ARRB1-related vector therapy in T cell Acute Lymphoblastic Leukemia (T-ALL)

Gene Therapy 2020/21

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BACKGROUND

ACUTE LYMPHOBLASTIC LEUKEMIA:

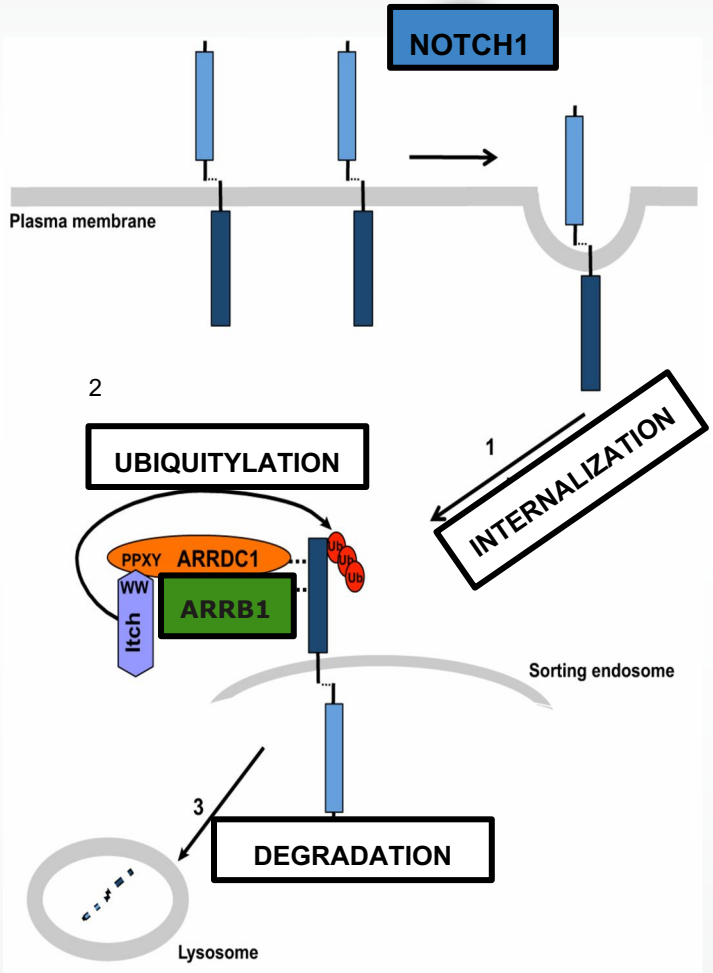
- Tumor characterized by malignant haematopoietic cells (lymphoblasts that couldn't complete the maturation into T-cells)

NOTCH1:

- Mutations occur on >60% of ALL
- Most frequent mutation on *NOTCH1*: JME, HD, PEST

ARRB1:

- Negative regulator of *NOTCH1*
- Oncosuppressor
- It helps in *NOTCH1* ubiquitylation and consequently degradation



Puca & Brou, *Cell Science* 2014

AIM OF THE PROJECT

Combined therapy
‘’*Ex vivo* Gene Therapy + Chemotherapy’’

- Increase the efficiency

- Decrease the side effects due to other therapy like only chemotherapy and allogeneic bone marrow transplant

OBJECTIVES	STRATEGIES
1. Increase the ARRB1 expression in T-ALL patients in order to degrade mut-Notch1	1. Treating patients’ lymphoblasts with Baculoviral vector
2. Repopulate patients blood with lymphoblasts that can complete their maturation	2. Lymphoblasts-treated reinjection into leukemia patients’ blood

TOOLS

BACULOVIRAL VECTOR

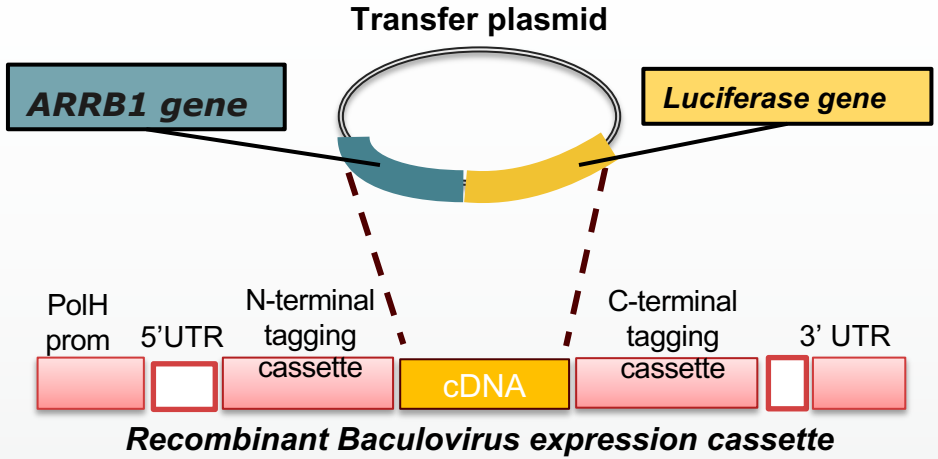
- Low immunogenicity
- Non-pathogenic
- Non-replicating

CELL LINE

- Sf9 cells
- Jurkat Cells

ANIMAL MODEL

- NOD/SCID mice

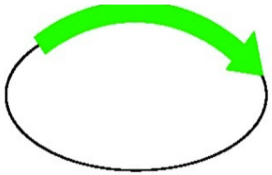


Recombinant Baculovirus expression cassette

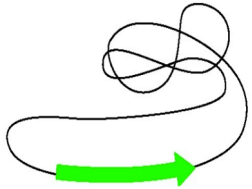
Meyer and Debatin, *Cancer Res Rev.*, 2011

TIMELINE

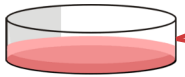
1 Creation of the Baculoviral Vector



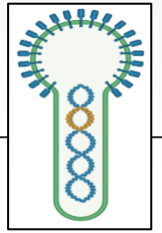
Transfer plasmid containing target genes



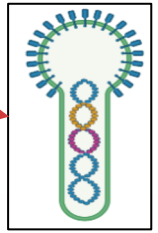
Recombinant baculovirus



Sf9 cells



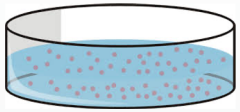
BacLuc



BacLuc+ ARRB1

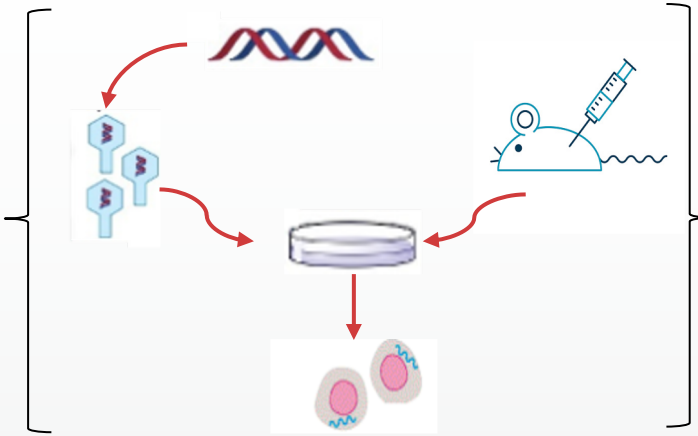
2 Experimental plan

In vitro



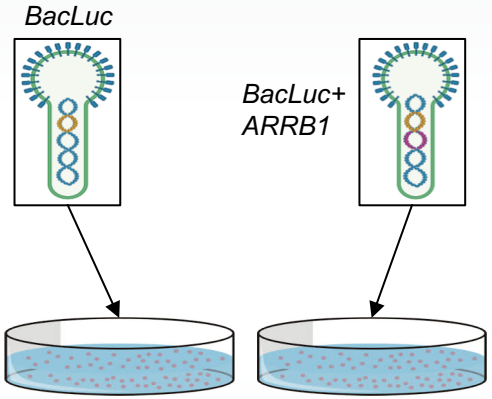
Jurkat cells

Ex vivo

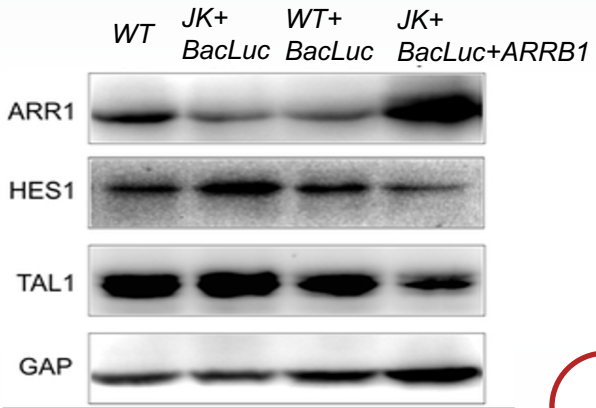


Controls

EXPERIMENTAL PLAN – *In vitro*



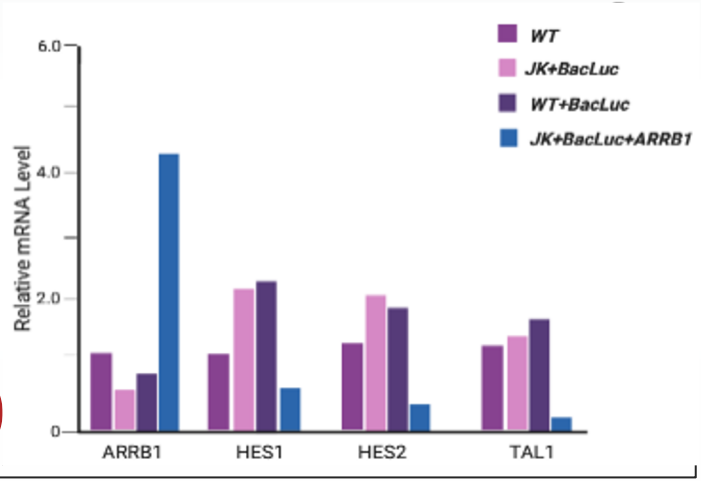
Jurkat cells



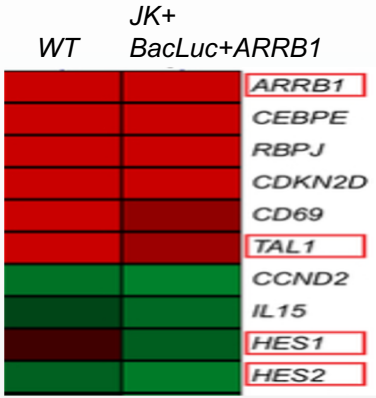
Shu & Wang, *Cancer Res.*, 2020

Western blot

RT-PCR



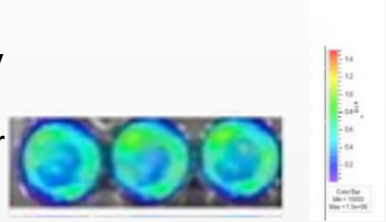
RNA-seq heat map



Shu & Wang, *Cancer Res.*, 2020

1

Luciferase Assay to demonstrate successful vector entry



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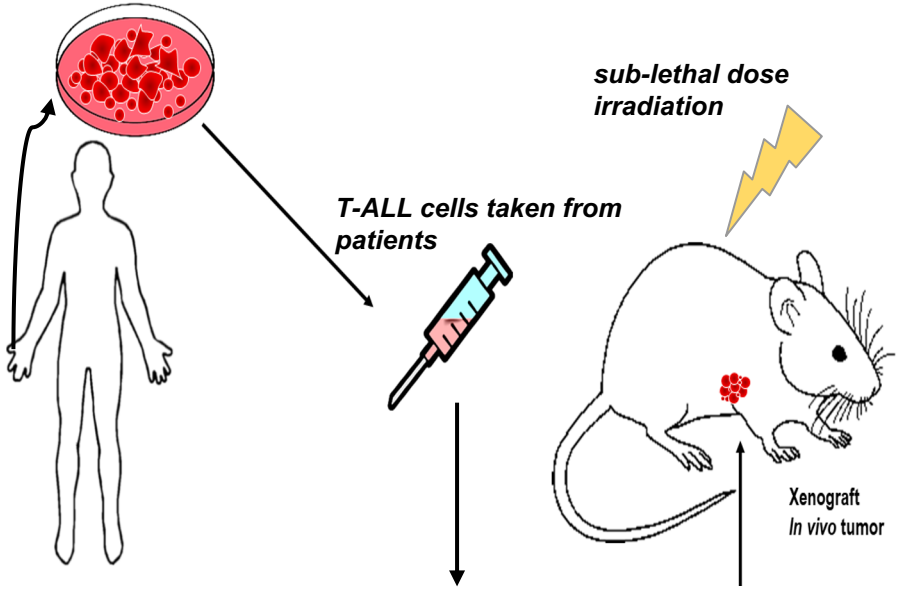
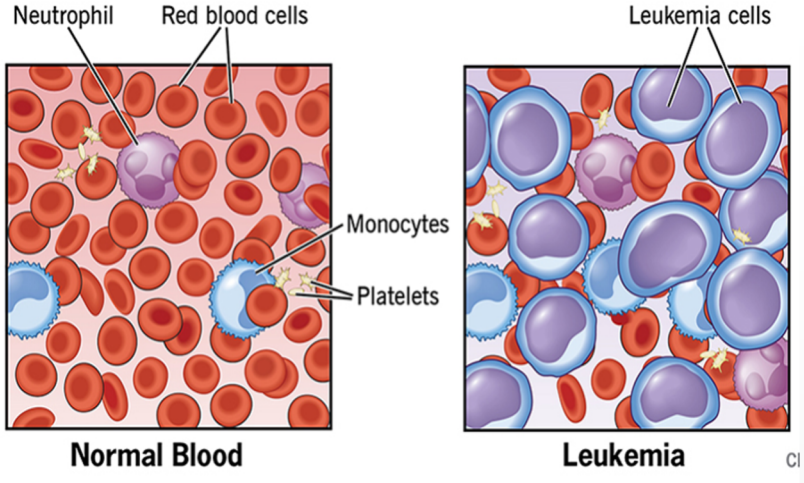
EXPERIMENTAL PLAN:

Induction of the T-ALL in mice

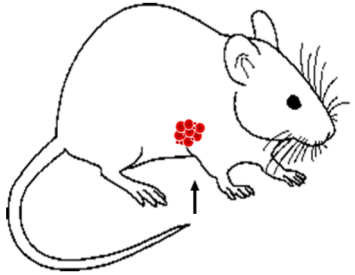


Analysis of blood smears



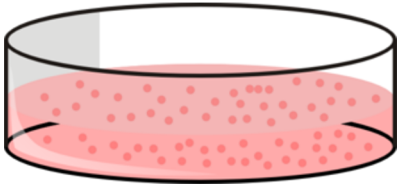
EXPERIMENTAL PLAN:

Isolation of T-ALL lymphoblasts

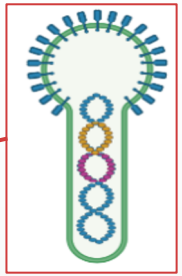


Mice with T-ALL

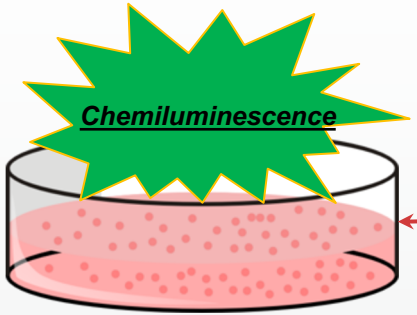
Blood Sample



Isolated T-ALL Lymphoblasts



BacLuc+ARRB1



Luciferase Assay
to check the successful entry of the vector in the cells

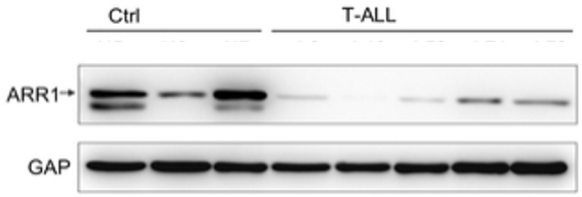
EXPERIMENTAL PLAN:

Ex vivo gene therapy

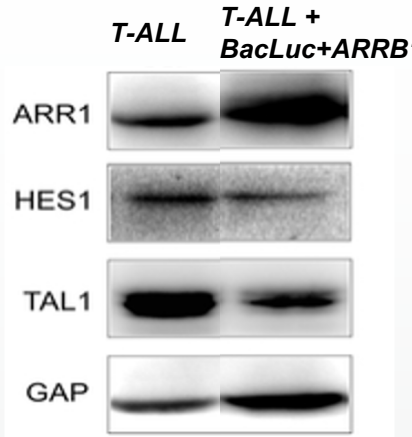
CHECK POINTS BEFORE INJECTION:

1. Western blot

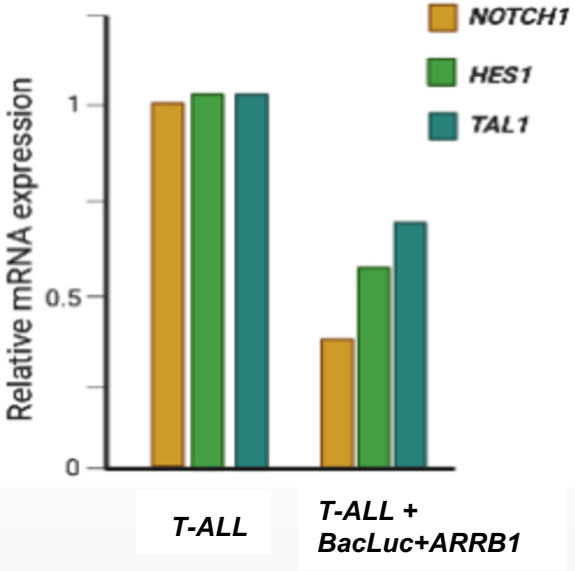
Before treatment



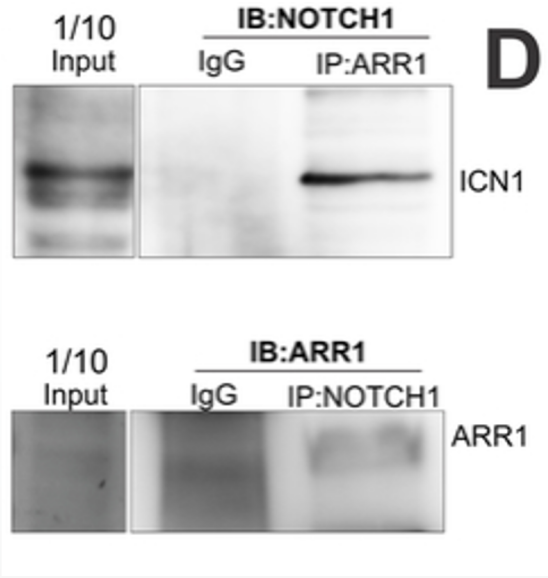
After treatment



2. RT-PCR



3. Co-immunoprecipitation



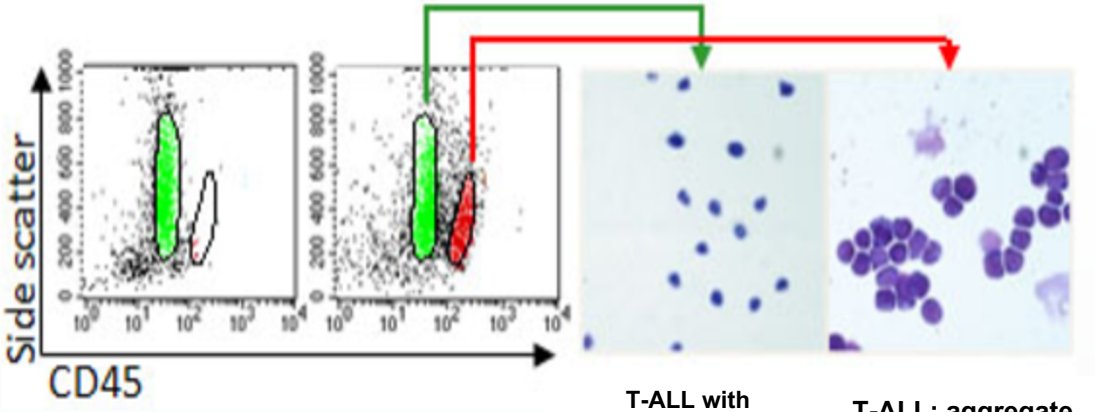
All pictures are adapted from Shu et al. Cancer Research 2020

EXPERIMENTAL PLAN: *Ex vivo* gene therapy

4. Flow cytometry, CCK-8 and Morphological analysis

T-ALL with BacLuc+ARRB1

T-ALL

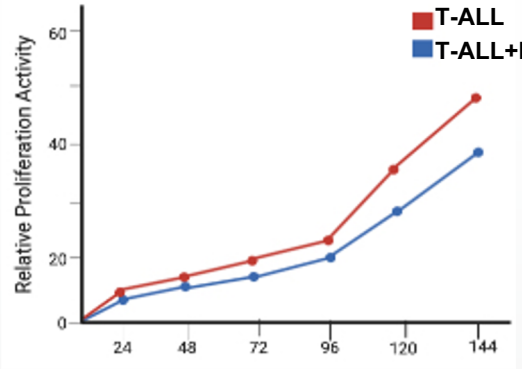


Wei Guo & Hong Wu, *Nature*, 2008

T-ALL with BacLuc+ARRB1: Evenly distributed

T-ALL: aggregate

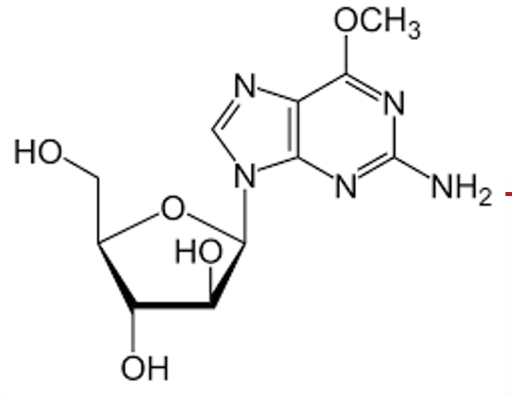
CCK-8



EXPERIMENTAL PLAN:

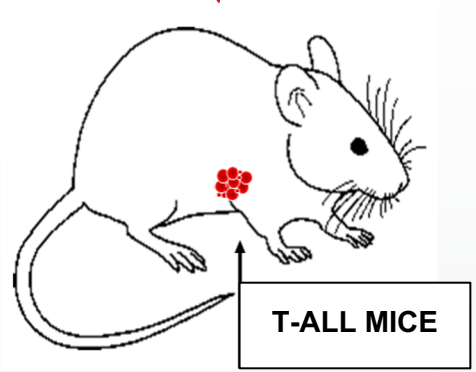
Chemotherapy

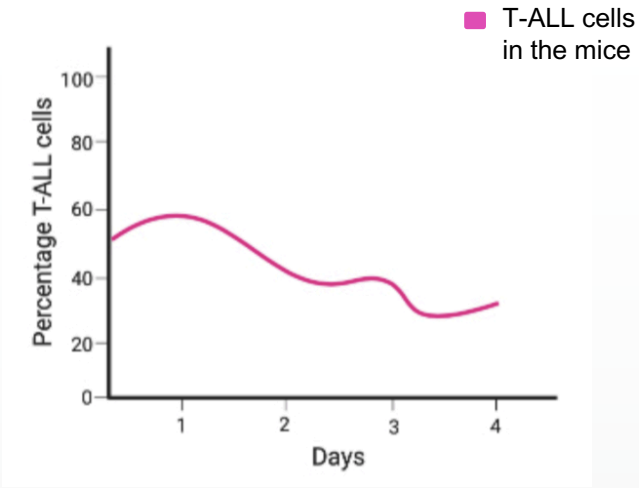
In the meanwhile, T-ALL mice are treated with a specific T-ALL chemotherapeutic drug: ***Nelarabine***



Nelarabine

Intravenous dose:
369 mg/m²

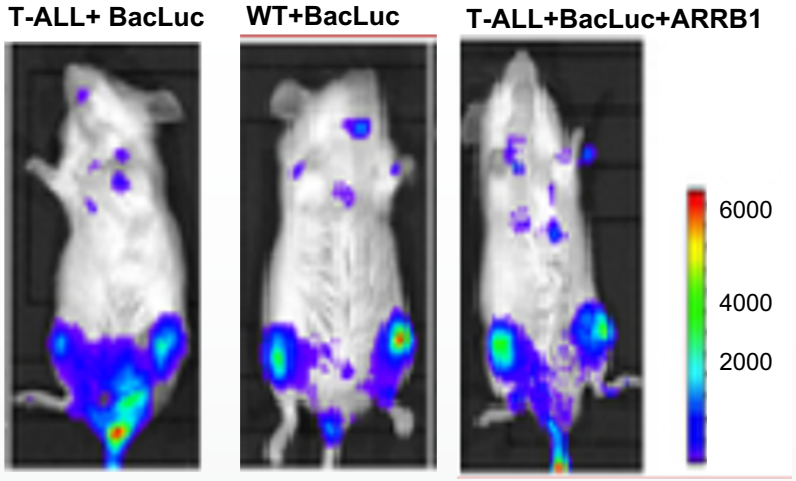




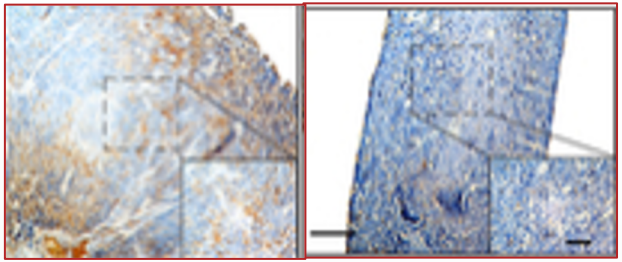
EXPERIMENTAL PLAN: Injection of treated-T-ALL into the T-ALL mice

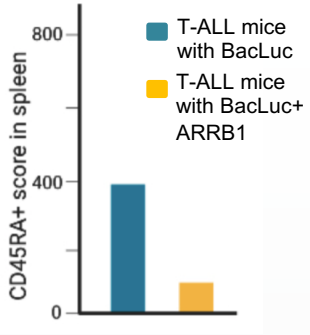
1. Injection of Cells+BacLuc+ARRB1 → **2. Controls**

Luciferase Assay *after 21 days*



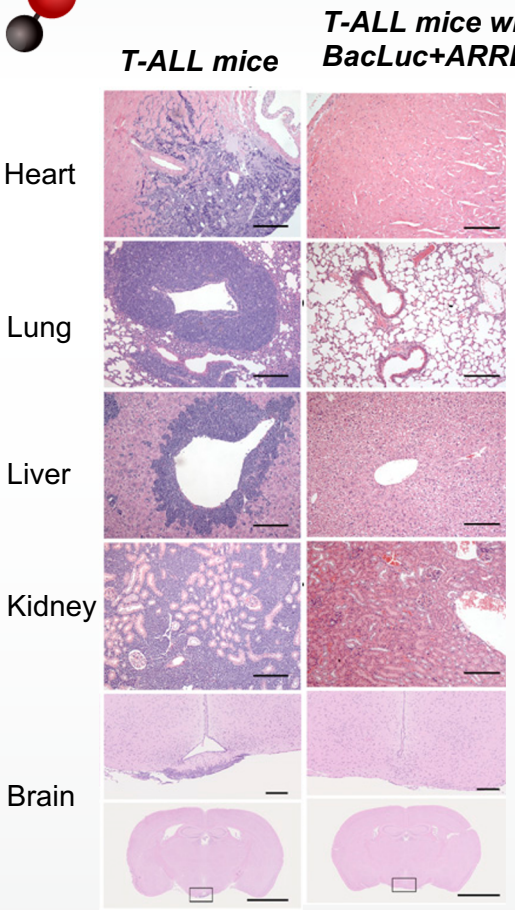
Spleens spliced and stained with ***anti-CD45RA antibody***





Both are adapted from Shu et al. *Cancer Research* 2020

EXPERIMENTAL PLAN:



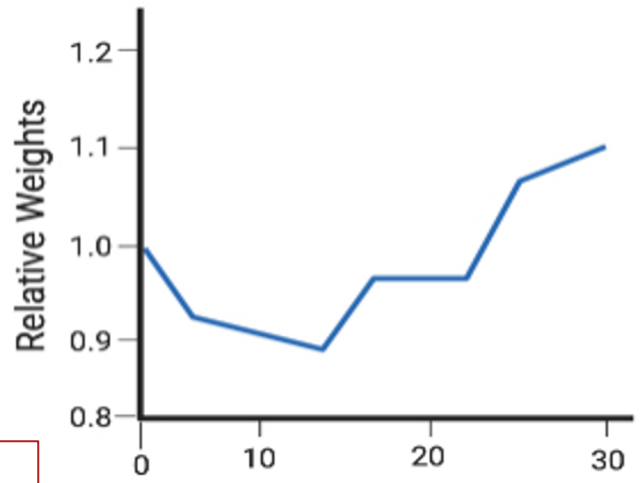
Ballesteros-Arias et al. *J Immuno* 2019

Metastasis

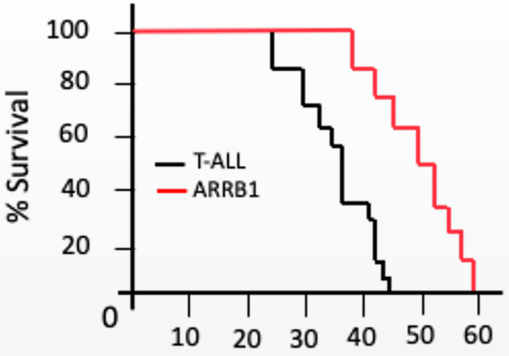
Weights of mice monitored for 4 weeks

Long-term controls

Survival curve



Shu & Wang, *Cancer Res.*, 2020

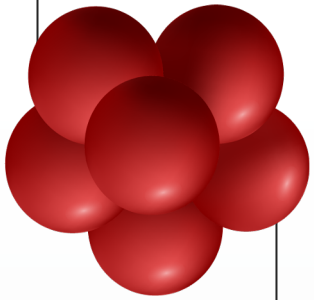


Shu & Wang, *Cancer Res.*, 2020

MATERIALS AND METHODS

VECTORS AND MODELS

- **Viral vectors**: Baculovirus vectors containing *ARRB1* gene with its promoter and Luciferase in order to check if vectors is entired into T-ALL; control vector with Luciferase to evaluate the efficiency of the therapy
- **T-ALL cells** taken from leukemia patients (patients have to sign informed consent)
- **Humanized xenograft models**: immunodeficiency mice NOD/SCID with T-ALL cells taken by leukemia patients
- **Humanized WT NOD/SCID mice**: immunodeficiency mice with T-cells taken from healthy patients (patients have to sign informed consent)



METHODS *EX VIVO*

- **Luciferase** fluorescence assay to check the successful infections of the cells by the BEVS
- **Western blot** is used to highlight the amount of ARRB1 protein into T-ALL cells before and after the baculoviral vectors treatment
- **RNA-seq** to analyse the transcriptome variations and underline the oncosuppressor activity of ARRB1 protein
- **RT-PCR** to evaluate different expression of treated and T-ALL cells measuring mRNA level in the cells; to check degradation of mut-NOTCH1 receptors
- **Flow cytometry** analysis to identify the presence of any cancerous cells before mice injection
- **Co-immunoprecipitation** to demonstrate the interaction between ARRB1 protein and NOTCH1 receptor

PROJECT BUDGET

VIRUS VECTOR PRODUCTION: €1.843,02 / year

- Vector containing ARRB1 and luciferase
- Vector containing Luciferase
- Plasmid DNA Preparation
- Bacmid Generation
- Bacmid DNA Preparation
- Baculovirus packaging

CELL LINE: €595 / year

- Jurkat Cells

ANIMAL MODEL: €1.968 / year

- 20 NOD/SCID mice

SALARIES: €122.121 / year

- 2 post-DOC
- 2-3 PhD

TEST: €16.146,66 / year

- Luciferase
- Western Blot
- RNA Seq
- Q-PCR
- RT-PCR
- Co-Immunoprecipitaion
- CCK8
- Nelarabine

Total: €142.673,68 / year

PITFALLS and SOLUTIONS

Object	Limitations	Improvements
Baculovirus vector	Fragility of Budded Virus	Recombinant baculovirus Construction with a hexahistidine (His6) tag displayed on the viral envelope, which enables virus purification by a simple immobilized metal affinity chromatography (IMAC)
	Inactivation by Serum Complement	Using an *ex vivo* therapy to avoid the serum complement
Gene Target	Incapacity of Recognition between *ARRB1* and Pest domain of *Notch1* due to Pest mutation	More understanding of the Pest Sequence and correction of mutation using Crispr-Cas

CONCLUSIONS

We proved that using *ARRB1*-related Baculoviral vectors increase the *NOTCH1* degradation in the humanized xenograft mice without side effects. Furthermore, analyzing the transcriptome in the T-ALL cells treated, we noticed that genes expression has changed, providing that Notch1 may not activate other oncogenes, such as TAL-1/HES1/HES2, and it may not prevent the lymphocytes maturation.

FUTURE PERSPECTIVES

This therapy could be applied directly to leukemia patients since as proved, by previous experiments, that gene expression has been changed by *NOTCH1* non-degradation in human T-ALL cells (compared to WT T cells). It suggests that increasing the *ARRB1* expression in humans might restore WT conditions.

References

1. Invitrogen life technologies Instruction Manual '*Guide to Baculovirus Expression Vector Systems (BEVS) and Insect Cell Culture Techniques*', Invitrogen Corporation
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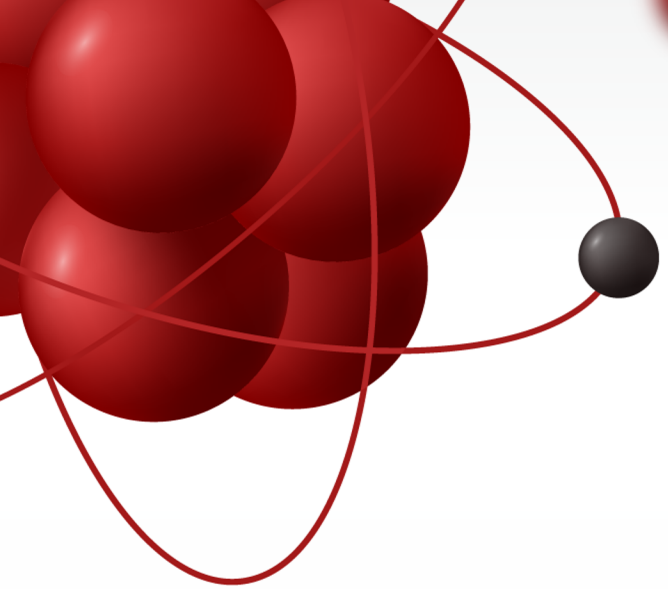
13. "T-cell acute lymphoblastic leukemia as a consequence of thymus autonomy" Luna Ballesteros-Arias, Joana G. Silva, Rafael A. Paiva, Belén Carbonetto, Pedro Faìsca and Vera C. Martines. J immunol 2019; 202: 1137-1144.
14. *'Approval Summary: Nelarabine for the Treatment of T-Cell Lymphoblastic Leukemia/Lymphoma'* Martin H. Cohen, John R. Johnson, Tristan Massie, et al. Clin Cancer Res 2006;12:5329-5335.



THANK YOU
for your attention!