The selective IKZF2 molecular glue degrader, PVTX-405, counters T_{reg} immune suppression, shows significant tumor growth delay as single agent and synergistic response with immune checkpoint therapies (ICTs)

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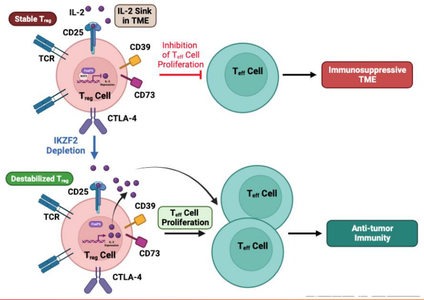
Background & Introduction

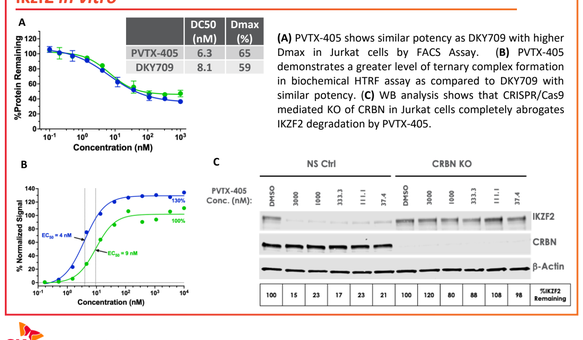
- Immunosuppressive regulatory T-cells (Tregs) are key modulators of tumor immune evasion and resistance to ICTs
- IKZF2 (Helios) is a transcription factor that is selectively expressed by Treg and is essential for maintaining Treg function
- IKZF2 promotes an immunosuppressive tumor microenvironment (TME) by modulating IL-2 expression in Tregs and suppression of effector T-cell (Teff) proliferation.
- We hypothesize that IKZF2 depletion will destabilize Tregs in the TME leading to increase proliferation of Teff and improved efficacy of ICTs



Key Findings

- PVTX-405 is a potent, selective, and orally bioavailable IKZF2 glue degrader with improved off-target activity against SALL4 and improved hERG IC50 (5X) compared to the clinical compound DKY709
- Oral, once daily administration of PVTX-405 significantly delays the growth of MC38 tumors *in vivo*
- PVTX-405 in combination with ICTs, anti-PD1 or anti-LAG3, significantly increases animal survival and durable tumor regressions compared to ICTs alone

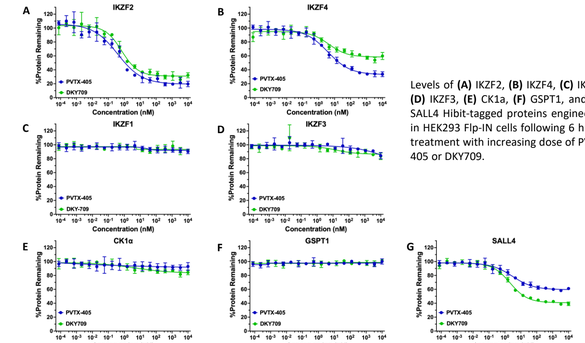
Figure 1. PVTX-405 shows CRBN dependent potent degradation of ikzf2 *in vitro*



	DC50 (nM)	Dmax (%)
PVTX-405	6.3	65
DKY709	8.1	59

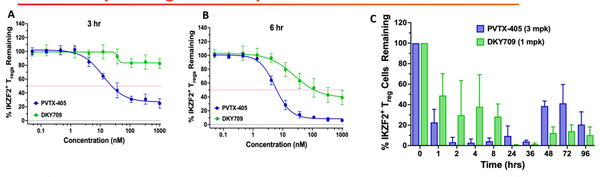
(A) PVTX-405 shows similar potency as DKY709 with higher Dmax in Jurkat cells by FACS Assay. **(B)** PVTX-405 demonstrates a greater level of ternary complex formation in biochemical HTRF assay as compared to DKY709 with similar potency. **(C)** WB analysis shows that CRISPR/Cas9 mediated KO of CRBN in Jurkat cells completely abrogates IKZF2 degradation by PVTX-405.

Figure 2. PVTX-405 shows selectivity against CRBN neosubstrates of concern



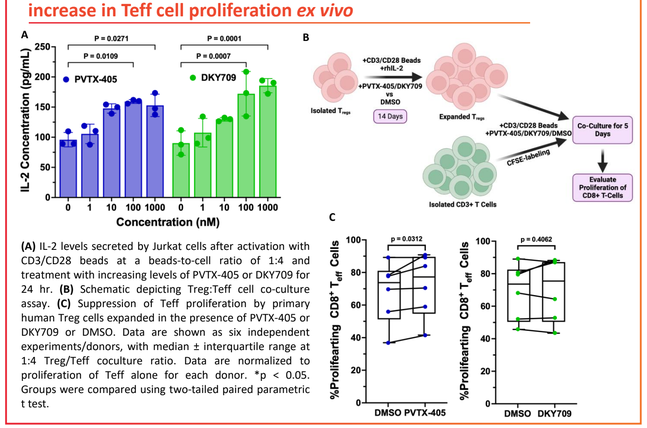
Levels of **(A)** IKZF2, **(B)** IKZF4, **(C)** IKZF1, **(D)** IKZF3, **(E)** CK1a, **(F)** GSPT1, and **(G)** SALL4 Hibit-tagged proteins engineered in HEK293 Flp-IN cells following 6 hrs of treatment with increasing dose of PVTX-405 or DKY709.

Figure 3. PVTX-405 shows potent degradation of IKZF2 in human Tregs *ex vivo* & in cynomolgus monkeys *in vivo*



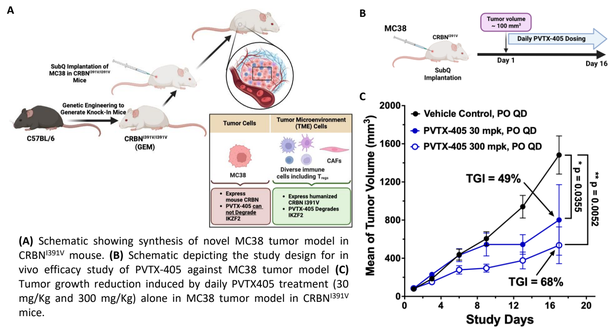
(A) & (B) PVTX-405 shows significantly higher potency and maximal degradation of IKZF2 compared to DKY709 after 3 and 6 hr treatment in primary human Tregs *ex vivo*. **(C)** PVTX-405 and DKY709 shows similar IKZF2 degradation in cynomolgus monkeys *in vivo* at indicated times after administration of one oral dose.

Figure 4. Degradation of IKZF2 results in IL-2 induction *in vitro* and increase in Teff cell proliferation *ex vivo*



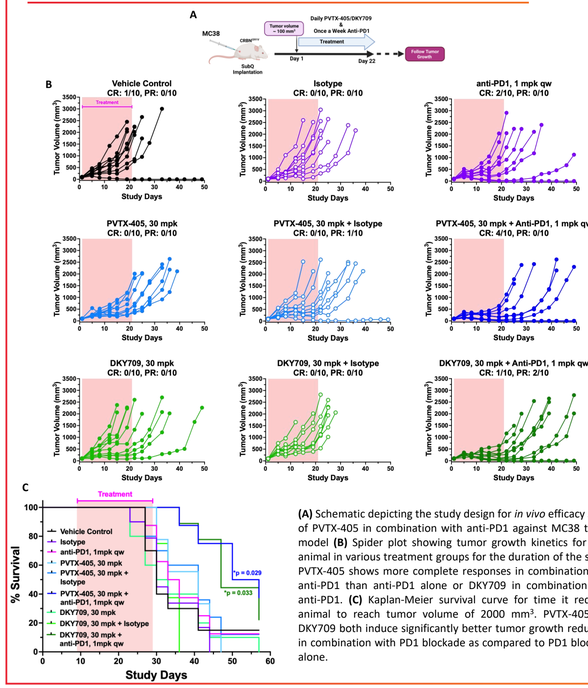
(A) IL-2 levels secreted by Jurkat cells after activation with CD3/CD28 beads at a beads-to-cell ratio of 1:4 and treatment with increasing levels of PVTX-405 or DKY709 for 24 hr. **(B)** Schematic depicting Treg:Teff cell co-culture assay. **(C)** Suppression of Teff proliferation by primary human Treg cells expanded in the presence of PVTX-405 or DKY709 or DMSO. Data are shown as six independent experiments/donors, with median ± interquartile range at 1:4 Treg/Teff coculture ratio. Data are normalized to proliferation of Teff alone for each donor. *p < 0.05. Groups were compared using two-tailed paired parametric t test.

Figure 5. PVTX-405 inhibits the growth of MC38 tumors in CRBN^{I391V} (humanized CRBN) mice *in vivo*



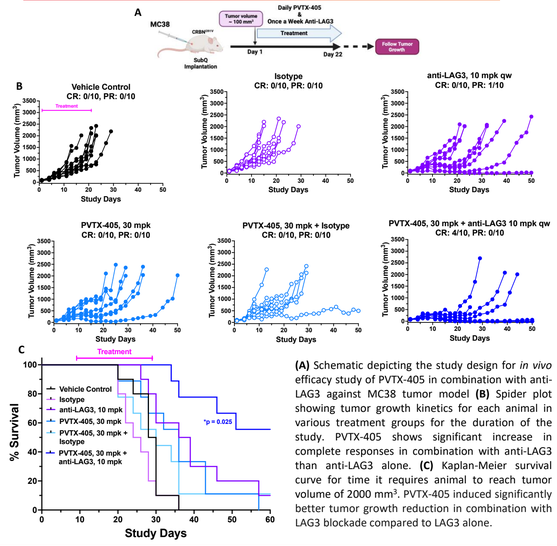
(A) Schematic showing synthesis of novel MC38 tumor model in CRBN^{I391V} mouse. **(B)** Schematic depicting the study design for in vivo efficacy study of PVTX-405 against MC38 tumor model **(C)** Tumor growth reduction induced by daily PVTX405 treatment (30 mg/Kg and 300 mg/Kg) alone in MC38 tumor model in CRBN^{I391V} mice.

Figure 6. PVTX-405 in combination with anti-PD1 significantly improves animal survival and durable tumor regression *in vivo*



(A) Schematic depicting the study design for *in vivo* efficacy study of PVTX-405 in combination with anti-PD1 against MC38 tumor model **(B)** Spider plot shows tumor growth kinetics for each animal in various treatment groups for the duration of the study. PVTX-405 shows more complete responses in combination with anti-PD1 than anti-PD1 alone or DKY709 in combination with anti-PD1. **(C)** Kaplan-Meier survival curve for time it requires animal to reach tumor volume of 2000 mm³. PVTX-405 and DKY709 both induce significantly better tumor growth reduction in combination with PD1 blockade as compared to PD1 blockade alone.

Figure 7. Combining PVTX-405 with a next-generation ICT, anti-LAG3, demonstrates synergistic efficacy *in vivo*



(A) Schematic depicting the study design for *in vivo* efficacy study of PVTX-405 in combination with anti-LAG3 against MC38 tumor model **(B)** Spider plot showing tumor growth kinetics for each animal in various treatment groups for the duration of the study. PVTX-405 shows significant increase in complete responses in combination with anti-LAG3 than anti-LAG3 alone. **(C)** Kaplan-Meier survival curve for time it requires animal to reach tumor volume of 2000 mm³. PVTX-405 induced significantly better tumor growth reduction in combination with LAG3 blockade compared to LAG3 alone.

Conclusions

- PVTX405 promotes a productive ternary complex with the E3 ubiquitin ligase substrate receptor CRBN to selectively and potently degrade IKZF2 with (DC_{50} = 6.3 nM) while sparing other CRBN neosubstrates (IKZF1/3, GSPT1, CK1a).
- PVTX-405 mediated degradation of IKZF2 leads to increase in production of IL-2 *in vitro* and reduction in the suppressive activity of Tregs *ex vivo*.
- Once daily oral administration of PVTX-405 as single agent significantly delays the growth of MC38 tumors in CRBN^{I391V} (Humanized CRBN) mice.
- PVTX-405 in combination with ICTs, anti-PD1 or anti-LAG3, significantly increases animal survival and led to durable tumor regressions compared to ICTs alone *in vivo*.
- In summary, these results demonstrate a strong synergy between ICTs and Treg destabilization through pharmacological degradation of IKZF2, representing a promising combination strategy for cancer treatment.

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