#250: DRIVING NK CELL IMMUNOTHERAPY AGAINST NSCLC, IN THE CONTEXT OF HYPOXIA, USING TRI-SPECIFIC ENGAGERS

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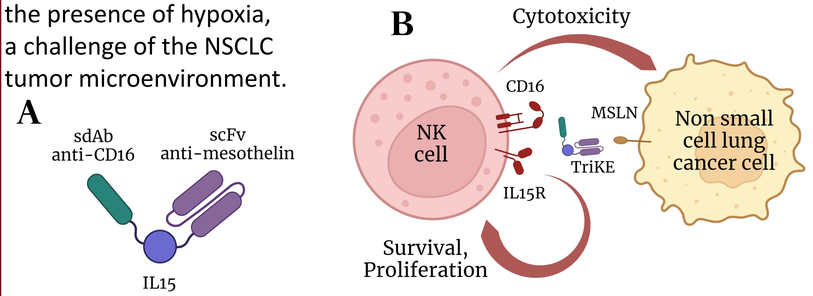
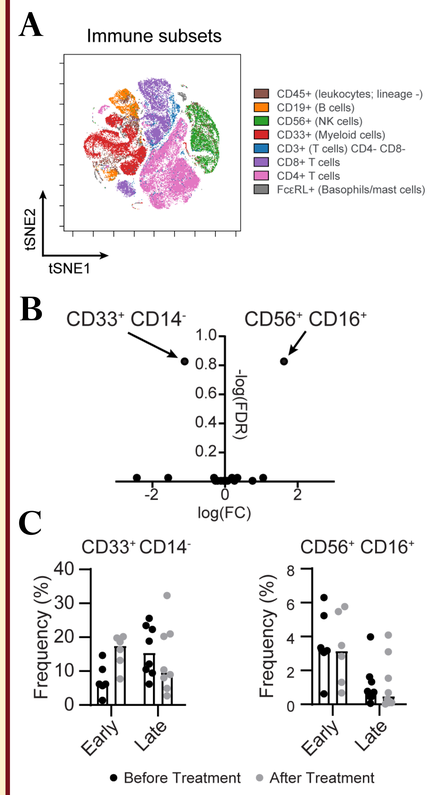
OVERVIEW: Tri-specific killer engagers (TriKE®) are being tested in the clinic to treat leukemia and lymphoma. These biologics consist of a single domain antibody (sdAb) that binds CD16/FcγRIII on natural killer (NK) cells, a single chain variable fragment that binds a tumor antigen and an IL15 moiety that links the two domains (A). Cross-linking CD16 and the tumor antigen drives cytotoxicity, and IL15 provides survival and proliferation signals to NK cells (B). Mesothelin (MSLN) is a tumor antigen being targeted in various cancers including non-small cell lung cancer (NSCLC). Using peripheral blood mononuclear cells (PBMC) collected from NSCLC patients, we tested whether a MSLN-targeted TriKE could drive cytotoxicity towards NSCLC cells at all stages of disease and treatment in the presence of hypoxia, a challenge of the NSCLC tumor microenvironment.



Patient Characteristics

	Feature/Group:	NSCLC – Early Stage	NSCLC – Late Stage
	Patients	6	8
	Age (median, years)	65	67
Staging	I-III	4	
	IVA	2	
	IVB		8
	Died	0/6	3/8
	Progression	3/6	7/7
Histology	Adenocarcinoma	5	7
	Adeno-squamous	1	
	Squamous		1
	%PDL1 (range)	<1-50	0-90
Best Response	CR	2/6	0/7
	PR	5/6	5/7
	PD	0/6	2/7
	SD	2/6	2/7
Treat-ment	Chemotherapy	6/6	5/8
	Radiation	5/6	6/8
	Checkpoint blockade	6/6	5/8

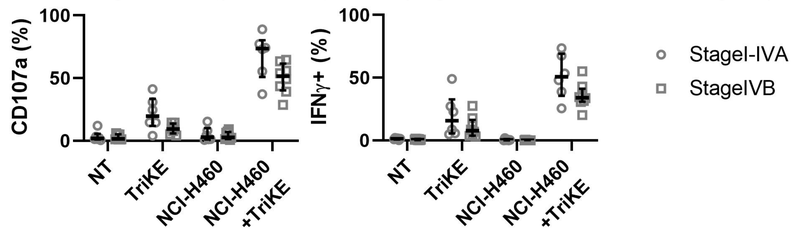
NSLC PATIENTS HAVE ALTERED NK CELLS:



PBMCs from NSCLC patients (described in the table) were collected (1) before patients started treatment, (2) after initial treatment and (3) at disease progression (where applicable). Time of flight mass cytometry was used to analyze the immune subsets present in these patients (A). Differential abundance analysis of immune subsets in early stage or late stage patient groups (see table) were performed using Astrolabe Diagnostics software. This revealed a greater abundance of CD56+ CD16+ NK cells and fewer CD33+ CD14- myeloid cells in early stage patients compared to late stage patients before treatment onset (B-C). The lack of CD16, which drives cytotoxicity, and the abundance of myeloid cells, that can suppress NK cell function, suggested late stage NSCLC patients may respond differently to biologics targeting NK cell cytotoxicity.

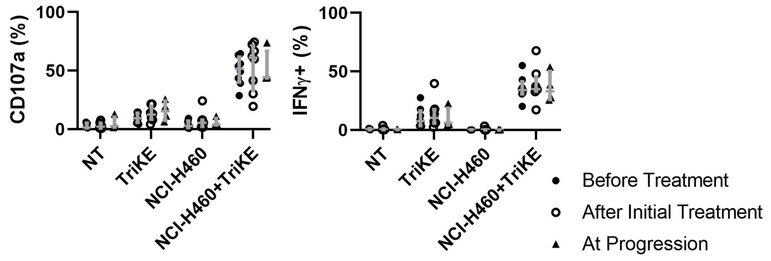
MESOTHELIN-TARGETED TRIKE DRIVES NK CELL FUNCTION REGARDLESS OF DISEASE STAGE:

We challenged patient PBMC with a NSCLC cell line (NCI-H460) for 5 hours in the presence of monensin and brefeldin A, measuring degranulation (CD107a) and cytokine production (IFNγ) by flow cytometry (live, single CD56+ CD3- cells). Compared to NK cells alone (NT); NK cells alone with drug ('TriKE'); or NK cells with tumor alone, the TriKE was able to induce significant ($p<0.0001$) activity against H460 cells for both groups.



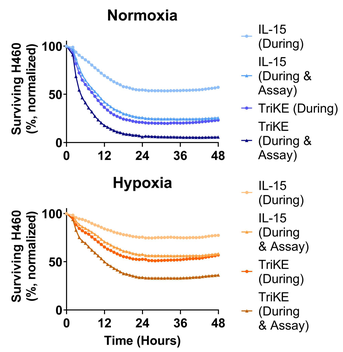
MESOTHELIN-TARGETED TRIKE DRIVES NK CELL FUNCTION AT ALL STAGES OF TREATMENT:

The previous panel shows functional responses of NK cells collected from patients before they started treatment. We therefore asked whether NK cells would be responsive to TriKE at all stages of treatment. The figures below show that TriKE induced degranulation and cytokine production in patient NK cells when in the presence of tumor cells (H460) at all stages of treatment (before treatment, after initial treatment and at progression).

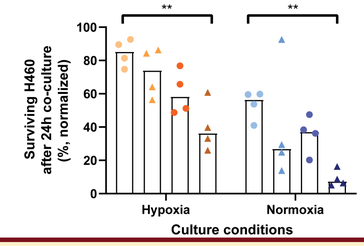


FUNCTION IS RETAINED UNDER HYPOXIA:

Hypoxia impairs NK cell cytotoxicity, but the TriKE enhances NK cell cytotoxicity of lung cancer cells (H460) after exposure to hypoxia for 7 days (during exposure to hypoxia and in the assay itself).



NK cells were isolated from healthy donor blood and analyzed by live cell imaging with a caspase 3/7 indicator of cell death.



CONCLUSIONS: This pre-clinical evidence suggests, despite the difference in circulating immune cells of Stage IVB NSCLC patients, mesothelin-targeted TriKE can work alongside current standard of care and provide benefit even in the hypoxic environment of a solid tumor.

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