PP 59

Two methods for labeling tyrosine kinase inhibitor sorafenib with carbon-11 to obtain a PET tracer for personalized cancer treatment

A. Poot, B. Van der Wildt, M. Stigter-van Walsum, R. Schuit, G. Van Dongen, A. Windhorst. *VU University Medical Center, Amsterdam, The Netherlands*

Background: Receptor tyrosine kinases play a pivotal role in the signal transduction of vital processes of the cell. Uncontrolled activation of these receptors is often related to tumor formation. Therefore, tyrosine kinases have become important drug targets in the treatment of cancer. Although several TKIs have demonstrated clinical value, most TKIs developed are only effective in a small subset of patients. In our center, PET imaging with radiolabeled targeted drugs is used as quantitative imaging strategy to speed up drug development and to facilitate patient selection. The aim of the present study was to develop radiolabeled [11C]sorafenib, to be used for the selection of patients who might benefit from treatment. Sorafenib was labeled at two positions to obtain [11C]methyl sorafenib or [11C]urea sorafenib. Both compounds are preclinically evaluated for there tumor targeting properties as well as their active metabolites.
Materials and Methods: [11C]methyl sorafenib was synthesized by reacting desmethyl sorafenib with [11C]MeI in a solution of TBAOH in DMF at elevated temperature. For the synthesis of [11C]urea sorafenib, a Rhodium promoted reaction between the corresponding azide and amine was performed at elevated pressure and temperature in the presence of [11C]CO. the crude mixtures were purified by HPLC and the isolated product fraction was formulated by solid phase extraction in ethanol and 2.5% polysorbatum in 0.9% saline solution (1:9 v/v).
Results: [11C]methyl sorafenib was synthesized in a decay corrected yield of 60% and [11C]urea sorafenib was synthesized in a decay corrected yield of 27%. Using analytical HPLC, the radiochemical purity of both products was determined to be higher than 99% and the identity of the product was confirmed by coinjection of the labeled product with reference sorafenib. A metabolite analysis in rats, revealed that the percentage of intact product in blood-plasma samples after 45 minutes are up to 90% for [11C]methyl sorafenib, and 96% for [11C]urea sorafenib, respectively.
Conclusion: Reliable labeling procedures were developed for the synthesis of [11C]methyl sorafenib and [11C]urea sorafenib. Both labeled products were obtained in a high yield and purity. Furthermore, both products have a high metabolic stability in rats and its tumor targeting properties are currently tested in renal cell carcinoma xenografts.

PP 64

Predictive assays for targeted therapeutics using image-based high content analysis of patient-derived tumor models

K.N. Praveen, R. Tseng, N. Streiner, S. Hoffmaster, C. Mirsaidi, T. Broudy, K. Anderes. *Molecular Response, San Diego, USA*

Background: The role of different cellular components of tumor microenvironment in dictating the sensitivity towards chemotherapy has been used in developing strategies to improve cancer therapy. However, most of the in vitro drug treatment studies use cell lines which lack many major components of the tumor environment. With more drug candidates being introduced for targeted therapies of cancers, it becomes important to test these compounds on a mixture of target positive and negative cell population to demonstrate the selective effect before being introduced into a Phase I clinical trial. Patient derived tumor models are superior to cell lines as representative of the actual tumor microenvironment. However, interpretation of the results from in vitro treatment studies using these cells becomes highly complicated due to the non-homogeneous nature of the cells. Molecular Response has an extensive collection of patient derived tumor cells which can be used to evaluate the therapeutic agents for their targeted action on cell populations. We have developed novel approaches to evaluate the anti-proliferative action of cancer drugs on cell population of specific interest.
Materials and Methods: This study investigated the utility of patient derived tumor cells to investigate selective anti-proliferative effects on cell populations carrying specific mutations. Using qPCR based assays, NSLC and melanoma samples were screened to identify samples with ALK-EML fusion and BRAF-V600E mutation status respectively. Using high content imaging platform, the selective anti-proliferative effects of crizotinib on cell populations with ALK-EML fusion as wells as effect of plx4032 on B-RAF mutated populations were assessed.
Results: We developed a semi-quantitative qPCR based screening strategy for cancer samples (N = 2000) to identify high expression of ALK. The fusion and ALK-EML was confirmed by FISH. PCR assays to detect BRAF mutations were used to screen a collection of melanoma samples (N = 900). Using a novel image-based high content screening assay, the targeted action of crizotinib as well as plx4032 were demonstrated in mutated cell populations in the context of a non-targeted cell population.
Conclusion: The use of patient-derived tumor cells as a tool to develop novel approaches to delineate the effects of therapeutics on targeted cell population has shown great potential in oncology drug development. This novel approach provides a means to evaluate variety of drug candidates used in personalized medicine.

PP 87

Molecular imaging demonstrates GLPG0187, a small-molecule integrin antagonist, binds to RGD-integrin receptors in vivo and is efficacious in tumor and metastasis models

P. Pujuguet, E. Berrocal, B. Heckmann, P. Wigerinck, G. Lorenzon, P. Clément-Lacroix, G. Bormans, C. Deroose. *Galapagos SASU, Romainville, France*

Background: GLPG0187, an integrin antagonist with nanomolar affinity for RGD-integrin receptors, shows potent anti-tumor activity in vivo. In a mouse model of human breast cancer bone metastasis, the combination of GLPG0187 with standard-of-care anti-resorptive (zoledronate) and chemotherapeutic (paclitaxel) agents shows superior efficacy when compared to each treatment taken separately providing further evidence that GLPG0187 could be an effective therapeutic for the treatment of tumors and metastases. The primary objective of this study was to evaluate whether GLPG0187 reaches target RGD-integrin receptors in vivo to identify an imaging biomarker suitable for patient selection. We also compared doses of GLPG0187 that compete for receptor binding with doses that are pharmacologically relevant.
Materials and Methods: Molecular imaging was performed by fluorescent tomography using Integrisense680and by μPET using 18F probes. Mice were bearing established human melanoma A375 xenografts and human MDA-MB-231/B02 breast cancer bone metastases.
Results: In the bone metastasis model, GLPG0187 administered at the pharmacologically efficacious dose of 30 mg/kg, p.o., b.i.d., displaced up to 70% of the binding of Integrisense680, thereby demonstrating its ability to bind RGD-integrin receptors in vivo. In melanoma xenografts, higher doses of GLPG0187 were needed to reach significant effects, both in receptor binding and efficacy studies. An assessment using [18F]-RGD and [18F]-FDG in melanoma xenografts indicated probe displacement and therapeutic efficacy, respectively, by GLPG0187.
Conclusion: These molecular imaging experiments show that GLPG0187 binds to target RGD-integrin receptors in tumors and metastases in vivo. Recent successful developments in oncology have relied on careful patient selection based on target expression. These preclinical data support the use of PET probes to select patients bearing RGD-integrin positive tumors in GLPG0187 clinical trials.

PP 102

Gene expression profiles obtained from mouse hair, xenograft tumor and ex vivo human scalp hair to determine the effects of drug response following treatment with an EGFR inhibitor

B. Reed, E. Grimes, T. Mefo, E. Harrison, L. Meyer-Turkson, G. Miele, G. Brady. *Epistem Ltd, Manchester, United Kingdom*

Background: Plucked human scalp hair represents an ideal surrogate sentinel tissue to enable non-invasive monitoring of drug response, both in clinical trials and also as a discovery platform ex vivo. Congruence of patterns of transcriptome activity show high concordance to that of primary target tissue. EGFR is often over expressed or deregulated in a variety of solid tumours and EGFR inhibitors are increasingly part of the therapeutic treatment of advanced lung, head-and-neck and colorectal carcinoma. Desirable non-invasive transcriptional biomarkers of EGFR inhibition demonstrate target engagement and define a PK/PD relationship, as well as enabling the monitoring of a well tolerated dose schedule with maximal biological effect.
Materials and Methods: In this study, we initially focused on a preclinical mouse model in which we analysed gene expression profiles in plucked hair following treatment with erlotnib. These biomarkers were then explored further in an erlotinib dose escalation study in mouse hair and tumour samples collected from a preclinical lung xenograft model. Epistem has also developed an ex vivo human hair culture platform in which we are able to model pharmacodynamic consequences of small molecules and biotherapeutics. We deployed our proprietary platform here to investigate the relationship of pre-clinical markers of erlotinib exposure in vivo to those discovered ex vivo in human hair treated with the erlotinib.
Results: We were able to demonstrate from the xenograft study, a panel of transcriptional markers that exhibited a similar response to drug treatment in both tumour and hair. The differentially expressed genes identified in both mouse and human hair samples were biologically relevant. The panels identified included genes in the EGFR signaling pathway or are known to be associated with this pathway. We also used our ex vivo human hair culture platform to further explore the relevance of pre-clinical markers of

erlotinib exposure in vivo to those observed ex vivo in human hair treated with erlotinib.
Conclusion: We conclude that plucked human scalp hair represents an ideal minimally invasive surrogate tissue, with which to monitor drug response in patients receiving treatment with EGFR inhibitors.

PP 97

The relevant role of angiogenesis pathway in BRCA1/2 breast cancers

C. Saponaro, A. Malfettone, R. Daprile, S. Tommasi, G. Simone, A. Paradiso, A. Mangia. *National Cancer Centre Giovanni Paolo II, Bari, Bari, Italy*

Background: Germline mutations in the BRCA1 and BRCA2 genes are related with familial predisposition to breast cancer. Tumor angiogenesis and its important in breast cancer has been extensively investigated. The most important regulator of angiogenesis is the vascular endothelial growth factor (VEGF), up-regulated during hypoxia by hypoxia-inducible factor-1 (HIF-1α), a protein released during oxygen stress. Few is reported about angiogenesis in hereditary breast cancer, but it is known that there is an overexpression of HIF-1α in BRCA1 related cancers respect to sporadic cancers; and any evidence exists about correlation of VEGF, HIF-1α and microvessels formation in these tumors. Aim of this study is verified a differential expression of VEGF and HIF-1α and micro-vessels development, by CD31 marker, and a possible correlation of these markers in BRCA1/2 related cancers, compared with familial and sporadic breast cancers.
Materials and Methods: We investigated the expression of VEGF, HIF-1α and CD31 in 18 BRCA1, 7 BRCA2 mutated cancers and in 94 familial and 93 sporadic cancers, by immunohistochemistry.
Results: VEGF resulted more expressed in BRCA1-related cancers than in familial ($p < 0.01$) and in sporadic cancers ($p < 0.001$). However, VEGF expression was higher in familial than in sporadic group ($p < 0.001$). Also the MVD was significantly higher in BRCA1 cancers than familial and sporadic group ($p < 0.001$). Further higher microvascular density was associated with elevated VEGF expression. HIF-1α expression was more intensive in BRCA1 than in familial cancers ($p < 0.05$). In addition, from the analysis of BRCA2 cancers was clear that VEGF expression was higher in BRCA2 mutated than in sporadic cancers ($p < 0.01$). On the contrary the MVD was stronger in BRCA2 mutated than both familial and sporadic ($p < 0.01$). Further also HIF-1α showed a more intensive expression in BRCA2 mutated than both familial and sporadic cancers ($p < 0.001$).
Conclusion: Our results showed an increased expression of VEGF, CD31 and HIF-1α in hereditary cancers. These findings suggest that the angiogenesis plays a crucial role also in hereditary breast cancers.

PP 37

Antitumor activity and antioxidant status of berberine against Ehrlich ascites carcinoma in Swiss albino mice

S. Saraswati, M. Pandey, S.S. Agrawal. *Delhi Institute of Pharmaceutical Sciences and Research, New Delhi, India*

Background: Berberine has anti-tumor properties in various cancer cells including breast cancer but the exact mechanisms and in vivo effects are unclear. We investigated anti-cancer activity of berberine in vivo in Ehrlich ascites carcinoma (EAC) tumor.
Materials and Methods: 15×106 EAC cells were implanted intraperitoneally (i.p., ascitic tumor) in Swiss albino female mice. Fifty mice were divided into five groups and received drug for 14 days: group (1) saline, group (2) Cyclophosphamide (CP, 10 mg/kg, ip), group (3) berberine (6 mg/kg/d, ip), group (4) berberine (12 mg/kg/d, ip), group (5) berberine (18 mg/kg/d, ip). On day 15, blood samples were collected for hematological assessment of hemoglobin (Hb %), RBCs, WBCs and PCV. Ascitic fluid was also collected by making an incision in the abdominal region of mice. All mice were then sacrificed; sections from and liver were cut and homogenized for biochemical analysis measuring glutathione (GSH), malondialdehyde (MDA) catalase (CAT) and superoxide dismutase (SOD) activity. Also sections from solid tumor formed at peritoneal wall were removed for pathological examination after staining with (H & E).
Results: Berberine significantly inhibited tumor growth, cell viability in Ehrlich ascites tumor growth in vivo ($p < 0.001$). Histopathological examination of tumor cells in the treated group demonstrated signs of apoptosis with chromatin condensation and cell shrinkage. Decreased peritoneal angiogenesis showed the anti-angiogenic potential. Berberine at 12 mg/kg dose significantly increased in SOD and CAT activity ($p < 0.01$). GSH and TBARS were increased by 46 and 58% compared with control group ($p < 0.001$). Furthermore, berberine increased total RBCs, WBCs as well as Hb% significantly ($P < 0.05$) compared to CP.
Conclusion: Administration berberine inhibited the growth of Ehrlich ascites tumor. The results indicate that berberine exhibited significant antitumor and antioxidant activity in EAC-bearing mice.

PP 17

Monitoring phosphorylation of p70S6K1 kinase (Thr421/Ser424), a biological marker for mTOR activity, improves Nottingham histoprognostic grading of invasive breast carcinomas

T. Sauri-Nadal, S. Del Barco, A. Vazquez-Martin, C. Oliveras-Ferraros, S. Cufí, B. Martin-Castillo, E. Lopez-Bonet, J. Menendez. *Catalan Institute of Oncology (ICO), Girona, Spain*

Background: Histological grade is one of the strongest prognostic factors in operable breast carcinomas (BC). 30−60% of BC are classified as histological Grade 2, which is associated with an intermediate risk of recurrence and is not informative for clinical decision making. The prognostic abilities of BC gene expression signatures such as the Genomic Grade Index (GGI), which can reclassify Grade 2 BC into 2 groups with high versus low risks of recurrence, are due mostly to the detection of proliferation activity. One of the strongest, yet simple and well-reproducible proliferation-associated prognostic factors is the mitotic activity index (MAI).
Materials and Methods: We have tested whether immunohistochemical assessment of MAI by monitoring phosphorylation of p70S6K1 (Thr421/Ser424) significantly impacts on the histopathological classification of Grade 2 BC. (1.) We validated the sensitivity of phospho-p70S6K1 Thr421/Ser424 (PP-S6K1) labeling in detecting & counting mitotic figures and also its usefulness for histoprognostic grading in a series of 144 BC biopsies; (2.) we investigated the correlation between PP-S6K1 MAI and the MAI determined by using the mitosis-specific marker phospho-Histone H3 Ser10 (PP-H3).
Results: PP-S6K1-labeled mitotic figures were easily seen and permitted a quick identification of the area of highest mitotic activity, even at low-power magnification. A statistically significant correlation was found between the mitotic counts obtained by using PP-S6K1 and those assessed by either standard Hematoxylin & Eosin [H & E] ($r = 0.680$) or PP-H3 staining ($r = 0.855$). PP-S6K1 MAI correlated also with tumor proliferative activity as measured with the Ki-67 labeling index ($r = 0.628$). Average mitotic counts were significantly higher when using labeling with PP-S6K1 (range = 0–141, mea n = 12) or PP-H3 (0–146, 19) than with the standard H&E protocol (0−60, 5). Importantly, when the global histoprognostic score of the Nottingham Grading System was re-evaluated on the basis of PP-S6K1 staining, there was a statistically significant shift from Grade 1 to Grade 2 in 7 cases, and from Grade 2 to Grade 3 in 17 cases. Indeed, PP-S6K1 was as efficient as PP-H3 at reclassifying BC patients with H & E-determined Grade 2 tumors.
Conclusion: Our findings reveal that immunolabeling with PP-S6K1 − a biological marker for activity of the mTOR signaling pathway − constitutes a simple and reliable method for quantifying proliferative potential that significantly improves Nottingham histoprognostic grading of BC.

PP 22

Analysis of HER-3, insulin-growth factor-1 (IGF-1), nuclear factor k-B (NF-kB) and epidermal growth factor receptor (EGFR) gene copy number (GCN) in the prediction of clinical outcome for K-RAS wild type colorectal cancer patients receiving irinotecan–cetuximab

M. Scartozzi, R. Giampieri, E. Maccaroni, A. Mandolesi, L. Giustini, R.R. Silva, E. Galizia, A. Falcone, I. Bearzi, S. Cascinu. *Oncologia Clinica, AO Ospedali Riuniti Ancona, Ancona, Italy*

Background: A large proportion of colorectal cancer patients does not benefit from the use of anti-EGFR treatment although in the absence of a mutation of the K-RAS gene. Preliminary observations suggested that HER-3, IGF-1, NF-kB and EGFR GCN might identify patients not likely to benefit from anti-EGFR therapy. We tested the interaction between HER-3, IGF-1, NF-KB, EGFR GCN and K-RAS mutational analysis to verify the relative ability of these variables to identify a sub-group of patients more likely to benefit from EGFR-targeted treatment among those harbouring a K-RAS wild type status.
Materials and Methods: We retrospectively collected tumours from 168 patients with metastatic colorectal cancer patients treated with irinotecan-cetuximab. KRAS was assessed with direct sequencing, EGFR amplification was assessed by chromogenic in situ hybridization and HER-3, IGF-1 and NF-kB were assessed by immunoistochemistry.
Results: In patients with K-RAS wild type tumours, the following molecular factors resulted independently associated with response rate: HER-3 (OR = 4.6, 95% CI: 1.8–13.6, $p = 0.02$), IGF-1 (OR = 4.2, 95% CI: 2–10.2, $p = 0.003$) and EGFR GCN (OR = 4.1, 95% CI: 1.9–26.2, $p = 0.04$). These factors also independently correlated with overall survival as follows: HER-3 (HR = 0.4, 95% CI: 0.28–0.85, $p = 0.008$), IGF-1 (HR = 0.47, 95% CI: 0.24–0.76, $p < 0.0001$) and EGFR GCN (HR = 0.59, 95% CI: 0.22–0.89, $p = 0.04$).
Conclusion: We believe that our data may help further composing the molecular mosaic of EGFR resistant tumours. The role of HER-3, IGF-1 and CISH EGFR GCN should be prospectively validated in clinical trials investigating anti-EGFR treatment strategies in colorectal cancer patients.