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Experimental Hematology / Oncology

Targeting the Thyroid-Stimulating Hormone Receptor in Poorly-Differentiated Thyroid Cancer with CD3-engaging Bispecific Antibodies or CAR T cells

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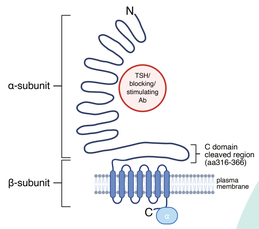
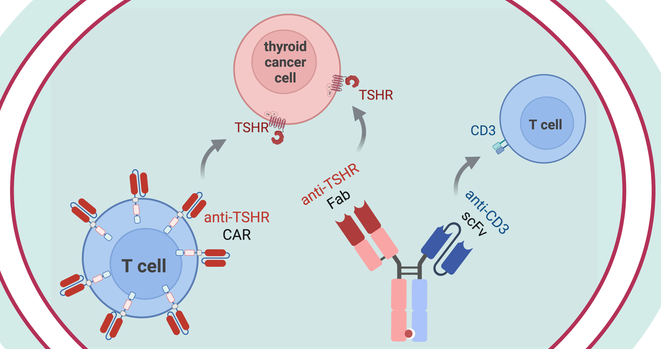
BACKGROUND:

- Poorly differentiated thyroid cancer (PDTC) is associated with unfavorable prognosis frequently exhibits resistance to available therapies such as radioactive iodine therapy (1). As treatment options are limited, developing novel treatment strategies is of high clinical relevance (1,2).
- Stable expression of the tissue-specific thyroid-stimulating hormone receptor (TSHR) has been observed in both differentiated thyroid cancer and PDTC, highlighting TSHR as a promising molecular target (3).
- In autoimmune thyroid diseases, anti-TSHR antibodies with blocking or neutralizing properties can lead to thyroid tissue destruction (4). Harnessing this TSHR-specific immune response could offer a novel therapeutic approach for eliminating TSHR-expressing thyroid cancer cells.
- Among the rapidly advancing immunotherapeutic strategies, bispecific antibodies (bsAbs) and chimeric antigen receptor (CAR) T cells have shown remarkable success, particularly in hematologic malignancies. Both approaches depend on the recruitment and activation of the patient's own T cells for targeted tumor cell elimination (5,6).

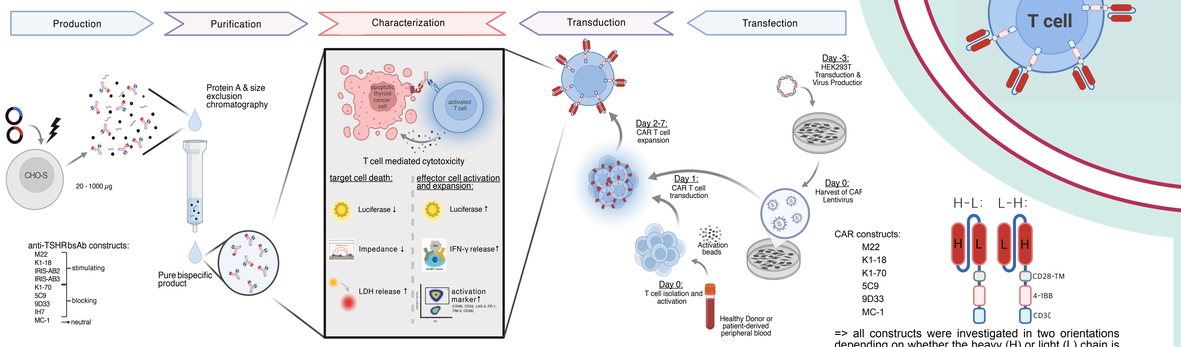
AIMS:

- To achieve specific targeting of TSHR-expressing poorly differentiated thyroid cancer (PDTC) using bispecific antibodies (bsAbs) or chimeric antigen receptor (CAR) T cells.
- To compare the cytotoxic efficacy of different anti-TSHR constructs and to investigate how stimulating, blocking, and neutral antibody properties influence their function in both bsAb and CAR T cell formats.





METHODS:

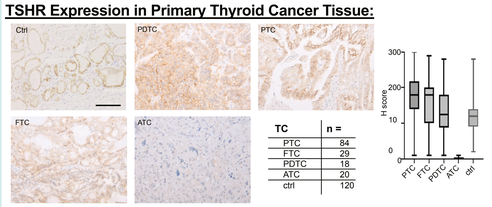


CAR constructs:
M22
K1-18
K1-70
5C9
9D33
MC-1

=> all constructs were investigated in two orientations depending on whether the heavy (H) or light (L) chain is attached to the transmembrane domain: H-L and L-H

RESULTS:

TSHR Expression in Primary Thyroid Cancer Tissue:

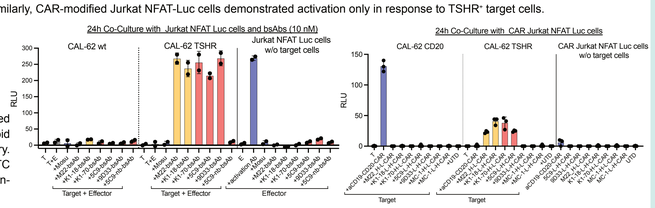


TC	n =
PTC	84
FTC	29
PDTC	18
ATC	20
ctrl	120

Formalin-fixed, paraffin-embedded thyroid tissue samples from patients diagnosed with differentiated thyroid cancer (follicular: FTC, papillary: PTC, poorly differentiated: PDTC), undifferentiated thyroid cancer (anaplastic: ATC) as well as non-neoplastic controls were analyzed by immunohistochemistry. TSHR levels were evaluated in a total of 271 specimens.. While TSHR expression was absent in ATC samples, differentiated thyroid cancers exhibited TSHR levels comparable to those observed in non-cancerous thyroid tissue, confirming TSHR as a potential antigenic target for immunotherapies.

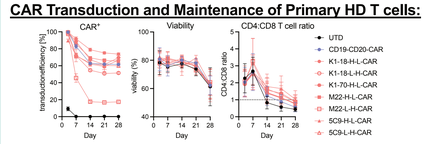
Activation of Jurkat NFAT Luc Reporter Cells via bsAb-Engagement or CAR Transduction:

For initial proof-of-concept experiments, TSHR-transduced target cells were co-cultured with Jurkat NFAT-Luc reporter cells serving as effector cells. These T cell leukemia cells are modified to function as reporter cells with luciferase production triggered upon activation. Co-culture of the thyroid cancer cell line CAL-62 with anti-TSHR bsAbs resulted in specific activation of effector cells exclusively in the presence of TSHR$^+$ target cells. Jurkat NFAT-Luc cells alone did not exhibit activation upon CD3 engagement in the absence of target cells, confirming the requirement of TSHR expression on malignant thyroid cells for bsAb-mediated activation. Similarly, CAR-modified Jurkat NFAT-Luc cells demonstrated activation only in response to TSHR$^+$ target cells.



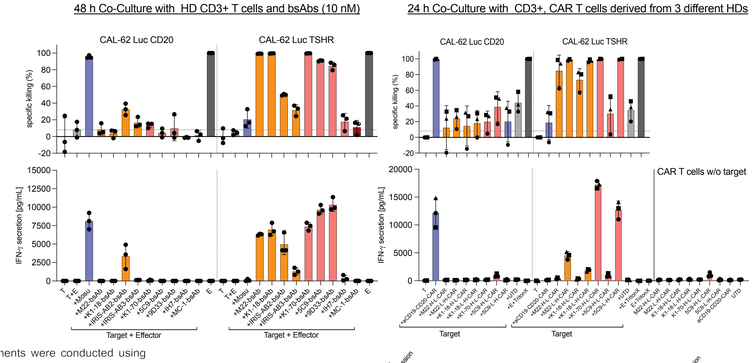
RESULTS:

CAR Transduction and Maintenance of Primary HD T cells:



T cells generated from healthy donor (HD) peripheral blood T cells were produced and monitored over a 28-day culture period. Across all constructs, transduction efficacy varied between 30 and 80% reaching stable levels approximately 14 days post-transduction. The expanded T cells maintained adequate viability for more than three weeks in culture. Notably, the CD4$^+$:CD8$^+$ T cell ratio gradually shifted in favor of the CD8$^+$ subset over time.

Comparison of CAR T cells and bsAbs Derived from Different anti-TSHR Antibodies:

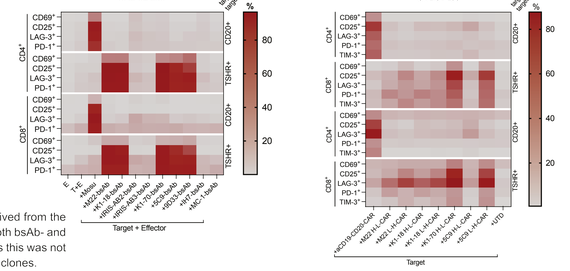


Co-culture experiments were conducted using either anti-TSHR bsAbs (left) or anti-TSHR CAR T cells with luciferase-expressing CAL-62 target cells, engineered to express or lack TSHR.

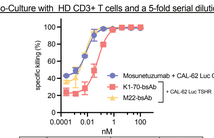
In both settings, several anti-TSHR constructs induced specific cytotoxicity against TSHR$^+$ target cells, accompanied by T cell activation and interferon-gamma (IFN-γ) secretion.

No such effects were observed in TSHR$^-$ target cells, confirming antigen-specific activity. Notably, the orientation of the heavy and light chains within the single-chain variable fragment (scFv) domain of certain CAR constructs critically influenced their functional efficacy.

Among the evaluated candidates, constructs derived from the K1-70 antibody demonstrated robust activity in both bsAb- and CAR-based immunotherapeutic formats, whereas this was not consistently observed for all anti-TSHR antibody clones.

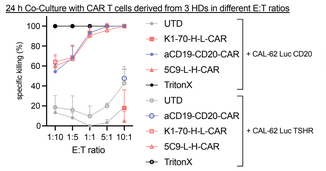


Selection and More in Depth Analysis of the Most Promising bsAb and CAR T Cell Candidates:



	Mosunetuzumab	K1-70-bsAb	M22-bsAb
IC50	0.008250	0.05982	0.006132

Serial dilution of bsAb showed high efficacy of the bsAbs K1-70-bsAb and M22-bsAb already in the 1 nM-range after 48 h of co-culture.



Anti-TSHR CAR T cells achieved approximately 60% target cell lysis after 24 hours at an effector-to-target (E:T) ratio of 1:10, which increased to nearly complete (100%) cytotoxicity with higher effector cell numbers.

CONCLUSION & OUTLOOK:

- Both TSHR-targeting bsAbs and anti-TSHR CAR T cells mediate TSHR-specific cytotoxicity and T cell activation through engagement with primary HD T cells.
- Cell lines lacking TSHR expression remain unaffected by either immunotherapeutic approaches, confirming target specificity.
- Stimulating, blocking, and neutral anti-TSHR antibody clones differ in their efficacy as bispecific antibodies, and the functional performance of corresponding CAR T cells does not always parallel that of their bsAb counterparts.
- The orientation of the heavy and light chains within the CAR single-chain variable fragment (scFv) is decisive for its functionality.

Outlook: Further *in-vitro*, *in-vivo* and *ex-vivo* characterization and final selection of the most prominent candidates

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