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,