C3 / Padres Pedal the Cause 2017

“Identification of genes critical for the production of T cells from human pluripotent stem cells for development of “off-the-shelf” T cell immunotherapies”

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SCIENTIFIC ABSTRACT
Many patients present with cancers refractory to conventional “standard-of-care” treatment. T cell-based immunotherapy using each patient’s T cells expressing chimeric antigen receptors (CAR-T cells) has become an exciting advance to treat refractory malignancies. T cells can also be produced from human embryonic stem cells (hESCs) or induced pluripotent stem cells (iPSC). However, the process by which these therapeutic cells are derived, as well as their functional nature, remains inefficient and poorly understood. In this proposal, we will use genetic CRISPR-Cas9 nuclease screening to identify genes that control differentiation of hESCs to effector T cells capable of tumor killing. Human ESCs will be transduced with lentiviruses encoding Cas9 and a guide-RNA library to disrupt a single gene per cell. Dual selected cells will undergo T cell differentiation via co-culture with OP9 stromal cells stably expressing the DL4 gene. Genes that contribute to derivation of a mixed population of T cell types will be disrupted and effector T cells expressing CD3, TCRαβ, and CD8αβ will be selected via flow cytometry and sequenced to determine which guide-RNAs they contain. These candidate genes will be targeted individually to produce hESC lines that will efficiently differentiate to effector T cells, which will be tested via in vitro killing assays. hESC lines lacking candidate genes can be further engineered to remove HLA genes, limiting host rejection. These universal donor cells will be ideal for engineering to recognize tumor cells for “off the shelf” effector CAR-T cell cancer immunotherapy.

LAY ABSTRACT
While anti-cancer therapies continue to improve, too many patients still do not respond to conventional therapies and die from their malignancy. A promising new therapy involves the use of the patient’s own white blood cells, known as T cells, being removed and engineered to specifically target tumors before being reintroduced to the patient. While this cancer immunotherapy approach appears to work well for some cancers, it is a laborious and expensive process. An ideal solution is to produce “off the shelf” T cells, which can be genetically altered to kill tumors. Human pluripotent stem cells can multiply without limit, differentiate into T cells, and be engineered to avoid being rejected by the immune systems of different patients, making them an ideal source universal donor cells. While previous studies have demonstrated derivation of T cells from human pluripotent stem cells, the process is inefficient. In this proposal we will use genetic screening to individually disrupt genes in stem cells and then derive specific T cells capable of killing tumors. We will select these tumor-killing cells and then use DNA sequencing to discern which genes are disrupted in the desired population. These genes can then be studied individually to derive stem cell lines, which will reliably give rise to T cells capable of killing tumors. These cells can then be modified to both avoid host rejection and target specific tumors. These universal donor “off the shelf” T cells will then be suitable to treat cancers in multiple patients.