In the tumor microenvironment, tumor associated macrophages (TAMs) support tumor growth, suppress anti-tumor immune response, promote angiogenesis, and are associated with poor clinical outcomes. In contrast to the classic phagocytic and cytotoxic pro-inflammatory phenotype (M1) of the macrophages, TAMs often adopt an immunosuppressive phenotype (M2), in response to colony stimulating factor 1 (CSF1), produced by tumor or stromal cells. Signaling through colony stimulating factor 1 receptor (CSFR1), 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, CSFR1 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/CSFR1/SRC with enzymatic kinase inhibition IC50s of 0.14, 0.71 and 0.12 nM, respectively. The activity of TPX-0022 against CSFR1 was demonstrated in cellular assays in an engineered Ba/F3 TEL-CSFR1 cell model. Furthermore, in the CSFR1/CSFR1 signaling-dependent M-NFS 60 model, TPX-0022 not only inhibited 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 M383 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 M383 tumors. These preclinical results demonstrate the potent inhibitory activity of TPX-0022 against CSFR1, 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 M-NFS-60 is a mouse myelogenous leukemia cell line, dependent on CSFR1 signaling pathway for survival, and sensitive to CSFR1 inhibitor pexidartinib treatment. 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 CSFR1 inhibition. The potency of Type I CSFR1 inhibitor TPX-0022 is less impacted by the CSF1 ligand than the Type II CSFR1 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.

**Conclusions**

- **In addition to its activity against MET/SRC, TPX-0022 is a unique macrocyclic Type I kinase inhibitor with potent activity against CSFR1 in vitro and 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 M383 syngeneic tumor model, TPX-0022 modulated TAM phenotype and promoted a more potent pro-inflammatory anti-tumor microenvironment.**
- **TPX-0022 has promising drug-like properties, and the novel polypharmacological profile of inhibiting MET/CSFR1/SRC has the potential to simultaneously target MET as an oncogenic driver and activate 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 I clinical trial initiation in the second half of this year.**

References:

**Model of TPX-0022 in complex with CSFR1 (based on PDB ID 3CLD)**

**Pexidartinib in complex with CSFR1 (PDB ID 4RTH)**

**Kinase inhibition IC50s of TPX-0022 at 10 µM of ATP**

<table>
<thead>
<tr>
<th>MET IC50 (nM)</th>
<th>CSFR1 IC50 (nM)</th>
<th>SRC IC50 (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPX-0022</td>
<td>0.14</td>
<td>0.71</td>
</tr>
</tbody>
</table>

**Note:** Kinase activity was determined at Resolute Biology, Inc.

**In vivo inhibition of CSFR1 kinase by TPX-0022 in vitro**

- **The constitutively activated TEL-CSF1 fusion protein in Ba/F3 cells doesn't have the CSF1R juxtamembrane domain.**
- **Type II CSFR1 inhibitor pexidartinib is much more active in this Ba/F3 cell line due to absence of the juxtamembrane domain for the interaction with Trp505 residue.**

**Inhibition of CSFR1 kinase by TPX-0022 in vitro**

- **Inhibition of CSFR1 auto-phosphorylation by TPX-0022 in Ba/F3 TEL-CSFR1 cells**
- **Inhibition of cell proliferation by TPX-0022 in an engineered Ba/F3 TEL-CSFR1 cell line**

**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 dissected and subjected to FACS analysis of tumor associated immune cells.**

**Conclusion: 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.**
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**Abstract #3749**

**Poster #1321**

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

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**TPX-0022, a Type I CSFR1 Inhibitor**

**TPX-0022 in complex with CSFR1**

**Pexidartinib in complex with CSFR1**

**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.**
- **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 CSFR1 inhibitor TPX-0022 is less impacted by the CSF1 ligand than the Type II CSFR1 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.**

**TPX-0022 at 15 mg/kg BID for 11 days**

- **Increased population of tumor infiltrated cytotoxic T cells**
- **A decreased TAM population in MC38 tumors**
- **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 CSFR1 in vitro and 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 M383 syngeneic tumor model, TPX-0022 modulated TAM phenotype and promoted a more potent pro-inflammatory anti-tumor microenvironment.**
- **TPX-0022 has promising drug-like properties, and the novel polypharmacological profile of inhibiting MET/CSFR1/SRC has the potential to simultaneously target MET as an oncogenic driver and activate 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 I clinical trial initiation in the second half of this year.**

**References:**

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