# SELECTED OPPORTUNITIES IN ONCOLOGY - IMMUNO-ONCOLOGY

PD-1 and Tim-3 expression as biomarkers for Predicting survival time and treatement response of a subject suffering from renal cell carcinoma (BIO15495)

# PD-1 and Tim-3 expression as biomarkers for Predicting survival time and treatement response of a subject suffering from renal cell carcinoma (BIO15495)

## Product factsheet

*Stage:*
*Pre-Analytic*
*Validation*

- **Biomarker:**
  - PD-1, Tim-3 on CD8+ T cell
- **Technology:**
  - IHC, Flow Cytometry
- **Sample:**
  - Biopsy
- **Information:**
  - Treatment Response
  - Prognosis
- **Scientific and Clinical Rationale:**
  - Tumor-infiltrate lymphocytes (TILs) populations distribution is not uniform between tumors types. Especially CD8+ T cells are located in the tumor core and the invasive margin where they have a better interaction with tumor cells.
  - CD8+ T cells responses are necessary for the control of tumors.
  - Immunotherapy is a new class of cancer treatment that works to harness the innate powers of the immune system to fight cancer. It targets PD-1 on T cells that normally help keep these cells from attacking other cells in the body. By blocking PD-1, the drug boosts the immune response against cancer cells.
  - In Renal cell carcinoma (RCC) cancer, the treatment based on the inhibition of PD-1 leads only about 30% clinical responses in cancer patients. Thus there is a need to identify and validate others biomarkers of treatment response.
- **POC:**
  - Retrospective cohort: 87 patients with renal carcinoma (clear cell carcinoma). Prospective cohort: 42 renal carcinoma patients
  - PD-1+Tim-3+CD8+T cells cannot be activated in vitro with a strong stimulus suggest that it could also be difficult to revigorate them after PD-PDL-1 blockade and thus constitutes a biomarker of resistance to immunotherapy
- **Selling points:**
  - **Priority:**
    - EP16 305 004.0 on 2016/01/04
    - PCT/EP2017/050088 on 2017/01/03
  - **Scientific Publication(s):**
    - Cancer Res, 2016 Nov 21, *Granier C. et al.*, doi: 10.1158/0008-5472.CAN-16-0274

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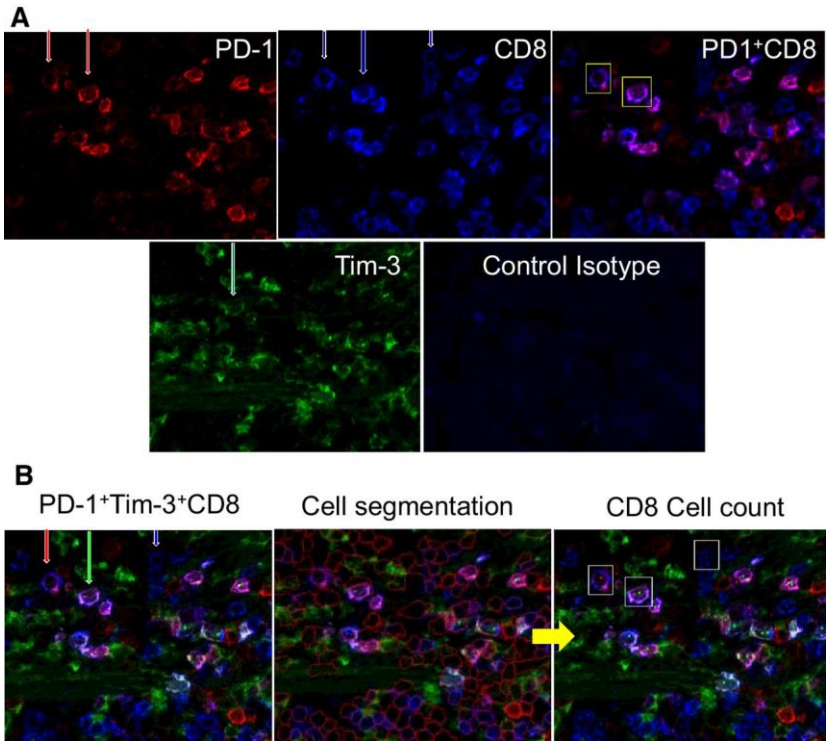
## Proof of concept

**Detection of PD-1 and Tim-3 expression on tumor-infiltrating CD8+ T cells from a patient with ccRCC**

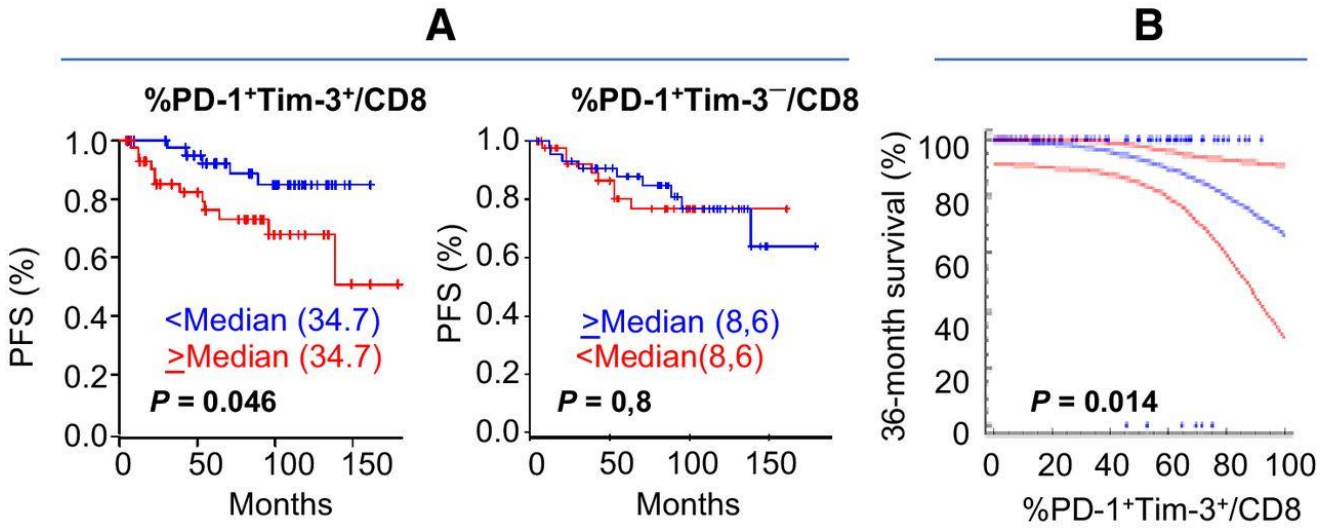
- **Pre-Analytic Validation:**
  - (A) Frozen tissue sections derived from RCC patients were stained by immunofluorescence with antibodies directed against human CD8 (blue), PD-1 (red), and Tim-3 (green). Yellow boxes, cells expressing both CD8 and PD-1. Stexperimentaining with isotype controls was included for each.
  - (B) Triple costaining for CD8, PD-1, and Tim-3 (merged) is shown on the left, with the green arrow indicating CD8+ T cells coexpressing PD-1 and Tim-3, the red arrow corresponding to CD8+ T cells expressing PD-1, and the blue arrow identifying CD8+ T cells not expressing PD-1 or Tim-3.
  - For automated counting, inForm software allows cell segmentation based on DAPI staining of the nucleus and morphometric characteristics (middle). An automated count based on a user-defined algorithm was then performed (right), which generated green dots corresponding to CD8+ T cells coexpressing PD-1 and Tim-3, red dots corresponding to CD8+ T cells expressing PD-1 without Tim-3, and blue dots corresponding to CD8+ T cells not expressing PD-1 or Tim-3.
  - (original magnification, ×200).



# PD-1 and Tim-3 expression as biomarkers for Predicting survival time and treatement response of a subject suffering from renal cell carcinoma (BIO15495)

## Proof of concept

- **<u>Pre-Analytic Validation:</u> Clinical significance of relationships between the *in situ* co-expression of PD-1 and Tim-3 on CD8⁺ T cells and clinical outcome**




- (A) RCC patients (n = 87) were divided into two groups depending on whether the percentage of PD-1 without Tim-3 coexpression (right), PD-1 and Tim-3 coexpression (left) on CD8+ T cells was above or below the median (34.7). Kaplan–Meier curves for PFS for the two groups of patients are shown.
- (B) The correlation between the percentage of PD-1 and Tim-3 coexpression on CD8+ T cells selected as a quantitative variable and the 36-month OS is shown (probit regression model). The blue line corresponds to this correlation, whereas the red line represents the upper or lower limits of the 95% CI. Blue squares on the top indicate that the corresponding patients are alive, whereas blue squares on the bottom correspond to deceased patients.

- **<u>Highter rate of PD-1⁺ Tim-3⁺ coexpressing cells is correlated with worse prognosis of patients.</u>**

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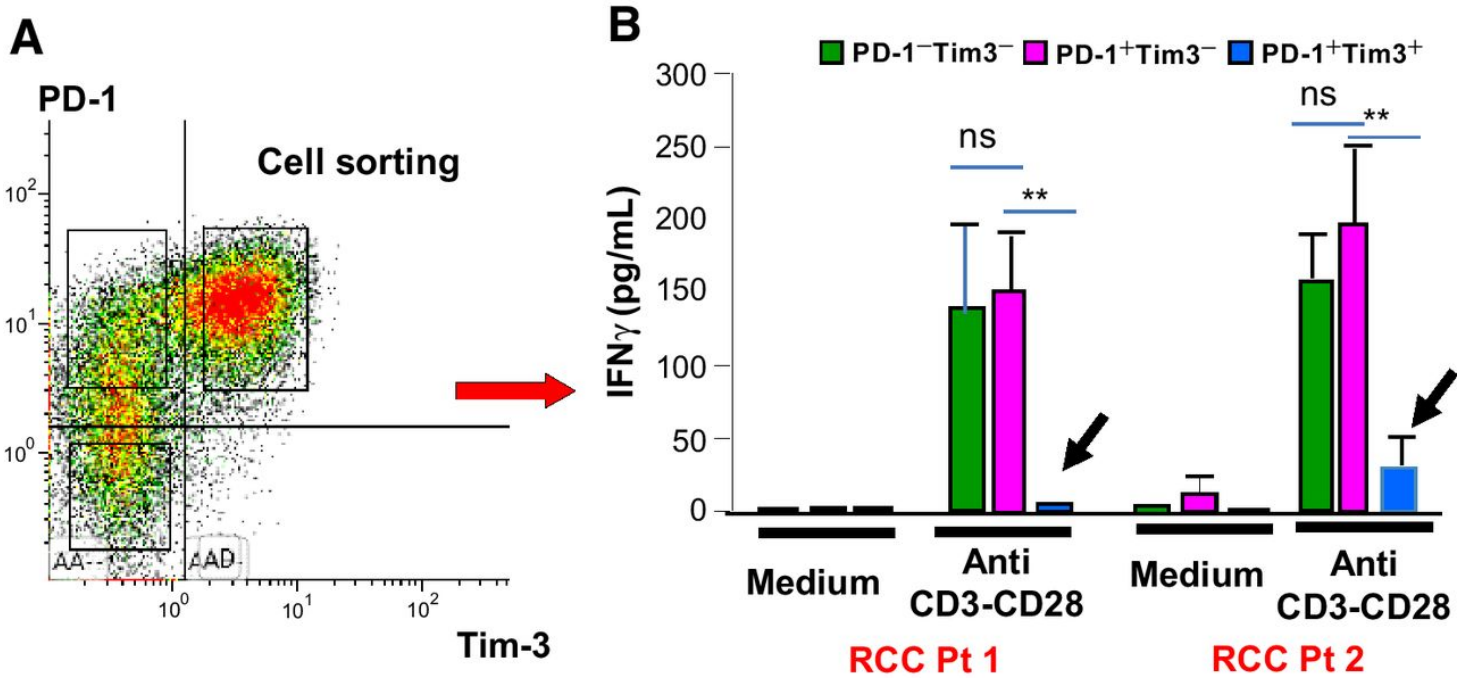
## Proof of concept

- **<u>Pre-Analytic Validation:</u> Functional analysis of CD8+ T cells depending on their expression of PD-1 alone or combined with Tim-3**
  - (A) CD8+CD3+ T cells were sorted on the basis of their PD-1 and Tim-3 expression into three cell populations: PD-1+Tim-3+, PD-1+Tim-3−, and PD-1−Tim-3−.
  - (B) Cells collected after sorting (105/well) were activated or not by anti-CD3 and anti-CD28 (2.5 millions beads per five 106 cells) for 24 hours, and IFNγ was then measured by ELISA in the supernatant. **, P < 0.01 (Wilcoxon test). ns, not significant.
- **<u>PD-1⁺ Tim-3⁺ CD8 T cells express less IFNγ *in vitro*, suggesting less reactivation through PD-1/PD-L1 blockade and thus possible resistance to immunotherapy</u>**



## Proof of concept

- **<u>Pre-Analytic Validation:</u> Co-expression of PD-1 and Tim-3 on CD8+T cells correlate with clinical parameters of RCC aggressivity.**

  - The percent of PD-1+Tim-3+ on CD8+T cells selected as a continuous variable and measured by in situ imunofluorescence technique was plotted against various clinical parameters defined as a binary (TNM, Fuhrman grade, UISS score) or a continuous variable (tumor size). TNM was divided in two groups : localized disease (pT1 and pT2) and advanced disease (pT3, pT4, N+ or M +). The Fuhrman grade was defined as low (grade I or II) and high (grade III or IV) and the UISS score into 3 classes (0,1,2).