International Winter School Molecular Medicine

14-18/12/2020

Sapienza University of Rome, department of Biology and Biotechnology

CAR-T cells fitness improvement through RINF Knock down

Mattia Fumagalli

Laurea Magistrale Genetica e Biologia molecolare

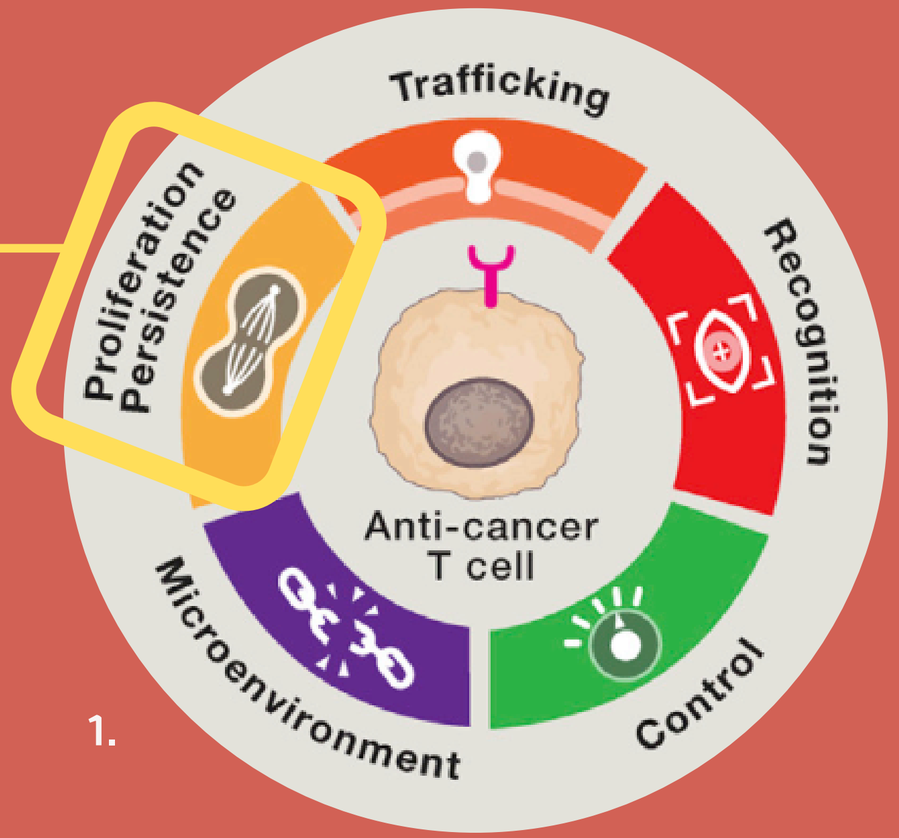
Magistère Européen de Génétique

Background

1. General context

CAR-T cells theraphy against cancer has shown impressing results, yet its efficiency and safety need to be further improved. The project I will be working on focuses on one possible strategy to improve CAR-T cells proliferation and persistance.

- Anti-PD1 therapy
- T-Reg depletion
- Anti-TGFbeta therapy
- .....



1.

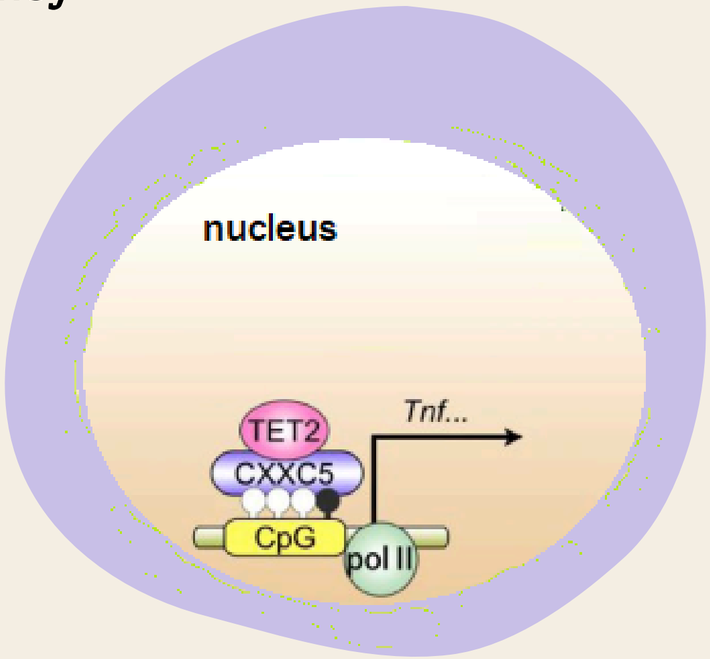
2.

2. The experiment's rationale

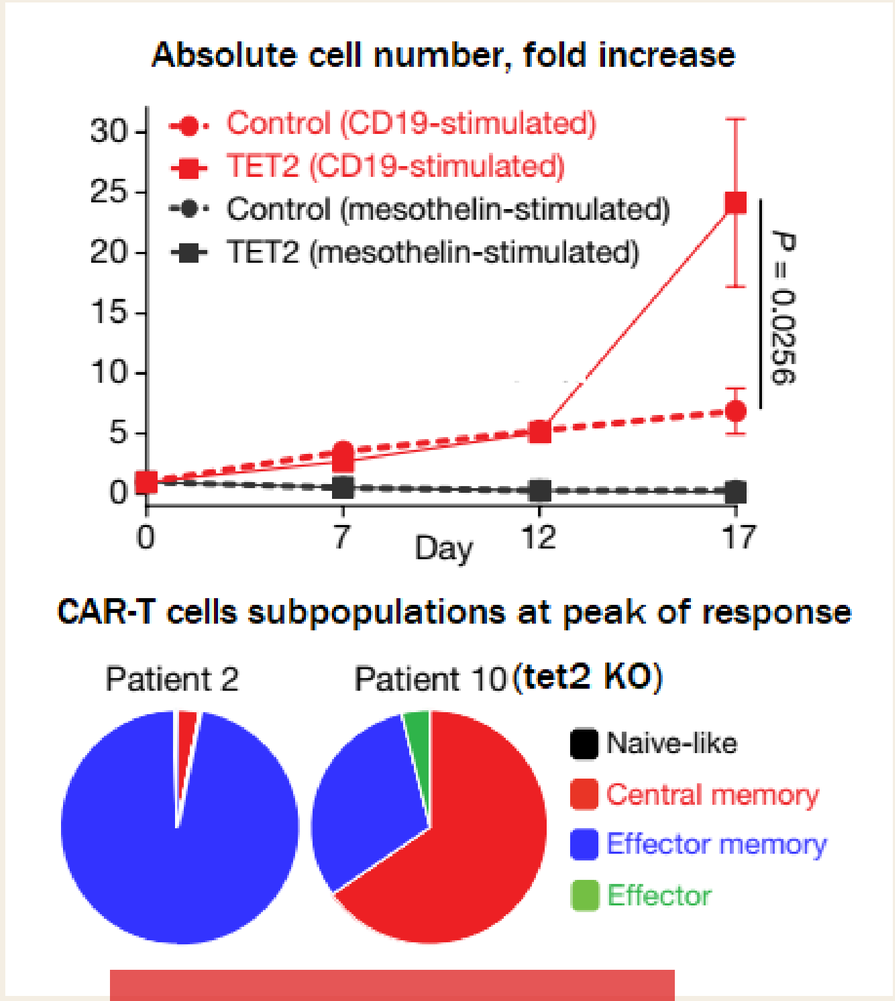
This project bases on 2 main recent findings, that justify the pursuing of RINF KD strategy for CAR-T cells improvement:

- RINF is essential to TET2 demethylase activity in several cell lines, for its docking function

- TET2 Knock-down can skew CAR-T cells to a more central memory phenotype, incresing the overall anticancer efficiency



3.



4.

Aim of the project :

verify and characterize RINF KD effect on CAR-T cells efficiency for the cure of solid tumour

To fullfill this goal, is pivotal to answer to some central questions:

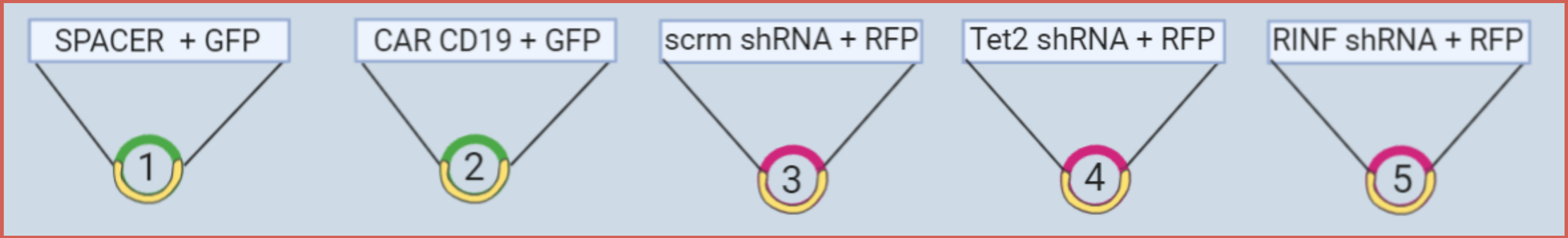
- Do RINF KD improve CAR-T cells fitness?
- Do RINF KD improve CAR-T cells therapeutic efficience in solid tumour models?
- What pathways could explain such an improvement?
- What similarities and differences with Tet2 KD?

Building the right tools

Our study bases on recent works, in a domain which is far from being fully understood, for this reason some specific attentions have to bo dedicated to the design of the experiment.

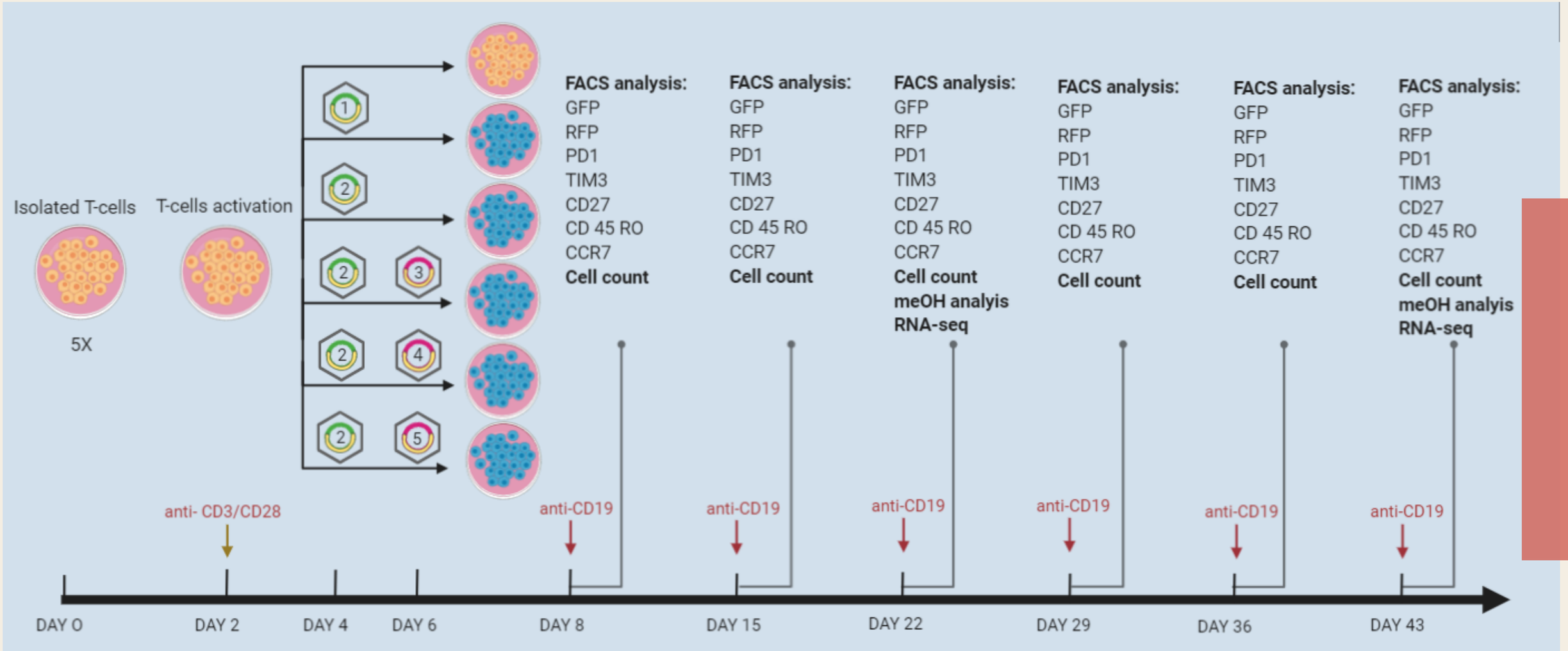
To obtain clear responses and data comparable with other studies results, we will reproduce at best the experimental conditions from the *fraietta et al* paper, concernig chiefly: the CAR to use, CAR-T cell culture conditions and stimulation and re-stimulation techniques.

As well, to rule out any possible interference, we seek to use transduction vectors of our own production for shRNA products to obtain Tet2 and RINF KD.



Chronic stimulation assay

Basing on previous works we developed a pipeline to asses the effects of RINF and TET2 KD on CAR-T cells proliferation, exhaustion and memory phenotype over chronic antigen exposure. We also intend to asses the effects on the hydroxymethilome, since both TET2 and RINF are fundamental for DNA hydroxymethylation

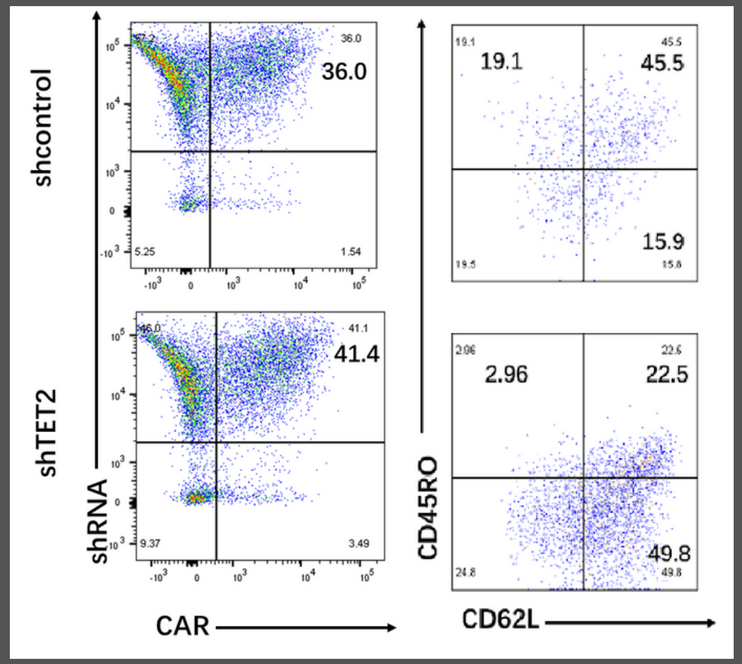


Analyses

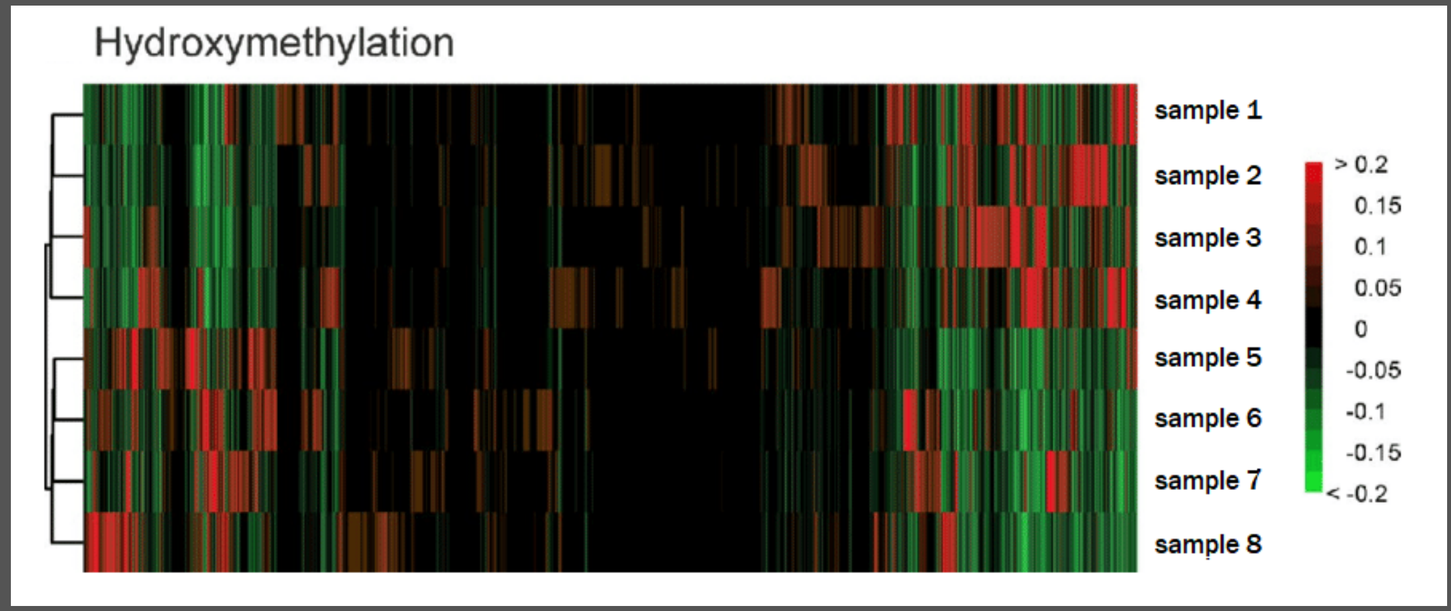
Cell count will allow us to immediately grasp eventual hyperproliferation trends

We will perform FACS analysis for CAR and shRNA expression, that will allow us to see if these, subpopulations increase through time. We will as well check for exausthion[PD1, TIM3] and memory markers [CD27, CD45 RO, CCR7] in the different subpopulations.

Hydroxymethylome analysis and RNA-seq are expected to better characterize the CAR-T phenotypes and give us important informations about the mechanism behind the phenotype, by revealing changes in gene regulation that could have been caused by RINF and TET2 KD.

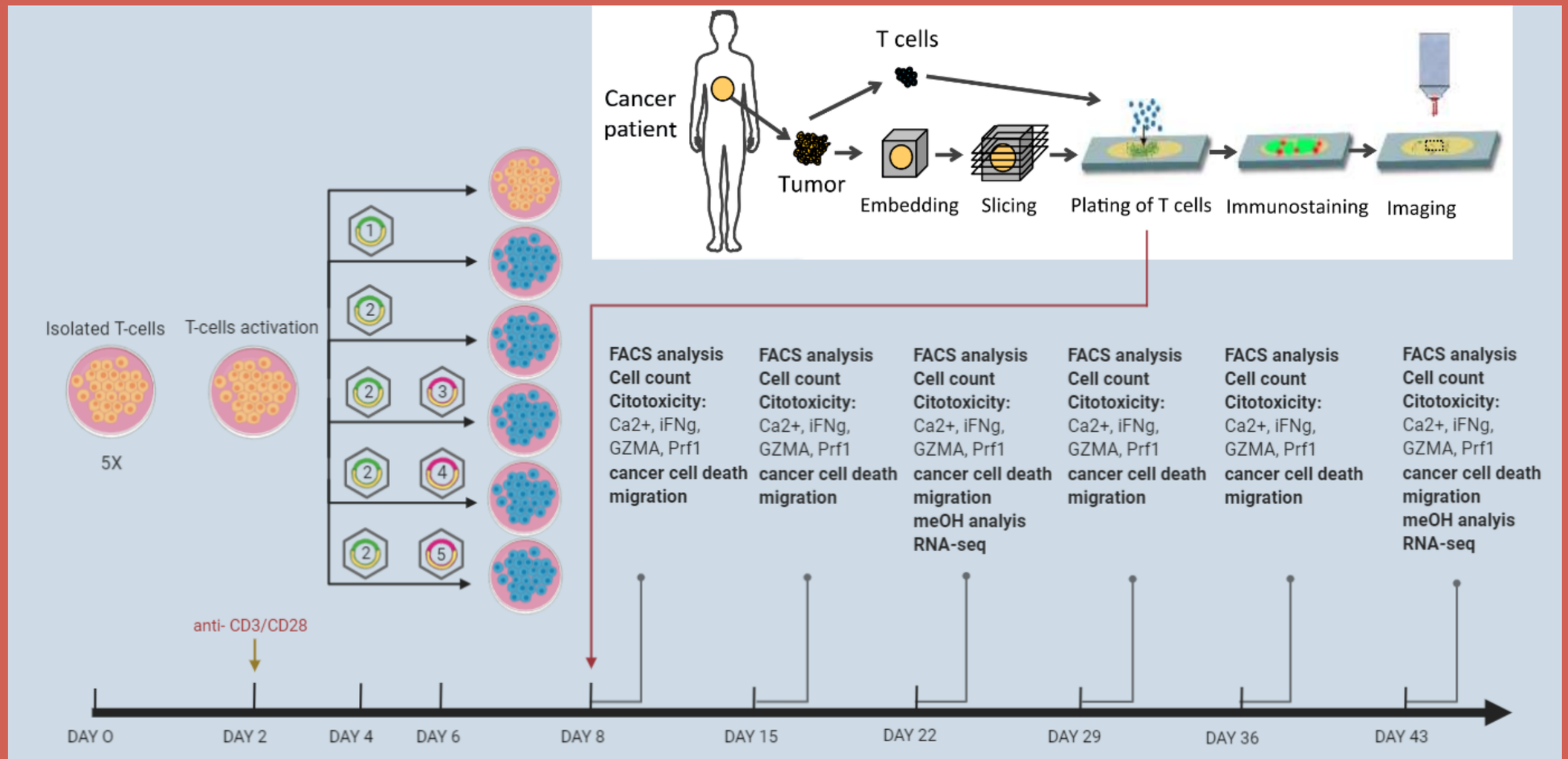


5.



Tumour slices assay

The Donnadieu team developed and patented a new pre-clinical model in the recent years, fundamentally based on human solid tumour slices as models of an in vivo like environment. We are thus going to asses the effects of RINF and TET2 KD in CAR-Ts exposed to this environment, checking as well their antitumor efficacy



Pitfall and solutions

Both TET2 KD and RINF KD show no effect in vitro chronic stimulation assay

We will plan our experiment in a way to obtain statistically significant data, in total adherence to fraietta at al study, so we should reproduce the Tet2 KD result.

RINF KD show "mixed" results in chronic stimulation assay

Is possible that we obtain a different effect by RINF KD, with respect with TET2 kd, or even no effect at all in vitro

RINF KD show no evident result for CAR-T cells improvement

It is possible anyway that RINF KD has no effect on T-cells proliferation, memory phenotype, cytoxicity or memory phenotype

If technical errors can be ruled out, we will put to discussion findings on Tet2 KD

An impossibility to reproduce the Tet2 KD in the same exact conditions, if no error is made, would fundamentally prove wrong previous conclusions.

We do expect differences

A different phenotype is expected from RINF KD and Tet2 KD. Moreover a more 'in vivo-like' condition could give different results, justifying anyway to continue with the tumour slices assay.

RINF and Tet2 KD methylome and RNA-Seq will still provide useful informations

In any case the comparison of the effects of these KDs on the methylome and transcriptome, will allow to provide some explanations on the possible pathways behind the phenotypic effects observed.

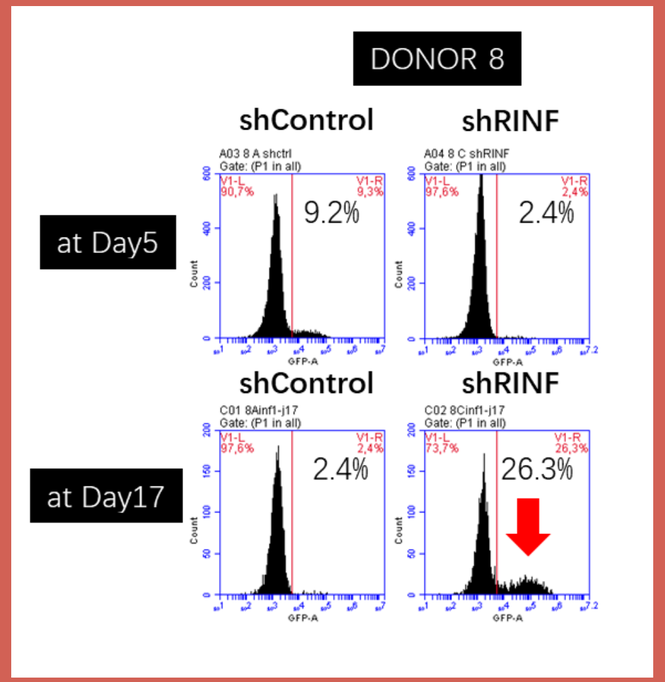
Conclusions

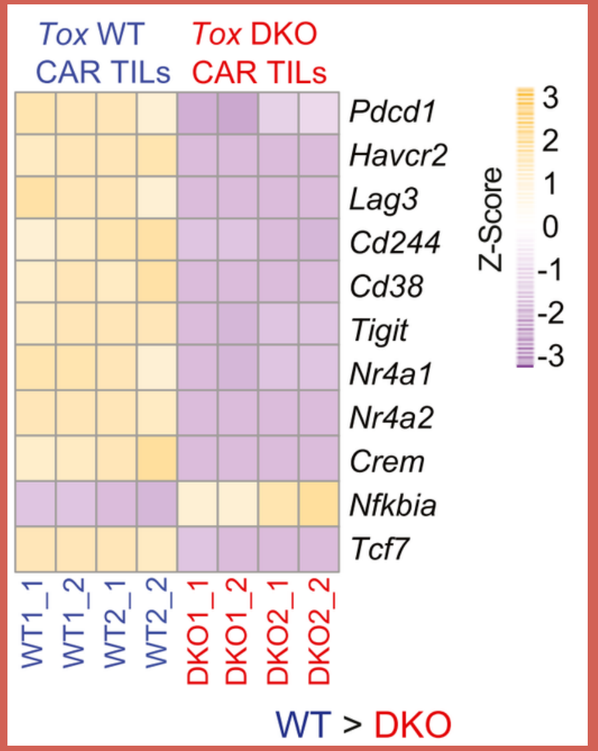
We believe that following a strict protocol, the answer to the question: do RINF KD increase CAR-T cells efficiency, will be found, whatever it will be.

At the same time the analysis of the changes in the hydroximethylome and transcriptome of CAR-T cells will help to characterize the role of RINF and Tet2 in these cells, in their exausthion as in their differentiation.

In case of positive results, the molecular mechanism of RINF and/or Tet2 mediated action will need to be further clarified.

Such clarification will allow to plan combinations of these strategies with other ones that are being proposed for the increase of CAR-T persistance, as c-jun overexpression or TOX and TOX2 DKD. The aim should be to obtain combinatorial effects and avoid uneffective redundacies.





References

- *Disruption of TET2 promotes the therapeuticefficacy of CD19-targeted T cells* A. Fraietta, C. L. Nobles, M. A. Sammons et al. Nature 558 (7709), 307 (2018).
- *Epigenetic regulator CXXC5 recruits DNA demethylase Tet2 to regulate TLR7/9-elicited IFN response in pDCsS*. Ma, X. Wan, Z. Deng et al., The Journal of experimental medicine 214 (5), 1471 (2017)
- *Rinf Regulates Pluripotency Network Genes and Tet Enzymes in Embryonic Stem Cells* M. Ravichandran, R. Lei, Q. Tang et al., Cell reports 28 (8), 1993 (2019).
- *Functional involvement of RINF, retinoid-inducible nuclear factor (CXXC5), in normal and tumoral human myelopoiesis* F. Pendino, E. Nguyen, I. Jonassen et al., Blood 113 (14), 3172 (2009).
- *Chimeric antigen receptor T cell persistence and memory cell formation* A. D. McLellan and S. M. Ali Hosseini Rad, Immunology and cell biology 97 (7), 664 (2019).23 J.
- *TOX and TOX2 transcription factors cooperate with NR4A transcription factors to imposeCD8+ T cell exhaustion* Hyungseok Seo, Joyce Chen, Edahí González-Avalos, D. Samaniego-Castruita, A. Das, Y.H. Wang, I. F. López-Moyado, R. O. Georges, W. Zhang, A.Onodera, C. Wu, L. Lu, P. G. Hogan, A. Bhandoola and A. Rao Proceedings of the National Academy of Sciences Jun 2019, 116 (25) 12410-12415
- *c-Jun overexpression in CAR T cells induces exhaustion resistance*, A. C. Lynn, E.W. Weber, E. Sotillo, D. Gennert, P. Xu, Z. Good, H. Anbunathan, J. Lattin, R. Jones, V. Tieu, S. Nagaraja, J. Granja, C. F. A. de Bourcy, R. Majzner, A. T. Satpathy, S. R. Quake, M. Monje, H. Y. Chang & C. L. Mackall

EXTERNAL IMAGES REFENCES

1. *The Principles of Engineering Immune Cells to Treat Cancer* W. A.Lim, C. H.June, Cell (2017)
2. *Principle of CAR-T cells* Memorial Sloan Kattering Cacer center
3. (EDITED) *Rinf Regulates Pluripotency Network Genes and Tet Enzymes in Embryonic Stem Cells* M. Ravichandran, R. Lei, Q. Tang et al., Cell reports 28 (8), (2019).
4. *Disruption of TET2 promotes the therapeuticefficacy of CD19-targeted T cells* A. Fraietta, C. L. Nobles, M. A. Sammons et al. Nature 558 (7709), 307 (2018).
5. (EDITED) I*nhalation exposure to cigarette smoke and inflammatory agents induces epigenetic changes in the lung* . C. L. Seiler, J. Min Song, D. Kotandeniya, J. Chen, T. J. Y. Kono, Q.Han, M. Colwell, B. Auch, A. L. Sarver, P. Upadhyaya, Y. Ren, C.Faulk, S. De Flora, S. La Maestra, Y. Chen, F. K. & N. Y. Tretyakova Scientific reports (2020)
6. TOX and TOX2 transcription factors cooperate with NR4A transcription factors to imposeCD8+ T cell exhaustion Hyungseok Seo, Joyce Chen, Edahí González-Avalos, D. Samaniego-Castruita, A. Das, Y.H. Wang, I. F. López-Moyado, R. O. Georges, W. Zhang, A.Onodera, C. Wu, L. Lu, P. G. Hogan, A. Bhandoola and A. Rao Proceedings of the National Academy of Sciences Jun 2019, 116 (25) 12410-12415