Evaluation of the antitumor effect of Natural Killer cells expressing Chimeric Antigen Receptors (CAR) against murine melanoma cells

Mariana Saldanha Viegas Duarte[1], Luiza de Macedo Abdo[1] Luciana Rodrigues Carvalho Barros[1], Martin Hernan Bonamino[1,2]
1. Instituto Nacional do Câncer, INCA.
2. Fundação Oswaldo Cruz, FIOCRUZ.

INTRODUCTION

Natural Killer (NK) cells are an important component of the innate immune system, having a classic role in providing anti-tumoral and antiviral immunity. Currently these cells are seen as potential effectors in allogeneic cancer immunotherapy, mediating antitumor effects without inducing potentially lethal alorreativity, such as graft versus host disease (GVHD) or other alloimmune and autoimmune toxicities. The lack of specificity and targeting of these cells is a limiting factor, which can be solved by inducing the expression of chimeric antigen receptors (CARs). CARs are chimeric receptors consisting of an extracellular domain based on the immunoglobulin variable region (scFv) and an intracellular signaling domain based on the TCR CD3 zeta chain, which may or may not have costimulatory sequences. Melanoma is a cancer with a high level of lethality, mainly in its metastatic form, requiring new therapeutic approaches aimed at increasing the survival of patients.

OBJECTIVE

Establish an immunotherapy model using NK cells expressing CARs via mechanism of transfer of the process of trogocitosis, against murine melanoma cells.

METHODOLOGY AND RESULTS

The B16F10 murine melanoma line is an important model for this tumor used to evaluate potentially therapeutic strategies. In search of a murine model of melanoma in immunodeficient animals aiming at future studies with the treatment of human NK CAR+, a model with NOD SCID mice and B16F10 cells was established. For this, experiments with different numbers of tumor cells were performed and the tumor growth kinetics of the parental B16F10, CD19+ B16F10, CD20 + B16F10 and CD19+ CD20+ B16F10 cells were evaluated. Human NK cells received CAR through trogocitosis, a mechanism of molecule transfer by cell to cell contact. In this case between the NK cells and the K562 19BBz (anti-CD19), K562 20BBz (anti-CD20) cells or both. The cytotoxic potential of NK cells expressing CAR 19BBz and/or 20BBz receptors against wild-type or modified B16F10 murine melanoma cells for expression of human CD19 and/or CD20 proteins was evaluated in in vitro cell lysis assays.

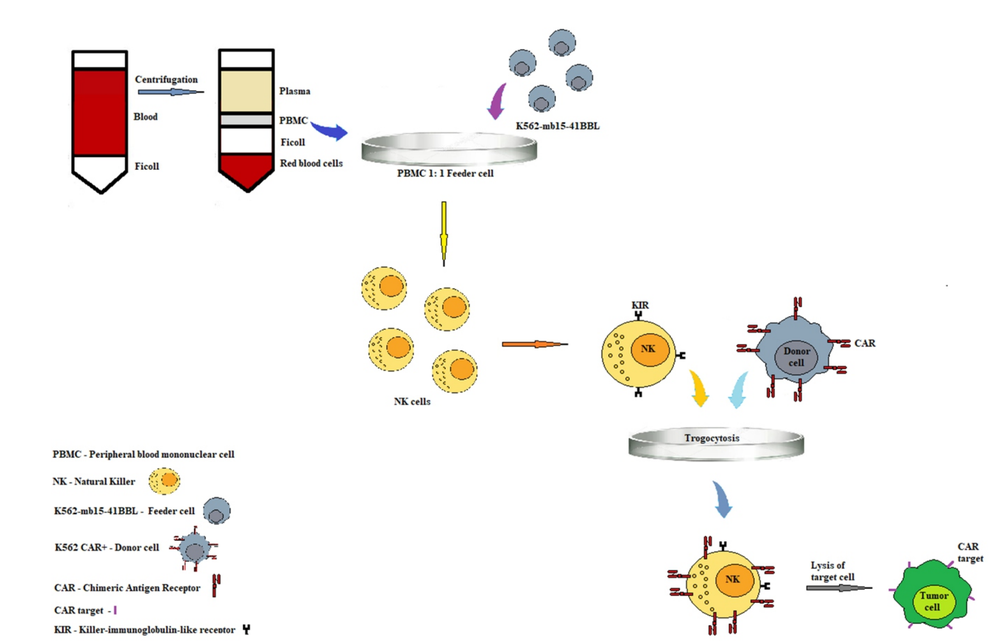

Figure 1. Expansion of NK cells and transfer of CAR by the mechanism of trogocitosis. Mononuclear cells were isolated from peripheral blood using density gradient separation (Ficoll) and subsequently incubated with K562-mb15-41BBL feeder cells and IL-2 with weekly replenishment. NK cells were modified by transient transfer of CAR from a donor cell to NK effector cells using the phenomenon of trogocitosis.

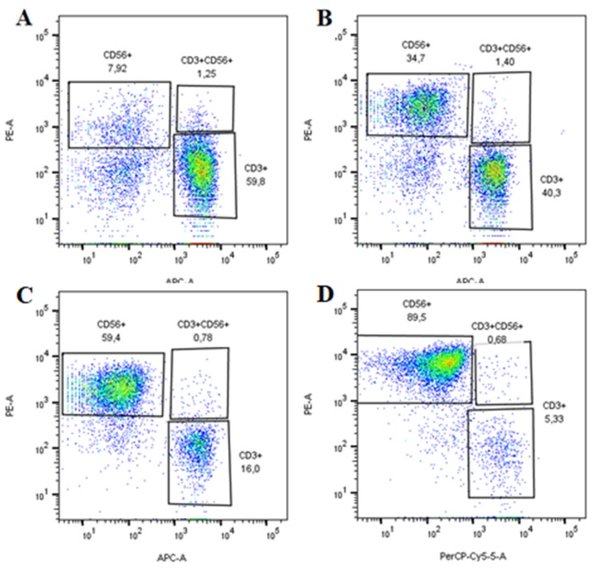

Figure 2. Expansion of NK cells. A) Cell populations after density gradient separation (Day 0). **B)** Day 7.**C)** Day 14. **D)** Day 21.

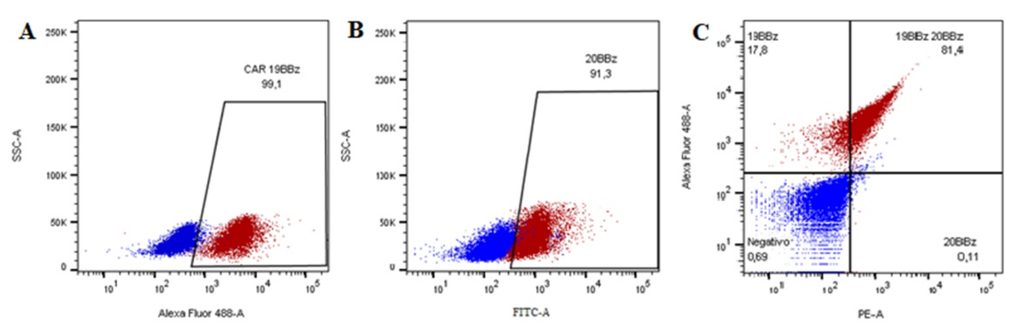

Figure 3. Flow cytometry analysis for identification of NK CAR+ cells. Comparisons of NK cells (CD16+ CD56+ or CD16+) before trogocitosis (blue) and after the process of trogocitosis (red). A) Transfer of CAR 19BBz. B) Transfer of CAR 20BBz. C) Transfer of CAR 19BBz and CAR 20BBz.

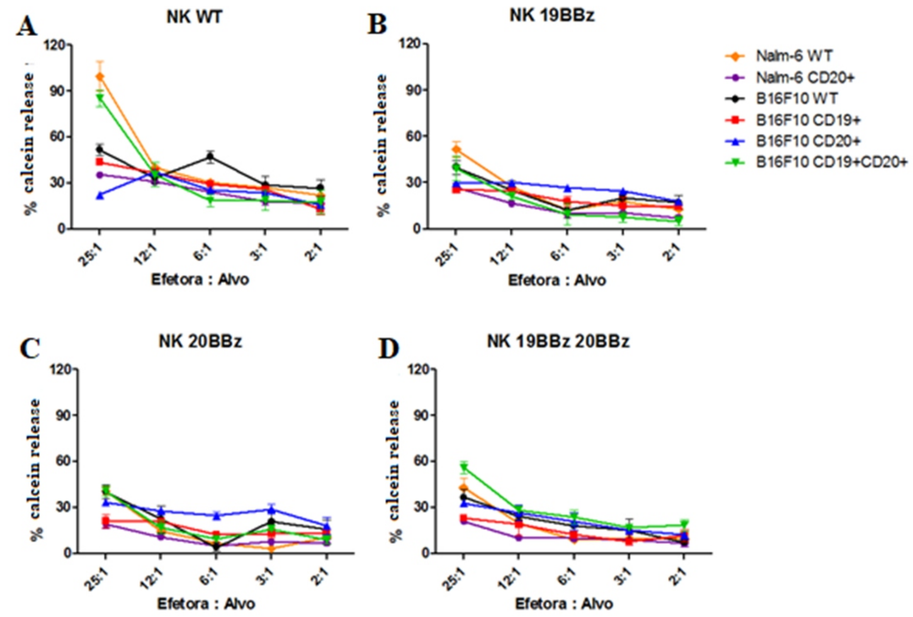

Figure 4. Analysis of the cytotoxic potential of NK cells in different conditions after trogocitosis. Calcein release profile by lysed target cells, using different ratios of effector cells per target, which were co-cultured for 4 hours. Target lines: Nalm-6 WT (yellow), Nalm-6 CD20+ (purple), B16F10 WT (black), B16F10 CD19+ (red), B16F10 CD20+ (blue), B16F10 CD19+CD20+ (green) A) NK WT. B) NK 19BBz. C) NK 20BBz. D) NK 19BBz 20BBz. The graphs show the cytotoxic profile of each effector cell against the different target cell lines.

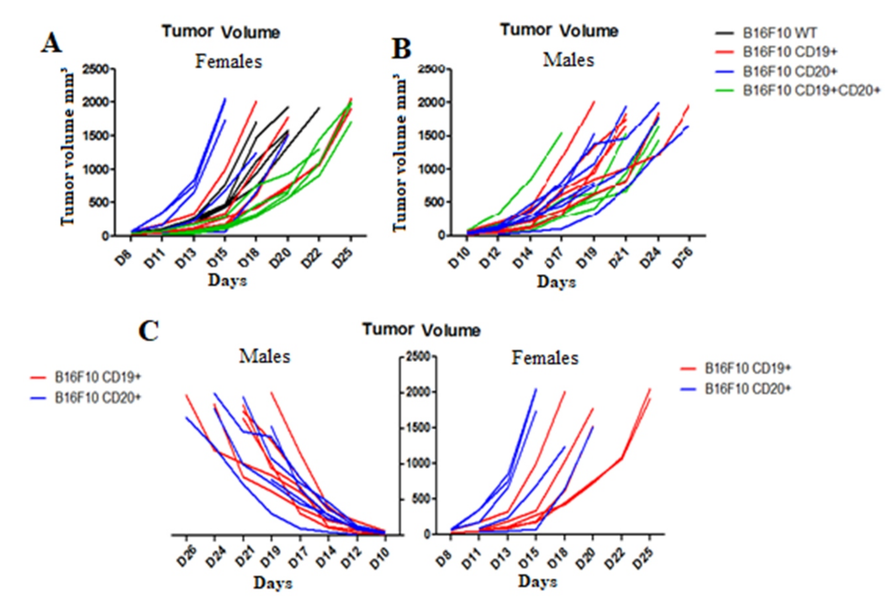

Figure 5. Comparative analysis of tumor growth in males and females. 1x10^5 cells of the B16F10 WT (black), B16F10 CD19+ (red), B16F10 CD19+CD20+ (green) and 5x105 cells from the B16F10 CD20+ (blue) lines were inoculated in the flank region in NOD SCID mice, subcutaneous route. A) Tumor volume in female mice. B) Tumor volume in male mice. C) Comparative tumor volume of B16F10 CD19+ and B16F10 CD20+ lines in females and males.

CONCLUSIONS

Trogocytosis showed to be an efficient method to load NK cells with CAR molecules. The model established in this work with melanoma in an immunodeficient murine model is an important tool for future evaluation of the anti-tumor potential of NK CAR+ cells.

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