

CHIMERIC ANTIGEN AND T CELL RECEPTORS AND METHODS OF USE

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 62/317,258, filed April 1, 2016, which is hereby incorporated by reference in its entirety.

5

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on February 28, 2019, is named K-1031_02US_SL.txt and is 450,469 bytes in size.

10

BACKGROUND OF THE INVENTION

[0003] Human cancers are by their nature comprised of normal cells that have undergone a genetic or epigenetic conversion to become abnormal cancer cells. In doing so, cancer cells begin to express proteins and other antigens that are distinct from those expressed by normal cells. These aberrant tumor antigens can be used by the body's innate immune system to specifically target and kill cancer cells. However, cancer cells employ various mechanisms to prevent immune cells, such as T and B lymphocytes, from successfully targeting cancer cells.

[0004] Current therapies T cell therapies rely on enriched or modified human T cells to target and kill cancer cells in a patient. To increase the ability of T cells to target and kill a particular cancer cell, methods have been developed to engineer T cells to express constructs which direct T cells to a particular target cancer cell. Chimeric antigen receptors (CARs) and engineered T cell receptors (TCRs), which comprise binding domains capable of interacting with a particular tumor antigen, allow T cells to target and kill cancer cells that express the particular tumor antigen.

[0005] A need exists for improved CARs and TCRs for targeting and killing cancer cells.

table providing the SEQ ID NO for each VH and CDR sequence illustrated in FIG. 2C. FIG. 2E shows a sequence alignment of example anti-BCMA binding molecules comprising a VH domain. Complementarity determining regions (CDRs) and framework regions (FRs) are shown, as determined by Chothia numbering (FIG. 2E). SEQ ID NOS 253-260, CDR1 in order from top to bottom; SEQ ID NOS 261-268, CDR2 from top to bottom, SEQ ID NOS 269-276, CDR3 from top to bottom. FIG. 2F is a table providing the SEQ ID NO for each VH and CDR illustrated in FIG. 2E. FIG. 2G shows a sequence alignment of example anti-BCMA binding molecules comprising a VL domain. CDRs and FRs are shown, as determined by Chothia numbering (FIG. 2G). FIG. 2H is a table providing the SEQ ID NO for each VH and CDR sequence illustrated in FIG. 2G. SEQ ID NOS 37-44, CDR1 in order from top to bottom; SEQ ID NOS 45-52, CDR2 from top to bottom, SEQ ID NOS 277-284, CDR3 from top to bottom.

[0056] FIG. 3 depicts CAR expression in primary human T cells electroporated with mRNA encoding for various CARs. Data obtained from CAR having a complete hinge domain (“CHD”) is shown and data obtained from CAR having a truncated hinge domain (“THD”) is shown.

[0057] FIGs. 4A-4X show IFN γ , IL-2, and TNF α production by electroporated anti-FLT3 CAR T cells following 16 hours of co-culture with the indicated target cell lines. FIGs. 4A-4B, 4G-4H, 4M-4N, and 4S-4T show IFN γ production following co-culture with Namalwa, EoL-1, HL60, and MV4;11 target cells, respectively. FIGs. 4C-4D, 4I-4J, 4O-4P, and 4U-4V show IL-2 production following co-culture with Namalwa, EoL-1, HL60, and MV4;11 target cells, respectively. FIGs. 4E-4F, 4K-4L, 4Q-4R, and 4W-4X show TNF α production following co-culture with Namalwa, EoL-1, HL60, and MV4;11 target cells, respectively.

[0058] FIGs. 5A-5H show cytolytic activity of electroporated anti-FLT3 CAR T cells against Namalwa (FIGs. 5A-5B), EoL1 (FIGs. 5C-5D), HL60 (FIGs. 5E-5F), and MV4;11 (FIGs. 5G-5H) target cell lines following 16 hours of co-culture.

[0059] FIGs. 6A-6B depict CAR expression in lentivirus transduced primary human T cells from two healthy donors.

[0060] FIGs. 7A-7F show IFN γ (FIGs. 7A-7B), TNF α (FIGs. 7C-7D), and IL-2 (FIGs. 7E-7F) production by lentivirus transduced CAR T cells from two healthy donors following 16 hours of co-culture with the indicated target cell lines.

[0061] FIGs. 8A-8D show the average cytolytic activity over time from two healthy donors expressing the anti-FLT3 CAR constructs co-cultured with Namalwa (FIG. 8A), EoL1 (FIG. 8B), MV4;11 (FIG. 8C), and HL60 (FIG. 8D) target cell lines for 16, 40, 64, 88, or 112 hours.

Suitable additional exemplary vectors include e.g., pBABE-puro, pBABE-neo largeTcDNA, pBABE-hygro-hTERT, pMKO.1 GFP, MSCV-IRES-GFP, pMSCV PIG (Puro IRES GFP empty plasmid), pMSCV-loxp-dsRed-loxp-eGFP-Puro-WPRE, MSCV IRES Luciferase, pMIG, MDH1-PGK-GFP_2.0, TtRMPVIR, pMSCV-IRES-mCherry FP, pRetroX GFP T2A Cre, pRXTN, pLncEXP, and pLXIN-Luc.

[0224] Any cell may be used as a host cell for the polynucleotides, the vectors, or the polypeptides of the present invention. In some embodiments, the cell can be a prokaryotic cell, fungal cell, yeast cell, or higher eukaryotic cells such as a mammalian cell. Suitable prokaryotic cells include, without limitation, eubacteria, such as Gram-negative or Gram-positive organisms, for example, *Enterobacteriaceae* such as *Escherichia*, e.g., *E. coli*; *Enterobacter*; *Erwinia*; *Klebsiella*; *Proteus*; *Salmonella*, e.g., *Salmonella typhimurium*; *Serratia*, e.g.,