CAR’s made it to the pancreas

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Despite intensive treatment, pancreatic adenocarcinoma still has the worst prognosis among all malignancies. Using clinically relevant models, we demonstrated the therapeutic efficacy of adoptively transferred T cells engineered with a carcinoembryonic antigen (CEA)- and ERBB2-specific chimeric antigen receptor against pancreatic carcinoma. Targeting CD24, a putative cancer stem cell antigen expressed by a minority of carcinoma cells, was likewise effective.

Pancreatic adenocarcinoma is often diagnosed at an advanced stage with a mean survival rate of less than 2 years and no primary therapy. In this situation, adoptive cell therapy with patient’s cytotoxic T cells redirected toward tumor-associated antigens may provide a therapeutic option. The “T-body” strategy pioneered and developed by our groups is based on engineering T cells with a chimeric antigen receptor (CAR) with antibody-defined specificity for a target expressed on cancer cells. Such genetically modified T cells showed remarkable efficacy against leukemia in recent trials, but their efficacy against solid neoplasms had not yet been investigated.

We have addressed this issue in a clinically relevant model of carcinoembryonic antigen (CEA) expression, which closely mimics the human situation with respect to physiological expression of the targeted antigen on healthy tissues, presence of the antigen in serum and competence of the immune system. Among several proteins, pancreatic carcinoma cells over-express CEA and ERBB-2 (HER2/neu), which are not tumor-specific but also expressed on epithelial cells of healthy tissues like lungs and the colon, although to a lesser degree. Adoptive therapy with CEA-specific T cells harbors the risk of damaging healthy CEA tissues, resulting in auto-immune reactions, as observed in a recent trial that is on hold due to severe auto-immune colitis and pneumonia.

The CEA-transgenic mouse expresses CEA in the gastrointestinal and pulmonary tract, secretes soluble CEA into the serum and is self-tolerant toward CEA. Mouse pancreatic adenocarcinomas with or without CEA expression were treated by the adoptive transfer of murine T cells engineered with an anti-CEA CAR. All mice showed profound regression of CEA+ pancreatic cancer, and six out of eight mice achieved sustained remissions. The antitumor response was CEA-specific and CAR-mediated.

We made several observations that add to our understanding of adoptive T-cell therapy:

(1) Following systemic administration, anti-CEA CAR T cells accumulated at the site of CEA+ pancreatic tumors, without inducing severe colitis nor infiltrating to major extents into the colon or lungs, although these tissues express CEA. Lack of autoimmune activation may be due to the fact that the epithelia of healthy tissues, as opposed to carcinoma cells, express CEA in a polarized fashion (to the lumen), hence making it less accessible to infiltrating T cells.

(2) The CAR that we used has a moderate binding affinity, i.e., 37 nM, that is lower than that of CARs used in clinical trials. CARs of different affinity differ in their activation threshold. Those of high affinity might lose the selectivity...
for cells with a high antigen load and may hence cause a greater degree of auto-immunity.

(3) The role of lymphodepletion, which is normally performed to facilitate the homeostatic expansion of transferred T cells, in causing autoimmunity pathology remains unclear. In our mouse model, lymphodepletion was neither required for achieving therapeutic efficacy nor did it result in auto-immunity.

(4) T-cell targeting to the tumor site occurred even in presence of soluble CEA at clinically relevant concentrations. Serum CEA binds to but does not activate engineered T cells, unless CEA is present in micro-domains on the cell surface to induce CAR clustering and formation of a signaling synapse.8

(5) Adoptively transferred CAR-expressing T cells had a central memory phenotype and became activated at the targeted tumor site. Nevertheless, they converted to an exhausted phenotype after prolonged persistence. Strategies to prevent exhaustion still need to be explored.

(6) Mice that successfully eliminated CEA+ carcinoma also rejected a secondary tumor cell challenge in a CEA-specific fashion, in line with previous observations obtained in other models.9 The process is accompanied by a humoral and a T\textsubscript{H}1 dominated CD4\textsuperscript{+} T-cell response, implying that T-bodies may act as mediators for a helper T cell-dependent humoral response.

(7) The observations made in the pancreatic adenocarcinoma model cannot be extended to colon carcinoma, a setting in which adjacent healthy CEA+ epithelial cells may become accessible to T cells, thereby increasing the risk of auto-immunity.

In a second report, we explored the selective elimination of “cancer stem cells,” rather than of bulk tumor cells, by targeting CD24 expressed on putative adenocarcinoma stem cells. Anti-CD24 CAR-expressing T cells could successfully eliminate orthotopic human pancreatic adenocarcinoma xenografts in which...
CD24⁺ tumor cells represent a minority. These xenografts resist final elimination by anti-ERBB2 T-bodies, in contrast to xenografts in which all cells expressed ERBB2. The treatment was highly effective in reducing the size of primary tumors, in particular in situations of high tumor burden, and in eliminating remote metastases.

The significant lesson of this study consists in the impact of CAR specificity. Targeting ERBB2, which is broadly, but not uniformly, expressed by pancreatic adenocarcinoma cells, did not endurably eradicate tumors whereas targeting CD24, which is expressed by a minority of cancer cells, arrested growth of freshly established orthotopic pancreatic carcinomas without inducing auto-immunity. Targeting putative cancer stem cells proved equally effective, although such cells represent a minority of the tumor mass. Both our studies provide a rationale for the adoptive T-cell therapy of this currently untreatable tumor.

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