432 Poster

Results from intermittent and continuous dosing schedules with sunitinib (SU) in previously treated, advanced non–small-cell lung cancer (NSCLC)

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Background In NSCLC, VEGF expression correlates with increased tumor angiogenesis and shortened survival. SU is an oral multitargeted tyrosine kinase inhibitor of VEGFRs, PDGFRs, KIT, RET, FLT3 and CSF-1R being evaluated in NSCLC as second-line therapy. The minimum plasma concentration of SU that inhibits the phosphorylation of VEGFR and PDGFR is ≥50 ng/mL (Mendel et al. Clin Cancer Res 2003;9:327-37). Single-agent activity of SU given via intermittent and continuous dosing schedules was assessed in advanced NSCLC. Methods This phase II study evaluated the efficacy, safety and pharmacokinetics (PK) of SU given in 2 patient (pt) cohorts: 50 mg/day for 4 wks followed by 2 wks off treatment (Schedule 4/2; 6-wk cycles) or 37.5 mg/day continuous daily dosing (CDD; 4-wk cycles). The primary endpoint was objective response rate. Eligible pts had stage IIIB/IV NSCLC and were ineligible or failed prior platinum-based chemotherapy. Results 110 pts were treated: 63 on Schedule 4/2 and 47 on Schedule CDD. Most pts (89%) had stage IV disease and adenocarcinoma (64% 4/2; 53% CDD). On Schedule 4/2, 7 pts (11%) had PRs, 18 pts (29%) had SD of ≥8 wks, median PFS was 12 wks and median OS was 23.4 wks. On Schedule CDD, 1 pt (2%) had a PR, 9 pts (19%) had SD of ≥8 wks, median PFS was 12.3 wks and median OS was 38.1 wks. Both regimens were generally well tolerated. Non-hematological grade 3/4 adverse events (AEs) of all causality included fatigue/asthenia (29% 4/2; 17% CDD), pain/myalgia (17% 4/2; 2% CDD), dyspnea (11% both) and nausea/vomiting (10% 4/2; 2% CDD). Hematological grade 3/4 AEs included thrombocytopenia (5% 4/2; 0% CDD) and neutropenia (5% 4/2; 9% CDD). At steady state, Schedule 4/2 median total drug trough concentrations (C_{trough}) were >80 ng/mL (nadir $_{trough}$ was ~50 ng/mL (44.4–65.3 ng/ml); both within the targeted range. Conclusions In heavily pretreated NSCLC pts, intermittent and continuous SU regimens showed promising single-agent antitumor activity with manageable toxicities, and achieved SU plasma concentrations predicted to inhibit VEGFR and PDGFR. Further evaluation of SU in NSCLC is ongoing in SU1087, a Ph III trial of SU 37.5 mg CDD plus erlotinib versus erlotinib alone.

433 Poster

HL-91 uptake and its correlation with molecular alterations in colon cancer cells. In vivo and in vitro studies

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Background: Colorectal adenocarcinoma is one of the main mortality causes by cancer. The decrease of oxygen concentration (hypoxia) constitutes a characteristic of solid tumors and has an important role in radiotherapy and in chemotherapy with some cytotoxic drugs. However, its quantification is only achieved by invasive methodologies which are difficult to use in routine basis. The nuclear medicine is able to give functional information after labeling specific molecules with gamma emitting radionuclides. The aim of this work is to correlate in vivo and in vitro hypoxia imaging with 99mTc- HL-91 homemade synthesized and molecular hypoxia alterations.

Material and Methods: A pharmaceutical formulation of HL-91 homemade synthesized was prepared in order to be labeled with 99mTc. Colon cancer cell cultures were incubated under hypoxic and normoxic conditions during 60 min. The 99mTc-HL-91 uptake was carried out during 120 min. The molecular analysis of tumoral cells was performed using flow cytometry in order to find out reactive oxygen species (ROS) (2,7-dichlorodihydrofluorescein diacetate), apoptosis (Annexin-V), and protein adducts reductively-activated by pimonidazole (Hypoxiprobe). Nude mice were injected with colon cancer cells. After three weeks, the animals were injected with pimonidazol. In order to perform a two phase dynamic acquisition the animals were injected with 37 MBq of 99mTc-HL-91. It was also performed the acquisition of static images until 240 min after radiopharmaceutical administration. The animals were sacrificed and several organs including tumor were excised and counted in a well count. The tumors were homogenized mechanically and chemically by collagenase IV in order to isolate tumoral cells. The isolated cells were submitted to the same molecular protocol referred above. Statistical analysis was made by a Spearman correlation.

Results: In vitro results show that 99mTc-HL-91 uptake is statistically different ($p<0.01$) at normoxic and hypoxic conditions. The molecular analysis show a few quantity of cells in apoptosis and an increase of ROS and pimonidazol-monoclonal antibody in cells incubated at hypoxic conditions when compared with normoxic ones. In vivo studies evidence a propensity for proportionality in 99mTc-HL-91 tumor/muscle and tumor/blood ratios and tumor size. The biodistribution studies show higher values of 99mTc-HL-91 tumoral uptake till 90 min after injection (%ID/g=4.4) and renal and hepatobilliary excretion. The molecular evaluation in the xenograft cells reveals also slight quantity of cells in apoptosis, increasing of ROS that correlates with tumor size, and increasing of pimonidazol-monoclonal antibody binding in the biggest tumors.

Conclusion: With our results we can conclude that the 99mTc-HL-91 is a good hypoxic imaging tracer what is confirmed by molecular studies, which can be translated to clinical use.

434 Poster

Relationship between tumor and plasma levels of telomerase reverse transcriptase mRNA in patients with colorectal cancer: implications for non-invasive monitoring of neoplastic disease

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Purpose. Colorectal cancer (CRC) is one of the most common cancers in western countries. Identification of circulating markers for CRC would optimize early stage diagnosis and the monitoring for disease recurrence. Expression of hTERT, the catalytic component of the telomerase complex, that extends telomeres at the end of eukaryotic chromosomes thus preventing cell senescence and death, is essential to the oncogenic process.Recent data suggest that hTERT mRNA in plasma may be a marker of neoplastic disease; however, no data are available comparing tumor and plasma hTERT levels.

Experimental procedures. 85 CRC tumors (25 stage I, 15 stage II, 15 stage III, and 30 stage IV), and the corresponding available adjacent non-cancerous mucosa (n=42(and plasma collected at the time of surgery (n=49) were analyzed. Control plasma samples were obtained from 43 age-matched healthy subjects. All hTERT transcripts (hTERT-AT) and transcripts encoding the functional protein (hTERT-FL) were quantified by real-time PCR. Results. hTERT-AT was found to correlate with hTERT-FL mRNA levels in tumors ($r=0.849$, $p<0.0001$) and both mRNAs increased along with tumor progression ($p<0.0001$). Conversely to controls, all but two samples from CRC patients were positive for hTERT mRNAs. Using a cut-off of 180 copies hTERT-AT/mL, the sensitivity and specificity of the assay for CRC were 92% and 100%, respectively. Furthermore, hTERT-AT mRNA levels in plasma significantly correlated with hTERT-AT mRNA levels in tumors ($r=0.702$, $p<0.0001$).

Conclusions. Overall, these findings indicate that quantification of circulating hTERT mRNA levels may be used as marker for the non-invasive monitoring of neoplastic disease.

435 Poster

Potencial prognostic and predictive factors in diffuse large B-cell lymphoma – the role of NFkappaB

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Introduction: Diffuse Large B-cell Lymfoma constitutes app.30-40% of non-Hodgkin lymphomas, Anon (1993), Armitage et al. (1998).The potencial prognostic and predictive factors in diffuse large B-cell lymfoma (DLBCL)

can be evaluated on clinical and histomorphologic grounds with contribution of immunoprofile and molecular profile (WHO 2001).

Design: Few studies used immunohistochemical expression detection for stratification of DLBCL, Barranas et al.(2002), Colomo et al. (2003), Linderoth et al. (2003), McClintock et al. (2003). Two major patterns of gene expression by gene array technology have been proposed, Alizadeh et al. (2000), Rosenwald et al. (2002), for dividing into prognostically significant subgroups in germinal centre (GC) a post-germinal centre (post-GC) DLBCL.

First publication discribing NFkappaB is mentioned in 1986 (Sen et al) and comprises family of transcription factors with important role in cell proliferation, antiapoptotic function and differentiation. NFkappaB signaling pathway is activated by numerous stimuli e.g. bacteria and viruses and is referred to as a central mediator of the immune response. NFkappaB signaling pathway regulates survival of normal and malignant B-cells by controlling the expression of cell death regulatory genes (Karin et al. 2002). Nuclear localization of NFkappaB leads to binding to the promoters of target genes, Li et al. (2002).

Summary: The sorting in GC and post-GC DLBCL group was used according the immunoprofile as decteted in GC and post-GC B-cells. CD10 is surface antigen that shows positive expression in non-neoplastic GC derived B-cells. Bcl6 is proposed protooncogeneic factor and shows nuclear localised positive expression in GC derived B-cells. MUM1 shows nuclear localised positive expression in post-GC B-cells. The GC group was signed if detected positive expression of CD10 and BCL6 and negative expression of MUM1. The post-GC group was signed if detected positive expression of MUM1 and negative expression of CD10 and BCL6.

Inactive NFkappaB heterodimers (c-REL/RELA, NFkappaB p50/p52, p65/RELA) reside in the cytoplasm, complexed with an inhibitor of IkappaB. The phosphorylation of IkappaB by IkappaK kinase results in the inhibitor`s dissociation from cytoplasmic NFkappaB heterodimer. Phosphorylated IkapaB is degraded via the proteasome. Free NFkappaB heterodimer translocates to the nucleus and induces the transcription of target genes. In our file of DLBCL we evaluated expression of NFkappaB (p50, p52, p65) and in some of cases in post-GC DLBCL group we detected it´s nuclear localisation.

Conclusion: Prognostic and predictive stratification of DLBCL in the group of post-GC DLBCL could be proposed to refine according to expression of NFkappaB family and also according to new available target of therapy, inhibitor of proteasome complex (bortezomib).

436 Poster

Mutations in the receptor tyrosine kinases in gastrointestinal stromal tumours from Russian patients

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BACKGROUND: Gastrointestinal stromal tumours (GISTs) are often show constitutive activation of either the KIT or PDGFRα receptor tyrosine kinases because of gain-of-function mutation. Aim of the study was to analyze KIT or PDGFRα mutations in GISTs from Russian patients and estimate their prognostic value.

METHODS: We have analyzed 90 DNA obtained from paraffin sections of GISTs in PCR with primers to KIT (exons 9, 11, 13, 17) and PDGRFA (exons 12, 14 and 18) followed with direct sequencing.

RESULTS: 96% of GISTs were CD117 positive. Seventy percents of GISTs harbor KIT mutations in exon 11, most of them were in-frame deletions or substitutions in the 5'-end of exon 11 in a region of 550-563aa. There was one gastric GIST with the deletion of 550-558aa that started in KIT intron 10 and involved the intron 10-exon 11 boundary. All GISTs with deletions in KIT exon 11 were highly malignant. Besides, 11% of GISTs had duplications of 1-12aa in the 3'-end of KIT exon 11 and were low malignant. These GISTs occurred predominantly in women over age 65. Mutations in KIT exon 9 (duplications of 502-503aa) were found in 27% of intestinal GISTs with aggressive behavior and metastases. Mutations in KIT exons 13 and 17 were found in one case each. PDGRFA mutations in exon 18 were found in 10% of GISTs. Typical substitution D842V was found only in two benign gastric GIST with epithelioid cell morphology, while other GISTs contain deletions, involving 842-846aa. There were wild-type KIT and PDGFRA in 13% of GISTs. We have found the additional mutations in KIT exon 17 (D820V and N822K) in GISTs treated with Gleevec that are associated with the secondary resistance to target therapy.

CONCLUSIONS: We have found some peculiarities in the variety of KIT and PDGFRA mutations in Russian patients, namely, high percent of GISTs with KIT exon 11 duplications, low percent of GISTs with D842V PDGFRA substitution, etc. The obtained results revealed some correlations between the type of KIT or PDGRFA mutation and clinico-pathologic parameters of GIST. They support the suggestion that mutational analysis of GIST is important for predicting GIST prognosis and the efficacy of target therapy.

POSTER SESSION

Oncogenomics

437 Poster

Breast cancer progression – genomic alterations in a continuum of stages

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Aim: To study the progression of genomic alterations in mammary epithelial cells from dense breast tissue to full blown cancers

Materials and methods: In total 127 breast tissue samples from three different series have been analyzed using 244K Agilent Human Genome CGH Microarrays (Santa Clara, CA). The samples comprise: 21 "normal" breast tissue (dense breast tissue, reduction mammoplasties, and normal tissue from mastectomies), 26 ductal carcinoma in situ (DCIS), and 75 breast carcinomas.

Results: Data analysis has been initiated using Nexus software from BioDiscovery (El Segundo, CA). Several of the "normal" samples show signs of alterations in areas known to be commonly altered in breast tumors. Hierarchical clustering revealed heterogeneity within each group of samples, suggesting further stratification. The "normal" samples clustered together with low aberrant tumor- and DCIS samples, while the highly aberrant tumor- and DCIS samples clustered together. Significance Testing for Aberrant Copy number (STAC) was applied to reveal common alterations within each group. Among the "normal" samples 118 genes were found to be in regions having significant frequency p-value ($p<0.05$) and being present in more than 35% of the samples, whereas the number was 105 for the DCIS and 245 for the tumors. Of these genes, 31 were overlapping between all groups. We identified group-specific events defined as at least 0.25-fold copy number change between two of the groups with a p-value < 0.05 using Fisher exact test. Genomic regions were found significantly altered in DCIS and breast cancer samples compared to the "normal" samples harboring 1341 and 2617 genes, respectively. Fewer genes (N=388) were identified in significantly altered regions when comparing breast cancer to DCIS. Enrichment analysis was performed to identify biological processes of significance.

Conclusion: Preliminary analyses reveal heterogeneity within each group and frequency of aberrations appears proportionally related to disease stage. Some genomic regions were found significantly changed in all groups. Most of these regions correspond to frequently observed copy number variations (CNVs) and might be candidate hotspots for early events of genomic rearrangements towards breast cancer development. More samples will be included and further stratification will be necessary to identify possibly important events that initiate and drive breast cancer carcinogenesis.

438 Poster

High frequency of copy neutral LOH in MUTYH-associated polyposis carcinomas

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Genetic instability is known to drive colorectal carcinogenesis. Generally, a distinction is made between two types of genetic instability: chromosomal instability (CIN) and microsatellite instability (MIN or MSI). Most CIN tumours are aneuploid, whereas MSI tumours are considered near-diploid. However, for MUTYH-associated polyposis (MAP) the genetic instability involved in the carcinogenesis remains unclear, as both aneuploid adenomas and near-diploid carcinomas have been reported. Remarkably, our analysis of 26 MAP carcinomas, using SNP arrays and flow sorting, showed that these tumours are often near-diploid (52%) and mainly contain