Results and discussions Minor allele frequencies for M420del were 0.18 and 0.1 in CML patients and controls; for M408V 0.4 and 0.27 respectively, closely paralleling those reported in western population.

No significant association between different genotypes of M420del and M408V was observed with either time to achieve complete haematological response (CHR) (p=0.341 for both SNPs),or presence of optimal/sub-optimal molecular responses(p=0.125, 0.629 for M420del and M408V respectively).

To analyse the combined effect of these two SNPs, CML cases were divided into 4 groups.Patients with mutant (homo/heterozygous) M420del and wild type homozygous M408V, failed to achieve an optimal molecular response to imatinib, unlike those with mutant genotypes (homo/heterozygous) for both SNPs (p=0.02).

Conclusion Mutant M420del allele may be linked to poor outcome of imatinib treatment in CML,however simultaneous presence of mutant M408V allele appears to circumvent this effect.These SNPs in *hOCT1* gene occur at reasonable frequencies in Indian population, to be of clinical interest as predictors of response to imatinib in CML.

PO-471   **EXOSOMES AND TRANSFERRING OF HORMONAL RESISTANCE OF BREAST CANCER CELLS**

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Introduction The main goal of the present work was the study of the molecular mechanism of acquired hormonal resistance of tumour cells, in particular - the study of the intercellular interactions and exosomes and their role in the progression of the resistance.

Material and methods Hormone responsive MCF-7 and hormone resistant MCF-7/T breast cancer cells were cultured in standard DMEM medium supplemented with 10% fetal calf serum. The transcriptional activities of NF-kB and AP-1 were determined by reporter luciferase assays. Then exosomes from cell supernatant were isolated by differential ultracentrifugation using standard protocol.

Results and discussions Here, using the estrogen-dependent MCF-7 breast cancer cells and estrogen-independent MCF-7/T cells we have demonstrated the ability of the resistant cells-derived exosomes to initiate the estrogen-independent growth of the parent MCF-7 cells. The subsequent analysis of the key signalling proteins revealed the ability of the exosomes of the resistant cells to inhibit the oestrogen signalling as well as to stimulate the Akt protein kinase and transcription factors AP-1 and NF-kB in the recipient cells. Importantly, the cell treatment with PI3K/Akt inhibitor wortmannin prevented the exosome-induced resistance giving the additional evidence for central role of this pathway in the mediating of exosomal resistance.

The data validation was performed on the resistant subline MCF-7/M which was selected under long-term cultivation of the parent MCF-7 cells with biguanide metformin and demonstrated the cross-resistance to metformin and tamoxifen. We have shown that the exosomes of the resistant MCF-7/M cells lead to the partial resistance of the parent cells to both drugs. Moreover, the acquired resistance was characterised with the

analogues signalling rearrangement: inhibition of the oestrogen signalling and activation of the Akt, AP-1 and NF-kB proteins - similar to the described above hormonal resistance. Advanced analysis of the exosomal microRNA revealed 27 microRNA differentially expressed in the exosomes of the resistant cells and associated with the progression of hormonal resistance.

Conclusion Totally, we demonstrated the new mechanism of horizontal transferring of hormonal resistance by exosomes, identified the possible intercellular targets of exosomes and revealed the main features of the exosomal transcriptome. This study was supported by Russian Science Foundation, grant 14-15-00362 M.K.

PO-472   **CHEMOTHERAPY RESISTANCE-ASSOCIATED EPITHELIAL TO ENDOTHELIAL TRANSITION IN GASTRIC CANCER**

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Introduction Gastric cancer (GC) is the fifth most common cancer worldwide and the third leading cause of cancer-related deaths. To date, gastrectomy and chemotherapy are the only therapeutic options, but drug resistance is the main cause for treatment failure.

Vasculogenic mimicry (VM) is a new model of neovascularization in aggressive tumours and has been correlated with poor prognosis in GC patients.

Our group has developed chemotherapy-resistant GC cells using the Caucasian adenocarcinoma cell line AGS and three drugs among the most used in clinic (5-fluorouracil, cisplatin and paclitaxel) henceforward denominated 5FUr, CISr, TAXr.

Our study has highlighted phenotypical differences among chemo-sensitive and chemo-resistant cell lines such as acquisition of stem-like phenotype and increased capacity to form vessels.

Material and methods Establishment of AGS resistant cell lines exposing cells to increasing dilution of drugs for over 9 months up to dilutions higher than IC50 values initially verified on AGS cells through MTT analysis.

Quantitative RT-PCR, flow cytometry and western blot analysis for stemness and VM markers.

Vasculogenic mimicryassay

Results and discussions AGS cells acquired chemoresistance as indicated by the increase of IC50 values in drug-treated cells with respect to AGS. Furthermore, MTT assay highlighted that there is not cross-resistance among 5FUr, CISr and TAXr. Supportive data is that cells are MDR1 negative.

Resistant cells showed an upregulation of Yamanaka factors either in qPCR and flow cytometer analysis, and particularly interesting is ALDH overexpression in 5FUr.

TWIST upregulation suggested the investigation of VM which resulted particularly enhanced in 5FUr cells which demonstrated their ability to form and sustain vessels up to 96 hours in the tube formation assay.

Markers of VM such Laminin γ2 and Ephrin A2 showed an increase in resistant cells and especially in 5FUr.

Conclusion One of the most interesting result is that 5FUr cells acquire stemness properties and are positive to the tube

formation assay suggesting that VM might be one mechanisms adopted by cells to avoid drugs exposure.

These findings suggest that acquisition of chemoresistance could cause a relapse of disease in which tumour cells take advantage of their capability to perform VM in order to self-sustain their growth and that may be cause of poor outcomes.

PO-473 QUANTIFICATION OF ERCC1-XPF COMPLEXES IN OVARIAN CANCER XENOGRAFTS WITH DIFFERENT SENSITIVITY TO CISPLATIN

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Introduction Epithelial ovarian cancer is the most lethal gynaecological cancer due to the development of resistance to a platinum based therapy. As DNA repair capacity is a key determinant for the cellular response to platinum (DDP) agents, DNA repair functional assays are required to study its relevance in DDP resistance. We set up a proximity ligation assay (PLA) to study the activity of nucleotide excision repair (NER) in patient derived ovarian carcinoma xenografts (PDXs) sensitive (S) and resistant (R) to DDP.

Material and methods Patient derived xenografts from fresh ovarian carcinomas were recently established in our laboratory. DDP antitumour activity was evaluated in most of the PDXs. Mice were sacrified when tumours reached 1,5–2 gr. Tumours were fixed in formalin and paraffin embedded (FFPE). PLA was performed on tumour slides, using DuolinkII reagents (Sigma-Aldrich) and following the manufacturer instructions. PLA detects the presence of the protein complexes ERCC1-XPF, that are quantified as foci per nucleus and represent a biomarker of NER activity. Images were acquired by Olympus Virtual Slider (Olympus) and analysed with ImageJ software. Statistical analysis was performed with GraphPad Prism7.

Results and discussions Our xenobank comprises PDXs with different response to DDP: MNHOC266 and MNHOC230 are very sensitive to the drug, while MNHOC315 is resistant. We also obtained three sublines resistant to DDP (MNHOC124R, MNHOC124LPR and MNHOC239R) starting from sensitive PDXs (MNHOC124S, MNHOC124LPS and MNHOC239S), after several *in vivo* drug treatments. Statistically significant higher level of ERCC1-XPF foci could be observed in MNHOC124R and MNHOC124LPR as compared to their sensitive counterparts. No differences were observed between MNHOC239S and R PDXs, even if the number of ERCC1-XPF foci in MNHOC239S were statistically higher than the ones observed in MNHOC124S and in MNHOC124LPS. MNHOC266 and MNHOC230 showed levels of foci comparable to those of MNHOC124S and MNHOC124LPS. mRNA and protein levels of the different isoforms of ERCC1 and of XPF were not different among the PDXs studied.

Conclusion PLA for the detection of ERCC1-XPF complexes was set up in FFPE xenograft tumour slides. These preliminary results highlight a possible link between DDP resistance and higher NER activity that need to be confirmed in a wider panel of PDXs. In addition, these data confirm the importance to develop functional assays to directly evaluate the activity of different DNA repair pathways to predict DDP activity.

Poster Presentation: Experimental/Molecular Therapeutics, Pharmacogenomics

PO-475 UNRAVELLING THE ROLE OF SIALYLATION IN TARGETED THERAPY RESISTANCE USING 3D CANCER MODELS

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Introduction In the scenario of personalised medicine, targeted therapies are currently the focus of cancer drug development. These drugs can block the growth and spread of tumour cells by interfering with key molecules of malignancy. Receptor tyrosine kinases, major targets for treatment of advanced gastric cancer, are transmembrane glycoprotein receptors whose glycan modifications have been shown to modulate the receptor activation. In this work, we have addressed the role of aberrant glycosylation, specifically of sialylation, in gastric cancer malignancy and therapy resistance.

Material and methods To mimic the *in vivo* tumour features, an innovative 3D high-throughput cell culture methodology has been developed for gastric cancer cells. After in-depth characterisation of the gastric cancer spheroids, we evaluated the resistance of cell models glycoengineered for key sialylation-related enzymes by subjecting the spheroids to tyrosine kinase inhibitors that are currently in clinical use and preclinical trials.

Results and discussions The phenotypical and functional parameters assessed disclose that cell sialylation leads to different cellular adhesive and invasive features. Furthermore, we demonstrate that by applying 3D cell culture methods, the cell glycocalix undergoes changes compared to the conventional 2D culture systems. Remarkably, our glycomodels display strikingly different cell cytotoxicity response to several inhibitors of major oncogenic receptors. Furthermore, distinct activation levels of cell receptors are observed by applying targeted therapy drugs, altogether suggesting sialylation as an important mechanism of cancer drug resistance.

Conclusion Our results demonstrate that cell glycosylation, in addition to being a key feature of tumour progression, plays a critical role in therapy resistance to tyrosine kinase inhibitors in gastric cancer. These findings shed new light on the mechanisms underlying cancer drug resistance and propose aberrant sialylation as new predictive biomarker for patients' treatment response.

PO-476 INCREASED ERK PHOSPHORYLATION AS A CANDIDATE DRIVER OF RESISTANCE TO THE EXPERIMENTAL CANCER DRUG AT13148

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Introduction The AGC family of serine/threonine protein kinases comprises a number of drug targets with therapeutic potential