DEVELOPMENT OF NOVEL ANTI-CHECKPOINTS STRATEGIES BASED ON INNOVATIVE LENTIVIRAL VECTORS.

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BACKGROUND

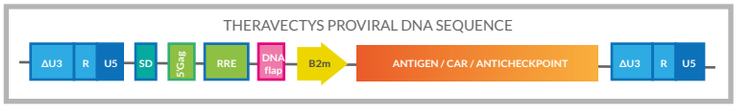
THERAVECTYS, a fully-integrated discovery and clinical development biotech company founded in 2005, develops a new generation of therapeutic vaccines and immunotherapies based on the lentiviral vector DNA Flap technology.

THERAVECTYS aims at developing innovative combined treatments in diseases against which efficient T-cell immune responses is required, such as viral, bacterial & parasitic infections and cancers.

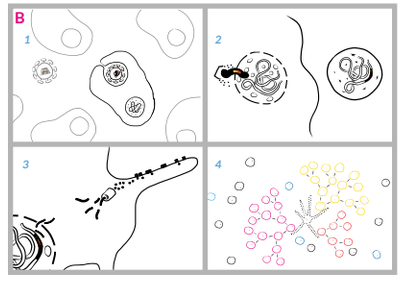
It is the first company to have launched a first in-human phase I/II clinical trial based on lentiviral vector technology with its THV01 product, a therapeutic vaccine targeting HIV.

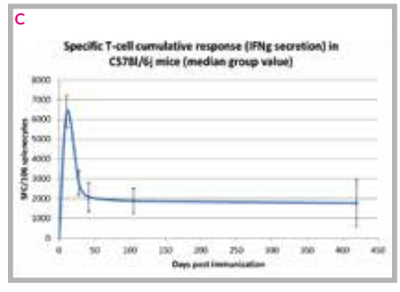
THERAVECTYS' TECHNOLOGY

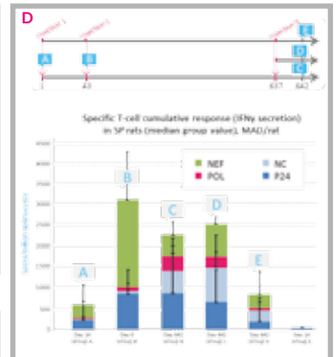
Integrative recombinant lentiviral vectors developed by THERAVECTYS are derived from the HIV-1 NL4-3 strain. They are non-replicative, non-pathogenic and self-inactivating (A). They are able to transduce both dividing and non-dividing cells thanks to dubbed DNA Flap, a 99 nucleotide long sequence identified in 1999 at Pasteur Institute, which is formed during the natural reverse transcription of HIV. This sequence is necessary for the virus' pre-integration complex to cross the nucleus membrane of the non-dividing cells it's infecting.



The ability of lentiviral vectors to transduce dendritic cells in a stable manner enables the presentation of antigens in an endogenous way to T-cells, thereby allowing the first vaccine applications to be developed (B). The antigens encoded by THERAVECTYS lentiviral vectors are under the regulation of THERAVECTYS' patented human promoter (THV-PROM) that is overexpressed in APC.







Characterization of vaccines developed by THERAVECTYS:

A. Vaccines proviral DNA sequence.

B. Mode of action :
1) Entry of the lentiviral vector in the immature dendritic cell. 2) After dendritic cell' transduction, the transgene is expressed and processed into epitopes. 3) A mature dendritic cell presents the epitopes at its surface to specific T lymphocytes. 3) Within 1 to 3 weeks, the transduced cells expressing the selected antigen are eliminated from the body.

C. Long lasting immune response induced inC57Bl/6j mice immunized by THV01-1 product (2.68 10^7TU/mouse).

D. Specific T-cell response after prime-boost regimens at the MAD (Maximal administrable dose) of THV01-01 and THV01-2 products, in Sprague Dawley rats.

Furthermore, THERAVECTYS has developed an efficient prime/boost regimen that enables iterative injections of the same product. Thus, vaccines developed by THERAVECTYS allow the generation of a long-lasting immune response that is both strong and specific (C&D).

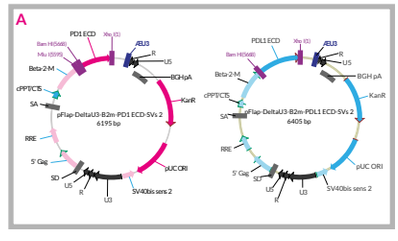
DEVELOPMENT OF NOVEL ANTI-CHECKPOINT STRATEGIES

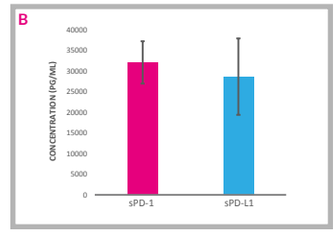
Tumors have evolved numerous mechanisms to escape immune surveillance, notably mechanisms linked to apoptosis, secretion of immunosuppressive molecules like IL-10 or TGFb, recruitment of immune suppressive cells such as regulatory T-cells or myeloïd-derived suppressor cells, downregulation or loss of MHC molecules to alter antigen presentation, expression or induction of molecules inhibiting T cell activation like HLA-G, IDO, CTLA-4 or PD-1/PD-L1.

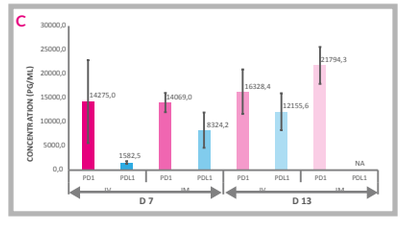
Among those mechanisms, the immune checkpoints PD-1/PD-L1 and CTLA-4 have been a focus of interest during the last years. CTLA-4 is expressed on T cells in the lymph nodes early during the immune response and acts as a physiological break to T-cell activation to maintain a normal immune homeostasis. PD-1 is expressed in the periphery later once T-cells have become activated to avoid auto-immune responses. These two mechanisms are used by tumors to dampen anti-tumoral immune responses. They are overexpressed in numerous tumor types and have thus become crucial therapeutic targets. Monoclonal antibodies directed against these molecules have been recently developed and show promising but still mitigated clinical results. More importantly, their toxicity is still very high.

THERAVECTYS is developing novel innovative strategies based on its proprietary lentiviral technology. It has been shown that soluble forms of PD-1 and PD-L1 corresponding to their extracellular domains, are able to bind to their corresponding partner, therefore blocking the access to the natural ligand. These soluble forms have shown anti-tumor efficacy in different *in vitro* and *in vivo* models. We have chosen to vectorize the extracellular domains of PD-1 and PD-L1 with a secretion signal in our lentiviral vectors (A).

We have made the proof of concept that these soluble forms are secreted at high levels in culture supernatants after *in vitro* transduction of HEK293 cells (B) and *in vivo* in C57Bl6 mice in terms of quantity and durability of the secretion (C).







Characterization of novel anti-checkpoints developed by THERAVECTYS

A. Lentiviral vectors maps.

B. Quantification of soluble forms of PD-1 and PD-L1 measured by ELISA in supernatants of HEK293 transduced with the corresponding lentiviral vectors.

C. Quantification of soluble forms of PD-1 and PD-L1 measured by ELISA in sera of mice injected with the corresponding lentiviral vectors.

NEXT STEP: COMBINED IMMUNOTHERAPIES

The anti-tumor or anti-infectious efficacy of these soluble forms will then be tested *in vivo* in combination with the different vaccines developed by THERAVECTYS.

THERAVECTYS is also developing nanobodies against PD-1, PD-L1 and CTLA-4. Nanobodies are antibody fragments made of a single monomeric variable domain derived from Llama heavy chain antibodies. They have numerous advantages compared to human monoclonal antibodies since they show high target specificity and affinity, low toxicity and they are easy to manufacture. Moreover they are very homolog to human antibodies, therefore avoiding any neutralization by human natural antibodies.

THERAVECTYS is currently selecting the nanobody clone with the best affinity for PD-1, PD-L1 and CTLA-4. The anti-tumor and anti-infectious activity of these selected clones will then be evaluated in *in vivo*. The vectorization of these nanobodies into our lentiviral vectors will also be assayed. These two strategies should overcome the problems of toxicities observed with monoclonal antibodies while showing optimal anti-tumor efficacy.