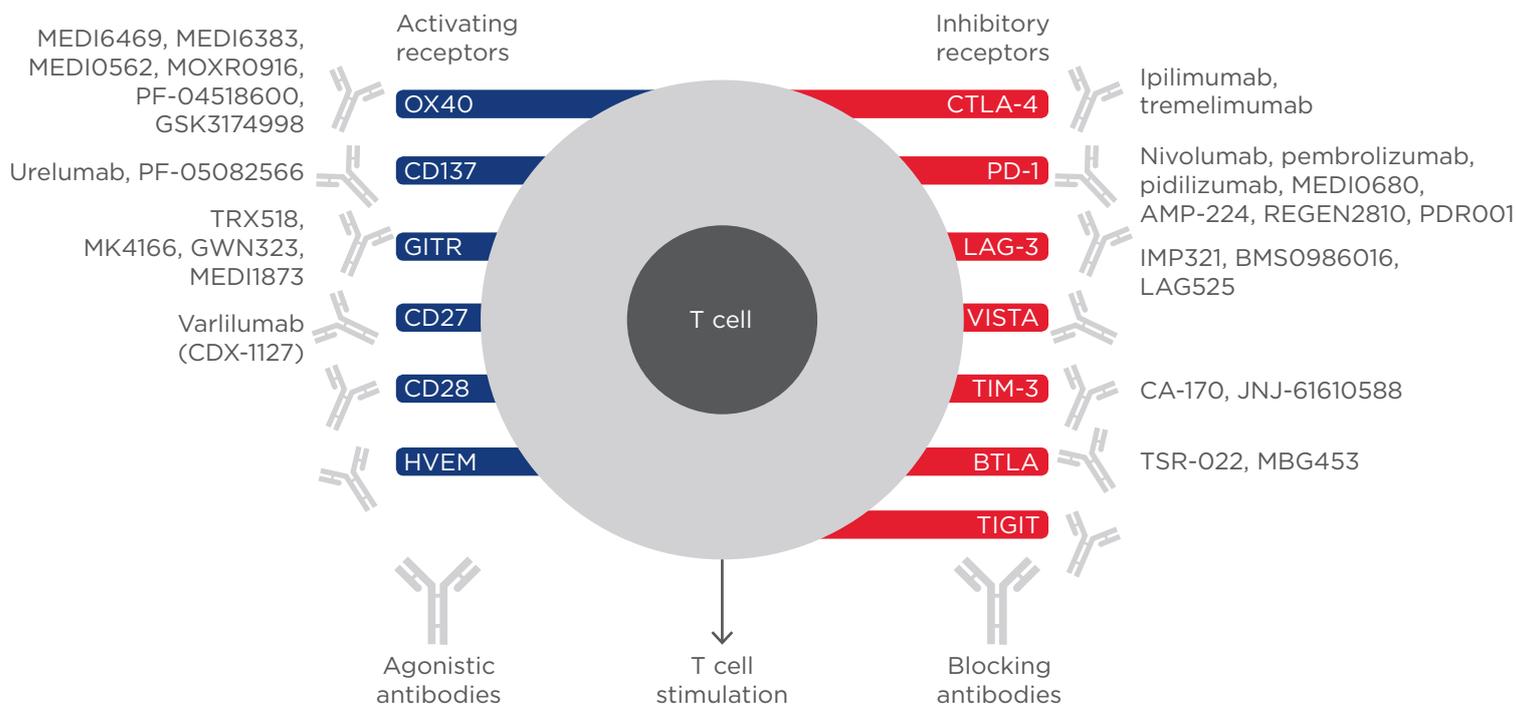
Figure 1: T cell targets for antibody-based immunotherapies.
CTLA-4: cytotoxic T lymphocyte-associated-protein 4; GITR: glucocorticoid-induced tumour necrosis factor-related; TIM-3: T cell immunoglobulin and mucin domain-3; LAG-3: lymphocyte-activation gene 3; PD-1: programmed cell death-protein 1; HVEM: herpes virus entry mediator; BTLA: B and T lymphocyte-associated; TIGIT: T cell immunoreceptor with Ig and ITIM domains.

In order to overcome immune exclusion, the mechanisms of exclusion must first be eliminated to allow infiltration of T cells into the tumour. These T cells could then be activated through immune checkpoint blockade, vaccines, personalised T cell adoptive therapy, etc. One potential mechanism for immune exclusion is through the action of the endothelial barrier. In a study of advanced ovarian cancer tumour samples, those without intratumoural T cells demonstrated increased expression of vascular endothelial growth factor.[58] A study of genomic and transcriptomic signatures in patients with melanoma patients treated with PD-1 blockade also showed that tumours resistant to PD-1 inhibition had upregulation of genes involved in angiogenesis.[65] The tumour endothelium may prevent circulating T cells from transmigrating into the tumour or it may attract T cells and then trigger T cell death through the expression of molecules such as Fas ligand.[66]

Overcoming immune ignorance or the existence of an immune desert within a tumour site represents a difficult challenge in the field of immunotherapy. Current investigations are trying to identify the key signalling pathways responsible for immune ignorance and examine targeted therapies that may reverse this effect. For example, *PTEN* loss is associated with an immune desert phenotype with complete silencing of immune and inflammatory signalling pathways.[67] In preclinical models, loss of *PTEN* led to upregulated expression of immunosuppressive cytokines and proangiogenic signalling, decreased T cell tumour infiltration, and poor response to PD-1 inhibitors. A selective PI3K-β inhibitor improved response to both PD-1 and CTLA-targeted therapy, suggesting a rationale for combinatorial therapy to overcome immune resistance. Radiotherapy may also represent an important strategy to convert a non-immunogenic tumour into a responsive tumour and is discussed below.

There is a clear need for algorithms to assess the potential for response and resistance to immunotherapy, but this will require considerable advances in bioinformatics and identification of reliable biomarkers. A cancer immunogram recently proposed by Blank et al.[68] suggests integration of multiple parameters to assess the capacity for an immune response. Variables include absence of checkpoint markers (PD-L1), immune

cell tumour infiltration, total lymphocyte count, mutational load, sensitivity to immune effectors (MHC expression and interferon gamma sensitivity), absence of inhibitory tumour metabolism (lactate dehydrogenase and glucose utilisation), and absence of soluble inhibitors (interleukin-6 and C-reactive protein). The relationships between these parameters and the hierarchal importance of each will need to be quantified in order to achieve a measurable readout useful for immunotherapy treatment decisions.

Immunotherapeutic Combination Strategies: Where Are We Now and Where Are We Going?

Doctor Ignacio Melero, Doctor Alexander M. M. Eggermont, Doctor Eric Deutsch

Combination of Different Immunotherapeutic Strategies

Integration of cancer immunotherapies into combination and sequential strategies represents an attractive therapeutic strategy to increase the anti-tumour immune response and improve long-term patient outcomes.[69] By carefully selecting agents or treatment modalities with complementary mechanisms of action, synergistic efficacy may be achieved. Tumours with strong endogenous anti-tumour immune response typically exhibit PD-L1 upregulation in the tumour and respond well to anti-PD-1 monotherapy.[52] Tumours with a weak endogenous anti-tumour immune response often lack PD-L1 upregulation and will usually not be responsive to PD-1 blockade. Utilising an inducer of tumour immunity such as a vaccine or co-stimulatory agonist can increase the endogenous anti-tumour immune response, leading to PD-L1 upregulation and reconditioning of the environment to become responsive to PD-1 blockade. This provides an important rationale for sequential or combinatorial immunotherapy strategies to stimulate or reactivate the immune system and boost tumour response.

The number of combination immunotherapy strategies under investigation continues to expand (Figure 2).[69] PD-1/PD-L1 blockade will likely continue to be the foundation for most combinatorial strategies given the efficacy and tolerability these agents have already demonstrated. PD-1/PD-L1 provides a unique common denominator for cancer therapy, with inhibitors of this immune checkpoint targeting a single molecular pathway that plays a role in many different tumour types.[50]
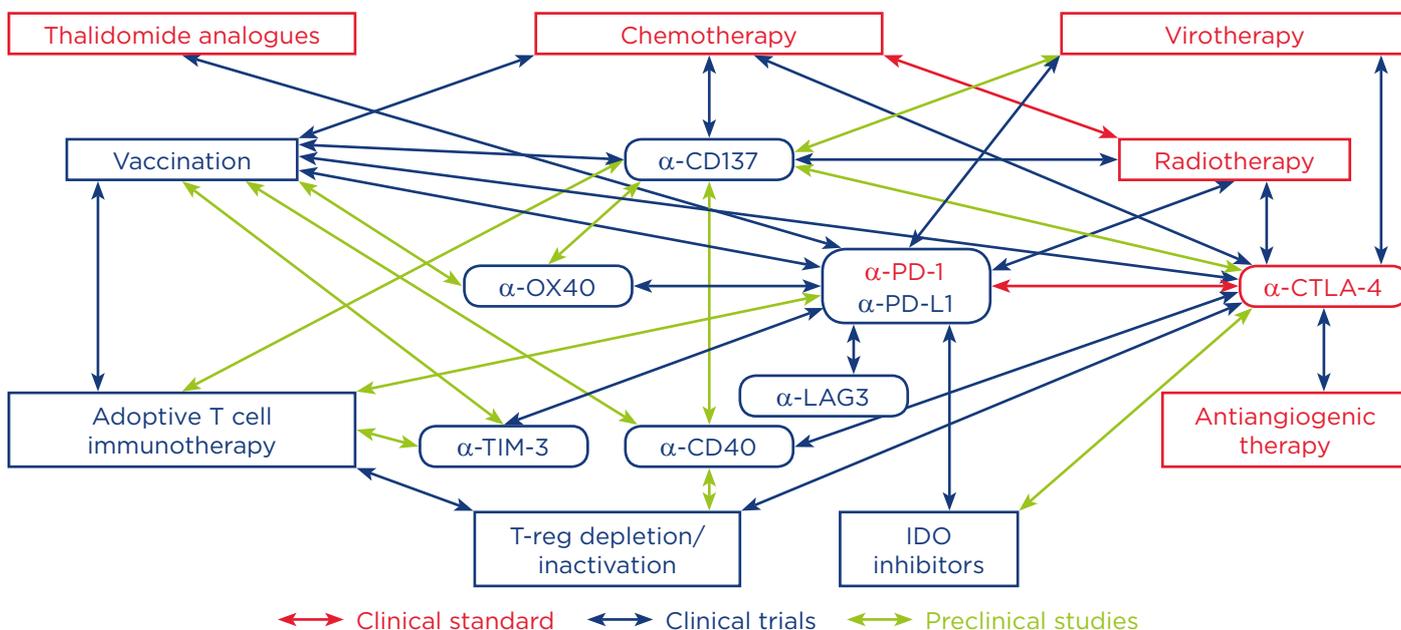

Figure 2: Combination therapy strategies involving cancer immunotherapy.[69]
CTLA-4: cytotoxic T lymphocyte-associated-protein 4; PD-1: programmed cell death-protein 1; PD-L1: programmed cell death-ligand 1; TIM-3: T cell immunoglobulin and mucin domain-3; IDO: indoleamine 2,3-dioxygenase.
Adapted with permission from Melero et al.[69]

PD-1/PD-L1 blockade can then be combined with blockade of other co-inhibitory molecules (CTLA-4, LAG-3, killer immunoglobulin-like receptors), agonists for co-stimulatory molecules (CD137, OX40, CD40, GITR), metabolic targets, vaccination strategies, or adoptive cell therapy.[4]

The success of the checkpoint inhibitors and their complementary mechanisms of action led to investigation of dual checkpoint blockade. As mentioned previously, the combination of ipilimumab and nivolumab demonstrated striking efficacy in patients with advanced melanoma, with durable responses and a significant improvement in PFS for the combination over ipilimumab or nivolumab monotherapy.[7] Importantly however, the superior efficacy of the combination came at the cost of excess toxicity, with 36% of patients discontinuing combination therapy due to treatment-related adverse events. Tolerability represents an important challenge in the use of immunotherapies, particularly when combinatorial strategies are employed. Replacing poorly tolerated immunotherapeutic agents with those that have a more favourable safety profile may improve the therapeutic index of future immune combinations. Sequencing of immunotherapies is clearly an important strategy to evaluate and may provide better disease control with good tolerability and preservation of quality of life.

The tolerability of agents such as ipilimumab may also be improved by re-evaluating the dosing and treatment schedule. For instance, recent data from the expansion cohort of the KEYNOTE-029 trial showed that combining standard-dose pembrolizumab with a reduced dose of ipilimumab (1 mg/kg for four doses) had robust activity and was well-tolerated.[70] Only 25% of patients experienced a Grade 3 or 4 irAE and very few had to discontinue ipilimumab therapy. Results from the ongoing Phase III trial comparing ipilimumab dosing of 3–10 mg/kg will also shed light on the optimum dosing for ipilimumab when given as sequential therapy.[71] Spacing out the doses of ipilimumab, such as administration every 3 months instead of every 3 weeks, may also improve tolerability of combination regimens, particularly for less fit patients.

A novel immunotherapy currently under investigation in combination regimens is the potent oral IDO1 inhibitor epacadostat. This agent demonstrated promising activity and tolerability in combination with ipilimumab in a Phase I/II trial

of patients with metastatic melanoma.[72] A second Phase I/II trial is evaluating epacadostat plus pembrolizumab in multiple solid tumour types.[73] The combination is well-tolerated, with only 11% of patients experiencing a Grade 3 adverse event and no Grade 4 events reported. Objective responses were observed in many tumour types, including advanced melanoma, RCC, NSCLC, TCC of the bladder, endometrial adenocarcinoma, and head and neck cancers. In 19 evaluable patients with advanced melanoma, 10 (53%) achieved an objective response, including 2 complete responses.

Another novel immunotherapy target with the potential to make a good partner for immunotherapy combinations is CD137 (also called 4-1BB), which is expressed on activated T cell and natural killer cells.[74] This co-stimulatory molecule binds to the CD137 ligand on APCs, promoting T cell proliferation, cytotoxic activity, and cytokine production, while inhibiting apoptosis. Anti-CD137 agonist therapy overcomes tumour antigen tolerance in a cytotoxic T lymphocyte-dependent manner and enhances natural killer cell antibody-dependent cellular cytotoxicity.[74,75] CD137 co-stimulation is synergistic with adoptive T cell therapy in preclinical models.[76] TILs can be selected for CD137 expression *ex vivo* to enrich for the most potent anti-tumour activity and/or cultured in the presence of anti-CD137 agonist to increase T cell activation. Administration of anti-CD137 agonist therapy at the time of TIL adoptive transfer can also increase T cell activation and tumour infiltration.

Combinations with OX40 targeted antibody therapy are also currently being explored and have shown activity in Phase I trials in solid tumours. For example, the OX40 agonist therapy MOXR0916 demonstrated promising activity and good tolerability in combination with atezolizumab in a Phase Ib dose escalation trial in advanced solid tumours.[77] The ongoing first-in-human ENGAGE-1 trial is examining the OX40 agonist GSK3174998 alone or in combination with pembrolizumab in patients with advanced solid tumours.[78]

The vast number of potential doublet and triplet immunotherapy combinations cannot all realistically be examined in clinical trials. Therefore, biological insight and robust preclinical studies are needed to identify the most promising combination strategies before moving into clinical studies. To assist in this investigation,

a humanised murine model has been developed that will allow the study of human tumours in an immunocompetent setting by transferring human lymphocytes into immunodeficient mice.[79] This will be a useful tool for the evaluation of immune checkpoint inhibitors and combination immunotherapy regimens. Murine tumour models are not ideal however, and due to the considerable differences between human and mouse immune systems, may underestimate or overestimate the potential for immunotherapies and combination regimens.

Combination Strategies with Immunotherapy and Radiotherapy

In addition to its direct anti-tumour effects, radiotherapy can trigger an immune response and mediate tumour regression not only locally but also at distant tumour sites, the so-called abscopal or 'away from the target' response.[80] In murine tumour models, radiotherapy increased PD-L1 expression in the tumour microenvironment and administration of PD-L1 blocking antibody synergised with radiotherapy to amplify the anti-tumour effect. The combination of radiotherapy and anti-PD-L1 therapy activated cytotoxic T cells and reduced the accumulation of myeloid-derived suppressor cells within the tumour, promoting an anti-tumour immune response. A similar study in murine cancer models using low-dose fractionated radiotherapy also showed upregulation of PD-L1 on tumour cells.[81] Interestingly, the synergy between radiotherapy and anti-PD-L1 therapy only prolonged survival when given concurrently, not sequentially. This may have implications for the use of combined immunotherapy and radiotherapy as this strategy moves into clinical practice. However, the half-life of immune checkpoint inhibitors is long enough that administering immunotherapy a few days prior to radiotherapy should be sufficient to ensure appropriate timing of immune stimulation.

The abscopal effect, associated with the combination of immunotherapy and radiotherapy, has been observed in an early proof-of-principle trial using local radiotherapy and GM-CSF in patients with metastatic solid tumours.[82] A total of 27% of the 41 patients demonstrated an abscopal response. A preclinical study in melanoma and RCC models showed that PD-1 expression inhibited the ability of single-dose radiotherapy to induce an abscopal effect.[83] This was reversed with PD-1 blockade therapy, demonstrating a synergistic anti-tumour effect in the primary tumour and an abscopal effect on non-irradiated tumours. A second study using a murine breast cancer model demonstrated similar synergistic responses with radiotherapy and CTLA-4 blockade, including abscopal responses in non-irradiated tumours.[84] However, unlike the previous study, the abscopal effect was seen only when fractionated radiotherapy was used, not single-dose radiotherapy. A small, retrospective melanoma study also demonstrated abscopal responses in patients who received ipilimumab followed by radiotherapy and suggested a survival benefit in patients who experienced abscopal responses compared with those who did not.[85] Larger, randomised trials will be necessary to fully elucidate the long-term benefit of an abscopal response to immunotherapy-radiotherapy combinations.

The largest randomised trial of immunotherapy combined with radiotherapy to date investigated single-dose radiotherapy followed by either ipilimumab or placebo in patients with castration-resistant prostate cancer.[86] Although dramatic, durable responses were observed in the ipilimumab arm: median OS was not significantly prolonged (10.0 months versus 11.2 months; hazard ratio: 0.85; p=0.053). The negative result may be attributed to factors such as suboptimal timing of radiotherapy or selection of the wrong immunotherapeutic agent for this tumour type.

Immunotherapy is also under investigation in combination with stereotactic ablative radiotherapy in several tumour types.[87] Triplet combinations incorporating radiotherapy, CTLA-4 blockade, and PD-1/PD-L1 blockade are also being explored based on data from preclinical murine tumour models.[88] Upregulation of PD-L1 and T cell exhaustion are a mechanism of resistance to radiotherapy and CTLA-4 blockade. The addition of anti-PD-L1 therapy appeared to reverse this effect and promote response and anti-tumour immunity. Combinations of stereotactic ablative radiotherapy with vaccine-based strategies are also under investigation.[89]

Numerous questions remain regarding the combination of radiotherapy with immunotherapies. Clinical trial data are needed to determine the optimal immunotherapy to combine with radiotherapy and the appropriate dose and fractionation schedule for radiotherapy. Further study will be necessary to truly determine whether radiotherapy can turn a non-immunogenic tumour

into an immunogenic tumour to maximise the anti-tumour immune response and careful attention paid to toxicities that may be exacerbated by these combination strategies.

Combination Strategies with Immunotherapy and Chemotherapy or Targeted Agents

Combinations with chemotherapy and targeted agents have also been explored in multiple tumour types, including melanoma and lung cancer.[69] In patients with untreated metastatic melanoma, the combination of ipilimumab with dacarbazine improved median OS compared to dacarbazine alone, but was associated with Grade 3 or 4 immune-mediated hepatitis in 31.6% of patients versus 2.4% with dacarbazine alone.[90] Dacarbazine does not lead to immunogenic cell death and may be a poor partner for novel immunotherapeutic combinatorial strategies. In breast cancer, ongoing studies are evaluating pembrolizumab in combination with agents such as paclitaxel, nab-paclitaxel, capecitabine, carboplatin/gemcitabine, and eribulin mesylate, as well as with poly(ADP-ribose) polymerase inhibitor therapy.[91]

Table 1: Selected ongoing trials of combination approaches with immune checkpoint inhibitors.[91]

Combination approach	Targets	Agents	Phase	Tumour types
Dual checkpoint blockade	PD-1, CTLA-4	Nivolumab + ipilimumab	I, II, III	Melanoma, lung, RCC, sarcoma, breast, colon, liver glioblastoma, gliosarcoma, MDS, lymphoma, myeloma
	PD-1, CTLA-4	Pembrolizumab + ipilimumab	I, II	Melanoma, RCC, lung
	PD-L1, CTLA-4	Durvalumab + tremelimumab	I, II, III	Breast, lung, HCC, gastric, H & N, bladder, melanoma, glioma, mesothelioma, prostate, pancreas
	PD-1, PD-L1	MEDI0680 + durvalumab	I	Selected advanced tumours
	PD-1, LAG-3	Nivolumab + BMS-986106	I	Solid tumours, glioblastoma
	PD-1, LAG-3	Pembrolizumab + IMP321		
	PD-1, LAG-3	PDR001 + LAG525	I, II	Solid tumours
	PD-1, TIM-3	PDR001 + MBG453	I	Advanced tumours
Checkpoint inhibitor plus co-stimulatory receptor agonists	CD137, PD-1	Urelumab + nivolumab	I, II	Solid tumours, glioblastoma
	CD137, PD-1	PF-05082566 + pembrolizumab	I	Solid tumours
	CD137, PD-L1	PF-05082566 + avelumab	I	Solid tumours
	OX40 + CTLA-4 or PD-L1 or CD20	MEDI6469 + tremelimumab or durvalumab or rituximab	I, II	Solid tumours, DLBCL
	OX40, PD-1	GSK3174998 + pembrolizumab	I	Solid tumours
	OX40, PD-L1	PF-04518600 + avelumab	I	Solid tumours
	OX40, PD-L1	MEDI6383 + durvalumab	I	Solid tumours
	OX40, PD-L1	MOXR0916 + atezolizumab	I	Solid tumours
	GITR, PD-1	MK-4166 + pembrolizumab	I	Solid tumours
	GITR, PD-1	GWN323 + PDR001	I	Advanced tumours
	CD27, PD-L1	Varilumab + atezolizumab	I, II	Advanced tumours
	CD40, CTLA-4	CP-870,893 + tremelimumab	I	Melanoma
Checkpoint inhibitor plus innate immune cell stimulators	KIR, CTLA-4	Lirilumab + ipilimumab	I	Solid tumours
	KIR, PD-1	Lirilumab + nivolumab	I	Lymphoma, myeloma, solid tumours
Checkpoint inhibitor plus IDO inhibition	IDO + PD-1 or PD-L1 or CTLA-4	Epacadostat + nivolumab, pembrolizumab, durvalumab, atezolizumab, or ipilimumab	I, II, III	Select advanced cancers, lung, melanoma
	IDO, CTLA-4	Indoximod + ipilimumab	I, II	Melanoma
	IDO, PD-L1	GDC-0919 + atezolizumab	I	Solid tumours

Table 1 continued.

Combination approach	Targets	Agents	Phase	Tumour types
Checkpoint inhibitor plus oncolytic therapy	Viral therapy, CTLA-4 or PD-1	T-VEC + ipilimumab or pembrolizumab	I, II, III	Melanoma
	Viral therapy, CTLA-4 or PD-1	CVA21 + ipilimumab or pembrolizumab	I	Melanoma
	Viral therapy, CTLA-4	HF10 + ipilimumab	II	Melanoma
Checkpoint inhibitor plus targeted therapy	HDAC, PD-1	Entinostat + pembrolizumab	I, II	Lung, melanoma
	HDAC, PD-1	Vorinostat + pembrolizumab	I, II	H & N, salivary gland
	HDAC, PD-1, CTLA-4	Entinostat + nivolumab + ipilimumab	I	Solid tumours, breast
	HDAC, PD-L1	Entinostat + atezolizumab	I, II	Breast
	EGFR, PD-1	Erlotinib or gefitinib or afatinib + pembrolizumab	I, II	Lung
	VEGF, PD-L1	Bevacizumab + atezolizumab	I, II, III	RCC
	VEGFR, PD-1	Axitinib + pembrolizumab	I	RCC
	VEGFR, PD-L1	Axitinib + avelumab	I, III	RCC
	HER2, PD-L1	Trastuzumab/pertuzumab or T-DM1 + atezolizumab	I	HER2+ breast
	PARP, PD-1	Niraparib + pembrolizumab	I, II	Breast
	CDK, PD-1, ER	Palbociclib + pembrolizumab + letrozole	II	ER+ breast
	BRAF, MEK, PD-L1	Dabrafenib + trametinib + durvalumab	I, II	Melanoma
Checkpoint inhibitor plus chemotherapy	PD-1, cytotoxic targets	Nivolumab + platinum doublet chemotherapy	I	Lung
	PD-L1, cytotoxic targets	Atezolizumab + carboplatin/paclitaxel +/- bevacizumab	III	Lung
	PD-1, cytotoxic targets	Pembrolizumab + paclitaxel, nab-paclitaxel, eribulin mesylate, carboplatin/gemcitabine, capecitabine,	I, II	Breast
	PD-L1, cytotoxic targets	Atezolizumab + nab-paclitaxel	II, III	Breast
Checkpoint inhibitor plus radiotherapy	PD-L1, PD-1	Chemoradiotherapy + consolidation durvalumab or atezolizumab or nivolumab	II, III	Lung
	PD-L1	Atezolizumab	0	Lung
	CTLA-4	Ipilimumab	I, II	Lung
	PD-1	REGN2810, pembrolizumab	I, II	Lung

BRAF: v-raf murine sarcoma viral oncogene homolog B; CTLA-4: cytotoxic T lymphocyte-associated-protein 4; DLBCL: diffuse large B cell lymphoma; EGFR: epidermal growth factor receptor; GITR: glucocorticoid-induced tumour necrosis factor-related; H & N: head and neck; HCC: hepatocellular carcinoma; HDAC: histone deacetylase; IDO: indoleamine 2,3-dioxygenase; KIR: killer cell immunoglobulin-like receptor; LAG-3: lymphocyte-activation gene 3; MEK: mitogen-activated protein kinase; PD-1: programmed cell death-protein 1; PD-L1: programmed cell death-ligand 1; RCC: renal cell carcinoma; VEGF: vascular endothelial growth factor; VEGFR: VEGF receptor; MDS: myelodysplastic syndrome; HER2: human epidermal growth factor receptor 2; ER: estrogen receptors; PARP: poly(ADP-ribose) polymerase.

Atezolizumab is being investigated in combination with nab-paclitaxel, HER2-targeted agents, and HDAC inhibitors. The combination of atezolizumab and nab-paclitaxel demonstrated promising tolerability and efficacy in a recently reported Phase Ib trial in triple-negative breast cancer.[92]

The combination of ipilimumab with paclitaxel/carboplatin was explored in two Phase II trials in patients with chemotherapy-naïve advanced NSCLC or extensive disease SCLC.[93,94] In both trials, 'phased' ipilimumab (two doses of chemotherapy plus placebo, followed by four doses of chemotherapy plus ipilimumab) significantly improved immune-related PFS compared to chemotherapy alone, which was not observed when ipilimumab and chemotherapy were initiated concurrently. In the NSCLC trial, 'phased' ipilimumab with paclitaxel/carboplatin also improved median PFS and ORR compared with chemotherapy alone.[93] A Phase I study of nivolumab in combination with different platinum-based doublet chemotherapy in advanced NSCLC showed ORRs of 33–47%, similar to the efficacy of doublet chemotherapy alone.[95] Another Phase Ib trial evaluated atezolizumab plus carboplatin in combination with either paclitaxel, nab-paclitaxel, or carboplatin as first-line therapy for advanced NSCLC.[96] While the patient numbers are small, the combination of atezolizumab with first-line chemotherapy had a tolerable safety profile and generated durable responses, with ORRs ranging from 60–75%. The combination of pembrolizumab with platinum-based doublet chemotherapy demonstrated ORRs of 48–71% as front-line therapy for advanced NSCLC.[97]

Several ongoing trials of immunotherapy combined with chemotherapy or targeted agents are ongoing or planned in lung cancer, including the Phase III KEYNOTE-189 trial evaluating pembrolizumab with platinum-based doublet chemotherapy as first-line therapy for metastatic non-squamous NSCLC.[91,98] Phase I/II trials are also evaluating checkpoint blockade in combination with erlotinib, gefitinib, or afatinib in patients with *EGFR*-mutated NSCLC.[91] Combinations of immunotherapy with chemotherapy or targeted agents are often associated with considerable toxicity, suggesting these strategies may be more beneficial administered as sequential therapy rather than concomitantly.

Conclusions

Future development of cancer immunotherapy will undoubtedly focus on combination strategies, including combinations of checkpoint inhibitors with other therapies (Table 1).[91] Robust, well-designed clinical trials will be essential to ensure continued progress in cancer immunotherapy. As novel combinations move into early-phase trials, it will be both challenging and critical to identify efficacy signals and detect potential synergy between therapies. Appropriate patient selection is critical, as treating unselected populations with these combinations is unlikely to give a robust survival signal. Emerging biomarkers and algorithms should be rapidly incorporated into clinical trials to increase the probability of detecting an efficacy signal if one is present. It may also be beneficial to initially test novel immunotherapy agents or combinations in patients who do not respond to PD-1/PD-L1 blockade to identify those with significant activity that are worth pursuing. The most promising agents can then be explored in treatment-naïve patients and earlier lines of therapy.

We have only scratched the surface with regard to the potential for cancer immunotherapy. Continued identification of the critical regulators of immune evasion and response will open the door to development of novel immunotherapy strategies and eradication of tumours that currently lack effective treatment options. The next few years will undoubtedly witness a massive expansion in cancer immunotherapies and combination regimens. Only by carefully evaluating these emerging strategies will we be able to effectively incorporate them into clinical practice and improve patient outcomes.

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