HIPI
Human Immunology
Pathophysiology
Immunotherapy

Identification of CAR-T cell persistance-associated genes in Multiple Myeloma

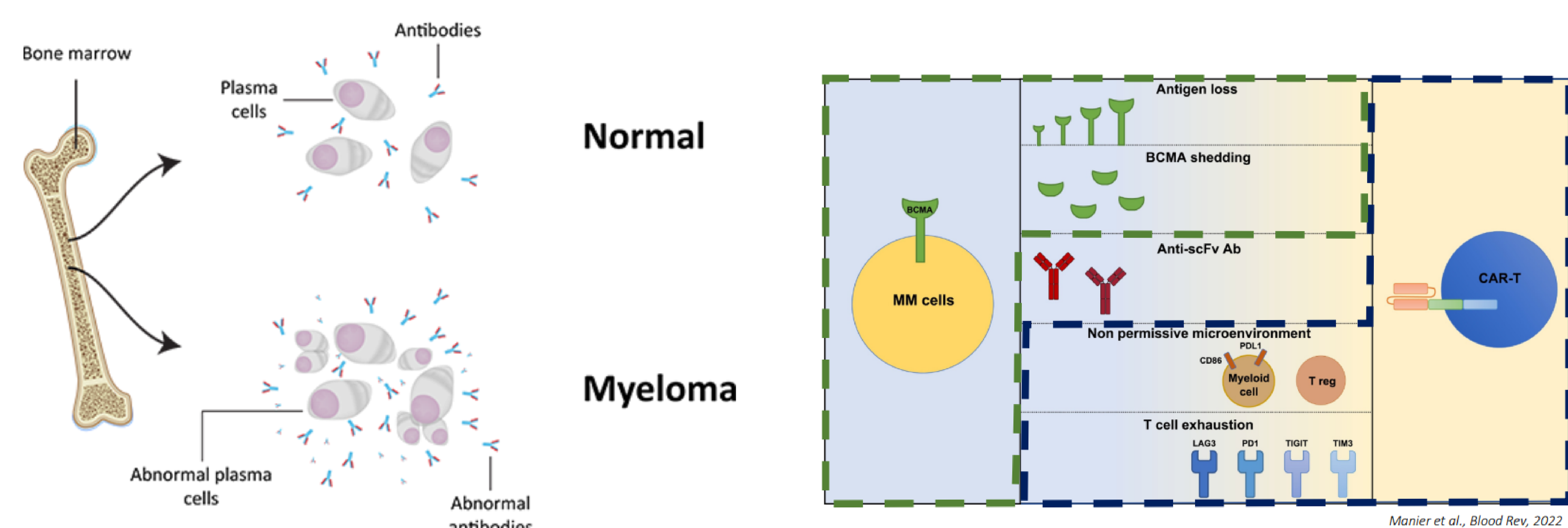
Jean-Christophe BORIES, Fabio RAINERI

ABSTRACT

Chimeric Antigen Receptor engenireed T- lymphocytes (CAR-T cells) have been proven as an innovative and efficient therpay in numerous hematological malignancies. In Multiple Myeloma (MM), the identification of B cell maturation antigen (BCMA) on the surface of tumorous plasma cells has led to the development of anti-BCMA CAR-T cell therapy. However, this FDA-approved treatment practically always leads to relapse of patients, and an optimization of BCMA CAR-T therapy is needed. For that, the goal of my internship will be to identify the genes involved with CAR-T cells persistance during MM, via a large-scale screening of candidate genes selected thanks to the previous works of my host-lab. The identifaction of these genes will be performed via a CRISPR/Cas9 Knock-Out of 237 genes targeted by a lentiviral sgRNA library. The validated genes will be functionnaly tested to confirm their potential for optimized survival in KO CAR-T cells.

BACKGROUND

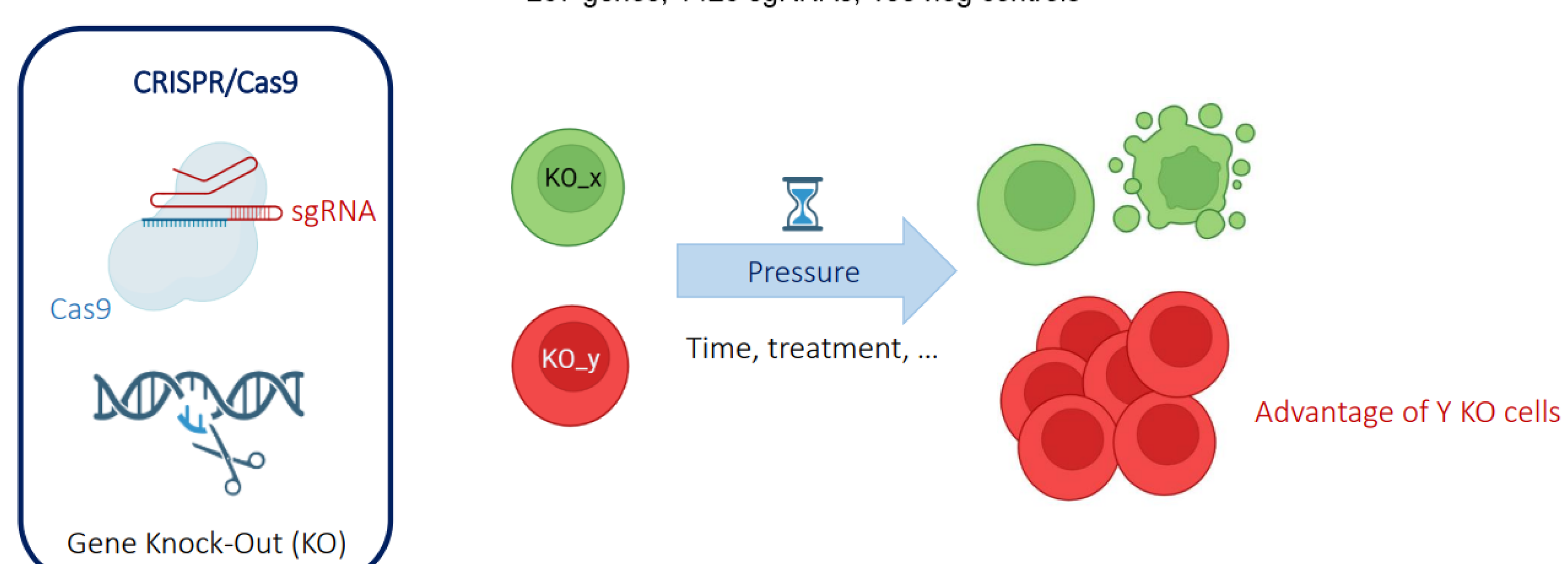
Multiple Myeloma is a hematological cancer caracterized by the malignant proliferation of abnormal plasma cells in the Bone Marrow (BM), producing monoclonal antibodies. Despite recent therapeutic advances, multiple myeloma remains an incurable disease and the current therapeutic arsenal is insufficient in refractory patients. Lymphocytes T expressing a chimeric antigen receptor (CAR) constitute a form of innovative cellular immunotherapy by reprogramming T lymphocytes (CAR-T) to induce an anti-tumor response. After their success in the treatment of B-cell malignancies, the identification of B cell maturation antigen (BCMA) on the surface of tumor plasma cells has allowed their development in myeloma where they are booming. However, their main limit is that a relapse is always observed after a certain treatmenet time. In order to fight the mechanisms of relapse and optimize the efficiency of BCMA CAR-T Cells, we can try to hinder the resistance of MM cells, or improve the persistance of CAR-T cells.



HYPOTHESIS & AIMS

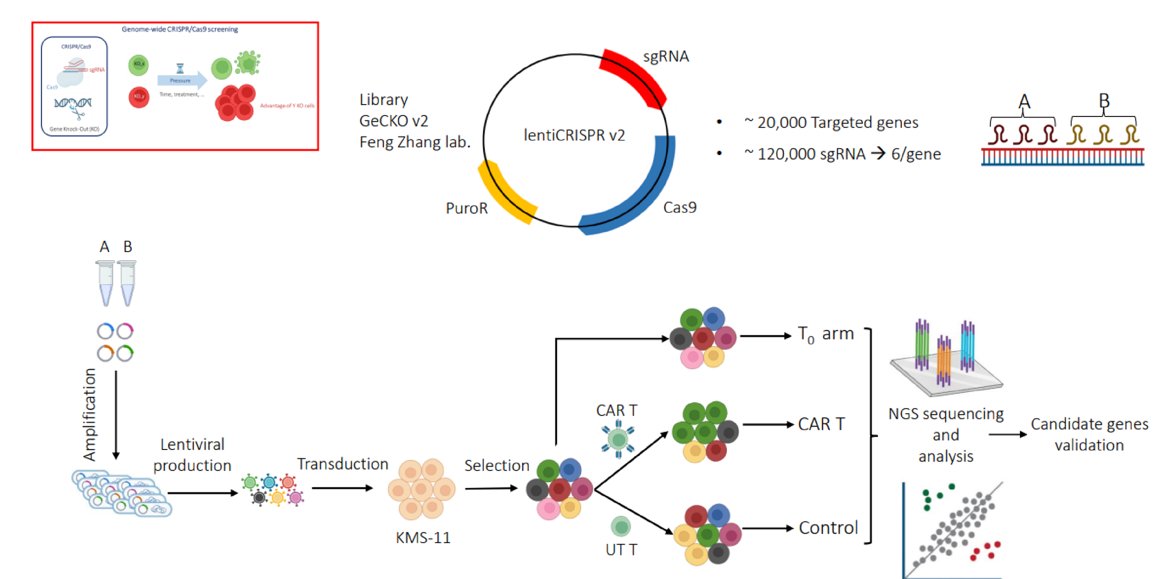
To optimize BCMA CAR-T cells efficiency, we chose the strategy of finding the mechansims improving their survival during MM. For that, the goal of the project will be to identify genes associated with a lower CAR-T cells survival rate. We will perform a knockout of candidate genes in CAR-T cells, in vivo and in vitro, with a lentiviral vector library. These 237 candidate genes are selected from previous genome-wide KO data from the lab. We will then add a selective pressure to model MM environment, and perform a sequencing and gene analysis after several days in order to validate the candidate genes. Enriched edited CAR-T cells compared with control will allow to validate the deleterious impact of the gene on CAR-T cell survival. Finally, the edited CAR-T cells will be functionnaly tested to validate if the gene-KO CAR-T cell has a higher peristance and thus an optimized efficiency.

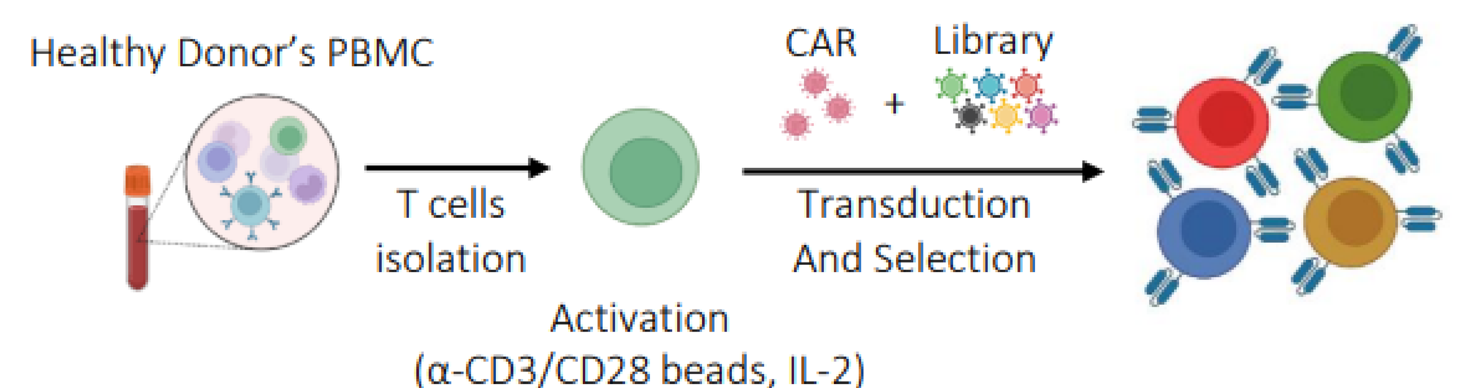
Minipool CRISPR/Cas9 screening
237 genes, 1423 sgRNAs, 100 neg controls

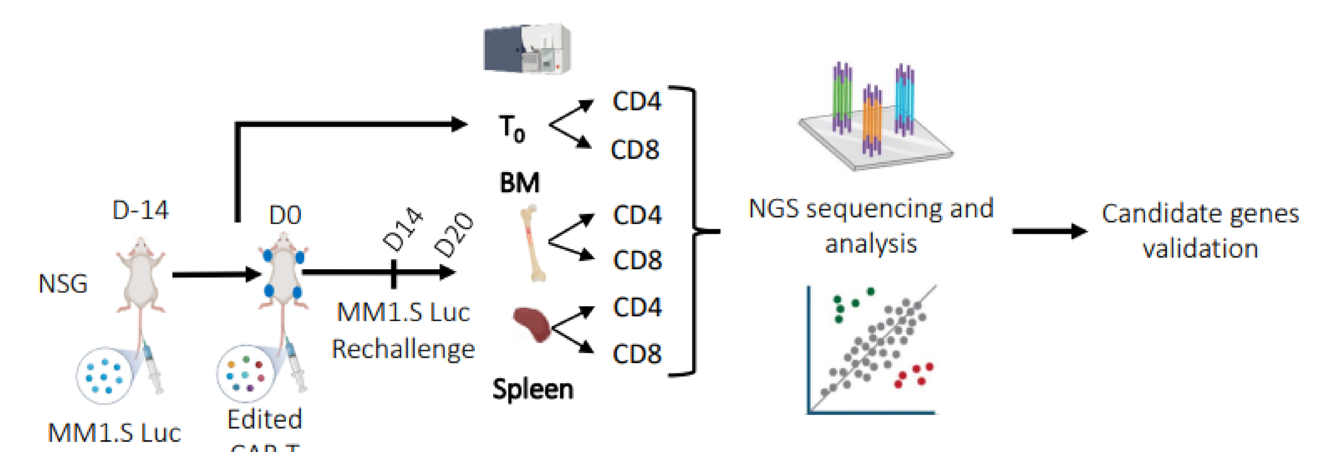


METHODS

A : Previous method from the lab for MM resistance screening, that has inspired the workflow for the CAR-T persistance study project.
B : *in vitro* screening of BCMA CAR-T cells, that will be selected through co-culture with MM cell line
C : *in vivo* screening, that will be done in parallel with the *in vivo* study. In both cases, candidate genes will be validated via cytotoxycity assays.







CONCLUSIONS

This BCMA CAR-T cell persistance study will allow to identify which genes are associated with CAR-T cell survival when knocked out. This could lead to optimization of CAR-T therapy in MM thanks to the use of edited KO BCMA CAR-T cells, with an ultimate goal of lowering the relapse rate in patients.

REFERENCES

Principal Investigator : Jean-Christophe Bories
Tutor : Fabio Raineri (Post-doc)
Lab : Lymphocyte development and lymphoid disorders
Unit : "Human Immunology, Pathophysiology, Immunotherapy" *INSERM 976*
Institut de Recherche de Saint-Louis (IRSL), Paris

Created with BioRender Poster Builder