DEVELOPMENT OF AN ANTI-HTLV-1 VACCINE FOR THE TREATMENT OF ADULT T-CELL LEUKEMIA/LYMPHOMA

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

Ana Bejanariu[*], Lamya Loussaief[*], Julien Mafille, Abdel Zemmar, Emmanuelle Sabbah-Petrover, Karima Belkhiria, Téreza Coman[1], Julien Rossignol[1], Olivier Hermine[1] and Déborah Revaud.

BACKGROUND

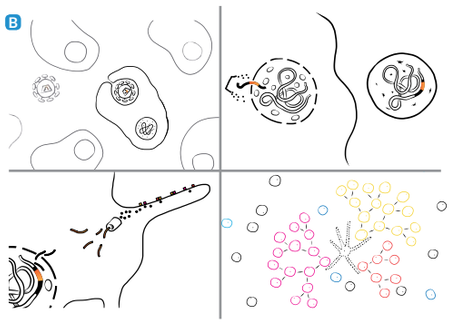
THERAVECTYS is a privately-owned, fully-integrated discovery and clinical development biotech company originating from the Pasteur Institute. Based on its lentiviral vector technology, THERAVECTYS develops therapeutic vaccines and immunotherapies to fight diseases against which efficient T-cell immune response is required (ie viral, bacterial and parasitic infections, cancers).

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

THERAVECTYS' TECHNOLOGY

Vaccine candidates developed by THERAVECTYS are integrative recombinant vectors derived from the HIV-1 NL4-3 strain. They are non-replicative, non-pathogenic & self-inactivating A.

They have the unique ability to transduce both dividing and non-dividing cells such as dendritic cells, thanks to dubbed DNA Flap, leading to a broad, intense and long lasting cellular immune response B.



The transgene encompassed in the lentiviral vectors are under the regulation of a proprietary patented human promoter (beta-2 microglobulin) overexpressed in antigen presentating cells.

[*] *co-authors.*

[1] *Téreza Coman, Julien Rossignol, Olivier Hermine: Department of Haematology, Necker Children's Hospital, Assistance Publique-Hôpitaux de Paris (APHP), Paris, France; Sorbonne Paris Cité, Paris Descartes University, Imagine Institute, Paris, France; Centre de référence des déficits immunitaires héréditaires (CEREDIH), Necker Children's Hospital, Assistance Publique-Hôpitaux de Paris (APHP), Paris, France; INSERM UMR 1163, CNRS ERL 8654 IMAGINE Institut, Paris, France.*

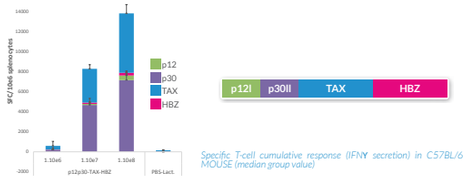
PRECLINICAL DEVELOPMENT OF THE ANTI-HTLV-1 VACCINE CANDIDATE: THE THV02 TREATMENT

Part1: Immunogenicity

The THV02 treatment is composed of 2 therapeutic vaccines, THV02-1 and THV02-2, encoding for a unique polypeptide derived from Tax, HBZ, p12I and p30II proteins, involved in HTLV-1 pathogenicity and known to be recognized by the immune system of HTLV-1 infected patients.

Preclinical results have demonstrated that THV02 products can induce an intense and diversified cellular immune response in rodents as demonstrated by IFN-Y Elispot assays.

The diversity of the response depends of the strain of animals.



Specific T-cell cumulative response (IFNY secretion) in C57BL/6 MOUSE (median group value)

Part3: Efficacy

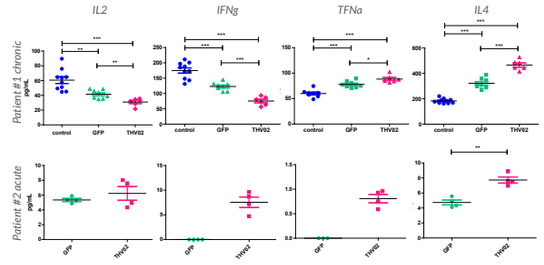
As no relevant ATL immunocompetent animals model could be used to assess the efficacy of a direct injection of the THV02 treatment according to the expression pattern of HTLV-1 proteins observed in patients, Theravectys has developed in collaboration with Pr Olivier Hermine from Necker hospital (Paris), an *ex vivo* efficacy model using blood samples of ATL patients A.

Briefly, monocyte-derived dendritic cells (MDDC) from blood of ATL patients, transduced with THV02-1, are co-cultured with autologous CD8+ T-cells for stimulation of the cellular immune response. Autologous CD4+CD25+ are then co-cultured with activated CD8+ cells and the cytotoxic activity is monitored by flow cytometry and Luminex® analyses.

THV02 preclinical development has received a positive feed-back from the EMA during a Scientific Advice request in february 2014.

Four assays has been performed in 2 different patients, 1 chronic and 1 acute, in order to optimize the model and determine the accurate time of the different co-cultures.

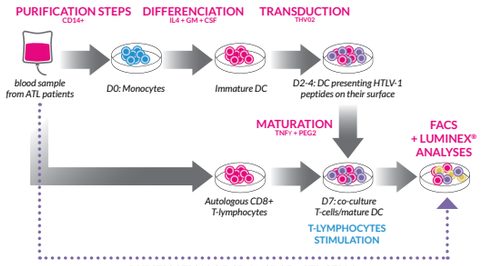
B LUMINEX® ANALYSES OF CYTOKINE SECRETED IN THE MEDIA AFTER 2 DAYS OF MDCC- CD8 CO-CULTURE PLUS CD4CD25 AUTOLOGOUS PURIFIED CELLS



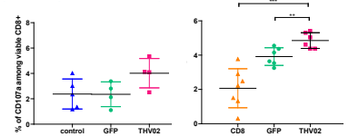
Media were removed from the co-cultures to be analyzed using Luminex® Technology for a determined cocktail of cytokines.
For patient #1, media were removed 2 days after MDDC-CD8 co-culture and 1 day after the seeding of CD4CD25 cells in the MDDC-CD8 co-culture.
For patient #2, media were removed 1 days before MDDC-CD8-CD4CD25. In the two patients, a specific increase of cytokines of stimulation IL4 and TNFa due to THV2 treatment, compare to untreated control or to GFP lentiviral vector. The profile of other cytokines depends of the patient.

These assays demonstrated: i) specific stimulation of the cellular immune response attested by secretion of cytokines of activation in the media B, ii) specific activation of the cytotoxic activity of CD8+ cells attested by the increase of CD107a marker in FACS analyses C.

A PROTOCOL:



C CD107a FACS ANALYSES ON ATL PATIENT BLOOD SAMPLE #1 (CHRONIC SUBTYPE) AND #2 (ACUTE SUBTYPE).



Expression of CD107a has been studied in viable CD8+ cells of the different co-cultures, 6 hours after seeding feshly purified CD4+CD25+ cells.

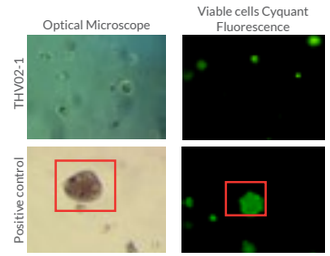
Part2: Safety

As the THV02 antigen is derived from HTLV-1 oncogenic proteins, in vitro and in vivo oncogenicity studies were performed, in primary mouse & human cells and in immunodeficient NOG mice respectively, in order to assess the THV02 safety.

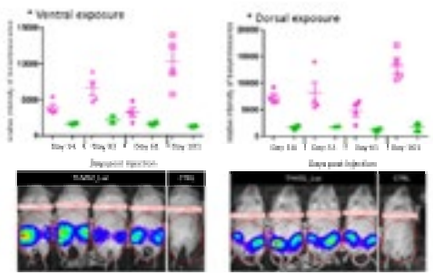
In vitro, clonogenic assays demonstrated no transformation of immortalized mouse primary cells as well as human and mouse non immortalized primary cells after transduction with THV02-1 product A.

In vivo, carcinogenicity study demonstrated no appearance of tumor phenotype in NOG mice 6 months after *in vivo* intramuscular injection of the THV02-1 product B.

A IMMORTILIZED MEF, 21 DAY OF INCLUSION



B 3 MONTHS IN VIVO CARCINOGENICITY: BIOLUMINESCENCE ASSAY USING THV02_LUCIFERASE



MUSCLE INJECTION SITE

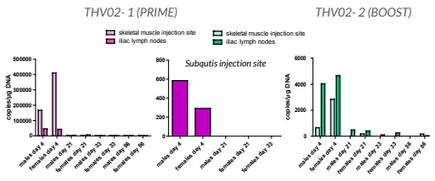
Part4: Regulatory preclinical study

GLP regulatory preclinical studies have been performed using a prime/boost regimen of the THV02 treatment (THV02-1 and THV02-2) to evaluate: i) the toxicity of the treatment , ii) the biodistribution and the shedding of the THV02-1 and THV02-2 products.

These studies were performed on Sprague-Dawley rats with the Maximum Achievable Dose (MAD) of the products.

Results demonstrated absence of toxicity following treatment, as well as a fast clearance of the product in positive organs (injection sites and draining lymph node only) and no dissemination in the environment A.

A RESULTS OF REGULATORY BIODISTRIBUTION STUDY



Median of copies/µg DNA in muscle injection site, subcutis injection site and iliac lymph node.

**Statistical analyses: unpaired t-test with *p<0,05; 0,001 ≤ **p < 0,0001 and ***p<0,0001*

CLINICAL DEVELOPMENT

THERAVECTYS plans to perform a clinical trial in ATL patients to assess the safety and the immunogenicity (cellular immune repsonse) of the THV02 treatment as co-primary objectives.

This trial will be open-label, up to 24 patients will be enrolled and will receive a direct intramuscular injection of THV02-1 and THV02-2 products in a prime-boost regimen.

Patients will be assigned to 2 different doses (2.10e8 TU low dose or 1.10e9 TU high dose). The escalation to the next level of dose will be allowed by the safety review board depending of the DLT (Dose Limiting Toxicity).

The coordinating investigator will be Pr. Olivier Hermine (Necker Hospital, Paris).

SPONSOR / CODE	THERAVECTYS / THV02
STUDY PHASE	Phase I/II
STUDY TITLE	An open-label, dose-escalation, Phase I/II study to assess the safety and immunogenicity of the THV02 vaccination as treatment of patients with Adult T-cell Lymphoma-Leukemia (ATL).
RECRUITMENT	Up to 16 patients (8 per group) with Adult T-cell Lymphoma-Leukaemia (ATL).

INCLUSION CRITERIA:	EXCLUSION CRITERIA:
• Patient with ATL, according to Shimoyama criteria *(Shimoyama, 1991)*;	• Patient treated by at least two lines treatment;
• Patient newly diagnosed or under first line treatment *(chemo; AZT-IFN or chemo+AZT-IFN)*;	• Patient who has received a BM transplantation;
• Patients untreated or with disease control (stable disease, PR or CR) during 4 weeks;	• Patient with uncontrolled and active infection other than HTLV *(HIV, HBV, HVC etc...)*
• Patient with adequate hematologic and hepatic function and normal calcemia;	• Patient diagnosed or treated for another malignancy within 3 years of enrolment *(exception of complete resection of melanoma or low-risk prostate cancer after curative therapy)*;
• Patient with ECOG 0 or 1.	• Patient who received other investigational drugs within 14 days or 5 half-life before baseline.

PRIMARY OBJECTIVES	Safety and cellular immune response
SECONDARY OBJECTIVES	Clinical response
EXPLORATORY OBJECTIVES	Humoral immune response, Antigen spreading, viral RNA expression, Clonality