A Phase I dose escalation trial evaluating the impact of an in situ Immunization strategy with Intra-Tumoral Injections of Pexa-Vec in combination with ipilimumab in advanced solid tumors with injectable lesions.

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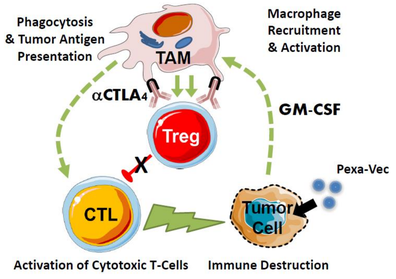
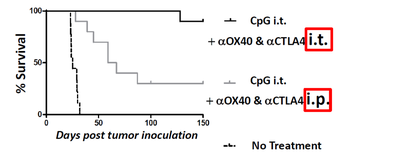
BACKGROUND

In situ immunization is a strategy where immunomodulatory products (such as PAMPs) are injected into one tumor site in order to use the tumor as its own vaccine and to trigger a systemic anti-tumor immune response.

We have recently shown that intratumoral (IT) PAMPs cure tumors via the activation of plasmacytoïd dendritic cells, one of the most efficient subset of antigen presenting cells, and change their phenotype from tolerogenic to immunogenic [1]. Of note, IT PAMPs synergize with anti-CTLA-4 to deplete tumor-specific T_{regs} and generate a systemic anti-tumor immune response able to eradicate distant, not injected, tumor sites, even in the central nervous system [3-4].

We formulate the hypothesis that IT treatment with an oncolytic virus (Pexa-Vec) could synergize with anti-CTLA4 therapy via oncolytic virus-induced tumor cell death & tumor-antigen release, GM-CSF-induced recruitment/maturation/activation of antigen presenting cells, and anti-CTLA4-induced T_{reg} blockade/depletion and reversion of T effectors inhibition.

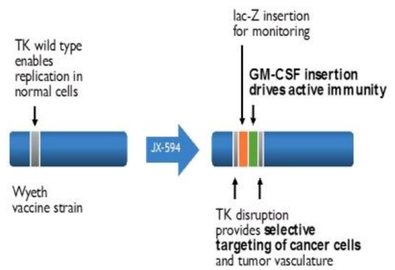




OVERVIEW OF STUDY DRUG

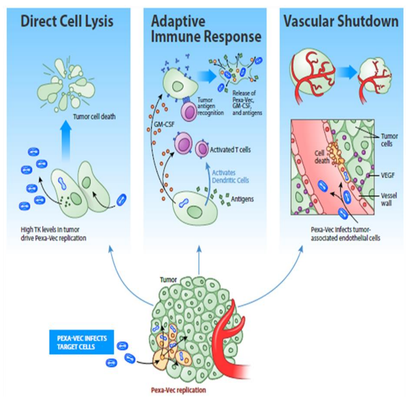
Pexa-Vec is a replication-competent, transgene-expressing therapeutic vaccinia oncolytic virus derived from the commonly used Wyeth vaccine strain (Dryvax®, Wyeth laboratories). Pexa-Vec, manufactured by Transgene S.A. (Illkirch-Graffenstaden, France) [6-8].

Three genetic modifications are included in Pexa-Vec: **Thymidine kinase (TK) gene deactivation, GM-CSF gene insertion** and **Lac-Z gene insertion**.



These genetic modifications allow a three-pronged mechanism of action with rapid onset including:

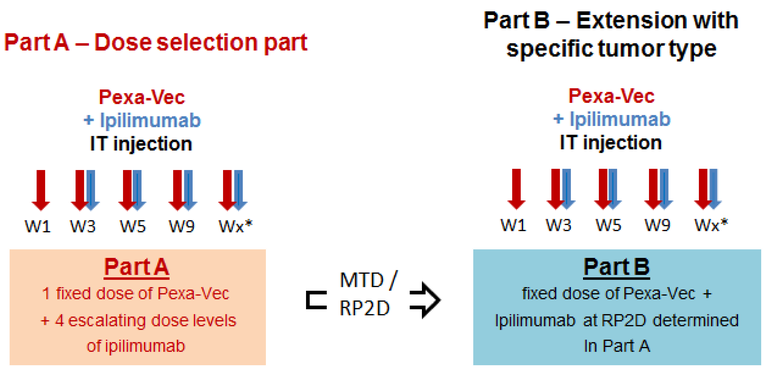
- **Oncolysis**: Pexa-Vec selectively replicates in and destroys cancer cells, while remaining inactive in normal cells.
- **Stimulation of a systemic anti-tumoral immune response** through the expression of its transgene, hGM-CSF, leading in the context of tumor lysis to DC expansion and increased tumor antigen presentation.
- **Shutdown of blood flow into tumor tissue** via infection of tumor-associated endothelial cells.



OBJECTIVES AND DESIGN

- **Primary objective :**
  - Escalation part : To define the MTD and RP2D of an in situ immunization strategy based on IT injections of ipilimumab combined with the oncolytic virus Pexa-Vec in metastatic cancer patients relapsing or refractory to standard therapy and with injectable tumor lesions. MTD and RP2D will be defined by observing the occurrence of Dose Limiting Toxicities (DLT) defined as the toxicities occurring during the DLT assessment window (the first 5 weeks) related to Pexa-Vec, Ipilimumab or both.
  - Expansion part : To assess the anti-tumor effect of the proposed therapeutic combination in specific tumor histological subtypes by evaluating the 3-month Objective Response Rate (ORR3m) defined by the percentage of patients having complete response (CR) or partial response (PR) according to immune related Response Criteria (irRC).
- **Secondary objectives :**
  - To evaluate the efficacy of the proposed therapeutic combination in terms of Objective Response Rate, Best Response, Duration of response, 3-month Disease Control Rate (CR + PR + SD), time to progression, Progression Free Survival and Overall Survival.
  - To further evaluate the safety of the proposed therapeutic strategy.

ISI-JX is a multicentric, Phase I dose escalation trial followed by an extension part aiming to evaluate the safety, feasibility, and anti-tumor effects of an *in situ* immunization strategy with IT injections of ipilimumab with Pexa-Vec in adults patients with solid tumors. The trial is conducted in patients with at least one injectable lesion (≥2 and ≤8 cm) and one distant non-injected measurable target site. Patients are treated with an IT injection of Pexa-Vec alone at Week 1 (W1) Day 1 (D1) followed by 3 IT injections of Pexa-Vec + ipilimumab at W3 D1, W5 D1 and W9 D1. The dose for Pexa-Vec is set at 1 x 10^9 pfu (fixed dose) and up to 4 escalating dose levels of Ipilimumab will be tested (see Table above)



* For patients with OR at W12 : a new IT injection with ipilimumab and Pexa-Vec could be considered at time of disease progression on a case by case basis

The dose escalation part follows a classical 3+3 design with 3 to 6 pts at each DL depending of the number of DTL observed (maximum of 24 pts). In the extension part, according to on the first stage of a Gehan design, 12 patients per cohort (3) will be enrolled (maximum of 36 pts).

Injections of Ipilimumab and Pexa-Vec will be made into 1–5 tumors by the radiologist or other trained physician using imaging-guidance. Both IMPs should be prepared separately and injected in the same lesions with switch of syringes. Different tumors may be treated on each treatment day.

	Ipilimumab (Part A)	Pexa-Vec Fixe dose
DL1: Starting Dose	2.5mg	10^9pfu
DL2	5mg	
DL3	7.5mg	
DL4	10mg	

STUDY POPULATION

- Male or female patients aged ≥ 18 years at time of inform consent signature
- Histologically confirmed, advanced/metastatic solid tumor refractory or relapsing to/after standard therapy or the patient has refused or does not tolerate standard therapy. Any tumor types can be considered in Part A except hepatocellular carcinoma (HCC). In part B, specific tumor histological subtypes will be considered.
- Tumor status (as determined by radiology evaluation):
  - At least one injectable site ≥ 2cm and ≤ 8 cm in diameter and
  - At least one distant non-injected measurable site (target site).
- Normal end organ functions .
- None of the following conditions:
  - Known significant immunodeficiency ;
  - History of auto-immunity or untreated wounds from infection or inflammatory skin conditions.
  - Experience of a severe systemic reaction or side-effect as a result of a previous smallpox vaccination ;
  - Ongoing severe inflammatory skin condition or history of severe eczema requiring medical treatment ;
  - Severe or unstable cardiac disease ;
  - Active brain metastasis ;
  - Any prior or planned organ transplant or allogeneic hematopoietic stem cell transplantation ;
  - Pregnant or breastfeeding women ;
  - Anticoagulant or anti-platelet medication that cannot be interrupted prior to IT injections or Inability to suspend treatment with anti-hypertensive medication
  - Hepatitis C virus therapy including interferon/pegylated interferon or ribavirin or by extension any other hepatitis C virus therapy that cannot be discontinued within 14 days prior to any Pexa Vec injection ;
  - Household contact exclusions for patients enrolled: women who are pregnant or nursing an infant, children < 1 year old, people with skin disease (e.g. eczema, atopic dermatitis and related diseases…), immunocompromised hosts (severe deficiencies in cell-mediated immunity, including AIDS, organ transplant recipients, hematologic malignancies).

TRANSLATIONAL PROGRAM

- Tumor and blood samples are collected with patient consent:
  - One archival FFPE tumor sample and de novo tumor biopsies just before the scheduled IMP IT injections at W1 D1, W5 D1 and W12.
  - If technically doable, patients are also biopsied in one of the non-injected sites at the same time points.
  - Blood samples are also collected (W1D1, W3D1, W5D1 and W12).
- The following assays will be performed to assess
  - The proportion of Tumor infiltrating immune cells & their activation phenotype by IF
  - The cytokinome and the frequency / activation status of circulating immune subpopulations by luminex and 10-12 color multiparametric flow cytometry, respectively.
  - The expression of gene involved in anti-tumor immune response mechanisms such as immune cell activation (OX40, CD137, ICOS, PD-L1, etc) and tumor cell reaction to infection (AIM2, DAI, RIG-I, etc.) by RNAseq.
  - T- cell and B-cell immune responses specific to Pexa-Vec and tumor-antigens (Elisa and tetramers by flow cytometry).

CURRENT STATUS

As of today, 8 patients have enrolled at Centre Léon Bérard (DL1 n =3, DL2 n=3 and DL3 n=2).
No DLT and no major safety issue were reported.
Two additional sites have been activated (Institut Bergonié [Bordeaux] and Centre Hospitalier Lyon Sud [Lyon]).

ACKNOWLEDGEMENTS

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