Eight Best Posters – Oral Presentations

OP 54

A genomic-based signature of response to chemotherapy in ovarian cancer fails to predict clinical outcome in two independent cohorts.

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Background: The development of numerous genomic signatures, from either pre-clinical assays or archived biospecimen, has suggested their potential clinical utility as biomarkers in cancer. However, due to (a) the technical precision of assays, (b) the potential for false discovery in high-dimensional data, and (c) tumor heterogeneity derived from multiple etiologies or from other key clinical factors; it is necessary to independently validate signatures in additional cohorts. Herein, we evaluate a previously-derived gene expression signature of response to chemotherapy (Dressman et al, 2007) in two ovarian cancer datasets.
Materials and Methods: The genomic signature for response to platinum-based therapy was evaluated in (1) 47 banked biospecimen at Duke University with known clinical response, and (2) 402 samples publicly available through The Cancer Genome Atlas (TCGA) project. Differences in labeling procedures and platform (Affymetrix U133a versus U133plus2.0 versus HT array) required normalization by gene standardization prior to validation. The association of genomic predictions to complete clinical response was evaluated using receiver operator characteristics and based on an a priori defined threshold. Time-to-death was evaluated by Kaplan–Meier plots and the logrank test. Differential expression within each cohort was evaluated using the empirical Bayes method, LIMMA. Signatures are evaluated using the resampling-based process, SAFE.
Results: The genomic prediction of chemosensitivity was not associated with response to treatment in the Duke ovarian cancer patients (AUC = 0.52, p = 0.8), nor in the TCGA samples (AUC = 0.48, p = 0.9). No difference in overall survival is observed (HR = 0.95, p = 0.7). Genes in the predictive signature do not show comparable levels of differential expression in the validation cohorts (SAFE p-value = 0.65 and 0.82) relative to the original test set (SAFE p = 0.004). A Venn diagram demonstrates almost no overlap in the patterns of differential expression within each cohort.
Conclusion: These results demonstrate the genomic signature of response to platinum-based therapy from Dressman et al (2007) failed to predict clinical outcome in two independent cohorts. Further, evaluation of differential expression from the three cohorts suggests the global patterns of gene expression from Affymetrix platforms are not supportive of developing a robust signature of chemo-responsiveness in ovarian cancer using untargeted approaches.

OP 50

Initial experience with the EANM accreditation procedure of FDG PET/CT devices

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Background: Quantitative FDG PET/CT studies in a multicenter setting are hampered by large variability in applied PET methodology, resulting in an up to 2 fold differences in results between centres. Therefore, in 2010 the European Association of Nuclear Medicine (EANM) published the European procedure guideline for PET tumour imaging with FDG. The guideline specifically aims at harmonizing quantification in multi-center studies. As SUVs are lesion size dependent, QC experiments measuring SUV as function of 'lesion' size are defined along with harmonizing criteria. To our best knowledge the European guideline is the first and only guideline with harmonizing performance standards and the EARL (EANM Research Ltd) accreditation program is the first initiative for implementation into practice.
Materials and Methods: A pilot accreditation program was launched by EARL from October 2010 till April 2011 in collaboration with and endorsed by the European Organisation for Research and Treatment of Cancer (EORTC). Eleven FDG PET/CT imaging sites (12 systems) that participated in an EORTC trial were included in the program. Accreditation QC included: (1) verification of PET/CT system calibration and uniformity using a uniform cylinder and (2) assessment of SUV recovery and image quality using a modified NEMA NU2 2007 phantom. After 3 months calibration QC was repeated to assess repeatability of calibration accuracy.
Results: After initial minor technical issues, e.g. related to data transfer, data entry errors and clock synchronization, all imaging sites met calibration accuracy requirements, i.e. global scanner calibration was within 10% without (visible) image artifacts. QCs for assessing SUV recovery allowed for harmonizing scanner performance to within the lower and upper (harmonizing) standards. Initially only for 2 imaging sites, recalibration or adjustment of reconstruction parameters was needed to achieve harmonized scanner performance.
Conclusion: The pilot study has shown the feasibility and successful execution of the EARL FDG PET/CT accreditation program in a multi-center setting. Retrospective analysis of clinical data collected in a Dutch trial demonstrated good correspondence in baseline SUV results between sites that were performing PET studies in accordance with the guideline, while SUV differed substantially (2 fold) for an imaging site that did not comply with the harmonizing standards. The results encourage to further spread the accreditation initiative across Europe.

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The co-development of a folate receptor molecular diagnostic imaging agent (99mTc-EC20) and folate receptor targeted drug conjugate (EC145) in the treatment of ovarian cancer patients

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Background: Molecular imaging provides a unique approach to assessing molecular markers for cancer treatment. Folate receptor (FR) is over-expressed in ovarian, breast, lung and colorectal cancers. Folate is required for cellular division and folate receptor expression is implicated as a negative prognostic marker for cancer. This study evaluates the potential use of a folate targeting SPECT imaging agent 99 mTc-EC20 to select ovarian cancer patients with high FR expression to be treated with EC145, a FR targeted-cytotoxic drug conjugate.
Materials and Methods: Women with platinum resistant ovarian cancer were scanned with 99mTc-EC20 to determine FR status and each measureable lesion was scored as either FR positive or FR negative. Patients were then randomized 2:1 to receive EC145 (2.5 mg IV t.i.w. weeks 1 and 3) + pegylated liposomal doxorubicin PLD (50 mg/m2 Ideal Body Weight (IBW) IV q 28 days) or PLD alone (50 mg/m2 IBW IV q 28 days). Progression free survival (PFS) was the primary endpoint. Exploratory analyses of the FR+ and FR− patient subgroups evaluated the use of 99 mTc-EC20 to select the patient population most likely to benefit from the treatment with EC145. The reproducibility of the EC20 reads was evaluated in an inter-reader agreement study.
Results: The majority of patients (80%) scanned with 99 mTc-EC20 were folate receptor positive. The final results on the reproducibility of the EC20 reads will be presented at the meeting. In the overall ITT patient population, PFS was statistically significant different in favor of the combination arm, 21.7 weeks compared with 11.7 weeks for patients treated with PLD alone (HR 0.626; 2-sided log-rank p value 0.031). The HR was improved to 0.547 (p value 0.044) in patients with at least one FR+ lesion and even further to 0.381 (p value 0.018) in patients with 100% FR+ lesions.
Conclusion: Women with platinum resistant ovarian cancer have very poor prognosis. No approved treatments have demonstrated an improvement in PFS or OS. 99 mTc-EC20 provides a reliable and reproducible imaging technology to select FR+ ovarian cancer patients that benefit most from the treatment with EC145. Statistically significant increase in PFS was seen in the combination of EC145 + PLD versus PLD alone, with increased benefit seen in patients with FR+ tumors. 99 mTc-EC20 and EC145 are the first folate receptor molecular imaging and drug combination to demonstrate a statistically significant improvement in PFS in platinum resistant ovarian cancer.

OP 29

Development of a genomic-clinical classifier model for predicting clinical recurrence in patients with localized prostate cancer

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Background: The efficient delivery of adjuvant and salvage therapy after radical prostatectomy in patients with prostate cancer is hampered by a

lack of biomarkers to assess the risk of clinically significant recurrence and progression. Better prognostic and predictive tools are required to guide clinical management and reduce overtreatment.
Materials and Methods: Patient specimens from the Mayo Clinic Radical Prostatectomy Registry were selected from a nested case–control cohort with 14 years median follow-up. RNA expression levels from FFPE tumor specimens were measured with 1.4 million feature oligonucleotide microarrays. Patients were divided into a training set (n = 359) for variable selection using cross-validated lasso logistic regression and model building with a Random Forest classifier. The final genomic-clinical classifier (GCC), a multivariate model consisting of 43 expressed markers (genes and non-coding RNAs) and pathology review Gleason score was compared to clinical variables and a multivariate clinical model (CM) combining age, PSA, Gleason score, stage and surgical margin status to predict early clinical recurrence (positive bone or CT scans within 5 years after biochemical recurrence). The receiver-operator characteristic area-under-the curve (AUC) metric was used to evaluate GCC and the clinical models in an independent validation set (n = 187) of prostatectomy patients.
Results: In the training subset, the GCC had AUC of 0.93, while Gleason and the CM models had AUC of 0.73 and 0.76, respectively. Overall in the validation set, the GCC model had an AUC of 0.77, which compared to the AUCs of 0.65 for Gleason and 0.67 for CM models in predicting clinical recurrence. However, in contrast to the clinical models only the GCC maintained consistent performance in high-risk (node negative and pT3 and/or positive margin) patients (n = 107). In this group, the GCC had a validated AUC of 0.81 whereas the Gleason and CM models had AUCs of only 0.56 and 0.66, respectively.
Conclusion: We have developed a combined genomic-clinical classifier that shows improved performance over clinical models alone for the prediction of clinical recurrence, notably in high-risk prostatectomy patients that are the most obvious candidates for adjuvant therapy. We are further testing the performance of this classifier and its usefulness in guiding decision-making for the adjuvant therapy setting in additional validation studies.

OP 16

Identification of JAK2/STAT3 as a novel therapeutic target in Kras mutant colorectal cancer models

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Background: STAT3 is activated by Janus kinases (JAKs), which are recruited and activated by numerous cytokine receptors, receptor tyrosine kinases (including EGFR) and non-receptor tyrosine kinases (such as Src). A recent study has shown that high STAT3 activation is positively associated with adverse outcome in colorectal cancer, supporting its potential role as a therapeutic target. Kras mutations occur in 40−45% of colorectal cancer (CRC) patients and confer resistance to EGFR targeted therapies. The aim of this study was to evaluate JAK2/STAT3 signalling as novel Kras synthetic lethal interactions in CRC.
Materials and Methods: STAT3 and JAK2 inhibition was obtained using siRNA and small molecule approaches. Analysis of cell viability was carried out using MTT assay, apoptosis was measured using Western blotting and Flow cytometry and migration using xCELLigence system. The isogenic KrasMT/WT HCT116 cell line model and a panel of KrasWT&MT CRC cells were used.
Results: Using different siRNA sequences, we found that silencing of STAT3 and JAK2 was lethal in KrasMT HCT116 cell line compared to its KrasWT clone, and these results were confirmed using the small molecule JAK2/STAT3 inhibitor 'cucurbitacin'. Similar data were obtained in our panel of KrasMT CRC cells. Interestingly, significant higher constitutive levels of pSTAT3 were observed in KrasMT HCT116 cells compared to its WT clone. Combination of STAT3 or JAK2 silencing with MEK1/2 inhibition or chemotherapy (5-FU, oxaliplatin) resulted in synergistic decreases in cell viability and increase in apoptosis in KrasMT HCT116 cell line and this was associated with potent increase in STAT3 activity following MEK1/2i or chemotherapy. Furthermore, STAT3 silencing resulted in strong decreases in cell migration in KrasMT HCT116 cell line.
Conclusion: These results indicate that KrasMT CRC models are dependent on JAK2/STAT3 pathway for survival. We are now further evaluating the effect of JAK2/STAT3 inhibition in combination with MEKi/chemotherapy in in vivo models.

OP 24

Somatic allelic selection in the tumor as an indicator of cancer relevance: insights from statistical mining of the Cancer Genome Atlas data

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Background: We sought to leverage the unprecedentedly rich, high-dimensional data available from the Cancer Genome Atlas (TCGA) to uncover genetic variants that are selected for somatically in tumor cell.
Materials and Methods: We hypothesized that certain genetic variants may give a proliferative advantage to the cell when be promoted somatically via copy number lesions or methylation-based silencing of a wild-type counterpart. To test for these phenomena, we integrated TCGA data from four platforms − single nucleotide polymorphism (SNP) arrays, expression arrays, methylation arrays, and "next-generation" sequencing − to query for preferential allelic selection of specific variants. Statistical tests for recurrent somatic promotion of one inherited parental SNP haplotype over another (via amplification, loss, or promoter methylation) were developed and applied to the SNP and methylation array data. Significant regions were tested for concordant effects on mRNA expression. Deep sequencing data enabled detection of somatic mutations and rare variants that may be the true targets of allelic selection.
Results: Our analysis uncovered evidence at multiple loci of strong selective pressures in the tumor environment. In many cases, copy-number aberration and promoter methylation were both utilized as selective mechanisms at the same genomic locus. Some germline cancer susceptibility variants reported by previously-published GWAS displayed signals of preferential somatic selection over their allelic counterparts, shedding light on the mechanisms-of-action for tumor predisposition loci. Furthermore, deep sequencing of the affected regions yielded new mutations in known and novel cancer-related genes. Included among the novel genes are intriguing candidates for follow-up functional studies that are now underway.
Conclusion: Our study demonstrates one approach to separating the "driver" molecular variants from the background "passenger" noise, pinpointing candidate diagnostic markers in cancer. More generally, our results exemplify insights into cancer biology that may be obtained via statistical mining of complex, multi-faceted data sets such as those generated by TCGA.

OP 75

An unbiased shRNA based lentiviral screen identifies tyrosine kinases that are important for survival and radioresistance in Head and Neck Squamous Cell Carcinoma

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Background: The radiation oncologist's interest in modulation of receptor tyrosine kinase (RTK) signaling in Head and Neck Squamous Cell Carcinoma (HNSCC) was recently invigorated by the therapeutic success of EGFR targeted therapy. Nonetheless a large part of the RTK-family remains uninvestigated. A high throughput screen was performed on two HNSCC cell lines (SCC61 and SQD9) to identify other tyrosine kinases with possible role in radioresistance or cell survival.
Materials and Methods: We used a pooled shRNAmir library containing over 270 lentiviral vectors targeting the tyrosine kinase family. In this way − after viral transduction − each cell in the cell population contained a different shRNA, knocking down the expression of a specific tyrosine kinase. Because each viral vector contains a barcode, the presence of each shRNA in the cell population could be tracked by sequencing before and after irradiation.
Results: Using this shRNA screen, we identified several tyrosine kinases with potential importance in the proliferation and/or resistance to irradiation of the SCC61 and SQD9 cell lines. Of note, FLT1 was identified as being important for radioresistance in both of our HNSCC cell lines. Expression of this kinase was demonstrated using qPCR. Its role in survival and radioresistance was validated using 2 different FLT1 siRNA's in a sulphorodamine B assay or clonogenic assay respectively. FLT1 silencing was associated with downregulation of phospo-ERK. Sunitinib malate, a TKI with known anti-FLT1 activity, resulted also in downregulated ERK-pathway activity and a corresponding decrease in cell survival. Sequencing revealed no mutations in this receptor, but overactivity could be explained by strong autocrine production of the receptors ligands VEGFA and VEGFB. In a next step, expression of FLT1 was examined in human tissue by immunohistochemistry. FLT1 expression was demonstrated in all 13 laryngeal cancers examined and autocrine ligand expression was also documented. Interestingly, the expression of FLT1 seemed to be more pronounced in the tumor than in the surrounding normal squamous epithelia.