**31** ORAL

**Temozolomide (TMZ) targets only glioblastoma with a silenced MGMT-gene. Results of a translational companion study to EORTC 26981/NCIC CE.3 of radiotherapy±TMZ**

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**Background:** We have demonstrated that the methylation-status of the *O-6-methylguanine-DNA methyltransferase* (*MGMT*)-promoter correlates with survival in glioblastoma patients treated with temozolomide (Hegi et al., Clin Cancer Res 2004). Here we test the relationship of MGMT silencing with outcome in.
**Material and Methods:** Paraffin embedded glioblastoma biopsies have been collected from patients treated within a prospective international phase III trial (Stupp et al. Proc Am Soc Clin Oncol 2004 { abstract #2}). The methylation status of *MGMT* in the tumor biopsies was determined in a subgroup of 191 patients undergoing resection for newly diagnosed glioblastoma. The epigenetic silencing of the *MGMT*-gene was determined using methylation specific PCR. All patients gave written informed consent.
**Results:** Inactivation of the *MGMT*-gene by promoter methylation was associated with longer survival in the patient group treated with TMZ/RT as compared to RT alone. In patients without *MGMT*-promoter methylation no difference in survival was found between the 2 treatment arms. A multivariate analysis is ongoing and final results will be presented.
**Conclusions:** This prospective trial will establish the value of MGMT determination as a predictive factor for GBM. This clear association between the epigenetic inactivation of the DNA repair gene *MGMT* and response to treatment will necessitate the determination of the methylation status of the *MGMT*-gene prior to choice of treatment. For the first time patients unlikely to respond can be identified and alternative treatments be proposed. The MGMT-methylation status determination is a step towards molecular diagnostics and tailored and individualized treatments.

## Wednesday 29 September 16:30–18:15

**PLENARY SESSION 3**

# AKT/PTEN/Survival pathways

**32** INVITED

**Functional analysis of PDK1 signalling pathway using knockout and knockin approaches; evaluation of PDK1 as a cancer target**

D. Alessi, E. McManus, B. Collins, A. Mora. *University of Dundee, MRC Protein Phosphorylation Unit, School of Life Sciences, Dundee, Scotland, UK*

The interaction of insulin and growth factors with their receptors on the outside surface of a cell, leads to the generation of a lipid "second messenger" termed PtdIns(3,4,5)P3 at the inner surface of the cell membrane. PtdIns(3,4,5)P3 activates several key signal transduction pathways which ultimately regulate all insulin responses, as well as promoting the proliferation and survival of cells. The key focus of our work has been to study signalling responses that are regulated by the PtdIns(3,4,5)P3 second messenger. The topic of my talk will focus on the characterisation of the master regulator of such signalling responses, a PtdIns(3,4,5)P3 binding protein kinase termed the 3-phosphoinositide-dependent kinase 1 (PDK1). PDK1 is the enzyme that phosphorylates and activates a number of protein kinases including PKB, S6K, SGK and PKC isoforms (collectively termed AGC kinases), which play important roles in mediating the diverse cellular effects of insulin and growth factors. In my talk, I will discuss our recent analysis of various of homozygous knockin ES cells expressing either a form of PDK1 with a mutation in its PH-domain that abolishes PtdIns(3,4,5)P3-binding or a form in which a substrate binding pocket termed the PIF-pocket is disrupted. These experiments establish the roles of the PDK1 regulatory domains and illustrate the power of knockin technology to probe the physiological function of protein-lipid and protein-protein interactions, without having to rely on overexpression of dominant negative or constitutive active mutants of proteins in cells to dissect roles of signalling pathways in cells. There is much evidence that in a significant number of cancers have elevated PtdIns(3,4,5)P3 levels and that the growth and survival of these cells is dependent upon high levels of PKB and S6K activity in these cells. I will discuss the evidence indicating that pharmacological inhibition of PDK1 may be effective in reducing growth and survival of cancers that are dependent upon AGC kinase activity.

**33** INVITED

**Approaches to inhibit the Akt pathway**

W.R. Sellers, M. Meyerson. *Medical Oncology, Dana-Farber Cancer Institute, Boston, USA*

Genetic alterations leading to the inactivation of the PTEN tumor suppressor gene trigger unregulated activation of PI3K signaling. Genetic studies in drosophila, studies in murine models and studies in human cancer cell models, all suggest that cancers lacking PTEN or harboring activated Akt may be susceptible to treatment with mTOR inhibitors. Our studies utilizing a mouse model bearing prostate restricted expression of Myr-Akt confirm the exquisite phenotype dependence on mTOR activity and show that such models can be utilized to understand the in vivo biologic response and for biomarker development. Collectively, the advances in the understanding of PTEN function encourage us to believe that an understanding of the somatic genetic alterations in cancer can lead to therapeutic insight. Our group is approaching the problem of detecting somatic genetic alterations through the use of high-resolution analysis of structural alterations in the cancer genome and through candidate exon-based resequencing. These efforts have led to the discovery of mutations in the EGFR receptor in lung adenocarcinoma. These and other emerging genetic alteration in cancer will be discussed.

**34** INVITED

**Novel targets that control protein translation downstream from Akt and PTEN**

N. Sonneberg. *Canada*

Abstract not received.

**35** INVITED

**Clinical experiences with mTOR inhibitors**

S. Faivre. *Institut Gustave Roussy, Medical Oncology, Villejuif, France*

The mammalian target of rapamycin (mTOR) is an intracytoplasmic serine-threonine protein kinase that drives cancer cell survival, proliferation and apoptosis. Activation of mTOR results in downstream phosphorylation of several effectors including the $p70^{S6}$ kinase and 4E-BP1. Specific inhibition of mTOR can be achieved using oral or intravenous rapamycin derivatives (CCI-779, RAD001 and AP23573) in clinical trials. Our first clinical experience using weekly dosing of CCI-779 showed that rapamycin derivative was safe with dose-limiting toxicities (DLTs) consisting of thrombocytopenia, bipolar disorders, asthenia, and stomatitis at very high doses. Further clinical trials with CCI-779, RAD001 and AP23573 were conduced using lower doses and consistently yielded to mild/moderate acne-like skin toxicity and mucositis, with no immunosuppressive effects. Pharmacokinetic analysis of CCI-779 showed its rapid and sustained biotransformation into sirolimus with minor interpatient variability at lower doses. Tumors that benefited from rapamycin derivatives as a single agent in phase I/II trials were renal clear cell carcinoma, non-small cell lung, and breast cancers. This sporadic antitumor activity was reported over a broad range of doses with no apparent correlation between exposure and clinical benefit. This suggested (1) that inhibition of mTOR may be achieved at doses well below the maximal tolerated dose and (2) that mTOR pathway is prevalent in some rapamycin-sensitive tumor types. Attempts to establish the biologically active doses were further based on surrogate molecular markers in our RAD001 phase I study in PBMCs. Modelization using a murine model and patient samples showed a correlation between the $p70^{S6}$ kinase dephosphorylation in human PBMCs and murine tumors, allowing dose recommendation. An important issue remains the identification of tumors addicted to mTOR signaling pathway for proliferation and apoptosis. Although several reports indicate that PTEN inactivation and MAPK-dependent AKT phosphorylation would drive mTOR activation, response to rapamycin derivatives would also depend on cell capacity to undergo apoptosis. For instance, bcl-2 expression was shown to prevent apoptosis induction by rapamycin and RAD001. Other studies showed that p53 status

may play a role in rapamycin-induced apoptosis. Identification of critical molecular markers in tumor cells will help to identify patients who shall benefit from mTOR inhibitors.

## Wednesday 29 September

# Poster Sessions

# New drug targets

**36** POSTER

**Transcriptional signature associated with sensitivity to ET-743 (Yondelis) in low passage sarcoma cell lines**

M. Sanchez Beato[1], N. Martinez[1], I. Fernandez[1], M. Navarrete[1], P. de la Cueva[1], A. Carnero[1], J.C. Tercero[2], J. Jimeno[2], M.A. Piris[1]. *[1]CNIO, Molecular Pathology, Madrid, Spain; [2]Pharmamar, Scientific Development, Madrid, Spain*

ET-743 (trabectedin, Yondelis) is a marine anticancer agent that has shown to induce long lasting objective remissions and tumor control in a subset of patients with pretreated/resistant soft tissue sarcoma. Drug induced tumor control is achievable in a 22% of such patients, but there is not clear indication of the molecular features correlated with clinical sensitivity/resistance to ET-743.

Nine low passage soft tissue sarcoma cell lines explanted from chemo naïve patients with different patterns of sensitivity (IC50 range, 0.4 to 100nM), have been profiled with a cDNA microarray containing 6700 genes relevant in cancer development and drug resistance. The molecular signature of these cell lines was analyzed at baseline and at 4 different time points after ET-743 exposure at the clinically relevant concentration of 10 nM. Additionally, association of p53 mutation and p73 expression levels with ET-743 sensitivity and cell cycle kinetics after treatment were also analyzed.

Gene expression profile analysis revealed upregulation of 86 genes and downregulation of 244 genes in response to ET-743, showing a strong inhibition of gene transcription by the drug. ET-743 gene expression signature reveals a group of genes related with cell cycle control, stress and DNA damage response, such as JunB, ATF3, CS-1. SAT, GADD45B, and ID2 that are upregulated in all the cell lines studied independently of its sensitivity and of the histological subtype.

Transcriptional signature 72 hrs after ET-743 administration, associated with ET-743 sensitivity, showed a more efficient induction of genes implicated in DNA damage response and apoptosis, such as Rad17, BRCA1, PAR-4, p21 and p53DINP1 in the sensitive cell lines group.

Flow cytometry studies showed cell cycle arrest and/or apoptosis in the sensitive cell lines. The presence of p53 mutations correlate with sensitivity. Data produced in this translational program provides with a rational to explore at the clinical level whether this signature can contribute to the identification of the subset of patients that can benefit from ET-743 therapy.

**37** POSTER

**Sensitivity and resistance of human leukemic blasts to aplidin; molecular signature by gene expression profiling (GEP)**

N. Martinez[1], M. Sanchez Beato[1], G. Kaspers[2], I. Fernandez[1], P. de la Cueva[1], J.C. Tercero[3], J. Jimeno[3], M.A. Piris[1]. *[1]CNIO, Molecular Pathology, Madrid, Spain; [2]VU University Medical Center, Pediatric Hematology/Oncology, Amsterdam, Netherlands; [3]PharmaMar, Scientific Development, Madrid, Spain*

Aplidin (APLD) is a marine anticancer drug discovered in the Mediterranean tunicate *A. albicans*. The antitumoral activity of APLD has been related to a cascade of events including cell cycle G1 arrest and G2 blockage, an acute apoptotis induction with JNK/p38 sustained activation; and inhibition of VEGF autocrine loop, reducing VEGF secretion and down regulating of the VEGFR-1. The phase I clinical program with APLD has been completed with evidence of a positive therapeutic index and lack of bone marrow toxicity. Phase II clinical studies are currently underway in hematological tumors with special focus on leukemia and multiple myeloma. In vitro and in vivo studies in leukemia models have demonstrated cytotoxicity at concentrations reachable in patients well below the recommended dose, and lack of cross-resistance with conventional agents. A translational program in pediatric acute lymphoblastic (ALL) and acute myeloid (AML) leukemia has produced evidence of variable in vitro sensitivity to APLD in blast from patients (Leukemia 2003, 17: 1338) at concentrations that do no affect normal bone marrow and peripheral blood samples.

Blast cells from 17 ALL and 12 AML patients with differential sensitivity to APLD have been analyzed by Gene Expression Profiling using a cDNA microarray that contains 6700 genes relevant in cancer development, apoptosis and drug resistance. The in vitro sensitivity to APLD of the patient blasts, measured as $IC_{75}$, ranged from 0.012 to 0.096 mM and 0.011 to 0.153 mM for primary and relapsed ALL, and 0.012 to 0.088 mM for AML, respectively. The $IC_{75}$ median values, used as cut off for classifying the samples as sensitive or resistant were 0.028, 0.014 and 0.045 mM for ALL, ALL-r and AML respectively. Gene expression profiles reveal a specific signature in AML and ALL samples that correlate with the extent of sensitivity to APLD.

AML samples sensitive to APLD presented high expression of genes related to signal transduction, metalloproteases and drug metabolism. Genes in APLD-resistant AML samples are involved mainly in NF-kB activation. In contrast, ALL samples sensitive to APLD presented higher expression of DNA damage response genes.

The GEP model generated in this study will be incorporated in the translational research studies within the phase II program with APLD in resistant leukemia.

**38** POSTER

**The role of sample preparation on gene expression profiling: impact on clinical use of microarray technology**

S. Debey, U. Schoenbeck, T. Zander, J.L. Schultze. *Molecular Tumor Biology and Tumor Immunology, Internal Medicine I, Cologne, Germany*

**Background**: Gene expression profiling is quickly entering the clinical arena in areas such as development of new diagnostic markers and pharmacoprediction. Although seminal findings have been reported using transcriptom analysis, numerous challenges remain for its routine usage. One of the foremost is that gene expression patterns in tissues, e.g. tumors or peripheral blood greatly depend on temporal and interindividual variations. In addition, technical aspects of sample processing, isolation of cellular components, RNA preparation methods and other facets such as time from biopsy or blood withdrawal to RNA-isolation and different experimental conditions have been suggested to affect gene expression patterns. However, these issues are poorly investigated in gene expression analysis using microarrays.

**Materials and Methods**: Peripheral blood from healthy individuals and cancer patients were used as a model to assess the influence of pre-analytical factors on gene expression profiles. Several methods to isolate different cell types and RNA (PAXgene, QIAamp, Ficoll, BD-CPT) and two different blood processing techniques (Buffy Coat vs venipuncture blood) were compared using Affymetrix HG-U133A microarrays. A total of 68 individual array experiments were included in this analysis. Furthermore, the influence of physical factors such as temperature (room temperature, 8ºC), cryopreservation and time delay in sample preparation were also analyzed.

**Results**: Overall, the pre-analytical conditions have a strong and significant impact on gene signatures outweighing e.g. interindividual differences. Particularly delayed sample handling revealed a striking impact on gene signatures. We observed an induction of genes related to hypoxia, concomitant with down regulation of genes associated with cell cycle, metabolism and apoptosis. Similarly, gene expression was strongly influenced by the choice of cell and RNA preparation technique: e.g. the use of the PAXgene system, solely providing stabilization of the gene expression profile of blood samples, revealed overall decrease of present calls, highest variability and decreased sensitivity for changes in expression patterns of lymphocytes and monocytes. Cryopreservation, different temperatures during cell isolation or the source of the blood sample introduced minor changes, nevertheless, they were biologically relevant as exemplified by regulation of the IL-8 gene by different temperatures during cell isolation.

**Conclusions**: Clinical utilization of microarray technology will require improved standardization. Careful annotation of sample collection, transportation or storage and of RNA isolation techniques needs to become a prerequisite during clinical use of this technology. Based on our results, we suggest immediate preparation of RNA prior prolonged sample transportation or storage.

**39** POSTER

**Discovery and development of multiplex angiogenesis inhibitors that target EphB4: validation with a novel chemical-genetics based in vivo model**

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EphB4 is a receptor tyrosine kinase (RTK) that plays a critical role in blood vessel development. EphB4 knockout mice die *in utero* from multiple