A backpack revs up T-cell activity

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Cytokine backpacks improve T-cell efficacy in the tumor microenvironment.

Turning a T cell infused into a cancer patient into a large population of tumor-killing T cells requires just the right combination of events. The T cell must recognize a tumor antigen on an antigen-presenting cell, its costimulatory receptors must be engaged, and it must encounter cytokines required for proliferation and sustained tumoricidal activity. But tumors often subvert the immune response against them, in part because the stromal cells that normally produce immunostimulatory cytokines are either absent in the tumor or have been reprogrammed to sequester the cytokines instead of releasing them. In this issue, Tang *et al.*[1] describe a nanoparticle "backpack" that can be loaded onto T cells and selectively release its cargo of cytokines only after the cells have recognized their cognate antigen in the tumor. The approach delivers high doses of cytokines to the immunosuppressive tumor microenvironment while minimizing systemic toxicity.

Adoptive T-cell therapy has proved more challenging for solid tumors than for hematologic malignancies because of immunosuppressive molecules in the tumor microenvironment that inhibit the proliferation, function, and survival of effector T cells. These molecules include PDL-1 and TGFβ, which inhibit T-cell activation and block T-cell responsiveness to immunostimulatory cytokines such as interleukin (IL)-7 and IL-15. Boosting cytokine signaling in the tumor microenvironment can promote T-cell persistence and enable T cells to counteract tumor escape mechanisms such as antigen downregulation[2].

Increasing cytokine concentration in tumors via intravenous infusion has been tested in animal models and patients. However, success has been limited by non-specific immune cell activation and systemic toxicity. Clinical trials of intravenous infusions of IL-7, IL-12, and IL-15 revealed dose-limiting toxicities and only modest enhancement of T-cell efficacy. In some studies, cytokine therapy even enhanced immune suppression through enrichment of regulatory T cells.

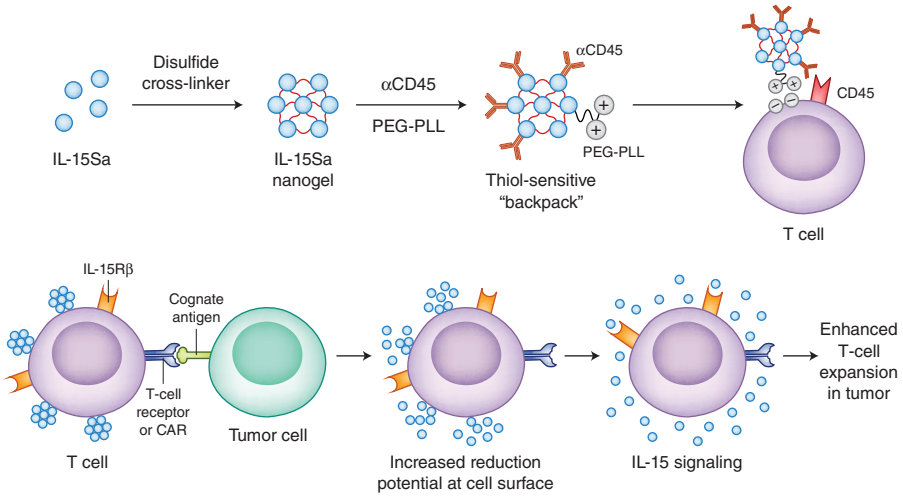

Figure 1 IL-15 backpacks selectively enhance T-cell activity at tumors. IL-15 cytokine molecules are aggregated into a nanogel with a synthetic crosslinker. PEG-PLL and anti-CD45 antibodies are adsorbed or conjugated onto the nanogel surface to facilitate electrostatic binding to T-cell membranes and to prevent nanogel internalization, respectively. The nanogel backpacks are then loaded onto T cells. The backpacks remain inert at the T-cell surface until the T cell encounters a tumor cell. Activation of the T cell induces a wave of thiol emission at the cell surface, creating a reduction potential that breaks the disulfide bonds in the synthetic crosslinkers and releases the IL-15 constituent molecules. The IL-15 molecules can then bind to cytokine receptors on the T-cell surface to enhance T-cell activity at the tumor site.

Another means of delivering cytokines is genetic modification of immune cells to constitutively express recombinant proteins. Genetic engineering of tumor-specific T cells restricts cytokine supplementation to a cell population that should track to tumor sites. Immune effector cells secreting IL-12 or IL-15 are currently being evaluated in clinical trials for patients with solid tumors (NCT02498912 and NCT03294954, respectively). Although this strategy has the potential to produce long-term activity, it will not be self-limited if there is toxicity and would require additional interventional measures to quench T-cell activity.

In related approaches, the signaling systems for the cytokines IL-7, IL-2, and IL-15 have been overexpressed in T cells[3–6] by various methods such as a novel chimeric antigen receptor design containing cytokine signaling elements, and a mutated IL-2 and IL-2-receptor system. More trials are likely to follow, and by necessity, targeted cytokine activity will have to be increased to achieve sufficient anti-tumor efficacy.

To address these challenges, Tang *et al.*[1] created an improved nanogel backpack to transport cytokines to tumors together with adoptively transferred T cells. An earlier

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version of the backpack developed by the same group used IL-15–bearing liposomes that adhered to the T cell membrane (ref. 7). Because the cytokine passively leaked out of the liposomes, T cells carrying the backpacks were continually stimulated with IL-15 until it was depleted, regardless of their location in the body. This could potentially induce T-cell expansion at non-tumor locations and reduce the effective dose of T cells trafficking to the tumor site.

In their new work, Tang *et al.*[1] replaced the liposome with a sophisticated nanogel architecture that allows situational release of the cytokine. They synthesized a novel chemical linker to aggregate cytokine proteins into functionally inert nanogel particles and included a reduction-sensitive disulfide bond in the linker that senses the reducing potential of the environment. The authors show that when T cells are activated, an increase in their cell surface reduction potential disintegrates the backpacks. Because the levels of free thiols in the bloodstream and the extracellular space of organs are low, the backpacks remain sealed while T cells circulate in the blood, and they release their cargo only after the T cells become activated by engaging their cognate antigen in the tumor (**Fig. 1**).

Tang *et al.*[1] show that the backpacks remain intact if T cells undergo apoptotic death. This rules out the possibility of toxicity due to non-specific cytokine release should the T cells die before reaching the tumor. They also demonstrate that tumor-specific T cells armed with backpacks selectively expand at the tumor site and exert anti-tumor activity without proliferating in lymph nodes or the bloodstream. Backpack-equipped T cells nearly doubled the survival of melanoma-challenged mice relative to control T cells receiving intravenously injected cytokine adjuvants. Similarly, backpack-assisted T cells eradicated glioblastoma tumors in 80% of mice, which remained cured weeks after tumors had reached terminal sizes in control mice.

Importantly, the systemic toxicity of the approach was low, despite the use of ALT- 803, an IL-15 superagonist with a relatively long serum half-life (7–8 h) in primate models[8]. A previous clinical trial evaluated an NFAT gene expression system in which T-cell activation triggered IL-12 secretion from modified T cells. However, systemic toxicity was found in some patients, possibly resulting from the long serum half-life of IL-12 (5–10 h)[9,10]. Given that nanogel backpacks show promising results in raising the therapeutic index of ALT-803, they may similarly improve the safety of cytokines such as IL-12.

Approaches that rely on introducing permanent transgenes into T cells are limited by the size of genetic constructs, which restricts the permutations of transgenes that can be expressed. This constraint does not apply to nanogel backpacks, which in principle can carry several different cytokines. The backpacks could be further diversified to include an assortment of cytokines, chemokines, and enzymes to facilitate entry of T cells around tumor stromal barriers[11], allowing T-cell therapies to rally increasingly effective immune responses at the tumor site while maintaining low systemic and off-target toxicity.

One drawback of nanogel backpacks is that their cytokine cargo will be depleted after a finite duration of T-cell activation. Repeated infusions will likely be necessary, using batches of patient cells manufactured in bulk and cryopreserved in dose aliquots that can then be thawed and administered to patients over the course of weeks or months. Fortunately, the authors have shown that the backpacks remain stably conjugated to T cells and maintain their performance even after a freeze/thaw cycle.

Overall, Tang *et al.*[1] convincingly demonstrate that the desired parameters of safety and efficacy can be achieved by sensor-guided cytokine release from nanogel backpacks. This novel drug delivery system has the potential to raise the therapeutic index for adjuvant molecules and provides yet another means to control the temporal and spatial parameters of T-cell activation. Smart cytokine-delivery technologies such as this could play an important role in enabling T-cell therapies to conquer solid tumors.

COMPETING INTERESTS
T.S. is co-author on a patent application for constitutively active cytokine receptors for cell therapy.

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Unexpected CRISPR on-target effects

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Cas9 can induce extensive on-target damage, including large deletions, inversions, and insertions.

Cas9 RNA-guided endonucleases, repurposed from CRISPR adaptive immune systems in bacteria and archaea, hold great promise in human therapy, and clinical trials using CRISPR–Cas9 are now underway in the United States and China[1]. A long-recognized potential limitation for the clinical use of CRISPR-based therapeutics is safety concerns associated with off-target effects that lead to the cleavage of unwanted DNA sites that are highly homologous to the target site. In this issue, Kosicki *et al.*[2] show that in addition to off-target effects, CRISPR–Cas9 on-target effects are also unpredictable, complex, and potentially problematic. This suggests that more caution may be needed when adopting CRISPR in human therapeutic applications.

Cas9 nucleases cleave chromosomal DNA in a guide RNA (gRNA)-dependent manner, producing site-specific DNA double-strand breaks (DSBs) in cells, the repair of which via homologous recombination or non-homologous end-joining (NHEJ) gives rise to desired genetic modifications[3]. In an attempt to disrupt the X-linked *PigA* gene, Kosicki *et al.*[2] first introduced Cas9 and gRNAs, targeted to intronic or exonic sites of the gene, into male mouse embryonic stem (ES) cells. Unexpectedly, single gRNAs targeted to intronic sites located hundreds to thousands of base pairs from the nearest exon, which could have served as negative controls, yielded PigA-deficient cells at frequencies of 5–20%, suggesting that DSB repair can involve large deletions extending up to several kilobase pairs in length. In addition,

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