Research Project Proposal

Academic year 2019-2020

Project Nº 49

Title: GAS6/PROS-TAM pathway inhibition to enhance immune response in T cells-based immunotherapy.

Department/ Laboratory

Immunology and Immunotherapy program

Lab 3.02. Immunomodulation Therapy

CIMA

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Summary

Adoptive cell therapy is a cancer therapy that involves administration to the cancer-bearing host of immune cells with direct anticancer activity. Recently, a new clinical trials have shown measurable responses in cancers lymphoma, melanoma. However, the translation of this technology to solid tumours has thus far been unsuccessful. The cause might be the physical barriers and the development of a highly immunosuppressive local tumour microenvironment. Tyro3, Axl, and Mer are membrane proteins that constitute TAM family. Growing evidence indicates that TAM receptors play an important role in anti-inflammatory responses through modulating the function of macrophages, dendritic cells, NK cells in the tumour context. In this proposal we investigate the potential of inhibition this immmunosuppressive pathway in CD8 cell-based treatment.

Goal

Evaluate the therapeutic role of GAS6/PROS-TAM pathways inhibition to enhance immune response in T cells-based immunotherapy.

Methodology.

1. **Plasmid constructions by molecular biology**
    a. Generate a new retroviral plasmids for expressing a soluble version of TAM receptors: Tyro3 and AXL
2. **Protein production:**
    a. Recombinant AXL and Tryo3 will be produced in e.coli and purified by FPLC (AKTA)
3. **Retroviral productions and mouse T cell activation and infection**
    a. New virus will be produced through transient transfection of packaged cell line, PlatE.
    b. CD8 from OTI transgenic mice (transgenic TCR specific for a peptide-SIINFEKL) will be isolated by magnetic beads activated using CD3/CD28 antibodies and infected with retrovirus by spin inoculation. At day 7 of expansion, the CD8 will be collected for in vitro and in vivo analysis.
4. **Characterization in vitro of the new cell product**
    a. The supernatant of modified CD8 will be collected to measure the secreted version of AXL and Tyro3.
    b. Functional analysis in vitro. Cytokine release such as IFNq or TNFa and proliferation will be measured to study the activity of modified AXL-OTI or Tyro-OTI CD8.
4. **Effect of TAM pathway inhibition in the antitumor activity of TCR specific CD8 (OTI model)**
    a. B16OVA-bearing mice will be treated with Tyro3-OTI or AXL-OTI CD8. The antitumor response will be analysed by measuring the tumour area twice a week.
    b. Further analysis to understand the mechanisms of this new T cell-based antitumor approach by flow cytometry

yes	**x**	**Does the project include the possibility of supervised animal manipulation to complete the training for animal manipulator?**
no		