CAR-iNKT CELLS: A NEW THERAPEUTIC APPROACH FOR MULTIPLE MYELOMA

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1. INTRODUCTION

- **Multiple myeloma** (MM) is a clonal plasma-cell neoplasm. It accounts for 10% of hematological cancers. It occurs due to gene translocations favored by the VDJ rearrangement in these cells.
- Current treatments are based on VRd therapy (Velcade, Remlimid, dexamethasone), which stops proliferation, followed by Autologous Stem Cell Transplantation. This improves survival, but all patients eventually **relapse.**
- The advent of monoclonal antibody therapies was a major breakthrough, but the fact that the therapy was directed against a target led to a large **clonal selection.**
- The discovery of many MM specific antigens has enabled the development of anti-BCMA CAR-T therapies. Although these therapies work well, CAR-T cell immunotherapy for B cell malignancies is still limited by disease relapse and tumour escape.

In this final thesis, **CAR-iNKT** cells are presented as a possible therapeutic substitute based on experimental data, describing their benefits, different antigens to target and ways to modulate them.

2. OBJECTIVES

- Describe MM disease, current therapies and why treatments fail.
- Understand the biology of **iNKT cells**, their role in cancer and propose them as an option for CAR-iNKT therapy in MM.
- Discuss the differences between **CAR-T and CAR-iNKT cells.**
- Compile a list **of multiple myeloma membrane antigens** and propose which ones may be a good target for CAR-iNKT therapy.
- Describe tools that can **modulate** the activation/inactivation of CAR-iNKTs.

3. MATERIAL, METHODS

- Bibliographic review of current MM treatments, new advances with CAR-modified-cells and discussion of the best options.
- Information obtained from journals, webs, PhD thesis, data bases. Main sources used for the research: **NCBI PubMed.**

Key words: Chimeric Antigen Receptor, iNKT, CAR-iNKT, Multiple Myeloma, immunotherapy, B-cell maturation antigen, cancer.

4. iNKT CELL ANTITUMORAL ACTIVITY

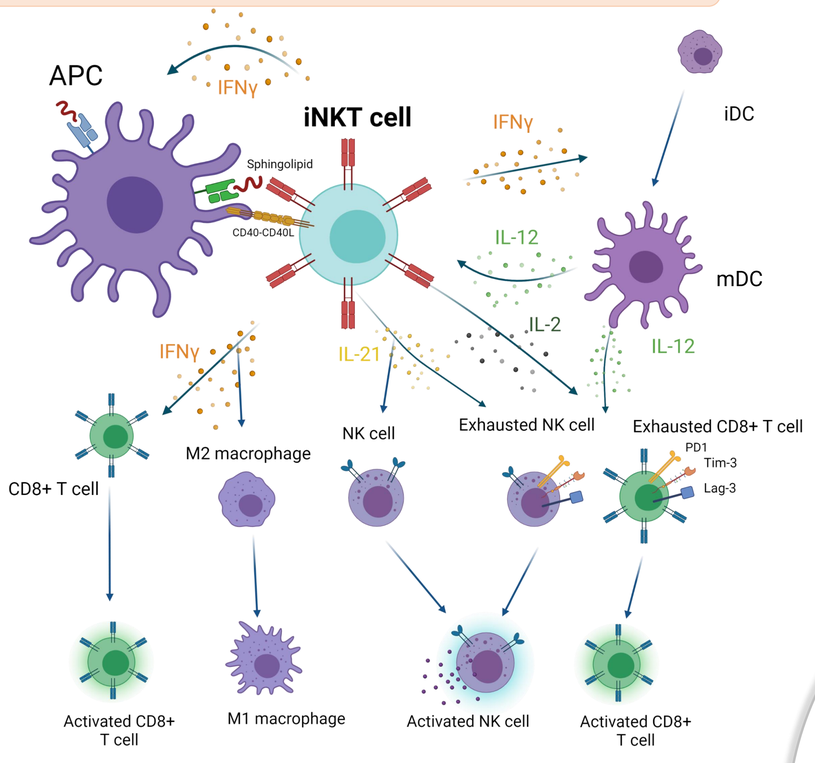
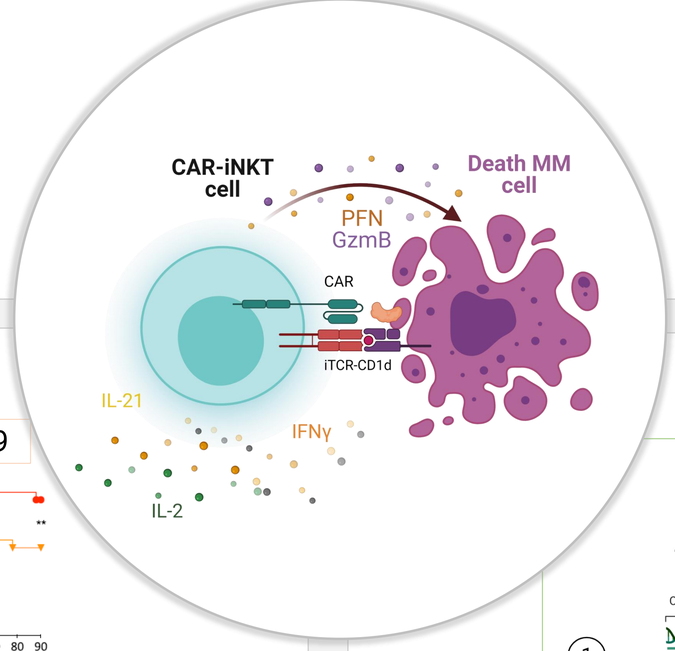


Figure 1. Role of iNKT cells in the physiological anti-tumour response.

5. CAR AND MM ANTIGEN SELECTION

Surface antigen	% of MM cells	Function	Tested on CAR-T cells	Possible Inconvenients
CD138	90-100%	Heparan sulphate proteoglycan	Clinical trial	Expressed in other tissues
SLAMF7	95%	Modulates activation/differentiation of some immune cells	CARAMBA project. Clinical trial	Expressed in other immune cells
CD38	80-100%	Regulates B cell proliferation	Clinical trial.	Expressed in other tissues
BCMA	60-100%	Differentiation and maturation of B cells	Many clinical trials	Clonal selection
CD19	0,05%	BCR co-receptor, signalling	Mostly in other haematological cancers	Low expression
Light chain of Ig	Low expression	Part of the immunoglobulin	Clinical trial	Low expression
CD56	70%	Phenotypic marker of natural killer cells	Preclinically	Expressed in other immune cells, as NK, iNKT
CD44v6	43%	Adhesion receptor	Preclinically	Expressed in monocytes
Lewis-Y	52%	Tetrasaccharide. Tumour associated antigen	Preclinically	-
NY-ESO-1	60%	Tumour associated antigen.	Preclinically	-
CD229	100%	Modulates activation/differentiation of some immune cells	Preclinically	Expressed in NK and iNKT cells

Figure 2. Selected MM antigens that are candidates for targeting by iNKT cells



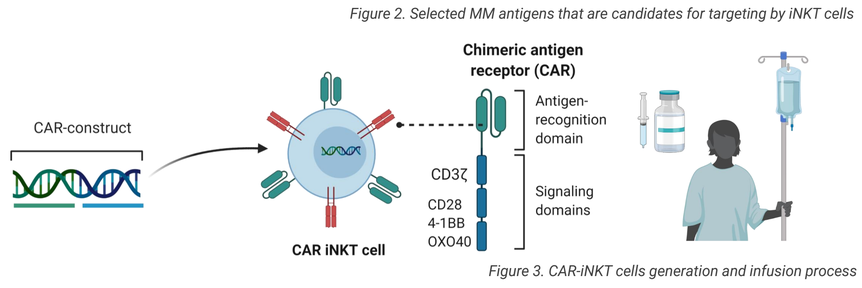
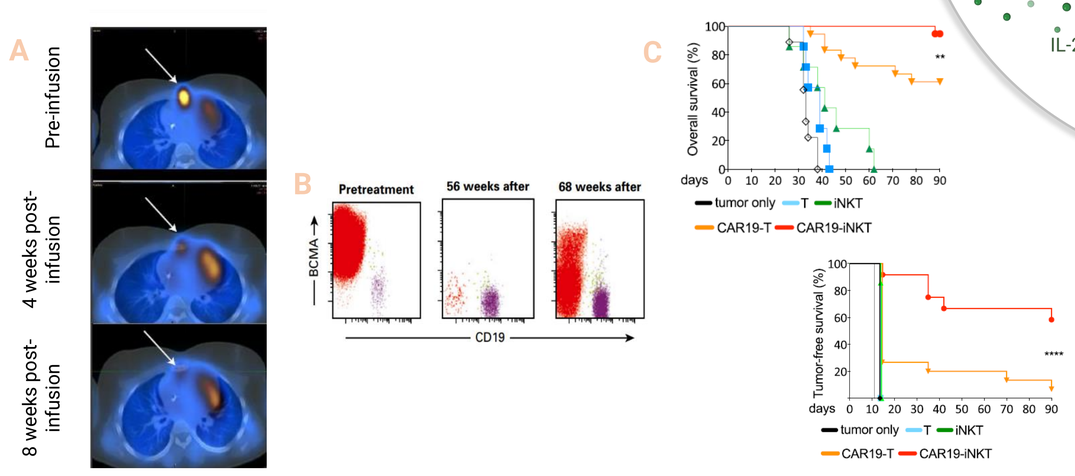


Figure 3. CAR-iNKT cells generation and infusion process

6. CAR-iNKT vs. CAR-T CELLS

CAR-iNKT anti-GD2 | CAR-T anti-BCMA | CAR-iNKT anti-CD19



A. First in human CAR-iNKT anti-GD2 cell therapy. Tumour regression was observed, and even bone metastases were eliminated in one patient. Xin Xu *et al.* (2019).

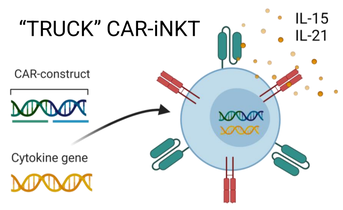
B. CAR-T anti-BCMA therapy in MM promotes clonal selection. Brudno *et al.* (2018)

C. Preclinical study by Rotolo *et al.* (2018), proposes the use of anti-CD19 CAR-iNKT cells to enhance the anti-lymphoma response . This article shows increased proliferation, cytokine secretion and higher cytotoxic activity of CAR19-iNKT cells compared to CAR19-T cells.

7. CAR-CELL REGULATION MECHANISMS

Enhancement

1. "TRUCK" CAR-iNKT



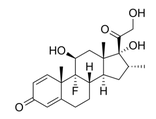
2. Anti-PD1 antibodies

3. Retinoic acid → increases CD1d expression

4. Pulsed DCs as an adjuvant

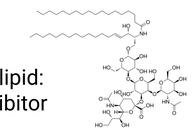
Control

1. Glucocorticoids (Dexamethasone)

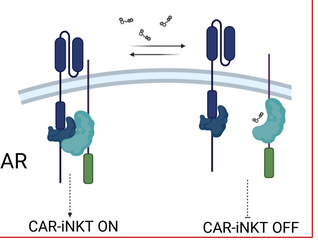


2. Desatinib: Tyrosine Kinase inhibitor

3. GM2 sphingolipid: iNKT cells inhibitor



4. STOP-CAR



8. CONCLUSIONS

1. CAR-iNKT cells target 2 proteins and could reduce resistant clones.

   CAR-iNKT cells are more effective than CAR-T cells in terms of exhaustion, proliferation and persistence in vivo.

2. BCMA seems a promising target.

   CD19, CD44v6, Lewis-Y or NY-ESO could be tested too.

   CD38, CD138 and CD56 could present off target problems.

3. Would be interesting to try the combination of 2 different types of CAR-iNKT cells or perhaps a simultaneous CAR-T and CAR-iNKT combination to reinvigorate exhausted cells.

4. The use of CAR-iNKT cells do not induce acute graft-versus-host-disease (aGVHD) among donors as iNKT cells are highly conserved in the population.

5. Care must be taken when over-activating the immune system. More research into such therapies.

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