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

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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