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BICKI®IL-7, optimized bifunctional anti PD-1/IL-7 drug, with good PK profile and preclinical anti-tumor efficacy

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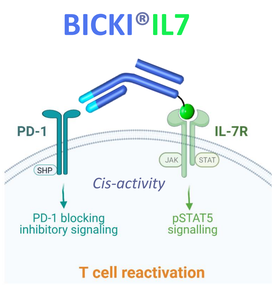
Introduction

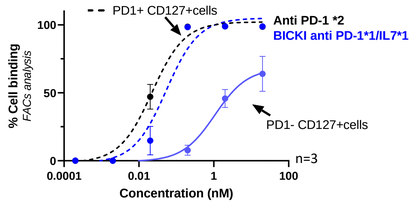
Despite the clinical success of PD-(L)1 therapy over other cancer treatments, most patients are resistant to the therapy. To counteract *de novo* and acquired resistance mechanisms, we **designed a second generation of PD-1 antibody: BiCKI®IL-7** by fusing IL-7 cytokine to the anti- PD-1 antibody Fc portion (BiCKI : **Bi**specific **C**he**c**K**i**point **I**nhibitor). In comparison with other cytokines fused to anti PD-1, e.g IL-2, IL-15 or IL21, BICKI®Anti PD-1 IL7 was the only bifunctional molecule able to induce synergistic activation of NFAT TCR signaling into PD-1+ T cells.

We have previously demonstrated (Morello et *al.*, AACR, 2020) that the BICKI®IL7 in vitro has superior efficacy than the anti PD-1 Ab to promote long-term proliferation of exhausted T cells as well as disarming Treg mediated immunosuppression. We designed various constructions of BICKI®IL7 and selected one constructed with one PD-1 valency and one IL-7 which demonstrated superior pharmacokinetics and in vivo anti-tumor efficacy compared to the BICKI®IL7 constructed with 2 anti PD-1 valences and 2 IL-7 cytokines.

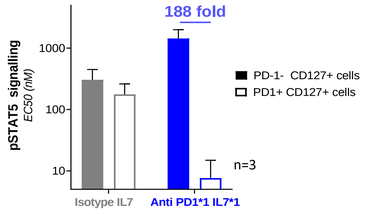
1/ BICKI®IL7 : Preferential targeting and synergistic activation of PD-1+ experienced T cells



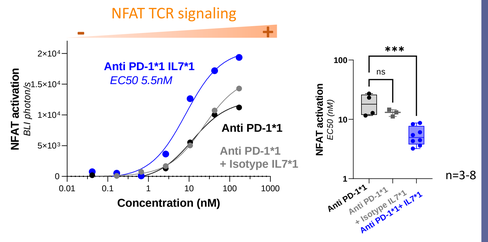
Preferential cis-targeting on PD1+ CD127+ over PD-1- CD127+ cells



Superior IL-7R cis-signaling into PD1+ CD127+ over PD-1- CD127+ cells

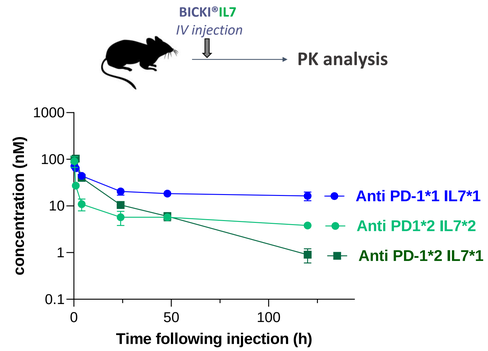


Synergistic reactivation of TCR signaling



Cis-activity and Cis-targeting was performed by co-culturing CPDe450 labeled U937 cells expressing hCD127+ only and CPDe670 labeled U937 cells co-expressing hPD1+ and hCD127+. Binding was measured on each cell type by flow cytometry using an anti hIgG-PE and by flow cytometry pSTAT5 activity (IL-7R) was quantified after 15 min incubation with traitement and intranuclear staining with the Anti pY694/STAT5-APC. TCR signaling activation (NFAT) was assessed using a PD-1 promega assay™. PD-1+ Jurkat cells coexpressing RE-NFAT-luc was co-cultured with aAPC CHO PDL1+ target cells +/- antibodies during 6 hours, then Bioluminescence measuring NFAT activation was quantified

2/ Improved pharmacokinetics

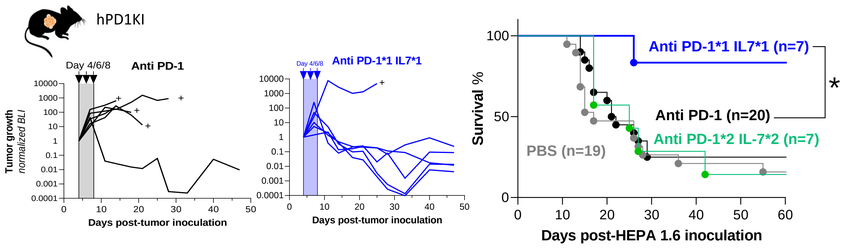


C57bl6JrJ mice were intraveously injected with one dose of BICKI®IL7 molecules (35nm/kg). Blood was collected after multiple time points and antibody concentration was quantified by ELISA using an anti-human Fc specific assay.

3/ Efficient *in vivo* anti tumor activity in orthotopic syngeneic models

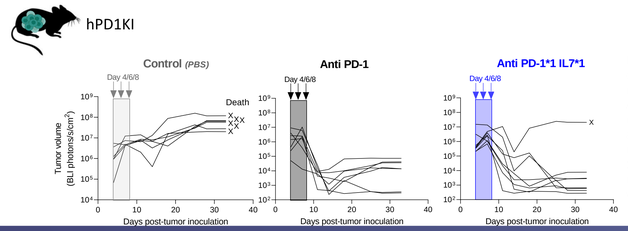
Significant efficacy in PD-1 resistant model

Hepatocarcinoma



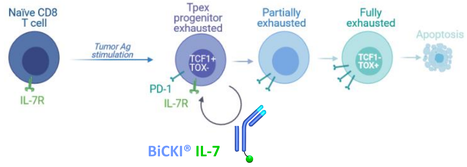
Conserved efficacy in PD-1 sensitive model

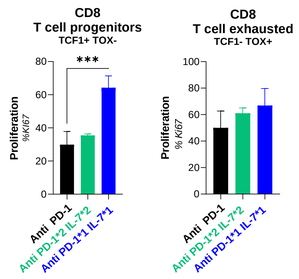
Mesothelioma



For Hepatocarcinoma model Hepa1.6 luc+ cells (2.5^6) were injected into the intraportal vein. For mesothelioma model, AK7 cells (3^6) were injected intraperitoneally. Tumor growth was assessed by Bioluminescence (BLI) after intraperitoneal injection of D-luciferin (37.5mg/kg). Anti PD-1 and BICKI IL7 were injected 3 times every 2 days at a dose with an equivalent drug exposure.

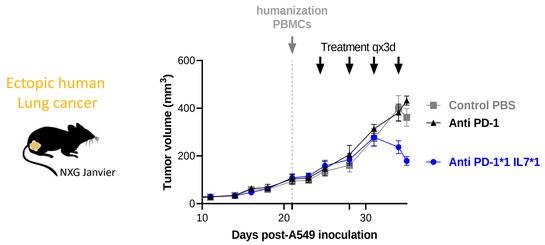
4/ BICKI®IL7 selectively expands stem-like Tpex cells *in vivo*

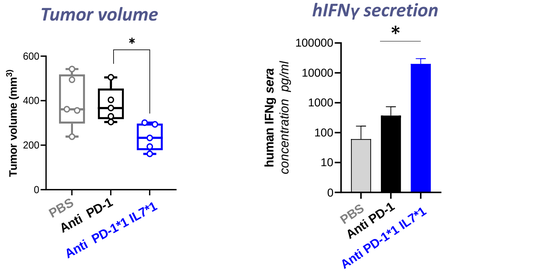




Ectopic MC38 bearing hPD1KI mice were treated with one dose of BICKI®IL7 or OSE279 (34nm/kg). On Day 4, MC38 tumor was harvested and CD8 T cell proliferation (Ki67) was quantified in the different subsets of exhaustion by flow cytometry. CD44 activation marker was used to differentiate naive and activated T cells. Tpex (CD45+CD3+CD8+CD44+TCF1+Tox-), T fully exhausted (CD45+CD3+CD8+CD44+TCF-1-TOX+).

5/ Efficient *in vivo* efficacy in humanized mice



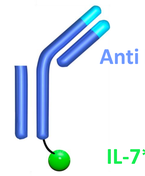


A549 were subcutaneously injected 5^6cells in NXG immunodeficient mice and humanization was performed on Day 21 with 10^6 PBMCs. Anti PD-1 and BICKI IL7 were injected 4 times every 3 days at a dose with an equivalent drug exposure. Comparison of tumor volume was assessed on Day 34 and human IFNγ secretion was quantified in the sera on Day 35.

Conclusion

BiCKI®IL-7

To promote long-term activation of PD-1+ T cells



- Conserved high PD-1 binding and PD-1/PD-L1 antagonist activity
- Higher biological activity with one IL-7 cytokine
- Allows a selective delivery of IL-7 on PD-1+ cells and synergistic activation of TCR signaling
- Good PK profile with the anti PD-1*1/IL7*1 construction
- Significant in vivo anti tumor efficacy in PD-1 sensitive and resistant syngeneic orthotopic model
- Confirmed preclinical efficacy in humanized model
- BICKI®IL7 boosts intratumoral proliferation of PD-1+ CD127+ TCF1+ progenitor T cells