HMPL-653, a highly potent and selective CSF-1R inhibitor, targets both tumor cells and tumor microenvironment

Abstract #34522

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INTRODUCTION

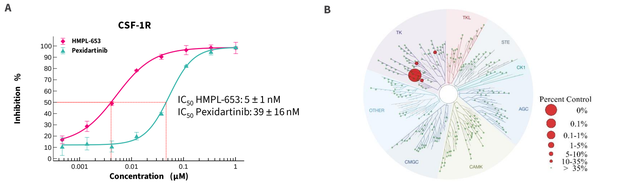
- Colony stimulating factor 1 receptor (CSF-1R) and its ligand CSF-1 signaling regulates the function and survival of tumor-associated macrophages (TAM), which are involved in tumor progression and suppression of anti-tumor immunity[1]. Moreover, activation of CSF-1R/CSF-1 axis including CSF-1 fusions or CSF-1R activating mutations has also been implicated in the pathogenesis of certain tumors such as tenosynovial giant cell tumor and histiocytic neoplasms[2,3]. Pexidartinib, the approved CSF-1R inhibitor, also targets other kinases, which may induce off-target toxicities and limit its clinical utility[4].
- HMPL-653 is a highly potent and selective CSF-1R small molecule inhibitor, discovered and being currently developed by HUTCHMED.

METHODS

- **Signaling pathway:** Ba/F3 cells stably expressed BCR-CSF-1R or CSF-1R mutations were incubated with a serial dilutions of compound for 1 or 2 hours and then were lysed for western blot assay. For signaling transduction in CSF-1 dependent mouse myelogenous leukemia cell, M-NFS-60, the similar protocol was applied except cells were stimulated with 100 ng/mL CSF-1 for 1 minute before cell lysate collection.
- **Cell viability assay:** Cell lines were treated with compound for 72 hours and cell viability was measured by CCK-8 assay.
- **M2 macrophage polarization:** Monocytes from human PBMCs were isolated and stimulated with 10 ng/mL CSF-1 to induce a M2-like phenotype. Cells were collected for CD163 (M2 macrophage marker) detection by flow cytometry after 72 hours treatment with a serial dilutions of compound.
- **Pharmacokinetics and pharmacodynamics (PK/PD) study:** Ba/F3$^{BCR\text{-}CSF\text{-}1R}$ tumor-bearing nude mice were treated with a single oral dose of HMPL-653 and euthanized at different time points. Tumor tissues were analyzed for p-CSF-1R (Tyr723) and CSF-1R by western blot assay. Meanwhile, plasma concentration of HMPL-653 was determined by LC-MS/MS .
- **In vivo anti-tumor efficacy study:** Ba/F3 tumor models were established by subcutaneous inoculation of tumor cells into Nu/Nu nude mice. Tumor volume was measured to assess tumor growth inhibition. M-NFS-60 ascites model was established by intraperitoneal injection of tumor cells into nude mice. Peritoneal fluid was collected and tumor cells were counted for anti-tumor efficacy evaluation. To demonstrate the immunoregulatory effect of HMPL-653, a murine tumor model, B16F10, was established in C57BL/6J mice, and the combination regimen, HMPL-653 plus PCP (poly (I:C)+CpG+anti-PD-1) was evaluated.
- **Flow cytometry:** Tumor tissue was dissociated into single cells suspension. After wash, cells were blocked with mouse Fc block, and stained with antibodies against surface markers. After fixation and permeabilization, cells were stained with antibodies against intracellular markers.

RESULTS

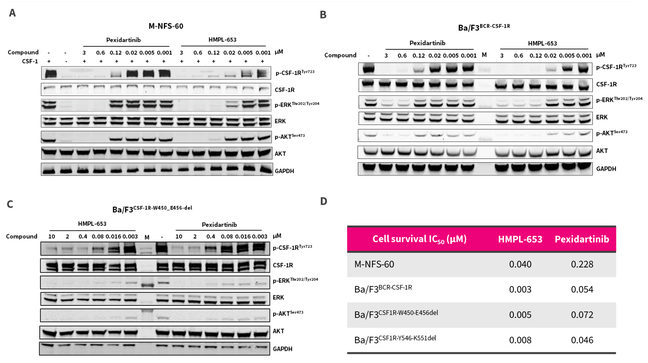
Biochemical Potency and Selectivity



A, *In vitro* biochemical IC_{50} value against CSF-1R determined with Z-LYTE™ kinase assay. Data was shown in mean ±SD; n=3. **B**, 375 wild-type kinases were profiled by Eurofins using 10 μM HMPL-653 and an ATP concentration at each enzyme's K_M. The sizes of dots indicated the remaining activity of each kinase after the inhibition by HMPL-653.

- HMPL-653 was highly potent only against CSF-1R, no other kinases hit by HMPL-653 at 10 μM with >80% inhibition.

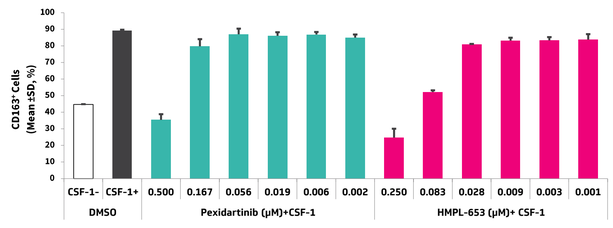
In Vitro Cellular Potency



Cell survival IC_{50} (μM)	HMPL-653	Pexidartinib
M-NFS-60	0.040	0.228
Ba/F3$^{BCR\text{-}CSF\text{-}1R}$	0.003	0.054
Ba/F3$^{CSF1R\text{-}W450\text{-}E456del}$	0.005	0.072
Ba/F3$^{CSF1R\text{-}Y546\text{-}K551del}$	0.008	0.046

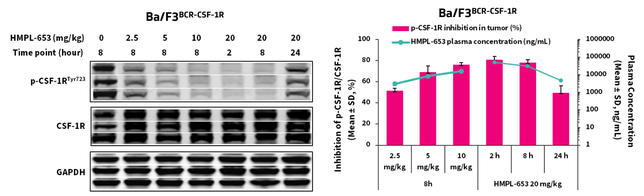
- HMPL-653 concentration-dependently blocked CSF-1R phosphorylation and downstream signaling, and strongly inhibited cell survival of CSF-1/CSF-1R dependent cell lines.

Function on Macrophage Polarization



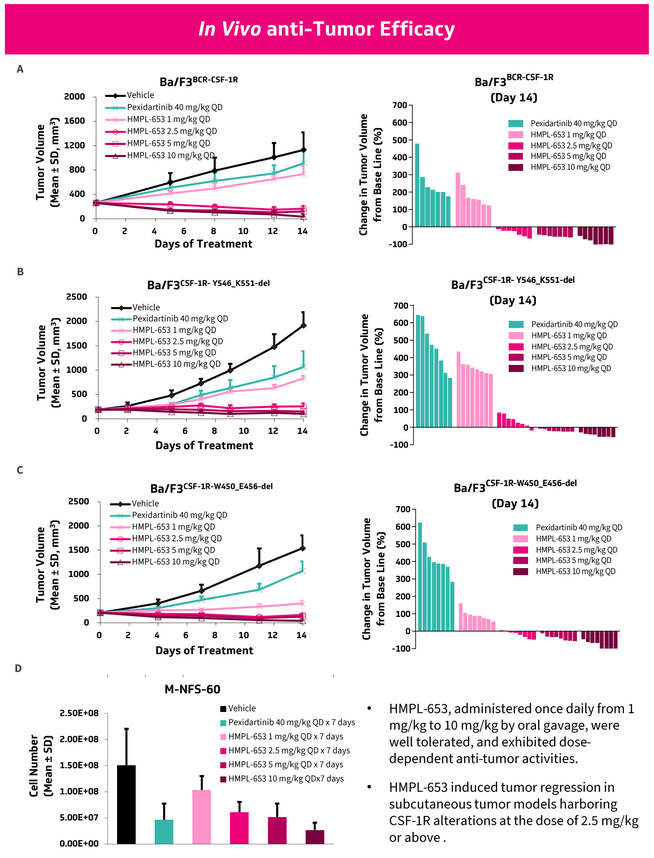
- HMPL-653 prevented human M2 macrophage polarization in a concentration-dependent manner.

In Vivo Pharmacodynamic Activity



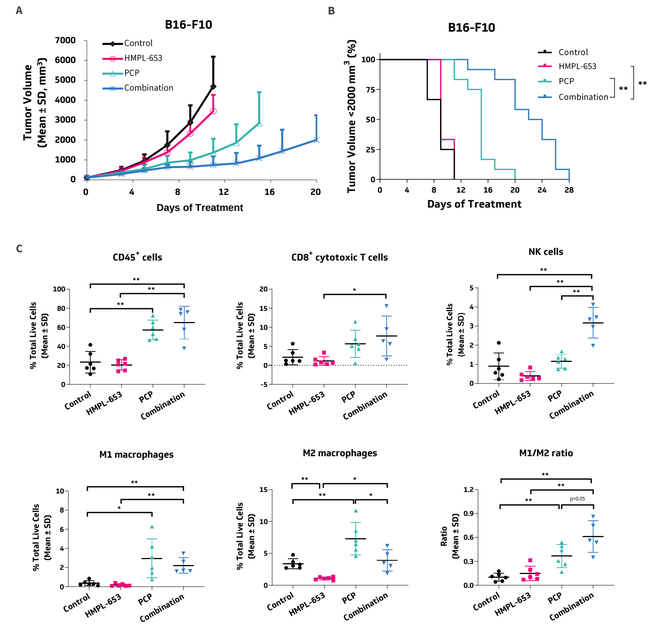
- PK/PD analyses of plasma exposure and target engagement in tumor samples demonstrated time- and dose-dependent inhibition on p-CSF-1R following a single oral dose of HMPL-653.

In Vivo anti-Tumor Efficacy



- HMPL-653, administered once daily from 1 mg/kg to 10 mg/kg by oral gavage, were well tolerated, and exhibited dose-dependent anti-tumor activities.
- HMPL-653 induced tumor regression in subcutaneous tumor models harboring CSF-1R alterations at the dose of 2.5 mg/kg or above .

Combination with Checkpoint Blockade Based Immunotherapy



A-B, The B16-F10 tumor bearing C57/BL6J mice (12 mice per group) received the treatment of control (vehicle of HMPL-653 and mIgG control antibody) , HMPL-653 (20 mg/kg, p.o., QD), PCP immunotherapy (peritumoral injection of 50 μg poly(I:C) and 50 μg CpG, intraperitoneal injection of 5 mg/kg anti-PD-1 antibody, twice a week) or the combination of HMPL-653 with PCP for 28 days. Tumor growth curve of each group and Kaplan-Meier curves were shown. **C**, In a separate biomarker study, after 8 days treatment, tumors were harvested 24 hours post administration of the last dose for flow cytometry analysis about tumor-infiltrating leukocytes. *: P<0.05 ; **: P<0.01.

- In the B16-F10 immunocompetent tumor model, HMPL-653 treatment enhanced response to PCP immunotherapy by decreasing tumor-infiltrating M2 macrophages and increasing the ratio of M1/M2 macrophages.

SUMMARY

- **HMPL-653 is a highly potent and selective CSF-1R inhibitor which displayed single agent anti-tumor activity in CSF-1/CSF-1R dependent tumor models.**
- **HMPL-653 exhibited synergy in combination with checkpoint blockade based immunotherapy.**
- **HMPL-653 is currently under clinical evaluations in phase I trial in solid tumors (NCT05277454).**

References

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