Abstract #3749
Poster #1325

TPX-0022, a polypharmacology inhibitor of MET/CSF1R/SRC inhibits tumor growth by promoting anti-tumor immune responses

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Introduction

In the tumor microenvironment, tumor associated macrophages (TAMs) support tumor growth, suppress antitumor immune response, promote angiogenesis, and are associated with poor clinical outcomes.[1] In contrast to the classic phagocytic and cytotoxic pro-inflammatory phenotype (M1) of the macrophage, TAMs often adopt an immunosuppressive phenotype (M2), in response to colony stimulating factor 1 (CSF1), produced by tumor or stromal cells.[2] Signaling through colony stimulating factor 1 receptor (CSF1R), a receptor tyrosine kinase normally expressed on the surface of mononuclear cells, is involved in the recruitment of TAMs and has been associated with tumor progression and suppression of the immune response. Thus, CSF1R represents a potential therapeutic target for immuno-oncology.

We have designed TPX-0022, a Type I kinase inhibitor with a novel macrocyclic structure, to inhibit MET/CSF1R/SRC with enzymatic kinase inhibition IC_{50}s of 0.14, 0.71 and 0.12 nM, respectively. The activity of TPX-0022 against CSF1R was demonstrated in cellular assays in an engineered Ba/F3 TEL-CSF1R cell model. Furthermore, in the CSF1/CSF1R signaling-dependent M-NFS-60 model, TPX-0022 not only exhibited potency under baseline conditions, but also potently inhibited the growth of M-NFS-60 cells in the presence of exogenous CSF1 at 1 ng/mL concentration, a condition mimicking elevated CSF1 levels often observed in the setting of advanced cancers. Finally, in the MC38 syngeneic mouse model, TPX-0022 effectively reduced TAMs, altered the polarity of TAMs toward a more M1 phenotype, increased cytotoxic T cells and inhibited the growth of MC38 tumors. These preclinical results demonstrate the potent inhibitory activity of TPX-0022 against CSF1R , the ability of TPX-0022 to inhibit tumor growth *in vivo* and to promote a pro-inflammatory anti-tumor microenvironment.

The activity of TPX-0022 against MET *in vitro* and *in vivo* will be presented at Poster # 1321 (Abstract #3719).

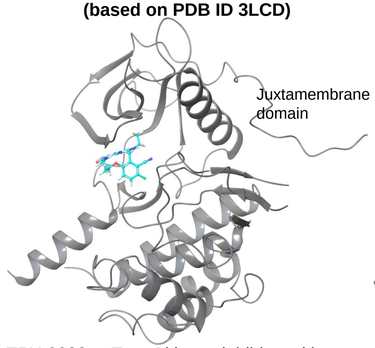
Kinase inhibition IC_{50}s of TPX-0022 at 10 μM of ATP*

	MET IC_{50} (nM)	CSF1R IC_{50} (nM)	SRC IC_{50} (nM)
TPX-0022	0.14	0.71	0.12

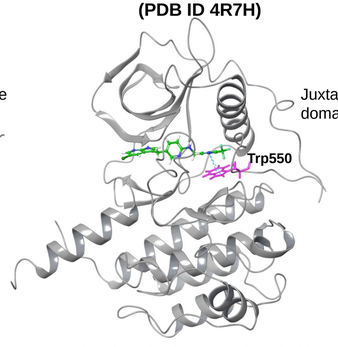
* Kinase activity was determined at Reaction Biology, Inc.

TPX-0022 is a type I CSF1R inhibitor

Model of TPX-0022 in complex with CSF1R (based on PDB ID 3LCD)



Pexidartinib in complex with CSF1R (PDB ID 4R7H)

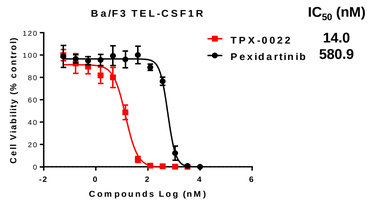


- TPX-0022, a Type I kinase inhibitor with a macrocycle structure that prefers the DFG-in CSF1R conformation
- Pexidartinib (PLX-3397), a Type II kinase inhibitor, extends to the back pocket of the kinase and has π-π stacking interaction with the juxtamembrane domain residue Trp550 (4R7H)[3]

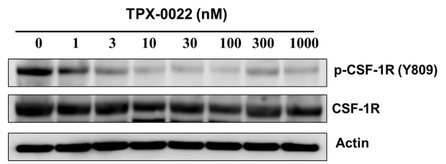
Inhibition of CSF1R kinase by TPX-0022 *in vitro*

- The constitutively activated TEL-CSF1R fusion protein in Ba/F3 cells doesn't have the CSF1R juxtamembrane domain
- TPX-0022 potently inhibited cell proliferation of Ba/F3 TEL-CSF1R cells and suppressed the auto-phosphorylation of CSF1R
- Type II CSF1R inhibitor pexidartinib[a] is much less active in this Ba/F3 cell line due to absence of the juxtamembrane domain for the interaction with Trp550 residue

Inhibition of cell proliferation by TPX-0022 in an engineered Ba/F3 TEL-CSF1R cell line



Inhibition of TEL-CSF1R auto-phosphorylation by TPX-0022 in Ba/F3 TEL-CSF1R cells

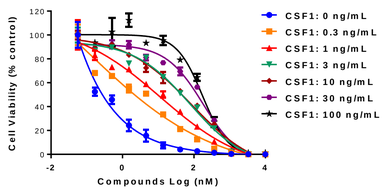


Ligand-dependent potency of CSF1R inhibitors

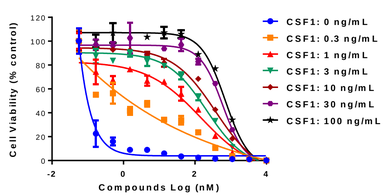
- M-NFS-60 is a mouse myelogenous leukemia cell line, dependent on CSF1R signaling pathway for survival, and sensitive to CSF1R inhibitor pexidartinib treatment[3]
- Both TPX-0022 and pexidartinib potently inhibited cell proliferation of M-NFS-60 cells in the absence of exogenous CSF1 ligand
- Exogenous CSF1 ligand decreased the sensitivity to CSF1R inhibition
- The potency of Type I CSF1R inhibitor TPX-0022 is less impacted by the CSF1 ligand than the Type II CSF1R inhibitor pexidartinib
- At a concentration of 1 ng/mL CSF1 that mimics the CSF1 concentration in advanced cancer patients, TPX-0022 is >10 fold more potent than pexidartinib

CSF1 ligand-dependent inhibition of M-NFS-60 cell proliferation

TPX-0022



Pexidartinib[a]

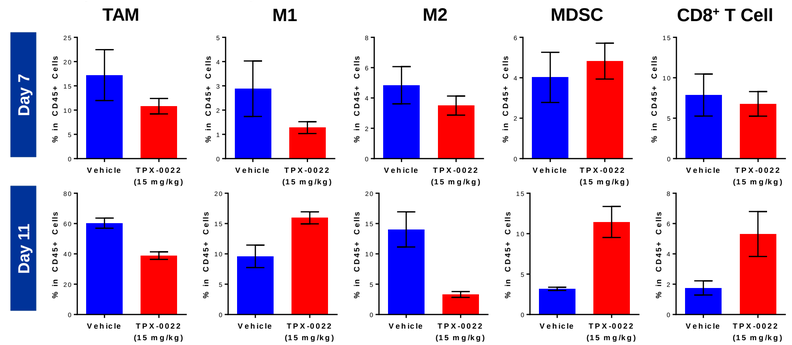


Compound	CSF1 (ng/mL)						
	0	0.3	1	3	10	30	100
TPX-0022	0.3	3	11.6	78.2	84.1	180.8	174.5
Pexidartinib[a]	<0.1	2	146.4	212.5	379.7	594.7	702.3

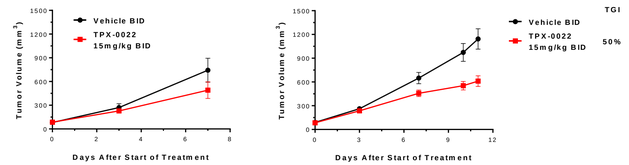
[a] Data based on evaluation of comparable proxy chemical reagent purchased from commercial sources

TPX-0022 modulates tumor associated immune cells and inhibits tumor growth in the MC38 syngeneic tumor model

- Two groups of C57BL/6 mice (10 each) bearing MC38 tumors were treated with Vehicle and TPX-0022 (15 mg/kg BID) for 11 days, respectively.
- On Day 7 and Day 11 five mice from each group were euthanized and tumors were dissociated and subjected for FACS analysis of tumor associated immune cells.



Day 7 Tumor Volume **Day 11 Tumor Volume**



- More than 7 days were needed to significantly modulate tumor microenvironment by TPX-0022
- The FACS analysis results on Day 11:
  - A decreased TAM population in MC38 tumors
  - Altered polarity of TAM by promoting the M1 phenotype and suppressing M2 phenotype
  - An increased population of tumor infiltrated cytotoxic T cells
- Tumor growth inhibition was achieved by TPX-0022 as a single agent

Conclusions

- In addition to its activity against MET/SRC, TPX-0022 is a unique macrocyclic Type I kinase inhibitor with potent activity against CSF1R in *in vitro* assays and in *in vivo* tumor models
- In the presence of exogenous CSF1 (≥1 ng/mL), TPX-0022, a type I inhibitor, inhibited cell growth of M-NFS-60 cells more potently than pexidartinib
- In the MC38 syngeneic tumor model, TPX-0022 modulated TAM phenotype and promoted a more pro-inflammatory anti-tumor microenvironment
- TPX-0022 has promising drug-like properties, and the novel polypharmacological profile of inhibiting MET/CSF1R/SRC has the potential to simultaneously target MET as an oncogenic driver and alter the tumor microenvironment, leading to enhanced anti-tumor activity
- TPX-0022 IND submission is planned for the first half of this year and a Phase 1 clinical trial initiation in the second half of this year

Reference
1. Hanahan D, Weinberg RA. *Cell.* **2011,** 144:646–674. 2. Yang L, et al. *J Hematol Oncol.* **2017**, 10, 58. 3. Tap WD, et al. *N Engl J Med.* **2015**, 373:428-37.

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