Introduction
Nowadays, a particularly attractive target for immunotherapy of several hematologic malignancies is CD123, the IL-3 receptor a chain. Indeed, the high expression levels of CD123 in acute myeloid leukemia (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 allogeneic 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.

Results

A) 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).

B) Flow-cytometry analysis demonstrated that haematopoietic HD precursor cells have a dim expression of CD123 antigen compared to leukemic AML cells. 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.

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.

Materials and Methods
Primary samples and cell lines: NK cells were purified from Peripheral blood mononuclear (PBMC) of Healthy 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) mononuclear cells were obtained after informed consent from the Department of Onco-Haematology, OPRG, (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 (FLuc-GFP) was used in selected experiments to label tumour cells (THP-1).

Xenograft in vivo leukemia models:
The NOD/SCID IL-2γnull (NSG) xenograft mice were grafted with AML TPH-1 FLuc and the tumour growth were assessed by the bioluminescence monitoring using IVIS Image System. Humanized mouse model were generated with irradiated NOD-scid IL2γnull-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.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6