Development of a Novel CAR T-Cell Therapy Targeting Thymic Carcinoma

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Background

Thymic carcinomas are rare anterior mediastinal malignancies with limited treatment options and modest responses to platinum-based chemotherapy. Immune checkpoint inhibitors have shown activity but often cause severe immune-related toxicities. Although CAR T-cell therapy has revolutionized hematologic cancer treatment, its application in thymic carcinoma is limited due to disease rarity, lack of tumor-specific surface antigens, and risks of on-target off-tumor toxicity, especially given the thymus's role in immune tolerance. Recent studies have identified overexpression of TROP-2, c-KIT, and CD70 in thymic carcinoma, presenting novel therapeutic targets.

Objectives

1. Profile TROP-2, c-KIT, and CD70 surface expression on thymic carcinoma–relevant cell lines.
2. Engineer CAR constructs targeting each antigen and validate CAR expression on T- cells.
3. Evaluate antigen-specific cytotoxicity of CAR T-Cells in vitro to guide development of multi-antigen CAR T strategies.

Methods

Candidate cell lines were screened for surface expression of TROP-2, c-KIT, and CD70 using quantitative flow cytometry. CAR T-cells were generated from healthy donor PBMCs via lentiviral transduction and expanded ex vivo. Antigen-specific cytotoxicity was evaluated through in vitro co-culture assays using selected patient-derived cell lines. Cytotoxic effects were quantified by 7-AAD via flow cytometry.

Results

Flow cytometry confirmed high surface expression of TROP-2 (83%), c-KIT (88%), and CD70 (99%) in T1889, Kasumi, and U266 cell lines, respectively. Lentiviral transduction produced Fab^+ CAR-T populations of 48.2% (TROP-2), 24.7% (c-KIT), and 47.5% (CD70). All three CAR-T constructs showed antigen-specific cytotoxicity significantly greater than GFP^+ $CD3^+$ controls.

Conclusions

This study provides early in vitro evidence supporting CAR T-cell therapy targeting TROP-2, c- KIT, and CD70 for thymic carcinoma. Results confirm strong CAR expression and potent, selective killing of antigen-positive targets.

Future Directions

Ongoing work includes FACS-based CAR T-cell enrichment, exhaustion marker analysis, development of a dual-antigen synNotch CAR, and in vivo validation in syngeneic mouse models to support clinical translation.