DEVELOPMENT OF AN INDUCIBLE CAR-T CELL PLATFORM USING LENTIVIRAL VECTOR

THERAVECTYS, 1 mail du Professeur Georges Mathé,
94800 VILLEJUIF, FRANCE

Sophie AGAUGUE, Emeline SARRY, Ana BEJANARIU, Klervi EVEN-DESRUMEAUX, Abdel ZEMMAR, Cécile BAUCHE

BACKGROUND

THERAVECTYS is a privately-owned, fully integrated discovery and clinical development biotech company originating from the Pasteur Institute. The company capitalizes on more than 15 years of fundamental research in the field of lentiviral vectors and owns over 20 patent families, either developed in-house or exclusively licensed from worldwide renown institutions from which the company has secured exclusive worldwide rights for the use of lentiviral vectors in vaccination and immunotherapy applications.

Based on its lentiviral vector technology platform, THERAVECTYS develops therapeutic vaccines and immunotherapies to fight cancers and infectious diseases, including a proprietary and differentiated CAR T-cell technology platform.

The company has recently completed the first-ever vaccination clinical trial conducted with lentiviral vectors confirming both the safety & immunogenicity of the lentiviral vector platform.

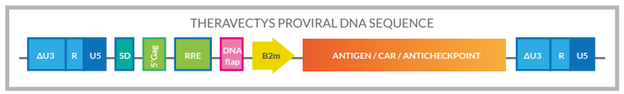
THERAVECTYS has recently been granted the status of a GMP pharmaceutical manufacturing establishment, by the French regulatory agency to produce lentiviral vectors for clinical development purposes and to manipulate human cells in the context of Chimeric Antigen Receptors (CARs) and T-Cell Receptor (TCR)-based cell therapies.

THERAVECTYS' TECHNOLOGY

Lentiviral vectors have the unique ability to transduce dividing cells - such as T-cells - but also non-dividing cells - such as dendritic cells in a stable manner.

The transgene encompassed in the lentiviral vector are under the regulation of a proprietary patented human promoter (beta-2 microgobulin) that is overexpressed in APC but also in T-cells and NK cells.

This integrative, non-replicative, non-pathogenic and self-inactivating recombinant lentiviral vector derived from the HIV-1 NL4-3 strain has been successfully used in a therapeutic clinical trial against HIV and will be used to initiate two additional phase I/II in oncology including one CAR T-cell trial.



GMP BIO-PRODUCTION & QC

THERAVECTYS has designed an innovative lentiviral vector manufacturing process combining high production yield, high immunogenicity and impurity profile compatible with direct injection into humans.

Furthermore, the lentiviral vectors produced by THERAVECTYS are also suitable for ex-vivo transduction of T-cells and NK cells in the context of adoptive T-cell therapies.

In parallel, THERAVECTYS has developed specific assays to control the quality requested for the release of lentiviral vectors (titration, RCL, residual DNA quantification…) and lentiviral vector-modified T-cells (MOI, integration profile…).

Moreover THERAVECTYS has set up a 750m2 bioproduction and QC facility designed in compliance with GMP and ISO standards. It consists in several independent production suites, including upstream and downstream process rooms and an aseptic filling suite (including a lyophilization step). The plant has an annual capacity of 24 active batches. Human cells are cultured in suspension in up to 1,000 liter bioreactors, using synthetic medium and disposable materials.

INNOVATIVE CARs

Chimeric antigen receptors (CARs) are usually composed of a scFv (single chain variable fragment) antigen binding domain linked to a spacer and intracellular signaling domains. CAR expression into T-cells allows the reinfusion of cells with optimal effector functions targeting a specific binding antigen.

Despite promising clinical results in hematological malignancies, CAR-T cells may be associated with some toxicity events (cytokine storm, on target/off-tumor). Improving safety while maintaining or improving anti-tumor efficacy of CAR-T cells therefore remains a key therapeutic challenge.

Based on its proprietary lentiviral vector technology, THERAVECTYS has decided to fully leverage its assets and to enter the CAR-T space with a differentiated program and to overcome the main limitations of current clinical developments: safety post-reinfusion, long-term toxicity, efficacy against solid tumors, reactivation capability, cost of the treatment.

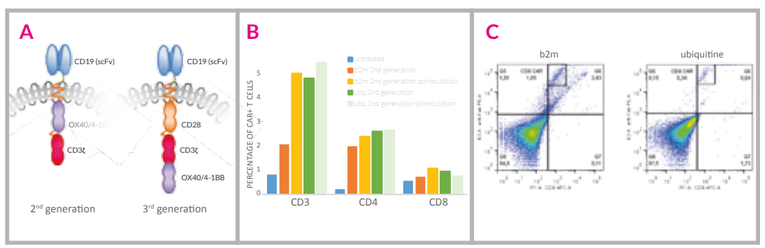
A BETTER CAR EXPRESSION

The potency of the CAR T-cells depends on the number of receptors expressed on their surface.

THERAVECTYS is developing a proprietary lentiviral vector coding for CARs under the regulation of the beta-2 microglobulin promoter. The overexpression of the CARs at the surface of the T-cells should allow a higher efficacy with a lower amount of cells to be re-infused.

Both the second (containing the CD3ζ and the 4-1BB co-signaling domains) and third generations (containing the CD3ζ, the CD28 and the 4-1BB domains) have been evaluated against hematological malignancies expressing CD19 (for CD19+ leukemias and lymphomas) used as a benchmark.

As shown in figures B and C, CARs are overexpressed under the control of the beta-2 microglobulin promoter when compared to ubiquitine. This overexpression of the CAR induced by the beta-2 microglobulin promoter does not depend neither on the CAR generation, nor on the targeted binding domain, nor on the blood donor.



A. The second and the third generation of CAR currently evaluated.

B. Percentage of CAR+ T-cells after 24h of transduction with the CAR constructs under beta2microglobulin or ubiquitin promoters.

C. FACS analysis of CAR expression at the surface of purified human CD8+ T-cells

A SWITCH FOR AN INDUCIBLE CAR TRAFFICKING

THERAVECTYS is developing an inducible CAR technology allowing an on-demand ON/OFF expression of the CARs at the surface of T-cells.

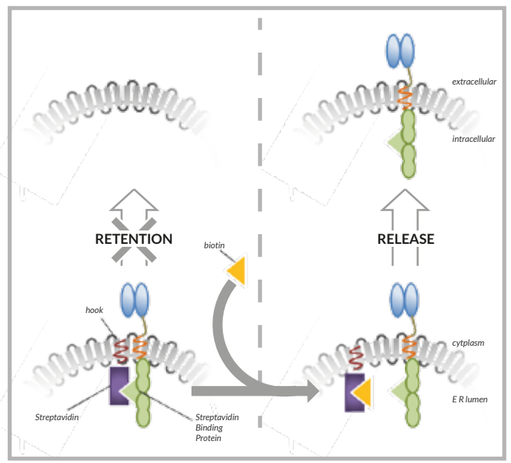
The switch developed by THERAVECTYS relies on the RUSH system (Retention Using Selective Hook): the CAR fused with the streptavidin binding protein (SBP) will be retained within the endoplasmic reticulum (ER) by an anchored hook coupled with the streptavidin protein; due to its greater affinity with streptavidin, biotin - orally administered or intravenously injected - will replace the SBP allowing the release from the ER of CARs which will be thus expressed at the surface of the cytoplasmic membrane of T-cells and NK cells.

The release of CARs can be turned OFF upon exhaution of biotin or following streptavidin administering - the antagonist of biotin. T-cells can be easily reactivated within a few days or a few months following further administering of biotin.

Hooks and CARs are vectorized within the same lentiviral vector (b2m-HOOK-IRES-CAR).

A CD19-CAR RUSH is currently under in-vitro evaluation - both on immortalized cells (HEK293T, Jurkat) and primary cells (T-cells) - as well as in-vivo evaluation to confirm the efficacy and the specificity of the construct. The program is expected to enter into the clinics within the next 12 months.

This system will increase safety of CAR-T cells and will allow the treatment of the patient at his bedside.



NANOBODIES

To screen and select new proprietary binding domains for its CAR T-cell program, THERAVECTYS has recently secured exclusive and worldwide access to a synthetic humanized nanobody library from Institut Curie.

The synthetic nature of the library will allow the identification of nanobodies in less than six months, as well as the engineering of their biochemical properties including affinity and stability.

Due to their low molecular weight, nanobodies can be easily vectorized allowing the expression of single or bi-specfic domains.

NEXT STEPS

THERAVECTYS technology platforms allow flexibility and reactivity in CAR design, production and evaluation, thus leading to the generation of optimal CAR-T cells. This proprietary and differentiated inducible and reversible (ON/OFF) CAR T-cell technology will enter the clinics within 12 months and is aimed to be delivered at-the-patient-bedside (automated process).

Alone or with partners, THERAVECTYS plans to advance its pipeline of oncology and infectious disease therapies using the company's integrated set of discovery, clinical development, and manufacturing capabilities.