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# Nanotherapeutics approaches to improve the efficacy of CAR-T cells in solid tumors

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**Abstract:** Adoptive cell therapy and Immune Checkpoint Blockade Inhibitors have recently revolutionized the field of oncology. However, these types of immunotherapeutic approaches have limited success in treating solid tumors. In particular, chimeric antigen receptor (CAR)-T cells efficacy is hampered by immunosuppressive signals in the tumor microenvironment (TME) and by a limited infiltration of re-infused T cells to the tumor site. The field of nanobiotechnology applied to oncology is also rapidly expanding. Nanoparticles-based delivery systems can be employed to modulate the activity of immune cells present in the TME enhancing the efficacy of CAR-T cells. Interestingly, nano-backpacks can be attached to CAR-T cells prior to re-infusion to support their homing to the tumor site and to slowly release immunopotentiators directly in the TME. Furthermore, nanovaccines can also be employed to support the *in vivo* expansion of CAR-T cells with consequent enhancement of their therapeutic potential. In this viewpoint, recent advancement in the field of nanobiotechnology to support CAR-T cell therapy will be discussed. The development of novel therapeutic CAR-T cells protocols together with nanotherapies is warranted in order to take full advantage of the high therapeutic potential of CAR-T cell therapy.

## Introduction

Despite the large success of adoptive cell therapy (ACT) in hematological cancers, its effectiveness in solid tumors remains limited due to acquired resistance to therapy and evasion of anti-tumor immunity (Saleh and Elkord, 2020). There are different intrinsic and acquired mechanisms of resistance to ACT: downregulation of MHC molecules in tumor cells, upregulation of immune checkpoints in the TME, loss of target antigens and secretion of immune suppressive signals by myeloid derived suppressor cells (MDSCs), tumor associated macrophages (TAMs) and T regulatory cells (Tregs) (Saleh and Elkord, 2020).

ACT-based cancer immunotherapy treatments mainly involve the re-infusion of genetically modified T cells (Laskowski and Rezvani, 2020). T cell based ACT can be divided into three sub-categories: 1) chimeric antigen receptors T cells (CAR-T), where T cells are modified with a single chain variable fragment able to recognize neo-antigen epitopes in a major histocompatibility complex (MHC) independent manner; 2) T cell receptor (TCR) engineered cells, where a TCR that is able to identify a specific tumor antigen, is added into the genome of T cells; 3) tumor-infiltrating lymphocytes (TILs), where patient derived T cells are simply expanded and re-infused (Leon *et al.*, 2020).

To enhance the impact of immunotherapies in solid tumors multiple therapeutic strategies could be employed at the same time to effectively attack cancer cells, while simultaneously reducing the immunosuppressive molecular signals in the TME. For example, standard treatments (chemotherapy and radiation) can be combined effectively with ACT to reduce immunosuppressive cells in the TME, and enhance immunotherapy (Murciano-Goroff *et al.*, 2020). Other novel approaches comprise the use of nanoparticles (NP) to deliver immunomodulatory molecules to the TME or to further boost the anti-tumor immune response in the case of cancer nanovaccines (Bai *et al.*, 2019; Musetti and Huang, 2018).

NP-based delivery systems can be designed to take advantage of the aberrant vasculature, the hypoxic or acidic TME, to induce the release of therapeutic drugs directly in the tumor milieu, reducing off-target side effects (Thomas *et al.*, 2020). The recent discovery of novel bio-compatible nano materials has impacted on the field of nanobiotechnology. For instance, novel stimuli-responsive polymers have been used to develop advanced nanostructures

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with the ability to improve the pharmacokinetic properties of many drugs used in oncology (Yang *et al.*, 2020).

The production of CAR-T cells requires *ex vivo* manipulation, expansion and subsequent reimplantation. To reach a clinically meaningful number of T cells, the expansion phase requires long periods of time, leading to delays in the treatment schedule and high costs of production. In order to reduce both the production time and the costs involved, Parayath *et al.* (2020) have developed a NP-based strategy to transiently induce CAR expression on T cells *in vivo*. An mRNA transcript encoding the CAR gene, was condensed to the cationic polymer PBAE-447 to form NP targeted to CD8+ T cells. Interestingly, these NP were effective in mouse models of human leukemia, prostate cancer and hepatitis B-induced hepatocellular carcinoma with comparable results to re-infused *ex vivo* engineered CAR-T cells. A Phase I clinical trial to treat patients with HBV-related hepatocellular carcinoma is currently ongoing. This strategy could potentially be applied for the *in vivo* generation of CAR-T cells specific for solid tumors.

To improve the expansion and effectiveness of transduced T cells *in vivo*, different NP-based "backpack" strategies have been devised to deliver immunomodulating agents together with T cells in the TME. Protein nanogels targeted to CD45, which served as a stable, non-internalizing anchor, were employed to bind to T cells and slowly release an IL-15 superagonist complex in the tumor milieu, to improve T cell effector functions. This strategy increased the efficacy of CAR-T cells in B16F10 xenografts dramatically, leading to complete tumor eradication in 80% of treated mice, compared to only 20% in mice treated with standard CAR-T cells (Tang *et al.*, 2018). In another report, PEGylated immunoliposomes targeted to CD45 and loaded with a TGF-β inhibitor were used as a backpack prior to CAR-T cell infusion, leading to enhanced T cell efficacy compared to controls. Of note, the administration of immunoliposomes after T cell transfusion led to a further control of tumor growth control in B16F10 xenografts (Zheng *et al.*, 2017). Another backpack composed of cross-linked, multilamellar liposomal vesicles (cMLV) was developed to deliver the A2a adenosine receptor (A2aR) antagonist, SCH-58261. Adenosine in the TME suppresses T cell proliferation and IFN-γ secretion. Therefore, the blockade of this molecular pathway in infiltrating T cells improved the tumor-killing capacity of adoptively transferred T cells (Siriwon *et al.*, 2018). T cell backpacks can also be used to deliver cytotoxic drugs in the TME to slowly release the cytotoxic agent directly into the tumor milieu, enhancing its effectiveness while reducing off-target effects. Kim *et al.* (2020) developed a novel click-chemistry-based methodology to couple IL13-targeted NP to CAR-T cells prior to infusion. In this case, pH-sensitive NP were loaded with Doxorubicin (DOX) for the treatment of glioblastoma-bearing mice with backpacked CAR-T cells. The results showed increased tumor accumulation of DOX compared to the free drug, and strong accumulation of CAR-T cells in the TME showing that this strategy could be potentially implemented in other types of solid tumors.

CAR-T cells efficacy can also be enhanced by the treatment with immunomodulatory NP prior to T cell transfusion. This strategy can support CAR-T cells homing to the tumor lesion, leading to an increased anti-tumor function and expansion in the tumor milieu. In support of this hypothesis, 4T1-ROR1 tumor bearing mice treated with an integrin-targeted liposomes loaded with a combination of the PI3K inhibitor PI-3065, and the α-GalCer agonist 7DW8-5, showed enhanced efficacy of transplanted CAR-T cells which were able to eradicate tumors in 50% of treated mice, while CAR-T cells and NP alone were ineffective (Zhang *et al.*, 2018). In another interesting report, an mRNA-based nanovaccine (RNA-LPX), was developed to deliver the CAR target to lymphoid tissues to support the expansion of previously infused CAR-T cells. In this case, NP were used to deliver the CAR target to continuously stimulate the expansion of transplanted T cells. Treatment with RNA-LPX was effective in inducing the *in vivo* expansion of previously transplanted T cells, which showed an effector memory and a central memory phenotype. In addition, RNA-LPX treatment did not induce cytokine release syndrome, or depletion of antigen presenting cells (APCs) in the lymphoid tissues, and supported therapeutic tumor control in different murine tumor models mediated by a sub-therapeutic dose of infused CAR-T cells (Reinhard *et al.*, 2020).

These recent reports highlight multiple NP-based strategies that can be used to enhance the efficacy of CAR-T cells by increasing their tumor-homing and by slowly releasing immunomodulating or cytotoxic drugs directly in the TME to support T cell function. Furthermore, nanovaccines can be used to support *in vivo* T cell proliferation, to provide a strong, sustained activity of the implanted CAR-T cells to treat solid tumors.

The application of NP-based delivery systems to cancer therapy has already reached the clinical stage, with more than ten FDA-approved nanoformulations, mainly employed for the delivery of chemotherapeutics (DOX, daunorubicin, paclitaxel and irinotecan) (Anselmo and Mitragotri, 2019). Furthermore, nanovaccines designed to co-deliver antigen and adjuvants to APCs, have also been recently deployed for COVID-19, opening novel avenues for the use of nucleic acids-loaded NP for cancer therapy in the near future (Kim *et al.*, 2021).

In conclusion, the clinical translation of NP-based therapeutics should be accompanied by immunotherapies in order to attack advanced metastatic tumors from multiple and different angles to limit the strong immunosuppressive role of the TME and to support anti-tumor cytotoxic T cell function.

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