indicate that K562/IMA-3 cells are more effectively sensitized by silencing the *STAT5a* compared to the sensitive K562 cells. Non-transfected K562/IMA-3 cells showed 1.85- and 3.46-fold increases in caspase-3 enzyme activation in the presence of 5- and 10 μM of imatinib, while siRNA-transfected counterparts have shown 11.21- and 20.17-fold increases.
Conclusion: Observing these significant responses to imatinib after transfection with single gene-specific siRNA might provide new opportunities for dealing with the frequent occurrence of resistance to chemotherapeutic agents in leukemia.

202 Identification and evaluation of novel breast cancer related biomarker proteins by antibody proteomics technology

K. Nagano[1], T. Yamashita[2], S. Imai[1], Y. Abe[1], T. Yoshikawa[2], Y. Yoshioka[3], H. Kamada[1], Y. Tsutsumi[2], S. Tsunoda[1]. [1]*National Institute of Biomedical Innovation, Laboratory of Pharmaceutical Proteomics, Osaka, Japan,* [2]*Osaka University, Graduate School of Pharmaceutical Sciences, Osaka, Japan,* [3]*Osaka University, The Center for Advanced Medical Engineering and Informatics, Osaka, Japan*

Background: The identification of biomarkers is a promising approach for the diagnosis and effective therapy of cancer. In particular, disease proteomics is a potentially useful method for identifying such biomarkers. However, very few biomarker proteins for drug development have been discovered using this approach. The main difficulty is to efficiently select potential biomarkers from the many candidate proteins identified by the proteomics approach. To circumvent this problem, we have developed "antibody proteomics technology" that can screen for biomarker proteins by isolating antibodies against each candidate in a rapid and comprehensive manner. Here, we applied "antibody proteomics technology" to breast cancer-related biomarker discovery and evaluated the utility of this novel technology.
Material and Methods: *2D-DIGE analysis:* Cell lysates from breast tumour cells (SKBR3) and established normal breast cells (184A1) were labeled with Cy3 or Cy5 and analyzed by 2D-DIGE according to the manufacture's protocol. Non-labeled samples were also loaded in a normal gel for MS analysis and a modified gel that can be solubilized by sodium periodide for antibody isolation. Proteins of interest were extracted from the gel and subjected to MS analysis and used as targets for the phage antibody library.
Isolation of monoclonal antibody: Protein samples extracted from the solubilized gel pieces were immobilized onto a nitrocellulose membrane. Using these proteins as targets, phages displaying scFv antibody, which have affinity to the targets, were selected from non-immune scFv phage libraries.
Tissue microarray (TMA) analysis: Expression profiles of candidate proteins were analyzed by using breast tumour TMAs stained with the isolated scFv-phages.
Results and Conclusions: By 2D-DIGE analysis, 18 candidate proteins over-expressed in SKBR3 cells were identified. Using an *in vitro* scFv-phage affinity selection procedure, monoclonal scFvs binding to each of the 18 candidate proteins were successfully isolated within a few weeks. TMA analysis then identified novel biomarker proteins over-expressed in breast tumours and correlated with lymph node metastasis. Our data demonstrates the utility of the antibody proteomics technology for discovering and validating tumour-related proteins in pharmaceutical proteomics. We are currently analyzing the functions of the identified proteins as potential diagnostic markers or therapeutic targets.

203 Targeting the transcriptional activity of sarcoma specific chimeric fusion proteins

D. Herrero-Martin[1], B.W. Schäfer[1]. [1]*University Children's Hospital, Department of Oncology, Zurich, Switzerland*

Background: Alveolar rhabdomyosarcoma (aRMS) is an aggressive childhood sarcoma characterized by expression of the fusion protein PAX3/FKHR which is thought to drive malignancy of this tumour. Downregulation of the fusion protein induces apoptosis in aRMS cells supporting the notion that their survival depends on continuous expression of PAX3/FKHR. Hence, it could be hypothesized that small molecular drugs might already exist for the treatment of aRMS, but strategies to prioritize them have been lacking. We are trying to identify small molecules capable to specifically modulate the transcriptional activity of chimaeric oncogenic transcription factors in sarcomas.
Material and Methods: We screened a small compound library (LOPAC 1280, Sigma) which covers 1280 different drug-like and well annotated compounds covering all major drug types. We used an endogenous cellular model, Rh4, which represents a PAX3/FKHR bearing aRMS with a transcription profile very similar to tumour biopsies. As a read-out system we simultaneously assessed cell viability together with a well established and highly sensitive luciferase reporter assay based on the AP2beta target gene promoter to monitor fusion protein activity. Cells were plated into 96 well plates and treated with the compunds at a final concentration of 5 μM during 24 hours. We set as cut-off a 65% reduction in luciferase activity.
Results: We have obtained a list of nearly 53 promising compounds affecting the activity of PAX3-FKHR. Among them there are different types of molecules with a broad role of functions such as kinase inhibitors, topoisomerase II inhibitors or compounds related to nitric oxide. We have chosen a final list of 11 compounds that we have tested using different concentrations (5, 1 and 0.5 μM) and time points (24, 48, 72 hours) and finally we have decided to focus our attention in two compounds: fenretinide (retinoic acid p-hydroxyanilide) and the kinase inhibitor IC261.
Interestingly, both fenretinide and IC261 reduced the mRNA expression level of PAX3-FKHR and some well known target genes such as AP2β, fibroblast growth factor receptor 2 (FGFR2) or fibroblast growth factor receptor 4 (FGFR4).
Then, we analyzed the effects of both compounds in additional rhabdomyosarcoma cell lines, both alveolar and embryonal, measuring again the cell viability and expression levels of PAX3-FKHR and its target genes. Both compounds were effective in all aRMS cell lines (Rh41, RMS13) but not in cells of embryonal origin (Ruch-2, RD) that lack PAX3-FKHR expression.
Conclusion: Unlike most other experimental strategies which reason that an increased understanding of the biology (target genes) would lead to identification of active compounds, our strategy is a reverse approach that has allowed us to identify compounds that are not tested yet for aRMS treatment such as fenretinide and IC261, and that then in turn are expected to help understanding the biology of sarcomas, identify pathways critical for aRMS progression and lead to the development of new therapeutic strategies.

204 Identification and pre-clinical validation of surrogate soluble biomarkers correlating with therapeutic response to met inhibition

D. Torti[1], A. Bertotti[1], F. Galimi[1], S. Gastaldi[1], F. Sassi[1], L. Trusolino[1]. [1]*IRCC, Molecular Oncology, Candiolo Turin, Italy*

Serum proteins may function as early and dynamic surrogate biomarkers of molecular sensitivity to targeted inhibitors in selected cohorts of patients. In a search for a panel of potential soluble biomarkers of response to Met receptor inhibitors in sensitive tumours, we pursued an unbiased screening approach followed by experimental validation using diverse Met-addicted models, both *in vitro* and *in vivo*. Two independent and complementary technological platforms – (1) a large-scale cDNA microarray profiling and (2) a medium-scale protein array – have been applied to the Met-addicted cell line GTL16 treated with the selective inhibitor PHA-665752. The intersection of the two datasets enclosed two candidate molecules robustly down-modulated upon treatment, interleukin-8 (IL-8) and urokinase-type plasminogen activator receptor (uPAR). By employing available ELISA kits to test for the concentrations of IL-8 and uPAR in the supernatants of GTL16 cells upon PHA treatment, we observed a significant reduction in the levels of IL-8 and of uPAR. Moreover, we performed the same experiments in two cellular models of resistance to Met inhibitors: (1) A549 cells and (2) GTL16 cells infected with the constitutively active Ras^{G12V}. In both models, Met inhibition negligibly influenced secretion of the two molecules. We performed serial blood sampling at day 0 (pre-treatment), 3, and 10, to assay the concentration of IL-8 and uPAR in the plasma of nude mice injected subcutaneously with GTL16 cells. We could detect a clearcut reduction in IL-8 levels over basal values at 72 hs, that persisted until the 10^{th} day of treatment; conversely, established xenografts of GTL16 expressing the Ras^{G12V} 'resistance gene' did not feature a similar reduction in IL-8 levels. We further extended our fishing analysis to six other molecules significantly modulated either in the microarray dataset (stanniocalcin-1 and REG4) or in the protein array (IL-6, GROα, MIF, and MCP-1) and in order to generalize the model, we gauged expression and dosage of such molecules in a tissue-specific panel of Met-addicted cell lines (MKN45, HS746T, and SNU5). ELISA-based analysis of such molecules indicated that two of them (GROa and IL-6) displayed consistent rmodulation in culture supernatants of Met-addicted cells following Met inhibition. These soluble proteins may warrant further investigation as surrogate plasma biomarkers of response to anti-Met targeted therapies in drug-responsive tumours.

205 In vivo delivery of siRNA to tumours and their vasculature by novel dendritic nanocarriers

P. Ofek[1], W. Fischer[2], M. Calderon[2], R. Haag[2], R. Satchi-Fainaro[1]. [1]*Tel Aviv University, Physiology and Pharmacology, Tel Aviv, Israel,* [2]*Freie University Berlin, Organic and Macromolecular Chemistry, Berlin, Germany*

Background: New targets for RNAi-based cancer therapy are constantly emerging. Nevertheless, *in vivo* delivery of siRNA remains a crucial issue for its therapeutic success. We propose to encapsulate the siRNA in a cationic carrier system, which can strongly improve its stability, cellular uptake and silencing efficacy. We developed novel polymerized dendrimer core shell structures to deliver siRNA *in vivo*. These water-soluble macromolecular carriers accumulate in the tumour environment due to the enhanced permeability and retention (EPR) effect and therefore, represent ideal delivery vehicles for antitumour biological agents.

Methods: Dendritic nanocarriers were synthesized and further characterized by dynamic light scattering, atomic force microscopy and electrophoretic mobility shift assay. Cellular internalization of polyplexes was monitored by confocal microscopy. The nanocarriers cytotoxicity profile was assessed by XTT and red blood cells lysis assay. The luciferase gene, ectopically overexpressed in human glioblastoma cell lines was used as a model system and its silencing efficacy was measured. mCherry and luciferase-labeled glioblastoma and mammary adenocarcinoma mouse models were established. The silencing efficiency of the nanocarrier luciferase-siRNA polyplexes was followed up by non-invasive intravital bioluminescence imaging.
Results: All dendritic nanocarriers synthesized had a mean hydrodynamic diameter of 10−40 nm. The novel nanocarriers entrap siRNA, neutralize its negative charge in a dose-dependent manner and significantly improve its cellular uptake. PG-Amine exhibited the optimal silencing efficiency and safety profile in additional *in vitro* biocompatibility and efficacy tests. Therefore, it was selected for further evaluation and *in vivo* gene silencing efficacy studies. A significant gene silencing effect was accomplished *in vivo* in both human glioblastoma and murine mammary adenocarcinoma mouse models. Within 24 hours, 85% and 68% silencing was achieved following intratumoural and intravenous treatment respectively, as measured by intravital non-invasive imaging of photon flux bioluminescence. No significant weight loss occurred following intravenous administration of the siRNA-nanocarrier complexes.
Conclusions: We show a proof of concept for siRNA delivery using a luciferase-based model. We predict that *in vivo* silencing of an important cell growth and angiogenesis regulator as Akt1 in a selective manner will warrant this approach as a successful anticancer therapy.

206 Downstream signaling pathways determine resistance of cancer cells against novel irreversible ErbB-targeting drugs

C. Brünner-Kubath[1], W. Shabbir[1], V. Saferding[1], R. Wagner[1], C.F. Singer[2], P. Valent[3], W. Berger[4], B. Marian[4], M. Grusch[4], T.W. Grunt[5]. [1]*Medical University Vienna, Division of Oncology Department of Medicine I, Vienna, Austria,* [2]*Medical University Vienna, Division of Special Gynecology Department of Obstetrics/Gynecology, Vienna, Austria,* [3]*Medical University Vienna & Ludwig Boltzmann Cluster Oncology, Division of Hematology/Hemostaseology Department of Medicine I, Vienna, Austria,* [4]*Medical University Vienna, Institute of Cancer Research Department of Medicine I, Vienna, Austria,* [5]*Medical University Vienna & Ludwig Boltzmann Cluster Oncology, Division of Oncology Department of Medicine I, Vienna, Austria*

Epidermal growth factor (EGF) receptor-related antigens (EGFR, ErbB1−4, HER1−4) represent emerging drug targets in oncology. However, resistance against ErbB-targeting drugs occurs frequently in cancer patients. Drug-resistant cells may exhibit drug-refractory phosphatidylinositol 3-kinase (PI3K) or mitogen-activated protein kinase (MAPK) signaling, but the relative impact and contribution of these two downstream pathways to drug resistance are still controversially discussed. We examined the effects of the two very potent, irreversibly binding ErbB receptor tyrosine kinase inhibitors (RTKIs) pelitinib (EKB-569) and canertinib (CI-1033) on PI3K- and MAPK activity in ErbB RTKI-sensitive and ErbB RTKI-resistant breast and ovarian cancer cells. Western blot analysis revealed that ErbB phosphorylation was abrogated by the inhibitors in both drug-sensitive and drug-resistant cells, whereas AKT- and GSK3b phosphorylation were drug-dependently downregulated only in drug-sensitive cells. ErbB RTKI sensitivity did not correlate with expression of wildtype PTEN or PIK3CA, nor was it associated with drug-dependent silencing of ERK1,2 in the breast and ovarian cancer cell lines examined. Moreover, exogenous AKT, but not MEK, significantly induced drug resistance. Our data demonstrate that blocking AKT phosphorylation is essential and sufficient, whereas abrogation of ERK phosphorylation is not required for ErbB RTKI anticancer efficacy. AKT phosphorylation may thus be a useful biomarker of ErbB RTKI sensitivity in breast and ovarian cancer cells. Supported by 'Medizinisch-Wissenschaftlicher Fonds des Bürgermeisters der Bundeshauptstadt Wien' (#08037) and 'Initiative Krebsforschung', Medical University Vienna.

207 Cellular mechanism of a novel CDK9 inhibitor CDKI-71

X. Liu[1], S. Shi[1], F. Lam[1], S. Wang[1]. [1]*University of Nottingham, School of Pharmacy and Centre for Biomolecular Sciences, Nottingham, United Kingdom*

Introduction: Human cancer cells resist apoptosis by relying on sustained expression of anti-apoptotic genes. Targeting transcription and suppress these anti-apoptotic proteins seem a promising strategy for anti-cancer therapy. CDK9-cyclinT1 activates RNA transcription by phosphorylating the CTD of RNAPII. CDKI-71, a potent CDK9 inhibitor, has been identified via our in-house drug discovery program. This compound inhibits RNAPII transcription, and effectively induces apoptosis in cancer cells. Hereby we report the detailed mechanism of action of CDKI-71.
Methods: Kinase inhibition was measured by radiometric assay. MTT assay was performed against the human tumour cell lines and non-cancerous cell line. Apoptosis was detected by Annexin-V and PI double stains. Phosph-RNAPII Ser-2, Ser-5, total RNAP II, Mcl-1, Bcl-2, p53, MDM-2, PARP, XIAP and g-H2AX were detected by western blots.
Results: CDKI-71 inhibits CDK9/T1 and CDK7/H with the K_i values of 6 nM and 114 nM respectively, having a similar potency and selectivity profile to the clinic CDK inhibitor flavopiridol (K_i = 3 and 113 nM for CDK9 and CDK7 respectively). Treatment of A2780 ovarian cancer cells with 0.5 mM CDKI-71 for 24 h blocked the phosphorylation at Ser-2 of CTD RNAPII, confirming its cellular CDK9 inhibitory activity. CDKI-71 is a potent anti-proliferative agent with an average GI_{50} of 0.55 mM against 12 human tumour cell lines. Interestingly, this compound shows a significantly reduced toxicity in the non-cancerous MRC-5 cells with a GI_{50} value of 4.3 mM. In contrast, flavopiridol exhibits little selectivity in the cancer and non-transformed cell lines. Treatment of HCT-116 and A2780 cells with CDKI-71 for 24 h resulted in a dose-dependent activation of caspase 3/7 and induction of apoptosis. However little affects on MRC-5 cells were observed by the same treatment. Blockage of Mcl-1 protein expression and increased nucleate p53 accumulation were also identified. To investigate whether CDKI-71 induces p53 protein is a consequence of DNA damage response we measured the level of γ-H2AX, the phosphorylated Ser-139 of H2AX, using western blots. Treating both A2780 and MRC-5 cells with vary concentrations of CDKI-71 for 24 h no γ-H2AX protein was detected. In contrast, exposure of the cells with 0.2 μM flavopiridol resulted in highly elevated level of γ-H2AX protein.
Conclusions: CDKI-71 is a potent and selective CDK9 inhibitor possessing excellent anti-proliferative activity. The compound induces apoptosis selectively in cancer cells over the normal non-transformed cells. CDKI-71 can be developed as an anti-cancer agent.

208 Downregulation of antiviral genes in prostate cancers affects the response to the DNA methylation inhibitor 5-aza-2′-deoxycytidine

W.A. Schulz[1], S. Bleckmann[1]. [1]*University Hospital, Urology, Duesseldorf, Germany*

Background: Mutations and polymorphisms in genes encoding antiviral proteins, notably RNASEL, are associated with an increased risk of prostate cancer. In addition, in many prostate cancers interferon response genes, like the prototypic MX1, are downregulated. We sought to elucidate whether changes in DNA methylation underlie this downregulation.
Experimental procedures and Results: Treatment with the DNA methylation inhibitor 5-aza-2′-deoxycytidine (aza-dC) for 3 days induced MX1 and several other interferon-responsive genes in the PC3 but not the LNCaP prostate cancer cell line. According to bisulfite sequencing, the major promoter of MX1 was unmethylated in either line. Induction of MX1 in LNCaP was restored neither by increasing the inhibitor concentration nor the length of the exposure. In contrast to PC3, LNCaP expresses significant levels of androgen receptor and contains mutant RNase L. Nevertheless, depleting LNCaP medium of steroids did not restore MX1 induction by aza-dC. Neither was MX1 inducibility in PC3 diminished by siRNA-mediated downregulation of RNase L. Intriguingly, compared to PC3, LNCaP cells also displayed a strongly muted response to exogenous interferon beta (8- vs. 90-fold), due to a 50-fold decrease in expression of JAK1, a protein kinase crucial for interferon signaling. Accordingly, interferon beta expression in PC3 became upregulated after 2 days of aza-dC treatment, preceding the increase in MX1. This suggests that aza-dC treatment elicits interferon production, JAK1 activation and induction of interferon-responsive genes like MX1 in PC3 cells. This sequence of events appears to be interrupted by lack of JAK1 in LNCaP. Downregulation of JAK1 is not a pecularity of this cell line, but was observed in a significant fraction of prostate cancer tissues as well.
Conclusions: Our data suggest that downregulation of interferon-responsive genes in prostate cancers is not caused by hypermethylation of their promoters, but occurs rather secondary to changes in interferon signaling pathways, such as JAK1 deficiency. Our findings have implications for the potential use of DNA methylation inhibitors in cancer therapy, in particular predicting that aza-dC will induce interferon responses in some prostate cancers, but not others. These differences are expected to affect the efficacy of aza-dC and other inhibitors of DNA methylation in this cancer type.