The HUH-7 cells were cultured in absence and presence of diferent concentrations of L-744832, MG-262 and everolimus, during 24−96 hours. The antiproliferative effect was assessed by the Alamar Blue assay and cell death by optic microscopy and flow cytometry, upon staining the cells with Annexin V and propidium iodide.
Our results showed that farnesiltransferase, proteasome and mTOR inhibitors had an antiproliferative and cytotoxic effects in monotherapy in a dose and time dependent manner, inducing cell death preferentially by apoptosis. On, the other hand, the combination of MG-262, L-744832 and everolimus with conventional anticarcinogenic drugs demonstrated a higher antiproliferative and cytotoxic effect for lower doses than the IC50 used in monotherapy (addition or potentiation synergism).
These results suggested that farnesiltransferase, proteasome and mTOR inhibitors may constitute a new potential therapeutic approach in HCC either in monotherapy or in association with conventional therapies.

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

S.S. Morgan[1], Z.J. Wang[2], P. Taverna[3], L.D. Cranmer[1]. *[1]University of Arizona, Arizona Cancer Center, Tucson Arizona, USA; [2]Children's Hospital of Michigan, Pediatrics, Detroit Michigan, USA; [3]Supergen Inc, Translational Pharmacology, Dublin California, USA*

Background: Although a rare disease, osteosarcoma (OS) primarily affects individuals 10 to 30 years old. While surgery, combined with chemotherapy, is effective in localized disease, five-year survival rates in the metastatic setting are less than 20%. Systemic therapeutic approaches for metastatic OS are desperately needed. Amuvatinib (MP-470) is an orally bioavailable, multi-targeted tyrosine kinase inhibitor that inhibits mutant c-KIT and PDGFR-α. In addition, MP-470 decreases levels of the DNA repair protein Rad51. Thus, we explored the ability of MP-470 to sensitize OS cells to DNA-damaging agents.
Materials and Methods: Osteosarcoma cell lines (U2-OS and P16T, previously characterized as being of high or low cisplatin-resistance, respectively) were treated with MP-470 alone or in combination with DNA-damaging agents (cisplatin, doxorubicin, and melphalan). 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.
Results: U2-OS exhibited relative resistance compared to P16T for all drugs tested. MP-470/cisplatin was antagonistic in U2-OS (CI = 3.06), but additive in P16T (CI = 1.05). MP-470/doxorubicin was synergistic in both U2-OS (CI = 0.58) and P16T (CI = 0.83). Interestingly, MP-470/cisplatin/doxorubicin was additive in U2-OS (CI = 0.92) but not in P16T (CI = 1.28). MP-470/melphalan was antagonistic in both U2-OS (CI = 1.5) and P16T (CI = 2.0) cells.
Conclusions: Therapeutic approaches using the combination of MP-470 and doxorubicin may lead to synergistic activity in OS. Additive effects may also have clinical relevance, since MP-470 may serve as a chemotherapy-sparing agent, reducing the dose-limiting toxicities associated with DNA-damaging agents. Based on these results, we hypothesize that MP-470 may also positively interact with topoisomerase inhibitors (e.g. etoposide and topotecan) that are used to treat OS.

Treatments	U2-OS	P16T
MP470 + Cisplatin	3.06	1.05
MP470 + Doxorubicin	0.58	0.83
Cisplatin + Doxorubicin	1.09	1.23
MP470 + Cisplatin + Doxorubicin	0.92	1.28
MP470 + Melphalan	1.5	2.0

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Characterization and preclinical development of LY2606368, a second generation Chk1 inhibitor

M. Marshall[1], C. King[1], D. Barnard[1], H. Diaz[1], D. Barda[2], A. Bence[3], E. Westin[3]. *[1]Lilly Research Laboratories, Oncology Research, Indianapolis IN, USA; [2]Lilly Research Laboratories, Medicinal Chemistry, Indianapolis IN, USA; [3]Lilly Research Laboratories, Oncology Business Unit, Indianapolis IN, USA*

Background: Many standard-of-care chemotherapeutics have as their mechanism of action the ability to cause DNA strand damage. In many cases the efficacy of these agents is limited by the ability of the tumor cells to arrest the cell cycle at specific 'checkpoints' in order to repair the inflicted damage. The Chk1 protein kinase is an essential regulator of the intra-S and G2/M DNA damage checkpoints. Genetic and biochemical interference of Chk1 results in an increased sensitivity of tumor cells to the cytotoxic effects of many DNA damaging agents including gemcitabine. LY2606368 was developed to be a second-generation inhibitor of the Chk1 protein kinase for use in combination with standard-of-care DNA damaging agents and as monotherapy.
Results: LY2606368 potently elicited a Chk1-knockout phenotype in cancer cell-lines as indicated by inhibition of DNA synthesis, premature entry into mitosis and mitotic catastrophe. LY2606368 inhibited doxorubicin and gemcitabine-activated Chk1 autophosphorylation in cells with an IC50 of <1 nM and abrogated the doxorubicin-induced G2M checkpoint with an IC50 of 11 nM. The *in vitro* anti-proliferative activity of gemcitabine was significantly increased by the presence of LY2606368 with the GI90 of gemcitabine reduced >50% by 0.3 nM LY2606368. In tumor xenografts, LY2606368 potently inhibited the protein kinase activity of Chk1 with an ED50 of 0.03 mg/kg, following induction by gemcitabine treatment. A single 10 mg/kg dose of LY2606368 blocked intra-tumoral Chk1 activity for 48 hours following gemcitabine administration, increasing DNA damage and abolishing the S and G2/M DNA damage checkpoints. Intermittent dosing of gemcitabine followed 24 hours later by LY2606368 significantly increased tumor growth inhibition over gemcitabine alone in multiple human tumor xenograft models. As a monotherapy, a single 10 mg/kg dose of LY2606368 was sufficient to cause long lasting DNA damage and checkpoint bypass in tumor xenografts. The same dose given BIDx3 followed by four days of rest for three cycles was highly efficacious in multiple tumor xenograft models. LY2606368 is currently in phase I clinical testing.

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Molecular bases of everolimus antiproliferative activity in mantle cell lymphoma

L. Rosich[1], G. Roué[1], S. Xargay[1], M. López-Guerra[1], E. Campo[1], D. Colomer[1]. *[1]Hospital Clinic of Barcelona-IDIBAPS, Hemathopathology Unit, Barcelona, Spain*

Background: Mantle cell lymphoma (MCL) is an aggressive B lymphoid neoplasm genetically characterized by the t(11;14)(q13;q32) leading to the overexpression of cyclin D1, which causes cell cycle deregulation at the G1-S phase transition. As a consequence of its poor response to conventional chemotherapy and relatively short patient survival, new therapeutic strategies are required.
The phosphatidylinositol 3-kinase/Akt/mTOR survival pathway is heavily deregulated in many hematologic malignancies. Interestingly, cyclin D1 cap-dependent translation is under mTOR regulation, thereby making the mTOR inhibition a strategy very attractive for the treatment of MCL. Our aim was to analyze in this model the antitumoral effect of everolimus (RAD001,Novartis), an oral rapamycin derivative.
Methods: The sensitivity to everolimus was analyzed in a set of 12 MCL primary cultures, MCL cell lines and peripheral blood lymphocytes from healthy donors. Cells were treated for up to 72 h with increasing doses of everolimus, followed by cytotoxicity quantification and western blot analysis. Apoptosis induction and drug cytostatic effect were determined by labeling cells with annexinV-FITC/propidium iodide and by MTT proliferation assay, respectively. Cell-cycle repartition was determined by flow cytometry analysis of ethanol-fixed, propidium iodide-labeled nuclei. A human phospho-kinase array was utilized to detect the phosphorylation profiles of 46 kinases in lysates from either untreated or everolimus-exposed (24 h, 5 μM) MCL cells.
Results: Everolimus heterogeneously exerts antitumoral effect on MCL cells while sparing normal cells. This effect was associated with G1 cell-cycle arrest and reduced phosphorylation of the mTOR downstream targets, 4E-BP1 and p70S6K. The phospho-kinase array showed that everolimus efficiently modulated several components of the mTOR signaling pathway, as well as the intracellular energy sensor phospho-AMPKα. Consistently, the combination of everolimus with the AMPKα activator acadesine demonstrated synergistic antitumoral activity in MCL cells.
Conclusions: This study suggests that everolimus is an effective cytostatic agent in MCL cells *in vitro* and provides the bases for its further combination with additional antineoplasic agents in MCL.
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