APC VACCINES IN iNKT CELL-MEDIATED ANTITUMORAL IMMUNOTHERAPY

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INTRODUCTION

- **Natural Killer T** (NKT) cells are a subset of T cells with properties of both T and natural killer (NK) cells.
- **Type I or invariant NKT** (iNKT) cells possess a semi-invariant TCR that recognizes glycolipid antigens presented by the **CD1d** molecule.
- iNKT cells can mediate **protective antitumor responses** by three mechanisms (**Figure 1**).
- The activation of iNKT cells via **antigen-presenting cell (APC) vaccines** loaded with the exogenous antigen **α-galactosyl-ceramide** (α-GalCer) has potential for cancer immunotherapy.

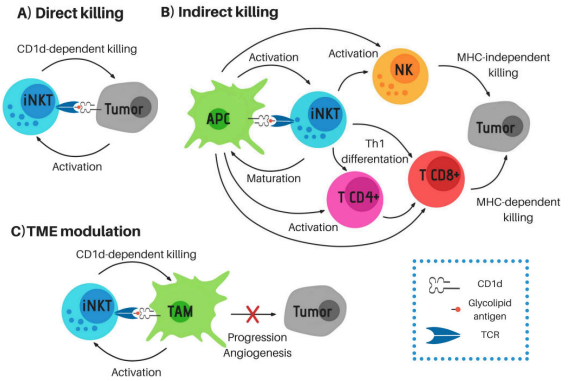

Figure 1. Mechanisms of antitumor immunity by iNKT cells.

AIMS

- Review the **APC vaccination strategies** described to harness iNKT antitumoral activity.
- Review the **results and limitations** of the most relevant preclinical and clinical trials.
- Review the **modifications introduced** to overcome the limitations described and the **new directions** NKT cell-targeted therapies could take.

METHODS

- Search for original and review articles in the **Pubmed database** using the keywords: natural killer T cells, iNKT cells, immunotherapy, dendritic cells, vaccination, trial.
- Selection of papers according to journal impact factor reported by **Scopus Scimago Journal Rank.**

RESULTS

1. First approach: Free α-GalCer administration

α-GalCer is a glycosphingolipid antigen derived from the marine sponge *Agelas mauritanus*. Direct intravenous administration of free α-GalCer can activate endogenous iNKT cells.

Preclinical Mouse Trials
- Tumor growth suppression
- Dose-dependent prolonged survival
- Regression of metastasis

Clinical Trial: Giaccone, et al. (1)
- No toxicity events → safe for use
- No dose-dependent antitumoral effects
- No partial or complete responses

LIMITATIONS DESCRIBED
- Cancer patients have **low levels** of iNKT cells
- iNKT cells suffer activation-induced **anergy**

→ **Lack of positive response to treatment in humans**

2. Optimized delivery system: DC vaccines

Using α-GalCer-pulsed dendritic cells as delivery vectors provides **co-stimulatory signals** to iNKT cells that avoid the induction of anergy. (**Figure 2**)

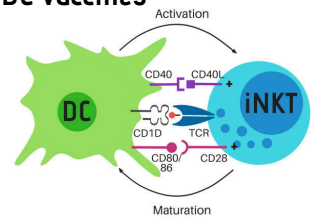

Figure 2. Signals of iNKT activation.

Clinical Trial: Nieda, *et al.* (2)
- iNKT, T and NK activation
- Increased IFN-γ and IL-12 levels
- Inflammatory exacerbation of tumor symptoms
- No tumor reduction

Clinical Trial: Nicol, *et al.* (3)
Intravenous vs Intradermal injection:
- iNKT activation
- Increased IFN-γ levels
- Stable disease

Clinical Trial: Chang, *et al.* (4)
In vitro maturation of DCs before pulsing:
- Sustained expansion of iNKT cells for +5 months.
- Stable disease

Route of administration and maturation status are relevant

3. Other professional APC vectors

Aside from DCs, the **CD1d molecule** is expressed on other cell types (monocytes, B cells..) that can also be used as cellular vectors for α-GalCer delivery.

Clinical Trial: Nagato, *et al.* (5)
Peripheral Blood Mononuclear Cell vector:
- Infiltration of activated iNKT cells into tumor site
- Increased IFN-γ-producing ability

Clinical Trial: Kurosaki, *et al.* (6)
Diverse APC population vector:
- Nasal submucosa injection: significant iNKT expansion
- Oral submucosa injection: no response

→ **Route of administration is relevant**

4. Increasing specificity: Tumor antigens

The **combination of α-GalCer and tumor antigens** in the same cellular vaccine can **increase the specificity** of the cytotoxic antitumoral responses generated (**Figure 3**).

Tumor antigens can be used in several strategies:
- Free tumor antigen administration.
- Tumor antigen-pulsed APCs.
- Irradiated whole tumor cells as antigen source.
- Transduction of APCs with tumor antigens.
- Tumor cell-derived exosomes pulsed on APCs.

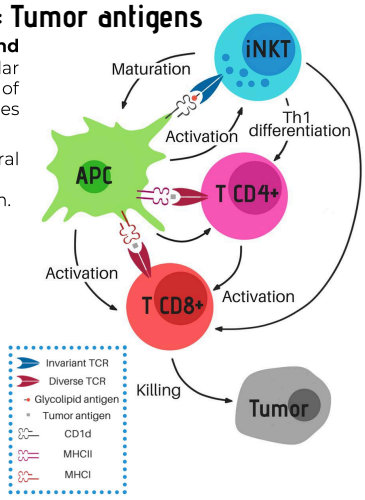

Figure 3. Mechanisms of tumor antigen + α-GalCer combined vaccination.

Clinical Trial: Gasser, *et al.* (7)
α-GalCer + NY-ESO-1 antigen - loaded DCs:
- No toxicity events
- Detectable NKT cell activity
- Increase in peptide-specific circulating T cells

5. Combination strategies

The combination of α-GalCer-pulsed APCs with other therapies has been developed in an attempt to obtain better clinical results.

- **Recombinant IL-21**: cytokine therapy can help to fully mature the NK population previously expanded by activated iNKT cells.
- **Lenalidomide**: LEN is an immuno-stimulatory drug that provides co-stimulation to T and iNKT cells.
- ***Ex vivo* expanded autologous iNKT cells**: can help overcome the low baseline levels of iNKT cells in cancer patients.
- **anti-PD-1 antibodies**: blocking the PD-1 receptor can stop the induction of anergy in activated iNKT cells.

Clinical Trial: Richter, *et al.* (8)
α-GalCer-pulsed APCs + LEN:
- Activation of NKT, NK, monocytes, and eosinophils
- Reduction in myeloma-associated immunoglobulin levels

Clinical Trial: Yamasaki, *et al.* (9)
α-GalCer-pulsed APCs + *ex vivo* expanded autologous iNKT cells:
- Expansion of iNKTs and infiltration into tumor site
- Stable disease / Tumor regression

Combination strategies show better results than monotherapy

CONCLUSIONS

- iNKT cells activate both innate and adaptive immunity and show great **potential for cancer immunotherapy**.
- Stimulation of iNKT cells with α-GalCer-pulsed APC vaccines shows **promising results in preclinical studies** but translating these findings to humans remains a major challenge. The use of **humanized animal models** and further **basic study** of human *vs* mouse NKT cells should be of great benefit.
- The main limitations described in trials are the **low number of iNKT cells** and their **activation-induced anergy**.
  - APC vaccines were introduced to reduce anergy induction.
  - Combination with *ex vivo* expanded autologous iNKT transfer was introduced to increase iNKT cell number.
  - Combination with tumor antigens, immunostimulatory drugs and cytokines was introduced to increase iNKT cell function.
- α-GalCer-loaded APC vaccines in **combination strategies** show better results than single therapy and remain the better alternative.

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