Mechanisms of resistance to immune checkpoint inhibitors

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Immune checkpoint inhibitors (ICI) targeting CTLA-4 and the PD-1/PD-L1 axis have shown unprecedented clinical activity in several types of cancer and are rapidly transforming the practice of medical oncology. Whereas cytotoxic chemotherapy and small molecule inhibitors (‘targeted therapies’) largely act on cancer cells directly, immune checkpoint inhibitors reinvigorate anti-tumour immune responses by disrupting co-inhibitory T-cell signalling. While resistance routinely develops in patients treated with conventional cancer therapies and targeted therapies, durable responses suggestive of long-lasting immunologic memory are commonly seen in large subsets of patients treated with ICI. However, initial response appears to be a binary event, with most non-responders to single-agent ICI therapy progressing at a rate consistent with the natural history of disease. In addition, late relapses are now emerging with longer follow-up of clinical trial populations, suggesting the emergence of acquired resistance. As robust biomarkers to predict clinical response and/or resistance remain elusive, the mechanisms underlying innate (primary) and acquired (secondary) resistance are largely inferred from pre-clinical studies and correlative clinical data. Improved understanding of molecular and immunologic mechanisms of ICI response (and resistance) may not only identify novel predictive and/or prognostic biomarkers, but also ultimately guide optimal combination/sequencing of ICI therapy in the clinic. Here we review the emerging clinical and pre-clinical data identifying novel mechanisms of innate and acquired resistance to immune checkpoint inhibition.

**CLINICAL RESPONSE AND RESISTANCE TO IMMUNE CHECKPOINT INHIBITORS**

Monoclonal antibodies targeting co-inhibitory immune checkpoints (e.g., PD-1 and CTLA-4) have demonstrated clinical activity in several malignances, including melanoma, non-small cell lung cancer, renal cell carcinoma, bladder cancer, head and neck squamous cell carcinoma, MSI-high colorectal carcinoma, Merkel cell carcinoma, and Hodgkin lymphoma, and have changed the practice of medical oncology (Pardoll, 2012; Topalian et al, 2015; Sharma et al, 2017). Immune checkpoint inhibitor therapy has been particularly successful in melanoma, for which approved treatments now include anti-PD-1 (nivolumab and pembrolizumab), anti-CTLA-4 (ipilimumab), and combination anti-PD-1/CTLA-4 regimens (nivolumab-ipilimumab). Long-term survival data for patients with melanoma treated with ipilimumab (anti-CTLA-4) indicates 20% of patients show evidence of continued durable disease control or response 5-10 years after starting therapy (Schadendorf et al, 2015). The response rate for melanoma patients treated with pembrolizumab (anti-PD-1) was 33% at 3 years with 70–80% of patients initially responding maintaining clinical response (Ribas et al, 2016a). Combination immunotherapy or dual immune checkpoint blockade (anti-PD-1 + anti-CTLA-4) has recently shown dramatic response rates in patients with metastatic melanoma (RR 58%); however, half of patients experienced significant toxicity from the treatment regimen (Larkin et al, 2015; Postow et al, 2015) and survival benefit for this approach has to be demonstrated.

Analysis of clinical trial data can identify three broad populations of patients – (1) those that respond initially and continue to respond (responders), (2) those that fail to ever respond (innate resistance), and (3) those that initially respond but eventually develop disease progression (acquired resistance) (Pitt et al, 2016; Restifo et al, 2016; O’Donnell et al, 2017; Sharma et al, 2017). Challenges remain in defining responders and non-responders especially given the heterogeneity in patterns of response that can be seen with ICI. Such heterogeneity can be spatial (i.e., different response in different lesions) and/or temporal.
Mechanisms of innate and acquired resistance to ICI therapy are not fully understood, owing in part to the incomplete understanding of the full complement of clinical, molecular, and immunologic factors associated with clinical response and long-term benefit to ICI therapy. In addition, few immune competent pre-clinical models exist in which tumour regression is induced by ICIs (Zitvogel et al, 2016), limiting the ability to recapitulate the diversity of tumour-immune interactions in patients. To frame the discussion of innate (primary) and acquired (secondary) resistance, we must first revisit the model of ‘response’ to ICI to focus on crucial steps that can be inhibited, bypassed, or blocked by the tumour, or co-opted by stromal and immune elements of the tumour microenvironment (TME), to subvert the efforts of the immune system to restrain tumour growth.

Although our understanding of the role of PD-1 and PD-L1 on tumour and immune cells continues to evolve (Juneja et al, 2017; Lau et al, 2017), it is generally accepted that successful anti-tumour immune responses following PD-1/PD-L1 blockade require reactivation and clonal-proliferation of antigen-experienced T cells present in the TME (O’Donnell et al, 2017; Sharma et al, 2017). Generation of tumour-reactive CD8 T cells requires successful processing and presentation of tumour-associated peptide antigens by antigen-presenting cells (APCs, e.g., dendritic cells) and recognition of these antigenic peptides displayed by MHC I/II. A unique T-cell receptor recognises MHC-bound tumour antigen providing the first signal for T-cell activation and full T-cell activation follows the engagement of the co-stimulatory CD28 receptor on T cells by B7 on the APC (Schumacher and Schreiber, 2015). Tumour-specific CD8 T cells subsequently differentiate into effector T-cells, undergo clonal expansion, traffic to the TME, and ultimately kill tumour cells displaying tumour-associated antigen on HLA, via release of cytolytic effector molecules (e.g., granzyme A/B and perforin) (O’Donnell et al, 2017). For long-term immunologic memory (and presumably durable disease control), a subset of effector T cells must differentiate into effector memory T cells (TEM) (Ribas et al, 2016b), under the guidance of CD4+ helper T cells and dendritic cells; these are maintained for life and respond to re-challenge with antigen (Harty and Badovinac, 2008; Farber et al, 2014).

Failure of ICI therapy can result from defects in any of the steps mentioned above, which can be thought of in three simple categories: (1) insufficient generation of anti-tumour T cells, (2) inadequate function of tumour-specific T cells (Marincola et al, 2000; Bronte et al, 2005), or (3) impaired formation of T-cell memory (O’Donnell et al, 2017; Sharma et al, 2017) (Figure 1). Lack of sufficient or suitable neoantigens, impaired neoantigen processing, and/or impaired presentation of neoantigens can all lead to impaired formation of tumour-reactive T cells (O’Donnell et al, 2017). Inadequate T-cell function can arise through diverse tumour-intrinsic and tumour-extrinsic innate suppressive components of the TME (Pitt et al, 2016), and recent studies have begun to elucidate mechanisms of durable ICI response (Pauken et al, 2016; Sen et al, 2016). We will explore each of these steps in the context of innate and acquired resistance, as well as strategies to overcome these mechanisms of resistance.

Mechanisms of Action of Immune Checkpoint Inhibitors

Insensitive anti-tumour T-cell generation. Tumours can evolve to evade both innate and adaptive arms of the immune system (Gajewski et al, 2013), thereby rendering ICI therapy ineffective (Pitt et al, 2016; Restifo et al, 2016). Tumour-intrinsic mechanisms of immune evasion include genetic and epigenetic alterations to influence neoantigen formation, presentation, and/or processing, as well as alterations in cellular signalling pathways that disrupt the action of cytotoxic T cells (Pitt et al, 2016). Tumour-extrinsic mechanisms involve non-cancerous stromal or immune cells, or other systemic influences (e.g., host microbiota) (Joyce and Fearon, 2015; Pitt et al, 2016) that can act in concert with cancer cells to promote growth and resistance to ICI.

Successful ICI treatment reactivates T cells directed at tumour-specific mutant proteins (Gubin et al, 2014), and lack of suitable neoantigens and alterations in antigen processing and/or presentation is associated with impaired anti-tumour immune response (Schumacher and Schreiber, 2015). Mutational burden is a tumour-intrinsic feature correlated with anti-tumour immune response and response to ICI, presumably by virtue of enhanced neoantigen formation from increased number of non-synonymous single nucleotide variants (Schumacher and Schreiber, 2015; Van Allen et al, 2015). Tumour types harbouring high levels of non-synonymous mutations (e.g., melanoma, lung, and bladder) (Lawrence et al, 2013) are among those with highest response rates to ICI. Consistent with this notion, DNA-mismatch repair deficiency leading to microsatellite instability is associated with enhanced response to PD-1 blockade (Le et al, 2015, 2017).

Importantly, alterations in genes encoding components of the antigen processing and/or presentation apparatus (e.g., class I MHC, β2-microglobulin (B2M)) can also lead to ICI resistance. Downregulation of HLA class I molecules and loss of (B2M) expression have been described. Loss of B2M expression results in impaired cell surface expression of MHC class I, which in turn impairs antigen presentation to cytotoxic T cells (Zaretsky et al, 2016). Neoantigen evolution may underlie aspects of acquired resistance (a) via outgrowth of tumour cell clones that never expressed the neo-Ag, despite effective killing of all other clones, or (b) acquisition of genetic changes that result in loss of neo-Ag expression. Although clonal neoantigens are associated with response to ICI therapy (McGranahan et al, 2016), evolution of the mutational landscape has been described in patients who developed acquired resistance to ICI (Anagnostou et al, 2016).

Strategies to promote immunogenic cell death (e.g., chemotherapy and radiation) or to enhance antigen presentation by stimulating innate immune responses and dendritic cell function (e.g., type I IFN, TLR ligands, LIGHT, and oncolytic viruses) may promote formation or presentation of suitable neoantigens in tumours with a non-inflamed, immune cell poor TME (Pitt et al, 2016; O’Donnell et al, 2017). In addition, promoting dendritic cell migration, maturation, and function via blockade of immunosuppressive factors (e.g., VEGF, IL-10, and TGF-β), may permit adequate T-cell priming and cooperate with ICI (Pitt et al, 2016; O’Donnell et al, 2017). HLA-independent tumour killing via natural killer cells (e.g., anti-KIR) (Guillerey et al, 2016) may be an option in tumours in which HLA neoantigen presentation is insufficient to promote cytotoxic T-cell killing.

Specific oncogenic signalling pathways can also influence the extent and type of intratumoural immune infiltration. Loss of PTEN is associated with increased levels of CCL2 and VEGF, diminished infiltration of T cells, and resistance to PD-1 blockade.
(Peng et al., 2016), and biallelic PTEN loss was recently reported in an isolated non-responding lesion in a patient with near complete response to PD-1 blockade (George et al., 2017). Alterations in β-catenin/WNT signalling caused decreased CCL4 production, which led to diminished infiltration of CD103+ dendritic cells and impaired anti-tumour immune responses (Spranger et al., 2015). The context of these mutations also influences that type of immune infiltration. For example, loss of STK11/LKB1 in the setting of an oncogenic KRas mutation promotes elaboration of IL-6, which recruits neutrophils, decreases T-cell infiltration, and was associated with higher levels of T-cell exhaustion markers (PD-1, CTLA-4, and TIM3), and lower expression of PD-L1 on tumour cells (Koyama et al., 2016a). The recently described innate PD-1 resistance (IPRES) gene signature identified a set of immune suppressive cytokines, EMT transcription factors, and proangiogenic factors associated with innate resistance to PD-1 blockade (Hugo et al., 2016). Of note, gene signatures enriched in non-responding patients also include signatures for wound-healing, EMT, and treatment/resistance to MAPK pathway inhibition (Hugo et al., 2015, 2016). Intriguingly, the receptor tyrosine kinase AXL, whose upregulation is associated with a reversible cell state marked by NF-kB activation and resistance to BRAFi/MEKi (Konieczkowski et al., 2014) is a component of the IPRES (Hugo et al., 2016). It is tempting to speculate that the IPRES may be part of a multigenic, reversible transcriptional program that could be modulated to influence sensitivity to ICI therapy.

Cell state changes are tumour intrinsic, epigenetic events that often result from reversible chromatin modification through removal or addition of methyl or acetyl marks to DNA or histones. Epigenetic modifying agents (EMAs), including DNA-methyltransferase inhibitors and histone modifiers, can act on tumour cells influencing expression of components of antigen-processing and presentation machinery (e.g., TAP, HLA class molecules, and B2M), novel tumour-associated antigens (e.g., cancer-testis antigens), and cytokines (Heninger et al., 2015). Restoration of Th1 cytokine production and enhanced responsiveness to checkpoint blockade has been demonstrated following treatment with DNMT or EZH2 inhibitors (Peng et al., 2015). Altered methylation of non-coding regions of the genome may also impact response to immunotherapy. Hypomethylating agents (e.g., 5-aza cytidine) can induce innate immune responses (Roulois et al., 2015), influence T-cell priming and effector function, modulate immune suppressive cells within the TME (Kim et al., 2014), and enhance response to ICI through induction of endogenous retroviral elements (ERVs) (Chiappinelli et al., 2015). Interestingly, tumour-specific ERVs have been associated with anti-tumour cytolytic activity and immune gene cell enrichment (Rooney et al., 2015). Although the strength and directionality of this relationship between ERVs and immune infiltration and activation, as well as the role for EMAs as adjuvants for ICI therapy via ERV modulation, requires further investigation, there is growing interest in the role of ERV induction as a mechanism to enhance response to PD-1 blockade (Goel et al., 2017).

**Inadequate anti-tumour T-cell effector function.** Following successful neoantigen presentation/cross-presentation and T-cell priming, the expanded repertoire of anti-tumour T cells faces an inhospitable TME that may preclude proper T-cell function, thereby limiting the efficacy of ICI therapy (Pitt et al., 2016; Sharma et al., 2017). These tumour-intrinsic and tumour-extrinsic factors...
JAK/STAT signalling may have a more complex role in mediating resistance to ICI therapy, suggesting that acquired resistance to ICI blockade could be driven by distinct different populations of PD-1 \(^+\) CD8 \(^+\) T cells that respond differently to anti-PD-1 treatment (Blackburn et al, 2008; Paley et al, 2012). For example, partially exhausted PD-1 \(^+\) CD8 \(^+\) T cells display a distinct chromatin landscape compared with effector T cells and T\(_{EM}\) cells (Pauken et al, 2016; Sen et al, 2016), and these epigenetically distinct T-cell states influence whether or not exhausted PD-1 \(^+\) T cells can be reprogrammed to avoid terminal exhaustion and dysfunction (Philip et al, 2017). Evaluation of the specific subsets of CD8 \(^+\) T cells that are expanded in response to PD-1/PD-L1 blockade identified a unique subset of CD8 \(^+\) PD-1 \(^+\) T cells that share features of T-follicular helper cells, CD8 memory precursors, and stem cells (Im et al, 2016), and resemble CXCR5 \(^+\) CD8 \(^+\) follicular T cells (He et al, 2016; Leong et al, 2016; Utschneider et al, 2016). Recent profiling of tumour-infiltrating T cells using mass cytometry revealed distinct mechanisms of action of PD-1 and CTLA-4 blockade, demonstrating that PD-1 blockade reinvigorates CD8 \(^+\) T-cell responses, and CTLA-4 blockade results in the expansion of Th1-like CD4 \(^+\) cells expressing the co-stimulatory ligand ICOS (Wei et al, 2017). Expression of alternative co-inhibitory immune checkpoints (e.g., CTLA-4, TIM-3, LAG-3, and VISTA) has been associated with resistance to PD-1 blockade (Thommen et al, 2015; Koyama et al, 2016b), and combination checkpoint blockade using LAG-3 \(^+\) PD-1 (Woo et al, 2012) and TIM-3 \(^+\) PD-1 (Sakuishi et al, 2010) has demonstrated improved responses in preclinical models. Although these studies suggest crucial roles for distinct sub-populations of PD-1 \(^+\) CD8 \(^+\) T cells, further investigation will be required to determine how to target specific CD8 and CD4 T-cell subsets to overcome primary and acquired resistance.

PD-L1-independent mechanisms of immune escape include alternate immune checkpoints or co-inhibitory receptors, immune suppressive cytokines, immune inhibitory metabolites, and immune suppressive cells (Pitt et al, 2016; O’Donnell et al, 2017; Sharma et al, 2017). Immune suppressive cell types that have been shown to influence ICI efficacy in pre-clinical models include Tregs, MDSCs, Th2 CD4 \(^+\) T cells, and M2-polarised tumour-associated macrophages (Pitt et al, 2016; O’Donnell et al, 2017; Sharma et al, 2017). These cell types individually and collectively promote an immune suppressive TME that prevent anti-tumour cytotoxic and Th1-directed T-cell activities, primarily through the release of cytokines, chemokines, and other soluble mediators (Pitt et al, 2016; Sharma et al, 2017). Depletion of these immune suppressive cell types (e.g., MDSCs and Tregs) has experimentally been shown to enhance anti-tumour immune responses overcoming innate resistance (Highfill et al, 2014; Ngjow et al, 2015). Myeloid- and cancer-cell-derived indoleamine-2,3-dioxygenase (IDO) catabolises tryptophan to the immune suppressive kynurenine (Platten et al, 2014). Interestingly, another immune suppressive enzyme, arginase 1, was recently shown to cooperate with the IDO pathway to inhibit dendritic cell function (Mondanelli et al, 2017). Recently, tumour-associated macrophages were demonstrated to directly limit PD-1 blockade by removing anti-PD-1 antibodies from PD-1 \(^-\) CD8 \(^+\) T cells in a FcyR-dependent manner (Arlackas et al, 2017). There is also emerging data that additional metabolic (e.g., glucose consumption, lactate production, and cholesterol metabolism) and inflammatory pathways (e.g., cyclooxygenase-2/prostaglandin E2) can simultaneously impact both tumour cells and immune cells (Pitt et al, 2016).
Impaired formation of T-cell memory. The most compelling clinical evidence for the success of ICI relates to the potential for long-term, durable clinical benefit. Thus, although ICI may temporarily re-invigorate CTLs to enhance tumour control, if formation of TEM cells is impaired then clinical response could fade leading to acquired resistance or recurrence of disease following discontinuation of therapy. Expansion of intratumoural TEM response to PD-1 blockade has been demonstrated, and is more pronounced in patients responding to therapy (Ribas et al, 2016b), suggesting a key role for TEM cells in anti-PD-1 action and clinical response (O’Donnell et al, 2017). The cellular and molecular mechanisms of TEM expansion following PD-1 blockade are not fully understood, however, recent studies have identified distinct transcriptional programmes associated with naive, acute effector, fully understood, however, recent studies have identified distinct transcriptional programmes associated with naive, acute effector, memory, and exhausted T-cell states (Pauken et al, 2016; Sen et al, 2016). There is emerging evidence that T-cell exhaustion is associated with epigenetic changes that promote a transcriptional landscape distinct from effector or memory CD8 T cells (Sen et al, 2016). Importantly, these epigenetic changes appear to limit the durability of CD8 T-cell function following PD-1 blockade (Pauken et al, 2016). Reacquisition of memory T-cell response may be limited during conditions if tumour antigen persists, as occurs in patients with higher tumour burden (Huang et al, 2017), indicating that future efforts to augment existing new T-cell responses or prime new populations of T cells may be required to generate durable anti-tumour T-cell memory.

SUMMARY AND FUTURE DIRECTIONS

Several clinical trials of combinations of immunotherapeutic agents with targeted agents, cytotoxic chemotherapy, and/or radiation are underway, all in the effort to provide long-lasting disease control to more patients (Buque et al, 2015) (Figure 2). Combination therapies to overcome innate resistance by targeting putative mechanisms of immune evasion within the TME are in various stages of development (Spranger and Gajewski, 2013; Smyth et al, 2016). Cancer vaccines (Ott et al, 2017; Sahin et al, 2017) are showing promise as a means of personalising cancer immunotherapy and potentially enhancing immune memory. Additional research efforts are underway to identify biomarkers associated with resistance and response to ICI, in parallel with early phase clinical testing of novel immune modulatory agents and novel combinations of immune modulators and ICI with other cancer therapies (Mahoney et al, 2015).

Figure 2. Combination therapies to overcome resistance to ICI therapy. Putative mechanisms of innate and/or acquired resistance to ICI therapy. Table listing select approaches for ICI combination therapeutic approaches (Sharma et al, 2017).
To date, precision medicine has largely been synonymous with use of molecular and largely genomic features of the tumour to administer specific targeted small molecules and biologics; however, novel and functional precision medicine platforms may offer additional opportunities to tailor therapies for individual patients or patient populations (Friedman et al., 2015). As the mechanisms of response and resistance to immune checkpoint inhibitors are further elucidated, molecular and functional technologies can and should be integrated to develop novel precision immuno-oncology platforms. In development, functional assays to evaluate response and resistance to ICI therapy and novel combinations may require bio-engineering expertise to appropriately model the native tumour immune microenvironment (Hirt et al., 2014). In the short-term, composite biomarkers (e.g., CD8 T-cell abundance + tumoural/stromal PD-L1 staining) will likely supplant individual biomarkers (e.g., PD-L1 staining), whereas next-generation molecular and/or functional diagnostics are in development. Ultimately, such precision mechanisms of response and resistance to immune checkpoint blockers are further elucidated, molecular and functional technologies can and should be integrated to develop novel precision immuno-oncology platforms. In development, functional assays to evaluate response and resistance to ICI therapy and novel combinations may require bio-engineering expertise to appropriately model the native tumour immune microenvironment (Hirt et al., 2014). In the short-term, composite biomarkers (e.g., CD8 T-cell abundance + tumoural/stromal PD-L1 staining) will likely supplant individual biomarkers (e.g., PD-L1 staining), whereas next-generation molecular and/or functional diagnostics are in development. Ultimately, such precision programs to immune checkpoint blockade in cancer. Cancer Discov 6(8): 827–837.

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