

# Boosting Anti-Tumour Immunity through Targeted Delivery of Interferon- $\alpha$

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**Immune checkpoint inhibitors (ICIs) have revolutionised cancer immunotherapy but their success is wholly dependent on amplifying an existing immune response directed against the tumour. A recent study (Tsuchiya *et al.*, *Cell Rep.*, 2019) suggests how the properties of induced pluripotent stem cells (iPSCs) may be exploited for the targeted delivery of interferon- $\alpha$  (IFN $\alpha$ ) to elicit an appropriate response.**

Recent years have witnessed the renaissance of tumour immunology driven, in part, by the advent of immune checkpoint inhibitors (ICIs), monoclonal antibodies (mAbs) specific for inhibitory receptors such as PD-L1 expressed within the tumour microenvironment, whose blockade releases the brakes on immune responses, maximising their anti-tumour activity. Success is, however, predicated on the existence of an ongoing immune response to the tumour which is itself dependent on high genomic instability, responsible for the generation of suitable neoantigens: those tumours that fail to elicit an effective immune response, so-called ‘cold’ tumours with a low mutational burden, are largely refractory to immune intervention [1]. However, it is not merely amplification of existing immune responses that ICIs seek to achieve but rather their vigorous initiation by dendritic cells (DCs): by altering the balance of stimulatory and inhibitory signals delivered to naïve T cells, the likelihood of initiating an immune response of appropriate magnitude is greatly increased.

But not all DCs are equally effective at eliciting anti-tumour immunity since the capacity to acquire exogenous tumour associated antigens (TAAs) and present them via MHC class I, a process known as antigen cross-presentation [2], is a pre-requisite for activation of CD8<sup>+</sup> cytotoxic T lymphocytes (CTLs). This property is confined to rare subsets of DCs that collectively express the chemokine receptor XCR1 [3], responsible for guiding them within

the tumour-draining lymph nodes (dLNs) towards CD8<sup>+</sup> T cells that secrete the ligand, XCL1. XCR1<sup>+</sup> DCs are surprisingly rare, especially within the tumour itself which they are known to actively infiltrate. Furthermore, they must undergo maturation before they are able to migrate to the dLNs and elicit a response to TAAs acquired *in situ* [4]. This process is normally dependent on the sensing of cytosolic DNA via the so-called stimulator of IFN genes (STING) pathway, or the local secretion of potent pro-inflammatory cytokines, such as IFN $\alpha$ , neither of which is abundant in the sterile microenvironment of a solid tumour, representing a significant weak link in anti-tumour immunity.

Various groups have sought to plug this gap by exploiting the unique properties of iPSCs. It has, for instance, proven feasible to direct the differentiation of human iPSCs into XCR1<sup>+</sup> DCs thereby providing potentially unlimited numbers for immunotherapy [5]. This novel source of cross-presenting DCs may be loaded with appropriate TAAs and induced to mature *in vitro* by exposure to inflammatory cytokines, thereby by-passing inherent deficiencies in the tumour microenvironment. However, Tsuchiya and colleagues have pursued an altogether different agenda, choosing, instead, to differentiate from mouse iPSCs so-called proliferating myeloid cells (pMC), myeloid-specified precursors induced to proliferate in a cytokine-dependent fashion through transduction with *c-Myc* [6]. The resulting iPSC-pMC retain the ability to differentiate into DCs [7] but surprisingly it is not their prowess as antigen presenting cells that Tsuchiya *et al.* exploit, but rather their capacity for sustained secretion of IFN $\alpha$  with which they had been transduced: targeted delivery of the IFN $\alpha$ -iPSC-pMC to the tissues surrounding tumours generated by injecting a melanoma cell line subcutaneously into the hind-limbs of mice, was found to control growth not only of the local tumour but a tumour introduced at a distant anatomical site as well as lung metastases.

Although IFN $\alpha$  may exert a direct anti-proliferative and apoptotic effect on tumour cells, a variant of the melanoma cell line deficient in the IFN $\alpha$  receptor (IFNAR1) was equally susceptible to control by administration of the IFN $\alpha$ -iPSC-pMC, suggesting an indirect effect, most likely through the induction of a tumour-specific immune response. Indeed, this prediction was supported by the accumulation of perforin<sup>+</sup> CTL within the tumour mass, the depletion of which abrogated any beneficial effect of immunotherapy.

However, it was elegant experiments using bone marrow chimeras that fully elucidated the underlying mode of action. Using IFNAR1<sup>-/-</sup> mice, either as recipients of wild type bone marrow or as donors of bone marrow for the reconstitution of lethally-irradiated wild type mice, Tsuchiya and colleagues showed unequivocally that IFN $\alpha$  signalling among hematopoietic cells is required to control distant tumours and metastases. Furthermore, by expressing the diphtheria toxin receptor in mice under control of the *Xcr1* promoter, the authors were able to achieve the specific depletion of DCs capable of antigen cross-presentation, an approach which formally identified XCR1<sup>+</sup> DCs as the principal cell type impacted by local secretion of IFN $\alpha$  (Figure 1). Interestingly, STING<sup>-/-</sup> mice were equally responsive to tumour control, confirming that provision of pro-inflammatory cytokines bypasses the need for direct sensing of tumour-derived cytosolic DNA. Perhaps most importantly, however, this novel approach worked synergistically with the administration of ICIs, rendering mice resistant to subsequent challenges with high numbers of tumour cells and developing long-lasting tumour immunity (Figure 1(c)).

A previous description by the same group of pMC differentiated from human iPSCs [8, 9] offers the tantalising prospect of clinical translation, however, a number of challenges

remain. What, for instance, is the identity of the pMC? Does an equivalent cell type exist *in vivo* or are they artefacts of the *in vitro* differentiation protocol that may prove unstable following administration? Furthermore, to what extent does the approach rely on sustained proliferation of the IFN $\alpha$ -iPSC-pMC and what safety issues might arise from the introduction of the oncogene *c-Myc*, which runs contrary to widespread efforts to circumvent its use for the generation of iPSC lines? Although IFN $\alpha$ -iPSC-pMC are dependent on GM-CSF for their survival, sustained proliferation for 3 months with a doubling time of ~16 hours risks selecting rare variants capable of shaking off the shackles of cytokine dependence. Nevertheless, the approach undoubtedly carries significant advantages: given that the mode of action is not dependent on antigen presentation by the pMC, a so-called ‘universal’ iPSC line might be used for their production, one that lacks MHC genes with the exception of HLA-E, capable of conferring protection from natural killer cell lysis [10]. Such an ‘off-the-shelf’ product is an especially attractive prospect that might ultimately be exploited for the targeted delivery of agents other than IFN $\alpha$ . Delivery of the anti-inflammatory cytokine TGF- $\beta$  may, for instance, serve to restore homeostasis at sites of localised autoimmune damage, potentially increasing the reach of this novel immunotherapy.

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## Conflicts of Interest

The authors have no financial conflicts of interest to declare.

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## Figure Legend

**Figure 1. Proposed Mode of Action of IFN $\alpha$ -iPSC-pMC.** (a) Under normal circumstances, XCR1<sup>+</sup> DCs may infiltrate a solid tumour and acquire TAAs *in situ*. However, their ability to cross-present TAAs to CTLs is hampered by their lack of maturation in the absence of appropriate inflammatory stimuli. (b) Local administration of IFN $\alpha$ -iPSC-pMC to the tissues surrounding the tumour provides a sustained source of IFN $\alpha$  that induces DC maturation and migration to the dLNs where clonal expansion of TAA-specific CTLs occurs. Migration of effector cells to local or distant tumours helps control tumour growth but expression of inhibitory receptors within the tumour mass, such as PD-L1, protects cells from cytolysis and limits tumour regression. (c) ICIs, such as anti-PD-L1, injected systemically, act synergistically with local IFN $\alpha$ -iPSC-pMC to effect a more sustained and vigorous anti-tumour immune response, characterised by well-developed immunological memory.