Cancer immunotherapy

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Taming CAR T cell therapy toxicity

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Post-infusion poly(ethylene glycol) surface modification of chimeric antigen receptor (CAR)-engineered T cells and a subcutaneous chemokine-adsorbing hydrogel address cytokine release syndrome and the neurotoxicity side effects of CAR T cell therapy against tumours.

Since 2017, the US Food and Drug Administration has approved eight adoptively transferred chimeric antigen receptor (CAR)-engineered T (CAR T) cell therapies against distinct tumours, and many others are currently under evaluation in clinical and preclinical studies[1]. Although CAR T cell therapies have demonstrated enduring clinical responses in the treatment of certain cancer types, clinical trials have revealed the presence of two major toxicities — cytokine release syndrome (CRS)[2] and CRS-associated neurotoxicity[1]. Recent studies in a humanized xenotolerant mouse model have shown that the systemic release of the cytokines interleukin-6 (IL-6) and interleukin-1 (IL-1) from human circulating monocytes is responsible for CRS and CRS-associated neurotoxicity[3,4]. The current approach to mitigate these side effects focuses on the use of IL-6 blockade antibodies[5]. Although effective for managing CRS in clinical settings, this strategy has proven ineffective in preventing CRS-associated neurotoxicity[6]. Now, two studies provide distinct solutions to alleviate CRS and CRS-associated neurotoxicity of CAR T cell therapy, while preserving its therapeutic effectiveness. Writing in *Nature Materials*, Ningqiang Gong and colleagues describe an approach to block the intense interactions between tumour cells and monocytes with CAR T cells, thus decreasing monocyte overactivation and IL-6 secretion[7]. In *Nature Biomedical Engineering*, Xianlei Li and co-workers report a thermoresponsive hydrogel conjugated with antibodies that capture

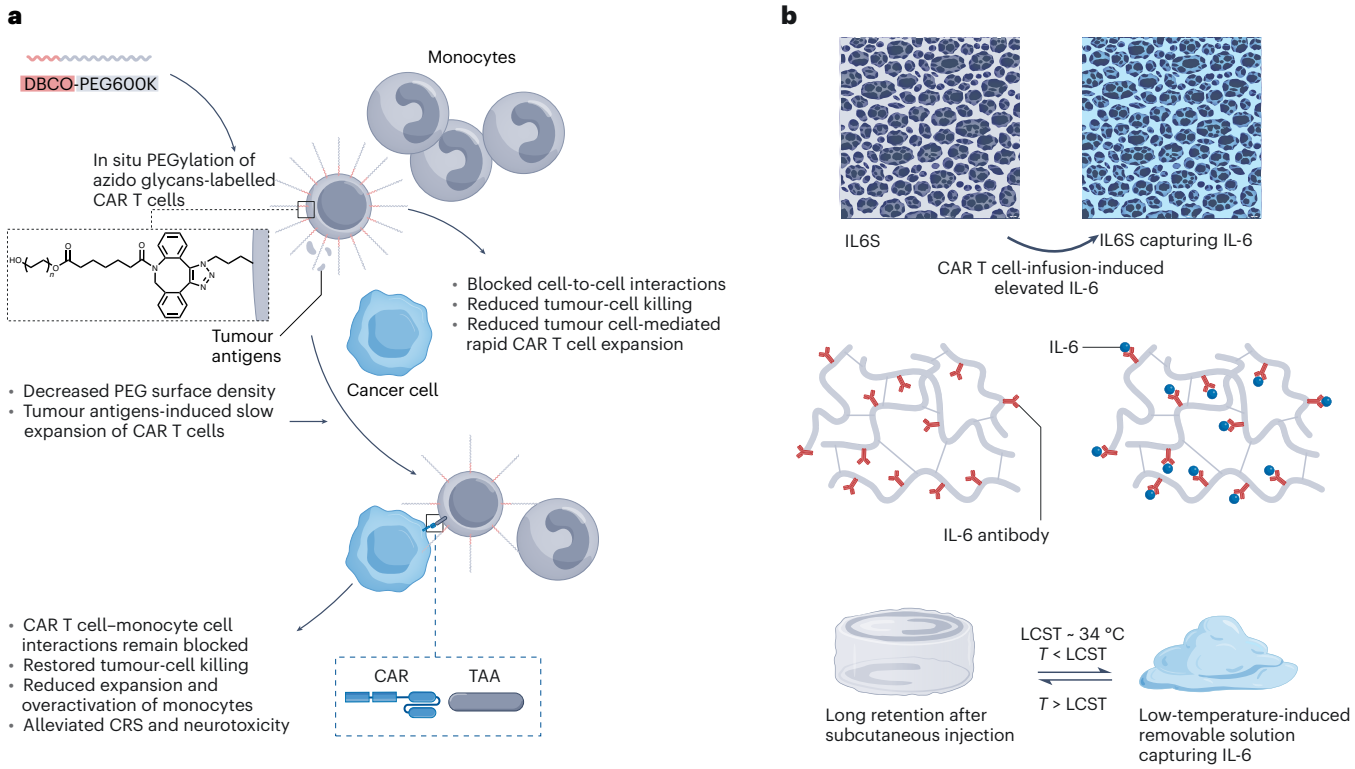

Fig. 1 | Solutions to mitigate the side effects of CAR T cell therapy, administered before or after CAR T cell infusion. a, In situ PEGylation of CAR T cells after they have been infused initially hinders cell interactions, reducing cytokine release and CRS symptoms. Subsequently, as CAR T cells expand, interactions are restored for efficient tumour targeting before monocyte activation, presenting a safer immunotherapy option with reduced neurotoxicity. **b**, A subcutaneous IL-6-adsorbing hydrogel (IL-6 sponge, IL6S), injected before CAR T cell infusion, selectively reduces IL-6 levels during CRS while preserving CAR T cell therapy's antitumour effect. Additionally, this removable temperature-sensitive hydrogel, triggered by a cooling-induced gel–solution transition (or lower critical solution temperature, LCST) offers a potential shift from CRS monitoring to prevention. TAA, tumour-associated antigen; *T*, temperature. Figure adapted with permission from: **a**, ref. 7, Springer Nature Ltd; **b**, ref. 8, Springer Nature Ltd.

IL-6 when subcutaneously implanted, thus effectively decreasing IL-6 serum levels[8].

To control the overactivation of monocytes by CAR T cells, Gong and colleagues introduce dibenzocyclooctyne (DBCO)-modified poly(ethylene glycol) (PEG) via intravenous injection, which can modify administered azido glycans-functionalized CAR T (CAR T-azide) cells in situ (Fig. 1a). This results in the destabilization of CAR T cell interactions with tumour cells and monocytes, and consequently lowers monocyte activation and IL-6 secretion. From the different DBCO-PEGs with varying molecular weights, the authors find that DBCO-PEG with a molecular weight of 600,000 (DBCO-PEG600K) effectively disrupts cell-to-cell interactions. The rapid in vivo conjugation of DBCO-PEG600K to CAR T-azide cells efficiently alleviates CRS in a humanized mouse model. By contrast, unmodified PEG600K and DBCO-PEG1K did not achieve CRS suppression, because these do not affect CAR T cell interactions. While DBCO-PEG600K effectively blocks CAR T cell interactions with both tumour cells and monocytes, it is gradually released from the surface of CAR T cells when in the presence of tumour-cell lysates. The restoration of CAR T cell–tumour cell interactions before the recovery of CAR T cell interactions with monocytes results in a therapeutic temporal window during which tumour eradication is achieved before monocyte overactivation. In practice, the side effects of CAR T cell therapy are mitigated by delaying IL-6 secretion. DBCO-PEG600K also lowers IL-1 levels, effectively mitigating neurotoxicity, a benefit not achieved by tocilizumab (which is currently used as an IL-6 blocker in patients). Moreover, ex vivo constructed DBCO-PEG600K-modified CAR T cells fail to inhibit tumour growth in mice because of fully interrupted cell-to-cell interactions, which highlights the importance of in situ PEGylation of CAR T cells.

Li and colleagues focused on controlling IL-6 serum levels with an IL-6 adsorption hydrogel sponge (IL6S), which is subcutaneously implanted before CAR T cell infusion. The material consists of a temperature-sensitive poly(*N*-isopropylacrylamide-*co*-methacrylic acid) matrix conjugated with IL-6-specific antibodies (Fig. 1b). The IL6S forms an in situ solid hydrogel that does not flow at the subcutaneous injection site, maintains its volume stability for more than 15 days, and demonstrates high biocompatibility and minimal IL-6 antibody leakage. The IL6S exhibits negligible IL-6 adsorption in healthy mice, whereas an equivalent intravenous injection of IL-6 antibodies reduces serum IL-6 levels. In an immunodeficient mouse model of CAR T cell-induced CRS, the IL6S effectively captures IL-6 in real time, promptly mitigating elevated cytokine levels, and effectively outpacing the development of CRS symptoms, which occur within 2–3 days. In humanized mice subcutaneously injected with the IL6S before CAR T cell infusion, the hydrogel substantially mitigates CRS-related symptoms and neurotoxicity-related meningeal thickening, surpassing the limited alleviation observed with free antibodies. Crucially, the IL6S does not compromise the antitumour efficacy of CAR T cells. Additionally, the IL6S can undergo a transition from a solid hydrogel to a flowable solution upon ice cooling, allowing for easy removal from the body.

Although the IL6S and DBCO-PEG600K offer solutions at different stages, before or after CAR T cell infusion, they both present innovative approaches to tackle the side effects linked to CAR T cell therapy. These findings hold promise for potential applications in the treatment of various diseases in the future, including antiviral therapies. Nonetheless, several considerations must be taken into account before these strategies can be effectively translated into future clinical applications. For instance, the requirement for azido glycans modification on CAR T cell surfaces and the potential issue of delayed tumour eradication with in situ PEGylation may pose challenges for the DBCO-PEG600K strategy.

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References

1. Morris, E. C., Neelapu, S. S., Giavridis, T. & Sadelain, M. *Nat. Rev. Immunol.* **22**, 85–96 (2022).
2. Lee, D. W. et al. *Blood* **124**, 188–195 (2014).
3. Norelli, M. et al. *Nat. Med.* **24**, 739–748 (2018).
4. Giavridis, T. et al. *Nat. Med.* **24**, 731–738 (2018).
5. Choy, E. H. et al. *Nat. Rev. Rheumatol.* **16**, 335–345 (2020).
6. Park, J. H. et al. *N. Engl. J. Med.* **378**, 449–459 (2018).
7. Gong, N. et al. *Nat. Mater.* https://doi.org/10.1038/s41563-023-01646-6 (2023).
8. Li, X. et al. *Nat. Biomed. Eng.* https://doi.org/10.1038/s41551-023-01084-4 (2023).

Competing interests

K.S. owns equity in and is a member of the Board of Directors of AMASA Therapeutics, a company developing stem cell-based therapies for cancer. K.S.'s interests were reviewed and are managed by Brigham and Women's Hospital and Mass General Brigham in accordance with their conflict-of-interest policies. C.L. declares no competing interests.