Abstract # B184

TPX-0005, a highly potent TRK inhibitor, effectively overcomes clinical-resistance TRK mutations including solvent front mutants TRKA G595R, TRKB G639R, and TRKC G623R

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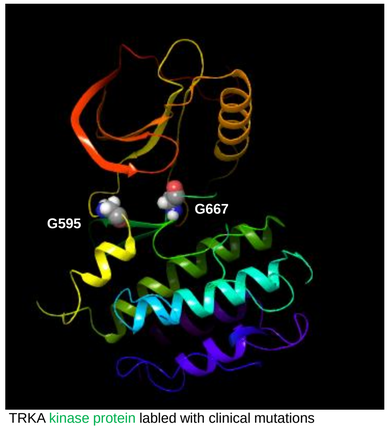
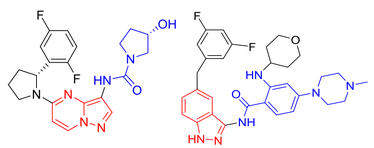
Abstract

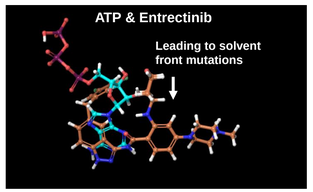
The tropomyosin receptor kinases (TRKs), including TRKA/B/C encoded by *NTRK1/2/3* genes, are high-affinity receptors for neurotrophins. Oncogenic rearrangement of *NTRK1*, *NTRK2*, and *NTRK3* have been identified in many solid malignancies. The use of TRK inhibitors entrectinib and larotrectinib has led to clinical benefit in patients with solid malignancies harboring oncogenic *NTRK* fusions. Similar to ALK and ROS1 inhibitor treatment, the solvent front mutations TRKA G595R and TRKC G623R (both analog to ALK G1202R) were reported in clinic from treatment resistant patients. A new generation of TRK inhibitor targeting both wild type and mutated TRKs is highly needed for effectively treating patients harboring fusion TRKs. TPX-0005, a novel three-dimensional macrocycle with a much smaller size than current TRK inhibitors in the clinic, was designed to overcome clinical resistance mutations systematically. TPX-0005 potently inhibited both wild type and mutant TRKs including solvent front mutations. TPX-0005 showed pico-molar activity against TRK kinases (IC_{50}s 0.83 nM, 0.05 nM, and 0.10 nM for TRKA/B/C, respectively) in Reaction Biology kinase assay. TPX-0005 is the most potent TRK inhibitor in clinic and effectively overcomes clinical resistance TRK mutations as shown in the table with cell proliferation assays. It was recently reported that LOXO-195 inhibited the phosphorylation of TRKA and ERK kinases in NIH3T3 cell line expressing ΔTRKA G595R or ETV6-TRKC G623R with IC_{50}s of 7 nM and 45.5 nM, respectively. TPX-0005 is more than 10 fold more potent than LOXO-195. In the xenograft tumor model studies, TPX-0005 dramatically caused tumor regression in the tumors carrying WT or solvent-front mutated TRK fusion gene. Overall, TPX-0005 demonstrated desired drug-like properties, good safety profile, and is a supreme TRK inhibitor against WT and mutated TRKs. A Phase 1/2 clinical trial of TPX-0005 is actively pursued (NCT03093116).

Introduction



TRKA kinase protein labled with clinical mutations

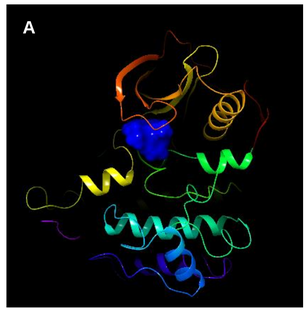


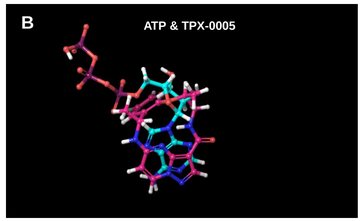


Entrectinib overlay with ATP

- There are 3 members of TRK family: TRKA, TRKB, and TRKC encoded by *NTRK1*, *NTRK2*, and *NTRK3*, respectively.
- Oncogenic rearrangements of *NTRK1*, *NTRK2*, and *NTRK3* that lead to constitutive activation of TRK fusion proteins have been identified in many solid malignancies.[1]
- Larotrectinib and entrectinib targeting TRK family kinases have led to clinical benefit in patients with solid malignancies harboring oncogenic *NTRK* fusions.[2,3]
- Solvent front mutations TRKA G595R and TRKC G623R have been reported in clinical from Larotrectinib and entrectinib refractory patients.[2,3]

Design of TPX-0005



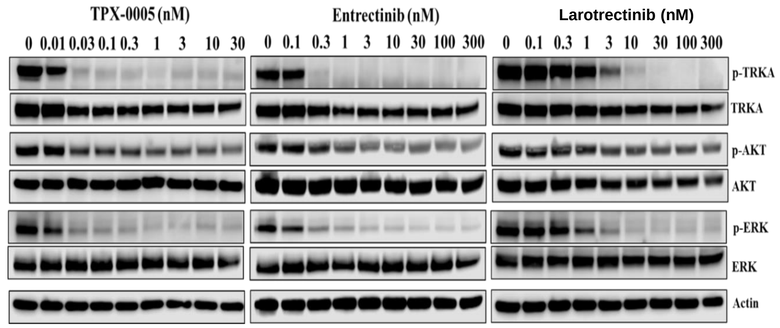


A. Modeling of TPX-0005 in the complex with TRKA kinase
B. TPX-0005 overlap with ATP

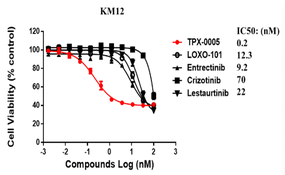
- TPX-0005 (MW 355.37) was designed to locate completely inside the ATP adenine binding pocket, without motifs extending to the back pocket or solvent front, and have higher binding affinity with the active kinase conformation.
- The macrocyclic structure of TPX-0005 anchors the three dimensional interactions with the protein with higher binding efficiency, and allows to target wild type (WT) and mutant kinases equally.

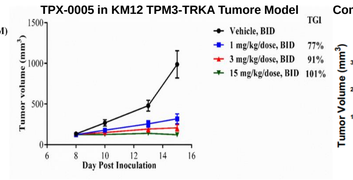
Inhibition of TPM3-TRKA in KM12 Cells

- **The effect of TPX-0005, entrectinib and larotrectinib on auto-phosphorylation of TPM3-TRKA and its downstream effectors AKT and ERK1/2 in KM12 cells**

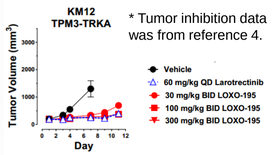


- **Effect of TPX-0005 on anti-proliferation in KM12 cells and anti-tumor growth in KM12 xenograft tumor model in athymic nude mice**





Comparison with Larotrectinib and Loxo-195[4]



* Tumor inhibition data was from reference 4.

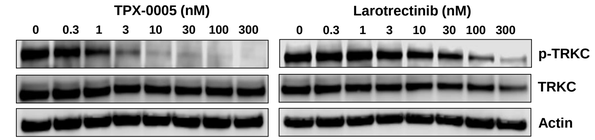
TPX-0005 Potently Inhibited WT and Mutant TRKs

- **Inhibition of cell proliferation in Ba/F3 cell lines**

Ba/F3 Cell Proliferation Assay IC_{50} (nM)

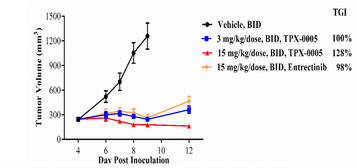
Inhibitor	LMNA-TRKA WT	LMNA-TRKA G595R	LMNA-TRKA G667A	LMNA-TRKA G667C	ETV6-TRKB WT	ETV6-TRKB G639R	ETV6-TRKB G709C	ETV6-TRKC WT	ETV6-TRKC G623R	ETV6-TRKC G696A	ETV6-TRKC G696C
TPX-0005	<0.2	0.4	0.5	7.7	<0.2	0.6	10.9	<0.2	3	0.2	7.4
Entrectinib	0.3	705	9.8	292.9	<0.5	1384	501.3	0.6	1000	16.6	313.1
Larotrectinib	3.5	1024	48.2	2244	10.9	3000	3179	10.2	1500	143.2	2569

- **Inhibition of TRKC phosphorylation in NIH3T3 ETV6-TRKC G696A cells**

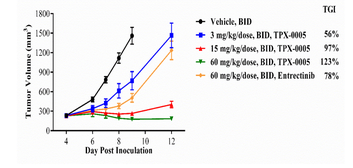


- **Efficacy of TPX-0005 against WT and mutant fusion TRKs in xenograft tumor models**

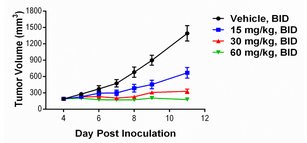
The anti-tumor efficacy in the NIH3T3 LMNA-TRKA WT xenograft model



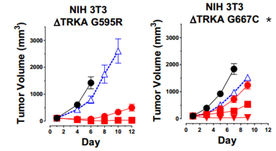
The anti-tumor efficacy in the NIH3T3 LMNA-TRKA G595R xenograft model



The Effect of TPX-0005 on the Growth of NIH3T3 LMNA-TRKA G667C Tumors



Comparison with LOXO-195 in similar models[4]



* Tumor inhibition data were from reference 4.

Conclusions

- TPX-0005 was designed to bind **WITHIN** the ATP binding boundary with high affinity and be able to target both wild type and mutant kinases.
- TPX-0005 potently inhibited WT and mutant TRKs *in vitro* and *in vivo*, especially the solvent front mutations which render common resistances to TRK inhibitors that are currently in clinic.
- TPX-0005 is the most potent TRK inhibitor against wildtype and mutant TRKs.
- A Phase 1/2 clinical trial is on-going for TPX-0005 for cancer patients with solid tumors harboring ALK, ROS1 or NTRK fusion gene (NCT03093116).

References
1. Vaishnavi A, et al. Cancer Discov. 2015, 5(1):25-34.
2. Russo M, et al. Cancer Discov. 2016;6(1):36-44.
3. Drilon A, et al. Ann Oncol. 2016, 27(5):920-6.
4. Drilon A, et al. Cancer Discov. 2017, 7(9):963-972

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