COMPOSITIONS AND METHODS FOR ENHANCING ADOPTIVE T CELL THERAPEUTICS

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4. Abstract: The present disclosure relates generally to compositions and methods for improving T cell therapy. In particular, the disclosure provides polypeptides and recombinant nucleic acid constructs and/or recombinant nucleic acids encoding polypeptides having mutations capable of altering T cell signaling, cytokine production, and/or in vivo persistence in tumors of therapeutic T cells comprising the mutation. The T cell signaling can be by NFAT, NF-κB and/or AP-1 pathways. The disclosure also provides vectors and cells including the polypeptides and/or recombinant nucleic acid constructs and/or recombinant nucleic acids of the disclosure as well as methods of preparing a T cell for use in cell therapy, and methods of identifying a mutation useful for improving T cell therapy.
5. BACKGROUND
    a. Adoptive T cell therapies, including chimeric antigen receptor (CAR) T cells, have revolutionized cancer therapy. However, impressive responses are limited to a subset of patients with hematological cancers and have not been unlocked in patients with solid tumors, which represent 90% of adult cancers. In both treatment-resistant hematological and solid cancers, adoptive T cell therapy is limited by a complex combination of factors including fitness of engineered T cells in tumors, T cell exhaustion, poor in vivo persistence and immunosuppressive environmental factors. Despite significant recent advances, rational design has failed to overcome the problems associated with such factors.
    b. Another approach to identify modifications that improve T cell function in vitro and in vivo, besides rational design, is unbiased screening. For example, the vast majority of screening efforts, have focused on genome-scale or genome-wide alterations which modify expression of endogenous wild-type genes via CRISPR-Cas9 or short hairpin RNA (shRNA) or cDNA overexpression.
    c. Chimeric antigen receptors (CARs) are synthetic receptors that include an antigen specific extracellular single chain variable fragment (scFv) attached to a flexible linker (hinge) region, transmembrane domain, and intracellular signaling domains. The intracellular portion of the receptor consists of T cell signaling domains such as 41BB, CD28 and CD3zeta, designed to mimic T cell receptor (TCR) stimulation and the immunological synapse upon engagement with the antigen specified by the scFv. CAR constructs do not require antigen presentation by MHC molecules, and therefore have been used to effectively redirect a patient's own T cells against a tumor specific cell surface antigen. To date, five CD19 targeted CAR-T cell therapies have been approved by the FDA for use against hematological B cell cancers. While

these therapies have proven highly effective in refractory B cell malignancies, CAR-T cell therapies have yet to provide robust, long-term efficacy against solid tumors. In the solid tumor setting, CAR-T cells can become exhausted and struggle to proliferate and perform effector function, ultimately resulting in the inability to control tumor growth or prevent relapse. Therefore, to create effective targeted cellular therapies against solid tumors the proliferative capacity, persistence and effector function of CAR-T cells needs to be improved.

d. An avenue under investigation is genetically modifying CAR-T cells to improve their functionality in solid tumors. A recent case study described a chronic lymphocytic leukemia (CLL) patient who experienced a delayed yet complete response after treatment with a CD19 CAR-T cell therapy. It was later discovered that, within a single T cell clone, the CD19 CAR cassette had integrated into the one allele of TET2, a known T cell lymphoma tumor suppressor, rendering it nonfunctional. Interestingly, the second TET2 allele of this patient was also mutated, resulting in a lack of function of TET2 in the CD19 CAR-T cells dosed to this patient. This single TET2 knockout CAR-T cell clone exhibited altered T cell differentiation and improved overall effector function. Ultimately, this clone expanded to become a majority of the CAR-T cell population, and mediated a complete response against the patient's relapsed CLL. In a second example, a similar complete response was mediated in a patient when the CD22 CAR cassette integrated into the T cell lymphoma tumor suppressor CBL. These case studies demonstrate that genetic knockout of T cell lymphoma tumor suppressors, such as TET2 and CBL, can have remarkable beneficial effects on CAR-T cell therapies. In preclinical studies, genome wide knockout assays have revealed genes, such as REGNASE, that upon knockout improve T cell fitness and anti-tumor efficacy in vivo. Additionally, other studies have found that the knockout of genes related to T cell exhaustion and memory formation, such as the NR4A family of genes, can result in improved and prolonged CAR-T cell response to tumors.

e. While these examples indicate that CAR-T cell functionality can be improved through genetic manipulation, particularly through manipulation of tumor suppressor genes, these studies are often extremely broad in their scope (examining the entire genome) and focus solely on the effect of constitutive genetic knockouts. Somatic single nucleotide variant (SSNV) mutations, translocations and gene deletions that naturally arise in cancers offer biologically rational candidates for genetic manipulation alongside CAR expression.

f. There remains a need in the art for alternative solutions to address the significant unmet need for effective adoptive T cell therapies and for enhancing engineered T cell fitness.