Immunology and Cell Biology (2015) 93, 323–325

www.nature.com/icb

NEWS AND COMMENTARY

Checkpoint inhibitors for cancer immunotherapy

Multiple checkpoints on the long road towards cancer immunotherapy

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Immunology and Cell Biology (2015) **93,** 323–325; doi:10.1038/icb.2015.12; published online 17 February 2015

Recently, five studies in the *Nature*[1–5] and two studies in the *New England Journal of Medicine*[6,7] explored the molecular determinants of responsiveness to the inhibition of programmed cell death-l (PD-1), its ligand PD-L1, and cytotoxic T lymphocyte antigen-4 (CTLA-4) for tumour immunotherapy. These prototypical checkpoint inhibitors have taken two decades to advance from discovery to the clinic and to demonstrate that while there has been progress, much remains to be done before we can realise Ehrlich's 'magic bullet' to treat all cancers.

The first putative case of tumour regression dates back to St Peregrine Laziosi (the 'Cancer Saint') in 1320 whose ulcerating cancer eventually healed after it became infected.[8] The hypothesis that infection can somehow stir a 'sleeping' immune system to control cancer was reinforced by anecdotal reports by Campbell de Morgan who observed cancer remissions in patients with postoperative streptococcal wound infections in 1875, and inconclusively tested by William Coley who developed bacteria-free extracts of streptococci (Coley's toxins) to treat sarcomas from 1881 to 1936. However, it was not until 1957 that MacFarlane Burnet and Lewis Thomas crystallised the immunosurveillance concept that a failure of the immune system to recognise and eliminate transformed neoplastic host tissues might have a central role in cancer pathogenesis (reviewed in Dunn *et al.*[9]). This cell-extrinsic theoretical viewpoint was ahead of its time and became overshadowed in the subsequent 50 years by major advances in understanding the cell-intrinsic mechanisms that drive cancer initiation, progression and spread. Utilising this knowledge, drugs designed to specifically target the mutated surface molecules, signalling pathways and gene networks involved in carcinogenesis have revolutionised the treatment of many cancers, with significant improvements in patient outcomes. Unfortunately, these molecularly targeted therapies are often limited by a narrow spectrum of sensitive cancers and the inevitable development of cancer drug resistance.

In the past few decades, clinical data showing increased rates of cancers in immunodeficient patients and transplant patients on immunosuppressive drugs, together with divergent results in immunocompetent and immunodeficient mouse models of cancer, have provided additional evidence highlighting the importance of immunosurveillance in cancer pathogenesis.[10] Furthermore, studies of the tumour microenvironment have revealed complex dynamic interactions between heterogeneous cancer cell clones and a range of innate and adaptive immune cells including tumour-infiltrating $CD8^+$ cytotoxic T lymphocytes (CTLs), $CD4^+$ helper T lymphocytes, natural killer (NK) cells, NK T cells, tumour-associated macrophages, myeloid-derived suppressor cells, dendritic cells and regulatory T cells. Thus, tumour antigens (altered self) that can be specifically recognised by CTLs are constantly under selective pressure to mutate. In addition, cancers can also actively suppress anti-tumour immune responses and promote cancer cell growth and metastasis by hijacking the physiological control mechanisms used by the host to enforce peripheral self-tolerance and to dampen chronic inflammation. This Darwinian tug-of-war between cancer and the immune system lead Schrieber and colleagues to propose the cancer immunoediting concept.[9]

Initial phase I studies have shown efficacy for PD-1–PD-L1 inhibition in metastatic melanoma, renal cell carcinoma and non-small-cell lung cancer.[11–13] It should be noted that only a proportion of patients responded in these trials and patients with castrate-resistant prostate, colorectal, breast, pancreatic and gastric cancers, which are classically considered less immunogenic, did not respond at all. Objective response correlated with PD-L1 expression by the tumour,[11] although PD-1 blockade improves survival regardless of PD-L1 expression by the tumour.[6] In the current studies, Herbst *et al.*[2] and Tumeh *et al.*[4] now report that PD-L1 expression by immune cells in the tumour microenvironment is also a key predictor of response. Furthermore, it was shown that the infiltration of CTLs with clonally restricted T-cell receptor repertoire and PD-1 and PD-L1 expression by immune cells at the tumour margins correlated with response.[4] These and other data point to the presence of a pre-existing immune response held in check by 'adaptive immune resistance' in those who respond to checkpoint inhibition.

Previous reports have indicated that cancers with high rates of somatic mutations respond better to checkpoint inhibitors, possibly because these cancers are more likely to generate neoantigens that can be recognised by CTLs. Therefore, Yadav *et al.*[5] developed algorithms to predict immunogenic mutations by combining mass spectrometry with whole exome and transcriptome sequencing data in two mouse cancer cell lines. Despite the fact that the screen identified >1300

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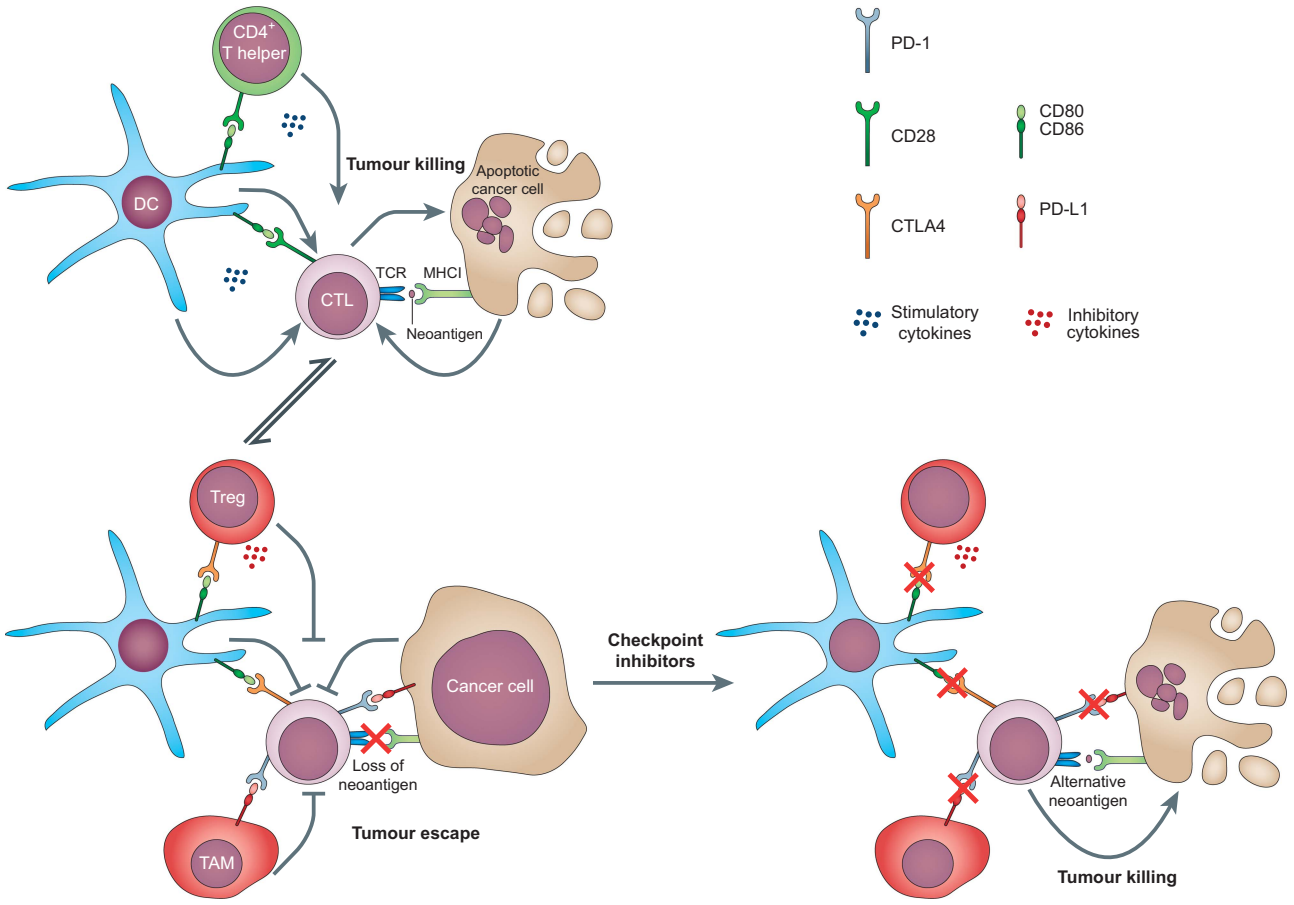

Figure 1 Overcoming adaptive immune resistance with checkpoint inhibitors. The immune system recognises neoantigens generated by somatic mutation to eliminate cancer cells. However, cancers have developed multiple strategies to evade and suppress this antitumour immune response. These include loss of neoantigen expression and hijacking normal control mechanisms used to enforce peripheral self-tolerance (such as CTLA-4-mediated suppression by regulatory T cells) and dampen chronic inflammation (such as PD-1–PD-LI-mediated exhaustion of antitumour CTLs). Checkpoint inhibitors block these inhibitory molecules to drive CTL killing of cancer cells expressing escape neoantigens.

amino acid substitutions, only a surprisingly small fraction of these mutant peptides was immunogenic when used as a cancer vaccine. These 'passenger mutations' were in genes and were not directly involved in carcinogenesis. Interestingly, peptide-major histocompatibility I dextramers used to identify antitumour CTLs also showed that they had an exhausted phenotype and expressed high levels of PD-1 and T-cell immunoglobulin mucin protein-3 (TIM-3). Gubin *et al.*[1] examined a mouse model in which cancers lose neoantigen expression and become resistant to immune rejection. When these mice were treated with anti-PD-1 and anti-CTLA-4, either singly or in combination, they rejected the tumour by recognising two different neoantigens. Vaccinating mice with these escape neoantigens also resulted in tumour rejection. Along similar lines, Snyder *et al.*[7] showed that patients with long-term clinical benefit from anti-CTLA-4 therapy had a high mutation load and that the predicted neoantigen landscape in these patients were dotted with a unique neoepitope signature. Notably, the neoantigens associated with long-term benefit were more likely to be homologous to viral and bacterial antigens than those associated with minimal or no benefit, suggesting that they may originate from memory CTLs. Collectively, these data reveal the tumour microenvironment as a dynamic ecosystem that can be tipped in favour of the host by boosting antitumour immunity (Figure 1).

However, despite its success, autoimmune toxicities are sometimes associated with the treatment with checkpoint inhibitors, particularly CTLA-4 inhibitors. So what can be done to further improve antitumour immune responses and minimise toxicity? Other checkpoint inhibitors (such as lymphocyte activation gene-3 and TIM-3), other approaches to cancer immunotherapy including co-stimulatory molecules (such as ICOS, CD40L and GITR), cytokines (such as interleukin-2 and interferon-γ), cancer vaccines, chimeric antigen receptors and other cell-based therapies are being developed, and these may well synergise with checkpoint blockade. Intriguingly, it was recently reported that targeted therapy with selective B-Raf and v-Raf murine sarcoma viral oncogene homolog B (BRAF) inhibitors induced a marked $CD4^+$ and $CD8^+$ T-cell infiltration and upregulated expression of granzyme B in human melanomas.[14] These and other data suggest that targeted therapies may cause acute inflammatory cell death and prime the immune system against the tumour, or even directly activate T cells expressing wild-type BRAF. In addition, radiation oncologists have observed rare abscopal effects, whereby localised radiotherapy is associated with regression of metastatic cancers distant from the irradiated site.[15] In the most well-documented case, regression was associated with the evidence of a strong immune response against the tumour. Thus, the combination of targeted therapy or radiotherapy and checkpoint inhibition may

improve responses in immunogenic cancers and may convert less immunogenic cancers into more immunogenic ones. These observations bring us back to St Peregrine and Coley's toxins—'the sleeper awakes'!

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