Enhancement of Anti-PD-1 Antitumor Efficacy in Syngeneic Preclinical Models by the Angiogenesis Inhibitor Lucitanib

Poster 1214

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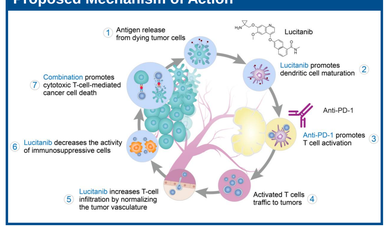
INTRODUCTION

- Lucitanib (E-3810) is an oral multikinase inhibitor whose targets are associated with angiogenesis and other key cancer and immune pathways[1]
- Angiogenesis plays a critical role in cancer cell growth through tumor vascularization and also promotes immunosuppression within the tumor microenvironment[2,3]
- Therapies targeting angiogenesis and immune checkpoint pathways enhance antitumor responses by modulating the tumor microenvironment[2,3]
  - For example, it is thought that inhibiting VEGF signaling may induce vascular normalization, increase T-cell infiltration into the tumor, promote dendritic cell maturation, modulate expression of cell adhesion molecules, and alter immune cell populations, whereas interfering with the PD-1/PD-L1 pathway can enhance T-cell activation and expansion

Proposed Mechanism of Action



- Combination therapies targeting angiogenesis and immune checkpoint pathways have demonstrated promising activity in preclinical and clinical studies[4-6]
  - For example, the combination of lenvatinib and pembrolizumab has shown enhanced activity in patients with renal cell and endometrial carcinomas[7,8]
- These data suggest that the combination of lucitanib and an immune checkpoint inhibitor may also demonstrate preclinical and clinical activity
- Preclinical studies were performed to investigate the antitumor activity and the mechanism of action of lucitanib in combination with anti-PD-1 in syngeneic mouse tumor models

METHODS

Kinase profiling: Enzymatic radioisotope filter binding assays were performed with 0.5 µM lucitanib and lenvatinib on 376 wild-type kinases in the presence of 10 µM ATP. Sunitinib was previously profiled under similar conditions,[9] and those results are used for comparison. To define IC_{50} values for lucitanib and lenvatinib against kinases that were inhibited by ≥50% at 0.5 µM, kinase inhibition was determined at 10 doses using 3-fold serial dilutions starting at 1 or 10 µM in the presence of 10 µM ATP. Kinases with a lucitanib IC_{50} <100 nM are shown.

Cell-based kinase assays: Cell lines, which exogenously (MEF-VEGFR3 [VEGFR3], MEF-SRC [SRC]) or endogenously (HUE [VEGFR2], NIH3T3 [PDGFRβ], KatoIII [FGFR2], M07e [c-KIT], and A431 [EGFR]) express high levels of the indicated tyrosine kinases, were cultured according to manufacturer's instructions. HUE, A431, M07e, and NIH3T3 cells were incubated in growth factor-deficient media overnight. All cell lines were treated with serial dilutions of lucitanib or lenvatinib in serum-free media for 1.5 hours. HUE, NIH3T3, M07e, and A431 cells were then stimulated for 3 minutes with 100 ng/mL VEGF-A, 100 ng/mL PDGF-BB, 100 ng/mL SCF, or 50 ng/mL EGF, respectively. Tyrosine kinase phosphorylation was detected in cell lysates by sandwich ELISA using a substrate-specific capture antibody and an anti-phosphotyrosine detection antibody.

In vivo phospho-VEGFR2 inhibition: BALB/c nude mice (n=4 per group) were treated with vehicle or a 10 mg/kg single dose of lucitanib or lenvatinib by oral gavage. Four hours later, a single IV bolus of 500 ng VEGF-A was administered to the mice. After 5 minutes, the mice were sacrificed and the lungs were collected. Tissue lysates were prepared in RIPA buffer and equal amounts of total protein from each sample were analyzed by western blot to detect levels of VEGFR and phospho-VEGFR.

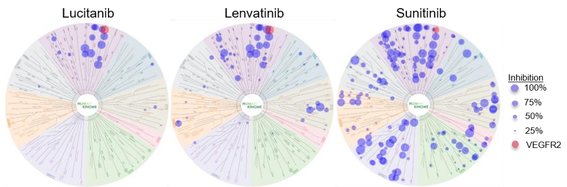
Murine syngeneic in vivo studies: CT26 (2×10^5), H22 (4×10^5), RENCA (2×10^5), 4T1 (4×10^5), and EMT6 (2×10^5) cells were subcutaneously implanted in BALB/c mice. MC38 (1×10^6), LLC1 (1×10^6), and B16F10 (8×10^4) cells were subcutaneously implanted in C57BL/6 mice, and P-BR5FVB1-Akt cells (7.5×10^5, provided by Sandra Orsulic, Cedars-Sinai Medical Center, Los Angeles, CA) were subcutaneously implanted in FVB/N mice. All models had 10 animals per group. Dosing of lucitanib (5-10 mg/kg PO QD), lenvatinib (10 mg/kg PO QD), anti-PD-1 (RPMI1-14; BioXcell) (5-10 mg/kg IP BIW), or the combination of lucitanib or lenvatinib and anti-PD-1 commenced once tumors were ≈50-100 mm^3 in size and continued for 21-38 days. Body weight and tumor volume were measured twice per week. Two-way ANOVA was used to compare tumor growth inhibition and median survival time between groups.

Gene expression profiling: Three syngeneic tumors from P-BR5FVB1-Akt-bearing mice were collected after 14 days of treatment with lucitanib (10 mg/kg PO QD), anti-PD-1 (RPMI1-14; BioXcell) (10 mg/kg IP BIW), or a combination of both. RNA was isolated from tumors using the RNeasy Kit (Qiagen) and analyzed using the NanoString nCounter Mouse PanCancer Immune Profiling Panel. Two-way ANOVA was used to compare cell type scores and gene expression changes between groups.

ANOVA, analysis of variance; ATP, adenosine triphosphate; BIW, biweekly; c-KIT, tyrosine-protein kinase Kit; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; ELISA, enzyme-linked immunosorbent assay; FGFR, fibroblast growth factor receptor; HUE, spontaneously immortalized human umbilical vein endothelial cell clone; IC_{50}, half maximal inhibitory concentration; IP, intraperitoneal; IV, intravenous; MEF, mouse embryonic fibroblasts; MST, median survival time; PDGF, platelet derived growth factor; PDGFR, platelet derived growth factor receptor; PD-1, programmed cell death receptor 1; PD-L1, programmed death-ligand 1; PO, oral; QD, daily; RIPA, radioimmunoprecipitation assay; SCF, stem cell factor; SD, standard deviation; SEM, standard error of the mean; SRC, proto-oncogene c-Src; TGI, tumor growth inhibition; TIL, tumor infiltrating lymphocyte; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

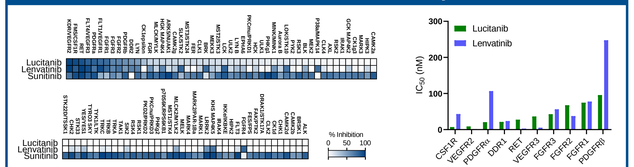
RESULTS

Lucitanib Is a Selective Angiogenesis Inhibitor



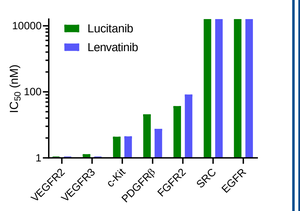
Whole kinome profiling (in ≥300 kinases) was performed using the indicated compounds. The results are displayed in Kinase Mapper plots, where the size of each spot reflects percent inhibition.

Lucitanib Exhibits Potent Kinase Inhibition Activity



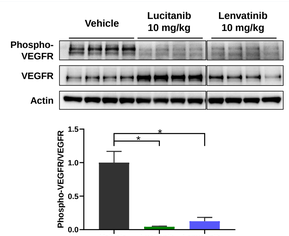
A heatmap depicting percent inhibition of kinases inhibited by ≥50% with 0.5 µM of at least 1 of the 3 inhibitors is shown (left). A bar graph compares lucitanib and lenvatinib IC_{50} values for kinases where the IC_{50} of lucitanib is ≤100 nM (right).

Lucitanib Inhibits In Vitro Cellular Phosphorylation



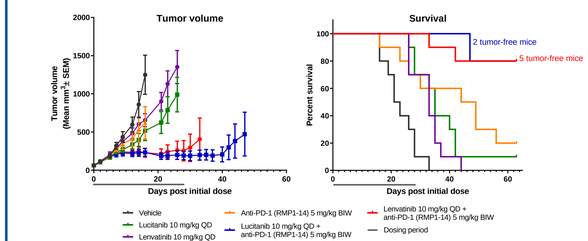
Cell-based phosphorylation assays were performed in the presence or absence of lucitanib or lenvatinib to determine the potency of each compound for inhibiting phosphorylation of the indicated kinases. Kinase phosphorylation was detected by ELISA from purified cell lysates.

Lucitanib Blocks VEGF Signaling In Vivo



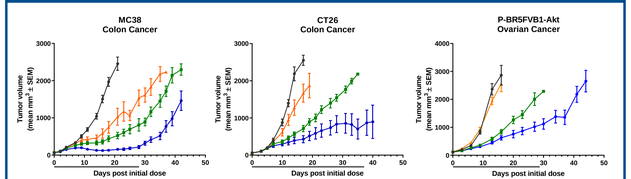
Mice were administered a single oral dose of the indicated compounds. Four hours later, mice were stimulated with VEGF-A for 5 minutes and lung tissues were collected for Western blot analysis (top). Ratios of phospho-VEGFR protein to total VEGFR protein detected are shown (bottom) (*P<0.05).

Anti-PD-1 Significantly Increases Tumor Growth Inhibition and Survival Effects of Lucitanib and Lenvatinib In Vivo



The effect on tumor growth (left) and survival (right) of single-agent lucitanib, lenvatinib, or anti-PD-1 compared with the combination of anti-PD-1 and lucitanib or lenvatinib was tested in H22 tumor bearing mice (n=10/group).

Lucitanib in Combination with Anti-PD-1 Demonstrates Antitumor Activity in Several Syngeneic Models

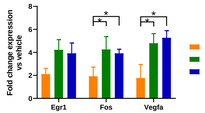


	Combination responder				Combination nonresponder				
Model	H22	MC38	CT26	P-BR5FVB1-Akt	LLC1	EMT6	RENCA	4T1	B16F10
Cancer type	Liver	Colon	Colon	Ovarian	Lung	Breast	Renal	Breast	Melanoma
Efficacy									
TGI anti-PD-1 (%)	48	64	43	19	6	5	0.6	0	-4
TGI lucitanib (%)	57	80	76	79	40	72	85	60	77
TGI combo (%)	81	97	84	85	47	68	90	55	81
P value	0.009	<0.0001	0.03	0.016	0.38	0.4	0.33	0.058	0.46
Survival									
MST vehicle (days)	22	19.5	14	29	16	16	21	20	14
MST anti-PD-1 (days)	46.5	29	19	43	16	17.5	19	19	14
MST lucitanib (days)	35	39	32	30.5	21	30	42	29.5	25
MST combo (days)	NR >63	45	40	55	21	31.5	43.5	31	26
P value	0.0067	<0.0001	0.0033	0.0004	1	0.31	0.069	0.17	0.58

In vivo assessment of the antitumor activity of lucitanib, anti-PD-1, or the combination in 9 syngeneic tumor models. Mean tumor volume over time is shown for combination responsive models (top). Efficacy and survival data for the 9 models evaluated are shown in the table (bottom). Statistical analysis comparisons between groups were performed using 2-way ANOVA. *P* values shown here are for the comparison of TGI or MST of the combination group vs the single-agent (lucitanib or anti-PD-1) group with the greatest TGI or MST, respectively.

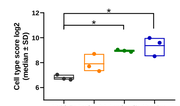
Lucitanib Plus Anti-PD-1 Enhances Immune Activity in a Syngeneic Ovarian Cancer Model

Increased Hypoxia-Related Gene Expression



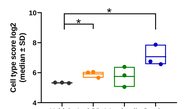
Syngeneic ovarian tumors treated for 14 days with lucitanib or the combination showed increased expression of hypoxia-related genes by NanoString analysis (*P<0.05).

Increased Dendritic Cells



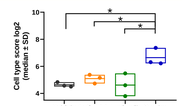
Increased dendritic cell numbers were seen in tumors treated for 14 days with lucitanib or the combination (*P<0.05).

Increased Tumor Infiltrating Lymphocytes



TILs were significantly increased in tumors treated for 14 days with the combination compared with those treated with the single agents (*P<0.05).

Increased Cytotoxic Cells



Cytotoxic immune cells were significantly increased in tumors treated for 14 days with the combination compared with those treated with the single agents (*P<0.05).

CONCLUSIONS

- In vitro enzymatic and cell-based kinase profiling show that lucitanib is a potent and selective inhibitor of receptor tyrosine kinases associated with angiogenesis, proliferation, survival, immune response, and metastasis
  - Lucitanib inhibits the enzymatic activity of VEGFR2 and CSF1R (IC_{50} <10 nM), as well as VEGFR1, VEGFR3, PDGFRα/β, DDR1, RET, and FGFR1-3 (IC_{50} <100 nM)
- The combination of lucitanib and anti-PD-1 enhances the antitumor activity of either single agent in multiple syngeneic mouse models
  - The mechanism of action is through both antiangiogenic effects and immunomodulatory effects on dendritic cells and T cells
- Lucitanib exhibits similar selectivity and activity to lenvatinib, which has demonstrated clinical activity in combination with pembrolizumab[7-8,10-12]
- These data provide preclinical support for planned clinical studies evaluating lucitanib in combination with PD-1/PD-L1 checkpoint inhibitors

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