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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

N. Nechiporchik[1], K. Lieb[1], L. Marquette[1], L. Polin[1], G.J. Peters[2], A. Chen[3], S.P. Ethier[1], P.M. LoRusso[1], A.M. Burger[1]. [1]*Karmanos Cancer Institute, Oncology, Detroit Michigan, USA;* [2]*Vrije Universiteit Medical Center, Medical Oncology, Amsterdam, The Netherlands;* [3]*National Cancer Institute, Cancer Therapy Evaluation Program, Bethesda Maryland, USA*

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

C. Hackl[1], S. Man[1], P. Xu[1], R.S. Kerbel[1]. [1]*Sunnybrook Health Sciences Centre, Molecular and Cellular Biology, Totonto, Canada*

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

G. Colella[1], G. Damia[2], M. D'Incalci[2], R. Cereda[1], E. Cavalletti[1], S. Spinelli[1], G. Camboni[1]. [1]*E.O.S., S.p.A, Milano, Italy;* [2]*Istituto di Ricerche Farmacologiche "Mario Negri", Oncology, Milano, Italy*

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

A.M. Araújo[1], S. Sousa Neves[2], A.L. Ferreira[1], J.D. Branco[1], A.B. Sarmento Ribeiro[2], J.M. Nascimento Costa[3]. [1]*University of Coimbra, Faculty of Medicine, Coimbra, Portugal;* [2]*Faculty of Medicine University of Coimbra, Center of Investigation on Environment Genetic and Oncobiology − CIMAGO, Coimbra, Portugal;* [3]*Faculty of Medicine/CIMAGO and Medicine Service and Hepatology Unity, Faculty of Medicine and University Hospital of Coimbra, Coimbra, Portugal*

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).