Production pré-Clinique de CAR-T cells IL-1RAP "GMP-like" en vue d'un essai Clinique de phase I Expérience Bisontine

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24 novembre 2021

EFS Bourgogne Franche-Comté Unit INSERM_UMR1098
Host-Tumor-Graft Interaction and Cellular and Genetic Engineering
Immuno-Molecular Cancer Therapeutics Team(TIMC)

Conflict of interest

- **Founder of CanCell Therapeutics**
- **Director manager of CanCell Therapeutics**

Brief information of Acute Myeloid leukemia (AML)

AML is an aggressive clonal expansion of myeloid blasts in bone marrow, blood or tissue
80% of adult acute leukemia and 3% of all cancers

(I De Kouchkovsky et al, blood cancer journal 2016)

- **Conventional Chemotherapy** (Cytarabine, Daunorubicine, idarubicine, mixantrone)

  (M. W. McCarthy et T. J. Walsh, Curr. Oncol. Rep., mars 2017)

- **Targeted therapy** (FLT3, BCL2 inhibitors)

  (Leick, M.B. and M.J. Levis, Curr. Hematol. Malig Rep., April 2017)
  (Souers, A.J., et al., Nat Med, 2013)

- **Cellular therapy** (Stem cell transplantation)

  (I De Kouchkovsky et al, blood cancer journal 2016)

- **Immunotherapy :**
  - **Monoclonal antibodies** (CD45-CD33…)
  - **Bifunctional antibodies** (CD3-CD33…)
  - **Checkpoints inhibitors** (CTLA-4, PD-1/PDL-1, TIM3, LAG3)
  - **Vaccines** (WT1)
  - **Dendritic cells**
  - **CTLs** (PR3)
  - **Transgenic TCR**
  - **Chimeric Antigen Receptors** (CAR-CD123, CAR-CD33…)

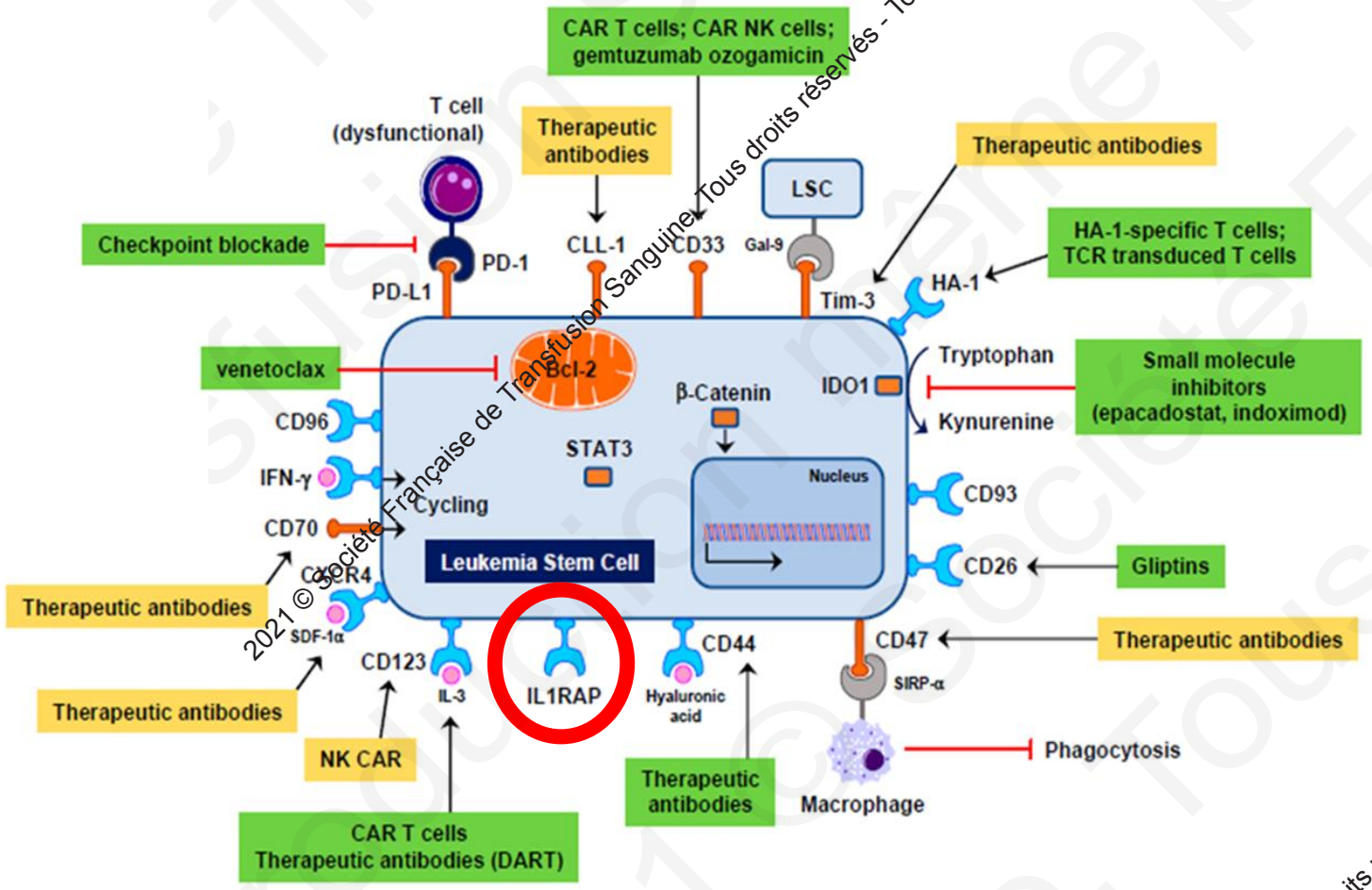
(Dolores A Grosso et al, Semantic Scholar, Cancer, 2015)

- **Relapse in 30 to 80%** of cases for patients not receiving allograft
  - Clonal heterogeneity of the LSC
  - Targets expressed non exclusively by Leukemic cells
  - Escape to the immune system

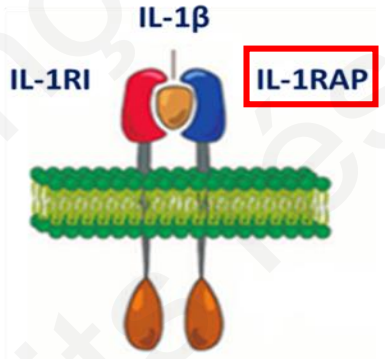
  → **Need to target leukemic stem cells (LSC) by affecting the least possible healthy tissues**

(Fumihiko Ishikawa, RIKEN Research, 2010)

IL-1RAP as target on AML leukemic stem cells



(K.Tasian et al, biomedicines,2018)



Interleukin-1 Receptor Accessory Protein

- **IL-1RAP is overexpress at the cell surface of Leukemic Stem Cell (AML, MDS, CML).**
  Jaras et al. PNAS 2010; Askmyr M et al. Blood 2013
- **IL-1RAP is not expressed in healthy tissue.**
  Zhang et al, Cancer Discovery, 2021
- **IL-1RAP potentiates multiple oncogenic signaling pathways in AML and promote leukemia.**
  Mitchell K et al. JEM 2018; De Boer et al, Haematologica 2020
- **KO of IL-1RAP inhibits Cancer (stomach carcinoma).**
  Qing et al, Tech Cancer Res 2021
- **Today, poorly targeted, excepted by using a monoclonal antibody in solid tumors.**
  clinical.gouv NCT03267316

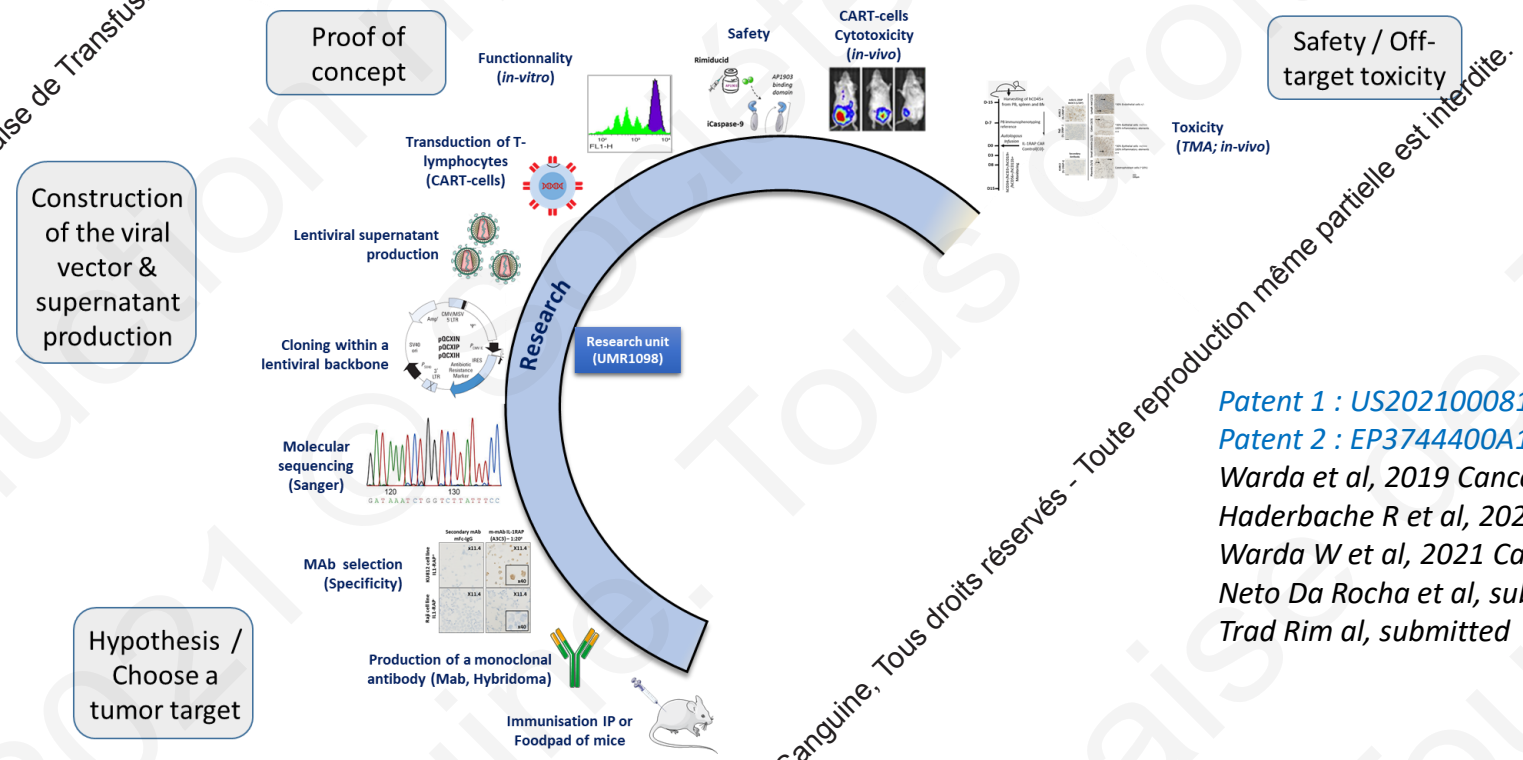
Research step: from hypothesis to the proof of concept

Based on Besançon UMR1098 (France) experience

Stages of development of CAR-T cells immunotherapy

Research *From hypothesis to the proof of concept (2014 to 2020)*

Validated



Patent 1 : US20210008108A1, field in 2017:11
Patent 2 : EP3744400A1, field in 2019/05
Warda et al, 2019 Cancer Res.
Haderbache R et al, 2021 J Transl Med
Warda W et al, 2021 Cancer Gene Ther
Neto Da Rocha et al, submitted
Trad Rim al, submitted

Preclinical step: Transfer of production process and up-scale

Based on Besançon UMR1098 (France) experience

Stages of development of CAR-T cells immunotherapy

Research

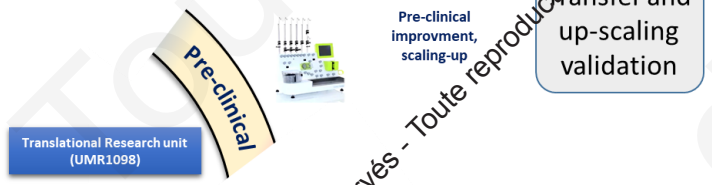
Validated

From hypothesis to the proof of concept (2014 to 2020)

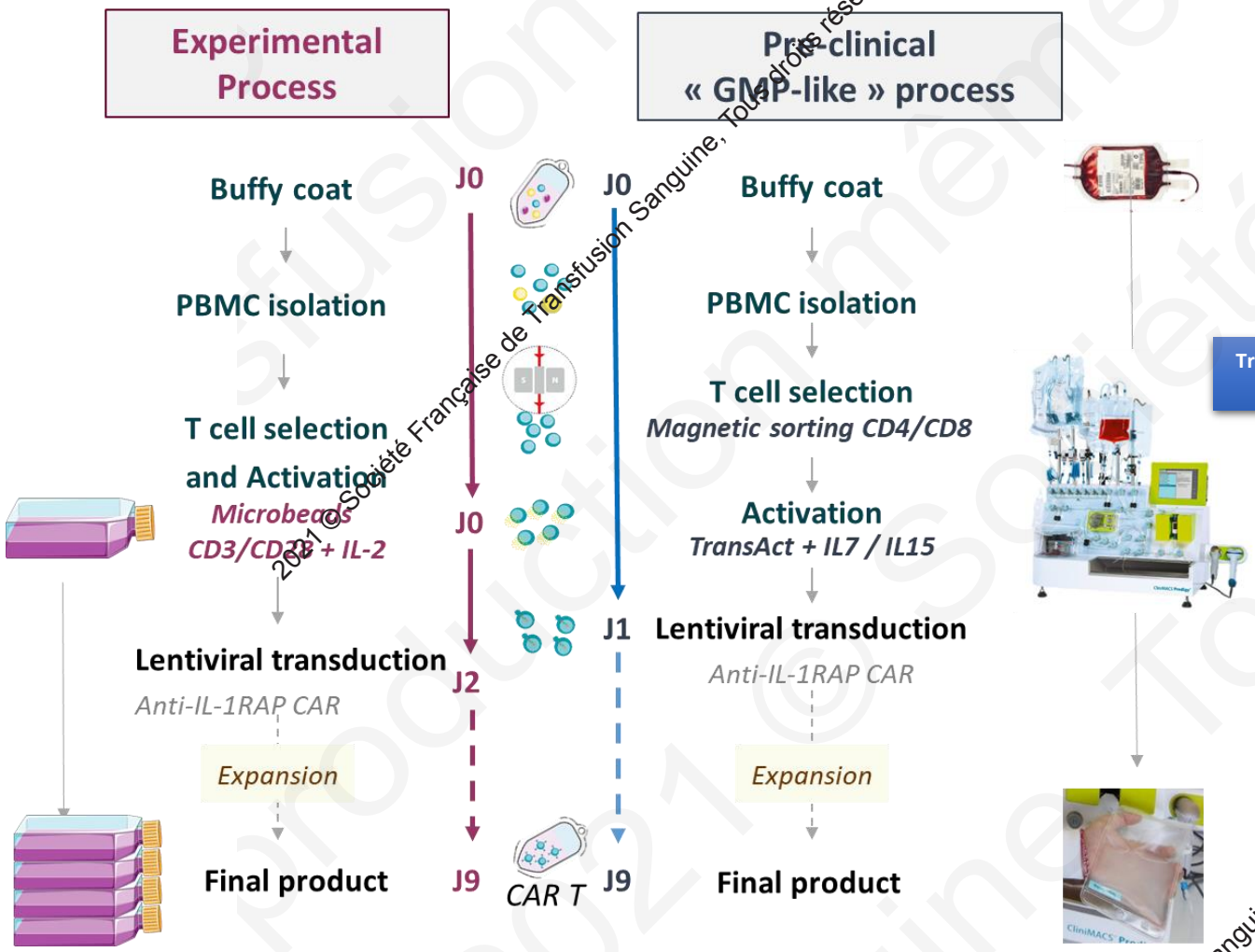
Pre-clinical development

On going

Preclinical « GMP like » process validation
Regulatory dossier – Awaiting GMP raw material



Preclinical step: Transfer of production process and up-scale



Outside clean room of pharmaceutical unit

- Transfer & up-scale all the process to GMP condition
- Automated and closed system production
- Characterize the final product
- Determine & validate QC delivery

Translational Research unit (UMR1098)

Pre-clinical

Pre-clinical improvment, scaling-up

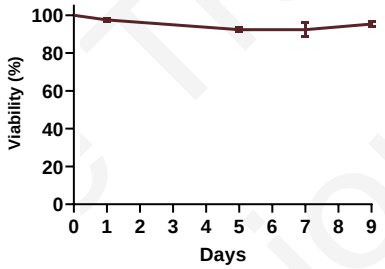
Transfer and up-scaling validation

Final product analysis

- **Viability** *(Flow cytometry, FvD)*
- **Expansion Fold** *(Trypan blue count)*
- **Transduction efficency** *(Flow cytometry)*
- **Cell count** *(Trypan blue count)*
- **Composition** *(Flow cytometry)*
- **Transgene copy number** *(ddPCR)*
- **Ratio CD4/CD8** *(Flow cytometry)*
- **T cell subpopulation Immunophenotyping** *(Flow cytometry)*
- **Exhaustion marker expression** *(Flow cytometry)*

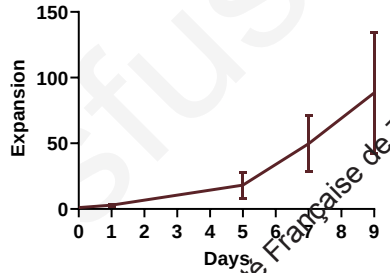
IL-1RAP CART-cells characterization in Final product:

Final product viability



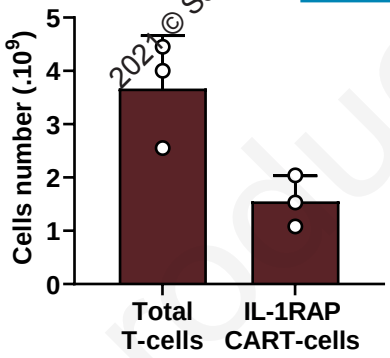
→ Viability of cells **>95%** (95,00±1,15%) in final product at day 9.

Cells expansion



→ Strong cell expansion: **88,394±46,323 expansion factor**

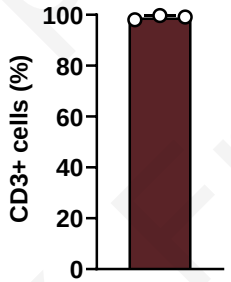
Cell count



→ Number of CART-cells: in final product: **1,55±0,48 X 10^9 cells**

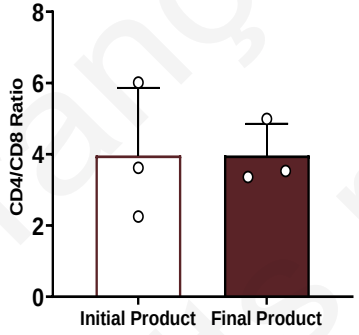
n=3

CD3+ purity



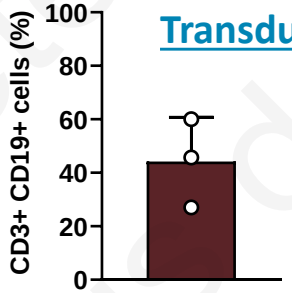
→ Product with high **CD3+ cell purity (98,93±0,86%)**

CD4/CD8 ratio



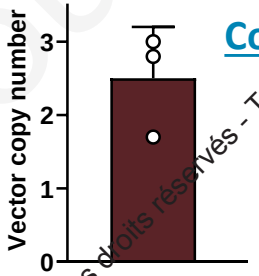
→ **CD4/CD8 ratio maintained** during the production process (3,96±1,90 vs 3,96±0,89)

Transduction efficiency



→ Transduction efficiency: **44,23±16,55%.**

Copy of transgene

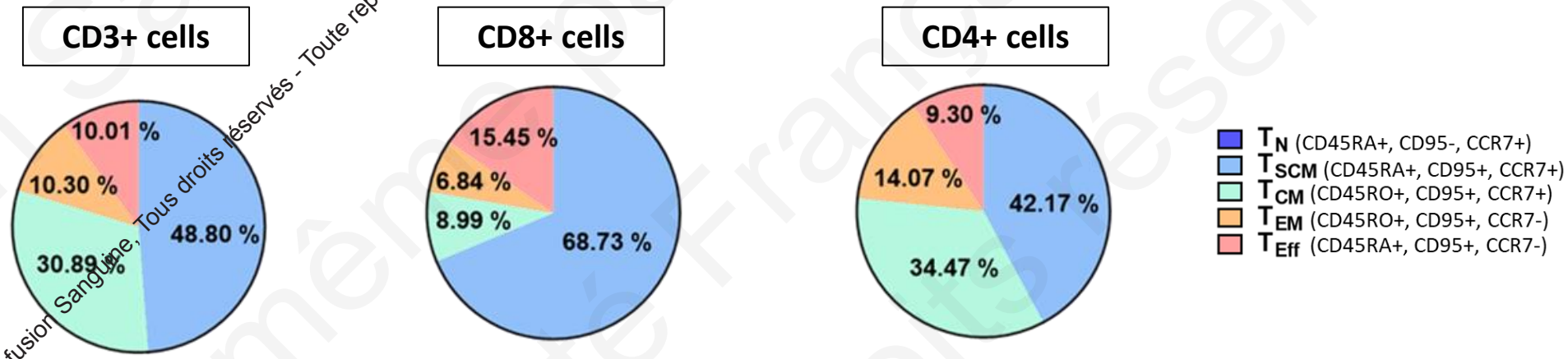


→ Number of transgene copies per cells: **2,50±0,70**

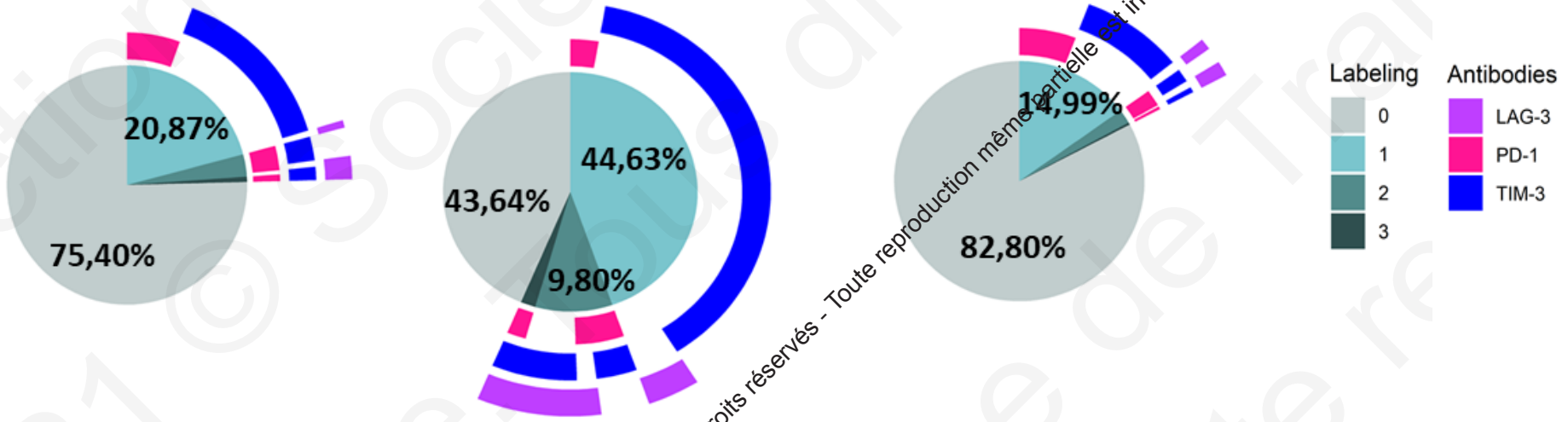
→ All parameters are in agreement for clinical application in a phase I/IIa clinical trial

Phenotypic profile of IL-1RAP CART-cells in Final product

Immunophenotyping (Memory/effector profile)



Exhaustion markers expression

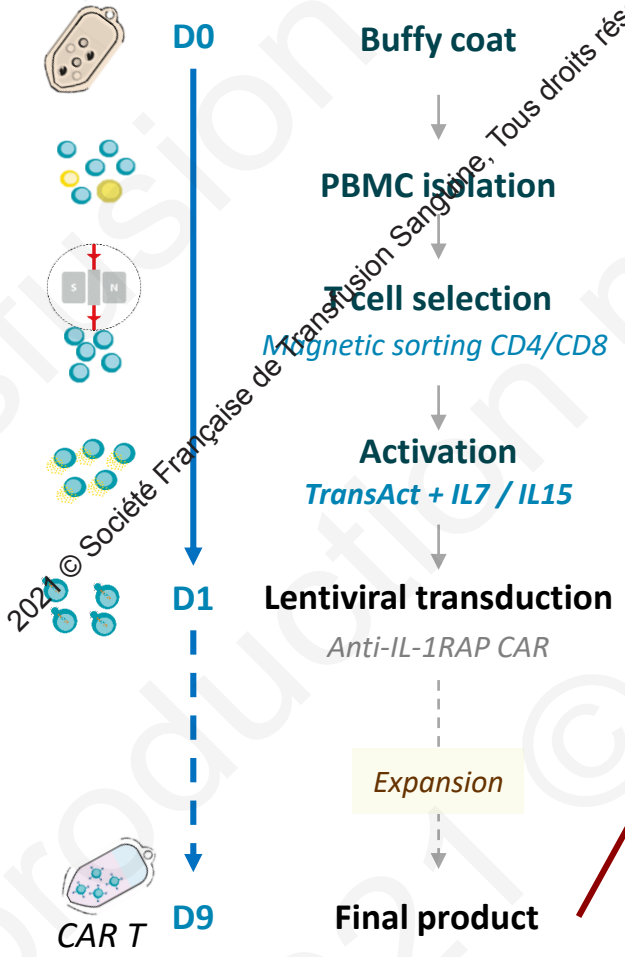


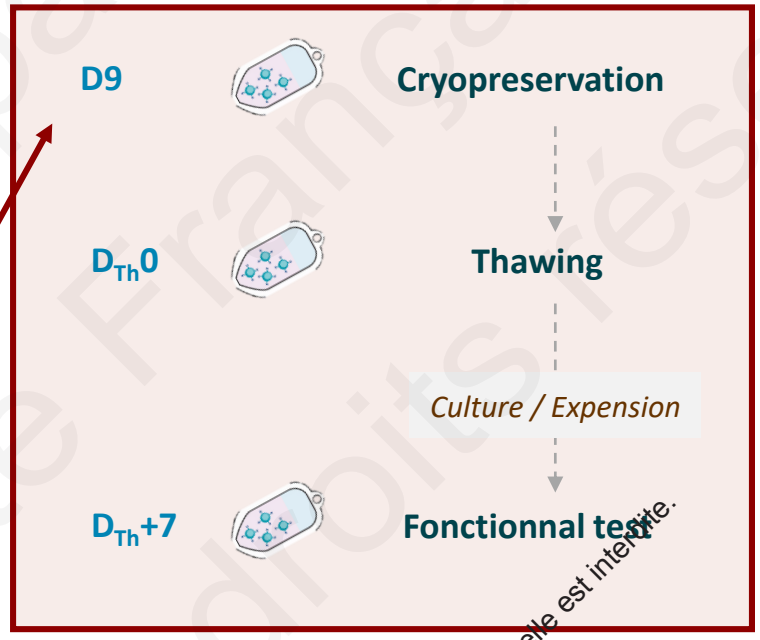
n=3

→ **The final product shows mainly a memory profile and a low expression of exhaustion markers.**

Functional validation of CART cells « GMP-Like » post thawing

Pre-clinical « GMP-like » process

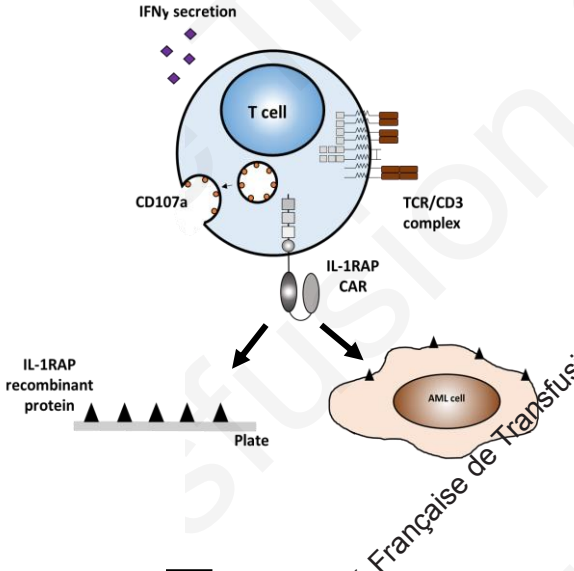




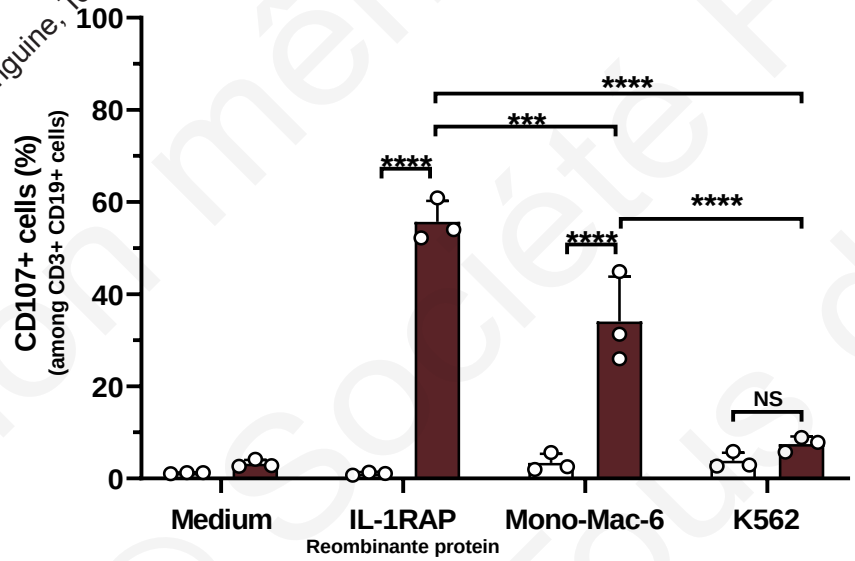
- **Degranulation : CD107a expression** (Flow cytometry)
- **IFNγ secretion** (Elisa)
- **Cell cytotoxicity** (Flow cytometry)
- **Metabolism analysis** (Seahorse)
- **Transcriptomic analysis** (Nanostring)
- **In-vivo cytotoxicity** (Xenographted NGS model)

Functional study : CD107a expression and IFNy secretion against leukemic cells

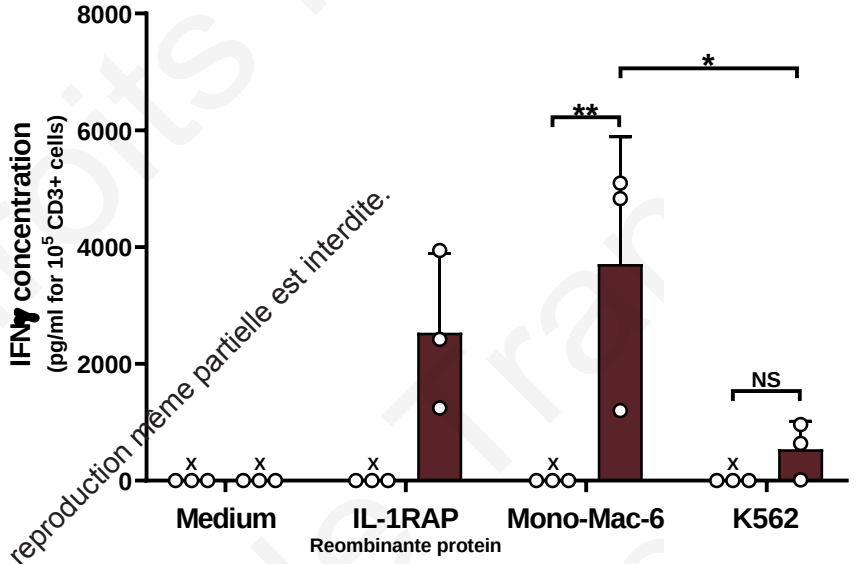
Stimulation with target : 6h
Mono-Mac-6 cell line : IL-1RAP +
K562 cell line : IL-1RAP -



Degranulation CD107a assay



IFNy assay

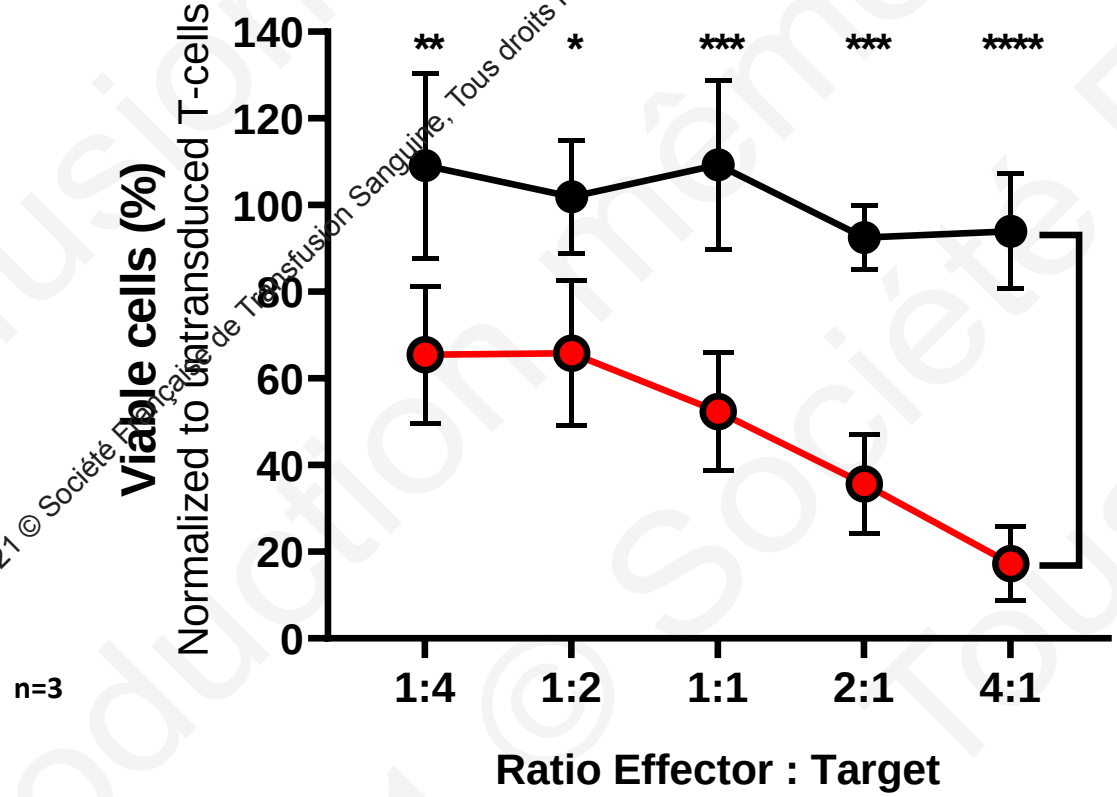


→ **IL-1RAP CART-cells degranulate and secrete IFNy after IL-1RAP target exposure**
(coated recombinant IL-1RAP Protein or co-culture with AML leukemic cell line)

Functional study : Cytotoxicity of IL-1RAP against leukemic cells

Co-culture: 24h
Mono-Mac-6 cell line : IL-1RAP +
K562 cell line : IL-1RAP -

Cytotoxicity
7-AAD staining



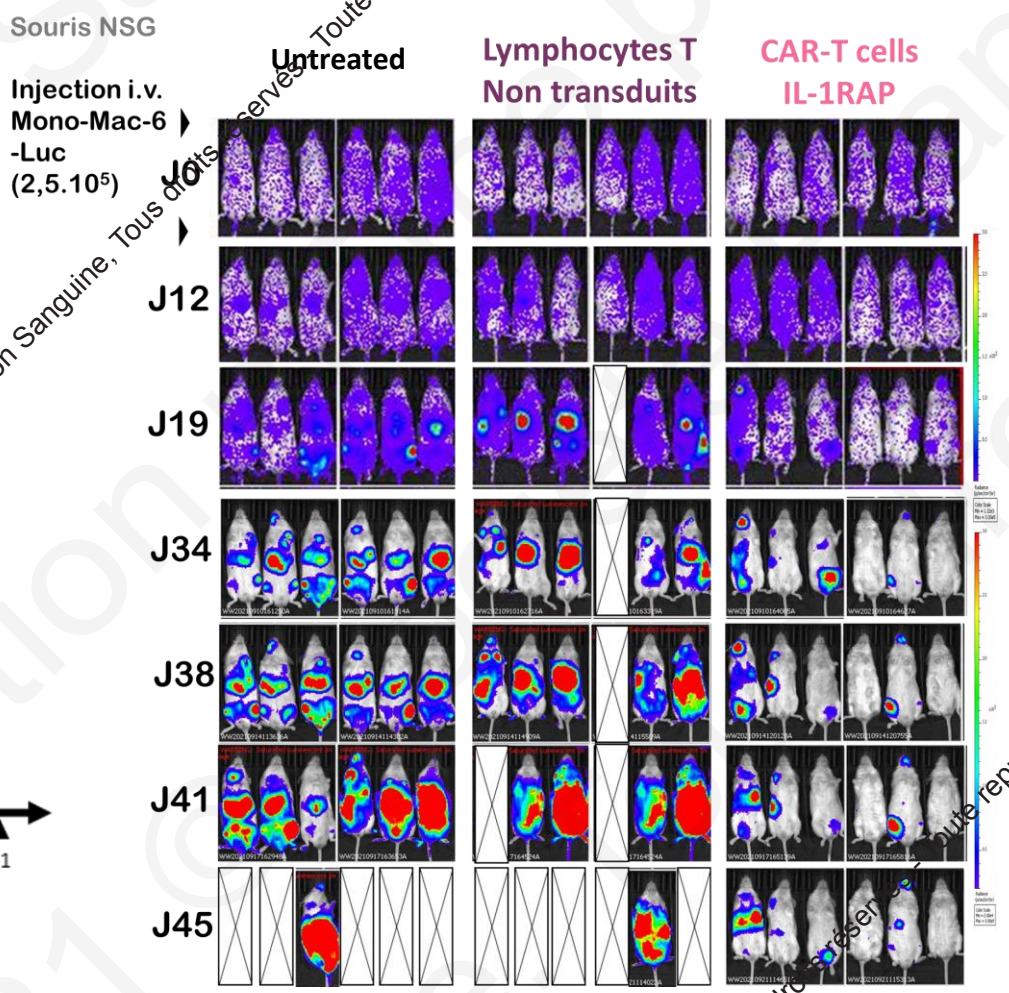
n=3

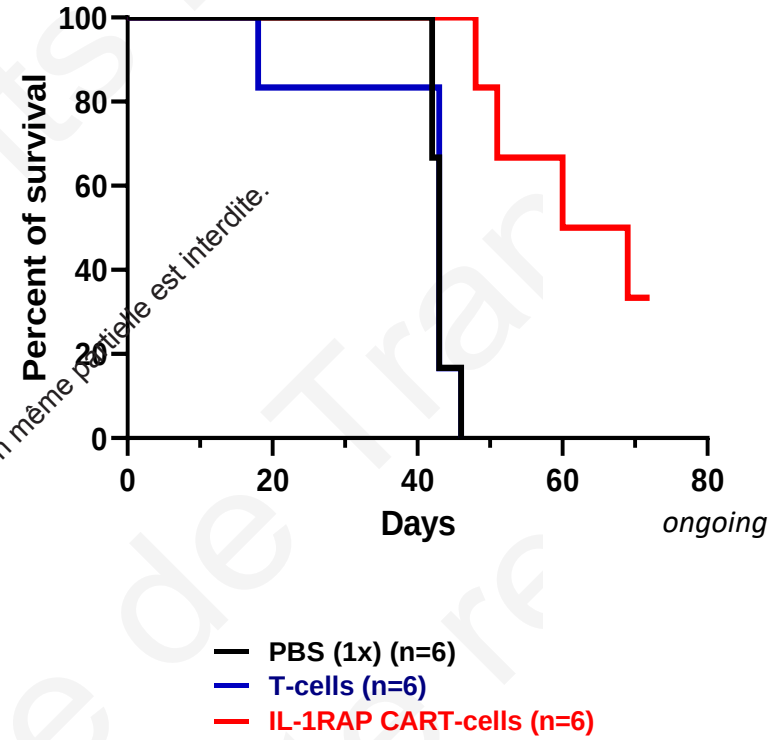
IL-1RAP CART-Cells vs K562
IL-1RAP CART-Cells vs Mono-Mac-6

n=3, **** P < 0.0001, *** P < 0.001,
** P < 0.01, * P < 0.05

→ **IL-1RAP CART-cells eliminate AML leukemic cells even at low Effector : Target ratio**

Functional study: xenograft murine model *(On going)*





→ **IL-1RAP CART-cells eliminate AML leukemic cells in xenograft murine model**

CanCell Therapeutics
Innovative Med

Stages of development of CAR-T cells immunotherapy

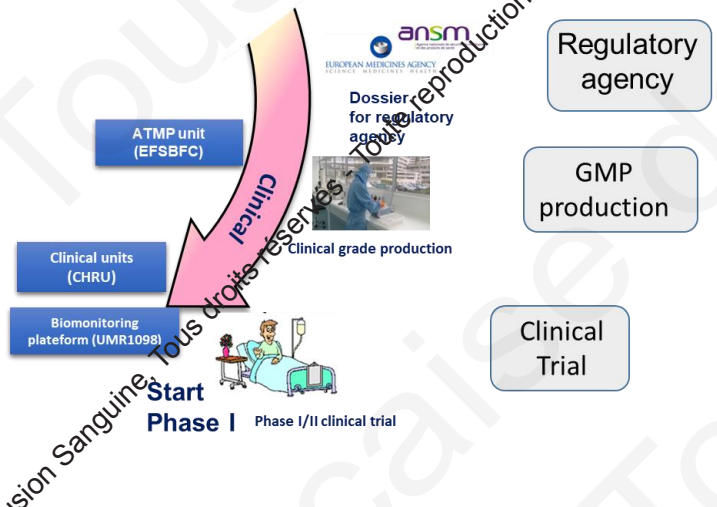
Research
Validated

From hypothesis to the proof of concept (2014 to 2020)

Pre-clinical development
On going

Preclinical « GMP like » process validation
Regulatory dossier – Awaiting GMP raw material

Clinical production of innovative drug
In comming



Next step - GMP production and clinical trial

Besançon UMR1098 (France) ATMP facilities

Clean and secure area: Airlocks and Grommets (biological material, consumables, waste)

Automated process

Trained and authorised staff

ATMP unit (EFSBFC)

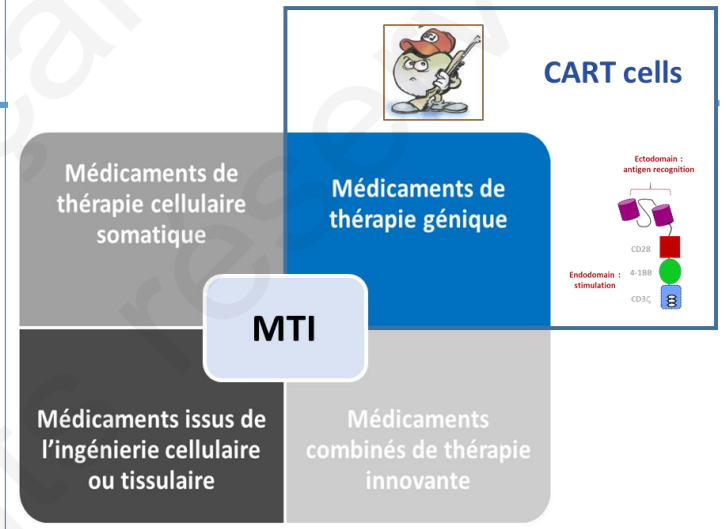
Dossier for regulatory agency

Clinical grade production

Regulatory agency

GMP production

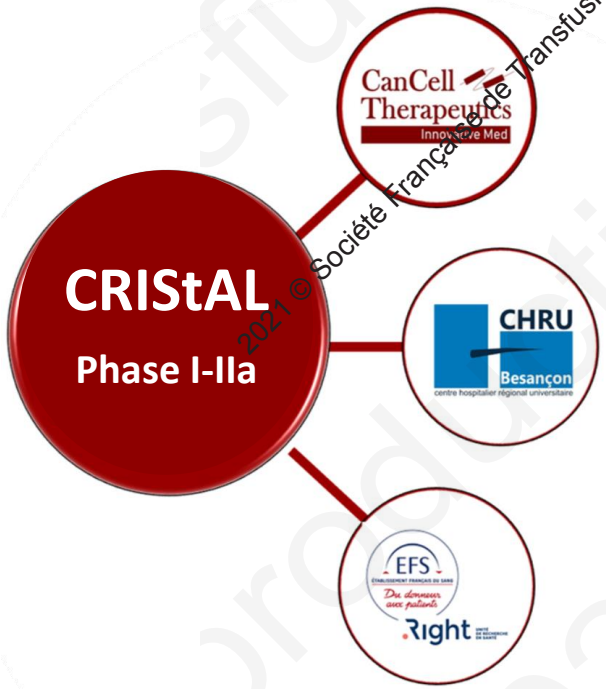
Règlementation européenne - CE 2007-1394



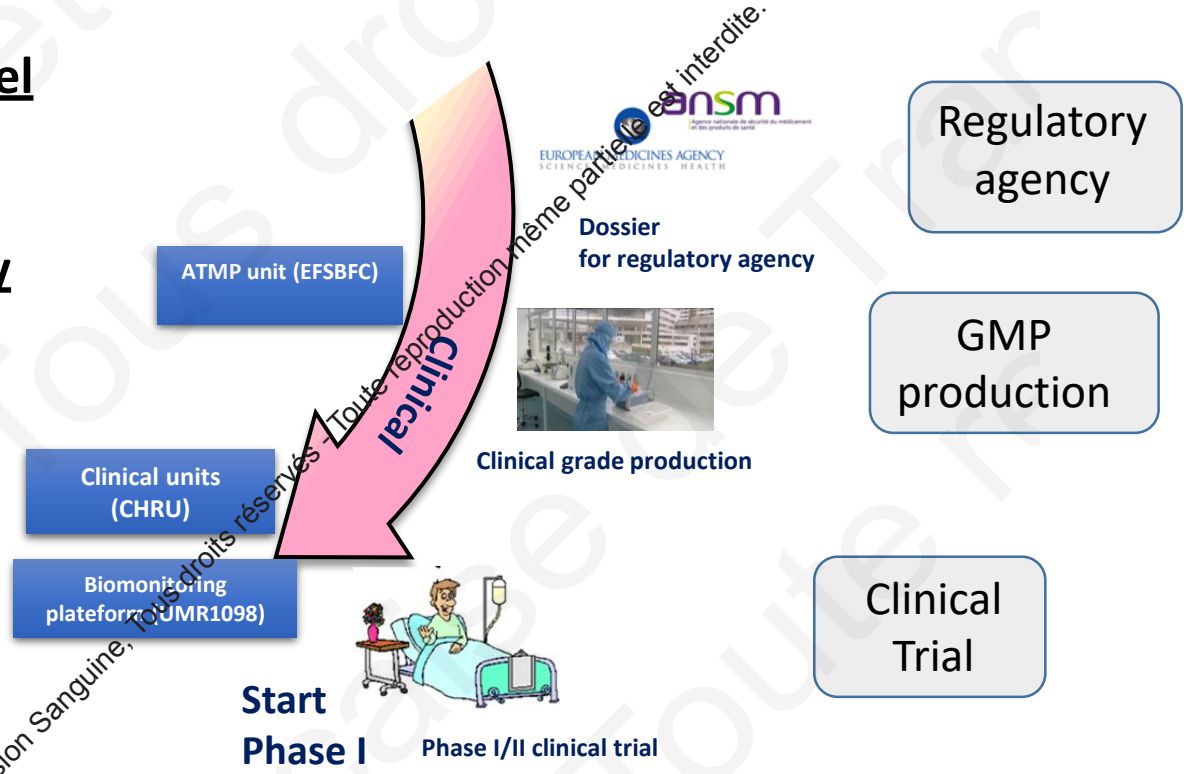
Next step - clinical trial CRIStAL

CRIStAL Clinical Trial **: C**himeric antigen **R**eceptor **I**L-1RAP, **S**afety and efficacy evaluation in relapsed and refractory **A**cute Myeloid **L**eukemia (AML)

Autologous engineered T-cells expressing anti-IL-1RAP chimeric antigen receptor, administered in adults patients with Relapsed/Refractory Acute Myeloid Leukemia (AML)



- **Phase I-IIa open label**
- **Dose-escalation**
- **First in human study**
- **Evaluation of the safety and clinical activity**



Academic CART Cell production: *From the target to the patient…*

Hypothesis / Choose a tumor target

Construction of the viral vector & supernatant production

Proof of concept

Safety / Off-target toxicity

Transfer and up-scaling validation

Regulatory agency approval

GMP production

Clinical Trial

Research

Pre-clinical

Clinical

2014

2019

2021

2023

Research unit

Translational Research unit

ATMP unit (EFSBFC)

Clinical units (CHRU)

Biomonitoring platform

Molecular sequencing (Sanger)

MAb selection (Specificity)

Production of a monoclonal antibody (Mab, Hybridoma)

Immunisation IP or Foodpad of mice

Cloning within a lentiviral backbone

Lentiviral supernatant production

Transduction of T lymphocytes (CART-cells)

Functionnality (*in-vitro*)

Safety

CART-cells Cytotoxicity (*in-vivo*)

Toxicity (*TMA; in-vivo*)

Pre-clinical improvment, scaling-up

Dossier for regulatory agency

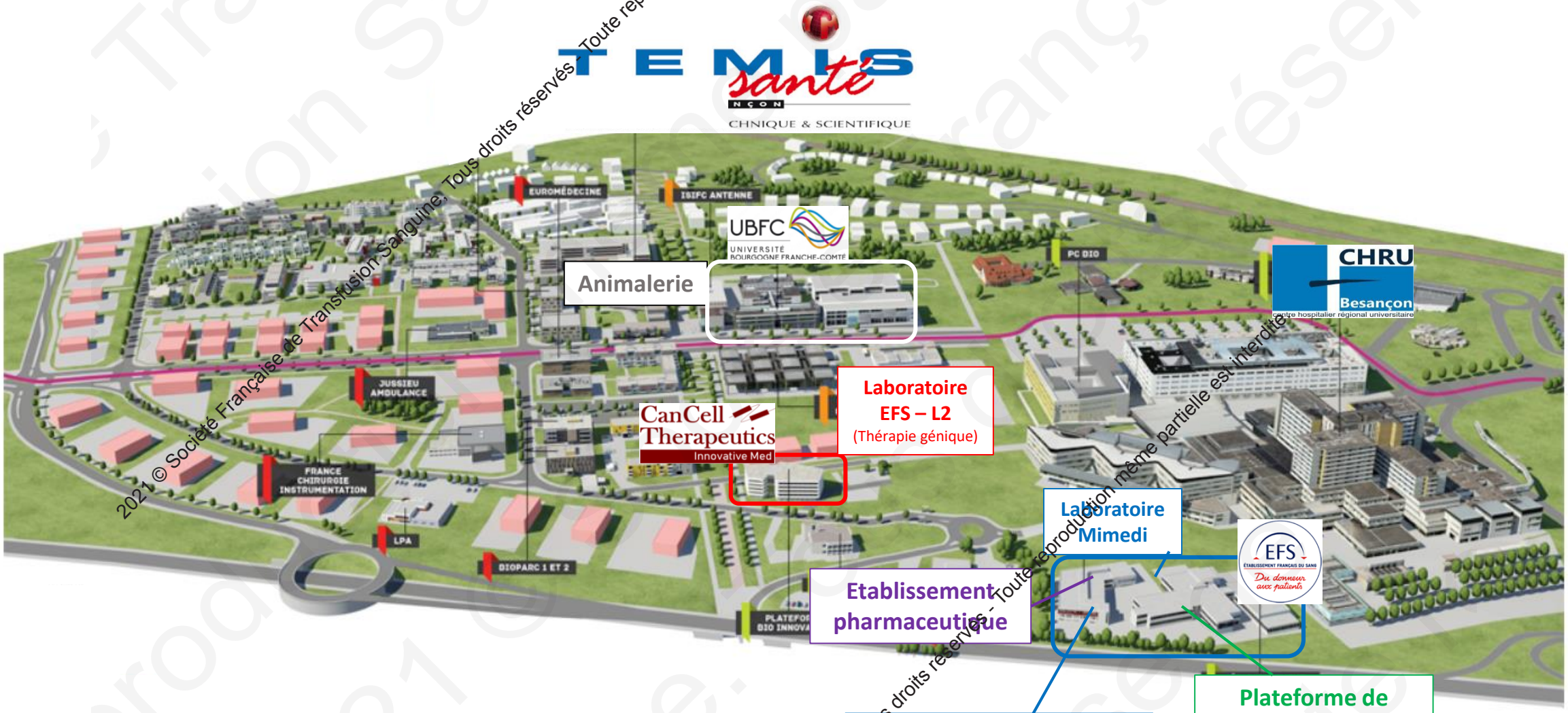
Clinical grade production

Start Phase I

Phase I/II clinical trial

Implantation au sein d'un environnement propice

L'ensemble des acteurs est réunis dans un périmètre réduit facilitant les interaction



FFDSB

Port sur Saone (70)
Saint Loup sur Semouse (70)
Saint Vit (25)
Union départementale (70)
Pont de Roide (25)
Fesche le Chatel (25)
Morez (39)
Beaune (21)
Le Creusot (71)
Maconnais sud (71)
Cousance (39)
L'isle sur le Doubs (25)
Besançon-Thise (25)
Saint Valliers (71)
Semur en Auxois (71)
Paray le Monial (71)
Sancey (25)
Nolay (39)
Le Russey (25)

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CanCell Therapeutics
Innovative Med

Grand Besançon Métropole

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Right — Unité de Recherche en Santé

MiMédi — Microtechniques pour les Médicaments innovants

UBFC Université Bourgogne Franche-Comté

Inserm — Institut national de la santé et de la recherche médicale

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ENGAGEMENT LEUCEMIE
Franche-Comté Engagement Leucémie

CANCÉROPÔLE DU GRAND-EST

LA LIGUE CONTRE LE CANCER

T²EVOLVE

INSTITUT CARNOT OPALE

Diaclone

nausicaa combat sa leucémie

Don du Sang