the most aggressive diseases with a 2-years survival rate of 32% with today's available standard treatments. Few biomarkers (serum and protein) such as GFAP, YKL-40 and S100b can be found in a proportion of human glioma samples yet due to their relative novelty and lack of a solid body of independent studies to date, none of these biomarkers have yet gained wide-spread acceptance in the neuro-oncological community. Importantly, it is unknown whether the cells, which express these markers are tumor-initiating cells or cells from the bulk representing the differentiated part of the tumor.
Materials and Methods: In order to evaluate the relevance of the above mentioned protein markers as specific glioma-initiating cells (GIC) biomarkers and potentially identify new biomarkers, we took advantage of a recently published procedure to discriminate glioma-initiating cells from the non-initiating cells within brain tumors (Clément et al, 2010).
Results: We revealed that known biomarkers such as GFAP, YKL-40 nor S100b showed low or almost no expression in the GIC compartment. We therefore screened for novel and specific biomarkers in purified initiating and non-initiating cell populations. Using Illumina microarrays, we identified 411 candidate genes (criteria: 2 fold-change and $p < 0.05$) and selected 11 candidate biomarker genes for the GIC compartment and 10 candidate biomarker genes for the non-GIC compartment. Using quantitative real-time PCR and immunochemistry/flow cytometry, we validated 2 novel biomarkers for GICs and some for non-GICs at the mRNA and protein levels.
Conclusion: Altogether our results demonstrate that pre-determined/known biomarkers cannot be used as biomarker for glioma-iniating cells. Furthermore, they point out the needs to have a better understanding of the molecular biology of this subpopulation of GICs within the tumor in order to identify and develop novel/specific biomarker for glioma-initiating cells.

PP 5

In vivo metabolic profiling of glioma-initiating cells using proton magnetic resonance spectroscopy at 14.1 Tesla

V. Clément-Schatlo, V. Mlynárik, C. Cudalbu, D. Marino, I. Radovanovic, R. Gruetter. *University of Geneva, Geneva, Switzerland*

Background: In the last decade, evidence has emerged indicating that the growth of a vast majority of tumors including gliomas is sustained by a subpopulation of cancer cells with stem cell properties, called cancer initiating cells. These cells are able to initiate and propagate tumors and constitute only a fraction of all tumor cells.
Materials and Methods: Human glioma cells were injected into the striatum of nude mice (Clement et al, 2010). These mice were examined on days 7, 14, 21, 28 and 35 after the cell injection on a 14.1 Tesla animal MR scanner (Mlynárik V et al. 2006; Gruetter R, 1993). Metabolite concentrations were estimated from the spectra using LCModel.
Results: We showed that intracerebral injection of cultured glioma-initiating cells (CGICs) into nude mice produced fast-growing tumors showing necrosis and gadolinium enhancement in MR images, whereas gliomas produced by injecting freshly purified glioma-initiating cells (FGICs) grew slowly and showed no necrosis and very little gadolinium enhancement. Using proton localized spectroscopy at 14.1 Tesla, a decrease of N-acetylaspartate, glutamate and glucose concentrations and an increase of glycine concentration were observed over time in the brain tissue near the injection site of the CGICs before solid tumors were detected by MRI. In contrast to the spectra of tumors grown from fresh cells, those from cultured cells showed intense peaks of lipids, increased absolute concentrations of glycine and choline-containing compounds, and decreased concentrations of glutamine, taurine and total creatine, when compared with a contralateral non tumor-bearing brain tissue. A decrease of concentrations of N-acetylaspartate and γ-aminobutyrate was found in both tumor phenotypes after solid tumor formation.
Conclusion: Our data show that this orthotopic mouse model of brain tumors seems to be suitable for studying glioma tumor as the changes in the metabolite concentrations at the injection site of the tumor cells are in an excellent agreement with metabolic changes observed in regions of tumor infiltration in patients (Stadlbauer A et al,. 2007). Further investigation are nevertheless needed to determine the cause of the dissimilarities between the tumors grown from cultured glioma-initiating cells and from freshly purified glioma-initiating cells, both derived from human glioblastomas. and precede the appearance of overt contrast on MR images.

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Clinical Assay Development Program

B. Conley, J. Jessup, T. Lively, M. Williams. *National Cancer Institute, Rockville, USA*

Background: For molecularly guided cancer therapy to become reality, appropriately validated molecular assays are necessary. However, discoverers of predictive or prognostic molecular features often do not have resources to analytically validate their findings into a "locked down" assay.
Materials and Methods: The Cancer Diagnosis Program, NCI, has initiated a program composed of contracted CLIA accredited laboratories (Clinical Assay Development Network), a research laboratory (Molecular Characterization and Clinical Assay Development Center) at NCI-Frederick, and contracted Tissue Resources. Eligible applicants (industry, academia and government)need to have one justified defined intended clinical use, at least a prototype assay applicable to human tissues, and information on prevalence of the molecular feature in the disease to which the assay will be applied. Applicants describe the clinical need, the current state of the assay and future plans for assay development (such as use in clinical trial) and request CADP services for address analytical validation, transfer to quality environment, specimens for clinical validation, platform migration, etc. The applications, are evaluated by outside experts (Special Evaluation Panel − SEP). Those applications recommended by the SEP are reviewed internally to match resources and NCI strategic direction to the application. The successful application is then overseen by a project management team (project manager, subject matter expertise from NCI, expertise from contracted resources, and assay submitter). After validation, the standard operating procedures are returned to the assay submitter.
Results: Twelve applications were submitted at the first submission; one has been approved for resources from the first submission date. Project management is beginning. Common issues of the initial applicants were lack of definition of single intended use, or marker still in discovery. Clarification of the application instructions and discussions with the initial applicants were implemented.
Conclusion: Continued education of the marker development community is necessary to encourage development of potential molecular assays from the research lab into clinical use. The initial results of the program have been promising and interest in this program from potential applicants has been increasing (http://cadp.cancer.gov).

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Utility of VEGF and IL-6 as biomarkers for response to PTC299, a novel antiangiogenic

G.K. Schwartz, J. Luke, M. Dickler, B.P. Schneider, A. Tiersten, L. Callahan, C.H. Darby, A.K. Ogden, C. George, T.W. Davis. *Memorial Sloan-Kettering Cancer Center, New York, NY, USA*

Background: Solid tumor growth is dependent on angiogenesis, a process mediated primarily by vascular endothelial growth factor (VEGF). Current antiangiogenic agents sequester VEGF or block activation of VEGF receptors. On-target adverse events commonly observed with such treatments include hypertension, bleeding, and proteinuria. PTC299 is an investigational new drug that differs from existing therapies by inhibiting tumor production of VEGF and other angiogenic cytokines, including interleukin-6 (IL-6). PTC299 avoids inhibition of physiologic VEGF expression, potentially offering an advantageous safety profile relative to current anti-VEGF therapies.
Materials and Methods: A Phase 1b studying patients (pts) with advanced cancer evaluated PTC299 monotherapy Stages 1 and 2, or combination therapy with docetaxel Stage 3. A separate Phase 1b, studying pts with metastatic breast cancer evaluated PTC299 monotherapy Stage 1, or combination therapy with aromatase inhibitor Stage 2. In both studies, serum VEGF and IL-6 levels were assessed at baseline and end of Cycle 1(EOC1). Normal and elevated VEGF levels were defined as <300 and $\geqslant$300 pg/mL, respectively. Moderately elevated and highly elevated IL-6 levels were defined as <10 pg/mL and $\geqslant$10 pg/mL, respectively.
Results: The two studies enrolled 72 patients (15 males, 57 females) with median [range] age 60 [26−82] years, and ECOG PS 0 (n = 39) or 1 (n = 33). Paired VEGF and IL-6 baseline and EOC1 specimens were available from 58 and 48 patients. Patients with normal VEGF at Day 1 (n = 37) had a small mean (SD) increase in VEGF at EOC1 of 18 (122) pg/mL. Patients with elevated VEGF at Day 1 (n = 21) had a significant mean (SD) reduction in VEGF at EOC1 of −150 (188) pg/mL ($p = 0.001$). Patients with moderately elevated IL-6 at Day 1 (n = 25) had a mean (SD) increase in IL-6 at EOC1 of 6 (11) pg/mL. Patients with highly elevated IL-6 at Day 1 (n = 23) had a significant mean (SD) reduction in IL-6 at EOC1 of −13 (16) pg/mL ($p < 0.001$). No incidence of grade 3 or 4 hypertension, bleeding, or proteinuria occurred.
Conclusion: PTC299 offers a novel mechanistic approach to antiangiogenesis by selectively inhibiting pathological VEGF and IL-6 expression. This hypothesis is supported by the reduction of VEGF and IL-6 in patients with elevated levels at baseline and the adverse event profile observed to date.