P9.5

Development of allogeneic gene-edited CAR T cells: from preclinic to clinical proof of concept

Agnès Gouble, Roman Galetto, Julianne Smith, Philippe Duchateau, David Sourdive, Laurent Poirot and Stéphane Depil

Cellectis, Paris, France & New York, NY, USA

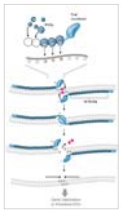
1 Abstract

Adoptive immunotherapy using engineered T-cells has emerged as a powerful approach to treat cancer. The potential of this approach relies on the ability to redirect the specificity of T-cells through *ex vivo* genetic engineering and transfer of chimeric antigen receptors (CARs) or engineered TCRs. The transduction of patients' blood cells with an anti CD19 CAR for the treatment of Acute Lymphoblastic Leukemia (ALL) have led to complete responses in the large majority of treated patients and early approval of two products. These autologous treatments require a complex manufacturing process and are dependent on the existence of a healthy T-cell population despite previous heavy chemotherapy lines of treatment. The use of allogeneic cells (derived from healthy donors rather than the patients themselves) allows preparation of cells ahead of a patient's need for treatment, in depth characterization of starting material, production and quality control of multiple treatment doses from one run and more affordable access to treatment.

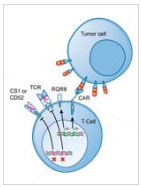
Using our proprietary nuclease-based gene editing technologies, we showed our capability to efficiently edit any gene in primary T-cells with very high precision. Here, we described how TALEN® gene-editing technology allows to create CAR T-cells that can be used in allogeneic setting but also empower them with additional safety and efficacy attributes. These new features include, among other possibilities, control properties, resistance to standard oncology treatments, and prevent fratricide killing of engineered CAR T-cells.

We were able to develop GMP-compliant manufacturing of TALEN® edited CAR T-cells for clinical use. Three allogeneic CAR T-cell products are now in the clinic. Preliminary data with UCART19 show expansion of those allogeneic cells associated with antitumor activity, providing first clinical proof of concept of the allogeneic approach. This technology offers therefore unparalleled possibilities to design next generation cell immunotherapies in hematological malignancies as well as in solid tumors.

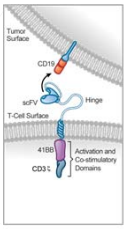
2 UCART: Allogeneic gene-edited CAR T-cells



Gene Editing
TALEN® Nuclease

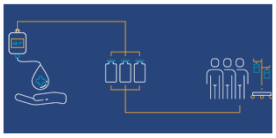


Allogeneic
CAR T cell



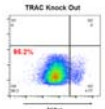
Chimeric Antigen Receptor
Tumor Recognition

Allogeneic CAR T-cells:
- *Immediately available to patients*
- *Can address T-cell deficiency*
- *Ability to re-dose*
- *Shipped worldwide, ahead of time*
- *Competitive CoGs and logistics costs*

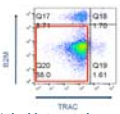


3 TALEN® high yield gene editing

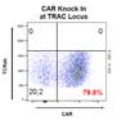
High Yield Multiplex Gene Editing using TALEN® (Knock Out and Knock In): Allogeneic CAR T-cells with additional safety and/or efficacy attributes



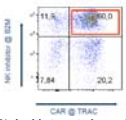
95.2% Single Knock-Out



88% double targeted gene inactivation
TRAC and B2M



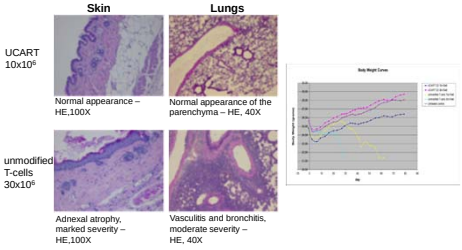
~80% Single Knock-In



60% double targeted gene insertion
CAR insertion at TRAC
NK inhibitor at B2M

4 UCART doesn't induce GvHD *in vivo*

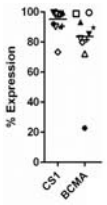
UCART cells do not induce Graft vs Host Disease in NSG mice: no body weight loss and no histopathological changes



Body weight and examples of histopathological findings of NSG mice intravenously injected with unmodified T-cells or UCART cells ($10x10^6$ or $30x10^6$ cells/mouse) 1 day post irradiation at 2Gy. Mouse body weight measured for a 80 day follow-up period. Histopathological analysis at sacrifice.

5 *CS1* inactivation using TALEN® allows efficient anti-CS1 CAR T-cells production

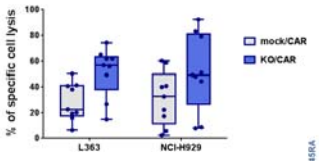
- *CS1 is highly expressed on multiple myeloma cells: CS1 is a promising target for CAR T-cell therapy*
- *CS1 is expressed on CD8+ T-cells: efficient production of anti-CS1 CAR T-cells requires CS1 knock-out to prevent CAR T-cells cross reactivity*



CS1 and BCMA expression on plasma cells from Multiple Myeloma patients (in collaboration with R. Mathur and S. Neelapu, MD Anderson Cancer Center, Houston, USA)

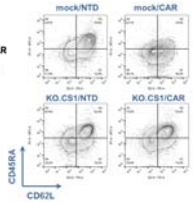
Increased activity of CAR T-cells following CS1 inactivation

Higher anti-tumor activity



In vitro cytotoxic activity was evaluated by flow cytometry following 4h co-culture of engineered T-cells with CS1+ (NCI-H929 and L-363) and CS1- (MOLM-13) cell lines. NTD: non-transduced.

Increased $T_{SCM}+T_{CM}$ / $T_{EM+TEMRA}$ ratio



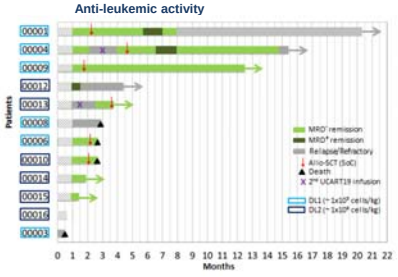
6 UCART19: Initial proof of concept of allogeneic CAR T-cells in ALL patients

- 1st patient dosed in June 2015 (compassionate); Phase I trials started in June 2016 in EU, in 2017 in the US
- 20 patients treated disclosed (12 adults and 8 pediatric, including compassionate)
- UCART19 is exclusively licensed to Servier and under a joint clinical development program between Servier and Allogene

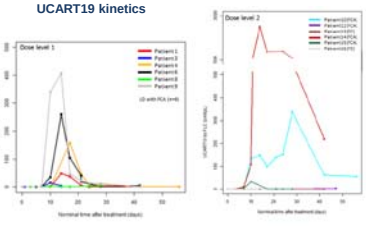
Interim Ph1 Dose Escalation In Adult ALL Patients (CALM study):

Patient median age	Lymphodepletion	Disease status before treatment	Dose level (CAR T-cells / kg)	Response	Adverse event > Grade 3	Follow up
29.5 years [range: 18-62]	FCA (10/10) FC (2/12)	12/12 R/R ALL 8/12 ≥ 3 prior treatment lines 7/12 with prior allo-SCT 6/12 > 25% blasts in bone marrow	DL1: $1x10^5$ DL2: $1x10^6$	2 patients non evaluable at D28 **8/10 evaluable patients achieved CR at D28 (88% MRD- CR)**	CRS 8.3% (1/12) Prolonged cytopenia 25% (3/12) Neutropenic sepsis 16.7% (2/12)	4 patients still MRD- CR at 12.4, 3.6, 1.8 and 1.3 months post UCART19

Anti-leukemic activity



UCART19 kinetics



- *Expansion of UCART19*
- *No severe GvHD, all patients but one experienced manageable CRS*
- *2 patients received a 2nd dose of UCART19 (off-protocol), whom both achieved MRD- CR at D28*
- *Preliminary sign of efficacy: 8/10 evaluable patients achieved CR at D28*

7 Industrialized manufacturing process

An integrated system combining gene-editing and CAR T-cells manufacturing process

- Successful GMP manufacturing of UCART19, UCART123, UCART22
- GMP manufacturing of UCARTCS1 started
- QC system in place, cleared for clinical trials by the FDA and in the EU

8 Conclusions

Allogeneic CAR T-cells using TALEN® gene-editing:
- *High yield multiplex gene editing using TALEN®*
- *GMP manufacturing process developed*
- *3 UCART products in clinic*
- *No significant GvHD in UCART19 and UCART123-treated patients so far*
- *PoC of allogeneic CAR T-cells: UCART cells engraft and expand and preliminary signs of efficacy were obtained with the first ALL patients treated with UCART19*