requiring a better selection of patients who should benefit from targeted therapies.
Methods: We evaluated the effects of the SRC tyrosine kinase inhibitor dasatinib (BMS-354825) on melanoma cell proliferation in relation with NRAS and BRAF mutation status and key proteins involved in melanoma signalling pathways.
Results: We examined 33 melanoma cell lines and found that 7 lines were highly sensitive to dasatinib (IC50 < 10^{-9}M), 13 were moderately sensitive (IC50 from 10^{-8} to 10^{-6}M) and 13 were resistant (IC50 > 10^{-5}M). All highly sensitive lines had no mutation on BRAF or NRAS, while 69% of the moderately sensitive and 69% of the resistant cell lines had activating mutations. All highly sensitive lines expressed high cKIT levels, whereas others had undetectable cKIT expression. Importantly, cKIT appeared as an effective target of dasatinib since the cell lines which were the most sensitive to dasatinib were also the most sensitive to the specific cKIT inhibitor ISCK03. Moreover, in all sensitive cell lines, dasatinib dramatically inhibited the phosphorylation of ERK and AKT, while it had not effects in the mutated lines, suggesting a selective effect on proliferation/survival of cKIT expressing cells, although NRAS/BRAF mutations are likely to render these cells much less dependant on cKIT signalling for their survival. We are currently evaluating this aspect as well as the effectiveness of dasatinib in combination with other agents in the case of tumor resistance.
Conclusions: We found that dasatinib was highly effective to inhibit cell proliferation in a subgroup of melanoma lines characterized by wild-type NRAS/BRAF and high cKIT expression, and this will be the basis of a clinical trial in a selected group of melanoma patients.

255 ORAL

Transcriptome sequencing of upper aerodigestive tract cancer cell lines to reveal potential therapeutic targets

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Background: We applied RNA-seq − a powerful technology that allows to obtain sequence and expression information simultaneously on a transcriptome-wide basis- to 30 upper aerodigestive tract cancer cell lines to conduct mutational profiling and enhance the knowledge of the underlying tumor biology.
Methods: RNA from 30 upper aerodigestive tract cancer cell lines was extracted and sequencing libraries constructed. Samples were analyzed using an Illumina Genome Analyzer with a paired end module (54 or 75 base read length). Raw data was processed with a proprietary data pipeline from the White Lab. Potential mutations were identified by subtracting common SNVs (e.g. dbSNP, population allele frequency), assessing evolutionary conservation, and evaluating ancestral alleles identified from multiple sequence alignments. These SNVs were then parsed via in house scripts to determine whether the SNVs were present in coding regions, 3′UTR, 5′UTR, or in splice acceptor/donor sites. The coding SNVs were further parsed to determine which SNVs result in non-synonymous changes. RNA-Seq expression data was analyzed using R scripts and Partek Genomics Suite. Pathway analysis was performed using GeneGO Metacore.
Results: 1 GB to 4 GB of data were obtained per sample. Between 700 and 3000 nsSNVs were identified, as well as a large number of alterations in the 3′ and 5′ untranslated regions. Genetic alterations in several commonly mutated genes were identified including TP53, ErbB2, and EGFR. Alterations were enriched in pathways commonly involved in cancer including cell cycle control, cytoskeleton, and receptor tyrosine kinases.
Conclusion: Cancer transcriptome sequencing is a promising approach for identifying mutations and obtaining expression analysis simultaneously. Transcriptome sequencing holds promise as a readily available platform for assessing potential treatment targets in a specific tumor.

3LB LATE BREAKING ORAL

MEDI-573, a dual IGF-1/-2 neutralizing antibody, blocks IGF-1R and IR-A signaling and maintains glucose homeostasis in a Phase 1 study for advanced solid tumors

For full abstract, see p. 4.

Thursday, 18 November 2010 16:30–18:30

PLENARY SESSION 7

Selected tumours as a niche for targeted therapies

256 INVITED

Emerging therapies in melanoma

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The development of systemic therapies with and impact on overall survival in melanoma has been stagnant for decades. Both in the non-targeted as well as in the targeted therapy arena a number of new drugs with completely different mechanisms of action are active in melanoma with excellent chances to be approved in the nearby future. The imminent candidates are anti-CTLA4 antibody ipilimumab, which has recently been demonstrated to significantly improve survival in melanoma patients with advanced metastatic disease, and the highly selective BRAF-inhibitor PLX4032, which causes significant regression of metastatic lesions in 80% of patients with BRAF-mutated melanomas, and is currently being evaluated for its impact on overall survival. So on the one hand significant developments in the field of immunomodulation and on the other hand in mutation driven signaling pathway inhibitors. Moreover in each class various other molecules are under development with very good perspectives. The new discoveries will bring an avalanche of trials and rational combination approaches unlike anything seen before. It's a new world in melanoma and the key developments in creating that world will be presented.

257 INVITED

Recent advances in the treatment of refractory thyroid cancer: the use of kinase inhibitors

M. Schlumberger. *France*

Abstract not received

258 INVITED

Biology and treatment of thymoma

G. Giaccone. *USA*

Abstract not received

259 INVITED

Non small cell lung cancer molecular subtypes: therapeutic implications

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NSCLC is currently being revisited on the basis of modern molecular portraits that allow the identification of new molecular subtypes.
Larges scale studies have identified frequent mutation mainly in TP53, RB1, CDKN2A, and STK11 tumor suppressor and in EGFR, KRAS and NRAS oncogenes. Many other molecular abnormalities have been reported at lower frequencies in genes such as PI3K, PTEN, AKT1, MDM2, APC, FGFR, HER2, KDR, MET, CTNNB1, ATM, BRAF, AKT1 and more recently ALK.
The most frequent kinase mutations were identified in EGFR receptor, a target of many recently developed molecules, in 10 to 20% of NSCLC. The majority of *EGFR* mutations occurred in exon 19 (small deletion) or in exon 21 (single point mutation, L858R). These activating mutations are associated to responsiveness to tyrosine kinase inhibitors. On the other hand, several mutations in exon 20 (T790M or small insertion) seem to confer resistance to such treatments. Patients harbouring EGFR mutation are highly sensitive to EGFR inhibitors, that have demonstrated a PFS advantage when compared to standard chemotherapy in the front-line setting.
Epidemiologic characterization of EML4-ALK translocations is ongoing but it seems to be a rare aberration, most common in non-smokers or light-smokers with the adenocarcinoma subtype of NSCLC (and signet ring features), forming a distinct subgroup from patients harbouring EGFR,

KRAS, mutations. The frequency of EML4-ALK fusion transcripts is around 5% or less in Caucasians patients. Crizotinib (PF-02341066) is an ALK and MET inhibitor that has demonstrated outstanding activity in monotherapy in patients with EML4-ALK translocations: 57% objective response in 82 patients with a median duration of treatment of 5,7 months and a predicted 67% PFS rate at 6 months.
Many other abnormalities linked to tyrosine kinase receptors could be used for selection of specific therapy such as amplification or activation mutation of HER2, HER3, HER4, FGFR1, FGFR2, KDR .... However correlation of presence of such abnormalities and clinical response are still not firmly documented, although some interesting case reports have been documented. Table 1 provides a summary of specific alterations and their potential corresponding drug. DNA repair markers are also potential predictors of chemotherapy bases therapies (ERCC1, MSH2, BRCA1, PARP).

Table 1

Molecular alteration	Potential Drugs
EGFR mutation	Erlotinib, gefitinib New pan-HER inhibitors
EML4-ALK translocation	Crizotinib
HER2 mutation or amplification	Trastuzumab Lapatinib
PI3K mutation or amplification	GDC-0941 XL-147 XL-765 PX-866 BEZ-235 BKM120
MET amplification	XL184 ARQ917
RAS and RAF mutations	Sorafenib AZD6244; GSK1120212; AS703026

Thursday, 18 November 2010

Poster Sessions

Animal models

260 POSTER

Multi-modality in vivo imaging of bone metabolism and tumor growth in a mouse model of bone metastasis

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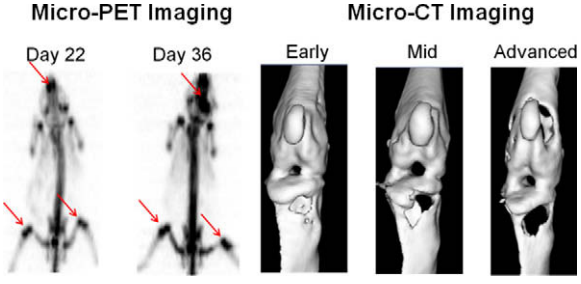
Background: The ability to visualize and quantify early stages of bone involvement in mouse models of bone metastasis would provide a platform for development of new agents targeted at inhibition or treatment of bone metastases. While micro-CT imaging provides a qualitative assessment of bone erosion and bioluminescence imaging using luciferase expressing tumor lines enables monitoring of tumor burden, there is a need to track bone lesion progression as well.
Materials and Methods: Optical imaging using biphosphonate fluorescent probes and 18F-NaF PET imaging both provide readouts for hydroxyapatite (HA) activity, a key feature of bone metastasis. Female nu/nu mice underwent intracardiac inoculation with MDA-MB-231-luc-D3H2LN human mammary adenocarcinoma cells (10^5 cells in 100 µl). On Day 14, all mice were imaged using bioluminescence and enrolled on study based on incidence of luciferase signals at bone sites. Subsequent bioluminescence imaging was used to monitor growth of bone metastases. Micro-CT (see figure: right panel) was used to assess the extent of corresponding bone lesions. 18F-NaF PET imaging (45 min uptake) (see figure: left panel), and fluorescence imaging (24 h after Osteosense 750 administration) were used to characterize HA activity.
Results: Both 18F-NaF PET imaging and fluorescent imaging using Osteosense highlighted localized bone signals that were associated with bioluminescent tumor signals and micro-CT visualized bone lesions from approximately Day 17. Bioluminescence imaging showed the greatest sensitivity to disease progression in both the mandible and hindlimb bones. The PET and fluorescence imaging approaches showed bone involvement (presumably through HA), indicating osteoblastic activity.
Conclusion: The combination of PET, fluorescent imaging of bone remodeling mechanism coupled with bioluminescent imaging of tumor growth and microCT imaging of bone anatomy, enables quantitative, non-invasive means for characterizing bone metastasis. Importantly, the use of complimentary imaging methods can provide assessment of novel therapeutics against both metastatic tumor growth and bone lesion progression.



261 POSTER

Effects of isoflurane anesthesia on bioluminescence measurements: impact on pharmacological assessment of anti-tumor activity of chemical entities

C. Schnell[1], S. Arnal[1], S. Barbé[1], M. Becquet[1], C. Garcia-Echeverria[1], R. Cozens[1]. [1]*Novartis Pharma AG, Oncology, Basel, Switzerland*

Bioluminescence imaging (BLI) has been used for several years in oncology research to quantify tumor growth. In order to allow immobilization during data acquisition, animals are usually anesthetized by isoflurane gas inhalation. However, anesthesia leads to modifications of many physiological parameters, and particularly decreases in heart rate, blood pressure and blood flow, leading to decrease of substrate availability. Thus, it can be postulated that anesthesia will impair bioluminescence read outs. This may in turn profoundly affect interpretation of compound activity based on BLI.
We have investigated the difference in BLI measurements performed in conscious and anesthetized animals using subcutaneous xenografts (HCT116 colorectal and U87MG glioblastoma) and orthotopic (U87MG cells injected directly in the brain) models. Moreover, functional tumor vessel mapping and hypoxia stage were obtained using casting technology and HypoxyProbe™, respectively. Anti-tumor activity of 5-fluorouracil and temozolomide were assessed in the HCT116 xenograft and U87MG orthotopic models, respectively.
Our results have clearly demonstrated that in all tested tumor models, tumor growth and efficacy of different compounds were not affected by the regular anesthesia procedure used for bi-weekly BLI assessments. We found a good correlation between caliper and BLI over a wide range of tumor size in conscious mice. When BLI was measured in anesthetized mice, signal intensity dropped significantly up to 70%, impairing the assessment of the antitumor efficacy of 5-FU. A clear correlation to functional tumor vessel density was evidenced.
In the U87MG tumors cells implanted intracranialy, we could clearly follow tumor growth over time using BLI readouts. Isoflurane did not impair BLI measurements or the efficacy profile of temozolomide.
In conclusion, we can say that BLI measurements in conscious tumor bearing mice offers a better alternative for resolving pharmacologic queries without the confounding effects of anesthesia. Obviously, more extended studies over a wide range of tumor types will be needed to reinforce these conclusions. Moreover, a more quantitative approach to assess vessel density and distribution in the casts using micro-CT technologies would be pivotal.

262 POSTER

Establishment of patient-tumor derived xenograft models for testing anticancer agents

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Human xenograft tumor models established by transplantation of human tumor cell lines into immunodifficient mice have been routinely used for preclinical test of anticancer agents. But such tumor models have a relatively low transplantability, limited number of cell lines available for certain tumor types, and limited correlation with clinical findings. Recently, we have developed patient-tumor derived xenograft tumor models by transplanting human fresh tumor tissues into nude mice, and which have been used for test of clinically used anticancer drugs as positive