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Preclinical activity of the poly (ADP-ribose) polymerase (PARP) inhibitor ABT-888 in combination with irinotecan in ovarian and triple negative breast cancers

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ABT-888 is a novel PARP inhibitor that is in phase I/II trials in combination with cytotoxic drugs, exploiting the principle of chemical synthetic lethality. Our institution is conducting a phase I of ABT-888 in combination with the topoisomerase I (topoI) poison irinotecan (CPT11). To establish response markers and identify target tumors for subsequent phase II studies, we have determined single agent and combination activity in a panel of 23 cell lines that were well characterized for DNA repair enzymes including PARP1 levels, γ-H2AX, topoI and ERCC1 expression; BRAC1/2 as well as p53 mutations. The methyltetrazolium assay was used to determine in vitro growth inhibition. In the combination studies, ABT-888 was added at a fixed concentration of 500 nM to increasing CPT11 doses. Molecular response analyses were done by quantitative real-time PCR and immunofluorescence staining. Human tumor xenografts established in nude mice were used to model the clinical treatment regimen and correlative endpoints. CPT11 was administered at 40 mg/kg i.v. on days 1, 8; ABT-888 was given at 5 mg/kg/d on days 3−15. Proliferation data showed that breast (n = 8) and ovarian (n = 3) cancer cell lines were the most sensitive to single agents and the combination. In particular, 4 triple negative breast cancer (TNBC) cell lines with BRCA1 mutations (MX1, HCC1937, SUM149, SUM1315) exhibited inhibitory concentrations 50% (IC50) for ABT-888 of 0.1−20 μM. The TNBC lines MDA-MB-231 and SUM159 with wild type BRCA1 were not sensitive. The ovarian line A2780, its cisplatin resistant subclone ADDP and BRCA2$^{+/-}$ IGROV-1 cells, showed also single agent ABT-888 activity but with IC50s between 17−60μM. CPT11 activity was enhanced by ABT-888 in all TNBCs, the A2780 and ADDP ovarian lines. The latter two were chosen for xenograft experiments. In A2780, neither single agent nor the combination showed significant tumor growth inhibition compared to control. ADDP xenografts were responsive to the ABT-888/CPT11 combination with marked tumor growth inhibition of 60% ($p < 0.05$). The single agents were not significantly active. Tumor biopsies taken from experimental groups at 24 and 4 hrs after treatment revealed that the combination strongly induced γ-H2AX foci as well as PARP mRNA in ADDP; in A2780 PARP and ERRC1 levels were increased. Together our data suggest that triple negative breast cancers and cisplatin resistant ovarian tumors might benefit from single agent ABT-888 and its combination with CPT11.

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Sorafenib effectively controls early-stage hepatocellular carcinoma but not visceral metastases in a preclinical orthotopic transplant model

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Background: In 2007, the small molecule receptor tyrosine kinase inhibitor sorafenib was FDA-approved for the treatment of advanced hepatocellular carcinoma (HCC). In contrast, little is known about the efficacy of adjuvant sorafenib for early stage HCC. As HCC is an intrinsically chemotherapy-resistant malignancy and as most patients suffering from HCC have reduced liver function thus not tolerating conventional chemotherapy, the impact of sorafenib-based regimens for this malignancy in earlier stages of disease progression may be important as a means to improve the clinical management of this highly lethal malignancy.
Methods: The human HCC cell line Hep3B was transfected with a hCG.pIRES vector and β-hCG expressing variants were obtained by puromycin selection. Analysis of β-hCG expression enables in vivo monitoring of relative tumor burden. Cells were orthotopically injected into the right lower lobe of the liver in a total of 50 CB17 SCID mice. Control vehicle or Sorafenib (15 or 30 mg/kg) was administered by daily gavage starting either immediately after wound healing (day 7) before circulating β-hCG was detected or after evidence of established tumors as determined by β-hCG analysis (days 14−21). Monitoring was carried out by analysis of β-hCG secretion, survival analysis and endpoint necropsy. Tissue was preserved for immunohistochemistry.
Results: All control animals needed to be sacrificed within 65 days due to primary tumor burden and ascites. No animal of this group showed local or distant metastasis. In contrast, all four dosing regimens of sorafenib significantly inhibited primary tumor growth, inhibited the formation of ascites and prolonged overall survival. However, possibly as a result of the prolonged survival, 56% (19/34) of the animals treated with sorafenib developed local, mesenteric and omental lymph node metastasis and 21% (7/34) developed secondary liver metastases. Metastatic cell lines were re-adapted to cell culture for future analysis.
Conclusions: Sorafenib prolongs survival and successfully controls primary tumor growth in an orthotopic model approximating early-stage HCC. However, it does not inhibit the development of secondary liver metastases or local and distant lymph node metastasis. The nature of these secondary growths will be addressed in follow-up experiments. Future analyses will also include adjuvant therapy of microscopic metastases following resection of the primary. Furthermore, experiments will be repeated using MHCC97-H as a second HCC cell line. Results of this ongoing study will be presented at the conference.

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E-3810, an inhibitor of the VEGF and FGF family receptors, inhibits the FGF-dependent growth of tumor cells

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Fibroblast growth factors (FGFs) and their cognate receptors (FGFRs) regulate fundamental development pathways and have recently became an interesting cancer therapeutic target. Compelling experimental evidence indicates that deregulated FGF/FGFR signalling is implicated in the pathogenesis of several tumors by directly driving cancer cell proliferation and survival and by promoting tumor angiogenesis. E-3810 is a novel small molecule that, in biochemical assay, selectively inhibits VEGFR1−3 and FGFR-1 tyrosine kinases with IC_{50} <30 nM; at higher nM concentrations it also inhibits FGFR-2. The compound has shown potent antitumour activity in human xenografts as well as strong antiangiogenic effects. Its effect on FGF-dependent tumour growth is being investigated. The mRNA expression of FGFR-1 and -2 was evaluated in a panel of 21 human tumor cell lines (9 ovarian, 9 breast, 1 hepatic and 2 prostate) by RT-PCR and levels were expressed as fold increase over the values obtained in human umbelical endothelial (HUVEC) cells. Overall, their expression levels were heterogeneous (ranging from 0.01 to 44 and from undetectable to 1107 fold over HUVEC for FGFR-1 and FGFR-2 respectively) and not correlated to each other. E-3810 cytotoxic activity was in the μM range when cells were grown in complete culture medium and no correlation to the expression level of FGFR-1 or FGFR-2 was seen. We then tested the FGF dependency of cell growth by culturing cells in FBS deprived medium with or without the FGF ligand. The addition of FGF stimulated growth in three out of the seven cell lines tested to date, without a clear relationship to the level of FGFR-1 and/or FGFR-2; the stimulatory effect of FGF ligand on cell growth was antagonized by E-3810 treatment. These data suggest that E-3810, in addition to its anti-angiogenic action, could have a direct antitumor effect in those tumors whose growth and survival depend on FGFR/FGF pathway activation. *In vitro* and *in vivo* experiments are ongoing to corroborate this hypothesis.

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New targeted therapies strategies in hepatocellular carcinoma − In vitro studies

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Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide, often diagnosed at an advanced stage when the most potentially curative strategies are no longer effective. Advances in the understanding of tumor biology are opening new paths for the prevention and treatment of HCC, through the development of new targeted therapies. Hepatocarcinogenesis is a multistep process and current evidence indicates that, in HCC development, both genetic and epigenetic mechanisms are involved contributing to alteration of numerous signaling pathways leading to deregulated cell proliferation and resistance to cell death. Despite the lack of profound understanding of the molecular mechanisms involved in liver carcinogenesis, the design of drugs that block different growth-promoting pathways, activate apoptotic pathways or modulate epigenetic mechanisms, as well as the combination of different targeted therapies, may also open new horizons in the treatment of HCC. The aim of this study was to test the efficacy of new targeted drugs involved in signalling pathways (survival and apoptotic), such as, farnesiltransferase (L-744832), proteasome (MG-262) and mTOR inhibitors (everolimus) in a HCC cell line (HUH-7 cells).

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

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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

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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

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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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