CML cells expressing IL-1RAP can be targeted by Chimeric Antigen Receptor–Engineered T Cells (CAR T-cells)

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INTRODUCTION

In CML, gene expression profiling studies have revealed a cell-surface biomarker, **IL-1 receptor accessory protein (IL-1RAP, IL-1R3), that is expressed by the leukemic but not the normal CD34+/CD38- hematopoietic stem cell (HSC)** compartment. Moreover, **IL1RAP expression has been correlated with tumor burden and the clinical phase of CML**. The tumor cell-surface expression makes **IL1RAP an ideal candidate to target and eradicate CML HSCs**, which are thought to be the origin of relapse. Thus, IL-1RAP is a promising cell-surface tumor-associated antigen for targeting with lower toxicity and higher efficacy in immunotherapy approaches, such as CAR T-cells. We hypothesized that **T-cells expressing a CAR directed against IL1RAP may eliminate leukemic stem cells**. Here, **we describe the proof of concept and the preclinical evaluation of an IL1RAP CAR in the context of CML**.

RESULTS I

1.Production of IL-1RAP CART-Cells.

The CAR lentiviral construct (pSDY-iC9-IL1RAPCAR-dCD19) was prepared by cloning the synthetically produced single-chain fragment variable (scFv; derived from the #A3C3 IL1RAP hybridoma, DiacloneSA, Besançon, FRANCE) into the SIN-pSDY backbone (***Fig.1A***). Lentiviral vector supernatant (SN) stock was harvested at 48 and 72 hours from subconfluent transfected 293T cells and overnight soft spin centrifugation. **Activated T cells were established from healthy donors or patients and transduced with lentiviral SN encoding the IL-1RAP CAR or Mock sequence** (missing the CAR sequence). **The *in vitro* production process with lentiviral SN allowed for 82.4% to 78.4% transduction of primary T-cells *(Fig.1B et C)***.

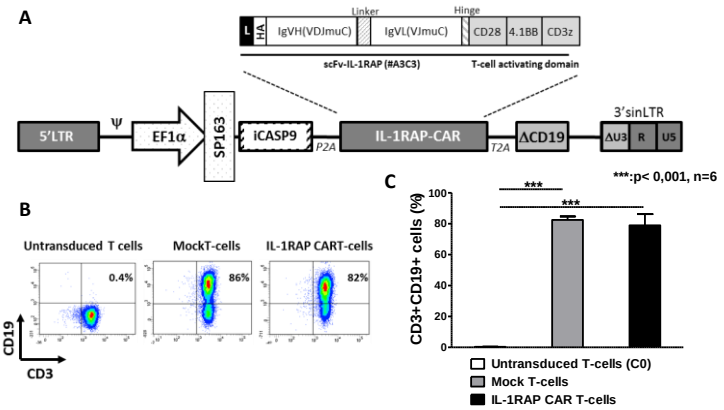

Figure 1 : Generation of IL1RAP CAR–expressing gene-modified T-cells.
A/ Lentiviral construct carrying the IL-1RAP CAR. ***B/*** Representative cytometry plot after CD3+/CD19+ staining of non transduced (C0), Mock-T, and IL-1RAP CAR T-cells. ***C/*** Results are presented as mean SD of 6 independent transductions of 6 different donor PBMCs (bottom right) using 100X concentrated SN.

RESULTS II

2. Inducible casp9 (iCASP9) safety.

In order to overcome potential toxicity of IL-1RAP CAR T-cells, **we demonstrated the functionality of the iCASP9/AP1903** (Rimiducid®) suicide system,**(*Fig.2A*)** included in our CAR construct (***Fig.1A*)**, *in-vitro* ***(Fig.2B)*** but also *in-vivo* in a NSG xenograft model (***Fig.2C,D*)** that, **when activate, is able to eliminate more than 90% of activated CART-cells, after 24hours of treatment with AP1903**.

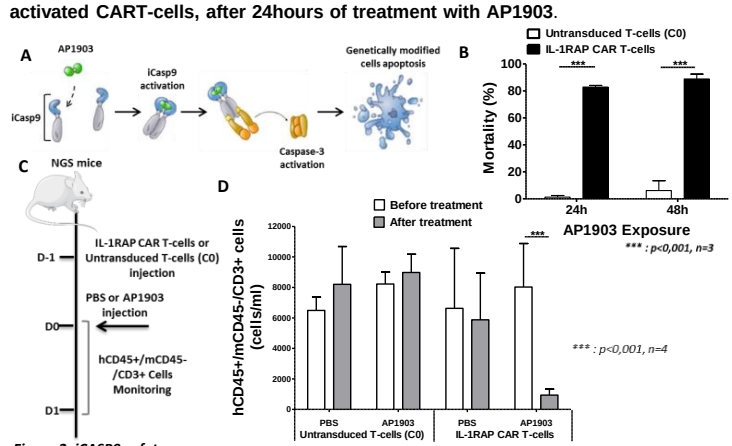

Figure 2: iCASP9 safety.
A/ *Icasp9 mechanism.* ***B/*** *Activation of iCASP9 in vitro.* ***C/*** *Activation of iCASP9 in vivo protocol.* ***D/*** *Activation of iCASP9 in vivo.*

RESULTS III

3. Functionality of IL-1RAP CAR T-cells.

The proliferative capability of IL-1RAP CAR T-cells triggered by IL-1RAP target-expressing cells was determined by cocultured (E:T 1:1) CFSE-stained (C0), Mock, or IL-1RAP CAR T-cells in the presence of K562, or KU812 cells. **Effector IL-1RAP CAR T-cells divided significantly only in response to the presence of cell surface IL1RAP expressing and KU812 cells, and divided at a lowest levels against K562 (*Fig.3A*)**. IL1RAP dependent cytolytic potency of IL1RAP CAR expressing T-cells in vitro was determined using fluorescent (eFluor) and 7-AAD staining to discriminate CAR T-cells and living cells, respectively. As expected, coculture at an E:T ratio of 1:1 and compared with C0 or Mock T-cells revealed **significant lytic activity characterized by the disappearance of cells in the 7-AAD-/eFluor- gate between IL-1RAP+ (KU812) target cells and IL-1RAP- (K562) target cells (*Fig.3B*)**. In a tumor xenograft murine model (***Fig.3C***), following tumor engraftment (D4), **IL-1RAP CAR T-cells (E:T 1:1) were allowed to target CML KU812/Luc+ (i.v) tumors until a decrease in size**. In contrast, we noticed tumor progression in untreated or Mock-T treated mice. Notably, tumors continued to grow in the absence of CAR T-cells in surviving mice in the untreated and Mock-T treated groups (***Fig.3D***).

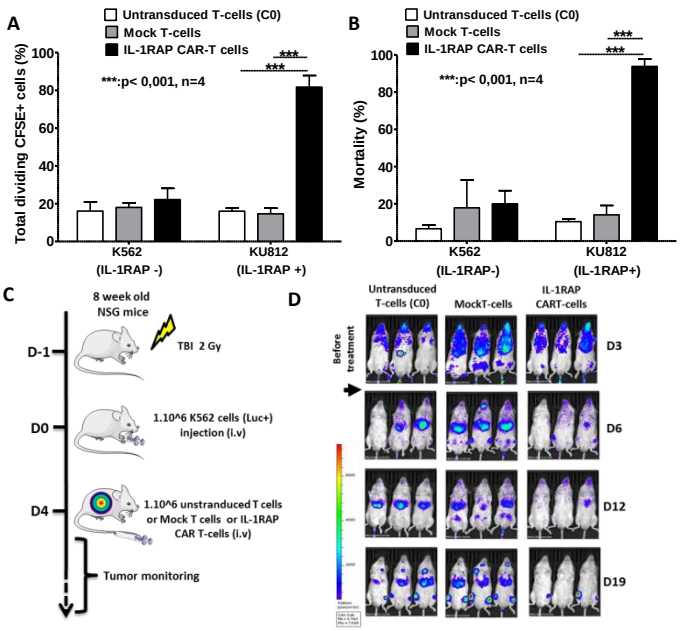

Figure 3 : Evaluation of proliferation and cytoxicity of IL-1RAP CAR T-cells in vitro and in vivo.
A/ Non transduced (C0) and Mock or IL-1RAP CAR-transduced T-cells were cultured with k562 or KU812 at an ratio E:T of 1:1. Effectors were labeled with 0.5 mmol/L CFSE. After 48 measurement of CFSE dye dilution allowed assessment of the division of live CD3+/CD19+ gated cells. ***B/*** Effectors, labeled with e-Fluor, were cultured at an ratio E:T of 1:5 in the presence of IL-1RAP+ or - target cells and the percentage of total killed target cells is determined. ***C/*** *Cytocycity in vivo protocol.* ***D/*** *In vivo tumor monitoring after xenograft (i.v) by KU812 and injection of CO, Mock T-cells or IL-1RAP CAR T-cells.*

CONCLUSION

This preclinical work demonstrates for the first time the whole production and validation process of CAR T-cells directed against IL-1RAP-expressing CML stem cells, from the development and characterization of the mAb to the final in vitro and in vivo functional studies of gene-modified CAR T-cells. Taken together, these findings based on the CML model make **IL-1RAP an interesting tumor-associated antigen for immunotherapy cell targeting using CAR T-cells.** In-depth studies are required to determine and/or reduce potential toxicities and side effects before phase I clinical trials.

This work was the subject of a patent application and a publication in American journal for Cancer research (Warda et al., 2019)[1].

This experimental approach may be applied in all CAR T-cells immunotherapies approaches developed at UMR1098.

[1]Cancer Res. 2019 Feb 1;79(3):663-675. doi: 10.1158/0008-5472.CAN-18-1078.