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# Engineered chimeric antigen receptor-expressing T cells for the treatment of pancreatic ductal adenocarcinoma

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**Abbreviations:** CAR, chimeric antigen receptor; CTLA-4, cytotoxic T-lymphocyte antigen-4; FDG, [18F]-fluorodeoxyglucose; PDAC, pancreatic ductal adenocarcinoma; PD-1, programmed cell death 1; PD-L1, programmed cell death 1 ligand 1; PET/CT, positron emission tomography/computed tomography; RECIST, Response Evaluation Criteria in Solid Tumors

Adoptive cell therapy with chimeric antigen receptor (CAR)-engineered T cells is under investigatation as an approach to restore productive T cell immunosurveillance in pancreatic ductal adenocarcinoma patients. Early findings demonstrate the relative safety of this cell-based therapy and the capacities of CAR-expressing T cells to mount exogenous tumor-specific immunity and induce native antitumoral immune responses.

Pancreatic ductal adenocarcinoma (PDAC) is an almost uniformly lethal disease with an abysmal 5-y survival rate that has remained static at ~5% for the past 2 decades despite significant effort. The resistance of PDAC to conventional forms of therapy has spurred investigations into novel treatment modalities, among which are immunotherapeutic regimens.

Leukocytes actively infiltrate the surrounding stromal microenvironment of pancreatic adenocarcinomas. However, tumor-infiltrating leukocytes are dominated by immunosuppressive cells, including macrophages, immature myeloid cells, granulocytes, and regulatory T cells. In contrast, immunostimulatory and potentially tumoricidal effector T cells are rarely observed to infiltrate tumor tissue.

Immunotherapy has recently demonstrated promise in the treatment of some solid malignancies, such as melanoma, non-small cell lung carcinoma, and renal cell carcinoma.[1-3] For example, reversing T-cell immunosuppression by infusion with blocking antibodies that recognize checkpoint molecules, such as those targeting cytotoxic T-lymphocyte antigen-4 (CTLA-4) and programmed cell death 1 (PD-1) or its ligand PD-L1, has produced impressive tumor regressions extending in some cases to long-term remissions. However, in the treatment of PDAC, single agent immunotherapy with checkpoint inhibitors, including anti-CTLA-4 and anti-PD-L1 antibodies, has yet to produce patient responses satisfying the trial's objective[1,4]. This finding may be due to a weak naturally occurring antitumor T-cell immune response against pancreatic cancer cells. Consistent with this hypothesis, promising results have recently been reported in PDAC patients with chemotherapy refractory disease by combinatorial treatment with anti-CTLA-4 antibodies and a vaccine designed to induce tumor-specific T cells.[5]

The induction of productive tumor-specific T-cell immunity is a multi-step process that requires effective processing and presentation of tumor-specific antigens by antigen presenting cells followed by the activation and expansion of tumor-antigen specific T cells. Several mechanisms can limit the productivity of this process leading to ineffective tumor-specific T cell immunity. For this reason, the adoptive transfer of T cells engineered to recognize tumor antigens has garnered recent attention. T cell adoptive therapy is already showing early promise in the treatment of hematologic malignancies.[6] However, the use of T cell transfer in the treatment of solid malignancies has been limited, partly due to concerns about on-target but off-tumor toxicities.

Mesothelin is a tumor-associated antigen that is overexpressed in the majority of PDAC that has been shown to be a spontaneous target of endogenous T cell immune responses.[7] In preclinical models, mesothelin-specific chimeric antigen receptor (CAR)-engineered T cells have demonstrated potent antitumor activity.[8] However, caution is warranted because mesothelin is present on normal peritoneal, pleural, and pericardial surfaces, and, as such, off-tumor toxicities are entirely possible. To circumvent this problem, we are currently exploring the use of autologous T cells, referred to as CARTmeso cells, engineered to transiently express (by virtue of mRNA electroporation) a mesothelin-specific CAR

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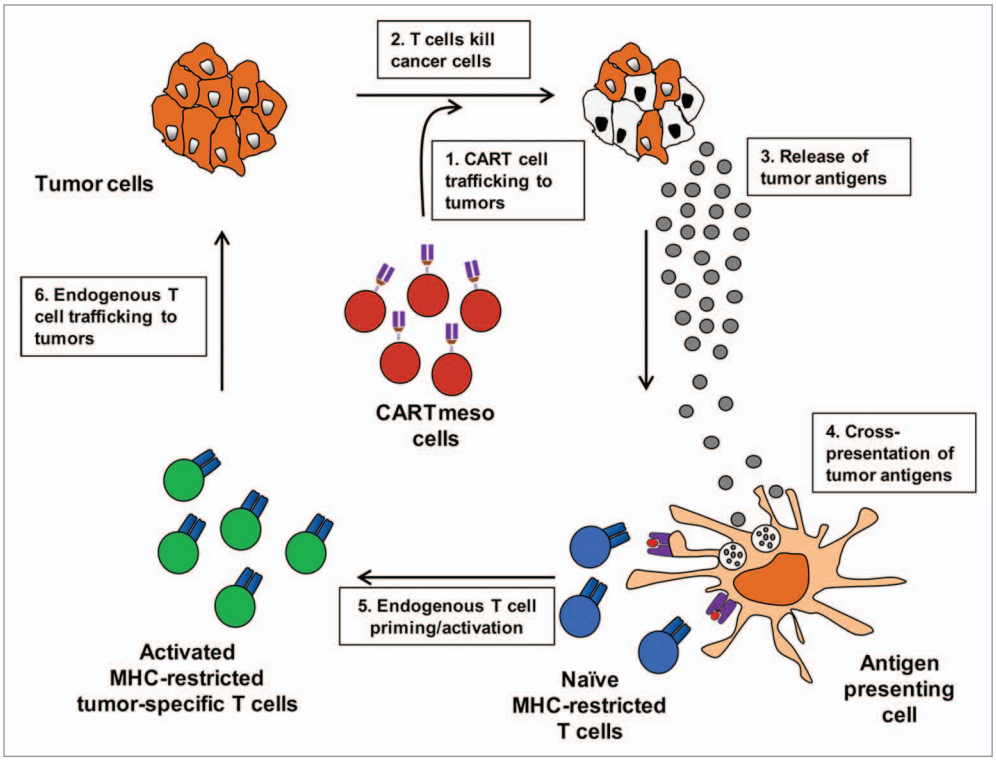


**Figure 1.** Chimeric antigen receptor modified T cell adoptive therapy induces an endogenous antitumor immune response through epitope spreading. Autologous chimeric antigen receptor (CAR)-engineered T cells induce the development of an endogenous antitumor immune response through a multi-step cyclical process as follows: 1) CAR-modified T (CART) cells infiltrate tumor lesions. 2) CART cells recognize tumor antigen expressed on the surface of cancer cells leading to tumor cell lysis. 3) Dying cancer cells release tumor antigens. 4) Tumor-associated proteins are engulfed by antigen presenting cells which process and present tumor-associated peptides in the context of major histocompatibility molecules (MHC) to endogenous T cells. 5) Tumor-specific T cells recognizing peptide/MHC complexes are primed and become activated. 6) Nascent, activated tumor-specific T cells infiltrate tumor lesions. Infiltrating tumor-specific T cells recognize tumor cells via T cell receptor engagement of peptide/MHC complexes present on tumor cells amplifing the initial antitumor T-cell response.

that incorporates the T cell receptor CD3ζ and tumor-necrosis factor receptor superfamily, member 9 (TNFRSF9, better known as 4–1BB) signaling domains (clinical trial #NCT01897415 and #NCT01355965).

We recently reported 2 case reports demonstrating the feasibility, safety, and preliminary efficacy of CARTmeso cells.[9] No overt pieces of evidence for off-tumor toxicities (e.g., peritonitis, pleuritis, and pericarditis) were observed following multiple CARTmeso cell infusions. However, one patient experienced an anaphylactic event when CARTmeso cell therapy was reinitiated after a 4-wk treatment interruption. This adverse event was determined to most likely result from patient anti-murine IgE antibodies mounted against the mesothelin-specific CAR that contains murine peptide sequences.[10] This finding prompted the modification of ongoing clinical trials evaluating mRNA CARTmeso cell therapy to prohibit infusion interruptions which could allow for IgE class switching.

In one patient with chemotherapy refractory advanced PDAC, we found that mRNA-engineered CARTmeso cell infusion produced a transient metabolic response measured by changes in [$^{18}$F]-fluorodeoxyglucose (FDG) uptake within tumor lesions detected on positron emission tomography/CT (PET/CT) imaging. In addition, analysis of ascites fluid consistently present throughout treatment revealed a marked decrease in tumor cell burden as measured by fewer cancer cells in the ascites fluid after CARTmeso cell infusion. These findings demonstrate the capacity of CARTmeso cells to mediate antitumor activity. However, stable disease was the best response determined by Response Evaluation Criteria in Solid Tumors (RECIST) 1.1, and unfortunately, disease control was transient with the patient ultimately experiencing cancer progression.

To understand the bioactivity of mRNA-engineered CARTmeso cells, we studied the persistence and trafficking of infused CARTmeso cells in vivo. CARTmeso cells transiently persisted within the peripheral blood after intravenous infusion and were found to traffic to tumor tissue. The transient presence of CAR-engineered T cells was expected based on preclinical data showing a rapid disappearance of CAR expression on T cells following their activation and proliferation.[8] Treatment was associated with the development of novel antibodies including transient antitumor immunoglobulin responses unrelated to the specificity of the CAR-engineered T cells. Similar findings were observed for a patient with advanced mesothelioma treated with CARTmeso cell infusions. These findings provide evidence for the capacity of CARTmeso cells to traffic to tumor tissue and facilitate tumor destruction leading to the release of self-proteins including tumor-associated antigens that are then cross-presented in the process of classical epitope spreading (**Fig. 1**).

The ability of such engineered T cells to stimulate endogenous antitumor immunity suggests that CAR-expressing T cells may also offer a personalized approach for inducing a vaccine effect. However, the success of CAR-engineered T cells may be restrained by immunosuppressive mechanisms present within the tumor microenvironment. As a result, combinatorial approaches incorporating other immunomodulatory strategies, such as immune

checkpoint blockade, may be necessary to optimize the potential of CAR-based T cell immunotherapy in the treatment of PDAC.

In summary, the feasibility, safety, and efficacy of mRNA-modified mesothelin-specific CAR-expressing T cells for the treatment of patients with PDAC, and other mesothelin-expressing advanced solid malignancies, is ongoing. Early clinical findings with CARTmeso cells have evinced the promise of clinically beneficial activity. For this reason, we believe that chimeric antigen receptor-engineered T cells are an attractive approach for the treatment of PDAC and to interrogate immune-resistance mechanisms established by PDAC to evade T cell immunosurveillance.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.


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