Amuvatinib (MP-470), a multi-targeted tyrosine kinase inhibitor and DNA repair suppressor, synergizes with Etoposide (VP-16) in Small Cell Lung Cancer (SCLC) cell lines and xenografts

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Background

- Amuvatinib is an orally bioavailable multi-targeted tyrosine kinase inhibitor specifically designed to be a potent inhibitor of mutant c-Kit and PDGFRα.
- Amuvatinib also decreases Rad51-mediated homologous recombination DNA repair and increases cancer cells' chemo- (Bristow et al., Mol Cancer Ther 2009;8(12 Suppl.): A122) and radio-sensitivity (Welsh et al., Radiat Oncol. 2009;4:69).
- In a Phase 1b clinical study of amuvatinib in combination with Etoposide (VP-16) + Carboplatin, responses were observed in Small Cell Lung Cancer (SCLC) (Tolcher et al., IASLC 13th World Conference on Lung Cancer, abst. 7936)
- To support a Phase 2 clinical study, we evaluated the effects of amuvatinib as single agent and in combination with VP-16 and carboplatin in a panel of 5 SCLC cell lines.
- Efficacy of amuvatinib + VP-16 combination was also studied in SCLC NCI-H146 xenografts.

Methods

- Viability of 5 SCLC cell lines (LB12-SCLC/OC2, LB13-SCLC/OC3, NCI-H146, NCI-H69 and NCI-H82) after treatment with amuvatinib, VP-16 and carboplatin as single agents or in combination was evaluated using the MTS assay and combination index (CI) was determined after simultaneous treatment for 72 hrs.
- Modulation of cell signaling pathways after amuvatinib treatment was evaluated in these SCLC cell lines and xenografts by Reverse Phase Protein Array (RPPA) and Western blot.
- Tumor growth inhibition after administration of amuvatinib and VP-16 was evaluated in NCI-H146 xenografts established in Swiss nude mice.

Results

- All 5 SCLC cell lines tested were sensitive to amuvatinib with LB12-SCLC/OC2 being the most sensitive (IC_{50}=4.79 μM).
- When amuvatinib and VP-16 were combined, effects produced were generally additive or synergystic (on 3 of the 5 cell lines tested)
  - Synergism was observed in NCI-H146 (Combination Index=0.68±0.18).
- When amuvatinib and VP-16 were combined to carboplatin, significant synergism was again evident in NCI-H146 (CI=0.72±0.12) and additivity was observed in NCI-H69 and LB12-SCLC/OC2.
- RPPA analysis of cell extracts showed a significant dose and time dependent modulation of phospho-S6 and phospho-4EBP1 after amuvatinib treatment.
- *In vivo* PO administration of amuvatinib in combination with IV VP-16 in NCI-H146 tumor-bearing mice at well tolerated doses and regimens produced a sustained reduction in T/C ratio < 39%.
- Modulation of Akt and 4EBP1 phosphorylation was observed in tumor extracts prepared from NCI-H146 xenografts after treatment with amuvatinib.

SCLC Cell Line Sensitivity

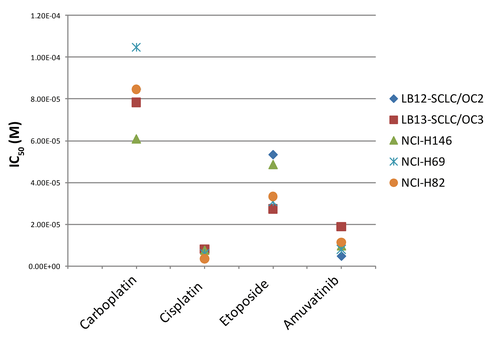
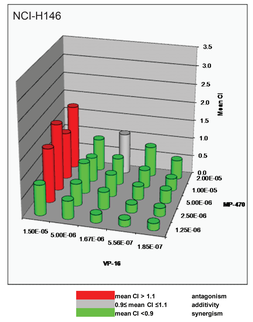


Figure 1. Sensitivity of five SCLC cell lines to amuvatinib

In Vitro Synergy



- Median Combination Index
- 3 different determinations
- 25 different combinations (5x5)

Cell Line	Median Combination Index
LB12-SCLC/OC2	1.08; 0.98; 0.98
LB13-SCLC/OC3	1.13; 4.24; 3.71
NCI-H146	0.94; 0.57; 0.53
NCI-H69	1.17; 1.04; 0.93
NCI-H82	1.27; 0.82; 0.96

Synergism in NCI-H146 also after VP-16 + MP-470 + Carboplatin.

Figure 2. *In vitro* synergy of amuvatinib and VP-16 in SCLC cell lines.

Reverse Phase Protein Array

- Treatment: amuvatinib 20-1.3μM
- Cell harvested at 24, 48, and 72 hr.

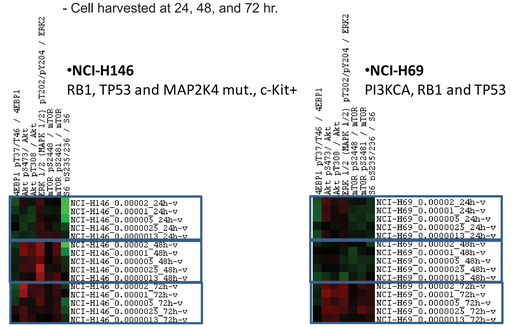


Figure 3: Reverse phase protein array (RPPA) of SCLC cell extracts after amuvatinib treatment. Data represented as ratio phospho/total protein - vehicle. Phospho-S6 and Phospho-EBP1 decrease appears to be more sustained in NCI-H146.

Rad51 Protein Reduction

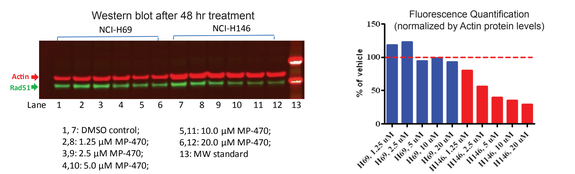


Figure 4: Rad51 protein reduction after amuvatinib treatment in SCLC cell lines

In Vivo Efficacy

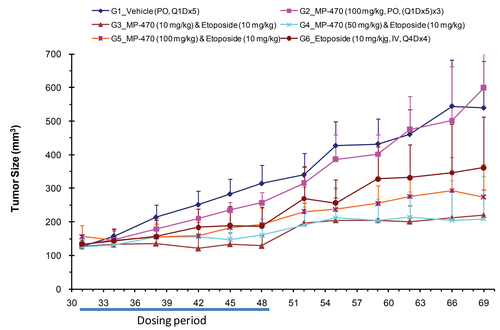


Figure 4: *In vivo* efficacy of amuvatinib + VP-16 in SCLC NCI-H146

Conclusions

Preclinical evidence supports a Phase 2 combination strategy of amuvatinib with VP-16 in SCLC.

- *In vitro*: 3 out of 5 SCLC cell lines amuvatinib + VP-16 → additive or synergistic effects
  - Synergism in NCI-H146
  - Synergism in NCI-H146 also after amuvatinib + VP-16 + Carboplatin
- *In vivo*: NCI-H146 xenograft tumors showed better in vivo antitumor activity after amuvatinib + VP-16 than after VP-16 alone
  - Exposures observed in mice were shown to be achievable in clinical studies
- Clinical Phase 2 study in SCLC with amuvatinib in combination with VP-16 + Platinum is being planned

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