A Novel Monoclonal Antibody for the Detection, Enrichment, and Activation of Cells Expressing scFv-based Chimeric Antigen Receptors

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INTRODUCTION

Chimeric Antigen Receptor (CAR)-T cell therapy is a highly innovative form of immunotherapy that has proven to be successful in the treatment of B cell malignancies and multiple myeloma. As this treatment modality continues to evolve toward the targeting of novel tumor antigens and engineering of CAR constructs that enhance cell persistence, there is a need for reagents that can be leveraged to selectively interrogate multiple aspects of CAR-T cell biology. Here, we report on a novel and highly versatile recombinant rabbit monoclonal antibody raised against the Gly_4Ser peptide linker, which is commonly integrated into single-chain variable fragment (scFv)-based CARs. This antibody can be leveraged for flow cytometry-based CAR detection, magnetic bead-based CAR-T cell enrichment, and selective activation of CAR transduced cells.

METHODS

The recombinant monoclonal antibody, E7O2V, was generated by immunizing rabbits with a synthetic peptide containing multiple repeats of the Gly_4Ser core pentapeptide. E7O2V was directly conjugated to a panel of fluorophores and validated for specificity in a live cell flow cytometry assay using primary human CAR-T cells. To assess its utility in cell enrichment protocols using bead-based sorting, a biotinylated conjugate of E7O2V was used in combination with streptavidin releasable magnetic beads to purify CAR-transduced cells from a mixed population. Finally, a plate-bound antibody stimulation assay was leveraged to assess the ability of E7O2V to selectively activate CAR-transduced cells.

RESULTS

Live cell flow cytometric analysis of CAR-transduced primary human T cells revealed that E7O2V could detect surface expressed CARs. No specific staining was observed on non-transduced cells. Furthermore, flow cytometric analysis of bead-free, purified cells revealed that biotinylated E7O2V could enrich CAR-transduced cells to a high degree of purity. E7O2V was found to selectively activate CAR transduced cells as demonstrated by upregulation of cell surface activation markers, including CD69.

CONCLUSIONS

Exploiting the commonly used Gly_4Ser linker of scFv-based CARs for antibody discovery, we identified a novel and highly versatile rabbit monoclonal antibody, E7O2V. This antibody binds to CARs of varying antigen specificity and can be leveraged in multiple assay formats to interrogate various attributes of CAR-transduced cells, including CAR expression, CAR signaling, and transcriptome profiling enabled by bead-based enrichment.

Detection of scFv-based CARs using G4S Linker (E7O2V) Rabbit mAb

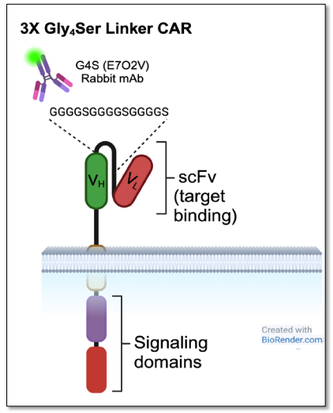
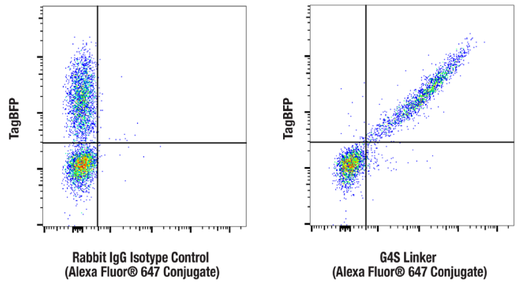


Diagram 1
Illustrated diagram showing the Gly_4Ser peptide linker within the scFV of a CAR that was targeted for discovery of recombinant rabbit monoclonal antibody, E7O2V (created with BioRender.com).

Figure 1: Detection of an Anti-CD20 (Leu16) CAR on the surface of primary human T cells



Flow cytometric analysis of live pan-CD3+ T cells engineered to express an scFv-based Anti-CD20 (Leu16) CAR containing a G4S linker, using G4S Linker (E7O2V) Rabbit mAb (Alexa Fluor® 647 Conjugate) (right) or concentration-matched Rabbit (DA1E) mAb IgG XP® Isotype Control (Alexa Fluor® 647 Conjugate) #2985 (left). Tag Blue fluorescent protein (TagBFP) is a transduction marker co-expressed with the CAR.

Magnetic bead-based immunoaffinity enrichment of CAR positive cells using G4S Linker (E7O2V) Rabbit mAb

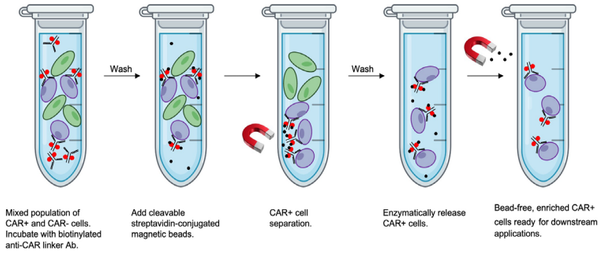
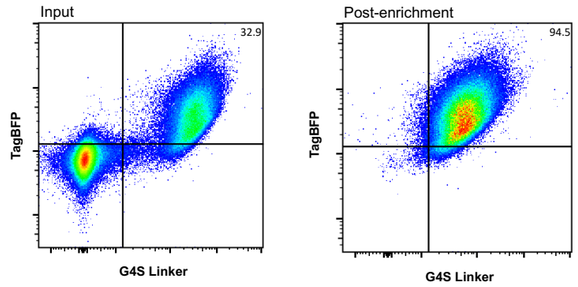


Diagram 2
Enrichment workflow diagram: Starting with a mixed population of CAR-transduced and non-transduced cells, incubate with biotinylated G4S (E7O2V) rabbit mAb. Wash cells to remove unbound antibody, then incubate cells with cleavable streptavidin-conjugated magnetic beads. Using a magnet to keep CAR+ cells in vial, wash to remove unbound cells. Incubate bead-bound CAR+ cells with DNase I to release beads from cells, use a magnet to bind beads, and remove enriched CAR+ cell fraction.

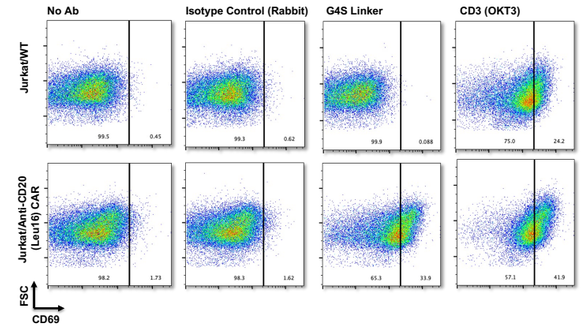
Figure 2: High purity enrichment of Anti-CD19 (FMC63) CAR positive cells



Flow cytometric analysis of live cells in the input (left) and post-enrichment fraction (right) for enrichment performed using biotinylated G4S Linker (E7O2V) Rabbit mAb. Input contains a mixture of wiltd-type Jurkat cells and Jurkat cells engineered to stably express an scFv-based Anti-CD19 (FMC63) CAR containing a G4S linker. Tag Blue fluorescent protein (TagBFP) is co-expressed with the CAR. Anti-Rabbit IgG (H+L), F(ab')$_2$ Fragment (Alexa Fluor® 647 Conjugate) #4414 was used as a secondary antibody to detect the biotinylated antibody. The post-enrichment sample shows a nearly pure population cells expressing the CAR on the cell surface. Engineered cell line was provided by the Lohmueller Lab, University of Pittsburgh.

Activation of CAR positive cells using G4S Linker (E7O2V) Rabbit mAb

Figure 3: G4S Linker (E7O2V) Rabbit mAb can activate Anti-CD20 (Leu16) CAR positive cells in a plate bound antibody stimulation assay



Wild-type Jurkat cells (Jurkat/WT) and Jurkat cells engineered to stably express an scFv-based Anti-CD20 CAR containing a G4S linker (Jurkat/Anti-CD20 (Leu16) CAR) were seeded for 24 hr in wells pre-coated with the indicated antibodies prior to collection for flow cytometric analysis. Flow cytometric analysis of live cells using CD69 (FN50) Mouse mAb (PE-Cy®7 Conjugate) was used as a readout of T cell activation. Engineered cell line was provided by the Lohmueller Lab, University of Pittsburgh.

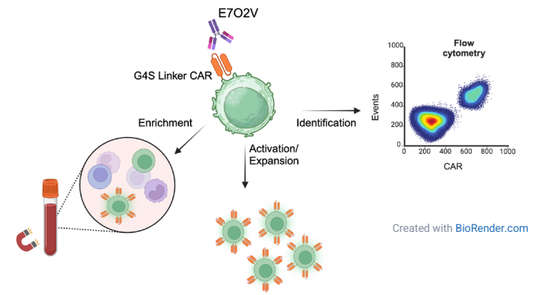


Diagram 3
G4S Linker (E7O2V) Rabbit mAb is a novel recombinant rabbit monoclonal antibody that can be leveraged across multiple workflows designed to characterize cells engineered to express a G4S linker-based scFv CAR (created with BioRender.com).

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