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Companion vaccines for CAR T-cell therapy: applying basic immunology to enhance therapeutic efficacy

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“With continued focus on leveraging principles of basic immunobiology and purposeful development of vaccines specifically designed to augment CAR T-cell efficacy *in vivo*, we may soon see the remarkable potential of cancer immunotherapy fully realized.”

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Over the past 20 years, momentum from decades of basic and clinical research has led to the development, testing, and US FDA approval of immunotherapies that are transforming cancer patient care such as surgery, chemotherapy, radiotherapy, and targeted therapies. In contrast to cytokines and immune checkpoint blockade which employ conventional pharmacological approaches to manipulate an immune system millions of years in the making to enhance immunity to cancer or remove barriers to endogenous cancer immunity, CAR T-cell therapy employs synthetic biology to generate *de novo* antitumor immunity. While remarkably effective for B-cell leukemia/lymphoma patients whose tumors are refractory to all other treatments, CAR T-cell therapy has not yet been successful for other malignancies, particularly solid tumors. Leveraging basic immunology principles, investigators are now uncovering ‘vaccine’ approaches to enhance CAR T-cell efficacy that could result in a significant leap forward for CAR T-cell therapy.

CAR T-cell therapy

In patients, T cells have been identified that recognize native and mutated antigens on the surface of cancer cells, resulting in cancer cell death via cytotoxic granule release, cytokine secretion and other mechanisms. Indeed, infiltration of effector/memory T cells into tumor nests has been established as a powerful positive predictor of patient survival [1]. Moreover, isolation of tumor-specific T cells from patient tumors followed by *ex vivo* activation and expansion and re-administration to the patient, a process known as tumor-infiltrating lymphocyte (TIL) therapy, has produced remarkable success in patients with certain tumors [2,3]. Together with other substantial data, these have established T cells as the primary mediator of antitumor immunity. While TIL therapy has been effective, it is difficult to successfully generate TILs from most cancer patients. As a solution, CAR T-cell therapy instead uses peripheral T cells of any (unknown) specificity obtained from blood and genetically modified to become cancer specific by delivery of a CAR. CARs are comprised of antigen recognition domains (typically a scFV from a cancer antigen-specific antibody) tethered in-tandem with T-cell signaling domains from various receptors (CD3 ζ , CD28, CD137, etc.). The first two CAR T-cell therapies were FDA approved in 2017 and 2018 to treat certain leukemias and lymphomas expressing the B-cell marker CD19 [4]. These therapies have unprecedented efficacy against cancers that are refractory to all other treatments. However, despite considerable effort, CAR T-cell therapy has not yet made substantial progress for the treatment of other cancers, particularly solid tumors. While several mechanisms have been hypothesized to explain this dichotomy, could the properties of the initiating normal cell (B cells) in the immune system be a significant contributor, suggesting possible solutions to improve CAR T-cell therapy for solid tumors?

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T-cell–antigen-presenting cell interactions

The immune system is a remarkably complex network of cell types, soluble and surface molecule interactions, and more, that relies on a multitude of stochastic events to convey host immunity to any microorganism that it may encounter [5]. Of importance here is the interaction between antigen-presenting cells (APCs) and T cells. Our T cell repertoire is extraordinarily diverse ($>10^6$ unique T-cell specificities [6]) and each T cell is maintained in an inactive ('naive') state. Naive T cells circulate through the blood and lymph nodes, waiting to encounter their cognate antigen to initiate a program of robust proliferation and production of 'effector' T cells which recirculate, infiltrate inflamed tissues, and eliminate infected cells. However, T cells are incapable of this process on their own. Instead, they rely on APCs which circulate into tissues to detect and acquire pathogens. Those APCs process microbial proteins into small peptides, display them on their surface in MHC molecules and travel to lymph nodes to activate T cells specific for the microbial antigen. Thus, lymph nodes are a vital 'meeting place' for APCs which have acquired microbial antigens and T cells that are poised and waiting for their cue to initiate an immune response [7]. However, this antigen 'handshake' is one of only two signals that are required. Remarkably, our immune system evolved several 'two-factor authentication' paradigms in which a second signal is required to initiate a response [8], not unlike the systems employed routinely on the internet to login to financial sites, social networks, etc. In the case of the immune system, the second 'handshake' comes via costimulatory molecules on the surface of APCs only after they have encountered microbial products. This system ensures that the T-cell response occurs only toward microbial antigens, but not harmless self or environmental antigens [9]. Indeed, APCs are uniquely capable of expressing these costimulatory molecules to engage T cells and initiate the cascades of proliferation, development of effector function, and long-term persistence (memory) characteristic of the vertebrate immune system. However, these interactions and signals are absent for CAR-T cells. Tumor cells do not express costimulatory molecules and CAR-T cells are incapable of interactions with APCs reflecting the absence of antigen presentation by APCs to CAR-T cells, except for the two FDA-approved CAR T-cell therapies that target CD19 found on certain leukemias/lymphomas and on normal B cells, one of the APC populations. Thus, it is possible that the efficacy of CD19-directed CAR-T cells could reflect, in part, promotion of CAR T-cell persistence and function through interactions with normal B cells and that recapitulation of native T-cell–APC interactions *in vivo* could substantially improve CAR T-cell therapy efficacy in solid tumors.

Vaccines for CAR T-cell therapy

Peptide-DC vaccination for pMHC-directed CAR-T cells

The first demonstration that a vaccine could be employed to enhance CAR T-cell efficacy came as a proof of concept in 2018 [10]. In that study, CAR-T cells were generated from an antibody directed against the 235–243 peptide of the WT1 antigen presented by MHC. Those CAR-T cells had antitumor activity in mice bearing human cancer cells with WT1 pMHC complexes on their surface. However, vaccination of CAR T-cell-treated animals with activated dendritic cells (DCs) pulsed with WT1 peptide enhanced CAR T-cell antitumor activity, resulting in greater delays in tumor progression. Moreover, DC vaccination enhanced CAR T-cell expansion in the blood and increased CAR T-cell infiltration and activation within tumors, associated with increased antitumor activity in two different tumor models.

Surrogate antigen delivery to APCs *in vivo*

While demonstrating proof of concept, DC-based vaccinations have significant challenges of their own, including sophisticated and expensive manufacturing processes, making them a poor approach to enhance CAR-T efficacy *in vivo*. Moreover, pMHC directed CAR-T cells are restricted by MHC type, negating the benefits of conventional, MHC-independent CAR-T cells. To overcome these limitations, Darrell Irvine's group generated a synthetic CAR ligand that is delivered *in vivo* to lymph node APCs, promoting CAR–APC interactions *in vivo* [11]. They employed an amphiphile CAR ligand (amph-ligand), that selectively trafficked to lymph nodes, decorating the surface of APCs to engage ligand-directed CAR-T cells. Employing T cells expressing a bispecific CAR containing a scFv directed to fluorescein isothiocyanate (FITC) in tandem with a scFv directed to the melanoma antigen TRP1, produced little antitumor activity against established TRP1-expressing B16F10 melanoma tumors in the absence of vaccination. However, delivery of the amph-FITC vaccine resulted in significantly improved antitumor efficacy, further supporting the premise that CAR–T–APC interactions could enhance efficacy.

Native antigen delivery to APCs *in vivo*

The above approaches required use of complex and expensive vaccine platforms (DC vaccines) paired with pMHC-directed CARs or production of novel bi-specific CAR constructs that can be activated with exogenously administered ligands (amph-ligands). Neither approach is ‘backward-compatible’ with conventional FDA-approved or experimental CAR T-cell therapies. In contrast, BioNTech Corp. recently tested a nanoparticle RNA vaccine approach that is fully compatible with conventional CAR-T cells [12]. The vaccine comprised intravenously administered liposomal antigen-encoding RNA (RNA-LPX) for body-wide delivery of RNA into the lymphoid compartment, resulting in selective uptake and antigen expression on DC surfaces. As a target antigen, the authors employed CLDN6, an oncofetal tight junctional protein expressed by several solid tumors. CLDN6-directed CAR-T cells were efficacious, but rapidly declined over time. In contrast, CLDN6 RNA-LPX resulted in CAR T-cell expansion, expression of memory markers, improved persistence, and substantially improved antitumor efficacy. Importantly, this approach resulted in native antigen expression on the surface of DCs as well as Toll-like receptor (TLR)-dependent Type I interferon driven DC activation. Thus, this strategy results in lymph node DCs with high levels of antigen on their surface and upregulation of T-cell costimulatory molecules (factors 1 and 2 of the two-factor authentication), leading to robust CAR T-cell stimulation *in vivo* and promotion of central memory phenotypes necessary for renewal of effector T-cell populations that infiltrate and eliminate tumors [13,14].

Conclusion

The three vaccine strategies above represent evolving approaches to harness native T-cell–APC interactions to generate CAR T-cell therapy for most cancers. Indeed, these may lead to the development of vaccines as a necessary companion to CAR T-cell therapy. The liposomal RNA approach is particularly intriguing, reflecting its compatibility with current CAR T-cell technologies; however, the immunogenicity of RNA-based vaccines has not yet been established in patients. With continued focus on leveraging principles of basic immunobiology and purposeful development of vaccines specifically designed to augment CAR T-cell efficacy *in vivo*, we may soon see the remarkable potential of cancer immunotherapy fully realized.

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