Thursday, 18 November 2010 08:00–09:45

PLENARY SESSION 4

Targeting checkpoints/DNA repair in cancer

242 INVITED

The concept of synthetic lethality

H. Calvert. *United Kingdom*

Abstract not received

243 INVITED

Targeting the Chk1/Cdc25A pathway in p53-deficient tumors

H. Piwnica-Worms[1], C. Ma[2], S. Cai[1], C. Ryan[1], Z. Guo[2], S. Li[2], M. Ellis[2]. *[1]Washington University School of Medicine, Cell Biology and Physiology, St. Louis, USA; [2]Washington University School of Medicine, Internal Medicine, St. Louis, USA*

Cancer cells frequently overproduce proteins that positively regulate the cell division cycle in order to maintain their proliferative capacity. The Cdc25A protein phosphatase is an example of a key cell cycle regulator that is overproduced in a wide variety of human cancers. Cdc25A drives the cell cycle forward by activating cyclin-dependent protein kinases. In addition to tightly controlling Cdc25A levels during a normal cell cycle, cells rapidly shunt Cdc25A for ubiquitin-mediated proteolysis when they are exposed to genotoxic stress. By eliminating Cdc25A, cells are able to temporarily arrest the cell division cycle to allow time for DNA repair. The Chk1 protein kinase targets Cdc25A for ubiquitin-mediated proteolysis during the S-and G2-phases of the cell division cycle. Importantly, p53-deficient cells are absolutely dependent on the integrity of this pathway to arrest cell cycle progression in response to DNA damage. This property makes the Chk/Cdc25A pathway a potential therapeutic target in p53$^-$deficient tumors. Results of a phase I clinical trial targeting the Chk1/Cdc25A pathway in resistant solid tumor malignancies will be described. In addition, a preclinical mouse model has been developed to evaluate combination therapies for treating p53$^-$deficient tumors. In this model, fresh tumor biopsies from breast cancer patients are engrafted into humanized mammary fat pads of immunodeficient mice. The initial xenograft tumor is transplanted into additional mice for experimental treatment and pathway studies that parallel our phase I studies. *TP53* is sequenced in each engrafted tumor explant and the integrity of the p53 pathway is determined by monitoring p53 stabilization and p21 induction following DNA damage. Three independent tumor explants, one wild-type and two mutant for *TP53* were analyzed for their response to irinotecan (to induce DNA damage) followed by Chk1 inhibition, with two independent Chk1 inhibitors. Results indicate that p53 status is a significant predictor of response to combination therapies involving DNA damage followed by Chk1 inhibition. Therefore, tumors lacking a functional p53 pathway may be effectively treated using this strategy.

244 INVITED

PARP inhibitors

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This session focuses on Poly(ADP-ribose) polymerase (PARP), which is an attractive antitumor target because of its vital role in DNA repair. The homologous recombination (HR) DNA repair pathway is critical for the repair of DNA double strand breaks and HR deficiency leads to a dependency on error-prone DNA repair mechanisms, with consequent genomic instability and oncogenesis. Tumor-specific HR defects may be exploited through a synthetic lethal approach for the application of anticancer therapeutics, including PARP inhibitors. This theory proposes that targeting genetically defective tumor cells with a specific molecular therapy that inhibits its synthetic lethal gene partner should result in selective tumor cell killing. The demonstration of single agent antitumor activity and the wide therapeutic index of PARP inhibitors in *BRCA1* and *BRCA2* mutation carriers with advanced cancers provide strong evidence for the clinical application of this approach. Emerging data also indicate that PARP inhibitors may be effective in sporadic cancers bearing HR defects, supporting a substantially wider role for PARP inhibitors. Drugs targeting this enzyme are now in pivotal clinical trials in patients with sporadic cancers. We review the evidence supporting this antitumor synthetic lethal strategy with PARP inhibitors, discuss evolving resistance mechanisms and potential molecular predictive biomarker assays and envision the future development of these agents.

245 INVITED

Targeting ATM and p53

M. Kastan[1], K. Guo[1], J. Chen[1], K. Guy[2]. *[1]St Jude Children's Research Hospital, Department of Oncology, Memphis, USA; [2]St Jude Children's Research Hospital, Department of Chemical Biology, Memphis, USA*

Significant progress has been made in recent years in elucidating the molecular controls of cellular responses to DNA damage in mammalian cells. Much of our understanding of the mechanisms involved in cellular DNA damage response pathways have come from studies of human cancer susceptibility syndromes that are altered in DNA damage responses. ATM, the gene mutated in the cancer-prone, radiosensitive disorder, Ataxia-telangiectasia, is a protein kinase that is a central mediator of responses to DNA double strand breaks in cells. Once activated, ATM phosphorylates numerous substrates in the cell that modulate the cell's response to the DNA damage. p53, one of the many targets of the ATM kinase, is a critical mediator of cell cycle changes and cell death signaling following DNA damage and other stresses. Mechanistic insights about these pathways provide us with opportunities to develop new approaches to target the pathway for patient benefit. Small molecule inhibitors of ATM lead to increased sensitivity to ionizing irradiation – I will discuss the development of ATM inhibitors as therapeutic enhancers in the treatment of malignancies. Conversely, inhibition of p53 induction has the potential to protect normal tissues from the ravages of chemotherapy and radiation therapy. Based on recent insights into a novel mechanism involved in regulation of p53 induction, new approaches to protecting normal tissues by blunting p53 induction will be discussed.

Thursday, 18 November 2010 10:15–12:00

PLENARY SESSION 5

New modalities of anticancer treatment

246 INVITED

New platforms of immunoconjugates

M. Sliwkowski. *USA*

Abstract not received

247 INVITED

Dual function BAFF receptor aptamers inhibit ligand induced proliferation and deliver siRNAs to NHL cells

J. Rossi[1], K. Tiemann[1], J. Zhou[1], J. Alluin[1], R. Chen[2], S. Forman[2]. *[1]Beckman Research Inst of the City of Hope, Division of Molecular Biology, Duarte, USA; [2]City of Hope, Hematology, Duarte, USA*

Non-Hodgkin's Lymphoma (NHL) killed 20,000 people in 2009 and 66,000 new cases were indentified. Currently patients suffering from NHL receive treatment in form of radio-, chemo-, or biological therapy. These interventions rarely cure and relapses within one year are all too common. Many types of NHL feature the constitutive expression of oncogenes such as transcription factors STAT3 or MYC, anti-apoptotic protein Bcl-2 or Cyclin family members such as Cyclin D1 (Monti et al. 2005; Shaffer et al. 2006). Over-expression of these genes causes not only uncontrolled cell proliferation and survival of malignant cells but it also provides protection against ionizing radiation and many commonly used chemotherapeutics (Domen et al. 1998; Makin and Hickman 2000). Hence, knockdown of these genes using RNA interference (RNAi) is a rationale strategy for therapeutic intervention.

We developed bifunctional siRNA duplexes that contain two fully target-complementary antisense strands. This technique can be used to down-regulate two critical B-cell lymphoma oncogenes simultaneously, promoting apoptosis or rendering lymphomas more susceptible to standard chemotherapy.

An algorithm predicts the sequence and efficiency of these bifunctional siRNAs. We tested the efficiency of bifunctional 27mer siRNAs against Cyclin E2, Cyclin D1, C-Myc, BCl2, Survivin, STAT3 and Syk in different combinations by qRT-PCR. We saw efficient target-gene down-regulation in HEK293, PC3 and HCT116 cells. CyclinE2/CyclinD1 bifunctional siRNA showed in all three cell lines approximately 60% down-regulation of both genes simultaneously as the algorithm predicted. No interferon response

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

C. Carlo-Stella[1], A.M. Gianni[1]. [1]*Istituto Nazionale Tumori and University of Milano, Medical Oncology, Milan, Italy*

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