Unlocking potential for enhancing anti-tumor immunity: Targeting Hematopoietic Progenitor Kinase 1 (HPK1) with novel small molecules

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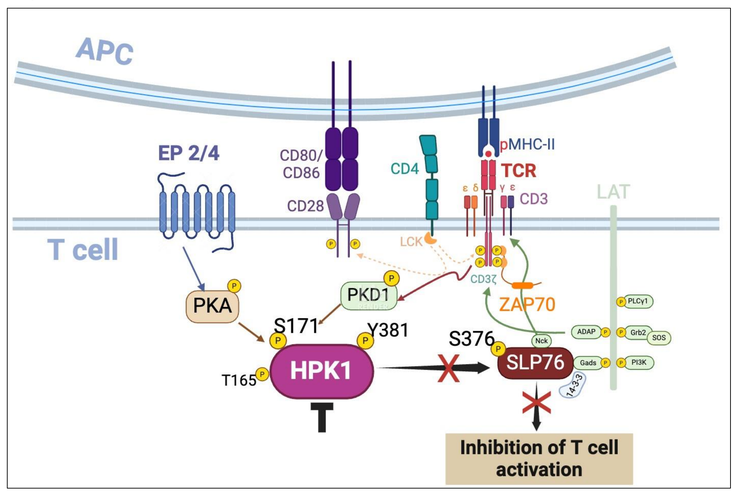
Introduction

HPK1, a MAP4K family serine/threonine kinase exclusive to hematopoietic cells, plays a pivotal role in dampening TCR and BCR signal cascades. Its influences IL-2 secretion, T-cell maturation and migration, tumor infiltration, and dendritic cell antigen presentation. Targeting HPK1 with potent small molecules presents a promising approach to enhance anti-tumor immunity and deliver clinical benefit to cancer patients.



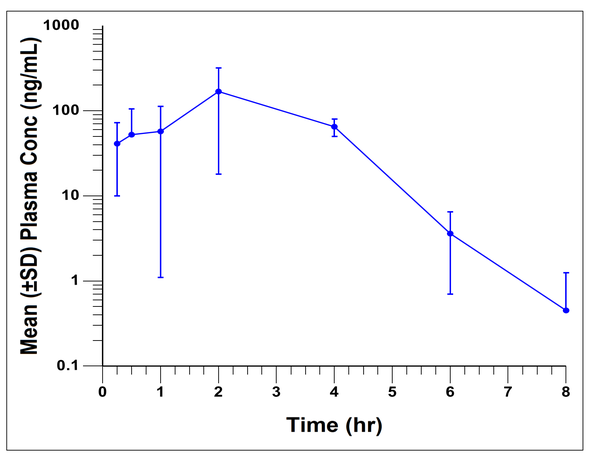
Structure-guided medicinal chemistry is used to design and optimize the potency against HPK1 kinase and selectivity against the MAP4K family using a panel of enzymatic assays. The identified novel leads are potent, selective, and orally bioavailable HPK1 inhibitors that demonstrate robust efficacy in suppressing tumor growth and enhancing the anti-tumor immune responses. These leads offer a potential therapeutic option for augmenting immunotherapy for several types of cancers. Further preclinical and clinical studies are warranted to validate their potential as therapeutic option for cancer patients.

Selectivity profile for MAP4K family and related kinases

Kinase IC_{50} (nM)	BWC3304	BWC4642	BWC4496	HPK-IN3
HPK1	3	1	2	1
GCK (ratio GCK/HPK1)	102 (34)	614 (614)	1804 (902)	66 (66)
IRAK4 (ratio IRAK4/HPK1)	58 (19)	394 (394)	668 (334)	ND
MAP4K3 (ratio MAP4K3/HPK1)	6 (2)	26 (26)	112 (56)	1 (1)
MAP4K4 (ratio MAP4K4/HPK1)	54 (18)	232 (232)	548 (274)	448 (448)
MAP4K5 (ratio MAP4K5/HPK1)	1 (0.3)	39 (39)	76 (38)	14 (14)
MINK (ratio MINK/HPK1)	26 (8.6)	469 (469)	893 (446)	685 (685)
LCK (ratio LCK/HPK1)	100 (33)	820 (820)	352 (176)	ND

- Improved HPK1 potency and selectivity among other isoforms.
- Lead compounds - BWC4642 & BWC4496 exhibit IC_{50} of 1-2 nM with a good selectivity window of >30-fold for MAP4K2 (GCK), MAP4K3, MAP4K4, MAP4K5 & MAP4K6 (MINK).

Oral Pharmacokinetics profile of BWC4496 in Male CD-1 Mice



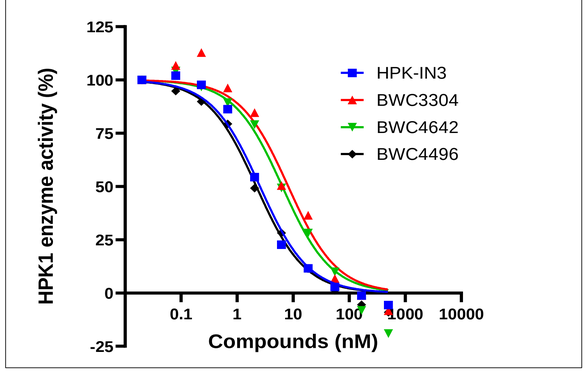
PK Parameters	Estimate
C_{max} (ng/mL)	175 ± 140
T_{max} (hr)*	2.0 (2.0-4.0)
AUC_{0-t} (hr*ng/mL)	410 ± 277
AUC_{0-inf} (hr*ng/mL)	NE

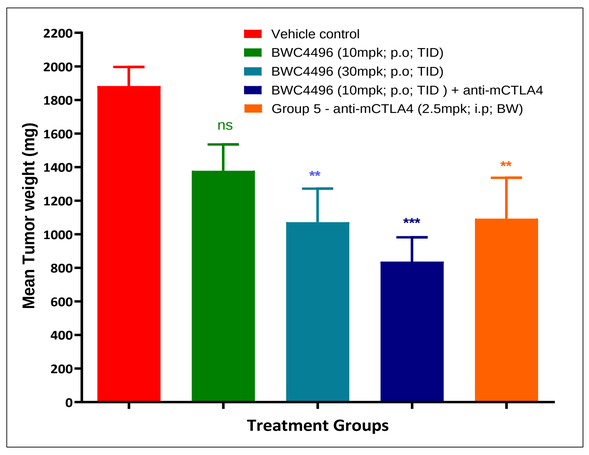
**Median (Min-Max);*
NE: Not estimated; n=3 mice

- BWC4496 is a potent compound inhibiting HPK1 with a IC_{50} <3nM.
- C_{max} achieved following 30mpk oral dose is 175ng/mL (90nM), 30x higher than the IC_{50} (3nM), enough to show the effect under in vivo conditions.
- Based on the pharmacokinetic data, 30mpk was determined to be the dose for efficacy studies in the CT26 Syngeneic mouse model.

Inhibition of HPK1 enzyme activity and SLP76 phosphorylation

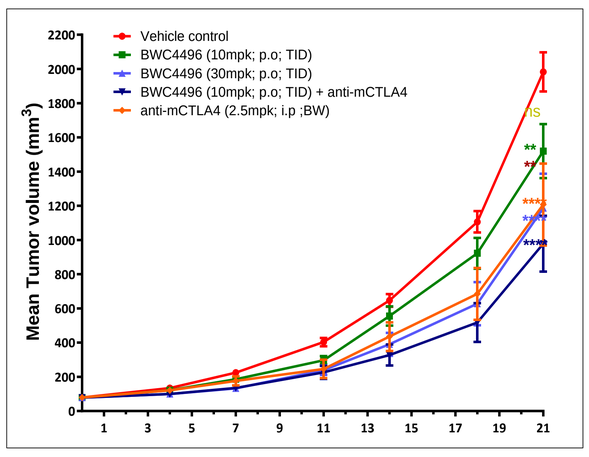
	HPK-IN3	BWC3304	BWC4642	BWC4496
IC_{50}(nM)	2.55	8.15	6.38	2.21



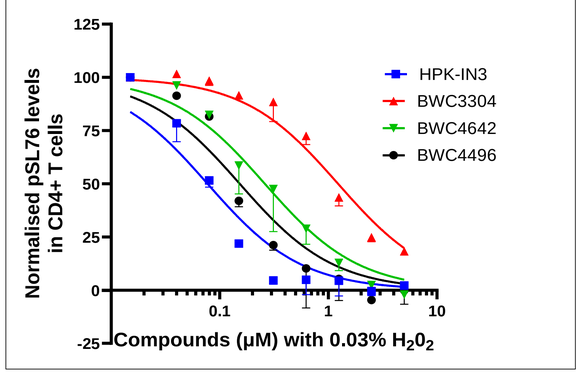


- A luminescence-based ATP quantification assay is used to evaluate the activity of HPK1 kinase using Myelin Basic Protein (MBP) as the substrate.
- Bugworks compounds demonstrate potent inhibition of HPK1 with IC_{50s} <10nM.
- Human PBMCs are treated with different concentrations of compounds for 1 hour and 0.03% H_2O_2 for 10 mins, followed by flow cytometric detection of phosphorylated SLP76.
- Bugworks compounds inhibit phosphorylation of SLP76 while total SLP76 levels remain unchanged, indicating the specificity of inhibition of serine 376 phosphorylation of SLP76.

Significant tumor growth inhibition in CT26 mouse model



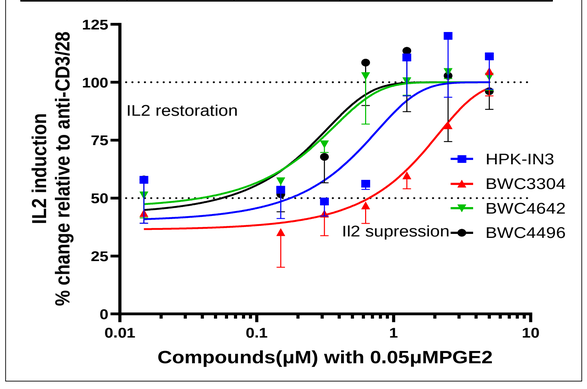
	HPK-IN3	BWC3304	BWC4642	BWC4496
IC_{50}(μM)	0.07	1.22	0.25	0.15



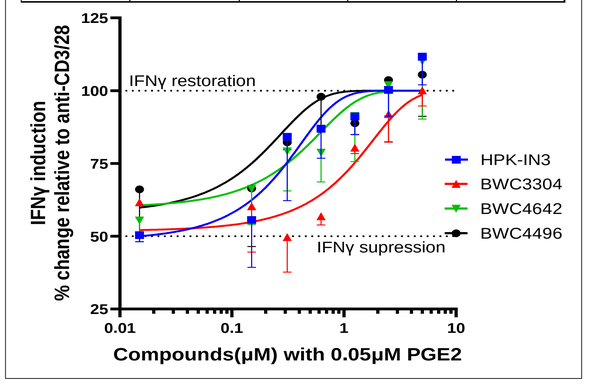
- Robust efficacy demonstrated for BWC4496 in CT26 syngeneic mouse model, both as a single agent and in combination with anti-CTLA-4 antibody.
- The efficacy achieved at a C_{max} of ~90nM is equivalent to the pSLP76 IC_{50} value.

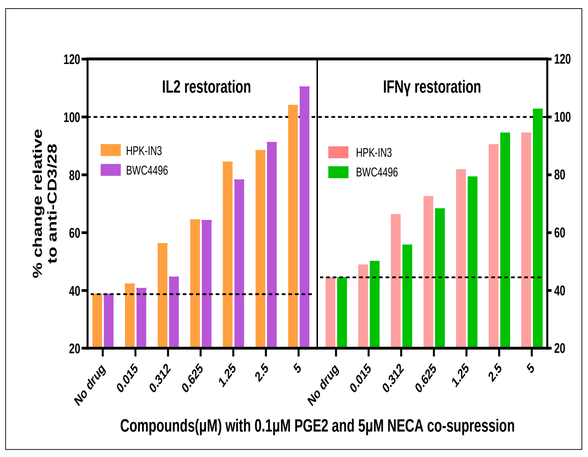
Cytokine restoration under immunosuppressive conditions

	HPK-IN3	BWC3304	BWC4642	BWC4496
EC_{50}(μM)	1.49	4.57	1.09	1.13



	HPK-IN3	BWC3304	BWC4642	BWC4496
EC_{50}(μM)	1.03	0.81	0.66	0.86





- Human PBMCs are stimulated by anti-CD3/28 in the presence of immune suppressive prostaglandin E2 and NECA (adenosine analog), treated with test compounds for 48 hours, followed by quantification of cytokines in cell-free supernatant using AlphaLISA.
- BWC4496 reverses the immune-suppression caused by both PGE2 and NECA, resulting in enhanced T cell function via secretion of IL-2 and IFN-γ.

Conclusions

- Bugworks optimized leads BWC4642 and BWC4496 demonstrate an IC_{50} <2 nM against HPK1 and >30-fold selectivity against other kinases of the MAP4K family.
- These leads effectively inhibit SLP76 phosphorylation in human PBMCs, indicating active target engagement.
- These leads restore immune function by reversing the suppression of IL-2 and IFN-γ secretion in human PBMCs, implying T cell activation.
- Lead compound, BWC4496 demonstrates good oral bioavailability and in vivo efficacy by inhibiting tumor growth as a single agent and in combination with anti-CTLA-4 therapy in the syngeneic CT26 mouse model.

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