Hypothesis:
Chimeric antigen receptor (CAR) cell therapy has had limited success in pancreatic cancer (PC) due to tumor microenvironment (TME) consisting of immune inhibitory Tregs and their secretary molecules such as TGF-β in malignant ascites. Signaling of TGF-β via Smad proteins results in the gene expression of additional immune inhibitory molecules such as IL-10. We have demonstrated mesothelin (MSLN)-CAR-cells with minicircle-DNA (for efficacy) and sleeping beauty transposons (for safety) with natural killer (NK) derived cell line NK-92MI (off-the-shelf therapy) with efficient elimination of PC cells and its inhibition by tumor conditioned medium (TCM) simulated as TME. TGF-β depletion reverses the inhibition; however, systemic use of neutralizing antibodies has severe toxicities. We posit that 1. the utilization of malignant ascites instead of TCM to mimic in vivo situations; 2. the development of mesothelin-CAR-NK cells co-expressing dominant negative TGF-β receptors within the tumor bed and 3. unraveling of the TGF-β signaling via Tregs depletion and Smad protein phosphorylation will bring clinical success to the therapy.

Goals:
We will (A) establish cultures of PC patient-derived malignant ascites with and without depletion of Tregs; (B) develop MSLN-CAR-NK cells vector with TGF-β dominant negative receptor and (C) analyze phosphorylation followed by nuclear translocation of Smad proteins in the presence of malignant ascites using the IL-10 expression as the end point. Experimental

Methods:
Construction of MSLN-CAR with dominant negative TGF-β receptor and electroporation into NK-92MI cells. MTT cytotoxicity assays with BxPC-3 cells. Electrophoretic mobility shift assays to assess TGF-β downstream signaling via Smad protein complex.

Relevance:
CAR-immunotherapy inactivating TGF-β within the tumor bed along with the utilization of the malignant ascites to simulate in vivo conditions will accelerate clinical translation.

Grant Coordinator

The grant coordinator listed should be the individual who oversees the grant projects for your institution.

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Project Plan / Budget

Project Plan & Preliminary Research

Project Plan