could be detected. This novel algorithm successfully designs and predicts efficiency of bifunctional siRNAs that can be used in the fight against NHL. A critical factor for any siRNA therapeutic is its effective and targeted delivery. Numerous B-cell malignancies show increased expression of BAFF receptor (BAFF-R). BAFF1 protein, a member of the tumor necrosis factor (TNF) family, trimerizes and binds to the BAFF-R on the cell surface where it is internalized by receptor mediated endocytosis (Lyu et al. 2007). This allows targeting of the BAFF-receptor for delivery purposes.
Non-Hodkin's Lymphoma cell lines such as Granta 519, Jeko-1, Rec-1, SUDHL4, Raji, Daudi and Z138 express BAFF receptor to different degrees. We produced BAFF receptor aptamers by using a nitrocellulose-filter based SELEX process. We chose two aptamers, designated as R1 and R14. Gel shift assays showed that the selected aptamers can specifically bind BAFF-R with nanomolar affinities (R1 K_d = 47.12 nM, R14 K_d = 95.65 nM). We have used live imaging confocal microscopy to visualize aptamers R1 and R14 selective binding and internalization in the B-cell lymphoma Jeko-1 cell line. Unlike the BAFF1 ligand, the aptamers do not enhance cell proliferation, and aptamer R1 is also able to block BAFF ligand mediated proliferation of these cells in MTS assays.
The ability of the aptamers to deliver functional siRNAs into B-cell lymphoma cells was examined using optimized R1 chimera-configurations to deliver an anti-Stat3 siRNA to Lymphoma cell lines. These aptamer siRNA chimeras internalize into Z138 and Jeko-1 cells and show target down-regulation.

248 INVITED

Targeting TRAIL receptors with genetically-engineered CD34+ stem cells

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Background: Preclinical studies demonstrating that tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) exerts a potent and cancer cell-specific cytotoxic activity prompted clinical development of recombinant soluble TRAIL. Despite a good toxicity profile shown in phase I/II clinical studies, limited evidences of antitumor activity have emerged for soluble TRAIL. Stem/progenitor cell-mediated gene delivery of anticancer therapeutics might represent an innovative approach to overcome the limitations inherent to TRAIL receptor targeting, i.e., pharmacokinetic of soluble TRAIL, pattern of receptor expression, tumor cell resistance.
Methods: We have envisaged the use of CD34+ cells engineered by adenoviral transduction to express membrane-bound TRAIL (CD34-TRAIL+ cells).
Results: Transduced cells efficiently act as TRAIL-presenting vehicles and exert a potent tumor cell killing activity against a variety of hematopoietic (e.g., multiple myeloma, non-Hodgkin lymphoma) and nonhematopoietic (e.g., breast cancer) tumors, both in vitro and in vivo in xenograft models of human tumors. Following intravenous injection, CD34-TRAIL+ cells home in the tumor peaking at 48 hours after injection. Tumor homing of CD34-TRAIL+ cells is largely mediated by vascular cell adhesion molecule-1 (VCAM-1) and stromal cell-derived factor-1 (SDF-1). Computer-aided analysis of TUNEL-stained tumor sections demonstrates significantly greater effectiveness for CD34-TRAIL+ cells in increasing tumor cell apoptosis and necrosis over soluble TRAIL. Proteome array analysis indicates that CD34-TRAIL+ cells and soluble TRAIL activate similar apoptotic machinery. In vivo staining of tumor vasculature with sulfo-NHS-LC-biotin reveals that CD34-TRAIL+ cells but not soluble TRAIL target endothelial cells expressing TRAIL-R2, as shown by apoptosis of endothelial cells, appearance of hemorrhagic areas and marked reduction of vessel density.
Conclusions: Overall, these results demonstrate that CD34-TRAIL+ cells induce potent antitumor effects by targeting tumor cells and tumor vasculature. Phase I/II clinical trials to test the safety and activity of CD34-TRAIL+ cells in patients with advanced solid tumors are planned to exploit the anticancer potential of cell-based TRAIL delivery.

249 INVITED

The state of nanotechnology for targeted treatment delivery

A. Urtti. *Finland*

Abstract not received

250 INVITED

Targeted imaging and radionuclide therapy of somatostatin receptor positive tumours

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Targeted radionuclide therapy is a rapidly growing field in nuclear medicine. Some years ago only radioiodine was available for targeted radionuclide therapy. Nowadays a number of approved radiopharmaceuticals are available (e.g. MIBG, phosphonates, anti-CD-20-antibodies). Beside these commercially available radiopharmaceuticals, radiopeptides are of particular interest. G-protein coupled receptors are overexpressed on certain tumors and radiopeptides do have the capability to bind these receptors. Radiopeptides feature highly suitable pharmacokinetics (rapid targeting, high diffusibility and fast clearance) for radionuclide therapy.
Over the last years most experience was acquired with radiolabeled somatostatin analogues. The somatostatin analogues show exemplary the development of a radiopharmaceutical with its way from an imaging agent to a therapeutic drug and the efforts that are made to improve the therapy further.
The peptides that are used for therapy can be radiolabelled with gamma- or positron-emitting radionuclides which makes them suitable for imaging thus allowing a very precise prediction of the biodistribution of the radiopharmaceutical.
For therapy a number of different somatostatin analogues as well as different radionuclides have been used. Generally, objective response rates of approximately 30% can be achieved in patients suffering from metastatic, gastro-entero-pancreatic neuroendocrine tumors (GEP-NET). Recently a median time to progression of >36 months has been published for GEP-NET patients. Usually the treatment is tolerated very well with only limited short term side effects. In the long term mainly the kidneys and the bone marrow are at risk of radiation damage which makes these organs the dose limiting organs.
In summary, targeted radionuclide therapy with radiolabelled somatostatin analogues appears to be the most effective therapy for patients with meatsatic, somatostatin receptor positive GEP-NET. An overview of the current status as well as future developments in field of targeted imaging and therapy using radiopeptides will be given.

Thursday, 18 November 2010 14:05−14:45

Special Lecture

251 INVITED

Lessons learnt of 20 years of targeted therapies

A. Awada[1], O. Metzger[1]. [1]*Institut Jules Bordet, Head of the Medical Oncology Clinic, Bruxelles, Belgium*

Small molecule tyrosine kinase inhibitors and receptors-targeted antibodies have emerged over the last two decades as "revolutionary" anticancer drugs. The possibility of blocking specific targets overexpressed or mutated involved in carcinogenesis generated a great scientific enthusiasm. A high therapeutic efficacy with limitted toxicity was expected due to the predicted selectivity of targeted drugs. The run for targeted therapy development led to important gains such as trastuzumab for breast cancer and imatinib for gastrointestinal stromal tumors. Nevertheless, an enormous amount of effort testing different targeted agents in large international studies turned to be negative or "marginally" positive.
Negative phase III clinical trials in diseases such as pancreatic cancer, non-small cell lung cancer and melanoma among others generated important informations: (1) The genetic complexity involved in carcinogenesis of solid tumors is a limiting factor for the identification of a unique target with the ability of blocking the oncogenic process − subpopulations are more likely to benefit. (2) Due to the "plasticity" of signaling pathways, pro-oncogenic signaling pathways can be unleashed upon blockade of a specific target − combination targeted therapy or multitargeted agents may be needed but "cumulative" side effects could be a limiting factor. (3) Defining the real implication of a specific target to the oncogenic process is needed − high throughput sequencing programs should be pursued to define the most important genetic abnormalities (e.g. mutations, translocations, . . .) for each tumor. A well performed preclinical and early clinical studies is a prerequisite step to predict the succes in the development of a new targeted agent.
Marginally positive phase III studies lead in limited circumstances to retrospective identification of subpopulations most likely to benefit or not from a given targeted agent. In advanced colorectal cancer, as an example, treatment with monoclonal antibodies targeting EGFR was restricted to a subpopulation known to be wild type KRAS after being studied in an unselected population. In fact, KRAS mutated tumors did not respond at all to this class of agent.
In the last years, the increasing sensibilisation towards upfront patient selection has already demonstrated strong positive results. In breast cancer, patients known to have defective DNA repair machinery due to BRCA gene mutation are particulary sensitive to PARP inhibitors. NSCLC patients with ALK gene rearrangements are highly senstitive to

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

J. Fletcher[1], T. Rege[1], C. Liang[1], C. Raut[1], K. Foley[2], D. Flynn[3], C. Corless[4], M. Heinrich[5], G. Demetri[6], Y. Wang[1]. *[1]Brigham and Women's Hospital, Pathology, Boston, USA; [2]Synta Pharmaceuticals Corp., In Vivo Pharmacology, Lexington, USA; [3]Deciphera Pharmaceuticals, Drug Development, Lawrence, USA; [4]Oregon Health and Science University, Pathology, Portland, USA; [5]Oregon Health and Science University, Division of Hematology and Oncology, Portland, USA; [6]Dana-Farber Cancer Institute, Medical Oncology, Boston, USA*

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

F. Journe[1], M. Wiedig[1], R. Morandini[1], F. Sales[1], G. Ghanem[1], A. Awada[2]. *[1]Institut Bordet, Lab Oncologie et Chirurgie Exp, Brussels, Belgium; [2]Institut Bordet, Oncologie Médicale, Brussels, Belgium*

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,