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Long-term anti-tumor efficacy of BICKI®IL-7, an optimized anti PD-1/IL-7 bifunctional antibody sustaining activation of progenitor stem-like CD8 TILs and disarming Treg suppressive activity

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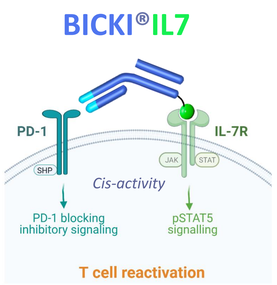
Introduction

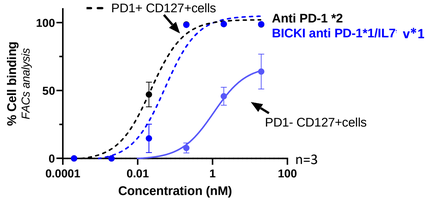
Despite the clinical success of PD-(L)1 therapy over other cancer treatments, most patients are resistant to the therapy. To counteract *de novo* and acquired resistance mechanisms, we **designed a second generation of PD-1 antibody: BiCKI®IL-7** by fusing IL-7 mutein cytokine (IL-7v) to the anti- PD-1 antibody Fc portion (BiCKI : **Bi**specific **C**he**c**k**p**oint **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.

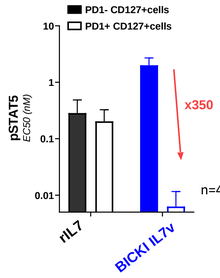
1/ BICKI®IL7 : Preferential targeting and synergistic activation of PD-1+ experienced T cells



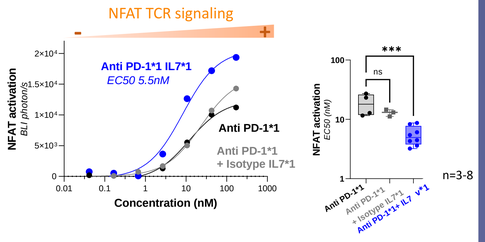
Preferential cis-targeting on PD1+ CD127+ over PD-1- CD127+ cells



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

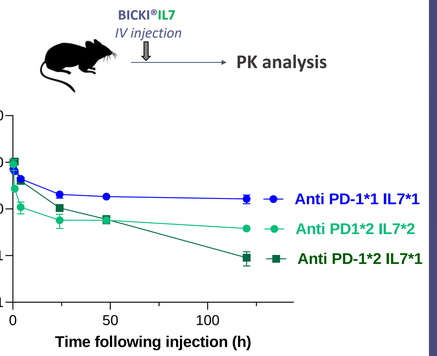


Synergistic reactivation of TCR signaling



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

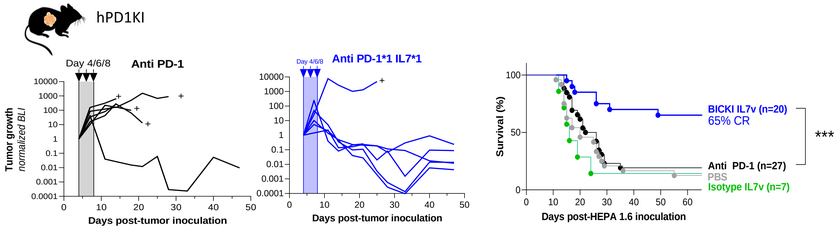


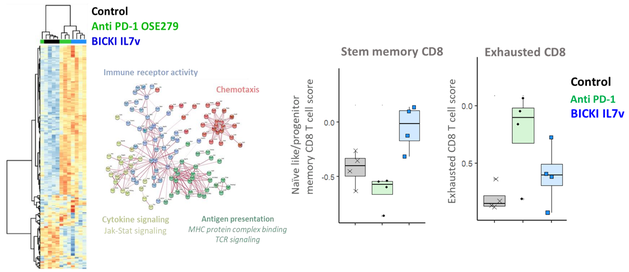
C57bl6JrJ mice were intraveously injected with one dose of BICKI®IL7 molecules (35nm/kg). Blood was collected after multiple time points and antibody concentration was quantified by ELISA using an anti-human Fc specific assay.

3/ Efficient anti tumor activity in refractory orthotopic tumor model

Significant efficacy in PD-1 resistant model

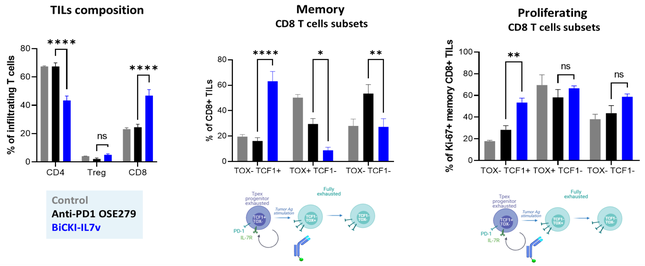
Hepatocarcinoma





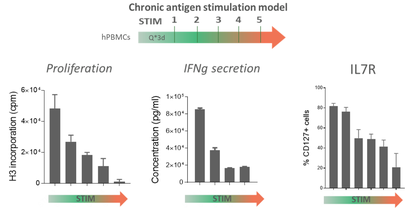
Liver transcriptomic analysis after 1 week of treatment shows 68 genes significantly downregulated and 160 genes significantly upregulated between treated animals and controls. BiCKI-IL7v group shows similar global profile compare to the anti-PD1 group excepting an inverse expression profile of stem-like memory CD8 gene signature increased and Exhausted CD8 T cells signature decreased as compared to anti-PD1 treated mice.

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

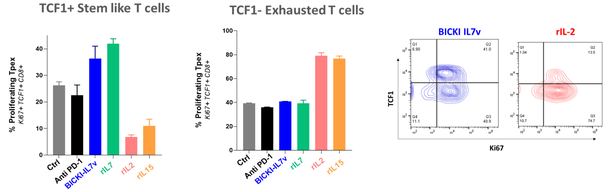


Orthotopic hepa1.6 bearing hPD1KI mice were treated with BICKI®IL7 or OSE279 as in (3). On Day 10, Liver were harvested and TILs were analyzed by flow cytometry. CD44 activation marker was used to differentiate naïve and memory T cells. Tox and TCF1 markers were used to analyzed Tpex progenitor (CD45+CD3+CD8+CD44+TCF1+Tox-), T partially exhausted (CD45+CD3+CD8+CD44+TCF-1-TOX+) and T fully exhausted (CD45+CD3+CD8+CD44+TCF-1-TOX-)

5/ BICKI®IL7 selectively expands human stem-like Tpex cells *in vitro*



Restimulation of fully human exhausted T cells (stim N°5)

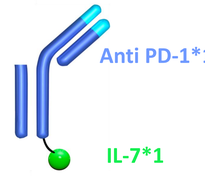


Human PBMCs were chronically stimulated with anti-CD3 + anti-CD28 mAbs every 3 days. Human T cells analyzed by flow cytometry after each stimulation loose progressively their capacity to proliferate, secrete IFNg as well as IL7R expression. After 5 stimulations, T cells are fully exhausted and were restimulated with IL2, IL7, IL15 or BiCKI-IL7. The phenotype of cells proliferating after cytokine incubation were characterized with TCF1 and Ki67 into CD8+ T cell pop.

Conclusion

BiCKI®IL-7

Promoting durable t PD-1+ T cells responses



- Conserved high PD-1 binding and PD-1/PD-L1 antagonist activity
- Higher biological activity with a single IL-7 mutein cytokine
- Allows a selective delivery of IL-7 on PD-1+ cells and synergistic activation of TCR signaling
- Improve 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 preferentially boosts the proliferation of PD-1+ CD127+ TCF1+ progenitor T cells in mouse and human.