NANOPARTICLE IMMUNOTHERAPY

Combo combat

Nanoparticle-enabled, sustained delivery of soluble hydrophilic cytokines and hydrophobic inhibitors engages the innate and adaptive immune systems to fight cancer.

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Stimulation of a patient's immune system to fight cancer would seem to be a pleasant alternative to systemic chemotherapy, which is painful and has enormous side effects[1]. For the immune system to respond to a tumour, however, it needs to recognize it as 'non-self' in an appropriate immunostimulatory environment. This is problematic, because cancer cells often express self-antigens[2]. Also, cancer cells overproduce immunosuppressive signalling molecules (cytokines), such as transforming growth factor-β (TGF-β), within the tumour microenvironment[2], decreasing the number and function of natural killer cells[3] and the cancer-killing function of cytotoxic T lymphocyte cells ($CD8^+$T cells)[4], while increasing the number of immunosuppressive regulatory T lymphocytes. These effects suppress both the innate and adaptive immune response and, as a result, immunotherapy using cytokines, such as interleukin-2 (IL-2), which activate adaptive T-cell response, are found often to be clinically unsuccessful for cancer treatment[5].

Writing in *Nature Materials*, Fahmy and colleagues[6] report a method that overcomes the immunosuppressive tumour microenvironment and engages the innate and adaptive immune systems in combating cancer. Using a synergistic, nanoparticle-based delivery approach, in which a TGF-β inhibitor and the immunostimulatory IL-2 are co-delivered, they show delayed tumour growth and increased survival of tumour-bearing mice.

As is now largely recognized, nanoparticle-based drug delivery has the potential to transform cancer treatment through packaging and protecting therapeutic cargos and cargo combinations, and delivering them selectively to the target cancer cells, or tumour environment, while avoiding normal healthy cells[7]. Doxil, in which doxorubicin is incorporated within a liposome, was approved in 1995 and since then, other liposomal, albumin-based and polymer-based nanocarriers have been approved or entered clinical trials. In this context, the nanoscale liposomal polymeric gel (nanolipogel) approach of Fahmy and colleagues — wherein a hydrogel core is encapsulated within a supported lipid bilayer conjugated with polyethyleneglycol (PEG) (Fig. 1) — stands out in several respects.

First, this core–shell construction allows the core to be designed to accommodate and release desired cargos, while the shell can be independently designed to achieve the stealth-like characteristics and advantageous pharmacokinetic properties of PEGylated liposomes[8]. Second, through gelation of acrylated cyclodextrins within a hydrophilic biodegradable polymer, the core can be simultaneously loaded with a hydrophobic TGF-β inhibitor (complexed with cyclodextrins) and soluble, hydrophilic IL-2, enabling the sustained, local delivery of this disparate cargo combination during subsequent polymer erosion. Third, rather than requiring internalization (and endosomal escape) of a cytotoxic cargo or cargo combination within the target cancer cell, the mechanism of action of the nanolipogel (Fig. 2) is the sustained concurrent delivery of a TGF-β inhibitor and IL-2 within the tumour microenvironment. This synergistic combination induces localized therapeutic immune responses, while reducing immunosuppression. Impressively, this combination treatment results in 100% survival rates for mice bearing subcutaneous melanoma tumours after 38 days, whereas delivery of the individual components within nanolipogels results in 0% survival after 25 days. Also, the intravenously injected nanolipogel combination therapy

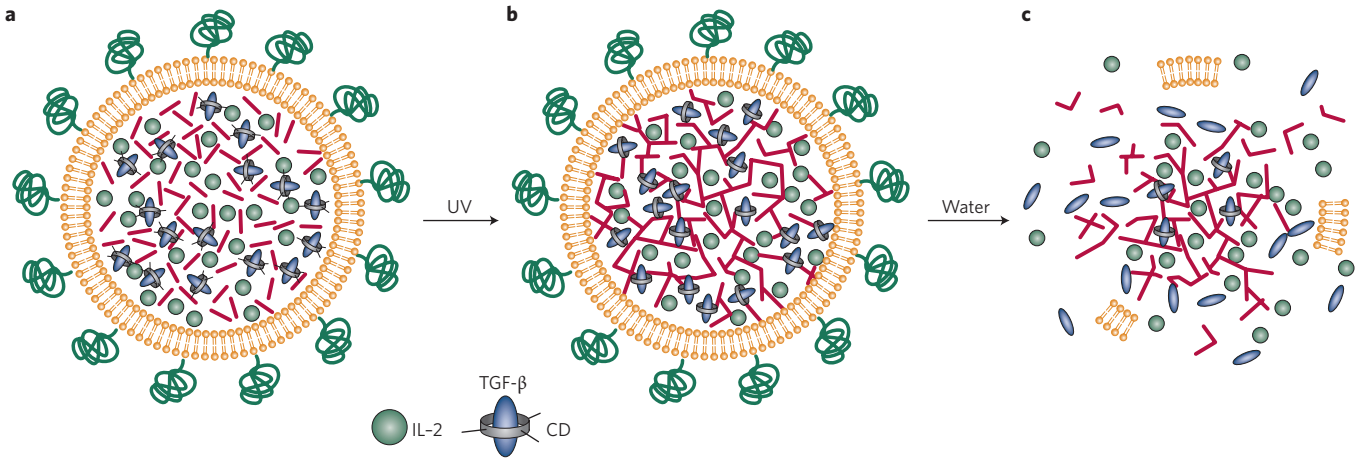

Figure 1 | The synthesis and biodegradation of nanolipogels. **a**, Liposomes are synthesized by extrusion of a poly(lactic acid) (PLA)–PEG crosslinker (red), a hydrophobic TGF-β inhibitor (blue) complexed with acrylated cyclodextrin (CD; grey), hydrophilic IL-2 (green spheres), phosphatidylcholine (yellow), PEGylated phosphatidyl ethanolamine (twisted green shapes) and cholesterol (not shown), followed by freeze drying and rehydration. **b**, Ultraviolet irradiation of the lyophilized liposomes photocrosslinks PLA–PEG with acrylated cyclodextrin-complexed TGF-β inhibitor, entrapping IL-2 within a 'nanolipogel'. **c**, Biodegradation of the nanolipogel in the tumour microenvironment allows the sustained simultaneous delivery of the TGF-β inhibitor and IL-2.

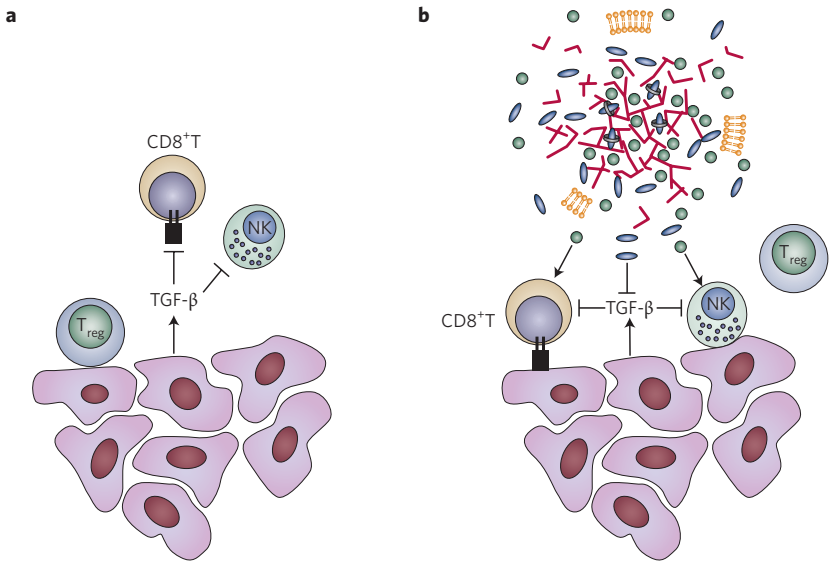

Figure 2 | The immunosuppressive tumour microenvironment and mechanism of action of nanolipogels. **a**, Production of immunosuppressive cytokines, including TGF-β, within the tumour (purple) microenvironment decreases the number and function of both natural killer (NK) and $CD8^+$T cells, suppressing both the innate and adaptive immune response, while increasing the number of immunosuppressive regulatory T lymphocytes (T_{reg}). **b**, Sustained combination release of the hydrophilic cytokine IL-2 and a hydrophobic TGF-β inhibitor through biodegradation of the nanolipogel (see Fig. 1 for description) increases the number of both NK cells and activated $CD8^+$T cells in the tumour environment, engaging both the innate and adaptive immune systems. The ratio of activated $CD8^+$T cells:T_{reg} is increased.

was significantly more effective in treating metastatic melanoma in the lungs than individually delivered TGF-β inhibitor or IL-2. This is noteworthy because improved co-delivery and biodistribution of short-lived cytokines and hydrophobic drugs is an unmet need for treating distant metastatic tumours.

The nanolipogels are synthesized by complexing a TGF-β inhibitor with acrylated cyclodextrins and then encapsulating this complex along with IL-2 and a biodegradable acrylated crosslinker within ~120-nm-diameter liposomes (Fig. 1a). Ultraviolet irradiation of the lyophilized liposomes polymerizes the crosslinker and cyclodextrin complex, entrapping the TGF-β inhibitor and IL-2 uniformly throughout the hydrogel core (Fig. 1b). Administered intravenously, nanolipogels accumulate preferentially in solid tumours by the enhanced permeation and retention effect. Confocal imaging of a surrogate cyclodextrin-complexed fluorescent cargo, rhodamine, contained within a fluorescently labelled bilayer shell showed sustained release in the tumour microenvironment for up to one week, presumably mediated by the biodegradation of the hydrogel core (Fig. 1c).

Harvesting of tumours after treatment with nanolipogels containing IL-2 showed increased lymphocyte infiltration and that IL-2, delivered alone or in combination with the TGF-β inhibitor, increased the percentage and absolute number of activated $CD8^+$T cells and the ratio of $CD8^+$T cells to regulatory T cells — clear evidence of activation of the adaptive immune system. This did not explain the synergistic effects of the combined treatment, however, and further analysis showed that nanolipogel delivery of IL-2 in combination with the TGF-β inhibitor (but not alone) resulted in an increased percentage and absolute number of natural killer cells in the tumour bed (Fig. 2). Thus, it is the nanolipogel-enabled, sustained, simultaneous delivery of a hydrophilic cytokine and hydrophobic inhibitor that allows the activation and engagement of both the innate natural killer cells and adaptive $CD8^+$T cells to overcome cancer immunity (Fig. 2), greatly enhancing the immunotherapeutic effect and the survival of tumour-bearing mice. As TGF-β plays multiple pivotal roles in regulation of the growth and differentiation of tumour cells as well as regulation of the tumour microenvironment[2], a further beneficial effect of the TGF-β inhibitor could be an increase in the enhanced permeation and retention effect[9].

Fahmy and colleagues suggest that improvements in survival index might result from higher and more frequent doses, delivery of additional cytokines such as interleukin-15, and surface derivatization with tumour-retention ligands to achieve selective delivery and retention within the tumour microenvironment. Such benefits might be realized using alternative targeted nanoparticles with higher capacity amphipathic cores. In this regard, mesoporous silica nanoparticles are of interest[10,11], as their high internal surface area allows high-capacity loading and retention of multiple, diverse cargos by simple immersion, and the cargo release rate can be tuned by the pore size, surface charge and dissolution rate of the silica core, or triggered by stimuli-responsive molecular valves[12]. Wrapping a mesoporous silica nanoparticle with a PEGylated lipid bilayer — essentially creating an inorganic analogue of the nanolipogel[11] — could be used to test this strategy. A further potential benefit of mesoporous silica nanoparticles is that they could function as both a nanocarrier and adjuvant[13]. Conceivably such a combination strategy could serve to further activate the adaptive immune system within the tumour microenvironment. Another nanoparticle-enabled strategy is the delivery of gold nanorods to a tumour followed by irradiation with near-infrared light, resulting in local heating and extravascular coagulation[14]. Coagulation in turn amplifies the recruitment of targeted therapeutic nanoparticles administered in a second step, mimicking the features of an inflammatory response.

Regardless of the ultimate delivery vehicle and the various improvements that can be envisioned, Fahmy and colleagues' work emphasizes both the power of nanoparticles to achieve simultaneous delivery of synergistic drug combinations and the appealing prospect and potential of engaging the patient's innate and adaptive immune systems to combat cancer. ❐

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