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

FRANCESCO MAININI*

Immunotherapy and Innovative Therapeutics Unit, Department of Medical Oncology, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, 20133, Italy

Key words: Nanoparticle, Immunotherapy, Cancer, Adoptive cell therapy, CAR-T, Tumor microenvironment

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...

*Address correspondence to: Francesco Mainini, francesco.mainini@gmail.com
Received: 08 May 2021; Accepted: 07 June 2021

Doi: 10.32604/biocell.2021.017399
This work is licensed under a Creative Commons Attribution 4.0 International License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
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 1 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 increase in the production of CAR-T cells (Siriwon et al., 2018). T cell backpacks composed of core-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 development to deliver the A2a adenosine receptor (A2aR) linked, multilamellar liposomal vesicles (cMLV) was in vivo transduced T cells of treated mice, compared to only 20% in mice treated with the PI3K inhibitor PI-3065, and the α-GalCer agonist 7D18-5, 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.

**Authors’ Contribution:** The author confirms sole responsibility for the following: study conception and design, and manuscript preparation.

**Funding Statement:** The author received no specific funding for this study.

**Conflicts of Interest:** The author declare that he has no conflicts of interest to report regarding the present study.

**References**

Anselmo AC, Mitragotri S (2019). Nanoparticles in the clinic: An update. Bioengineering & Translational Medicine 4: e10143. DOI 10.1002/btm2.10143.
Bai Y, Wang Y, Zhang X, Fu J, Xing X, Wang C, Gao L, Liu Y, Shi L (2019). Potential applications of nanoparticles for tumor microenvironment remodeling to ameliorate cancer immunotherapy. *International Journal of Pharmaceutics* 570: 118636. DOI 10.1016/j.ijpharm.2019.118636.

Kim GB, Aragon-Sanabria V, Randolph L, Jiang H, Reynolds JA et al. (2020). High-affinity mutant Interleukin-13 targeted CAR T cells enhance delivery of clickable biodegradable fluorescent nanoparticles to glioblastoma. *Bioactive Materials* 5: 624–635. DOI 10.1016/j.bioactmat.2020.04.011.

Kim J, Eygeris Y, Gupta M, Sahay G (2021). Self-assembled MRNA vaccines. *Advanced Drug Delivery Reviews* 170: 83–112. DOI 10.1016/j.addr.2020.12.014.

Laskowski T, Rezvani K (2020). Adoptive cell therapy: living drugs against cancer. *Journal of Experimental Medicine* 217: e20200377. DOI 10.1084/jem.20200377.

Leon E, Ranganathan R, Savoldo B (2020). Adoptive T cell therapy: Boosting the immune system to fight cancer. *Seminars in Immunology* 49: 101437. DOI 10.1016/j.smim.2020.101437.

Murciano-Goroff YR, Warner AB, Wolchok JD (2020). The future of cancer immunotherapy: microenvironment-targeting combinations. *Cell Research* 30: 507–519. DOI 10.1038/s41422-020-0337-2.

Musetti S, Huang L (2018). Nanoparticle-mediated remodeling of the tumor microenvironment to enhance immunotherapy. *ACS Nano*. American Chemical Society. DOI 10.1021/acsnano.8b05893

Parayath NN, Stephan SB, Koehne AL, Nelson PS, Stephan MT (2020). In vitro-transcribed antigen receptor MRNA nanocarriers for transient expression in circulating T cells *in vivo*. *Nature Communications* 11: 6080. DOI 10.1038/s41467-020-19486-2.

Reinhard K, Rengstl B, Oehm P, Michel K, Billmeier A et al. (2020). An RNA vaccine drives expansion and efficacy of claudin-CAR-T cells against solid tumors. *Science* 367: 446–453. DOI 10.1126/science.aaay5967.

Saleh R, Elordi E (2020). Acquired resistance to cancer immunotherapy: Role of tumor-mediated immunosuppression, *Seminars in Cancer Biology*. DOI 10.1016/j.semcancer.2019.07.017.

Siriwon N, Kim YJ, Siegler E, Chen X, Rohrs JA, Liu Y, Wang P (2018). CAR-T cells surface-engineered with drug-encapsulated nanoparticles can ameliorate intratumoral T-Cell hypofunction. *Cancer Immunology Research* 6: 812–824. DOI 10.1158/2326-6066.CIR-17-0502.

Tang L, Zheng Y, Melo MB, Mabardi L, Castaño AP et al. (2018). Enhancing T cell therapy through TCR-signaling-responsive nanoparticle drug delivery. *Nature Biotechnology* 36: 707–716. DOI 10.1038/nbt.4181.

Thomas RG, Surendran SP, Jeong YY (2020). Tumor microenvironment-stimuli responsive nanoparticles for anticancer therapy. *Frontiers in Molecular Biosciences* 7: 610533. DOI 10.3389/fmolsb.2020.610533.

Yang F, Shi K, Jia YP, Hao Y, Peng JR, Qian ZY (2020). Advanced biomaterials for cancer immunotherapy. *Acta Pharmacologica Sinica* 42: 844. DOI 10.1038/s41401-020-0372-z.

Zhang , Stephan SB, Ene CI, Smith TT, Holland EC, Stephan MT (2018). Nanoparticles that reshape the tumor milieu create a therapeutic window for effective T-cell therapy in solid malignancies. *Cancer Research* 78: 3718–3730. DOI 10.1158/0008-5472.CAN-18-0306.

Zheng Y, Tang L, Mabardi L, Kumari S, Irvine DJ (2017). Enhancing adoptive cell therapy of cancer through targeted delivery of small-molecule immunomodulators to internalizing or noninternalizing receptors. *ACS Nano* 11: 3089–3100. DOI 10.1021/acsnano.7b00078.