Evaluating U5 snRNP200-Targeting CAR-T Therapy for Acute Myeloid Leukemia: A novel multiplex immunoassay for analyte discrimination in mouse models

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Introduction

Multiplex immunoassay kits are essential for profiling immunomodulatory proteins, including cytokines, chemokines, and growth factors in humans and animal models. Humanized mice, engineered to express human genes or incorporate human cells, allow for the study of human immune responses and vaccine performance. The ability to distinguish human and mouse homologues in the same sample is valuable for researchers. A novel multiplex immunoassay was developed containing both mouse-specific and human-specific antibody pairs, utilizing a rigorous screening protocol specifically designed for the selection of effective human and mouse-specific capture antibodies. Specificity was confirmed by testing individual recombinant proteins, ensuring that mouse serum and plasma samples were detected exclusively by mouse-analyte capture beads, while human-analyte capture beads showed no reactivity. Using this novel multiplex immunoassay, we evaluated the safety and performance of IL-18 secreting CAR-T cells targeting U5 snRNP200, a ribonucleoprotein that is specifically expressed on the surface of AML cells in a syngeneic mouse model. Firstly, IL-18 secreting CAR-T-cells in the recipient mice showed both prolonged survival and marked proliferative ability. Cytokine profiling of serum collected 14 days post-transplant revealed elevated levels of murine IL-18, IFN-gamma, CXCL9, and CXCL10 while corresponding human cytokines remained undetected, confirming mouse specificity and validation of our model. This dataset establishes a baseline for future humanized experiments, demonstrating the kit's utility in validating mouse models before transitioning to human applications.

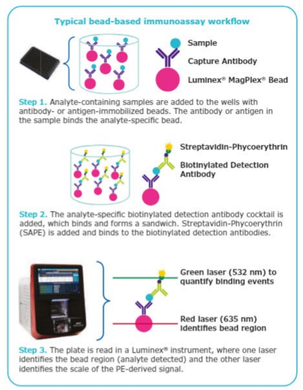

Figure 1: MILLIPLEX® format.
MILLIPLEX® assays use magnetic microspheres (beads) conjugated to capture antibodies. Each set of beads is distinguished by different ratios of two internal dyes yielding a unique fluorescent signature to each bead set, allowing researchers to simultaneously measure the analytes targeted by the capture antibodies. Native protein is analyzed by means of a sandwich immunoassay, pairing the capture beads with a biotinylated detection antibody.

Methods

Multiplex assays: The MILLIPLEX® Humanized Mouse Panel (Cat. No. HUMU-210K) was assayed in 96-well plates according to the product manual. All assays were run on the Luminex® 200™ instrument and data was acquired via xPONENT® v. 4.3 software. Data analysis was performed for all immunoassays using the Belysa® Immunoassay Curve Fitting Software (Cat. No. 40-122).

CAR-T model: CAR-T cells were generated via introduction of a transgene construct encoding an anti-U5 snRNP200 single-chain variable fragment and constitutively expressed murine IL-18.

Mouse model: Irradiated CD45.1 mice on a C57BL/6J background were injected with $1x10^6$ luminescent RN2 cells at day 0. On day 3, mice were treated with $3x10^6$ CAR-T cells either with or without the U5 snRNP200-targeting construct. Mice were subjected to bioluminescence imaging beginning on day 6. Serum was collected on day 14 for cytokine analysis.

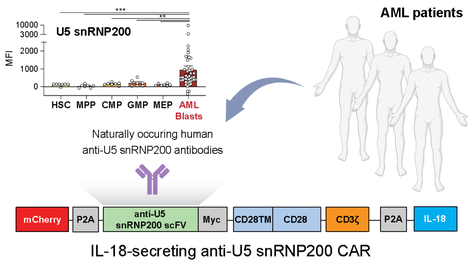

Figure 2: CAR-T strategy.
Observations of U5 snRNP200 reactivity in AML samples (but not the hematopoietic precursors of healthy donors) led to the development of a CAR construct designed to bind to the U5 snRNP200 present on AML cells and secrete murine IL-18 protein, which enhances CAR-T activity.

Results

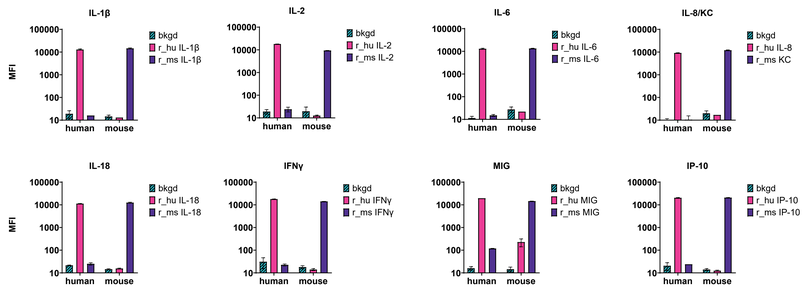

Figure 3: Specificity of human and mouse assays in the MILLIPLEX® Humanized Mouse Panel.
The MILLIPLEX® Humanized Mouse Panel contains 41 analytes total, including 18 targets with matched human-specific and mouse-specific antibody pairs. To evaluate the fidelity of species selectivity, the 41-plex assay was run with single standards for the recombinant human and mouse proteins, used at the highest concentration for each respective standard curve. MFI data is shown, with background MFI included for comparison. Eight representative analytes are shown, including the four analytes elevated in the CAR-T model.

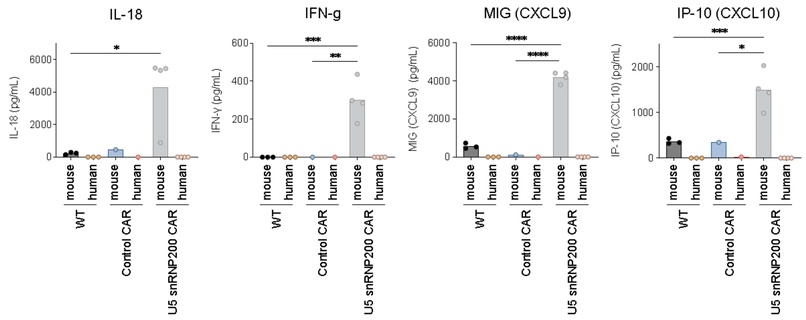

Figure 4: U5 snRNP200-targeting CAR-T cells stimulate cytokine expression.
Serum collected 14 days after injection of luminescent AML cells was evaluated with the MILLIPLEX® Humanized Mouse Panel. Human and mouse cytokine levels were compared between the treatment groups and the concentrations of 4 analytes elevated by the U5 snRNP200 CAR-T therapy are displayed above.

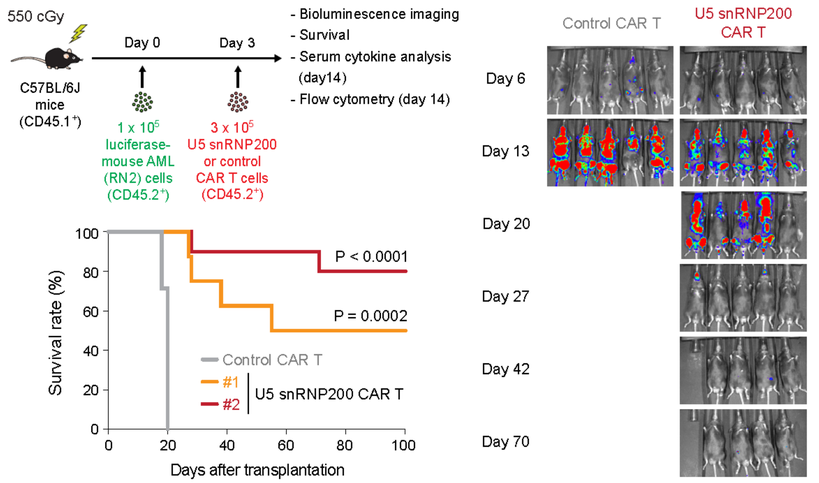

Figure 5: U5 snRNP200-targeting CAR-T cells stimulate clearance of luminescent AML tumors and survival in a mouse model.
The schematic above (upper left) illustrates the protocol for testing this CAR-T therapy in a mouse model. The survival curve is shown below. Bioluminescent imaging (right) shows the AML tumor burden for each of the CAR-T cohorts over the experimental time course.

Summary

Acute Myeloid Leukemia is frequently characterized by the aberrant expression of U5 snRNP200 on the cell surface, revealing a potentially actionable target for CAR-T therapy. Here we characterize a mouse model in which U5 snRNP200-targeting CAR-T therapy stimulates cytokine expression, reduces tumor burden, and increases survival.

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