head-and-neck, and kidney. We have previously shown that SPARC expression appeared to correlate with response to nanoparticle albumin-bound (*nab*) paclitaxel (Abraxane) in head-and-neck as well as pancreatic cancer patients, and increases in SPARC suggested improved response to *nab*-paclitaxel in tumor models. To further define the role of SPARC, recombinant human SPARC (rhSPARC) was characterized to identify its angiogenic domain and its albumin-binding domain.
Materials and Methods: Recombinant human SPARC and genetically engineered variants were expressed and purified using HEK 293 cells maintained in hollow fiber bio-reactors. The albumin binding site on SPARC was defined using a solid phase albumin binding assay with Alexa 488-labeled BSA binding to rhSPARC immobilized onto PVDF in presence of increasing concentration of various SPARC derived peptides. The angiogenic activity of rhSPARC and its variants was evaluated using a HUVEC tube formation assay.
Results: The SPARC albumin binding assay revealed a pattern of saturable and specific binding with an estimated Kd of 700 mM, very near the known plasma concentration of albumin (600 μM). Cathepsin K-digested SPARC and competitive binding with SPARC-derived peptides identified the albumin-binding domain to be at aa209–223 of the SPARC C-terminus. In the HUVEC tube formation assay, wild type SPARC was angiogenic at 1 and 10 μg/ml and anti-angiogenic at 100 μg/ml. A proteolytic degradation product of SPARC (SPARC-d) lacking the C-terminus of SPARC was anti-angiogenic at 1, 10, and 100 μg/ml, demonstrating the angiogenic domain was located to the SPARC C-terminus.
Conclusions: SPARC exhibited an affinity for albumin at physiologic concentrations, potentially leading to the targeting of nanoparticle albumin-bound drugs *in vivo*. The identification of SPARC angiogenic domain supports the role of SPARC in tumor angiogenesis and invasiveness. This finding provides useful information in the use of SPARC as a therapeutic target.

1004 ORAL

SPARC may be a predictive biomarker of response to nab-paclitaxel

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Background: *nab*-Paclitaxel (Abraxane®), an albumin-bound nanoparticle form of paclitaxel, increases tumor accumulation of paclitaxel through binding of albumin to SPARC. Recently we have shown that SPARC overexpression may correlate with response to *nab*-paclitaxel monotherapy in head and neck cancer (Desai et al. 2009, *Trans Onc* **2**, 59−64). Here we described the further development of SPARC IHC as a companion biomarker test for *nab*-paclitaxel using data from three different clinical trials: 1) CA-040: phase I/II metastatic pancreatic cancer trial of gemcitabine plus *nab*-paclitaxel; 2) N057E: phase II unresectable stage IV melanoma trial of carboplatin and *nab*-paclitaxel; and 3) BRE73: phase II neoadjuvant breast cancer trial of gemcitabine, epirubicin, and *nab*-paclitaxel.
Materials and Methods: A panel of antibodies against SPARC were evaluated by IHC in a CLIA approved laboratory and scored by an independent pathologist. The data were analyzed to evaluate a correlation between therapeutic responses and SPARC positivity. Level 3 SPARC staining by immunohistochemistry was considered SPARC+.
Results: For pancreatic cancer, 63 pts received treatment. In 27 pts with both tumoral SPARC staining and evaluable response data, SPARC+ pts (8/27, 30%) were more likely to be responders (6/8, 75%) than SPARC- pts (5/19, 26%), $P = 0.03$, Fisher's exact test. Median progression-free survival (PFS) increased from 4.8 months for SPARC- pts (22 pts) to 6.2 months for SPARC+ pts (9 pts). For melanoma, 76 pts received treatment with 41 pts having SPARC staining. Of these 83% (34/41) pts were positive for tumoral SPARC, with some evidence that OS may be longer with tumoral SPARC positivity (10.0 vs 12.8 mo; SPARC- vs SPARC+). For neoadjuvant breast cancer, 123 pts received treatment with 72 pts having SPARC staining. A breakdown of SPARC staining revealed that the improvement in PFS was associated with tumoral SPARC (25% failure at 601 vs. >950 days, SPARC- vs. SPARC+).
Conclusions: Preliminary data from three different clinical trials including melanoma, pancreatic, and neoadjuvant breast cancer are supportive of the hypothesis that SPARC may be a predictive biomarker of response to *nab*-paclitaxel. Further validation of this hypothesis is being conducted in phase III clinical studies.

1005 ORAL

High resolution microarray copy number analysis (array CGH) suggests that determination of HER2 amplification by FISH (FISH+) is inaccurate in human breast cancer specimens that are HER2 2+ by immunohistochemistry (IHC2+)

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Background: Although 20−30% of human breast cancers are HER2 "positive" by IHC and FISH, these methods have well-documented limitations. Furthermore, clinical studies evaluating HER2 status as a predictor of sensitivity to HER2-targeted, anthracycline-containing, and taxane-containing regimens have been inconsistent, perhaps because of inaccuracies in HER2 measurement. For this reason, we compared HER2 status by array CGH with FISH in 99 archived, formalin-fixed, paraffin-embedded primary breast cancer specimens that were previously determined to be IHC2+. The advantages of array CGH include its ability to distinguish broad (such as whole-arm duplication) from local amplification events, and to distinguish true HER2 amplification from loss of chromosome 17 centromeres as an etiology for altered HER2/CEP ratios.
Methods: The specimens were evaluated by IHC and by FISH at Memorial Sloan-Kettering Cancer Center and by array CGH on Agilent 244K feature arrays at the Cold Spring Harbor Laboratory in a double-blinded manner. FISH+ was defined as a HER2: chromosome 17 centromere signal $\geqslant 2.0$.
Results: Array CGH and FISH demonstrated a high degree of concordance in specimens without HER2 amplification by FISH (Table). However, only 10 of the 22 FISH+ specimens were also HER2 amplified by array CGH. Furthermore, none of the 5 specimens with FISH scores of 1.8 to 2.2 demonstrated amplification by array CGH.
Conclusions: Our results suggest that when examined by the higher resolution technique of array CGH the assessment of HER2 amplification by FISH is inaccurate in IHC 2+ breast cancer. Improved HER2 assessment should permit more efficient use of clinical resources, enhanced development of newer targeting agents, and decreased uncertainty and anxiety on the part of both clinicians and patients. A larger series and correlation of HER2 status by array CGH with trastuzumab response are underway.

Table: HER2 status by ROMA versus FISH in HER2 2+ by IHC human breast cancer specimens

HER2 by FISH (N = 99)	HER2 by array CGH (N = 99)	
	Amplified	Non-amplified
Amplified	10	12
Non-amplified	2	75

1006 ORAL

The anti-IGF-IR antibody figitumumab (CP-751,871) is active in patients with lung adenocarcinoma undergoing epithelial-to-mesenchymal transition

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Background: Invasiveness is a hallmark of carcinomas mediated by Epithelial-to-Mesenchymal Transition (EMT). Growth factor receptors have been implicated in the regulation of EMT in NSCLC. Figitumumab (F), a specific IGF-IR inhibitor, has shown phase 2 activity in NSCLC in some histologies, i.e. squamous cell (SqCC) and adenocarcinoma (AD), but not others, i.e. large cell/NOS (LC/NOS). This ancillary study was undertaken to investigate these findings and inform the design of phase 3 trials of F in advanced NSCLC.
Methods: Tissues samples were obtained from 217 NSCLC pts with known outcome, including 52 pts treated in F trials. A total of 536 serial blood samples from 159 F pts were analyzed. Gene expression profiling was conducted in 35 NSCLC cell lines. Protein expression was assessed using an automated quantitative analysis (AQUA®) system. Plasma marker levels were determined by ELISA.

Results: IGF-IR expression ($p = 0.02$) and decreased IGFBP-3 ($p = 0.009$) were independent predictors of sensitivity to F in NSCLC cell lines. These pharmacologically defined groups matched 2 of 3 phenotypes identified by tissue and plasma marker profiling of NSCLC pts: Epithelial (differentiated) and EMT. A third phenotype, mesenchymal (undifferentiated), did not appear responsive to F. The epithelial high E-cadherin-expressing phenotype included most (73%) SqCC tumors. These tumors exhibited high levels of IGF-IR ($p = 0.05$), low vimentin levels, low free (unbound to IGFBPs) plasma IGF-I (fIGF-I), and an association between IGF-2 and its inhibitor, the IGF-2R ($p = 0.02$). Mesenchymal-like NSCLC was represented by LC/NOS that expressed the highest levels of vimentin ($p < 0.001$) and low receptor and ligand levels. The transitional EMT phenotype was observed in the majority of AD pts (63%) who had high plasma fIGF-I levels ($p = 0.06$). fIGF-I correlated directly with vimentin ($R = 0.475$, $p = 0.03$) and inversely with E-cadherin ($R = -0.524$, $p = 0.02$), indicating ligand-driven EMT, and it was predictive of F clinical benefit. Median PFS were 2.73 and 6.53 months for chemotherapy alone and chemotherapy with F 20 mg/kg, respectively, in pts with high fIGF-I levels ($p = 0.001$) while no significant treatment effect of F was observed in the low ($\leqslant$0.54 ng/mL) fIGF-I group.
Conclusions: High IGF-IR expression characterizes SqCC while IGF-I driven EMT is a key element in the biology of AD NSCLC. Both IGF-IR and fIGF-I levels may contribute to the identification of NSCLC pts who could benefit from F therapy.

1007 ORAL

Myelosuppression and kinase selectivity of multikinase angiogenesis inhibitors

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Background: Vascular endothelial growth factor receptor (VEGFR) tyrosine kinase inhibitors often inhibit other kinases, besides VEGFRs, which may contribute to their adverse event profiles. Myelosuppression has been observed with several multikinase angiogenesis inhibitors in clinical studies, although the frequency and severity varies among the different agents. The present study evaluated differences in kinase selectivity of pazopanib, sorafenib and sunitinib and their effects on ligand-induced human bone marrow colony formation unit (CFU).
Methods: Kinase selectivity of pazopanib, sorafenib and sunitinib was evaluated using Upstate kinase profiler against 242 kinases at 0.3 and 10 mM. Ki^{app} was determined against key tyrosine kinases for all 3 compounds. Cellular potency against VEGFR2, PDGFRb, c-Kit and Flt-3 was measured using receptor autophosphorylation assay. Inhibition of CFUs in the presence of GM-CSF, SCF and Flt-3 ligand was evaluated and correlated with their kinase selectivity profile.
Results: In the Upstate kinase profiler assay, sunitinib inhibited 49 additional kinases, besides VEGFR, PDGFR and c-Kit, at IC_{50} within 10-fold of VEGFR2, whereas pazopanib and sorafenib inhibited 7 and 10 additional kinases, respectively. Sunitinib was more potent against Flt-3 compared to VEGFR2 in both enzyme and cellular assays. Pazopanib was 25 to 100-fold less active against Flt-3 compared to VEGFR2 in enzyme and cellular assays. Sunitinib inhibited the human CFUs induced by SCF and/or Flt-3 ligand at 7 to 16-fold lower IC_{50} than that required for inhibition of VEGFR2 autophosphorylation in endothelial cells. Pazopanib and sorafenib had >10-fold higher IC_{50} in the CFU assays compared to VEGFR-2 autophosphorylation.
Conclusion: Sunitinib inhibits c-Kit and Flt3 tyrosine kinases at potency $\geqslant$ to VEGFR2, whereas sorafenib has similar potency against the 3 kinases and pazopanib is less potent against Flt3 compared to VEGFR2 and c-Kit. Sunitinib inhibits proliferation of bone marrow cells in the presence of SCF and Flt-3 ligand more potently than VEGF-induced VEGFR2 phosphorylation in endothelial cells. These results provide a potential explanation for the observed differences in myelosuppression observed with various multikinase angiogenesis inhibitors in the clinic.

Poster discussion presentations
(Wed, 23 Sep, 17:00–18:00)
Basic Science/Translational research

1008 POSTER DISCUSSION

Mesenchymal Stem Cell (MSC) secretion of TGFβ and VEGF stimulates Epithelial to Mesenchymal Transition (EMT) in breast cancer cell lines

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Background: Adult Mesenchymal Stem Cells (hMSCs) are bone marrow-derived multipotent cells that have the ability to self renew and differentiate into multiple cell types including bone and cartilage. Chemokines and growth factors secreted by MSCs have been reported to have a significant effect on tumor growth and development. The aim of this study was to examine the effect of MSC secreted factors on breast cancer cell proliferation and gene expression, and to potentially identify the factors mediating these effects.
Materials and Methods: MSCs were harvested from healthy volunteers and grown in a six-well plate format for collection of conditioned medium, containing all factors secreted by the cells. Indirect co-culture was established by culturing breast cancer cell lines (T47D, MDA-MB-231, SK-BR-3) in MSC conditioned medium. Cell proliferation was assessed at 72hrs using an Apoglow® assay and cells were harvested for analysis of gene expression by RQ-PCR. Factors potentially mediating observed changes in gene expression were identified by repeating the experiments in the presence of antibodies targeting Vascular Endothelial Growth Factor (VEGF) and Transforming Growth Factor b-1 (TGFb-1).
Results: Following indirect co-culture with MSCs, all three breast cancer cell lines displayed a downregulation of proliferation, with the greatest decrease seen in the T47D cells. Analysis of gene expression revealed a significant increase in expression of a panel of genes associated with Epithelial to Mesenchymal Transition (EMT) in both T47D and SK-BR-3 cell lines. In both the SK-BR-3 and T47D cell lines there was significant upregulation in expression of the mesenchymal marker Vimentin (range 158–276 fold), the anti-apoptotic transcription factor Snail (range 4–7 fold) and N-Cadherin (range 9–32 fold). Inclusion of an antibody to VEGF in the MSC- conditioned media significantly reduced the change in Vimentin expression in both cell lines. MSC secreted TGFb-1 was also shown to play a role in upregulation of N-Cadherin expression in the SK-BR-3 cell line.
Conclusion: Mesenchymal stem cells have a distinct paracrine effect on breast cancer epithelial cells, which is mediated at least in part by VEGF and TGFb-1. These factors play an important role in the metastatic cascade and may represent potential therapeutic targets to inhibit MSC-breast cancer interactions.

1009 POSTER DISCUSSION

A role for auxiliary TGF-beta receptor endoglin as a modulator of tumor progression

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Background: We and others have provided evidence for a direct role of endoglin in malignant progression. Thus, reduction of endoglin expression in endoglin heterozygous ($Eng^{+/-}$) mice had a double effect on two-stage chemical skin carcinogenesis, by inhibiting the early appearance of benign tumors (papillomas), but increasing progression to spindle cell carcinomas (SpCC).
Materials and Methods: Swiss albino mice were used for induction of tumors by initiation with DMBA and promotion with TPA for 15 weeks. Endoglin expression has been checked by qRT-PCR, Western-blot and immunohystochemistry. Luciferase reporter genes have been used to study TGFbeta pathway status. Cell growth assays "in vitro", and "in vivo" to study tumorigenicity in immunodeficiency mice
Results: Our finding that endoglin is expressed both in epidermal basal keratinocytes and in their appendages (hair follicles and sweat glands), led us to study the expression of endoglin during the different stages of chemical mouse skin carcinogenesis: benign papilloma, squamous cell carcinoma (SCC), and spindle cell carcinoma (SpCC). Endoglin undergoes a proteolitic cleavage (shedding) during the SCC to SpCC progression,