Effects of combining amuvatinib (MP-470) with DNA-damaging agents in osteosarcoma cell lines

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Updated Abstract

Background: Although a rare disease, osteosarcoma (OS) primarily affects individuals 10 to 30 years old. Surgery, combined with chemotherapy, is effective in localized disease, but five-year survival rates in the metastatic setting are less than 20%. New systemic therapeutic approaches for metastatic OS are desperately needed. Amuvatinib is an orally bioavailable, multi-targeted tyrosine kinase inhibitor that inhibits mutant c-KIT and PDGFR–α. Amuvatinib also decreases levels of the DNA repair protein Rad51. We explored the ability of amuvatinib to sensitize OS cells to agents that may cause DNA damage.

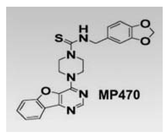
Materials and Methods: OS cell lines (U2-OS, M189, and P16T) were treated with amuvatinib alone or in combination with DNA-damaging agents (doxorubicin, melphalan, etoposide, and topotecan). After 72 hours, cell viability was measured using the Cell Titer 96 cell proliferation assay. Combination Index (CI) values were calculated to determine synergism, antagonism, or additivity for the various combinations. Western blot analysis was used to assess alterations in Rad51 protein levels in response to amuvatinib treatment.

Results: U2-OS exhibited relative resistance compared to M189, and P16T for all drugs tested. Amuvatinib/doxorubicin was synergistic in both U2-OS (CI=0.58) and P16T (CI=0.83), but antagonistic in M189 (CI=1.59). Amuvatinib/melphalan was antagonistic in both U2-OS (CI=1.5) and P16T (CI=2.0) cells, but additive in M189 (CI=0.99). Amuvatinib/etoposide was synergistic in both U2-OS (CI=0.61) and P16T (CI=0.78), but additive in M189 (CI=1.07). Finally, amuvatinib/topotecan was synergistic in U2-OS (CI=0.54) and additive in P16T (CI=0.98), but antagonistic in M189 (CI=1.53). Western blot analysis demonstrated that amuvatinib reduced Rad51 protein levels in all three cell lines.

Conclusions: Therapeutic approaches using the combination of amuvatinib with doxorubicin, etoposide, or topotecan may lead to synergistic activity in OS. Additive effects may also have clinical relevance, since amuvatinib may serve as a chemotherapy-sparing agent, reducing the dose-limiting toxicities associated with DNA-damaging agents.

Background

- Amuvatinib is a novel small molecule that modulates a variety of signaling molecules (e.g. c-KIT and PDGFR–α).



- These signaling pathways play important roles in osteosarcoma cell proliferation, survival, and progression.
- Thus, amuvatinib may have significant activity in osteosarcoma.
- Amuvatinib has been previously shown to sensitize cells to DNA-damaging agents by reducing protein levels of the DNA repair molecule Rad51.
- Amuvatinib phase I studies have been completed and Phase II clinical trials are currently being planned.

Project Goal

Our goal is to assess the sensitivity of osteosarcoma cell lines to the combination of amuvatinib and DNA-damaging agents.

Results

Cell Culture: U2-OS was purchased from the American Type Culture Collection and maintained in McCoy's 5A medium. M189 and P16T were obtained from the Children' Hospital of Michigan at Wayne State University and maintained in RPMI 1640. Media were supplemented with 10% fetal bovine serum. Cells were cultured at 37°C in an atmosphere supplemented with 5% CO_2.

Table 1.
The range of calculated IC_{50} for each agent alone

Cell Proliferation Assay:
Cells were seeded in quadruplicate in 96-well plates at a density of 4.0 x 10^3 cells per well for 24 hours followed by incubation with vehicle or drug(s) for 72 hours. Cell viability was measured using the MTS CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay (Promega).

Treatments	U2-OS	M189	P16T
Amuvatinib	34.9 – 100 μM	1.3 – 2.9 μM	1.18 – 3.57 μM
Doxorubicin	250 – 559 nM	64.5 – 91.3 nM	29.2 – 196 nM
Melphalan	32.7 – 50 μM	4.5 – 7.8 μM	1.1 – 1.9 μM
Etoposide	8.8 – 19.2 μM	0.75 – 3.0 μM	0.35 – 1.6 μM
Topotecan	6.5 – 17.3 μM	32 – 86.5 nM	3.3 – 29 nM

Table 2. Summary of the combination index (CI) values for each treatment combination

Treatments	U2-OS	M189	P16T
Amuvatinib + Doxorubicin	*0.58*	1.59	*0.83*
Amuvatinib + Melphalan	1.5	0.99	2.0
Amuvatinib + Etoposide	*0.61*	1.07	*0.78*
Amuvatinib + Topotecan	*0.54*	1.53	0.98

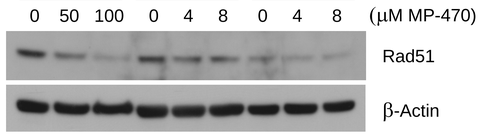
Calculation of Combination Index (CI) values: CI was calculated using the median-effect analysis method of Chou and Talalay.

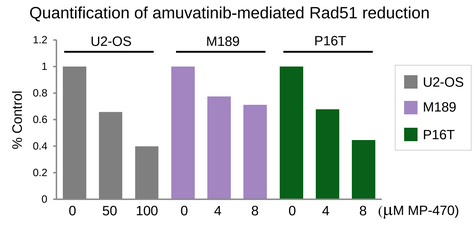
$$CI = \frac{D_1}{(D_x)_1} + \frac{D_2}{(D_x)_2}$$

D_1 and D_2 are doses of drugs 1 and 2 that have x% effect when used in combination, and $(D_x)_1$ and $(D_x)_2$ are doses of drugs 1 and 2 that have the same x% when used alone.

CI < 0.90 Synergistic | CI = 0.9–1.1 Additive | CI > 1.10 Antagonistic

Figure 1.
Amuvatinib reduces Rad51 protein levels in all three OS cell lines





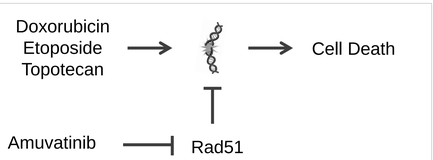
Western Blot Analysis: Cells were seeded in 6-well plates at a density of 0.5 x 10^6 per well for 24 hours, followed by incubation with vehicle or amuvatinib (MP-470) for 72 hours. Cells were rinsed and then scraped in lysis buffer. Lysates were centrifuged for 5 minutes at 13,000 × *g* and quantified using the BCA Protein Assay (Pierce). Equal amounts of proteins (35 μg), quantified using Protein Assay Reagent from Bio-Rad (Hercules, CA) were loaded onto 10% NuPage gels (Invitrogen). Following transfer, blocking, and incubation with primary and secondary antibodies, proteins were visualized by ECL reagents (Perkin-Elmer) and exposed to HyBlot CL films (Denville Scientific). Densitometric quantification of bands was conducted using the Image J software.

Conclusions

- The osteosarcoma cell lines tested exhibit varying degrees of sensitivity to amuvatinib, DNA-damaging agents, and topoisomerase inhibitors.
- Combination of amuvatinib and topoisomerase inhibitors (doxorubicin, etoposide, and topotecan) lead to synergistic activity or additive effects in osteosarcoma cell lines. These effects may have significance in the clinical setting.
- Amuvatinib reduces Rad51 levels in U2-OS, M189, and P16T.

Discussion

- Potential Model for synergistic effects of amuvatinib:



Amuvatinib reduces Rad51, thus sensitizing cells to the effects of DNA-damaging agents and subsequently cell death

- Synergistic and additive effects may have clinical relevance, since amuvatinib may serve as a chemotherapy-sparing agent, reducing the dose-limiting toxicities associated with a particular agent. This may be especially important with doxorubicin, a backbone therapy for OS, but hampered by dose-limiting cardiotoxicity.
- Since amuvatinib reduces Rad51 levels in all 3 cell lines, other mechanisms may be involved in mediating synergism of amuvatinib with DNA-damaging agents and topoisomerase inhibitors.

Future Directions

1. Characterize the effects of combining amuvatinib and the DNA-damaging agents on Rad51 protein levels.
2. Investigate the role of Rad51 on the effects of amuvatinib combinations with other agents.
3. Unravel the crosstalk between signaling mechanisms induced by amuvatinib and other agents (doxorubicin, etoposide, and topotecan).
4. Test amuvatinib drug combinations in a panel of cell lines representing other sarcoma subtypes.

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Acknowledgements

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