Development of a pharmacological platform to study in real time immune checkpoints signaling pathways: validation with therapeutic mAbs and small molecules.

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INTRODUCTION

- **bioSensAll™** : a powerful pharmacology BRET-based platform that enables the analysis and monitoring in real-time of signal transduction occurring in several pathways (GPCR or RTK (1)).
- At Domain Therapeutics: development of a brand new and innovative pharmacological BRET platform dedicated to study Inhibitory Immune Checkpoint (ICP).
- Inhibitory Immune Checkpoint (PD-1 or CTLA-4): front-runner targets in immuno-oncology field (2):
  - involved in immune cells exhaustion,
  - lead to a blockade of the anti-tumor response.
- Blocking strategies targeting the PD-1/PD-L1 or CTLA-4 axis:
  - proven and reliable therapies to strike cancer.
  - more and more molecules such as blocking antibodies (Abs) or small molecule entities (SMEs) developed, and some of them are now in the drug market.

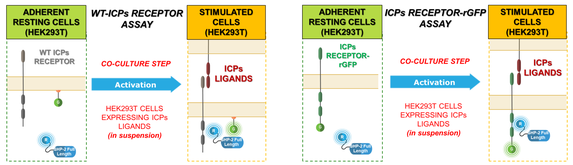
In Drug Discovery, to screen and characterize these therapies : New robust and reliable cell-based assays are urgently needed.

OBJECTIVES AND METHODS

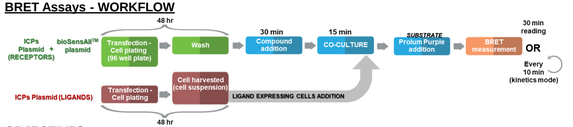
Development of BRET assays dedicated to either PD-1 or CTLA-4 axis:

- Wild type forms of ICP receptors,
- ICP receptors fused with rGFP,
- co-culture with PD-L1/PD-L2 or CD80-CD86 positive cells to activate effector cells. Inspired from *in vivo* process where pathways are triggered by cancer cells expressing ICPs ligands.

BRET Assays – PRINCIPLE



BRET Assays - WORKFLOW



OBJECTIVES

PD-1 and CTLA-4 BRET assay validation based on:

- monitoring the activation of PD-1 or CTLA-4 pathways in real time, and the specificity of the stimulation,
- assessing the inhibitory activity of know ICPs inhibitors (SMEs or blocking antibodies.)

Activation of PD-1 or CTLA-4 pathways in BRET assays

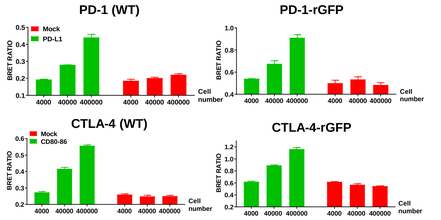


Figure 1. PD-1 and CTLA-4 BRET assays respond to ligand stimulation.

For both targets and both assays, a BRET ratio is observed in presence of ligand expressing cells. This activation of the pathway is dependent of the ligand quantity.

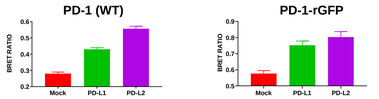


Figure 2. PD-1 BRET assays respond to PD-L2 stimulation.

Upon binding to its ligands PD-L1 or PD-L2, PD-1 or PD-1-rGFP engaged SHP2.

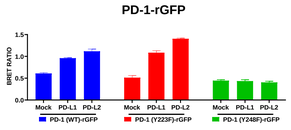


Figure 3. The phosphodeficient version of the key amino acid for SHP2 recruitment inhibits BRET signal.

As previously characterized (3), Y248F point mutation of PD-1 is not able to recruit SHP2 phosphatase. This is perfectly translated in our PD-1-rGFP assay by a complete loss of the BRET signal.

Monitoring of pathways activation in real time

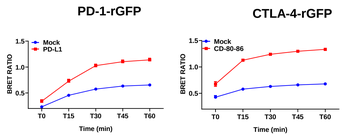


Figure 4. Kinetic of the pathway activation in PD-1 and CTLA-4 assays.

The kinetic activation of PD-1 and CTLA-4 pathways can be monitored in real-time. The maximal BRET signal is reached at 1H of incubation.

Specificity of pathway activation - PD-1

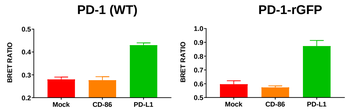


Figure 5. Only PD-L1 cells can stimulate PD-1 BRET assays.

CD86 positive cells are not able to stimulate PD-1 BRET assay.

Specificity of pathway activation - CTLA-4

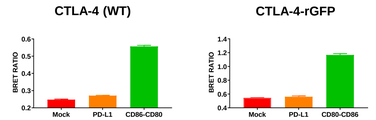


Figure 6. Only CD80-86 cells lead to an activation of the CTLA-4 BRET assays.

PD-L1 positive cells are not able to stimulate CTLA-4 assays.

PD-1 BRET assays validation with SMEs

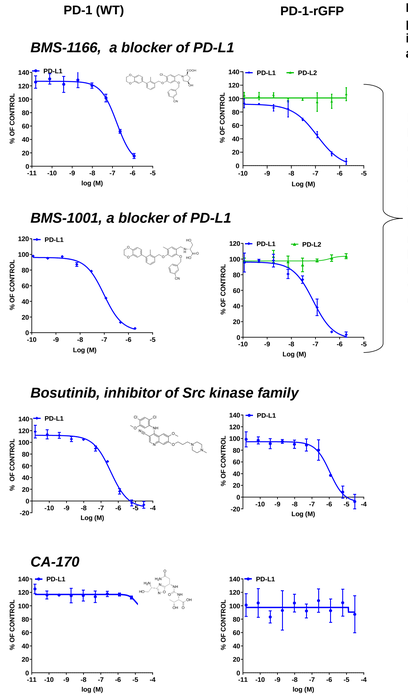


Figure 7. Pharmacology profile of PD-1/PD-L1 inhibitors into PD-1 assays.

Both assays are able to highlight the inhibitory effect of SMEs described to block PD-1 pathway: BMS-1166 and BMS-1001.
IC50 are similar compared to (4).
Interestingly, the assays can reveal the specificity of the ligand binding as no inhibitory effect is observed with a co-culture of PD-L2 expressing cells.

Inhibitor of the Src family: Bosutinib inhibits kinases involved in early PD-1 activation stage.
BRET signal in both assays is inhibited (also observed for other Src family kinase inhibitors: Dasatinid and Foretinib).

No inhibition of BRET signal was observed in the 2 assays. As already characterized (3,4), this molecule is not able to interact with PD-1/PD-L1 interface.

PD-1 BRET assays validation using PD-1 blocking antibody

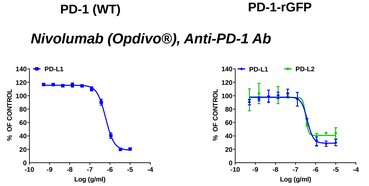


Figure 8. Pharmacology profile of well characterized PD-1 antibody: Nivolumab into PD-1 assays.

PD-1 neutralizing antibody Nivolumab inhibits BRET signal of PD-1 assays with PD-L1 or PD-L2 stimulation. BRET assays highlight the blocking activity of this antibody in both stimulations (PD-L1 and PD-L2).

CTLA-4 BRET assays validation using blocking CTLA-4 antibody

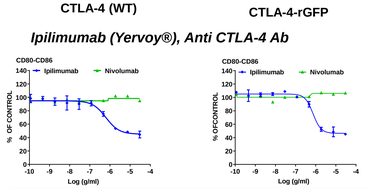


Figure 9. Pharmacology profile of CTLA-4 neutralizing antibody into CTLA-4 assays.

The two assays highlight the inhibitory effect of Ipilimumab antibody.
As a specific control, Nivolumab doesn't inhibit CTLA-4 pathway.

CONCLUSION

bioSensAll™ is a powerful pharmacological platform:

- A **versatile pharmacological platform** able to be highly adaptable for hot topic targets such as **Immune Checkpoint targets:**
  - **Primary and secondary assays** for PD-1 and CTLA-4 axis were developed,
  - Assays were able to **reveal the inhibitory activities of therapeutic molecules (SMEs, mAbs)**
- **Adaptably of the platform across several biosensors,**
- Capable to **monitor signaling pathways in real time kinetics for inhibitory ICPs, and will be assessed for stimulatory ICPs,**
- Can be companion tests to enhance drug discovery and also can be used as **quality control/batch release testing** for therapeutic molecules.

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