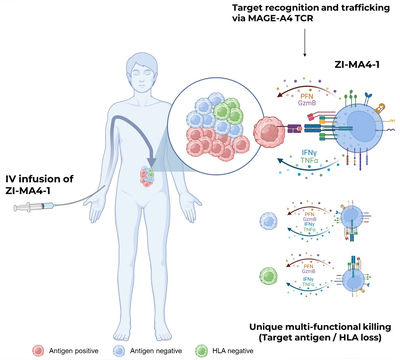
Preclinical development of ZI-MA4-1, the first optimized “off the shelf” MAGE-A4 targeting TCR-NK cells for advancement into the clinic for the treatment of solid tumors

Luise Weigand[1], Margherita Boieri[1], Zara Hannoun[1], Giulia Malachin[1], Julia Ino[1], Pranav Oberoi[1], Anders Holm[1], Emilie Gauthy[1], Namir Hassan[1], Sylvie Pollmann[1]

[1]Zelluna Immunotherapy, Oslo Cancer Cluster Incubator, Ullernchausséen 64, 0379 Oslo, Norway

TCR-NKs cells: Tackling tumor heterogeneity with a unique multi-pronged anti-tumor activity

- Combines TCR guided activity and innate NK activity
- Combines specific (TCR) with broad anti-tumor activity (NK)
- Will overcome the escape mechanisms used by tumor cells to avoid T cell recognition (loss of antigen and/or HLA)



Introduction

Background

T cell receptor (TCR)-T cell-based cell therapies have shown clinical efficacy across many cancer types and represent an attractive strategy for targeting solid tumors. However, the immunosuppressive tumor microenvironment, downregulation of target antigen and HLA, and the need for an autologous cell source, limit the efficacy and accessibility of TCR-T cell therapies. On the other hand, early clinical trials have shown the potential of Natural Killer (NK) cells in cancer therapy, supported by their favourable safety profile and suitability for off-the-shelf use. However, unguided NK cells exhibit limited potential against solid cancers due to their inability to infiltrate tumors effectively. TCR-NK cells are a novel and promising strategy combining advantages of approaches. By integrating antigen specific TCR recognition with the innate cytotoxicity and allogeneic potential of NK cells, TCR-NK therapies have the potential to overcome limitations of both TCR-T and NK cell-based therapies.
Here we present ZI-MA4-1, an optimised TCR-NK product comprising KVL-a, an AI affinity-enhanced TCR recognising MAGE-A4, a clinically validated tumor antigen expressed across several solid tumor indications.

Methods

ZI-MA4-1 is generated by transducing healthy peripheral blood-derived NK cells with the full CD3 complex, the KVL-a TCR, and a CD8 co-receptor. Our optimized manufacturing process enriches for TCR-NK cells thereby improving robustness and mitigating risks of donor variability, yielding a superior TCR-NK cell product.

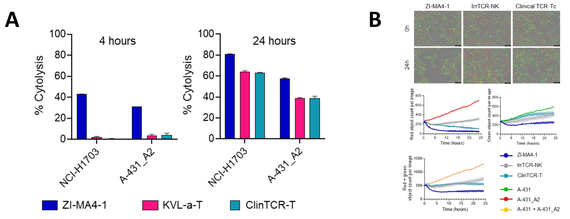
Results

We demonstrate that TCR-NK cells are faster and more potent killers than TCR-T cells. The introduction of the TCR does not hinder the innate functionality of NK cells against tumor cells and adds TCR-mediated specific killing of antigen-positive targets. Notably, the innate potential of the NK cells appears to be enhanced by the TCR engagement, creating NK cells with increased serial killing potential. In addition, the TCR endows NK cells with antigen-driven proliferation and increased cytokine and chemokine responses to cancer targets upon TCR engagement. Finally, TCR-NK cells show a safe profile, when co-cultured with a panel of normal cells derived from different tissue compartments.

We would like to acknowledge Nextera and Etcembly for their great work in engineering MAGE-A4 TCRs for high affinity to target.

Contact information: Luise Weigand, Chief Scientific Officer luise.weigand@zelluna.com www.zelluna.com

TCR-NK cells outperform TCR-T cells



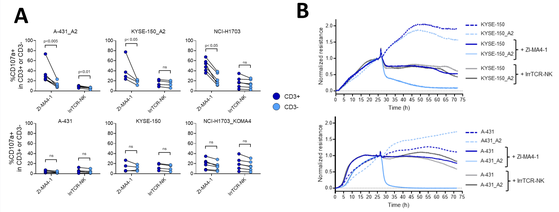
ZI-MA4-1 demonstrates accelerated and enhanced potency compared to TCR-T cell products

The ability of ZI-MA4-1 to kill heterogeneous tumors was compared to TCR-T cells expressing a MAGE-A4-specific TCR currently used in the clinic.

A) Percentage of specific target cell death measured by 7-AAD staining in a flow-based killing assay. Effector and target cells were incubated at E:T ratio of 4:1 for 4 and 24 h.

B) Cell death of single and mixed target cell cultures induced by ZI-MA4-1, an irrelevant TCR (IrrTCR)-NK, and a clinical TCR (ClinTCR)-T cells monitored over 24 h using Incucyte. Images illustrate the cell killing in one representative well, while quantification is displayed as graphs and represents the average of

KVL-a adds TCR specificity to NK cells and maintains innate activity

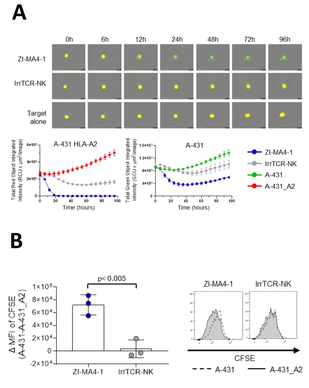


ZI-MA4-1a maintains innate NK cell activity while acquiring additional antigen specific potency.

A) Percentage of degranulating cells expressed as % of CD56+CD107a+ cells in CD3+or CD3− populations of the ZI-MA4-1 and IrrTCR-NK cells against different cancer cell lines at an E:T ratio of 1:1.

B) Cytotoxicity of ZI-MA4-1 against the KYSE-150, KYSE-150_A2, A-431, and A-431_A2 cell lines evaluated using an impedance-based assay. Normalized resistance indicates changes in impedance, indicative of target cell killing and detachment.

ZI-MA4-1 displays increased functional longevity

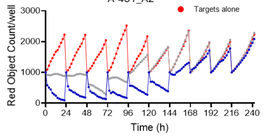


ZI-MA4-1 displays enhanced cytotoxicity, serial killing, and proliferation.

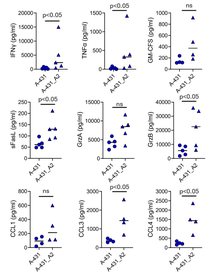
A) Kinetic of mixed spheroids killing by ZI-MA4-1 and IrrTCR-NK cells. The spheroids are composed of a 1:1 mix of A-431_A2_Nuclight Red and A-431_Nuclight Green cells. Cell killing is measured using Incucyte, based on changes in the fluorescence intensity of the total red and green objects. Images illustrate the growth of spheroids in one representative well, while quantification is displayed as graphs and represents the average of triplicate wells ±SD.

B) ZI-MA4-1 proliferates in response to tumor cell lines. Increased proliferation is observed in antigen positive targets activating the TCR in addition to the NK’s innate function. IrrTCR-NK cells show significantly lower proliferation in response to targets.

C) Serial killing ability of ZI-MA4-1 and IrrTCR-NK cells. Effector cells were co-cultured with A-431_A2_NLR cancer cell line at an E:T ratio of 4:1 during several rounds of 24h. Every 24 h, the TCR-NK were transferred to a new plate containing new target cells. Target cell growth was measured with Incucyte by imaging and quantifying the red fluorescent signal.



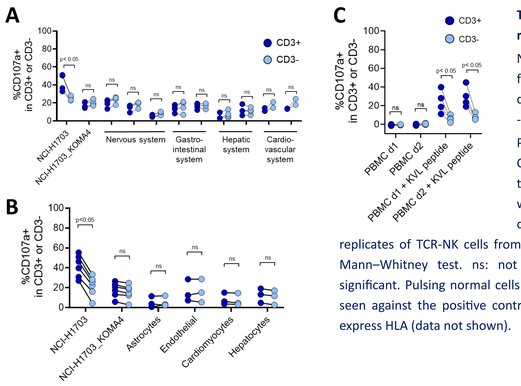
Increased CTK and chemokine release upon TCR engagement



Levels of the IFNγ, TNFα, sFasL, GrzB, and the chemokines CCL3 and CCL4 were significantly higher after co-culture with targets expressing MAGE-A4, enabling further immune system engagement through cytokine and chemokine secretion.

Cytokine and chemokine secretions of ZI-MA4-1 are measured after 6 h of co-culture with A-431 or A-431_A2 cells. The concentration of the analytes was measured in flow cytometry using the LEGENDplex assay. Each datapoint represents the average of three technical replicates from one ZI-MA4-1 batch. Statistical significance is calculated with Mann–Whitney test. ns: not significant. P value below 0.05 was considered significant.

ZI-MA4-1 demonstrates safety of affinity-enhanced TCR in NK cells



The addition of KVL-a does not introduce unspecific reactivity of ZI-MA4-1 toward normal cells.

No degranulation of ZI-MA4-1 to normal cells derived from critical organ systems beyond baseline degranulation of the TCR negative (CD3-) fraction of ZI -MA4-1.

Percentage of degranulating cells expressed as % of CD107a+CD56+cells in CD3+or CD3− populations of the ZI-MA4-1 after co-culture at an E:T ratio of 1:1 with (A) normal cells (B) iCells, and (C) PBMC. Each datapoint represents the average of two technical replicates of TCR-NK cells from one batch. Statistical significance is calculated with Mann–Whitney test. ns: not significant. P value below 0.05 was considered significant. Pulsing normal cells with peptide elicits responses close to the response seen against the positive control cell line NCI-H1703, demonstrating that the cells express HLA (data not shown).

Conclusion

Combining the innate cytotoxicity of NK cells with an affinity-enhanced MAGE-A4-specific TCR results in a potent and safe cellular product representing a promising and novel therapeutic off-the shelf paradigm for the treatment of multiple solid cancers. The clinical trial, ZIMA-101, assessing ZI-MA4-1 in ovarian cancer, squamous non-small cell lung cancer, synovial sarcoma and head and neck cancer has been initiated in the second quarter of 2026.