Redirecting NK cell cytotoxicity by CD16A-specific Innate Cell Engagers: A differentiated and innovative approach compared to CAR-NK cells

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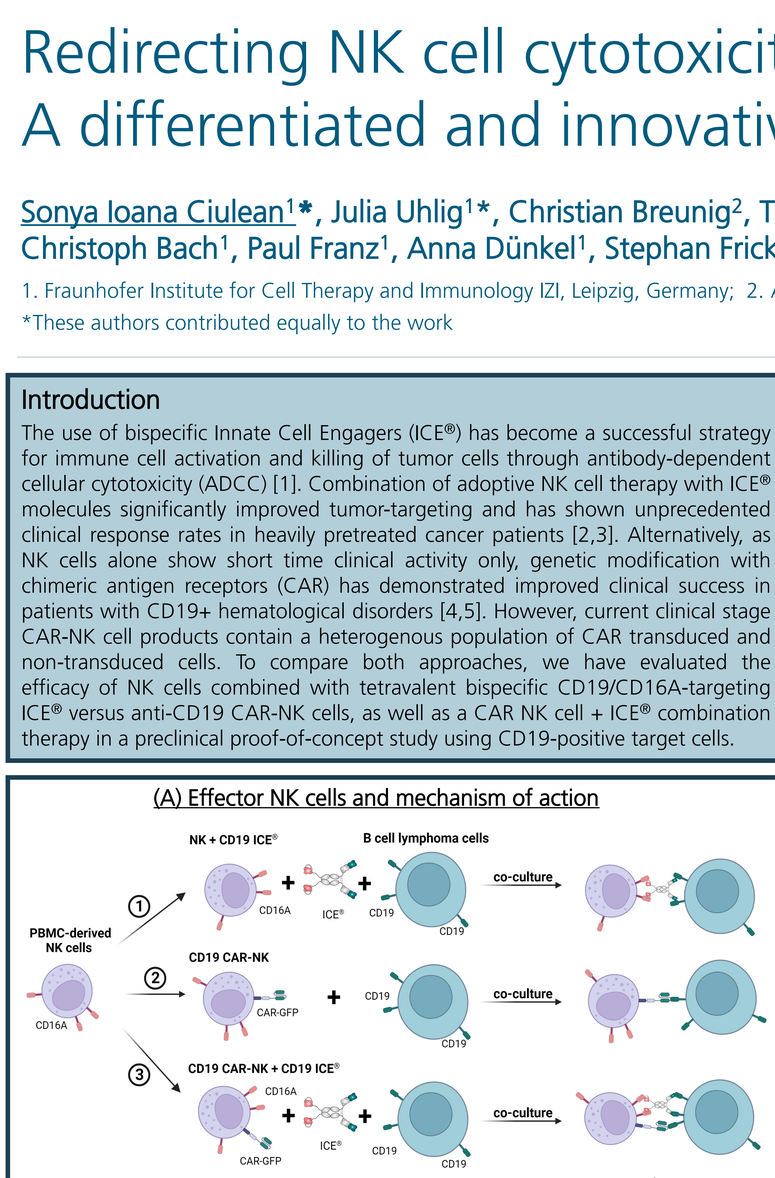
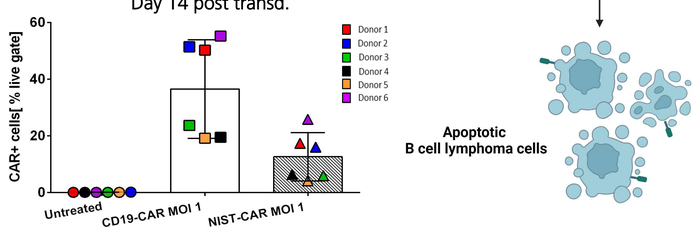
Introduction

The use of bispecific Innate Cell Engagers (ICE®) has become a successful strategy for immune cell activation and killing of tumor cells through antibody-dependent cellular cytotoxicity (ADCC) [1]. Combination of adoptive NK cell therapy with ICE® molecules significantly improved tumor-targeting and has shown unprecedented clinical response rates in heavily pretreated cancer patients [2,3]. Alternatively, as NK cells alone show short time clinical activity only, genetic modification with chimeric antigen receptors (CAR) has demonstrated improved clinical success in patients with CD19+ hematological disorders [4,5]. However, current clinical stage CAR-NK cell products contain a heterogenous population of CAR transduced and non-transduced cells. To compare both approaches, we have evaluated the efficacy of NK cells combined with tetravalent bispecific CD19/CD16A-targeting ICE® versus anti-CD19 CAR-NK cells, as well as a CAR NK cell + ICE® combination therapy in a preclinical proof-of-concept study using CD19-positive target cells.

(A) Effector NK cells and mechanism of action



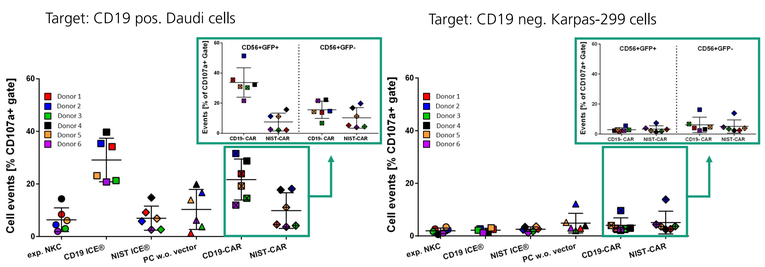
(B) CAR-NK transduction efficiency



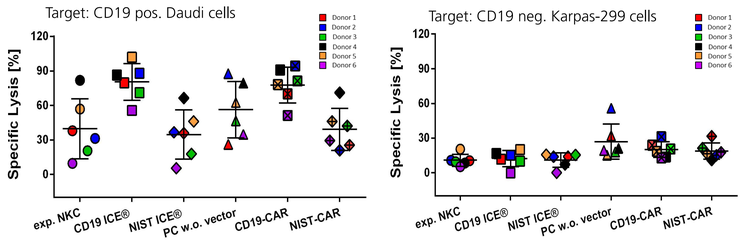
(A) Healthy-donor derived NK cells were expanded and either (1) combined with ICE®, (2) transduced on day 6 post isolation with gamma retroviral vectors encoding either an anti-CD19 or anti-NIST (MOCK control) CAR construct plus a Green Fluorescence Protein (GFP) (N=6 NK donors) or (3) first CAR-NK cells were generated and then combined with ICE®. These cells were co-cultured with B cell lymphoma cells and anti-tumoral effect was analyzed. Schematic figure was created with BioRender.com. (B) CAR transduction efficiency was heterogenous.

Combination of NK cells with ICE® induces anti-tumoral efficacy which is at least comparable to that of corresponding CAR-NK cells

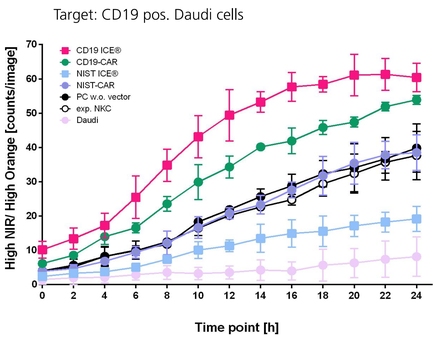
(A) Degranulation assay



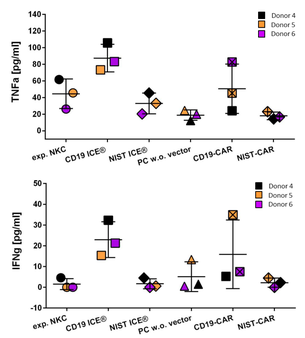
(B) Calcein release assay



(C) Incucyte - Kinetic killing assay



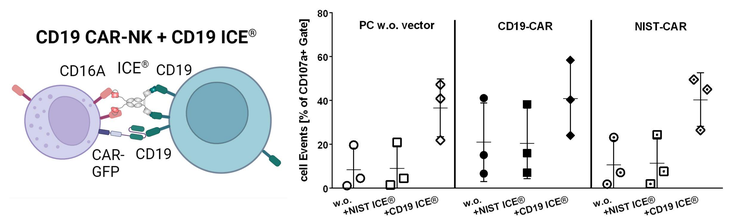
(D) Cytokine secretion



(A+B) Specific degranulation and killing (N=6 NK donors) were induced in CAR-NK cells as well as NK cells plus ICE® co-cultured with CD19 positive Daudi cells, but not with CD19 negative Karpas-299 cells. (C) The effector functionality of NK cells was assessed using a 24-hour kinetics Incucyte image-based assay (exemplary data of donor 6; CD19-CAR and GFP expression: 60%). (D) Higher levels of TNFα and IFNγ secretion were measured for NK cells + ICE® compared to CAR-NK cells co-cultured with Daudi cells (N=3 NK donors).

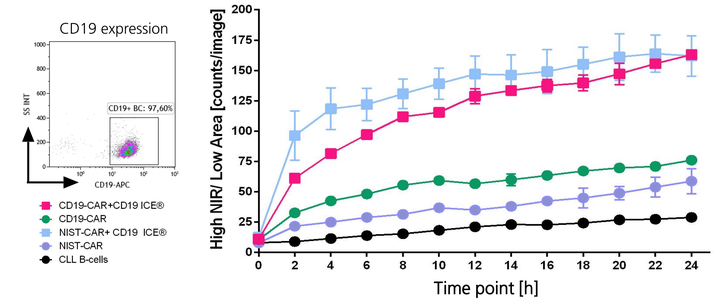
Combination of CAR-NK cells with ICE® is not superior in anti-tumoral efficacy when compared to NK cells with ICE®

(A) Degranulation assay with CD19pos. Daudi cells



(B) Primary Chronic Lymphocytic Leukemia B Cells – Incucyte assay

(preliminary data – validation with more donor samples ongoing)



Combination of CAR-NK cells together with ICE® were tested against (A) Daudi and (B) CD19+ primary B cells derived from a chronic lymphocytic leukemia (CLL) patient. Highest degranulation levels against Daudi cells (N=3 NK donors) and best killing efficacy using a 24-hour kinetics Incucyte image-based assay against primary CLL B cells (exemplary data; CD19-CAR expression <25%) were found when anti-CD19 ICE® was used for combination with either NK or CAR-NK cells.

Summary & Conclusions

- Combination of allogeneic NK cells with bispecific ICE® represent a versatile innovation with potent anti-tumoral activity providing advantages over engineered CAR-NK cells
- Combination of NK cells with ICE® induces anti-tumoral efficacy which is at least comparable to that of corresponding CAR-NK cells
- Combination of CAR-NK cells with ICE® is not superior in anti-tumoral efficacy when compared to NK cells with ICE®

Perspective

Combination of NK cells with ICE® represents the most straight-forward way to generate potent and targeted NK cells. In addition to forming an NK cell composite product (NK+ICE®) similar to CAR-NK cells, free ICE® can engage with other CD16A+ immune cells (e.g. NK cells, macrophages) mediating anti-tumoral ADCC and ADCP.

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References : 1)Pinto et al., *Trends in Immunology* (2022) 43:932-46; 2)Kerbauy et al., *Clin Cancer Res* (2021) 27:3744-3756; 3)Nieto et al., *Blood* (2022) 140 (Supplement 1): 415–416; 4)Gong et al., *Hematol Oncol* (2021) 14:73; 5) Liu et al., *N Engl J Med* (2020) 382:545-553