ALK inhibitors. Melanoma patients with BRAF mutation responded well to specific and selective agents. Clinical studies of targeted agents with a pre-treatment genetic screening program are ongoing and have a great chance of success. Moreover, modern drug development techonologies allows the development of targeted agents with a higher degree of specificity and selectivity when compared to some of the approved targeted agents.
Although significant advances have been achieved in the field of targeted therapy, focus is needed across critical "gray areas". Biomarkers with the ability to predict targeted therapy benefit and/or resistance have been evaluated acrosss different phase I to III clinical studies, but not subsequently validated. Several mechanisms of resistance to different drugs have been described, but assessment of their prevalence and importance for the clinical setting is lacking. In order to circumvent this research gaps, it is fundamental to prioritize clinical trials (statiscally powered) to answer important translational research questions. Prospective collection of biospecimens including tumor biopsies must become a standard for the upcoming clinical studies. A genetic screening of tumors (e.g. for mutations, translocations, other key genetic abnormalities) will probably select better those which will benefit most from targeted therapy as single agent or combination. A comprehensive drug development plan in advanced as well in the neoadjuvant setting including translational research questions have the potential to identify the most promising targeted drugs to be used in the adjuvant setting in pre-defined patient subgroups where the chance of cure will be much higher.
As a conclusion and for the upcoming 20 years it is expected that the scientific achievements will be greater when compared to the last two decades. The lessons learnt up to now should bring the concept of "personalized anti-cancer treatment" closer to the reality. A close collaboration of basic scientists, chemists, clinical investigators and statisticians is of utmost important to achieve quickly the ultimate goal of personalized medicine and cure of patients.

Thursday, 18 November 2010 14:45–16:15

PLENARY SESSION 6

Proffered papers

2LB LATE BREAKING ORAL

Anti-tumor activity of anti-RON antibodies and biomarker of response

For full abstract, see p. 3.

252 ORAL

Polyclonal resistance to kinase inhibition in GIST: Mechanisms and therapeutic strategies

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Background: Clinical progression of metastatic GIST, during tyrosine kinase inhibitor (TKI) therapy, is often multifocal. However, TKI resistance mutations are assessed in only single, or few, progressing metastases per patient. We used high-throughput screens to evaluate TKI resistance mechanisms in up to 40 progressing GIST metastases per patient.
Methods: Clinically progressing *KIT*-mutant GISTs were from patients formerly responding to imatinib and/or sunitinib. *KIT* exons 8 through 18 were sequenced at 2000-fold coverage (454 pyrosequencing) and these analyses were confirmed and extended (dHPLC and Sanger sequencing) to additional metastases from the same patients. Drug-response studies were performed by expressing mutant constructs in a KIT-negative GIST model.
Results: 454 *KIT* sequencing was performed in progressing GISTs (N = 50), untreated GISTs (N = 32), and non-GIST sarcomas (N = 5). DNA dilution series showed that *KIT* mutations were detectable by 454 when present in $\geqslant$1% of the *KIT* DNA from a given GIST. Secondary *KIT* mutations (in addition to the known primary mutation) were demonstrated in 3 untreated GISTs (9%), but were rare events (<4% of KIT sequences) in 2 of these. Secondary *KIT* mutations were found in 40 progressing GIST metastases (80%), of which 5 metastases had 2 or more resistance mutations in the same <2 mm^3 sample. Combined dHPLC and 454 analyses revealed a maximum of 7 different predominant secondary *KIT* mutations (each mutation found in >25% of *KIT* alleles from at least one metastasis) among 40 geographically discrete progressing metastases, from one patient. Novel sunitinib resistance mutations, in pts with *KIT* exon 9 primary mutation, involved *KIT* ex 11 (del-ins), ex 13−14 (N655S, N680K and F681L), and ex 18 (S840N). All *KIT* secondary resistance mutations were on the same allele (cis) as the primary mutation, and novel resistance mutations conferred constitutive KIT phosphorylation or KIT-ligand hypersensitivity. Nilotinib and sorafenib inhibited a subset of these mutations but were ineffective against others. However, all resistance mutations were inhibited potently by a KIT switch pocket inhibitor (DP-3636; Deciphera Pharmaceuticals), and a second generation HSP90 inhibitor (STA-9090; Synta Pharmaceuticals).
Conclusions: Systematic genomic evaluations demonstrate up to 7 TKI resistance mutations per patient, in different progressing GIST metastases. These complex molecular resistance mechanisms can be inhibited, *in vitro*, by novel therapeutic strategies.

253 ORAL

Screening for PIK3CA, RAS, and RAF mutations in trials with PI3K/AKT/mTOR signaling pathway inhibitors

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Background: Activating mutations of the p110α subunit of PI3K (*PIK3CA*) have been identified in many malignancies. Preclinical data suggest that these mutations may predict response to PI3K/AKT/mTOR inhibitors, but that concomitant *RAS* or *RAF* mutations may mediate resistance.
Methods: Patients with diverse cancers referred to the Phase I Program for targeted therapy from October 2008 to May 2010 were analyzed for *PIK3CA*, *RAS* (*KRAS*, *NRAS*), and *RAF* (*BRAF*) mutations using PCR-based DNA sequencing. Consecutive patients with any tumor type and *PIK3CA* mutations were treated whenever possible with agents targeting the PI3K/AKT/mTOR signaling pathway.
Results: Overall, 504 patients were tested and 54 (11%) had *PIK3CA* mutations (exon 9, n = 28; exon 20, n = 26). Patients with *PIK3CA* mutations in comparison to patients with wild-type *PIK3CA* had more frequently simultaneous *KRAS* mutations (38% vs. 16%; p = 0.001). *PIK3CA* mutations were most frequent in squamous cell cervical cancers (36%, 5/9 patients), endometrial cancers (24%, 7/29), breast cancers (21%, 6/29), colorectal cancers (17%, 17/103), squamous cell head and neck cancers (15%, 5/34), and ovarian cancers (12%, 7/60). Of the 54 patients with *PIK3CA* mutations, 40 (median number of prior therapies, 3) were treated on a protocol that included a PI3K/AKT/mTOR pathway inhibitor. Of these 40 patients, 8 (20%) achieved a partial response (PR) (2/5 squamous cell cervical cancers; 2/6 endometrial cancers; 1/3 squamous cell head and neck cancers; 2/7 ovarian cancers; 1/5 breast cancers) and 7 (17%) had stable disease (SD) for $\geqslant$4 months. Of the 40 treated patients, 17 (42%) had coexisting *RAS* and/or *RAF* mutations. Of these 17 patients (colorectal cancers, 10; ovarian cancers, 5; endometrial cancers, 2), only 2 patients with ovarian cancers had a PR.
Conclusion: *PIK3CA* mutations were detected in 11% of patients with various solid tumors. Fifteen (37%) patients had a PR (20%) or SD $\geqslant$4 months (17%). These preliminary results with PI3K/AKT/mTOR axis inhibitors are encouraging and although the number of patients is small, they suggest that coexisting *RAS* and/or *RAF* mutations may be associated with resistance to PI3K/AKT/mTOR axis inhibitors in colorectal and endometrial cancers, but not in ovarian cancer.

254 ORAL

cKIT overexpression and wild-type NRAS/BRAF predict response to the tyrosine kinase inhibitor dasatinib in melanoma cell lines

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Background: Patients with advanced melanoma have limited effective therapy. Thus, there is an urgent need to evaluate new targeted drugs. On the other hand, NRAS and BRAF mutations are described in about 25% and 50% of melanoma tumors, respectively, and are mainly responsible of the constitutive activation of the MAPK pathway independently of any growth factor-mediated tyrosine kinase receptor stimulation. Of note, both mutations are mutually exclusive. We hypothesised that the presence of these activating mutations should interfere with the efficacy of drugs,

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,