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CAR-NK cells effectively target CD123+ Acute Myeloid Leukaemia without on-target off-tumour effect against CD34+ bone marrow-derived healthy donor

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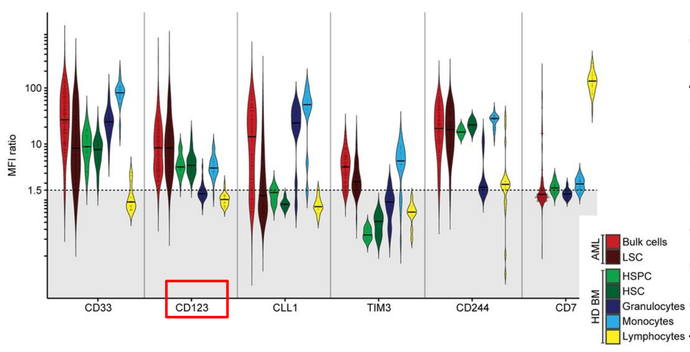
Introduction

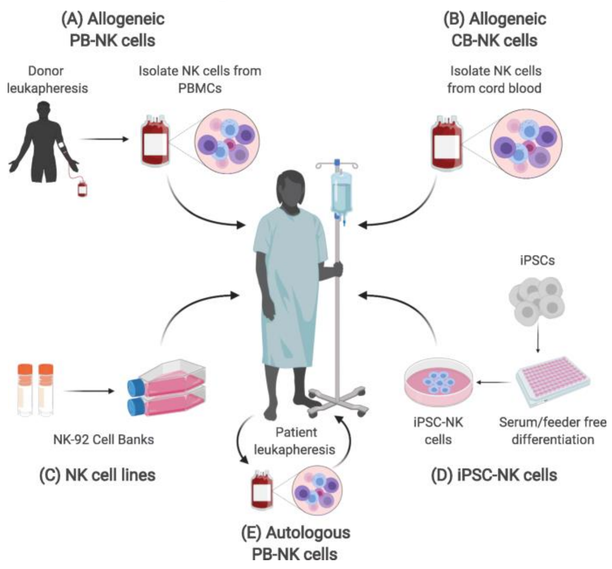
Nowadays, a particularly attractive target for immunotherapy of several hematologic malignancies is CD123, the IL-3 receptor α chain. Indeed, the high expression levels of CD123 in acute myeloid leukaemia (AML) highlights that CD123 is an attractive target for Chimeric Antigen Receptor (CAR) therapy to treat this heterogeneous clonal disorder, that nowadays is still characterized by treatment failure.

However, although T-cells genetically modified to express CARs represents an innovative approach for AML treatment, the toxicity related to Cytokine Related Syndrome (CRS) and an on-target off-tumour effect on haematopoietic stem cell remain the Achilles heel of immunotherapy. Moreover, CAR T-cell manufacturing is related to an expensive time-consuming production of autologous cell drug, which remains unfeasible especially in infant patients who cannot undergo autologous gene therapy, and very difficult to manage in general in the paediatric population, in case of failure of autologous production. In this scenario, an allogenic cell approach with Natural Killer (NK) cells could be the keystone to treat AML patients, thanks to their antitumor ability without graft-versus-host reactivity. In this project, we propose a generation of third-party bank of CAR NK cells genetically modified with CAR CD123 for an effective off the shelf immuno-therapy to treat AML patients.

Levels of cell surface expression of CD123 in AML



Allogenic NK cells source

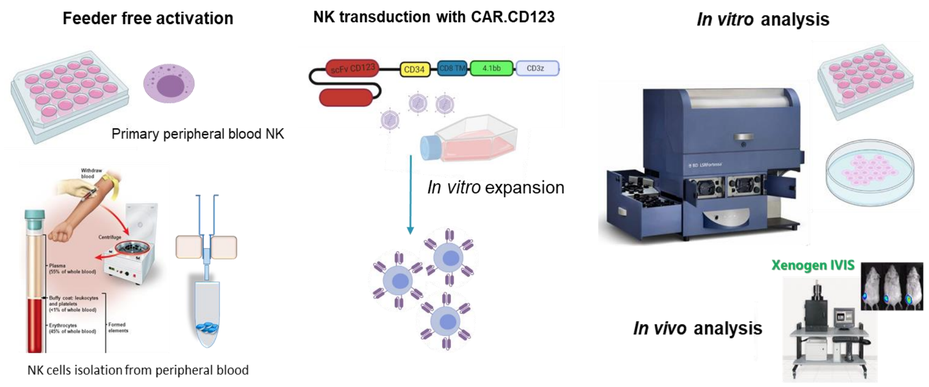


Materials and Methods

Primary samples and cell lines: NK cells were purified from Peripheral blood mononuclear (PBMC) of Healty Donor (HD) and activated in a feeder-free approach, to obtain a bulky NK population characterized by high degree of purity in the absence of CD3+ T-cell contamination.
CD123+ cell lines (THP-1, MOLM-13, OCI-AML3) were obtained by DMSZ. AML cells and adult bone marrow (BM) monuclear cells were obtained after informed consent from the Department of Onco-Haematology, OPBG, Rome (Italy).

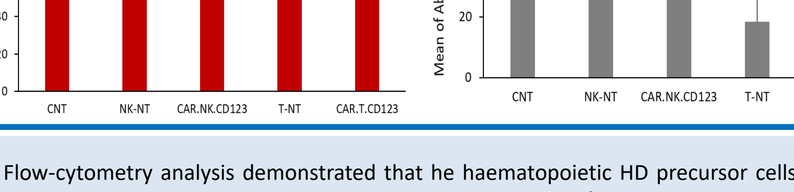
Plasmid construction and retrovirus production: Retroviral plasmid has been designed to carry the cassette of a second generation CAR with specificity for CD123, and 4-1BB as costimulatory domain. An additional retroviral vector encoding eGFP-Fireflyluciferase (FF.Luc-GFP) was used in selected experiments to label tumour cells (THP-1).

Xenogenic *in vivo* leukemia models:
The NOD/SCID IL-2Rγnull (NSG) xenograft mice were grafted with AML THP-1 FFLuc and the tumour grow were assessed by the bioluminescence monitoring using IVIS Image System. Humanized mouse model were generated with irradiated NOD-scid IL2Rgnull-3/GM/SF in which CD34+ Haematopoietic Stem Cells (HSCs) were engrafted in order to investigate the *in vivo* on-target off-tumour effect on primary haematopoietic stem cells.



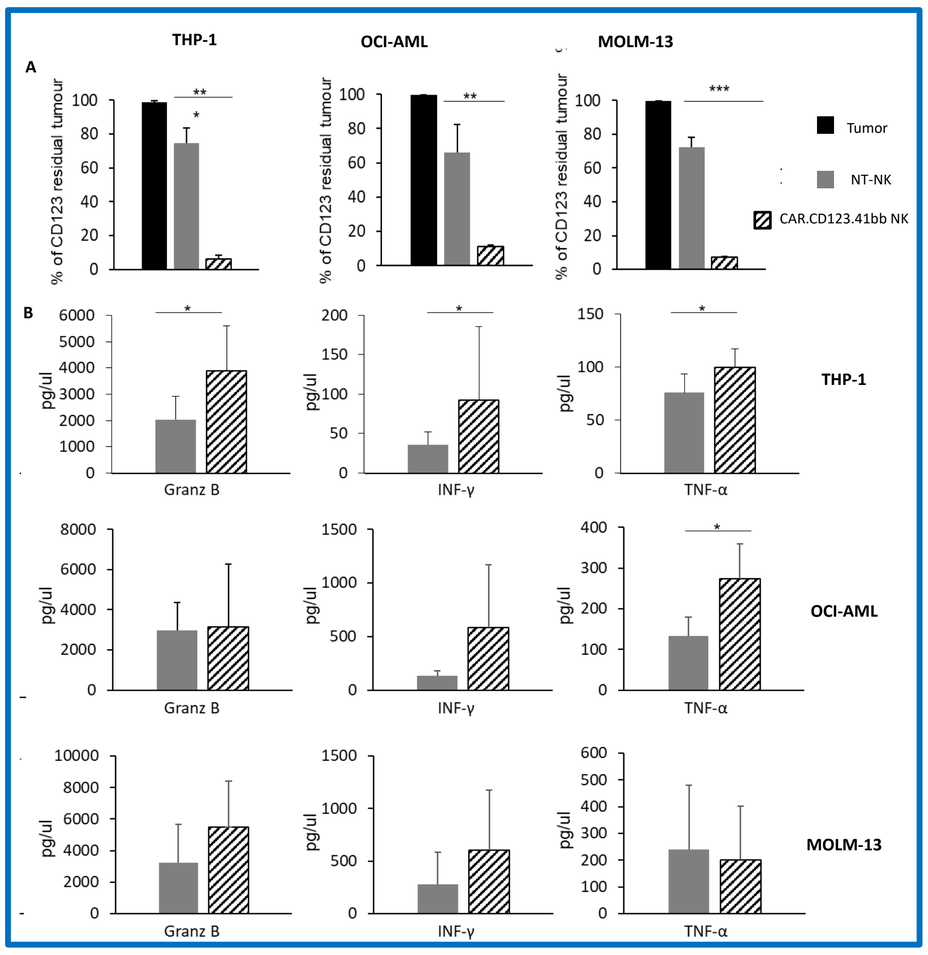
Results

Figure 1



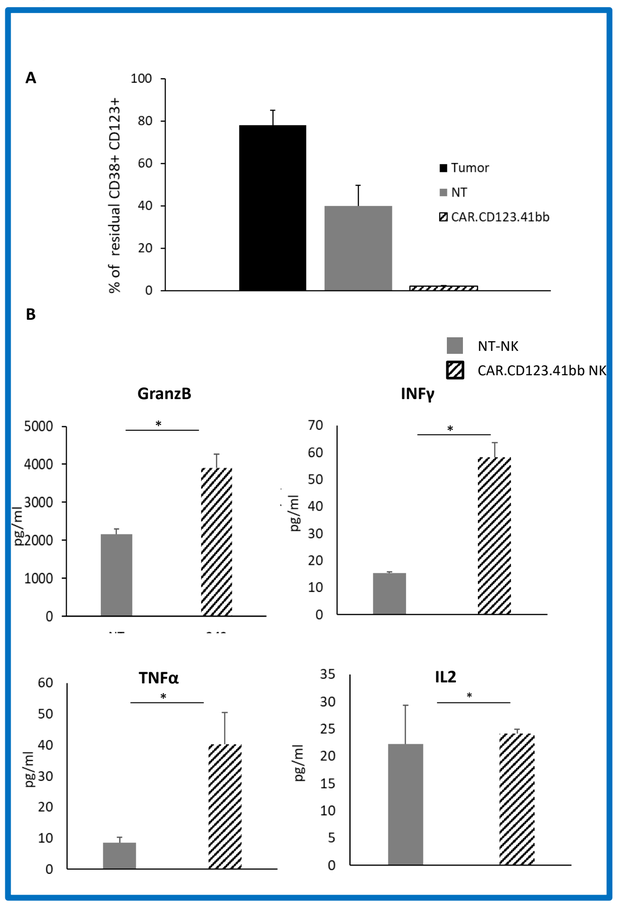
A-B) After genetic modification with a retroviral vector encoding a CAR specific for CD123 antigen, transduction of activated NK cells averaged 58%±21,9% and the CAR.CD123 expression was stable over extended *in vitro* culture (25 days).

Figure 2



A) In co-culture experiments CAR.NK cells show significant anti-leukaemia activity towards CD123+ tumour cell lines (6,5%±10%, 5,1%±5% and 7,7%±9% of residual THP1, OCI-AML3 and MOLM-13 cells). **B)** Those findings strictly correlated with cytokine quantification, indeed both Granz B, IFN-γ and TNF-α production of CAR NK during 24 hours of coculture with CD123+ leukemia were significantly higher than NK cells

Figure 3

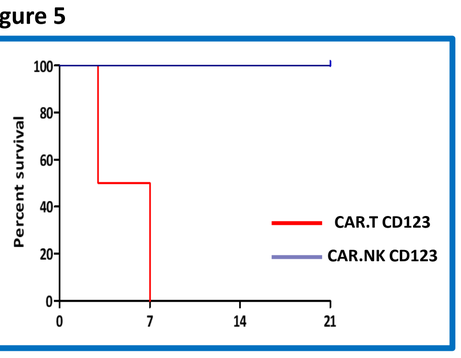


A) Leukemic CD123+ cells obtained from bone marrow samples of three patients affected by AML were co-cultured for 5 days with NT-NK or CAR.CD123-NK derived from HD (p=0,05). **B)** Cytokines production corroborate the *in vitro* antitumor activity.

Figure 4

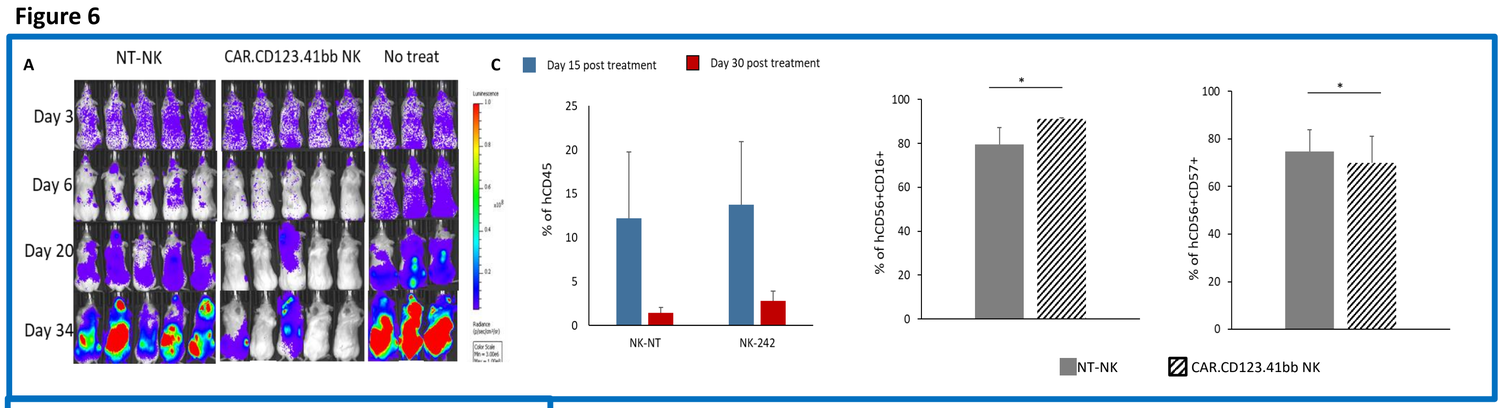
A-B) Flow-cytometry analysis demonstrated that he haematopoietic HD precursor cells have a dim expression of CD123 antigen compared to leukemic AML cells. **C)** The on-target off-tumour effect was analysed on CD34+ primary cells from HD that were exposed to un-modified NK cells and CAR.CD123 NK cells. Short term culture colony formation did not showed a significant effect on healthy BFU-E and CFU-GM colony growth.

Figure 5



We investigated the *in vivo* on-target off-tumour effect on humanized mouse model. Two mice groups were injected with both CAR.CD123-T or CAR.NK-CD123 cells and we observed a stronger toxicity on primary human BM cells in all mice treated with CAR.CD123-T showing aplasia and anaemia and an Overall Survival (OS) significantly lower compared to CAR.NK-CD123 treated mice (p=0.05).

Figure 6



(A-B) A xenograft immunodeficient mouse model of human AML CD123+ THP-1 cell line treated with NT-NK or CAR.NK-CD123 cells. We clearly demonstrated of CAR.NK-CD123 cells significantly improved mice OS, with 81% of the treated mice alive at the end of experiment. **C)** Flow-cytometry analysis showed a significant expression of CD16+ NK.CAR.CD123 population, while both NK.NT and CAR.CD123 NK cells showed a significant CD57+ expression.

Discussion and Conclusions

These data suggest that CAR.CD123.NK cells could be an innovative strategy for targeting leukemic CD123+ AML with lower toxicity profile compared CAR.CD123.T cells.