Modified Guide RNAs

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5. **Abstract: This disclosure relates to modified single and dual guide RNAs having improved in vitro and in vivo activity in gene editing methods.**
6. **Summary:**
    a. **This disclosure relates to the field of gene editing using CRISPR/Cas systems, a part of the prokaryotic immune system that recognizes and cuts exogenous genetic elements. The CRISPR/Cas system relies on a single nuclease, termed CRISPR-associated protein 9 (Cas9), which induces site-specific breaks in DNA. Cas9 is guided to specific DNA sequences by small RNA molecules termed guide RNA (gRNA). Guide RNA comprises trRNA (also known as tracrRNA) and crisprRNA (crRNA). The trRNA and crRNA may be contained within a single guide RNA (sgRNA) or in two separate RNA molecules of a dual guide RNA (dgRNA). Cas9 in combination with trRNA and crRNA or an sgRNA is termed the Cas9 ribonucleoprotein complex (RNP).**
    b. **Oligonucleotides, and in particular RNA, are sometimes degraded in cells and in serum by endonuclease or exonuclease cleavage. Improved methods and compositions for preventing such degradation, improving stability of gRNAs and enhancing gene editing efficiency is desired, especially for therapeutic applications.**
    c. **In some embodiments, therapeutic genome editing tools are provided comprising modified guide RNAs. The modified guide RNAs described herein may improve the stability of the guide RNA and the guide RNA/Cas9 complex and improve the activity of Cas9 (e.g., SpyCas9 and equivalents) to cleave target DNA. In some embodiments, the guide RNA is an sgRNA. In some embodiments, the guide RNA is a dgRNA. In some embodiments, the guide RNA is a tracrRNA. In some embodiments, the guide RNA is a crRNA.**

MEDIA AND METHODS FOR MAKING AND MAINTAINING PORCINE PLURIPOTENT STEM CELLS

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4. **Abstract: The present disclosure relates to methods for making porcine pluripotent stem cells. The present disclosure also relates to methods for gene-editing porcine pluripotent stem cells, generating animals from porcine pluripotent stem cells, and maintaining porcine pluripotent stem cells in culture over many passages. The present disclosure further relates to methods for making porcine induced pluripotent stem cells.**
5. **BACKGROUND: Genetically modified pigs can be created through the process of cloning or somatic cell nuclear transfer (SCNT) in which a cell is edited and then the whole cell or just its nucleus is fused into an enucleated zygote to then develop into an embryo proper. Cloning and gene editing can be done with fibroblast lines, which can be edited once before needing rejuvenation; thus, the cost to create a gene edited animal can be significantly higher when multiple edits need to be made. Stem cells can be used in regenerative medicine and cell therapy space because, e.g., of their ability to differentiate into various cell and tissue types. They can also desirable due to their longevity and capacity for multiple genetic edits, thereby reducing cost for animal models of disease and xenotransplantation. There exists need for new and improved methods and compositions for the development of pluripotent porcine stem cell lines.**

SEQUENCES AND PROMOTERS FOR USE IN PLANT CELLS AND METHODS OF MAKING AND USING SUCH SEQUENCES

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3. **Inventor Information:**
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4. Abstract: The present disclosure is directed to a novel sequence constructed from viral elements for use as a transgenic promoter; for example, in transgenic plants. More specifically, the present disclosure is directed to a chimeric transgenic promoter sequence comprising a portion derived from the Figwort Mosaic Vims (FMV/FiMV) genome and a portion derived from the Cassava Vein Mosaic Virus (CsVMV) genome. The present disclosure provides methods and compositions for the making and using such a transgenic promoter.
5. FIELD OF THE INVENTION: The present invention relates in general to nucleic acid sequences which may serve as promoters for transgenic expression. More specifically, the invention relates to sequence elements derived from viral promoters and the use of combinations of these sequence elements to express coding sequences or functional RNAs in plants.
6. BACKGROUND:
    a. One of the goals of plant genetic engineering is to produce plants with ergonomically preferable characteristics or traits, and for this aim-enhancing or reducing the expression level of a gene product (or products) or of functional RNAs. Such changes in expression commonly require the use of a non-endogenous promoter.
    b. Whereas for some plants and crops there is a wide set of promoters available for transgenic use, others, such as *Eucalyptus,* have but a few non-endogenous promoters which are well-characterized to be functional, even for use for constitutive transgenic expression. Thus, constructing promoters for such crops is valuable.
7. SUMMARY:
    a. In one aspect, the present disclosure provides (I) a nucleic acid sequence which comprises (i) a transcriptional regulatory element derived from the sub-genomic transcript (Sgt) promoter of the Figwort Mosaic Virus (FMV, FiMV), which does not include the promoter's TATA portion, and a (ii) transcription regulatory element derived from the genome of Casava Vein Mosaic Virus (CsVMV) promoter which does include a TATA portion, or (II) a nucleic acid sequence that comprises sequences substantially similar to the (i) and (ii) sequences described above.
    b. In an additional aspect, the present disclosure provides bacteria-clone propagated plasmids which include the nucleic acid sequence described above, and which can function as expression vectors in plant cells.
    c. The present disclosure also provides a transformed plant cell having in its genome the nucleic acid sequence described above, as well as transgenic plants or seeds including such plant cells.

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.