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Chemotherapy and immunotherapy: mapping the road ahead

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Cancer immunotherapy, and in particular checkpoint blockade, is now standard clinical care for a growing number of cancers. Cytotoxic drugs have been the primary weapon against cancer for a long time and have typically been understood because of their capacity to directly kill tumour cells. It is now clear that these drugs are potential partners for checkpoint blockade and different drugs can influence the immune response to cancer through a wide variety of mechanisms. Some of these relate to immunogenic cell death, whilst others relate to changes in antigen-presentation, tumour cell targeting, or depletion of immunosuppressive cells. Here, we review some recent advances in our understanding of the immunological changes associated with chemotherapy, discuss progress in combining chemotherapy with checkpoint blockade, and comment on the difficulties encountered in translating promising preclinical data into successful treatments for cancer patients.

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Introduction

Over the last five years, novel cancer immunotherapies have delivered a long-awaited translational breakthrough into the clinical setting [1]. Although these treatments have resulted in startling tumour regression in some people with advanced cancer, most patients do not respond, meaning that there is still much to be understood. Chemotherapy is now recognised as providing additional benefit to immunotherapy, with a large body of preclinical work providing mechanistic insight into the combinations that are most effective. However, translation of these partnerships has not been straightforward and we are still waiting for the benefits apparent in preclinical studies to be realised in the clinical setting. In this review we will summarise the mechanisms that mediate the adjuvant effect of cytotoxic chemotherapy, review recent developments in chemoimmunotherapy with a focus on checkpoint blockade in solid cancers and discuss the potential factors complicating the bench-to-bedside transition. Targeted therapies and those involving adoptive cell transfer are covered elsewhere in this issue, and therefore will not be discussed here.

Immunogenic cell death

Generation of an effective antitumour immune response requires several functional steps: availability of tumour antigen in the correct context (usually immunogenic cell death or ICD); uptake of antigen by antigen presenting cells (APCs); APC activation and antigen cross-presentation to T cells; infiltration of T cells into the tumour; minimal suppressive activity from myeloid-derived suppressor cells (MDSC) and regulatory T cells (Tregs); and continued cytotoxic activity of T cells against tumour cells without exhaustion induced by negative regulatory checkpoints [2••]. Immunotherapies are designed to target specific bottlenecks in this process, although in many instances more than one step in the immune response is targeted. This is particularly true in the clinic, where heterogeneity is present not only between patients with the 'same' cancer, but also between separate lesions in an individual patient, and even within different areas of a single tumour; hence it is imperative that multi-modality approaches (including chemotherapy, targeted therapy radiotherapy and surgery) be used to realise the full potential of immunotherapy. Some chemotherapies, through their ability to induce ICD, are particularly good at initiating an immune response, including the anthracyclines doxorubicin, epirubicin and idarubicin, the platinum based agent oxaliplatin, cyclophosphamide and others [3,4•]. ICD invokes a specific set of molecular pathways leading to the expression of damage-associated molecular patterns (DAMPs) that can cumulatively engage the immune system [5]. Three DAMPs are considered essential mediators of *bona fide* ICD. Firstly, the endoplasmic reticulum (ER) chaperone calreticulin is shuttled to the outer layer of the plasma membrane as a result of ER stress response [6,7]. Secondly, extracellular release of ATP occurs via induction of the cellular

autophagy pathway, acting through the P2RY2 receptor for recruitment and differentiation of DCs [8,9]. Thirdly, extracellular release of the chromatin-binding protein high-mobility group box 1 (HMGB1) stimulates Toll-like receptor 4 (TLR4). Tumours with low nuclear expression of HMGB-1 can respond more effectively to chemotherapy with anthracyclines or oxaliplatin when also treated with Dendrophilin, a lipopolysaccharide TLR-4 agonist [10]. Type I interferon (IFN) secretion in a TLR3-dependent manner was recently reported as a fourth DAMP requirement for ICD, at least in the case of anthracycline chemotherapy [11]. ICD-mediated DAMP signalling recruits and activates APCs, stimulating antigen uptake and presentation, facilitating downstream priming of antigen-specific T cells. In some cases, non-immunogenic cytotoxic chemotherapies that do not sufficiently promote either calreticulin exposure, ATP or type I IFN secretion, or HMGB1 release can invoke ICD when combined with strategies where the missing DAMP is exogenously added or induced by a further agent (comprehensively reviewed in [12]). Cancers in which current standard-care cytotoxic chemotherapies are not particularly effective may benefit from careful studies to identify whether non-ICD can be converted into ICD by the addition of a missing DAMP, for example, by cotreatment with repurposed agents.

Induction of ICD is of importance primarily in cancers with no defined neo-antigens, where the immune system requires assistance to identify and respond to low levels of peptide. However, in instances where a robust response to a known tumour antigen can be induced by therapeutic vaccination, chemotherapy can still provide synergistic activity. For example, vaccination with synthetic long peptides (SLPs) against viral oncoproteins in a preclinical model of HPV16 cancer was recently shown to synergise with cisplatin. SLP vaccination alone resulted in infiltration of HPV-specific TNFα- and IFNγ-producing T cells into the tumour, with cisplatin combination therapy decreasing tumour cell proliferation. Additionally, TNFα enhanced cisplatin-induced apoptotic tumour cell death [13].

Alternative mechanisms of immunogenicity

Alternatively, or in addition to ICD-dependent mechanisms, some drugs promote anti-tumour immune responses through myriad other pathways, many of which appear dose-dependent and remain poorly understood (recently reviewed in [14]). These effects can be mediated by various cell types but often operate by either positive regulation of APC activity or negative regulation of immune-suppressive cells.

Positive regulation of APCs

Mature DCs cross-present tumour-derived antigens, via the MHC class I pathway, to $CD8^+$ T cells, allowing them to proliferate and licensing them to kill their targets. This is a highly nuanced interaction that is subject to many positive and negative influences. An example of positive regulation of DCs is the promotion of cross-presentation by gemcitabine chemotherapy in a preclinical model of mesothelioma [15]. This study showed that DCs in the tumour (but not the lymph nodes) had a semi-mature phenotype and were defective in their ability to cross-present tumour antigen; this was reversed by gemcitabine [16]. When gemcitabine was combined with an agonistic anti-CD40 APC-activating antibody long term cures were seen in 80% of mice [17]. This immune-potentiating effect of chemotherapy at the level of the APC was confirmed in an orthotopic MB49 mouse model of bladder cancer, where 5-FU and a recombinant adenovirus-mediated CD40 ligand (both of which performed poorly as monotherapies) combined to induce effective systemic anti-tumour immunity [18]. Recent phase I clinical trials of agonistic anti-CD40 in combination with gemcitabine in pancreatic cancer, with pemetrexed/cisplatin in mesothelioma, and with carboplatin/paclitaxel in advanced melanoma and other solid tumours, have invoked at least transient tumour-specific T cell responses with some patients achieving long term survival [19–21]. Anthracycline chemotherapy has also been shown to modulate APCs, increasing CCL2 expression in tumours in response to treatment and resulting in recruitment and differentiation of $CD11b^+CD11c^+Ly6C^{hi}Ly6G^-MHCII^+$ DC-like APCs [8,22].

Negative regulation of immune suppressive cells

Several chemotherapies can abrogate Treg-mediated immune suppression. One explanation for this is that Treg are more likely to be in cycle when compared to effector T cells and many cytotoxics only affect proliferating cells. Nevertheless, docetaxel selectively depletes Tregs, particularly those with a $Foxp3^{hi}CD45RA^-$ activated phenotype [23]. Tregs also appear to be particularly susceptible to cyclophosphamide, which may relate to the activity of the ABCB1 transporter system and its ability to extrude this drug [24•]. As the cytokine TGFβ is known to promote Treg accumulation, Chen and colleagues investigated a TGFβ-neutralising antibody as a therapeutic in an orthotopic 4T1 mouse breast cancer model — but contrary to expectations Tregs increased. The authors proposed a model whereby TGFβ inhibits the proliferation of naturally occurring Tregs, whilst promoting differentiation of naïve $CD4^+$ T cells into Tregs. However, when given in combination with cyclophosphamide 3 days after tumour inoculation, anti-TGFβ therapy cured 80% of the mice [25]. To complicate matters, in mice at least, cyclophosphamide can also promote the expansion of immunosuppressive $CD11b^+Ly6C^{hi}CCR2^{hi}$ monocytic myeloid cells. If the functional activity of these cells was blocked by gemcitabine, by disruption of CCR2 signalling, or by inhibition of their suppressive effects by blockade of the PD-1/PD-L1 signalling axis, then this