Identification of PD1-IL2$_{TAP}$ – a PD-1 Blocking Immunoconjugate Harboring a Tumor-Activatable Masked IL-2 Payload Lacking Binding to IL2Rβ/CD122

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ABSTRACT

INTRODUCTION: Immunocytokines provide the opportunity to simultaneously address distinct and complementary mechanisms of action and to deliver cytokine payloads to specific immune cells ("*cis*-signaling") resulting in enhanced anti-tumor activity. However, there is increasing evidence that use of constitutively active IL-2 payload limits the therapeutic index of PD1-IL2 immunocytokines due to systemic toxicity resulting from potent IL-2 induced immune cell activation and release of pro-inflammatory cytokines in the periphery.
To overcome this issue, we have generated a conditionally activatable PD1-IL2 immunoconjugate (IC) consisting of an optimized and masked IL-2 payload conjugated to a human PD-1 blocking antibody. Using our novel chemical protein synthesis technology, we generated an IL-2$_{TAP}$ (Tumor Activated Payload) variant lacking binding to IL2Rβ/CD122. The IL-2$_{TAP}$ protein harbors a protease-cleavable sequence which would be processed specifically in the tumor microenvironment to yield the unmasked and activated IL-2 payload.

RESULTS: *In vitro*, efficient blockade of binding to CD122 translates into significantly reduced pSTAT5 induction by masked PD1-IL2$_{TAP}$ in PD-1 negative cells. Following protease-mediated processing and further enhancement by *cis*-signaling, activated PD1-IL2$_{TAP}$ exhibits a 10,000-fold increase in potency in cells expressing PD-1. We confirmed stability of the IL2$_{TAP}$ payload in plasma and observed efficient activation *ex vivo* by primary human tumor tissue but not by healthy or normal adjacent tissue. In line with this, we observed a long plasma half-life for PD1-IL2$_{TAP}$ in a PK/PD study performed in mice expressing human PD-1. The IC was very well tolerated at high doses and there was no evidence for TMDD indicating that PD1-IL2$_{TAP}$ was indeed stable in circulation. Importantly, PD1-IL2$_{TAP}$ was able to induce a strong local expansion of CD8+ T cells specifically within the tumor microenvironment, and intra-tumoral PD effects correlated with the induction of potent and durable anti-tumor efficacy.

CONCLUSION: Collectively, the data indicate that, using Bright Peak's chemical synthesis and ligation technology, we were able to generate a conditionally activatable PD1-IL2$_{TAP}$ immunoconjugate that exhibits a significantly increased therapeutic index, thus, mitigating the known safety risks associated with ICs harboring potent non-masked IL-2 payloads.

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Use of Bright Peak's Chemical Synthesis & Conjugation Platform to Generate a Structurally Unique Tumor-Activated IL-2 Payload (IL2$_{TAP}$)

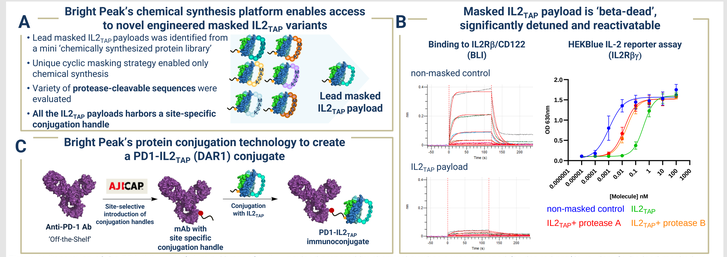


Figure 1: Generation of the PD1-IL2$_{TAP}$ IC A) A mini-library of IL-2$_{TAP}$ payload prepared by chemical synthesis was screened for desired profile to identify the lead masked IL2$_{TAP}$ payload with B) substantially decreased binding to the β-subunit of the IL-2 receptor (CD122) as shown by Bio-Layer Interferometry (BLI) and significantly less activity on cells as shown by an IL-2 reporter assay (Invivogen). IL2$_{TAP}$ payload can be reactivated with recombinant proteases; data presented as mean with SD, at least n=3. C) PD1-IL2$_{TAP}$ immunoconjugates (DAR1) are prepared using site-specific antibody modification technology to introduce a single-conjugation handle. Next, the IL2$_{TAP}$ payload is chemically conjugated to the modified antibody to generate these unique immunoconjugates.

Site-Specific Conjugation Does Not Affect the Properties of the Anti-PD-1 Antibody or the IL2$_{TAP}$ Payload

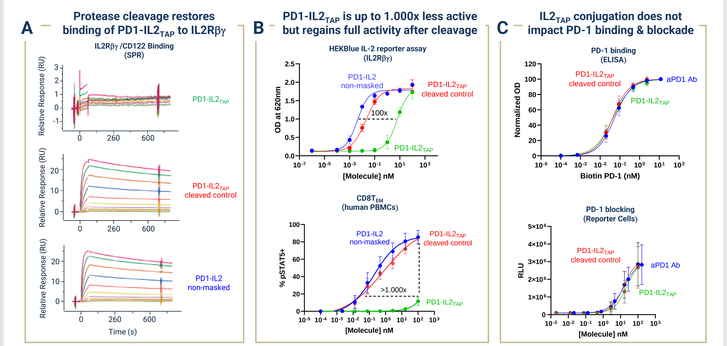


Figure 2: Characterization of the PD1-IL2$_{TAP}$ IC A) Surface Plasmon Resonance (SPR) measurement of binding to the IL-2 Receptor β/γ Heterodimer shows diminished binding for the PD1-IL2$_{TAP}$ molecule that can be regained as shown by the cleaved control compared to non-masked control. B) PD1-IL2$_{TAP}$ activity in cellular assays shows, that PD1-IL2$_{TAP}$ is up to 1000x less active when analyzed on cellular assays like an IL-2 reporter assay as well as STAT5 phosphorylation (pSTAT5) in CD8 T$_{mem}$ subpopulation in human PBMCs. C) Analysis of the PD-1 binding by ELISA as well as blocking activity (Invivogen) of the PD1-IL2$_{TAP}$ molecule shows, that site specific conjugation of the IL2$_{TAP}$ payload does not interfere with the aPD1 antibody characteristics. All data presented as mean with SD, at least n=3

PD1-IL2$_{TAP}$ exhibits >10.000x Difference in Potency in PD-1^{HIGH} vs PD-1^{LOW} NK92 Cells

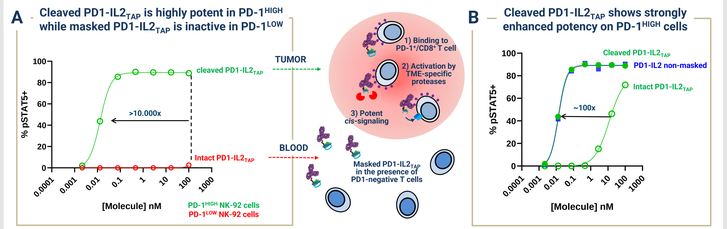


Figure 3: PD1-IL2$_{TAP}$ in vitro therapeutic window on NK92 cells +/-PD-1 A) Activity of PD1-IL2$_{TAP}$ analyzed by STAT5 phosphorylation in NK92 cells shows that PD1-IL2$_{TAP}$ is inactive on cells w/o PD-1 expression (PD-1^{LOW}) suggesting limited activity in periphery with a maximal window of >10.000x compared to cleaved PD1-IL2$_{TAP}$ on cells overexpressing PD-1 (PD1-1^{HIGH}) as in the TME. B) Activity of PD1-IL2$_{TAP}$ analyzed by STAT5 phosphorylation in NK92 PD-1^{HIGH} cells shows residual activity of PD1-IL2$_{TAP}$ and activity similar to a non-masked control when cleaved indicating high activity in the TME. All data presented as mean, at least n=3

PD1-IL2$_{TAP}$ is Stable in Human Plasma and More Efficiently Cleaved in Tumor vs. Normal Adjacent Tissue (NAT)

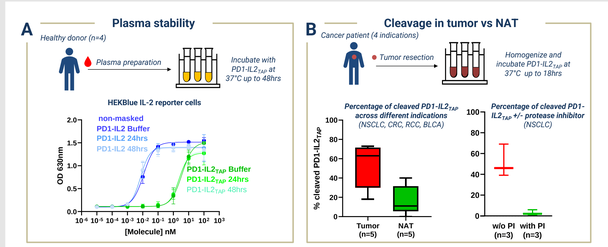


Figure 4: Plasma stability and reactivation in human tumors A) IL-2 reporter cells show comparable activity after incubation in plasma confirming stability of PD1-IL2$_{TAP}$. B) Tumor and matched NAT samples prepared as described (*Mansuror et al., 2022; protocol edited*). Left graph shows that PD1-IL2$_{TAP}$ is more efficiently cleaved in tumors than in matched NATs. Percentage cleaved is defined by the activity of PD1-IL2$_{TAP}$ compared to a cleaved control and a 0% cleaved control on IL-2 reporter cells. Right graph shows that cleavage is inhibited in a NSCLC sample by adding a broad protease inhibitor cocktail (PI) (*HALT protease inhibitory cocktail, Thermo Fischer*). All data presented as mean with SD. At least n=3

PD1-IL2$_{TAP}$ Shows an Antibody-Like PK Profile & Induces Strong CD8+ T Cell Expansion Specifically in the TME

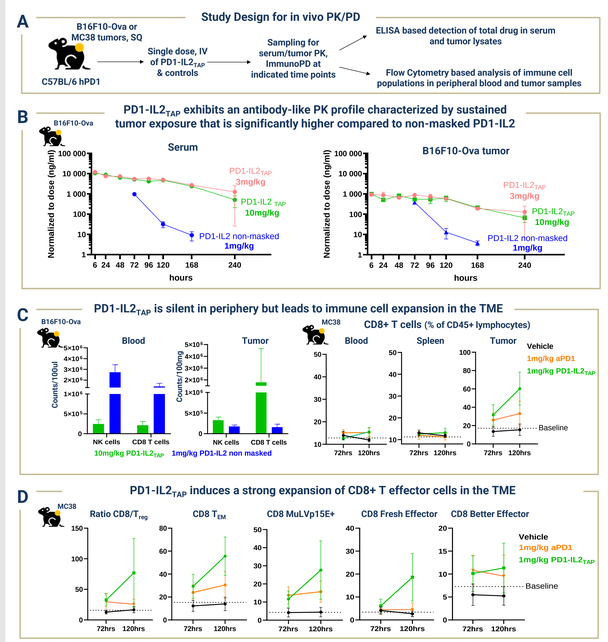
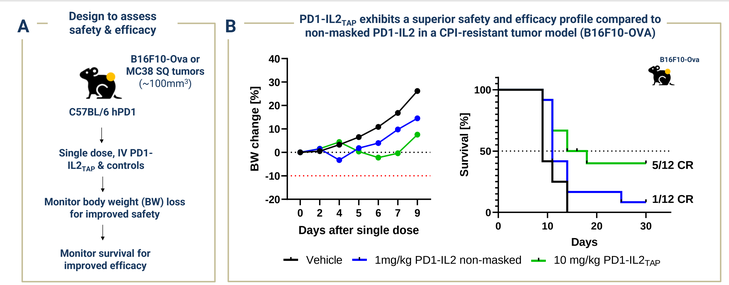


Figure 5: PK/PD analysis of PD1-IL2$_{TAP}$ A) In vivo study design to evaluate PK/PD characteristics of the PD1-IL2$_{TAP}$ molecule. Mice used were C57BL/6 mice transgenic for human PD-1. Controls include an anti PD-1 antibody (aPD1) and a non-masked PD1-IL2 IC. B) Left graph shows an improved serum PK of PD1-IL2$_{TAP}$ molecule compared to the non-masked molecule, right graph shows that antibody-like PK results in sustained exposure in the tumor (n=3/timepoint). C) Left group of graphs show FACS based analysis of NK and CD8 T cell number in the blood and tumor of B16F10-Ova tumor bearing mice (n=3) 120 hours after administration of the test articles. Right group of graphs show the percentage of CD8 T cells in the CD45+ population of peripheral blood, spleen and tumor samples after administration of a single dose of indicated molecules or Vehicle (n=5/timepoint). In both tumor models, PD1-IL2$_{TAP}$ is silent in periphery but leads to immune cells expansion in the TME. D) FACS based phenotyping of CD8 T cells in the TME of MC38 tumor bearing mice (n=5/timepoint) after administration of the indicated molecules shows that PD1-IL2$_{TAP}$ is superior to an aPD1 antibody in expanding CD8T cell subpopulation in the TME (MuLVp15E tumor antigen in MC38; fresh effectors are defined by PD-1+TIM-3+CD128a+; better effectors are defined by PD-1+TIM-3+, CD28a+); Baseline retrieved from n=3 on Day 0; All data presented as mean with SD

PD1-IL2$_{TAP}$ Shows an Increased Therapeutic Window *In Vivo* Compared to Non-Masked PD1-IL2



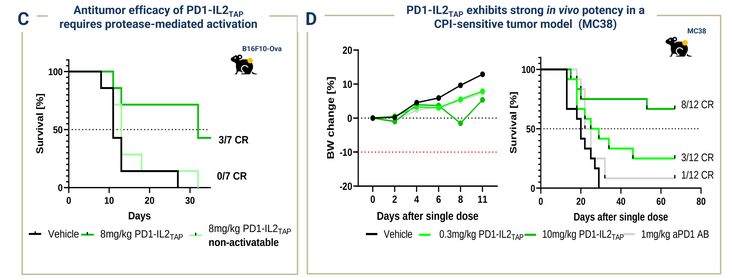


Figure 6: In vivo therapeutic window of PD1-IL2$_{TAP}$ A) Study design to assess safety and efficacy of PD1-IL2$_{TAP}$. Mice used were C57BL/6 mice transgenic for human PD-1. Controls include an anti-PD-1 antibody (aPD1), a non-masked PD1-IL2 IC and a PD1-IL2$_{TAP}$ with a non-activatable linker. B) Left graph shows change in BW in B16F10-Ova tumor bearing mice (n=12/group) after a single dose of the indicated molecules. PD1-IL2$_{TAP}$ enables a 10x higher dose compared to non-masked control. Right graph shows corresponding survival curve indicating that a 10x higher dose translates into a better survival compared to the non-masked control. C) Survival curve in B16F10 –Ova tumor bearing mice (n=7/group) show that an equal dose of a PD1-IL2$_{TAP}$ and PD1-IL2$_{TAP}$ with non-activatable linker differ in survival outcome confirming the need for reactivation of the IL-2$_{TAP}$ payload in the TME. D) In MC38 tumor bearing mice (n=12/group), BW change on the left and survival curve on the right suggest a therapeutic window of 30x (MED 0.3mg/kg, MTD 10mg/kg) exceeding the therapeutic index of a dose limited non-masked PD1-IL2 molecule as shown in B.

Summary & Conclusions

- Using its chemical synthesis & conjugation platform, Bright Peak succeeded in generating a structurally unique Tumor-Activated IL-2 Payload (IL2$_{TAP}$) masked by a protease-cleavable intramolecular loop preventing its binding to IL2Rβ/CD122.
- Site-specific conjugation of the IL2$_{TAP}$ payload to an anti-PD-1 Ab generates a PD1-IL2$_{TAP}$ immunoconjugate without affecting the properties of the PD-1 Ab or the IL-2 payload.
- *In vitro*, masked PD1-IL2$_{TAP}$ is inactive in the absence of PD-1 expression. In contrast, in PD1^{HIGH} cells, PD1-IL2$_{TAP}$ exhibits moderate potency due to *cis*-signaling which is further enhanced following protease-mediated activation.
- *Ex vivo*, PD1-IL2$_{TAP}$ is stable in plasma while being efficiently activated specifically by tumor homogenates.
- In mice, PD1-IL2$_{TAP}$ exhibits an Ab-like PK profile characterized by a long plasma half-life resulting in sustained high exposure within the TME.
- In contrast to non-masked PD1-IL2, PD1-IL2$_{TAP}$ shows minor activity on NK and CD8+ T cells in the periphery while a significant expansion of CD8+ T effector cells in the TME.
- *In vivo*, PD1-IL2$_{TAP}$ exhibits a superior safety profile compared to non-masked PD1-IL2 translating into an increased therapeutic window in both CPI-sensitive and CPI-resistant tumor models.