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.

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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.