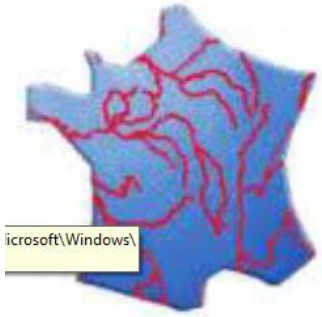


Techniciens Associés des Centres de Transfusion

PROGRAMME
RENCONTRES TACT Dijon
24 et 25 Mars 2022

- **CART-cells Généralités-** *Dr Christophe Ferrand*
- **Développement d'un CART-cells ciblant IL-1RAP : une nouvelle alternative dans le traitement de la LAM (2014-2021) -** *Dr Marina Deschamps*

EFS Bourgogne Franche-Comté Unit INSERM_UMR1098 RIGHT

Host-Tumor-Graft Interaction and Cellular and Genetic Engineering

Immuno-Molecular Cancer Therapeutics Team (TIMC)

Techniciens Associés des Centres de Transfusion

PROGRAMME
RENCONTRES TACT Dijon
24 et 25 Mars 2022

- **CART-cells Généralités-** *Dr Christophe Ferrand*
- **Développement d'un CART-cells ciblant IL-1RAP : une nouvelle alternative dans le traitement de la LAM (2014-2021) -** *Dr Marina Deschamps*

EFS Bourgogne Franche-Comté Unit INSERM_UMR1098 RIGHT

Host-Tumor-Graft Interaction and Cellular and Genetic Engineering

Immuno-Molecular Cancer Therapeutics Team (TIMC)

The best way to describe an innovative therapy drug «CAR T-cells»

The incredible and unexpected story of Emily Whitehead (USA)

May 2010
B-ALL (@ 5 years)

October 2011
1st relapse

February 2012
Programmed bone marrow transplant

March 2012
2d Relapse!
Cancelled bone marrow transplant

April 2012
Phase I Clinical Trial
Genetically modified T-cells (CAR-T cells)
targeting leukemia cells CD19+

May 2012
Complete remission

Children's Hospital of Philadelphia, Upenn, (USA)

Leukemia cells not detected ▶

Mai 2021
Already « free of cancer »

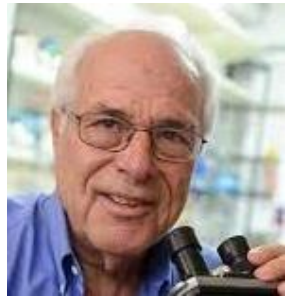
Beginning of the true clinical history of CART-cells
Power of this Personalized precision medicine : living/dynamic drug with immune memory

History of CAR T cells and its progress and milestones

From 1989 to 2013…and after

Founding principle

Zelig Eshhar
Weizmann Institute of Science
Tel Aviv Sourasky Medical Center



Expression of immunoglobulin-T-cell receptor chimeric molecules as functional receptors with antibody-type specificity.
Gross G, Waks T, **Eshhar Z.**
Proc Natl Acad Sci U S A. 1989 Dec;86(24)

Second signal

Dario Campana
St Jude institute US
National University of Singapore

Chimeric receptors with 4-1BB signaling capacity provoke potent cytotoxicity against acute lymphoblastic leukemia.
Imai C, Mihara K, Andreansky M, Nicholson IC, Pui CH, Geiger TL, **Campana D.**
Leukemia. 2004 Apr;18(4)

Preclinical POC

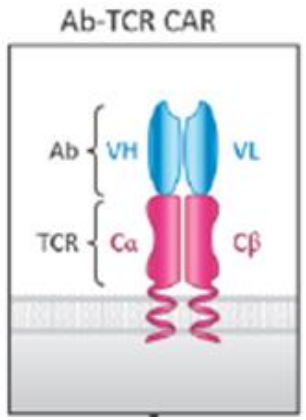
Carl June
Department of Pathology and Laboratory Medicine, UPenn

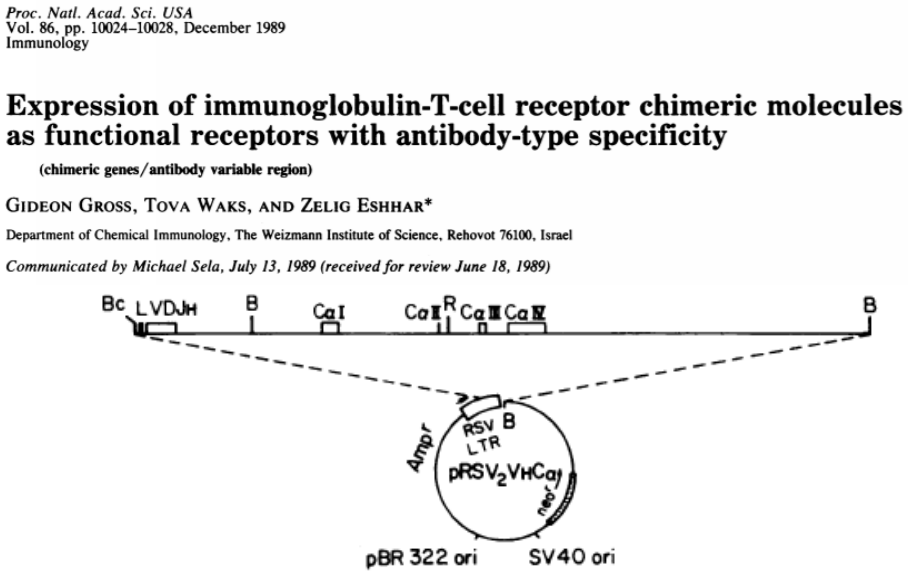
T cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemia.
Kalos M, Levine BL, Porter DL, Katz S, Grupp SA, Bagg A, **June CH.**
Sci Transl Med. 2011 Aug 10;3(95)

Clinical Trial

Steve Grupp
CHOP, Philadelphia

Chimeric antigen receptor-modified T cells for acute lymphoid leukemia
Stephan A Grupp, Michael Kalos, David Barrett, Richard Aplenc, David L Porter, Susan R Rheingold, David T Teachey, Anne Chew, Bernd Hauck , J Fraser Wright, Michael C Milone , Bruce L Levine, Carl H June
N Engl J Med. 2013 Apr 18;368(16):1509-1518.





Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 10024–10028, December 1989
Immunology

Expression of immunoglobulin-T-cell receptor chimeric molecules as functional receptors with antibody-type specificity

(chimeric genes/antibody variable region)

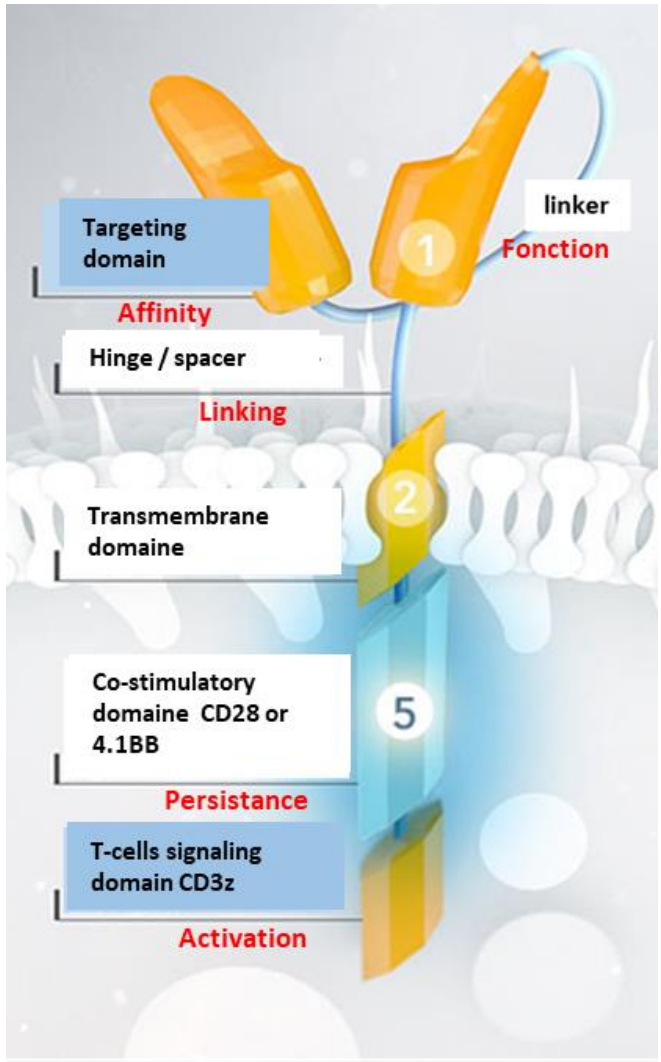
GIDEON GROSS, TOVA WAKS, AND ZELIG ESHHAR*

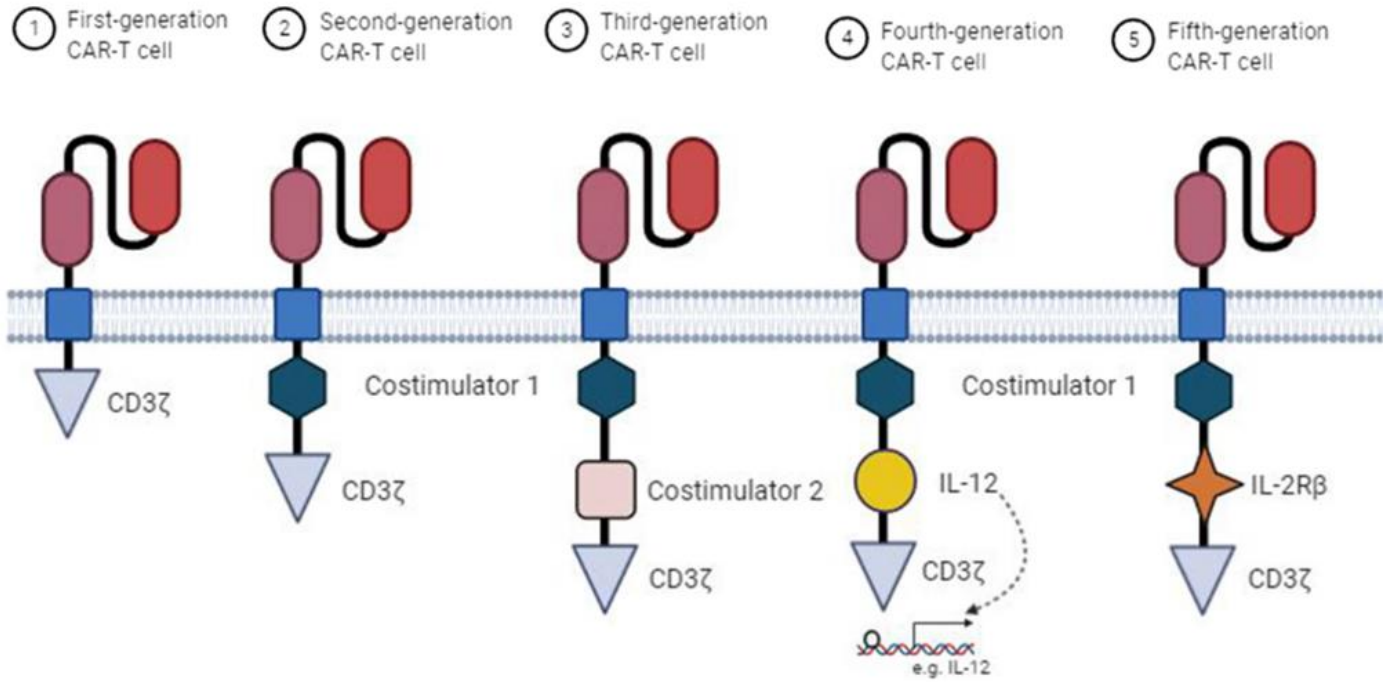
Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot 76100, Israel

Communicated by Michael Sela, July 13, 1989 (received for review June 18, 1989)

Structure of Chimeric Antigen Receptor (CAR)

From the first to the next generations





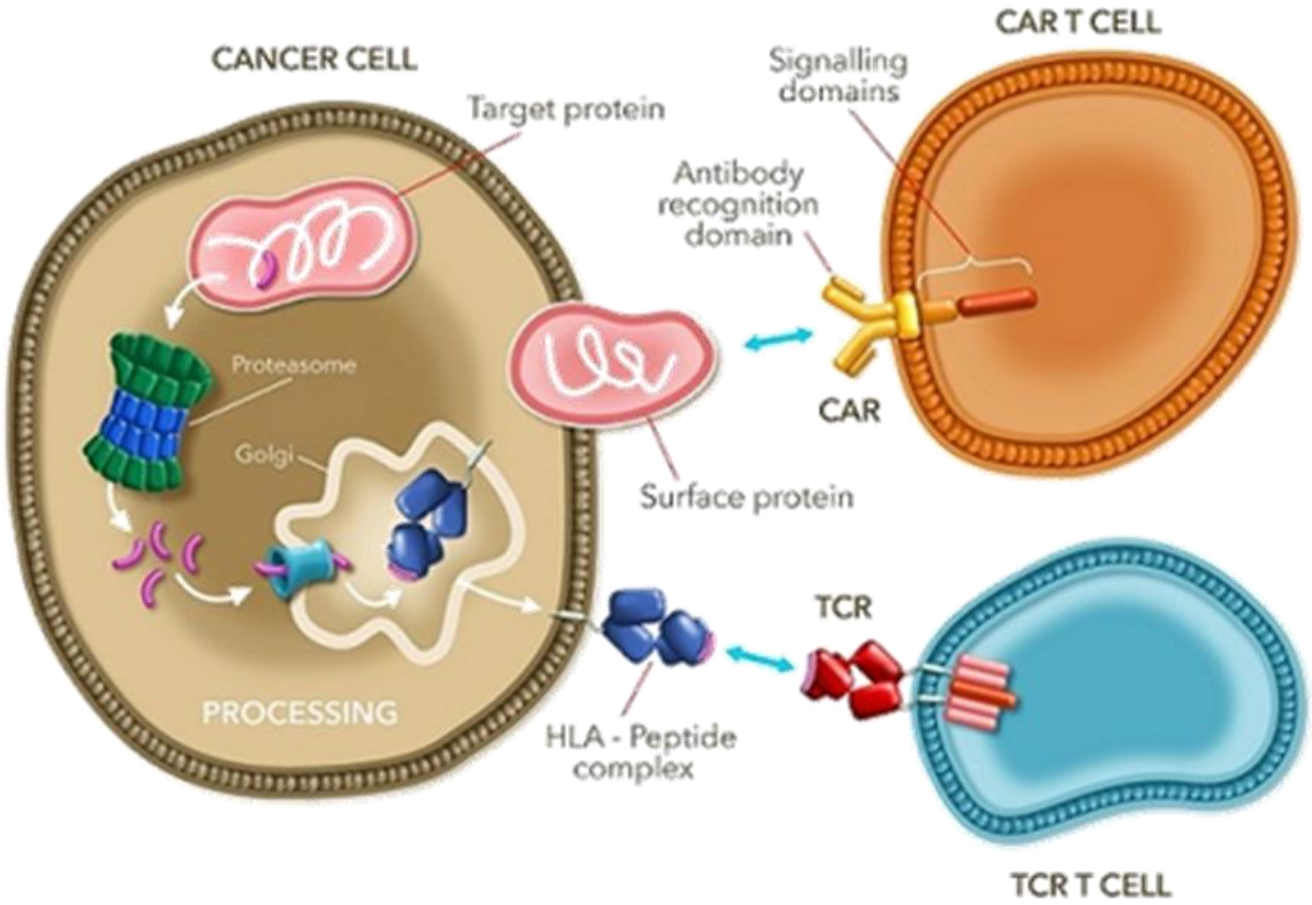
Cytotoxicity	*CD3z*	1
Proliferation	*CD28*	2
Survival/persitance	*4.1bb*	3
Improvement / Inducible / Safety / Specificity		4-5

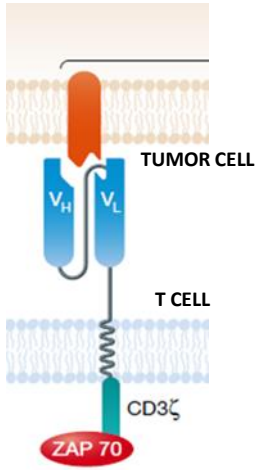
iCASP9 – IL-15

(Jin et al., Cancer cell international, 2021)

Bypassing Central Tolerance and generate potent T-Cells to Self Antigens

Two approaches : CART-cells or TgTCR



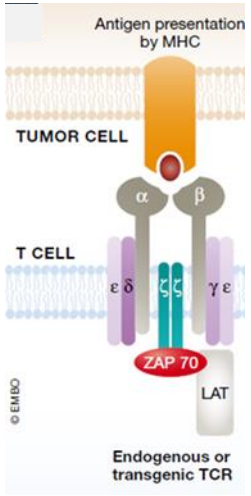


- Cell surface antigens
- Persistence in-vivo?

- MHC independent
- Ac/Ag +++

Advantages

Inconvenients

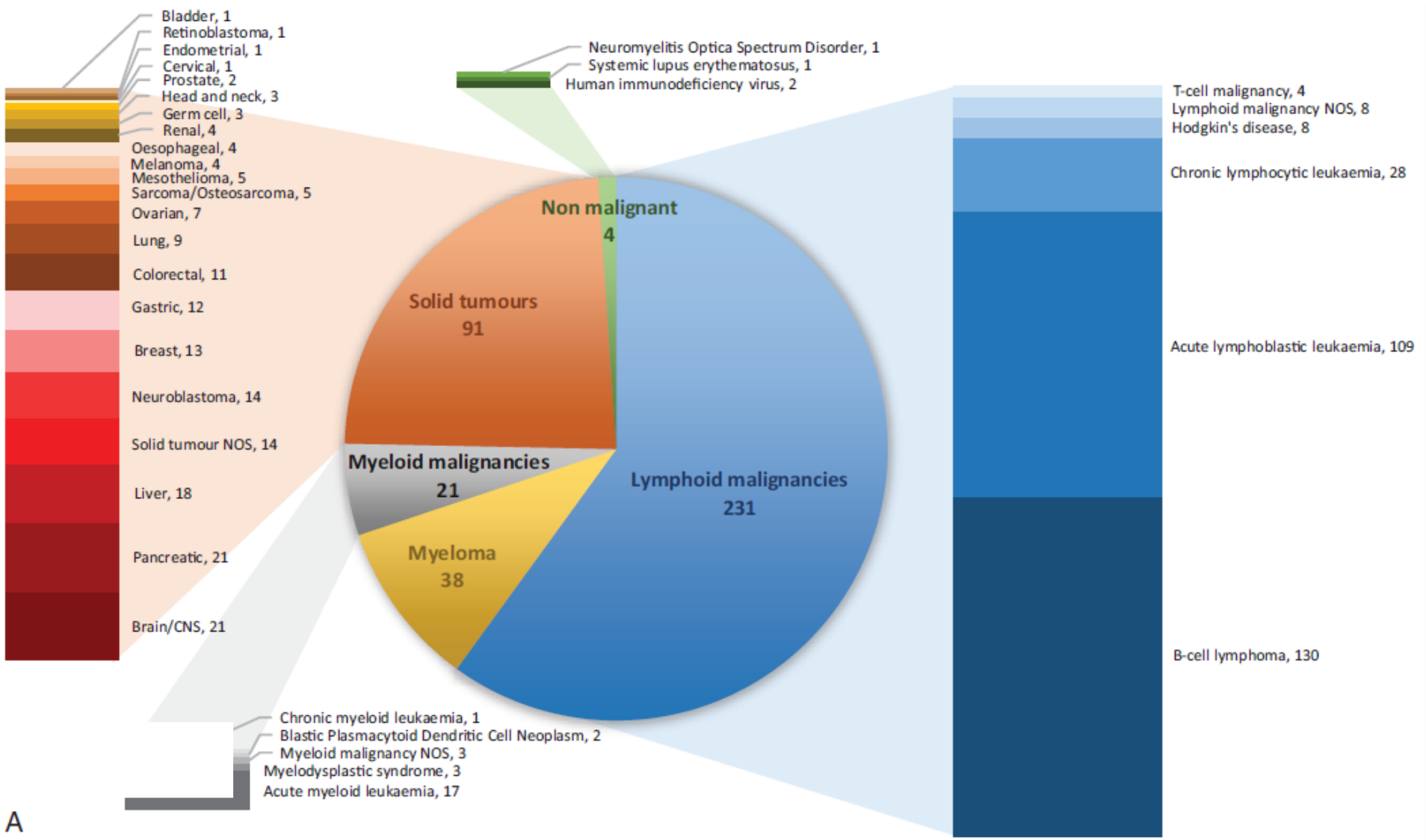


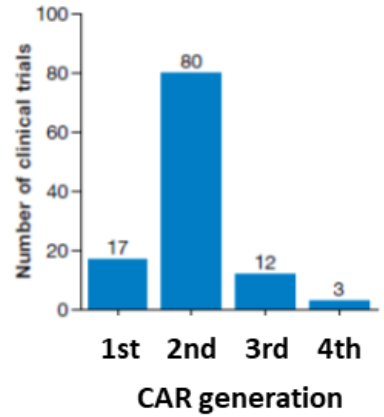
- Intracellular antigens

- MHC dependent
- TCR/Ag +
- Recombination TgTCR/endogen TCR

Distribution of CART-cells clinical trials

75% in Onco-Hematology diseases, 25% in solid cancers





Hartmann_et_al-2017-EMBO_Molecular_Medicine
Charrot et al, Hemasphere 2019

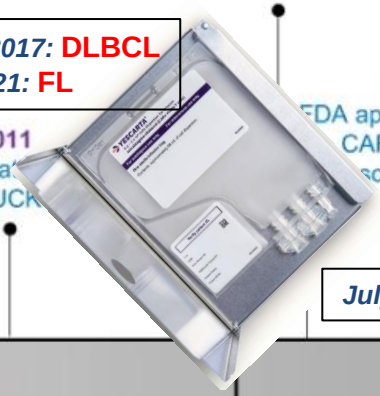
Developpement des CAR T-cells

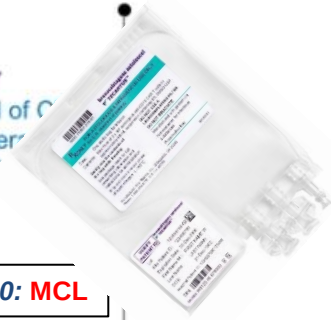
Approbation FDA & EMA

August 2017: **Ped. and AYA B-ALL**
May 2018: **DLBCL**

February 2021: **DLBCL**

October 2017: **DLBCL**
March 2021: **FL**





July 2020: **MCL**

1989

1990 — **1993** First-generation CAR (CD3ζ)

1995 — **2003** 2nd generation CAR (CD28- CD3ζ)

2000 — **2004** 2nd generation CAR (4-1BB- CD3ζ)

2005 — **2007** First IND for CD19 CAR

2010 — **2011** 4th genera… (TRUCK…

2015 — **2017** FDA approval of CD19 CAR T therapy (Kymriah) for ALL

2017 FDA approval of C… CAR T ther… …scarta) …

2020 — **2021** FDA approval of CD… CAR T therapy (Liso-cel, Breyanzi) for NHL

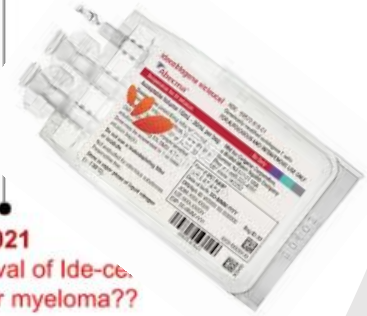
2013 Demonstration of BCMA as a functional CAR target in myeloma (Preclinical)

2014 First in-human BCMA CAR T therapy clinical trial (NCI)

2017 Ide-cel (bb2121) granted Breakthrough-therapy designation award by FDA and PRIME by EHA

2019 Cilta-cel (JNJ4528) granted Breakthrough-therapy designation award by FDA

2021 FDA approval of Ide-ce… (bb2121) for myeloma??



Mars 2021: **MM**

Taux de patients traités par CAR-T

Nb de CAR-T Nationaux FR

FDA and/or EMA approved CART-cells

more than 30 years later …

Prefix
Suggest by Manufacturer

Infix
Gene : for gene modification
Cabta : for **C**ells expressing **A**nti**b**ody and **T**-cells **A**ctivation
Leu : for Leucocytes

Suffix
Cel : For cell used for Cell therapy

Kite Pharma / GILEAD **Axi-cabta/gene-Cilo leucel** *(Yescarta) = axi-cel*

NOVARTIS **Tisa-gene-Lec leucel** *(Kymriah) = tisa-cel*

Drug			Provider	Chimeric Receptor					Disease indication	Cells	Pivotal Clinical trials
							Extracellular domain				
Market	Generic	Abbreviation		Generation	Co-stimulary signal	Transduction	Target	MAbs			
KYMRIAH®	Tisagenlecleucel	**tisa-cel**	Novartis	Second	4.1BB	Lentiviral	**CD19**	FMC63	Acute lymphocytic leukemia (ALL)/Diffuse large B-cell lymphoma (DLBCL)	Bulk PBMC --> CD3+/CAR+	**Eliana (ALL)** **Juliet (DLBCL)**
YESCARTA®	Axicabtagene ciloleucel	**axi-cel**	Kite/Gilead	Second	CD28	Retroviral	**CD19**	FMC63	Diffuse large B-cell lymphoma (DLBCL)	Bulk PBMC --> CD3+/CAR+	**Zuma-01 (DLBCL, PMBCL)**
TECARTUS®	brexucabtagene autoleucel	**brexu-cel**	Kite/Gilead	Second	CD28	Retroviral	**CD19**	FMC63	Mantle cell lymphoma (MCL)/ALL	T-cells --> CD3+/CAR+	**Zuma-02 (MCL)** **Zuma-03 (ALL)**
BREYANZI®	lisocabtagene maraleucel	**liso-cel**	Celgene/BMS	Second	4.1BB	Lentiviral	**CD19**	FMC63	Large B-cell lymphoma (LBCL)	CD4+/CD8+ --> CD4+/CD8+ [1:1]CAR+	**Transcend**
ABECMA®	idecabtagene vicleucel	**ide-cel**	Celgene/BMS	Second	4.1BB	Lentiviral	**BCMA**	bb21	Multiple myeloma (MM)	Bulk PBMC --> CD3+/CAR+	**Karmma**

Heterogeneity for comparison between CART-cells use

CART constructs, production, cells, administration, patients, clinical trials, real life, companies…

Intra-CAR-T heterogeneity

- **Previous Therapy** — *Impact of T-cell compartment Auto/Allo T-cells*
- **Patients** — *Adults/Children Comorbidity*
- **Bridging Chemotherapy** — *Waiting time Impact of patient condition*
- **Disease status** — *Relapse/Refractory (R/R) Complete remission (CR) MRD neg*
- **Toxicity management** — *Steroids Anti-IL-6 mAb Anakinra*

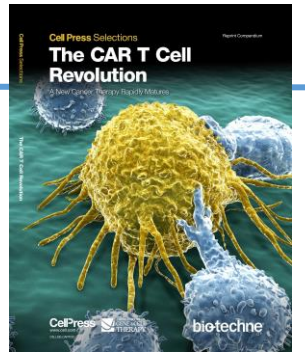
Inter-CAR-T heterogeneity

- **CAR structure** — *Target scFv Costimulation (2d)*
- **Vector** — *Retro / Lenti Transposons RNA*
- **T-cells source / subset** — *Autologous/universal donor Unselected T-cell or CD4:CD8 ratio*
- **Lympho-depletion** — *Fludarabine, Cyclophosphamide, Bendamustine, Alemtuzumab (CD52)*
- **Infusion schedule** — *Single vs split dose Adapted to tumor burden*

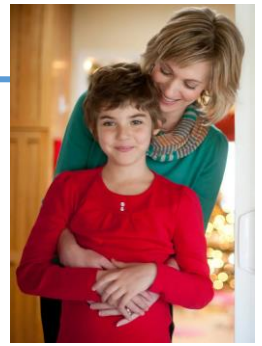
Acute Lymphoid Leukemia (ALL)

First ALL childhood CD19 CART-cells treatment

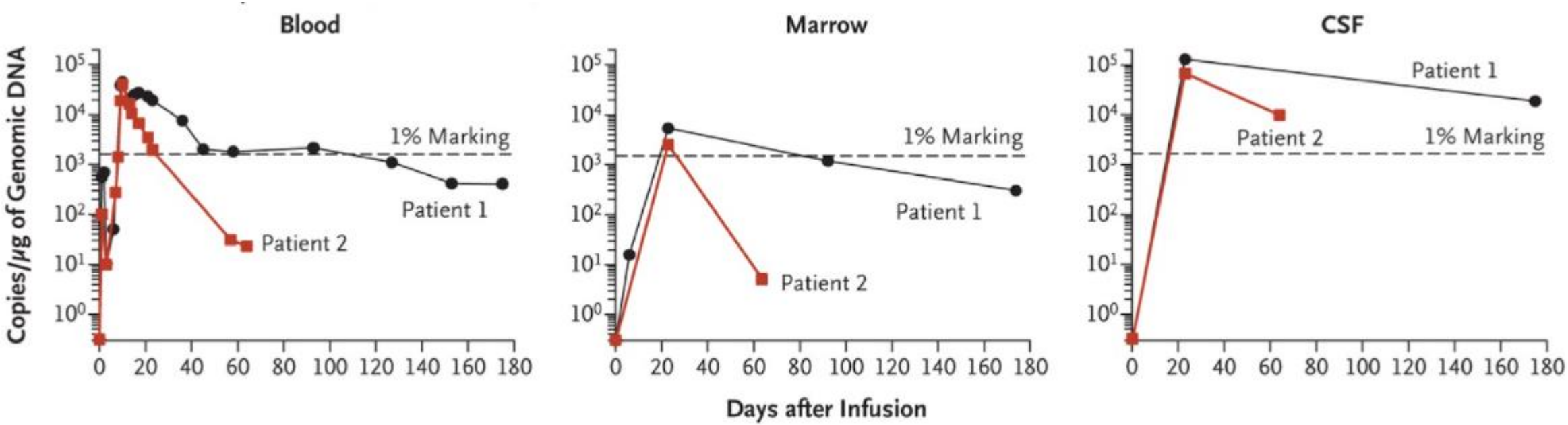
The New York Times



Jan 2015, 30th



Dec 2012, 9th



Main keys of success :

- 100x to 1000x fold expansion and proliferation
- Persitence of CART-cells
- CRS Management (IL-6 / Tocilizumab)

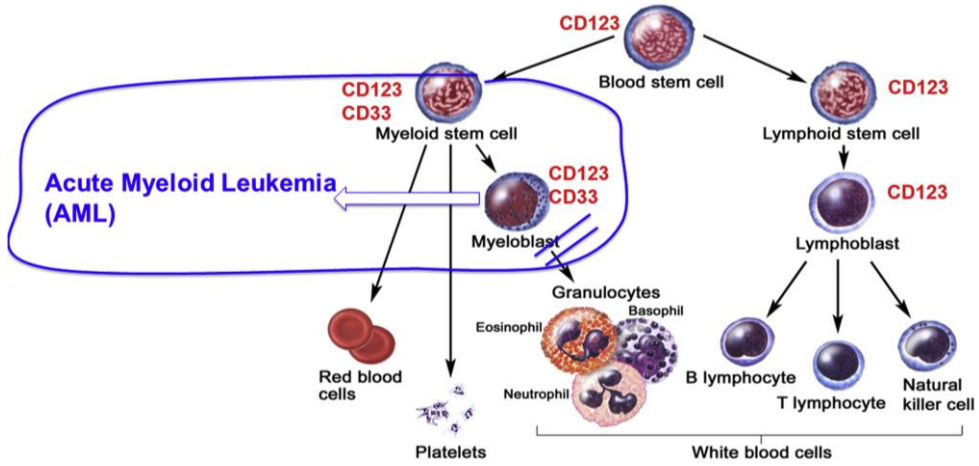
Grupp et al, NEJM 2013

More complicated in Acute Myeloid Leukemia (AML) …

Saar Gill et al, Best Pract Res Clin Haematol 2019
Michelozzi et al, Cancers 2021

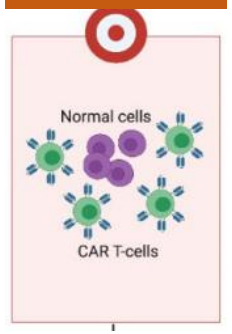
Most of cell surface tumor (associated) antigens are expressed by normal hematopoietic stem cells

- No leukemia-specific surface marker exists in all AML patients
- Lack of a Leukemia-Specific Antigen for Use as Target for CAR T Cells
- In AML all CAR T-cells are at an experimental development (early-stage clinical trials)
- Most common AML cells surface markers targeted : **CD33-CD123**-CLL1-TIM3-CD44v6-CD7-FLT3…
- Myeloablation is a likely consequence of potent anti-myeloid CAR T-cell therapy

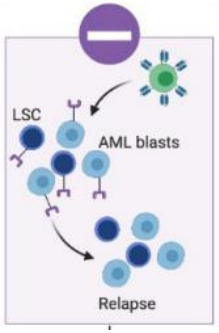


Challenges

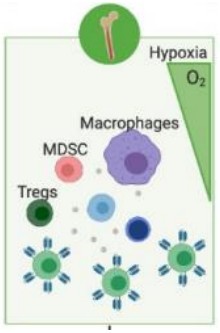
On-target/Off-tumor toxicity



Clonal Heterogeneity



BM Niche



Solutions

- Neoantigens targeting
- Ideal target
- AND and NOT logic gated CART
- Modulation affinity scFv
- Combination with KO HSC transplantation (ie CD33-/-)
- Limiting persistence of CART
- Dual with dissociated activating system

- Personalized approaches
- Multi targeting (dual, bi, CART..)

- Personalized approaches
- Switch receptor CAR
- CAR Trafficking
- Niche targeting (CAF, MSC..)
- Combination with hypoxic sensors
- Allogeneic CAR

Conclusion (1)

CART-cells generalities

- CART-cells (but also immunotherapy) are a **revolutionary technological advance** that change the therapeutically practice and offer new alternatives for **refractory / relapsed (R/R) patients**
- Autologous CART-cells have been approved in **B-cell malignancies (ALL, DLBCL, FL, PMBCL)** and also in **Multiple Myeloma (MM)** for R/R patients
- Toxicity of CART-cells : Adverse events **(CRS and neurotoxicity) are better understand** are now better managed by physicians. What's about financial toxicity?
- Currently, they are experimented in early lines of treatment **(Soon in first line?)**
- There is a need to find an ideal target and approaches in Acute Myeloid Leukemia (AML)
- Remain to be **improve for solid tumor Cancers**
- There are multiple (unlimited) possible improvements that can be imagine (Dual, 4th generation, universal, combination, effectors ..)
- **Allogeneic Gene Edited (GE) CART-cells** or NK-CAR can be a promising approach, although that GE need to be more secure

Techniciens Associés des Centres de Transfusion

PROGRAMME
RENCONTRES TACT Dijon
24 et 25 Mars 2022

- CART-cells Généralités- *Dr Christophe Ferrand*

- **Développement d'un CART-cells ciblant IL-1RAP : une nouvelle alternative dans le traitement de la LAM (2014-2021) -** *Dr Marina Deschamps*

EFS Bourgogne Franche-Comté Unit INSERM_UMR1098 RIGHT

Host-Tumor-Graft Interaction and Cellular and Genetic Engineering

Immuno-Molecular Cancer Therapeutics Team (TIMC)

Brief informations 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)

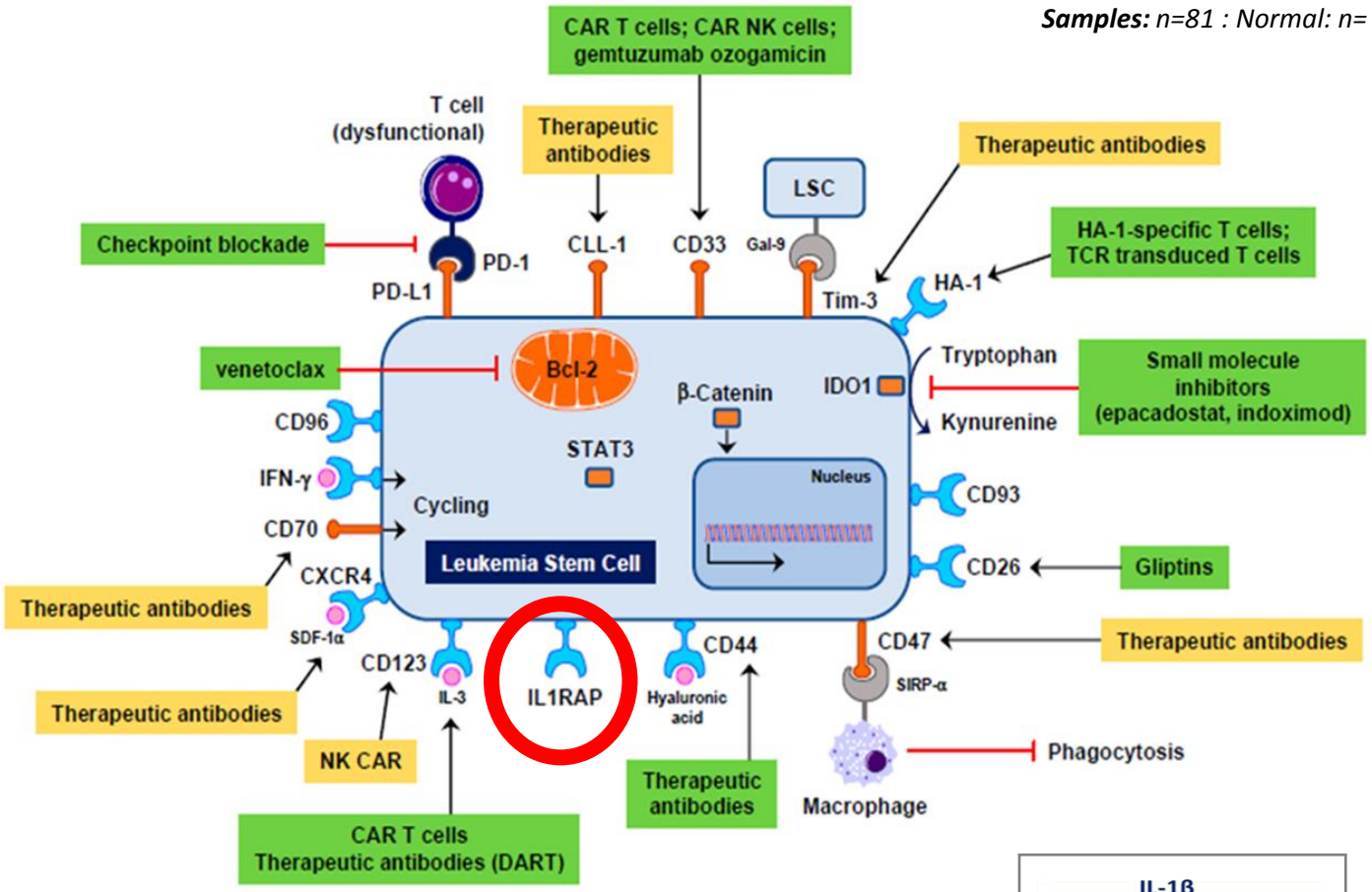
- **Relapse in 30 to 80%** of cases for patients **not receiving allograft**
  - Clonal heterogeneity of the LSC
  - Targets expressed non exclusively by Leukemic cells
  - Escape to the immune system

(Fumihiko Ishikawa, RIKEN Research, 2010)

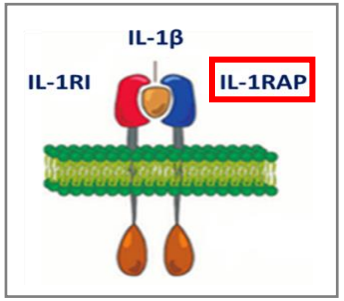
→ **Need to target leukemic stem cells (LSC) by affecting the least possible healthy tissues**

IL-1RAP as target on AML leukemic stem cells

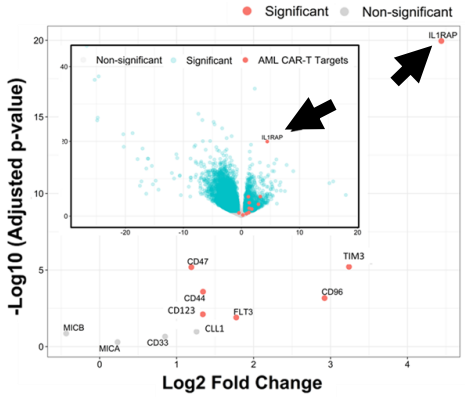
Data: *Corces R. et al, Nature Genetics 2016 (GSE: GSE74246 & SRA: SRP065216)*
Method: *RNA seq on sorted population*
Samples: *n=81 : Normal: n= 8 donors / 49 samples; AML: n= 12 patients / 32 samples (AML at diagnosis & High risk +++)*

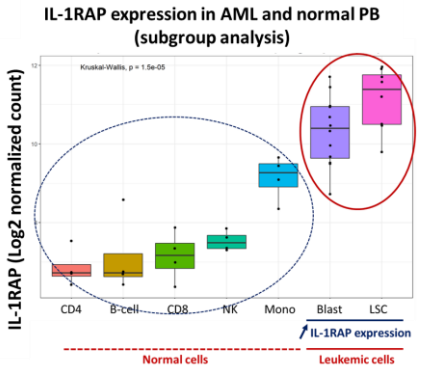


(K.Tasian et al, biomedicines,2018)



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*

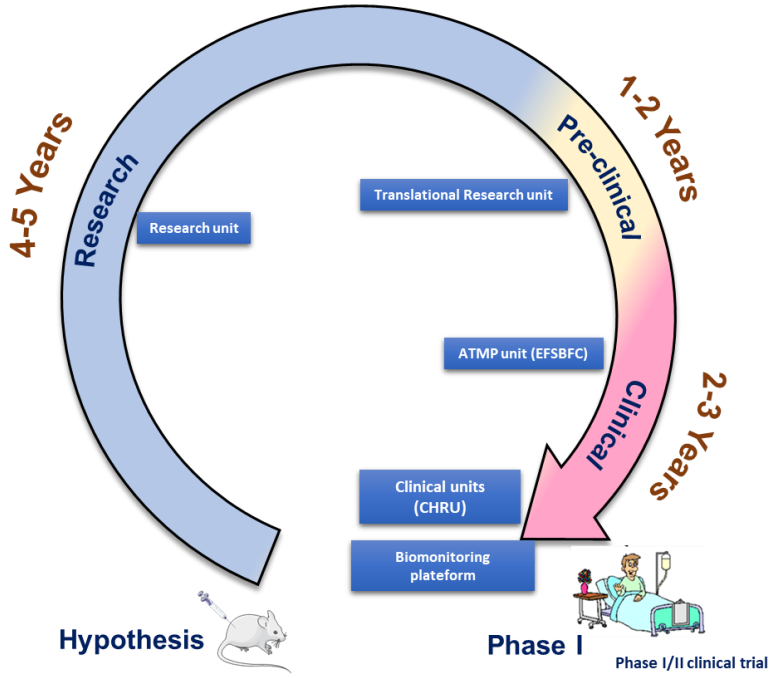
Requirements for academic production of CAR-T cells in accordance with Good Pharmaceutical Practice (GMP). Guidelines from the Francophone Society of Bone Marrow Transplantation and Cellular Therapy (SFGM-TC)

Stages of development of CAR-T cells immunotherapy

Research — *From hypothesis to the proof of concept*

Pre-clinical development — *Transfer of production process and up-scale*

Clinical production of innovative drug — *GMP production for clinical trial*



Bulletin du CANCER

Deschamps et al. Bull Cancer 2019 Sep 20

Research step: hypothesis

Based on Besançon UMR1098 (France) experience

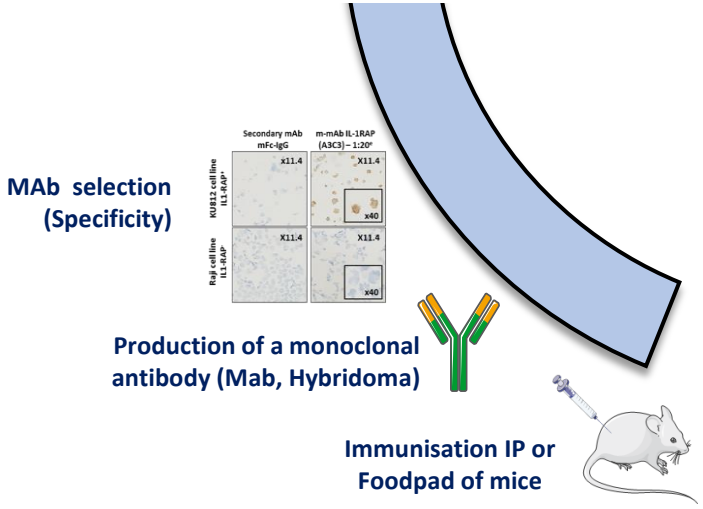
The development of CAR-T cell immunotherapy starts by the hypothesis with the choice of antigen to target.

What is the ideal antigen?

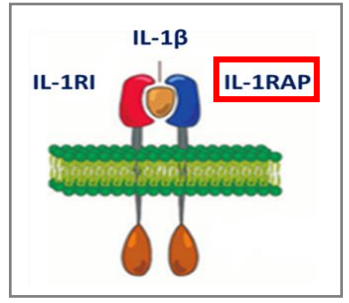
- Should be expressed by the tumor cell and not at all by the healthy cells
- Should not affect healthy tissue,
- Should be express at the cell surface
- Its expression must remain stable

Antigen is a key selection with regard to specificity & safety

Hypothesis / Choose a tumor target



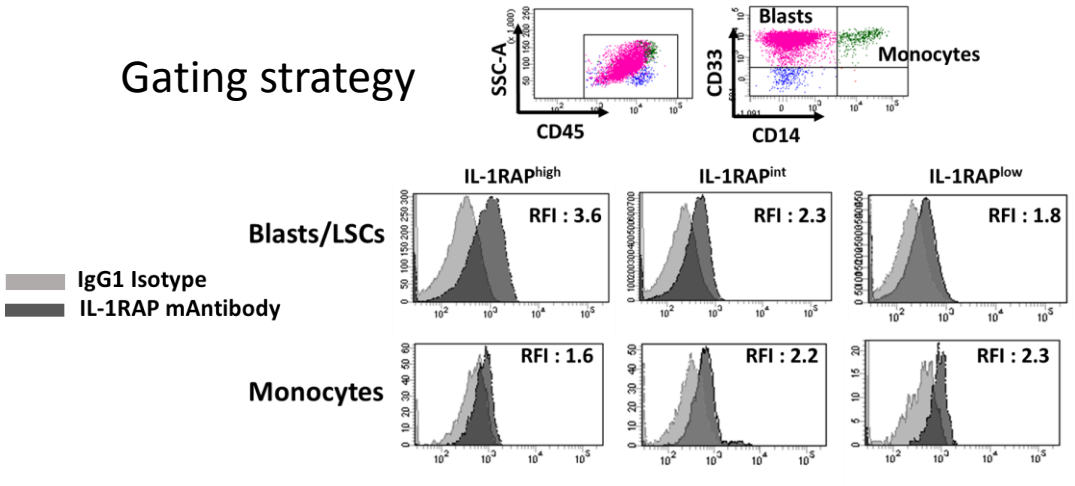
Interleukin-1 Receptor Accessory Protein

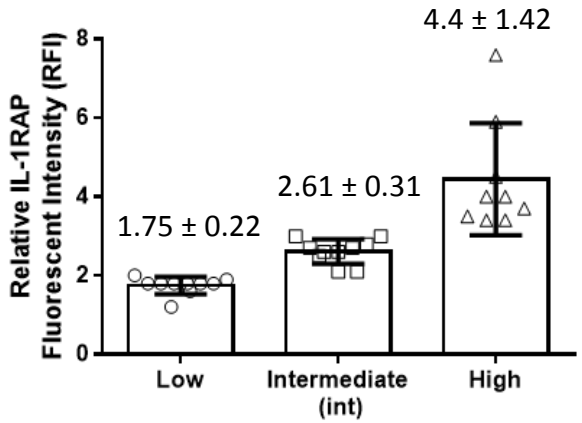


IL-1RAP mAb (#A3C3) staining in AML primary samples

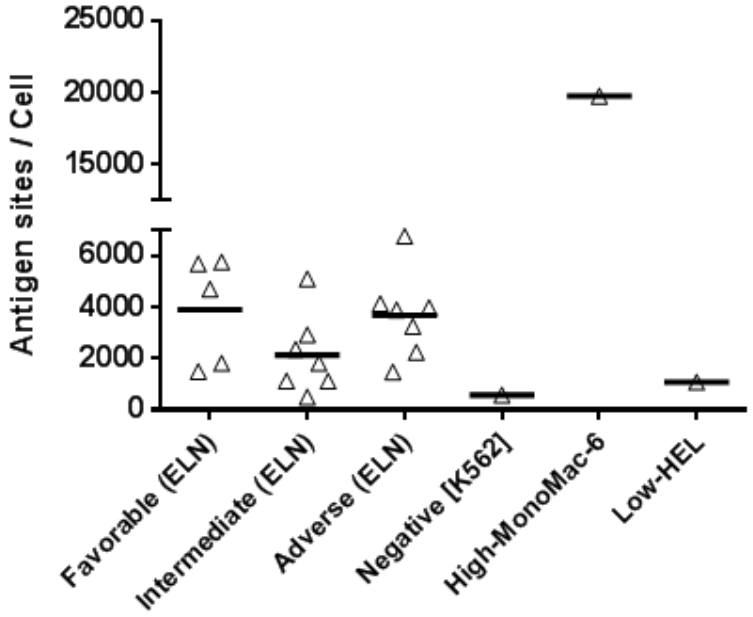
Filothèque Cohort n=30 *10 patients in each group of cytogenetic stratification (ELN) of AML*

Gating strategy





Number of IL-1RAP antigenic sites

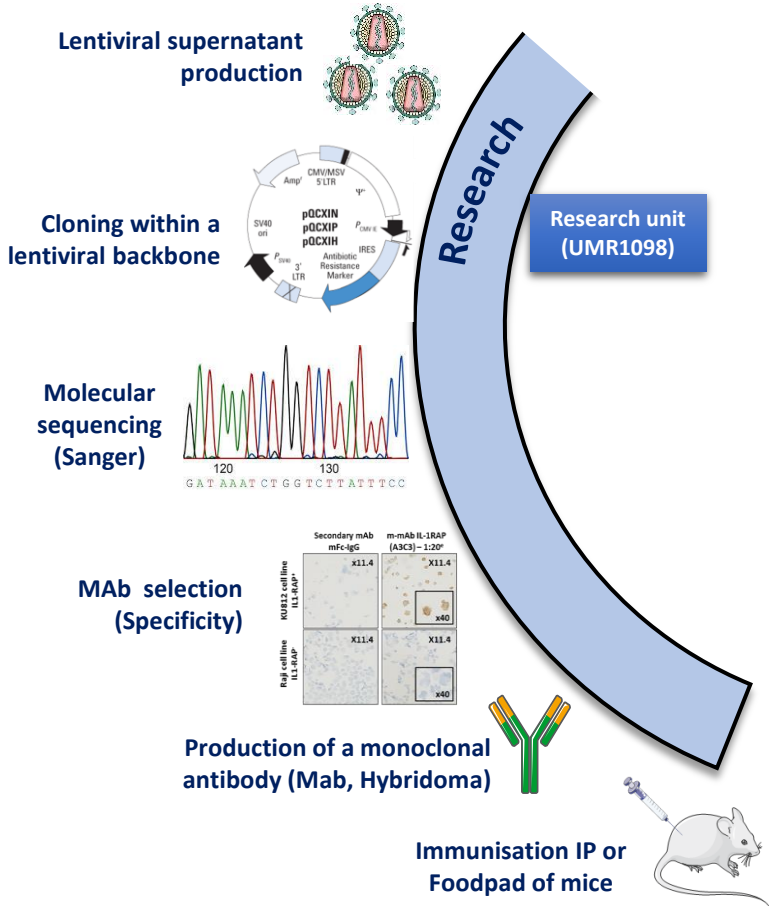


IL-1RAP is expressed on primary cells of AML patients also at 3 levels « Low », « Intermediate » and « High »

Research step: Construction of the viral vector

CAR are engineered membrane protein that consist of three main components:

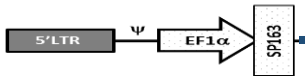
Construction of the viral vector & supernatant production



Hypothesis / Choose a tumor target

Production of a 3rd Chimeric Antigen receptor from IL-1RAP mAb (#A3C3)

Viral vector



Secure
iCASP9 suicide gene

Elimination of the drug in 24-48h

Targeting
CAR IL-1RAP / 3e génération

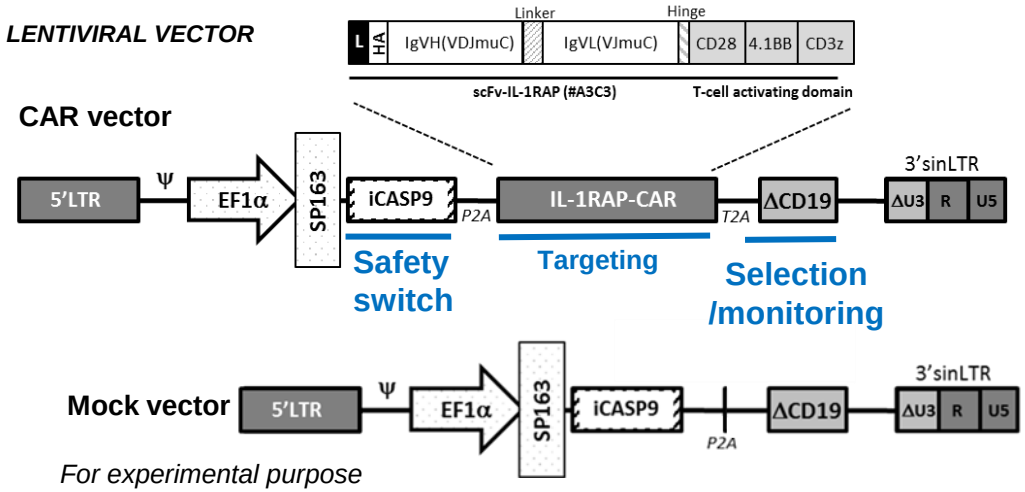
Specific recognition of leukemia cell

Surface marker
Membrane protein ΔCD19

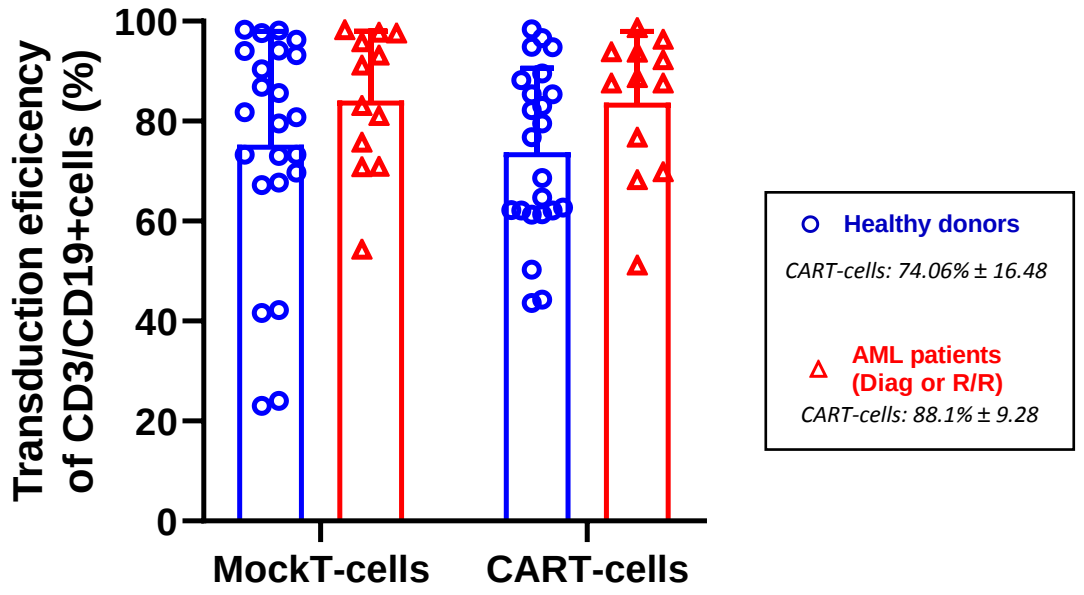
In-vitro monitoring/selection (production) and In vivo follow-up (patients)

Molecular characterization of the coding sequence

→ 3tr generation: single chain linked to the CD28-41BB and CD3z T cells activation signal



Transduction efficiency on primary T cells



Research step: the proof of concept

Hypothesis / Choose a tumor target

Construction of the viral vector & supernatant production

Proof of concept

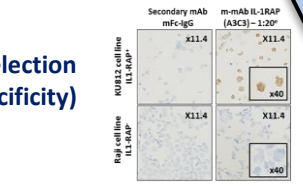
Research

Research unit (UMR1098)

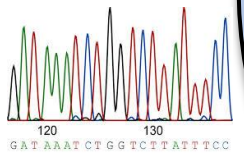
Immunisation IP or Foodpad of mice

Production of a monoclonal antibody (Mab, Hybridoma)

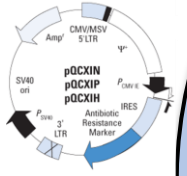
MAb selection (Specificity)



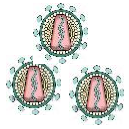
Molecular sequencing (Sanger)



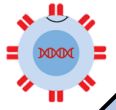
Cloning within a lentiviral backbone



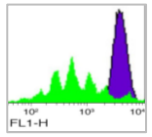
Lentiviral supernatant production



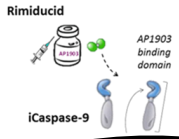
Transduction of T-lymphocytes (CART-cells)



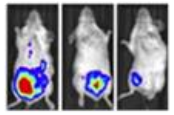
Functionnality (*in-vitro*)



Safety



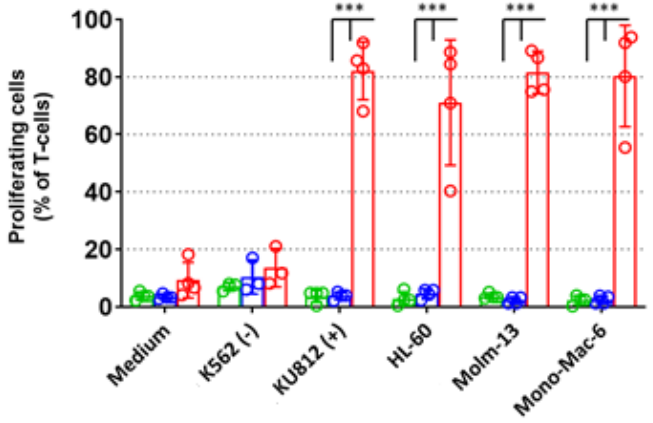
CART-cells Cytotoxicity (*in-vivo*)



In-vitro functionality analysis of IL-1RAP CAR-T cells : Activation (CFSE), IFNγ secretion, CD107 staining, co-culture cytotoxicity

Activation / Prolifération

3 Days of co-culture
Ratio Effector : Target (E:T) = 1:3

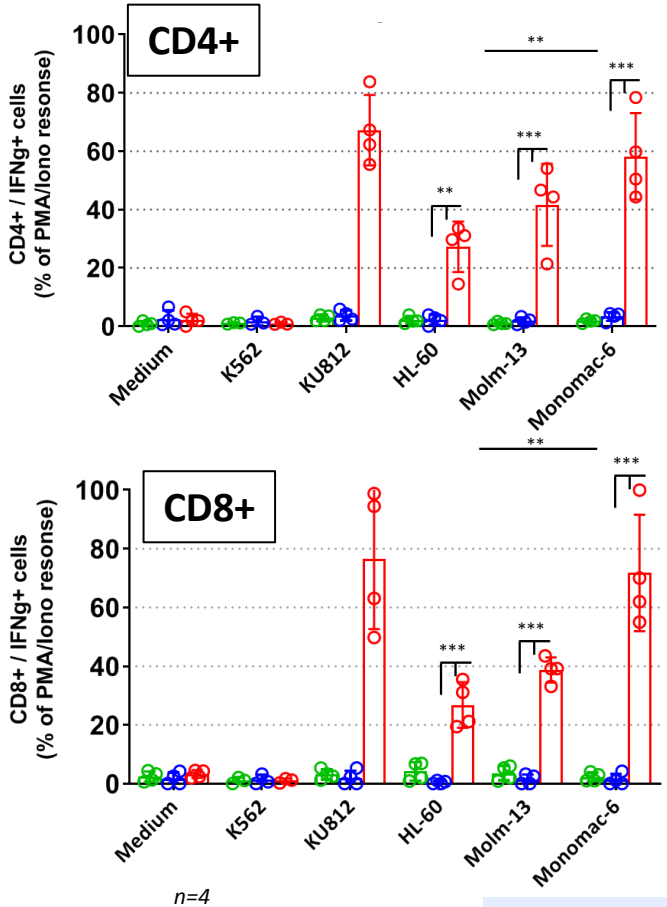


n=4
**** : p<0.001*

UnT T-cells (C0)
Mock T-cells
IL-1RAP CAR T-cells

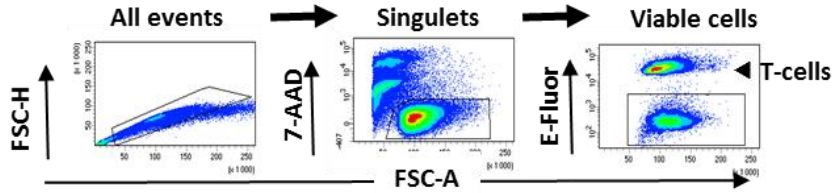
IFNγ Secretion

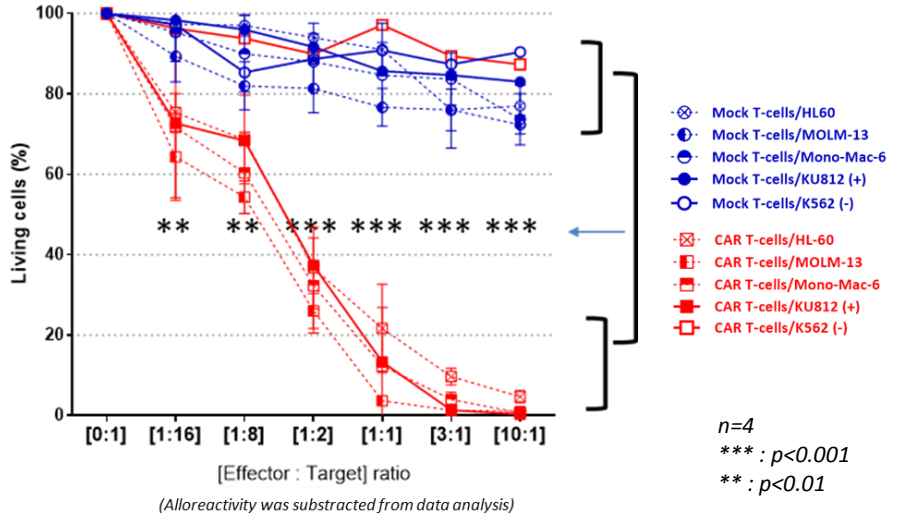
Over night co-culture
Ratio E:T = 1:5



n=4
**** : p<0.001*
*** : p<0.01*

Cytotoxicity Cells lines





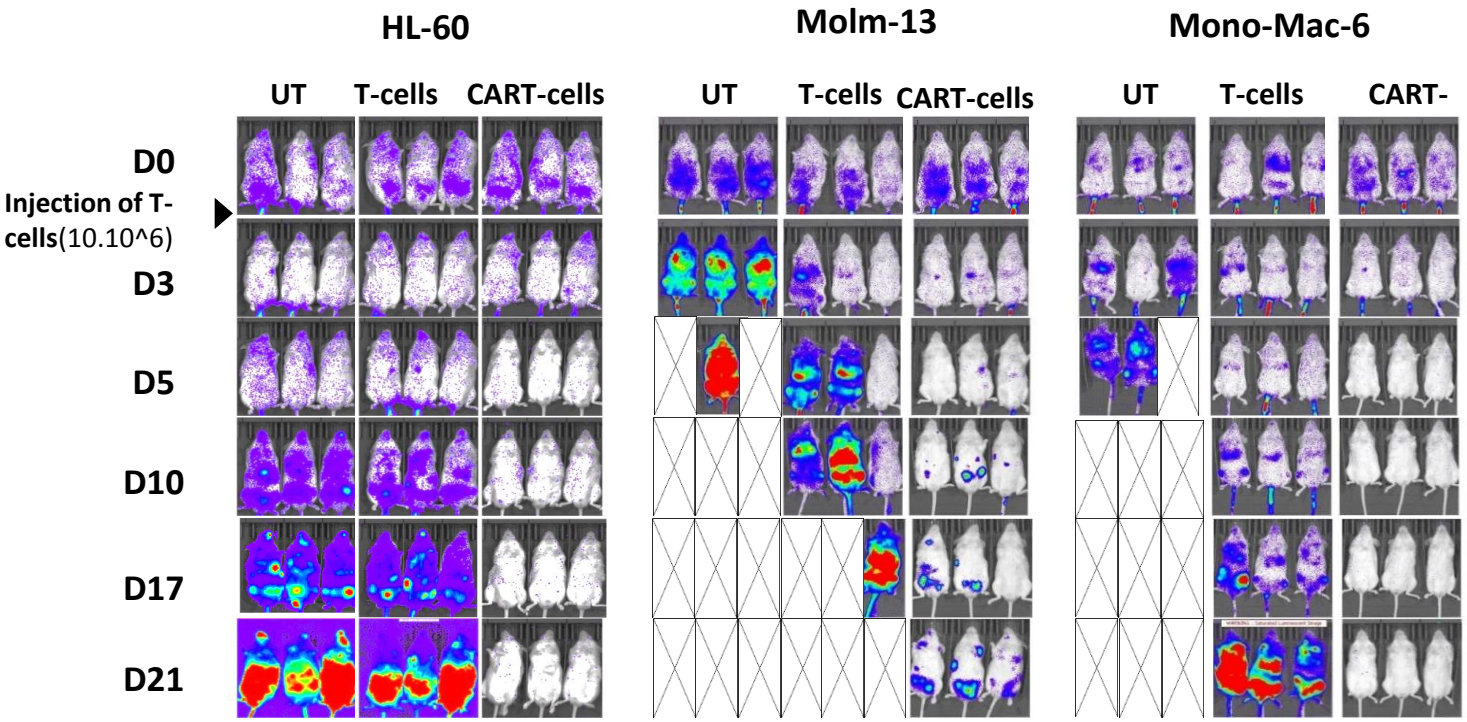
(Alloreactivity was substracted from data analysis)

n=4
**** : p<0.001*
*** : p<0.01*

These results show that CAR-T cells are functional

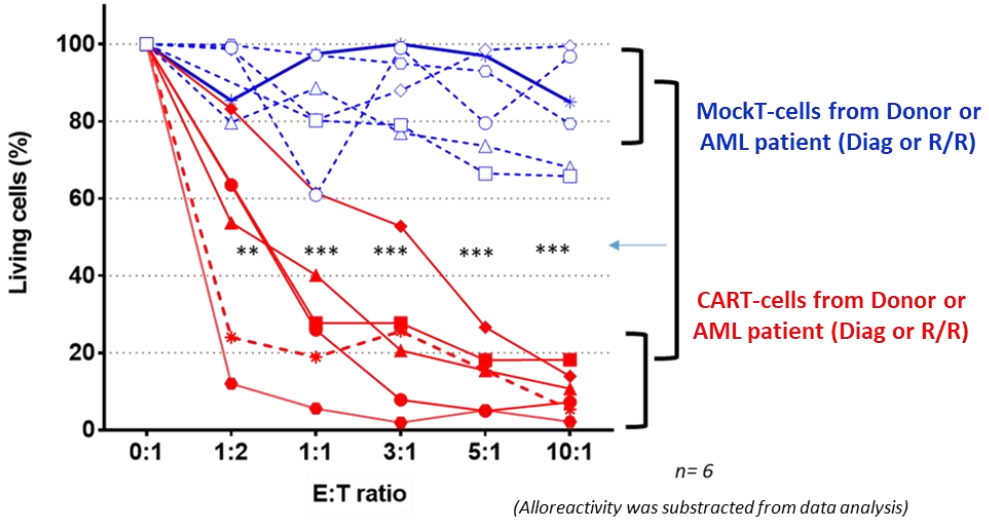
Validation of proof of concept IL-1RAP CART-cells in AML

Xenograft murine model



→ Efficacy of IL-1RAP CART-cells against AML cells in R/R patients (*In vitro*).

Settings	Symbols	Combinations *Donor (D) Patient (P)*	IL-1RAP CART-cells *Diag (D) or Relapse (R)*	AML blastes *Diag (D) or Relapse (R)*
Allo	○	D / P	Healthy donor #A	Patient #1 **(D)**
Allo	□	D / P	Healthy donor #B	Patient #2 **(D)**
Allo	⬢	D / P	Healthy donor #C	Patient #4 **(R)**
Allo	△	P / P	AML patient #6 **(D)**	Patient #7 **(D)**
Auto	◇	P / P	AML patient #5 **(D)**	Patient #5 **(D)**
Auto	✳	P / P	AML patient #7 **(R)**	Patient #7 **(R)**



(Alloreactivity was substracted from data analysis)

(Trad et al SFH2020)
(Trad et al submitted 2021)

Research step: Safety & Toxicity

Warda et al, Cancer Res. 2019 Feb 1;79(3):663-675
Patent 1 : PCT/EP2018/081273
Patent 2 : 1H318460 5EP / May 2019

Hypothesis / Choose a tumor target

Construction of the viral vector & supernatant production

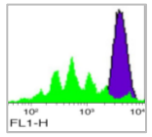
Proof of concept

Safety / Off-target toxicity

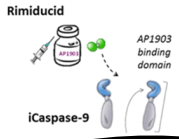
Research

Research unit (UMR1098)

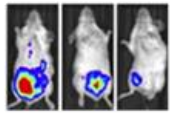
Functionnality (*in-vitro*)



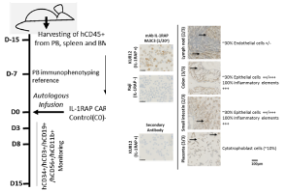
Safety



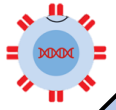
CART-cells Cytotoxicity (*in-vivo*)



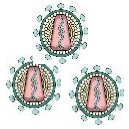
Toxicity (*TMA; in-vivo*)



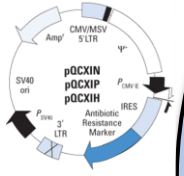
Transduction of T-lymphocytes (CART-cells)



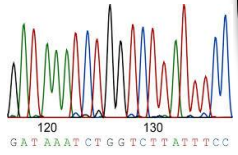
Lentiviral supernatant production



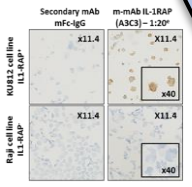
Cloning within a lentiviral backbone



Molecular sequencing (Sanger)



MAb selection (Specificity)

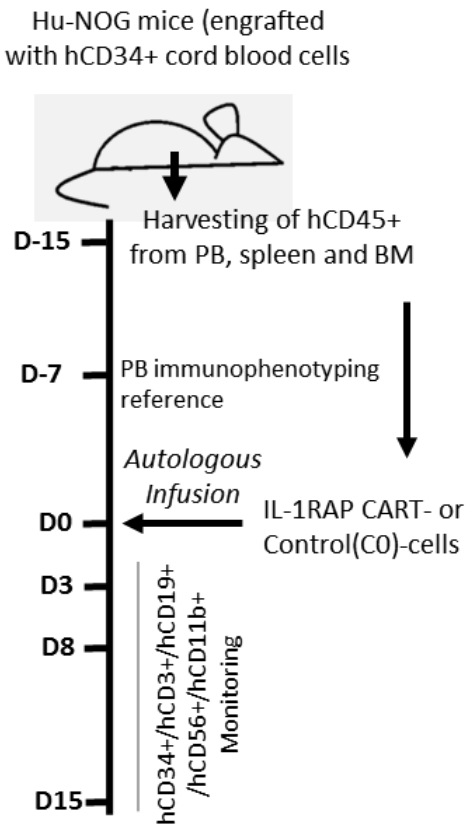


Production of a monoclonal antibody (Mab, Hybridoma)

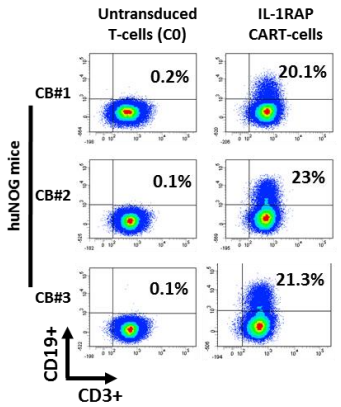
Immunisation IP or Foodpad of mice

IMMUNOSAFETY on human CD34+ cord blood engrafted mice model (hu-NOG)

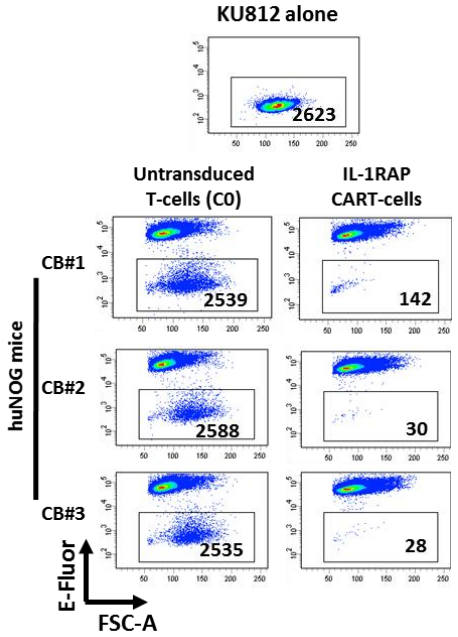
CD34+ xenograft model (hu-NOG)

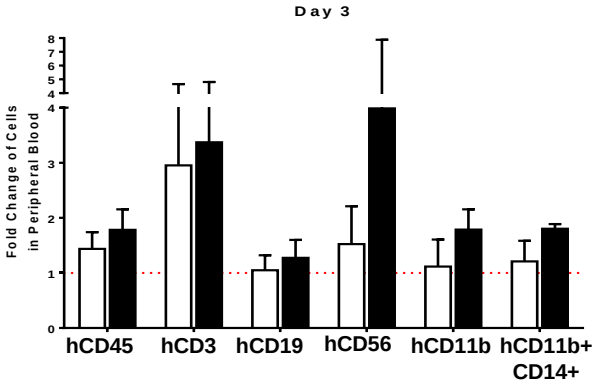


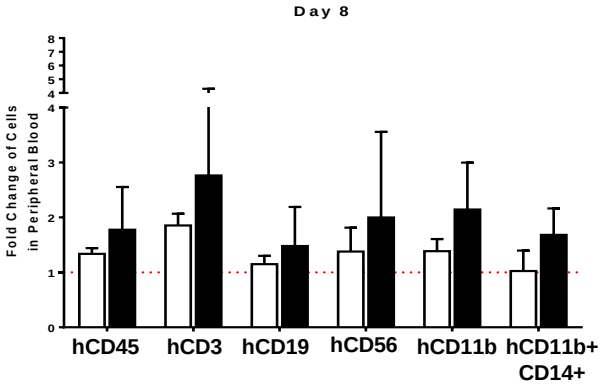
CAR-T Transduction efficiency

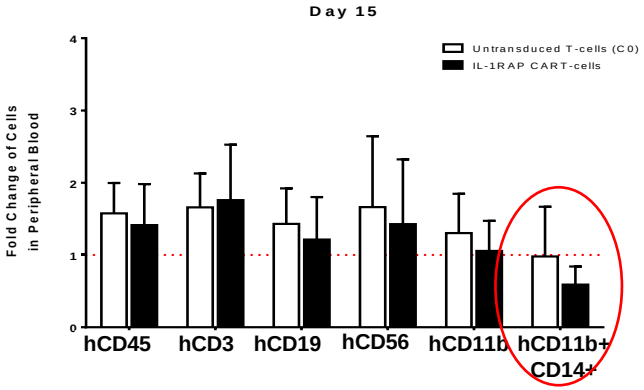


CAR-T functionality again cell line



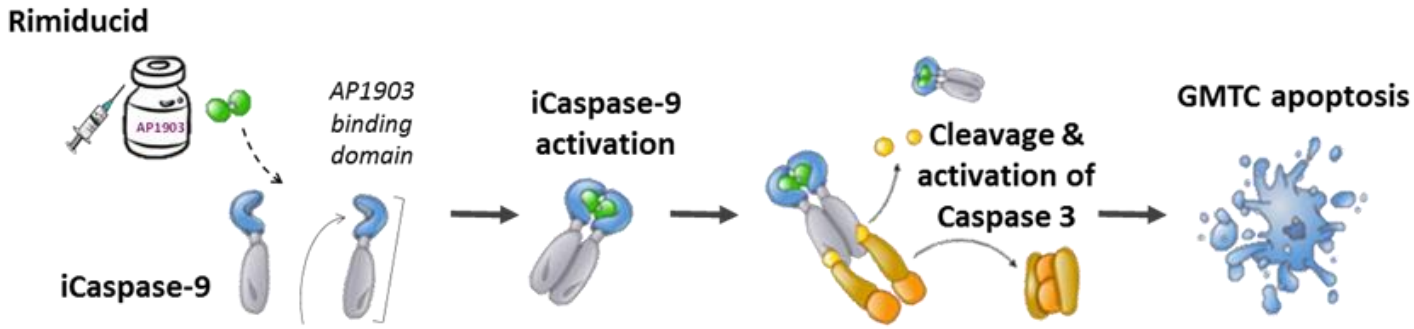




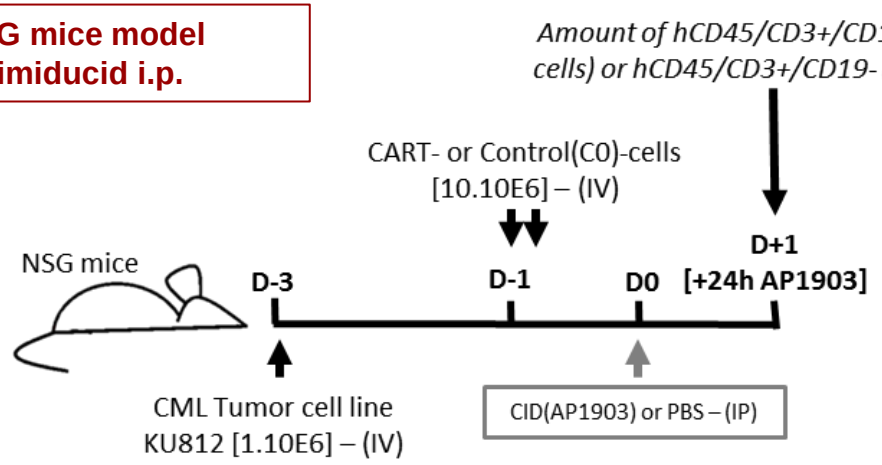


Results predict low toxicity on Healthy Hematopoietic System

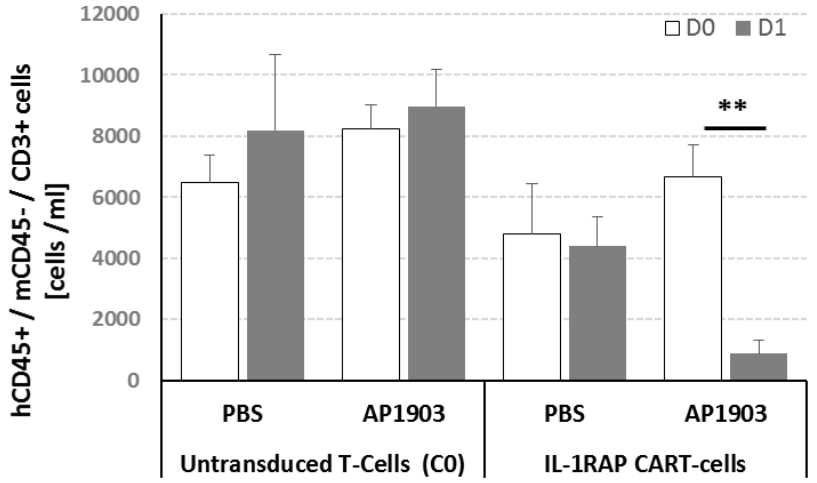
Safety suicide gene iCASP9 / AP1903 [Rimiducid®] switch – in-vivo



NSG mice model
Rimiducid i.p.



Amount of CAR-T cells depleted after Rimiducid injection.



** : p<0.01, n=3

Activation of suicide switch allows to kill more than 80% of IL-1RAP CAR-T cells in 24h (88,9% after 48h), n=3

Status of IL-1RAP CART-cells development in AML

RESEARCH → 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
Neta Da Rocha et al, submitted
Trad Rim al, submitted

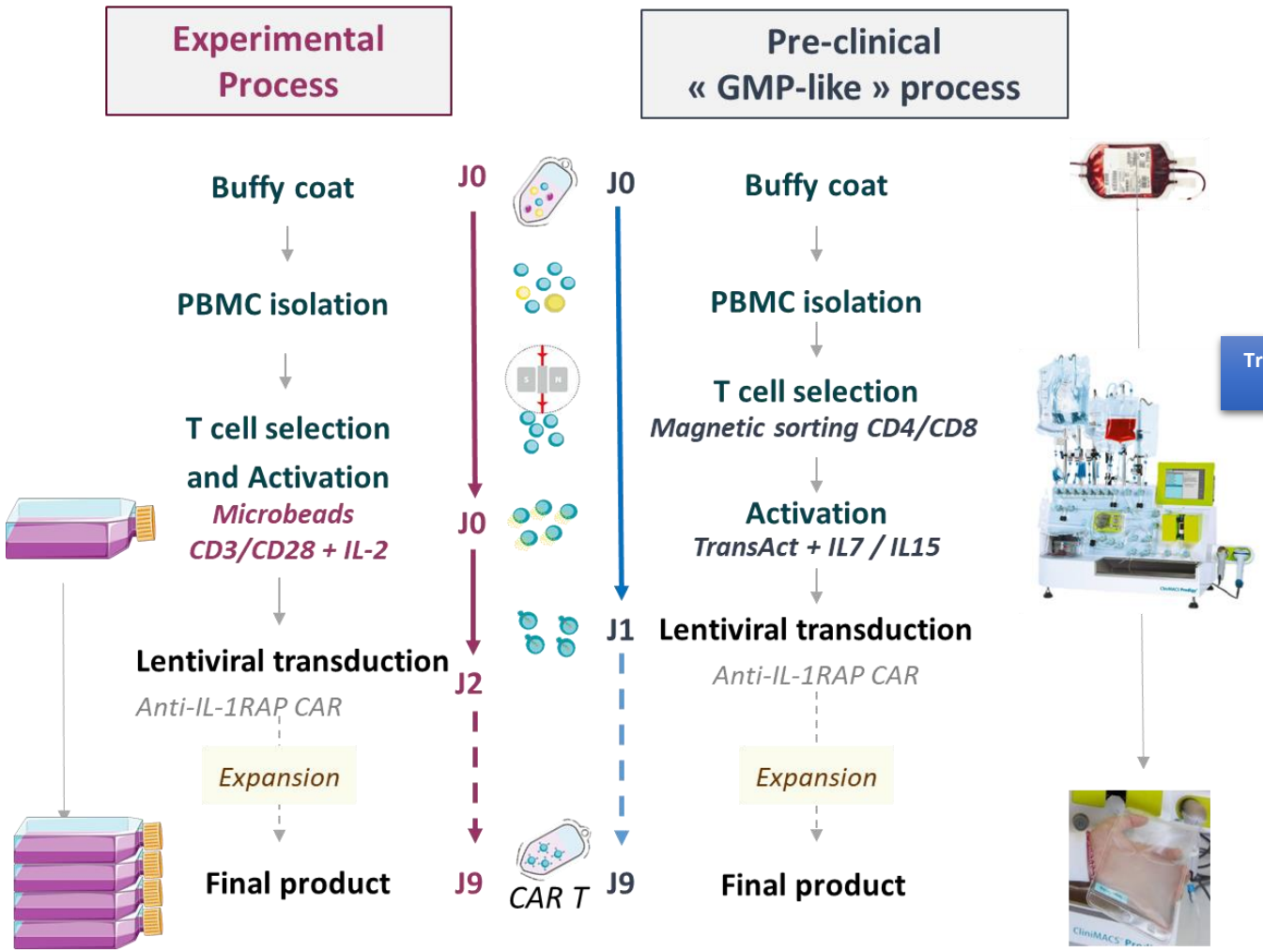
PRECLINICAL VALIDATION →

- **Preclinical « GMP like » process validation** - *In progress*
- Preclinical GMP process validation / Regulatory dossier – *Awaiting GMP raw material*

CLINICAL TRIAL → Awaiting GMP preclinical validation

INITIATION OF A PHASE I/IIA DOSE ESCALATION CLINICAL TRIAL FOR CAR IL-1RAP

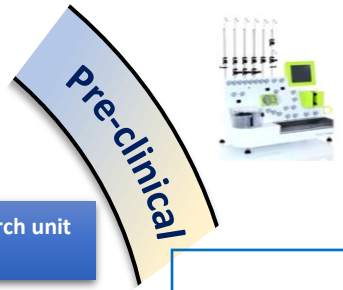
Preclinical step: Transfer of production process and up-scale



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

Transfer and up-scaling validation



Translational Research unit (UMR1098)

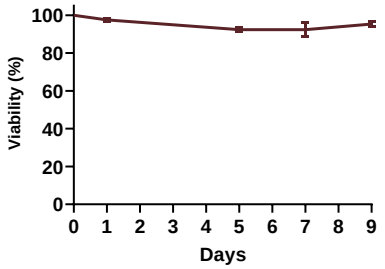
Pre-clinical improvment, scaling-up

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)*

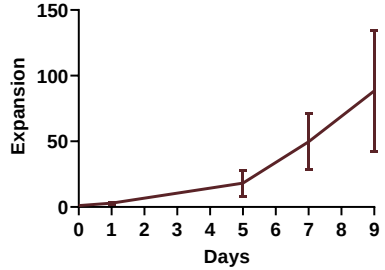
IL-1RAP CART-cells characterization in Final product:

Final product viability



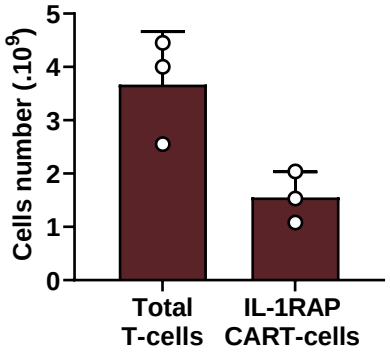
→ Viability of cells **>95%** (95,00±1,15%) in final product at day 9.

Cells expansion



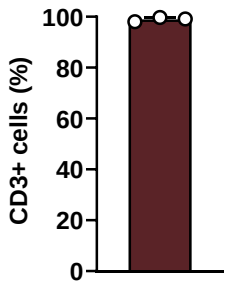
→ Strong cell expansion: **88,394±46,323 expansion factor**

Cell count



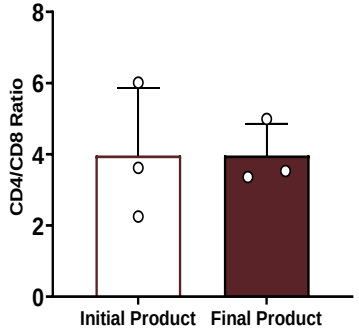
→ Number of CART-cells: in final product: **1,55±0,48 X 10^9 cells**

CD3+ purity



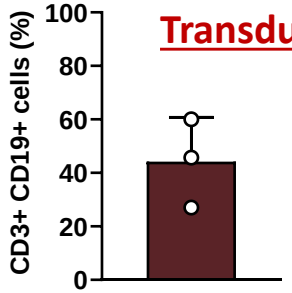
→ Product with high **CD3+ cell purity (98,93±0,86%)**

CD4/CD8 ratio



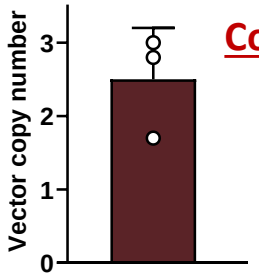
→ **CD4/CD8 ratio maintained** during the production process (3,96±1,90 vs 3,96±0,89)

Transduction efficiency



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

Copy of transgene

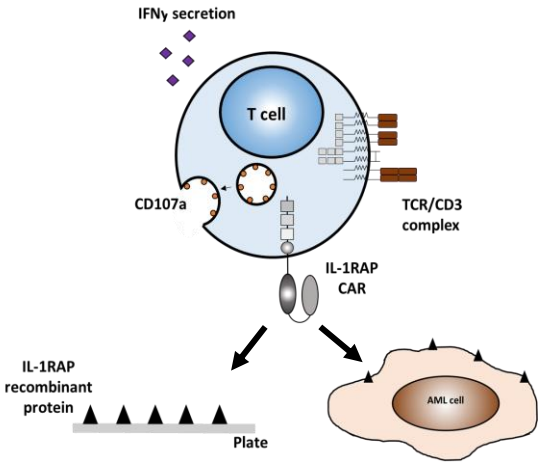


→ Number of transgene copies per cells: **2,50±0,70**

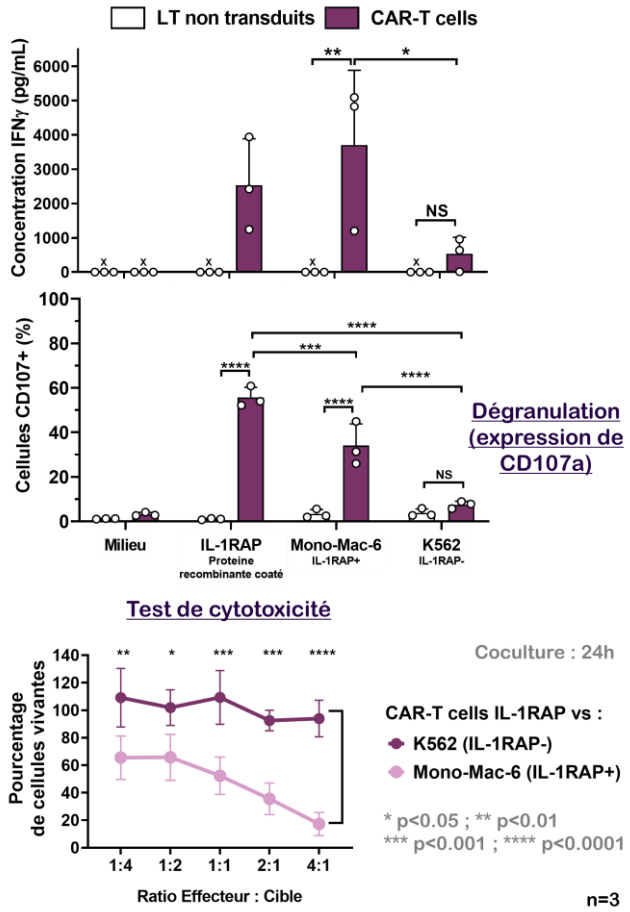
n=3

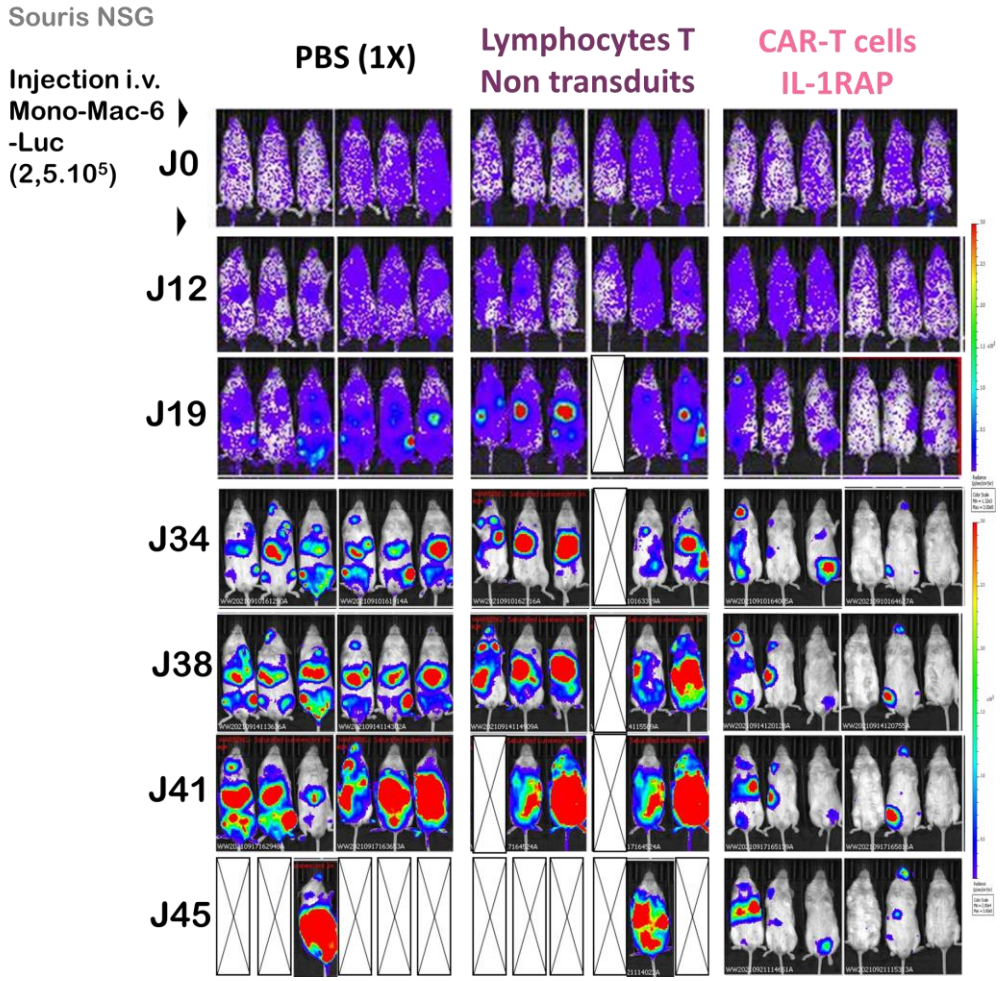
→ All parameters are in agreement for clinical application in a phase I/IIa clinical trial

Functional study of IL-1RAP CAR-T cells



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





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

Next step - GMP production and clinical trial

Besançon UMR1098 (France) ATMP facilities

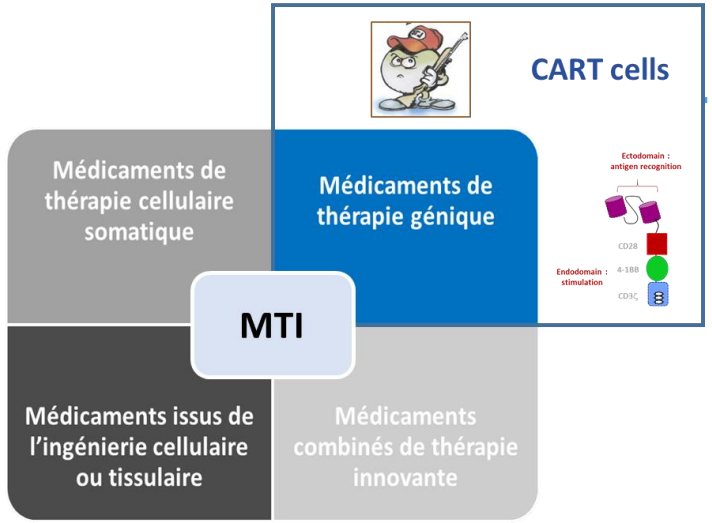
Clean and secure area: Airlocks and Grommets (biological material, consumables, waste)

Automated process

Trained and authorised staff

ATMP unit (EFSBFC)

Règlementation européenne - CE 2007-1394



EUROPEAN MEDICINES AGENCY

Dossier for regulatory agency

Regulatory agency

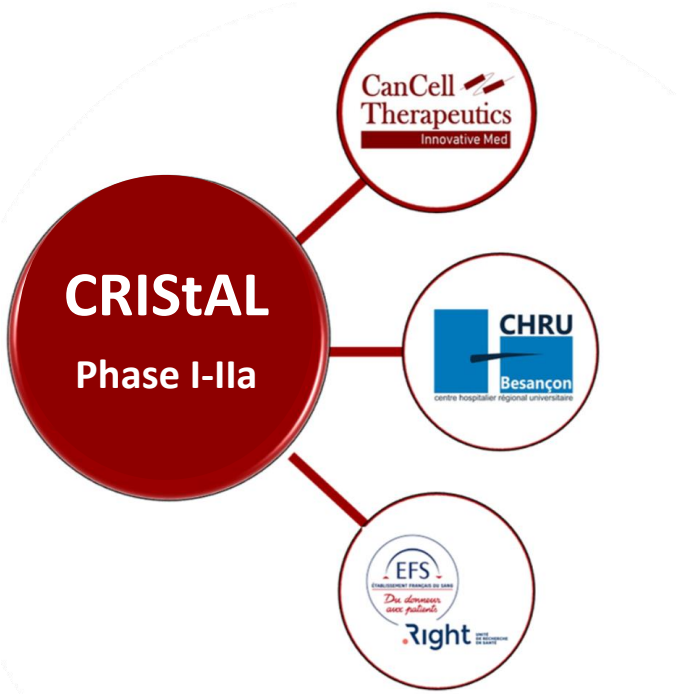
GMP production

Clinical grade production

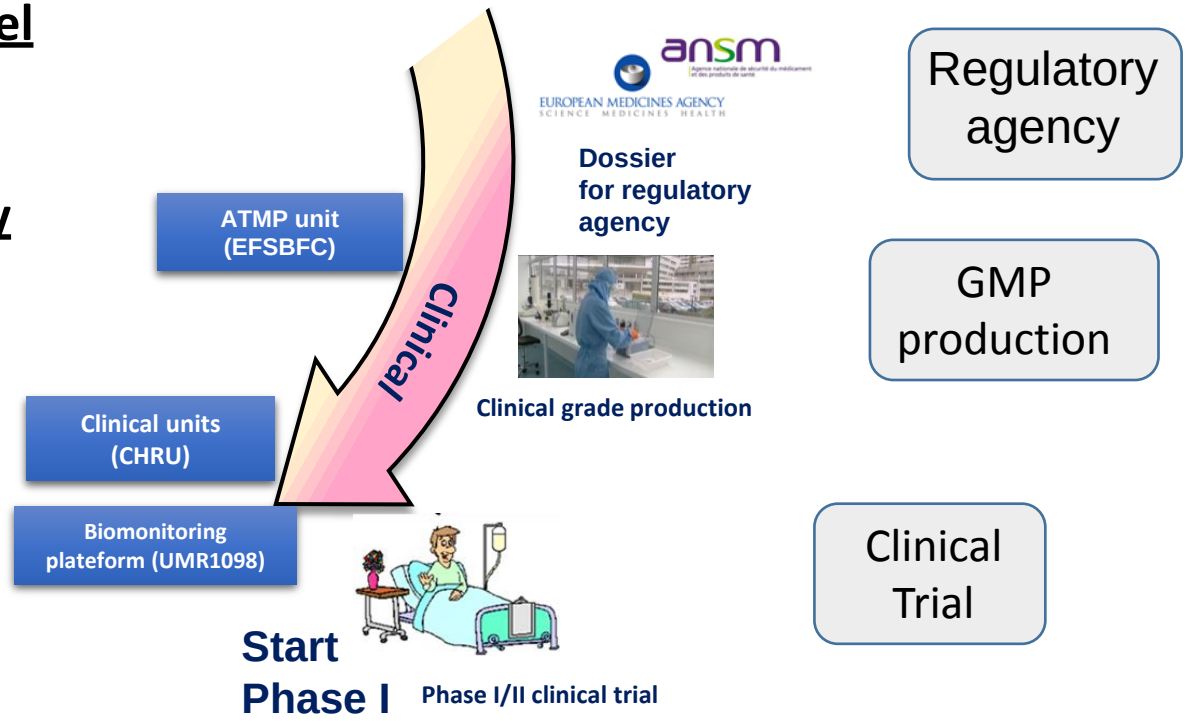
Next step - clinical trial CRIStAL

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)



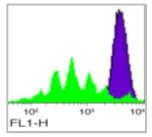
- **Phase I-IIa open label**
- **Dose-escalation**
- **First in human study**
- **Evaluation of the safety and clinical activity**



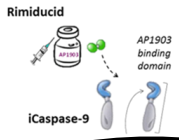
Academic CART Cell production: *From the target to the patient…*

Proof of concept

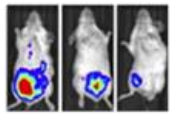
Functionnality (*in-vitro*)



Safety

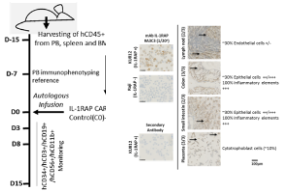


CART-cells Cytotoxicity (*in-vivo*)



Safety / Off-target toxicity

Toxicity (*TMA; in-vivo*)

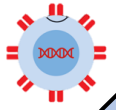


Transfer and up-scaling validation

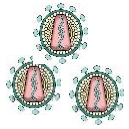
Pre-clinical improvment, scaling-up

Construction of the viral vector & supernatant production

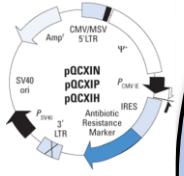
Transduction of T-lymphocytes (CART-cells)



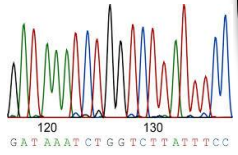
Lentiviral supernatant production



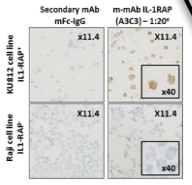
Cloning within a lentiviral backbone



Molecular sequencing (Sanger)



MAb selection (Specificity)



Production of a monoclonal antibody (Mab, Hybridoma)

Immunisation IP or Foodpad of mice

Research

Research unit

2014

Pre-clinical

Translational Research unit

2019

2021

Regulatory agency approval

Dossier for regulatory agency

GMP production

ATMP unit (EFSBFC)

2023

Clinical

Clinical grade production

Clinical units (CHRU)

Biomonitoring plateform

Clinical Trial

Start Phase I

Phase I/II clinical trial

Hypothesis / Choose a tumor target

UMR1098 Director
Pr Philippe SAAS

TIM-C team Director
Pr Olivier ADOTEVI

Team CAR IL-1RAP
Dr Christophe FERRAND
Dr Marina DESCHAMPS
Dr Walid WARDA
Rim TRAD
Lucie BOUQUET
Clémentine NICOD
RafiK HADERBACHE
Mélanie GUIOT
Marie-Charlotte LAUDE
Léa BOSDURE
Eva Loren CHARAVNER
Evan SEFFAR
Mathieu GONCALVES

Margaux POUSSARD
Maxime FREDON
Dr Fanny ANGELOT-DELETTRE
Pr Francine GARNACHE-OTTOU
Sabeha BIICHLE
Patricia LETONDAL
Dr Florian RENOSI
Dr Yann GODET

MTI-EFS/BFC
Dr Fabienne POUTHIER
Dr Stéphane ROUX
Dr Jeanne GALAINE

CHU Besançon
Dr Xavier ROUSSEL
Dr Etienne DAGUINDAU
Dr Ana BERCEANNU
Dr Yohann DESBROSSES
Pr Eric DECONINCK

Rafik HADERBACHE *Mathieu GONCALVES* *Clementine NICOD* **Eva loren CHARAVNER**

Walid WARDA **Mathieu NETO DA ROCHA** **Christophe FERRAND** **Marina DESCHAMPS** *Lucie BOUQUET* *RIM TRAD*