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

Mathieu Nerto-Da-Rocha1, Walid Warda1, Rim Trad1, Maxime Frona1, Sabeha Bichile1, Fabrice Larosa2, Etienne Daugudiau2, Francine Garnache-Ottou2, Olivier Adotevi2, Marina Deschamps2 & Christophe Ferrand1

1 INSERM UMR1098, EFS BFC, University of Bourgogne Franche-Comte, Besançon, France.
2 Department of Hematology, University Hospital of Besancon, Besancon, France.

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, IL-1RAP expressing cells has been correlated with tumor burden and the clinical phase of the CML. The tumor cell-surface expression makes IL-1RAP 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.

A lentiviral construct carrying the IL-1RAP CAR was generated (pSDF-G3-IL1RAPCAR-G3CD19) by transducing the synthetic scFv fragment (scFv: derived from the CH1 domain of the anti-CD38 812 monoclonal antibody) into the SIN-Pseudotyped virus backbone (Fig. 1A). Lentiviral vector supernatant (SN) was harvested at 48 and 72 hours from subconfluent transduced 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 62.4% 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+ (CD3+) staining of non transduced (C0), Mock-T, and IL-1RAP T-cells. C) Results are presented as mean SD of 6 independent transductions of 6 different donor PBMCs (both 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/IPAP1903 (Rimiducid/9i) suicide system (Fig. 2A) included in our CAR construct (Fig. 1A), in vitro (Fig. 2B) but also in vivo in a NOD_SCID/IL2Rγc−/− mouse model (Fig.2C,D). In this study, iCasp9 was shown to increase by more than 90% of activated CAR-T-cells, after 24 hours of treatment with IPAP1903.

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 tumor cells determined by coculture (ET 1:1) CFSE-stained (C0), Mock, or IL-1RAP CAR T-cells in the presence of K562 or KUB12 cells. Effectors IL-1RAP CAR T-cells divided significantly only in response to the presence of cell surface IL1RAP expressing and KUB12 cells, and divided at a lower levels against K562 (Fig. 3A). IL-1RAP dependent cytokine potency of IL-1RAP CAR expression in vitro was determined using fluorescent (effluor) and 7-AAD staining to discriminate CAR T-cells and living cells, respectively. As expected, coculture at an ET 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/-Fluor- gate between IL-1RAP + (KUB12) target cells and IL-1RAP - (K562) target cells (Fig. 3B). In a xenograft murine model (Fig. 3C), following tumor engraftment (D4), IL-1RAP CAR T-cells (ET 1:1) were allowed to target CML KUB12/Luc+ (luc) 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 cytotoxicity of IL1RAP CAR T-cells in vitro and in vivo. A) Non transduced (C0) and Mock or IL-1RAP CAR-T-cells were cultured with K562 or KUB12 at an ratio E T of 1:1. Effector were labeled with 0.5 mmol/L CFSE. After 48 hours, the cells were stained with 7-AAD and propidium iodide. (C) Representative cytometry plot after CD3+ (CD3+) staining of non transduced (C0), Mock-T, and IL-1RAP T-cells. C) Results are presented as mean SD of 6 independent transductions of 6 different donor PBMCs (both right) using 100X concentrated SN.

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