downstream substrates of TORC2. Expression levels of cell cycle regulating proteins and activation status of related pathways were also characterized. In mouse xenograft model, INK128, rapamycin and Nexavar show strong activity in inhibiting tumor growth, however, differ in the mechanisms underlying their anti-tumor activity. INK128 inhibits phosphorylation of AKT, NDRG1, S6 and 4EBP1; rapamycin only inhibits S6 phosphorylation and induces AKT phosphorylation whereas Nexavar has little effect on the PI3K/AKT/mTOR pathway. INK128 induced autophagy and decreased cyclin D1 expression. INK128 and rapamycin both inhibit expression of HIF-1a and VEGF which contributes to their anti-angiogenesis activity. We conclude that rapamycin and Nexavar exert anti-tumor activity mainly by attacking the tumor microenvironment while the anti-tumor effect of INK128 is derived from both cell-autonomous (direct inhibition of tumor cell growth) and non-autonomous (anti-angiogenesis) activities. Combination of INK128 with Nexavar displayed enhanced activity in RCC tumor model. In preclinical tumor model, INK128 induced dose-dependent pharmacodynamic inhibition of phosphorylation of S6, AKT and 4EBP1 in PBMCs, skin and tumor tissue at plasma concentrations leading to tumor growth inhibition.

Conclusion: In summary, INK128, a potent, selective, and orally active dual TORC1/2 inhibitor demonstrated anti-tumor activity in preclinical models of RCC by a distinct mechanism. INK128 is currently being studied in a phase I clinical trial.

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Pleiotropic stromal effects of VEGFR2 antibody therapy in renal cell carcinoma models

I. Duignan[1], E. Corcoran[1], A. Pennello[1], M.J. Plym[1], J.D. Schwartz[2], H. Youssoufian[2], L. Witte[1], D. Surguladze[1], J.R. Tonra[1]. *[1]ImClone Systems a wholly-owned subsidiary of Eli Lilly and Company, Research, New York NY, USA; [2]ImClone Systems a wholly-owned subsidiary of Eli Lilly and Company, Clinical Science Investigation, New York NY, USA*

The benefits of targeting vascular endothelial growth factor A (VEGF) signaling in cancer patients are frequently attributed to effects on tumor endothelial cells. Targeting non-endothelial cell stromal components to further impact tumor cell growth and survival is being pursued through combination strategies or through cross-reactivity of VEGF receptor targeting small molecules with other growth factor receptors important for the survival and/or proliferation of non-endothelial stromal cells. However recent data points to the potential for targeting lymphatic vessels and pericytes, in addition to blood vessels, with VEGF specific targeting agents. Here in fact we demonstrate the ability of 7 days treatment with an antibody specific to one of the VEGF receptors, VEGFR2 (DC101, 40 mg/kg IP, 3×/wk), to significantly reduce CD31 expressing blood vessels, α-smooth muscle actin (αSMA) positive pericytes and LYVE-1 expressing lymphatic vessels in the tumor stroma of subcutaneous (SKRC-29) and orthotopic (786-O-LP) models of renal cell carcinoma (RCC), a cancer for which VEGF targeted therapy has known efficacy. DC101 decreased CD-31 positive blood vessel density by 72% in the SKRC-29 model and 78% in the 786-O model ($p < 0.001$ for all comparisons versus control). LYVE-1 and αSMA positive vessel like structures were reduced by 58% and 37%, respectively, in the SKRC-29 model, and 73% and 60% in the 786-O-LP model ($p < 0.05$ for all). Sunitinib (40 mg/kg PO, daily), a tyrosine kinase inhibitor targeting VEGFR2 and several other growth factor receptors, also caused a significant loss of tumor blood vessels in the SKRC-29 and 786-O-LP models (63% and 61% decrease, respectively) after 7 days of therapy, but had a weaker effect than DC101 on LYVE-1 and αSMA staining density at the doses utilized; 24 and 9% reduction in the SKRC-29 model, respectively, and 45 and 23% reduction in the 786-O-LP model. Our data have important implications for combination therapy design, supporting the conclusion that targeting VEGFR2 alone in renal cell carcinoma has the potential to have pleiotropic anti-cancer effects on the tumor stroma.

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Angiogenesis-related gene profiles with predictive value in advanced ovarian carcinoma (AOC)

M. Mendiola[1], J. Barriuso[2], A. Redondo[2], I. Sánchez-Navarro[1], M. Miguel-Martín[1], R. Madero[3], A. Hernández[4], E. Espinosa[2], J. De Santiago[4], D. Hardisson[5]. *[1]La Paz University Hospital IdiPAZ, Pathology and Oncology Lab, Madrid, Spain; [2]La Paz University Hospital IdiPAZ, Medical Oncology, Madrid, Spain; [3]La Paz University Hospital IdiPAZ, Biostatistics Unit, Madrid, Spain; [4]La Paz University Hospital IdiPAZ, Obstetrics and Gynecology, Madrid, Spain; [5]La Paz University Hospital IdiPAZ, Pathology, Madrid, Spain*

Background: Multimodal therapy with cytoreductive surgery plus chemotherapy is the standard of care in AOC. Parameters such as age, extent of residual disease after surgery, and the histopathological subtypes are imperfect predictors of response. Several genes involved in angiogenesis proved to have prognosis capacity in advanced ovarian carcinoma. The aim of this study is to build profiles with a predictive value of response to treatment in patients of AOC derived from genes involved in angiogenesis.

Materials and Methods: 61 patients with III/IV FIGO stage ovarian cancer who underwent surgical cytoreduction and received a carboplatin plus paclitaxel regimen were included. Clinical response was evaluated using CT after the completion of multimodal therapy. A second look laparotomy was performed in 34 of them, defining optimal debulking as $\leqslant 1$ cm (diameter) residual disease. RNAs were collected from formalin-fixed paraffin-embedded AOC samples. Expression levels of 82 angiogenesis related genes were measured using quantitative real time polymerase chain reaction. A logistic regression method was used to build multiple models based on the significant genes in the univariate analysis. The accuracy of the models was evaluated using Receiver Operating Characteristic (ROC) curves. The Akaike Information Criterion based selection was used to find the most accurate one. And Leave-one-out Cross Validation (LOOCV) method was applied to avoid overoptimistic predictions.

Results: All patients had advanced disease (FIGO stages III/IV). Most of them had FIGO stage III (51, 83.6%), grade 3 tumors (35, 57.4%), and serous histology (42, 68.9%). Two different predictive models were generated for clinical or pathological response to treatment. The first one, predictor of clinical response, comprises 8 genes with an AUC of 0.955 ($p < 0.001$). Leave-one-out cross validation was applied to avoid overfitting of the model, obtaining a corrected AUC of 0.880, 95%IC: 0,776−0,985. The second one, for pathological response, comprising 5 genes with an AUC of 0.950 ($p < 0.001$). When LOOCV was applied, a corrected AUC of 0.846, 95%IC: 0,717−0,976 was obtained.

Conclusions: It is feasible to identify gene expression profiles related to angiogenesis with predictive value for response in AOC. However, their predictive value should be further evaluated in prospective studies of patients with AOC.

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Restoration of paracrine signalling within the tumour microenvironment increases tumour growth and activation of c-Met

R. Argent[1], R. Kumari[1], P. Clarke[2], D. Onion[1], D. Lobo[3], S. Watson[1], A. Grabowska[1], P. King[4], B. Hall[4], M. Page[4]. *[1]University of Nottingham, Ex vivo Pharmacology Centre Division of Pre-Clinical Oncology, Nottingham, United Kingdom; [2]University of Nottingham, Division of Pre-Clinical Oncology, Nottingham, United Kingdom; [3]University of Nottingham, Division of Surgery, Nottingham, United Kingdom; [4]OrthoBiotech, Oncology Research & Development, Beerse, Belgium*

Introduction: Paracrine signalling between tumour epithelial cells and the stroma in the tumour microenvironment is a pre-requisite for tumour growth and may determine responsiveness to therapeutic inhibition. Hepatocyte growth factor (HGF) and the HGF receptor, c-Met, are reported to be up-regulated in colorectal liver metastases (CRLMs) and considered to be markers of metastatic potential. They also involved promote epithelial–mesenchymal transition (EMT), an important developmental process which is frequently activated in carcinogenesis resulting in metastatic spread.

Methods: CRLMs ($n = 27$) were grown as sub-cutaneous xenografts in nude mice, and subsequently treated with c-Met inhibitors. Tissues were used for RNA extraction and quantitative PCR analysis, were enzymatically disaggregated to isolate epithelial and mesenchymal cells, and were used for immunohistochemistry.

Results: Analysis of primary human CRLMs showed that c-Met was overexpressed and accompanied by decreased E-cadherin, and increased EMT-related gene expression. Enrichment of individual cell types from primary CRLMs showed that HGF was mesenchymal, whereas c-Met was epithelial. Growth of tumours as sub-cutaneous xenografts revealed that human stroma, and thus paracrine signalling, was rapidly lost, and treatment of tumour-bearing mice with c-Met inhibitors had a generally poor response. Co-implantation of primary CRLM tumour epithelial cells with tumour-conditioned mesenchymal cells increased tumour growth, decreased necrosis, and increased c-Met activation. Implantation of tumour-associated fibroblasts into xenografts was also found to increase response to a c-Met inhibitor and to standard of care agents.

Conclusions: Paracrine HGF:c-Met signalling is rapidly lost in CRLM xenograft models indicating the need to restore human stroma, and its loss may reflect the poor response to inhibition. Recapitulation of the human tumour microenvironment in xenografts by implantation of human mesenchymal cells improves the therapeutic response, and therefore provide improved models to assess drug efficacy.