

# World Federation of Neuro-Oncology Societies magazine

Neurology • Neurosurgery • Medical Oncology • Radiotherapy • Paediatric Neuro-Oncology  
• Neuropathology • Neuroradiology • Neuroimaging • Nursing • Patient Issues

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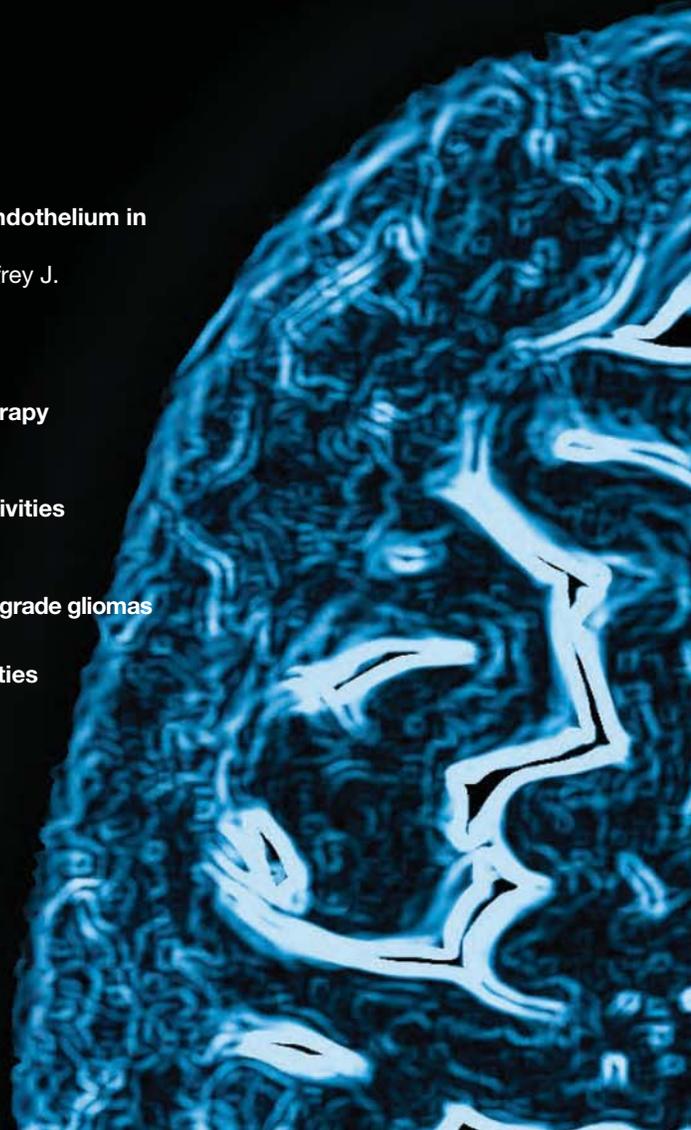
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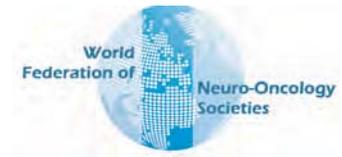
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# World Federation of Neuro-Oncology Societies



# magazine

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## Editorial

On behalf of the leadership of the Society for Neuro-Oncology (SNO) and the European Association of Neuro-Oncology (EANO), we have the pleasure to publish the first issue of the *World Federation of Neuro-Oncology Societies* (WFNOS) magazine.

This new magazine is a transition from the EANO magazine and pursues the mission to become a central platform for communication for all those interested in neuro-oncology, including professions involved in the diagnosis and care of brain tumor patients across the world. Although initiated from Europe and the US, we hope very much that this magazine will gradually involve support and contributions from all parts of the world. This global outreach is

supported by a contribution from the Indian Society of Neuro-Oncology, which has readily accepted our invitation for a presentation of its activities in this first issue of the WFNOS magazine.

Further topics covered include a review on lung cancer cell biology relating to brain invasion, the role of angiogenesis inhibition for the prevention of brain metastases, radiosensitization in glioblastoma, assessing activities of daily living in brain tumor patients, a controversy on the use of temozolomide in MGMT promoter-unmethylated glioblastoma as well as contributions from the sections *Nurses Corner* and *Patient Advocacy*. Furthermore, we present the concept of a clinical trial involving a vaccine targeting mutant

isocitrate dehydrogenase 1. As a further tradition from the EANO magazine, we will maintain the *Neuro-oncology Highlights* corner.

We are grateful to Oxford University Press for engaging in this joint activity of EANO and SNO and we invite our membership and readership to provide us with feedback and suggestions on how to develop and improve this magazine so it can serve its purpose and mission best.

Kind regards,  
on behalf of EANO & SNO

**Michael Weller, MD**  
President, EANO & WFNOS

**E. Antonio Chiocca, MD, PhD**  
President, SNO



# NSCLC Cell Adhesion to and Transmigration through Brain Endothelium in Brain Metastasis

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Approximately 33% of intracranial tumors are secondary metastases originating from primary non-CNS cancers. Indeed, 20%–40% of patients with systemic cancers develop secondary brain tumors<sup>1</sup>. Brain metastasis is the most important fatal complication of systemic cancer, especially in diagnoses of breast cancer<sup>2,3</sup>. There have been great advances in the diagnosis and treatment of breast cancer, with a 5-year survival rate >90% (when a diagnosis is made early). While many patients with early diagnoses will live their lifetimes without metastasis, >90% of the half million patients with metastasis will succumb to this cancer per year, with a majority due to brain metastasis<sup>3</sup>. Metastasis involves a series of multiple steps, and determining which of these steps are optimal in terms of targeted therapies for metastatic seeding is not clear<sup>4–6</sup>. For example, could blocking breast cancer circulating tumor cells (CTCs) from binding to brain endothelial cells prevent the seeding of breast cancer in the brain, and could this be used as preventive therapy<sup>7</sup>? The highest incidence of brain metastasis is seen in lung cancer patients (40%–50%), followed by breast cancer (20%–30%) and melanoma (5%–10%) patients<sup>8</sup>. In particular, the brain is known to be a key target for metastasis from the lung; 20%–40% of patients with non-small-cell lung cancer (NSCLC) have been reported to develop secondary brain tumors<sup>9</sup>. Brain metastasis is of considerable clinical importance and correlates with poor prognosis. Although the mechanisms underlying brain metastasis are still not yet fully elucidated, what is known is that there are 5 key biological elements which characterize the process of metastasis to the brain (Fig. 1):

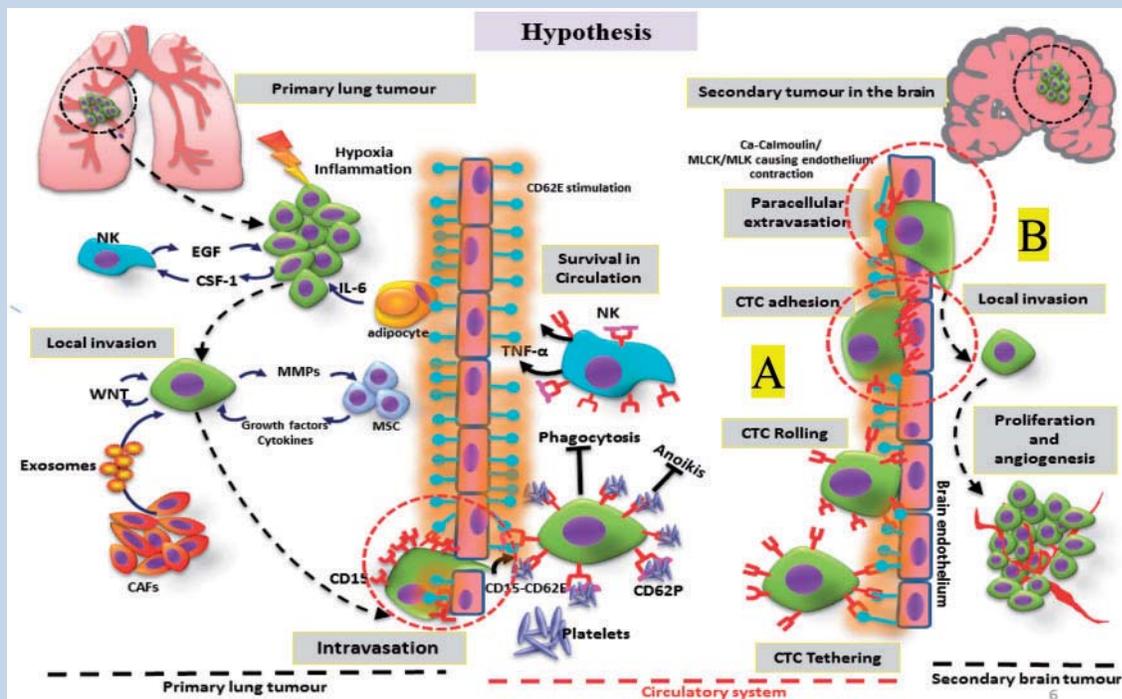
- (a) Intravasation from within the primary cancer
- (b) Travel within the blood circulation
- (c) Adhesion to the target organ (brain) vascular endothelium
- (d) Extravasation into brain tissue
- (e) Colonization and growth within the brain

Each one of these mechanisms brings with it obstacles for the metastasizing cancer cell to overcome and involves a complex communication between and interaction with the cellular components of the host environment. While intravasation of cancer cells from malignant primary cancers may be aided by damaged vascular channels, a complex process of cancer cell attachment to the extracellular matrix and/or endothelial cells via specialized cell adhesion molecules and proteases is required to gain entry into the blood circulation. Once into the circulation, circulating cancer cells (CCCs) face a hostile environment<sup>10</sup> of rapid flow, which may prevent adhesion to the luminal surface of target organ metastasis as well as destruction through the aggressive immune surveillance cells of the blood. Indeed, it has been suggested that less than 0.01% of total CCCs will form a secondary cancer. Those that do survive the journey must engage with their endothelial pairing partner (cell adhesion molecules/selectins/integrins) on a host blood vessel—a process of homophilic or heterophilic

binding which may be under the regulation of solutes such as cytokines and growth factors for either cancer or endothelial cells and involves chemoattraction, rolling adhesion, and tight adhesion; once firmly adherent, they must then transmigrate across the endothelium by either paracellular diapedesis (between adjacent endothelial cells which have high resistance junctions in the case of the brain vasculature) or transcellular diapedesis (directly through the endothelial cell cytoplasm itself). Once this transmigration process is complete, yet another potential hazard for the metastatic cancer cell is encountered: possible challenge from the macrophage population and acquisition of suitable growth conditions within the new microenvironment provided by the brain parenchyma. Since the first 2 stages of metastasis to the brain are known to occur through disruption of the intratumoral blood vessels, we decided to address the next two—critical—stages: cancer cell adhesion to the brain vessels and their subsequent transmigration into the brain parenchyma. Indeed, the importance of brain endothelial cells in brain metastasis was recently highlighted in the early stages of cancer cell seeding<sup>11</sup>. Our initial studies were aimed at NSCLC, but we are currently extending this to include breast cancer metastasis to the brain and even possible spread through vascular, rather than leptomeningeal/CSF, pathways of medulloblastoma from cerebellum to spine locations.

In man, cancer cells tend to metastasize into the brain via the capillaries, which comprise part of the blood–brain barrier (BBB). In our laboratories we have recently engineered an *in vitro* model of the BBB based upon use of human cells (brain microvascular endothelial cells, astrocytes, and pericytes) grown in 2D and 3D culture systems under human serum supplementation conditions<sup>12</sup>. We have further adapted this model to incorporate it into a system for interrogating both drug delivery and cancer cell metastasis via both static assay and dynamic assay conditions; in the latter, vessels can be configured where flow rates can be manipulated to assess their influence on CCC adhesion to and transmigration through the vessel.

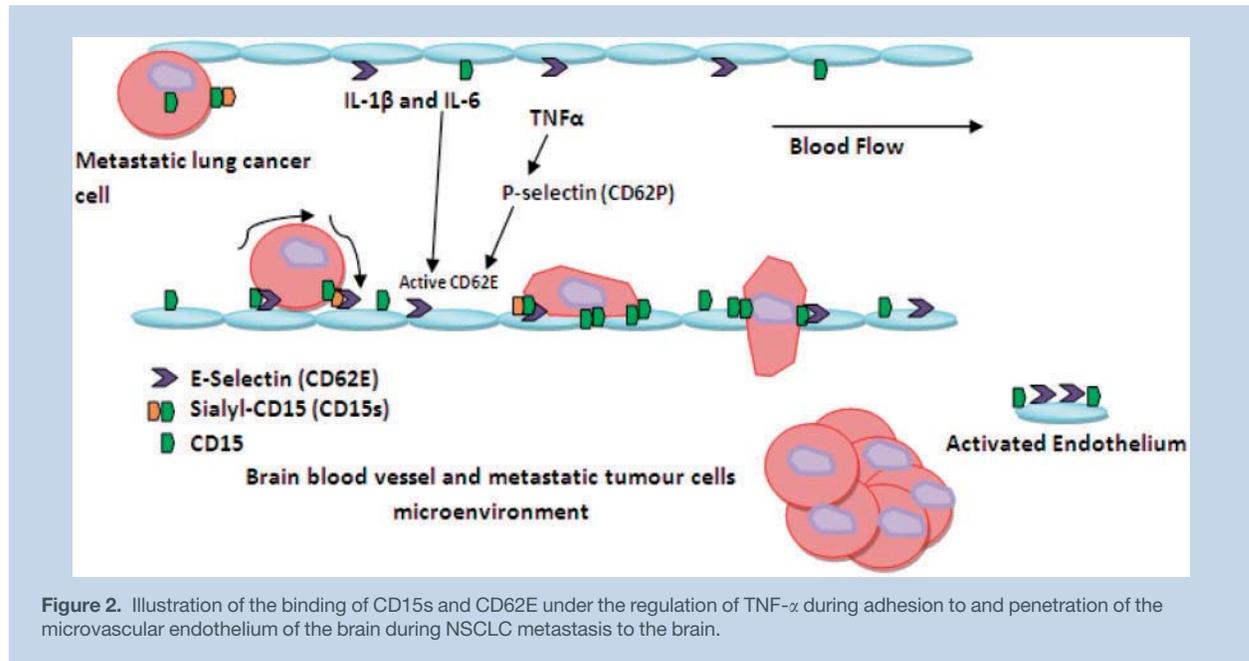
CCCs have specific cell-surface glycosides (specialized surface epitopes) that function in brain endothelial cell adhesion as well as provide protection from immune system attacks<sup>13–15</sup>. CD15 and CD15s (which differs from CD15 only by the addition of a sialic acid) are 2 such epitopes overexpressed in many metastatic cancers<sup>16,17</sup>. CD15 (Lewis X), also known as stage-specific embryonic antigen-1, is a carbohydrate cell adhesion molecule<sup>18,19</sup>. It is expressed on glycoconjugates of different cells during specific developmental stages. CD15 is associated with human polymorphonuclear granulocytes and various tumor cells, such as those of lung, colon, and breast carcinomas. Although CD15 and CD15s have been shown to be important cell-cell adhesion molecules which are implicated with metastasis in many non-CNS malignancies<sup>20,21</sup>, their functional role in metastasis to the brain has not been determined. Conversely, in fact, the



**Figure 1.** A hypothetical illustration of the sequence of events from the local spread of NSCLC, through intravasation at the lung, passage via the blood circulation, adhesion and intravasation at the cerebral blood vessels, diapedesis, and colonization of the brain.

nonexpression of CD15 and binding partner selectins in human primary brain tumors was considered to be a factor in the rarity of extraneural metastases from glioblastoma<sup>22</sup>. We, therefore, determined expression of CD15/CD15s in a range of primary and metastatic human NSCLCs by immunohistochemistry using the commercially available CD15 and CD15s antibodies and conducted our analysis using the Ariol microscope with the quantitative morphometric image analysis system. This showed that there was a consistent, elevated expression of both CD15 and CD15s at regions of vessel/brain intersection. CD15/CD15s expression was then evaluated using a range of primary and metastatic human NSCLC cell cultures with immunocytochemistry, flow cytometry, and Western blotting and confirmed that these antigens were more highly expressed in metastatic lung cancer cells (from brain and lymph node) than in those cells from the primary lung cancer lesions. CD62E is an important ligand of CD15 and CD15s, and it was reported that adhesion of lymphocytes to endothelial cells was regulated through the heterophilic interaction between these 2 molecules<sup>23</sup>. We therefore hypothesized that metastatic lung cancer cells may mimic this behavior, so we investigated CD62E expression in brain endothelial cells, after stimulation with tumor necrosis factor (TNF)- $\alpha$ . Results showed that CD62E was highly expressed in human brain endothelial cells, while stimulation with TNF- $\beta$  was also used to confirm the specificity of TNF- $\alpha$ . We then investigated the role of CD62E and CD15 in adhesion of lung cancer

to brain endothelial cells using the CytoSelect Tumor-Endothelium Adhesion Assay Kit (Cell Biolabs), which demonstrated that CD62E plays a key role in the adhesion of cancer cells to brain endothelium (Fig. 2). We then proceeded to demonstrate that CD15 and CD62E were co-localized at the site of adhesion of metastatic lung cancer cells adhering to the human cerebral microvascular endothelial cell (hCMEC)/D3 human brain endothelial monolayer using 3D confocal imaging generated by Z-stacks. Metastatic lung cancer cells were more adherent than the primary lung cancer cells, and immunoblocking of CD15 significantly reduced adhesion ability of cancer cells in a static assay. Similarly, immunoblocking of CD15 reduced the dynamic adhesion of highly metastatic lung to brain cancer cells onto hCMEC/D3 brain microvascular endothelial cells<sup>24</sup>, which were investigated under a live cell imaging flow system. For these dynamic flow studies we used Vena8 Endothelial+ biochips (channel volume: 2.69  $\mu$ L; Cellix). The chips were connected to a microfluidic pump (Cellix), and we kept the whole unit overnight in an incubator at 37°C, 5% CO<sub>2</sub>, under shear stress flow on perfusion mode with a 15mL/h volumetric flow rate (2.5 dyn/cm<sup>2</sup>). For live cell image analysis, the biochip was connected to a Zeiss Axiovert 200M inverted live cell (time lapse) microscope at 37°C, 5% CO<sub>2</sub>. NSCLC cells (green fluorescently tagged) were pumped into the system at 2.5 dyn/cm<sup>2</sup> controlled by a Mirus Evo nanopump (Cellix) and analyzed via VenaFlux Assay software. Live cell images were collected and movie



sequences generated using Volocity software v 5.4 (PerkinElmer). This initial work demonstrated the feasibility of targeting CD15/CD15s/E-selectin interaction between CTCs and brain endothelial cells<sup>25</sup>. More recently we have focused on the fucosyltransferases, a family of enzymes of which FUT4 and FUT7 aid in the synthesis of CD15 and CD15s, respectively. Here, we used both small hairpin RNA knockdown and CRISPR (clustered regularly interspaced short palindromic repeat) knockout of NSCLC cell-specific fucosyltransferases in a sophisticated series of all-human in vitro models of the BBB and its various components with state-of-the-art assessment tools to measure transendothelial electrical resistance (TEER) as well as live cell imaging with the capacity to mimic physiological blood flow to evaluate the influence of FUT4/FUT7 silencing on NSCLC adhesion and transmigration. TEER analysis using both 2D and 3D BBB models was carried out using Electric Cell-substrate Impedance Sensing (ECIS) for 2D and Endothelial Voltammeter (EVOM) impedance spectroscopy (CellZscope) for 3D studies. A transient reduction in TEER values indicated passage of cells through the BBB; silencing of both FUT4 and FUT7 in NSCLC cells reduced the transmigration potential of cancer cells, but overexpression of FUT4/CD15 and FUT7/CD15s increased and accelerated the transmigration of cancer cells (Jassam et al, submitted).

Although this points to the value of possible CD15/CD15s in therapeutic targeting to prevent brain metastasis of NSCLC and indeed other cancers of the lung and breast, additional factors may influence the propensity of CTCs to adhere to and transmigrate through brain capillaries. One other variable to consider is cell cycle phase in relation to CD15/CD15s, a little-investigated

area. We therefore sought to establish whether any such relationship existed. We initially found in nonsynchronized cells a prominent expression of CD15 in metastatic brain tumor cells (28%–36%) and a similar high expression of CD15s (34%–47%). We then went on to synchronize NSCLC tissue cultures at G0/G1 phase by serum starvation; at S phase by hydroxyurea (1 mM), since this inhibits ribonucleotide reductase activity<sup>26</sup>; and at G2/M phase by nocodazole (2  $\mu$ g/mL), as this inhibits the polymerization of microtubules<sup>27,28</sup> and found that, using a fluorescent cell cycle indicator system (FUCCI) and flow cytometry analysis, CD15 and CD15s overexpression, respectively, was correlated with cell arrest at G1 phase (Jassam et al, submitted).

These investigations not only underline the merits of using 3D in vitro modeling with human cells and serum when in tandem with contemporary analytical equipment in dissecting out pathways underlying critical brain tumor biology and evaluating potential therapeutic approaches, but, taken together, the new findings suggest that CD15/CD15s may provide a suitable target for the prevention of metastatic spread to the brain. Timing of therapy, however, would have to be planned to account for the variation in CCC cycle phases.

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# Prevention of Brain Metastasis by Inhibition of Angiogenesis?

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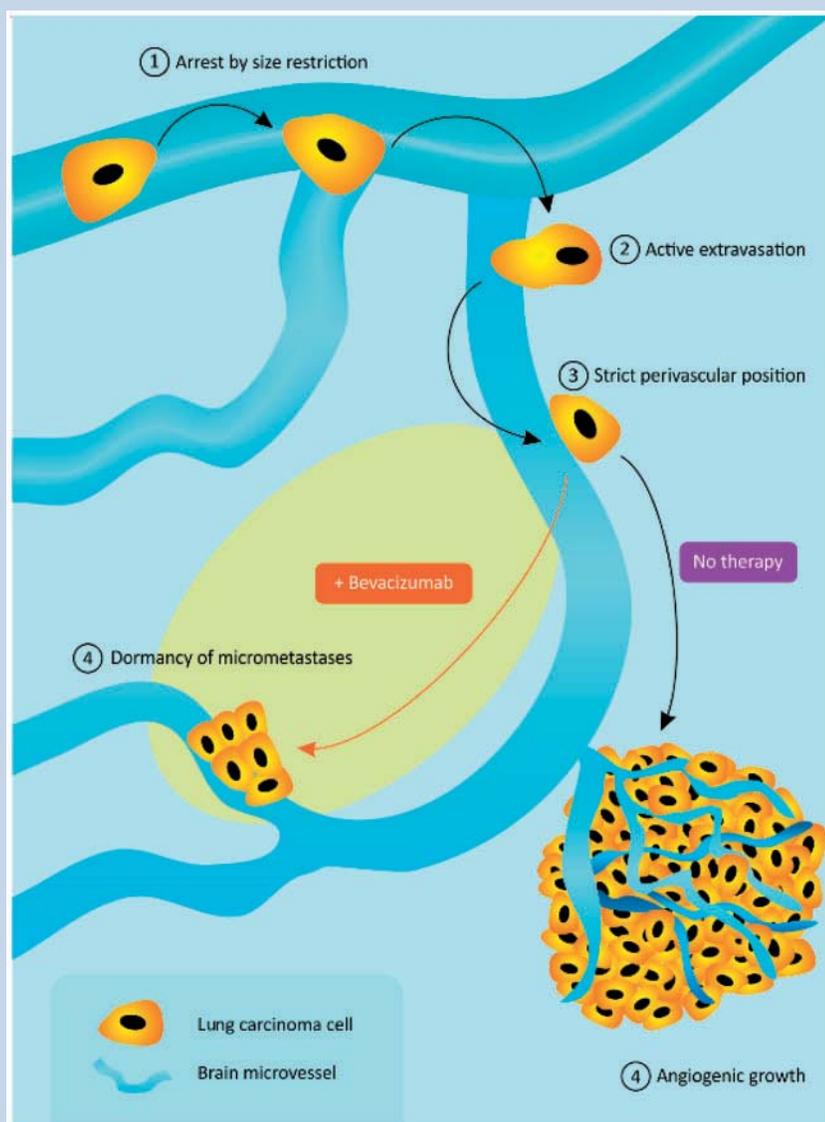
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Brain metastasis (BM) is frequently observed in distinct tumor entities, including nonsquamous non-small-cell lung cancer (nsNSCLC, mainly lung adenocarcinoma) and triple negative and human epidermal growth factor receptor 2 (HER2)-positive metastatic breast cancer (mBC)<sup>1</sup>. Lung cancer patients show by far the highest risk to develop BM during their disease: a current population-based study demonstrated that ~20% of all lung cancer patients will develop BM, compared with 6.5% of renal carcinoma, 6.9% of melanoma, and 5.1% of breast carcinoma patients<sup>2</sup>. Given its high incidence, lung cancer is therefore responsible for about 60% of all BM<sup>2</sup>.

BM is inevitably associated with high morbidity and mortality [2] and is a considerable burden for patients and caregivers. For example, for patients with stage III nsNSCLC, survival rates at 5 years are below 20%, mostly due to subsequent spreading of the tumor to the brain and other distant sites<sup>3</sup>.

There are limited treatment options for BM. One in 3 BMs can be locally treated by surgery and radiosurgery; if multiple metastases occur, whole-brain radiotherapy (WBRT) is applied, which prolongs life by 2–5 months but is associated with unwanted neurotoxic effects<sup>4</sup>. However, despite these multimodal therapies, recurrence rates are



**Figure 1.** Mechanism of prevention of BM by administration of anti-VEGFA agents like bevacizumab via induction of chronic dormancy of micrometastases (for details, see Kienast et al <sup>13</sup>).

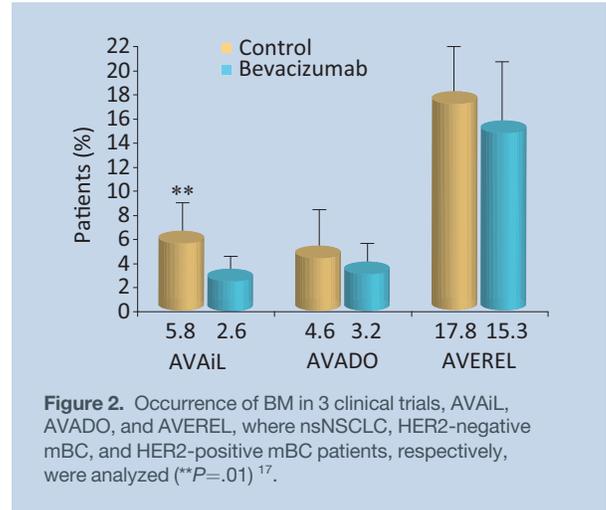
high, and median survival after BM occurrence is still below 1 year.

Remarkably, despite metastasis being the leading cause of death in patients suffering from solid tumors, there exists little clinical evidence regarding metastasis prevention<sup>5</sup>. For BM in small cell lung cancer, prophylactic WBRT has been shown to result in a decreased incidence of future BMs and improved overall survival<sup>6</sup>. For NSCLC, a large phase III study investigating prophylactic WBRT in stage III patients failed to enroll a sufficient number of patients, although BM was reduced by more than 2-fold, but without any detectable survival benefit<sup>7</sup>. There might be several explanations for this failure, including relevant neurotoxicity of WBRT<sup>4</sup> and the inclusion of squamous NSCLC patients, who are known to have a lower risk of developing BM<sup>8,9</sup>.

The treatment of BM as well as targeting of early metastatic formation in the brain are hindered by the blood–brain barrier, which compromises drug penetration<sup>10</sup>. Anti-angiogenic agents such as bevacizumab circumvent this problem; they target signaling at the cerebral endothelial cell without the need to fully cross the blood–brain barrier<sup>10</sup>. There exist several clinical and preclinical hints that chronic anti-angiogenic therapy with bevacizumab could prevent formation of metastasis in lung cancer. A subgroup analysis of the ECOG 4599 trial suggested that absence of a macroscopic residual tumor might improve overall survival in favor of bevacizumab<sup>11</sup>. A similar trend was demonstrated in the second large phase III study of bevacizumab efficacy in stage IIIB versus stage IV<sup>12</sup>. This suggests that the greatest benefit of bevacizumab should be expected for patients who do not have metastatic disease (ie, a high tumor burden already).

In our preclinical experiments using a novel mouse model where single metastasizing cancer cells were tracked by intravital microscopy, we demonstrated that early angiogenesis was mandatory for successful macrometastasis formation in the mouse brain, and prolonged anti-vascular endothelial growth factor A (VEGFA) treatment forced small micrometastases (5–10 nsNSCLC cells only) into a state of chronic dormancy without any signs of further growth over many weeks<sup>13</sup> (Fig. 1). In contrast, outgrowth of melanoma cells in the brain, which grew by co-option of preexisting brain vessels, was not influenced by bevacizumab treatment.

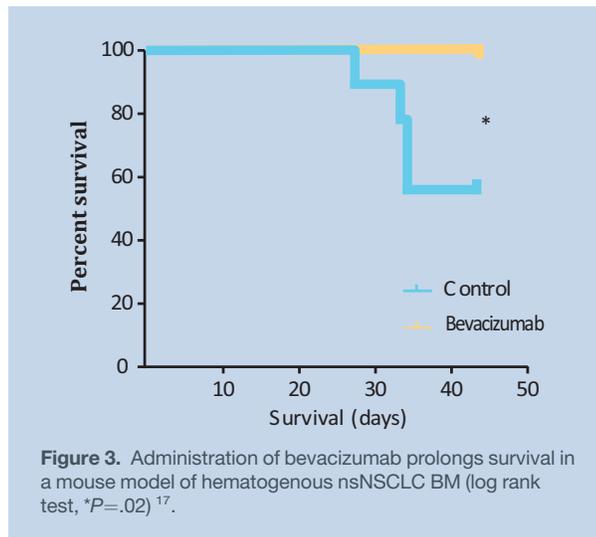
To characterize the role of bevacizumab on BM prevention, we retrospectively analyzed 3 phase III clinical trials regarding the incidence of BM in patients with nsNSCLC (the AVAiL trial<sup>12,14</sup>) and HER2-negative and -positive mBC (the AVADO and AVEREL trials, respectively<sup>15,16</sup>), where bevacizumab was part of the standard treatment<sup>17</sup>. Among the patients with nsNSCLC (AVAiL trial), BM as a first site of recurrence was significantly lower in the bevacizumab arm compared with the control chemotherapy arm (2.6% vs 5.8%;  $P=.01$ ; Fig. 2), with a lower risk of BM development over time (hazard ratio [HR], 0.36,  $P=.001$ ). No significant effect of bevacizumab regarding



BM occurrence was observed in patients with HER2-negative and -positive mBC. This suggests that bevacizumab might prevent or delay the formation of BM in nsNSCLC patients, whereas no effect was seen for BM prevention in mBC and for nsNSCLC metastasis outside of the brain. In all 3 trials, preexisting BM was an exclusion criterion for study entry; this criterion led to a bias for patients with a metastatic pattern preferentially involving sites other than the brain (ie, a low number of total BM events over time) but allowed exploration of the effects of bevacizumab on the development of new BM during and after therapy.

As a next step, we attempted to confirm the clinical data obtained from retrospective investigation of the 3 phase III trials<sup>17</sup>. For this purpose, different doses of bevacizumab were investigated in mouse nsNSCLC metastasis models. Using subclinical bevacizumab (5 mg/kg, twice weekly, i.p. administration) in brain-seeking lung adenocarcinoma cells, a BM preventive effect could be achieved, which also translated into a survival benefit in these mice (Fig. 3). No effects could be observed on the incidence of non-BM, confirming a brain-specific preventive effect. Together these data speak for the potential of anti-VEGFA therapies to prevent metastatic outgrowth, which appears to be specific for the brain, and particularly relevant for nsNSCLC.

Remarkably, further support for the concept of chronic anti-angiogenesis in suppression of early tumor outgrowth comes from 2 recent studies, both of which demonstrated that chronic impairment of angiogenesis is the reason why tumor growth is inhibited or prevented in 2 different mouse models of Down syndrome (trisomy 21)<sup>18,19</sup>. Chromosome 21 harbors 225 genes only, of which many are now identified as endogenous angiogenesis inhibitors, partly via the VEGF pathway—and a mild increase of 50% gene dose by the third chromosome appears sufficient to suppress the growth of solid tumors with high efficacy<sup>18–20</sup>. Most importantly in this context,



it is well known that the age-corrected mortality from solid tumors in individuals with Down syndrome is very low, less than 10% than expected<sup>21</sup>. Together with the preclinical data, this argues for an effective suppression of early tumor outgrowth by anti-angiogenesis therapy in humans, which should be achieved with considerably low doses of anti-angiogenic agents.

Combined, these data provide a strong biological and clinical rationale to test chronic anti-angiogenic therapy for its potential to prevent the occurrence of BM in patients. A controlled, prospective clinical trial is warranted, with stage III nsNSCLC patients being today the most plausible that should be included in such a trial. These patients are at particularly high risk of developing BM and to suffer from morbidity and mortality caused by them.

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# Glioblastoma: Example of Translational Research in Radiotherapy and Radiobiology

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## Abstract

Glioblastoma multiforme (GBM) is an aggressive brain tumor known to be highly radioresistant due to modulation of various signal transduction pathways involving tumor cells and their microenvironment. We describe in this paper our research experience in order to better understand and encompass this radioresistance.

We chose to focus particularly on all factors controlling angiogenesis, hypoxia, and invasion, but also on the intrinsic radioresistance of glioblastomas. Thus, we decided to study the pathway of fibroblast growth factor 2 (FGF-2), a potent angiogenic factor highly expressed in glioblastoma and its receptors (FGFR), as well as the pathway of  $\alpha v\beta 3$  and  $\alpha v\beta 5$  integrins expressed in both GBM cells and endothelial cells. We investigated their role in the control of the hypoxia pathway, known to be involved in tumor radioresistance. Moreover, one of the hypotheses that could explain the phenomenon of resistance to radiotherapy is the presence of tumor stem cells known to be radioresistant and dependent on FGF-2 and hypoxia. In this way, our team is also implicated in the deciphering of GBM stem cell radioresistance. Finally, our team is involved in the field of GBM advanced imaging to better assess the radioresistant features of such tumors in an attempt to improve local control by adapting a more ballistic radiotherapy.

The aim is to define some relevant therapeutic targets in association with targeted and personalized radiotherapy in order to develop adapted clinical trials, which could validate such targets and would lead to improved outcome of GBM patients.

**Keywords:** Glioblastoma, Radiotherapy, Radiobiology, Radioresistance, Angiogenesis, Integrins

## Introduction

Glioblastoma multiforme (GBM) is an aggressive and hypoxic brain tumor, with poor prognosis because of almost systematic local relapse despite appropriate treatment combining surgery and chemoradiotherapy<sup>1,2</sup>. This tumor's radioresistance is due to modulation of various signal transduction pathways and to crosstalk between tumor cells and their microenvironment, controlled by growth factors and their receptors.

Tumor response to radiotherapy is influenced by several intracellular tumor biological factors but also by the tumor microenvironment, such as the extracellular matrix and its components as well as tumor angiogenesis. Our team has been involved for several years in understanding the complex molecular mechanisms involved in the regulation of tumor microenvironment and radioresistance. Our approach was first to establish in the laboratory the proof of concept of a specific target as a key factor of radiosensitization and then to validate this target *in vivo* in orthotopic xenografts. The next step was to extrapolate our data to human patients through early clinical trials in order to validate these targets as surrogate markers of response to radiotherapy and as relevant therapeutic targets with the development of adapted targeted therapies.

We chose to focus particularly on all factors controlling angiogenesis, hypoxia, and invasion, but also intrinsic radioresistance of glioblastomas as well as factors controlling GBM stem cell radioresistance, including radiation-induced tumor cell plasticity. We focused on the pathway of fibroblast growth factor 2 (FGF-2), a potent angiogenic factor that is highly expressed in glioblastoma and its receptors (FGFR), but also the pathway of  $\alpha v\beta 3$  and  $\alpha v\beta 5$  integrins expressed in both GBM cells and endothelial cells<sup>3,4</sup>.

## FGF-2 Pathway

Fibroblast growth factors are in a wide range of cancers involved in a variety of cellular processes, such as proliferation, anti-apoptosis, drug resistance, and angiogenesis<sup>3</sup>. FGF-2 (also known as basic FGF) and its receptors (FGFR1, 2, 3, 4) turned out to be strongly expressed on glioblastoma cells, as well as on endothelial cells from tumor vasculature. Depending on the isoform, localization, or cell conditions, FGF-2 can undergo several alternate signaling pathways<sup>5</sup>.

Several data have shown that FGF-2 constituted a strong prognostic factor among adults and children with glioblastoma who were treated with radiotherapy, suggesting that FGF-2 could be strongly correlated with tumor response after radiotherapy<sup>6,7</sup>. One of our study targets was the impact of FGF-2 on GBM radioresistance.

## FGF-2 Increases DNA Radiation Damage Repair

FGF-2 is found in several isoforms. Five known isoforms of FGF-2 exist via alternative initiation of translation. Classically, we distinguish the low molecular weight secreted form (18 kDa) from the high molecular weight intracellular or intranuclear forms (22, 22.5, 24, and 34 kDa). First of all, our data showed that the 24-kDa nuclear isoform could increase the expression of DNA-dependent protein kinase catalytic subunit (DNA-PKcs), an enzyme involved in double-strand DNA break repair, through nonhomologous DNA end joining<sup>8-10</sup>, as is also described with epidermal growth factor receptor, which after irradiation is translocated to the nucleus and then activates DNA-PKcs<sup>11</sup>.

## FGF-2–Mediated Radioresistance Is Controlled by RhoB through Intracellular Radioresistance, but also Hypoxic Pathways and Angiogenesis

In a further step, we hoped to better understand this radioresistance mechanism from the FGF-2 pathway. We then found that this mechanism was mediated by RhoB, a small GTP-ase, also known to be activated by several stress agents, such as ultraviolet as well as ionizing radiation, hypoxia, or other growth factors (epidermal growth factor). Moreover, given that RhoB could undergo several posttranslational modifications (such as farnesylation or geranylation), we then studied which protein form was involved in radiosensitivity modulation. Our findings highlighted the farnesylated RhoB as the only key actor to inhibit radiation-induced mitotic cell death through control of radiation-induced centrosome overduplication<sup>12</sup>.

The next step was to analyze the impact of RhoB pathway inhibition on glioblastoma radiosensitivity. Our data first suggested that RhoB biological pathway inhibition could lead to *in vitro* radiosensitization of radioresistance in glioblastoma U87 cell lines<sup>13</sup>. Then we demonstrated that inhibiting RhoB pathways through *in vivo* induction of the negative dominant of RhoB could modify the

*in vivo* radiosensitivity of human GBM cell lines by controlling intrinsic radioresistance, hypoxia, and angiogenesis. Indeed, after RhoB inhibition, we first could observe an oxygenating effect and a normalization of vasculature<sup>13</sup>. Furthermore, this radiosensitizing effect could also be explained by the degradation of the hypoxia-inducible transcription factor 1 $\alpha$  (HIF1 $\alpha$ ) by the proteasome via glycogen synthase kinase (GSK) 3 $\beta$ <sup>14</sup>. These data strongly suggested that RhoB should be a major determinant of cellular resistance to ionizing radiation.

We then investigated the ability of the farnesyltransferase inhibitor R115777 (tipifamib), a pharmacological inhibitor of RhoB farnesylation, to radiosensitize human gliomas. We have shown that inhibition of RhoB farnesylation before irradiation led to significant radiosensitization of several GBM cell lines. This radiosensitizer effect was due to the induction of postmitotic cell death<sup>15</sup> but also to a significant oxygenation through a decrease of HIF1 $\alpha$  expression in U87 xenografts. We observed a normalization of vascularization, which was associated with inhibition of the expression of matrix metalloproteinase 2. These data strongly suggest that R115777 could increase tumor oxygenation, suggesting the involvement of RhoB in controlling GBM radiation resistance and its microenvironment<sup>16</sup>.

We then conducted a phase I and a phase II trial combining continuous infusion of the farnesyltransferase inhibitor tipifamib (R115777) with radiotherapy in patients with glioblastoma. This phase I allowed us to define the dose-limiting toxicity, which was obtained at the second planned dose level. These preliminary data then suggested the feasibility and the excellent tolerance of the association of tipifamib (200 mg/d) with standard radiotherapy in patients with glioblastoma<sup>17</sup>. Moreover, we observed a normalization of tumor perfusion 10 days after the start of radiation associated with tipifamib in patients in phase I who were followed by <sup>15</sup>O-H<sub>2</sub>O PET<sup>18</sup>. We confirmed in a very recent study this normalization of perfusion after association of radiotherapy and tipifamib using MRI perfusion<sup>19</sup>. The results of the phase II trial testing the association of 200 mg tipifamib with radiotherapy confirmed that this combination was well tolerated and showed promising overall survival rates but without increase in time to progression compared with historical data<sup>20</sup>.

## FGFR Inhibition, a New Strategy to Optimize the Efficiency of Radiotherapy in Glioblastoma

After having established the role of FGF-2 in tumor cell radioresistance, we wanted to investigate whether

inhibiting the FGF-2 pathways by targeting FGFR might represent a new strategy to optimize the efficiency of GBM radiotherapy.

We first investigated the role of an extracellular acting small molecule, an allosteric inhibitor of FGFR signaling at nanomolar concentrations (SSR128129E). We then demonstrated that targeting FGFR with this specific FGFR blocker decreased glioblastoma cell radioresistance by modulating tumor cell survival after irradiation. Moreover, SSR128129E given prior to irradiation in U87 orthotopic xenograft-bearing mice improved their neurological-signs-free survival rates. This inhibitor of FGFR signaling may represent an interesting radiosensitizing agent for glioblastoma<sup>21</sup>.

Thereafter, we focused more precisely on FGFR1. We first showed in our phase I-II clinical trials that expression of FGFR1 on GBM cells is an independent prognostic factor of overall survival and of progression-free survival after chemoradiotherapy<sup>20</sup>. These results led us to hypothesize that FGFR1 could be involved in the radioresistance of GBM and that inhibiting FGFR1 in tumor cells might increase the efficiency of radiotherapy. We thus showed that inhibition of FGFR1 could radiosensitize glioblastoma U87 cells by significantly increasing mitotic cell death after irradiation and decreasing phospholipase C gamma phosphorylation. We then performed an in vivo approach by generating subcutaneous limb xenografts with 2 different FGFR1 silenced U87 clones, irradiated with 2 fractions of 3 Gy, showing that silencing FGFR1 led to a significant in vivo radiosensitization. Moreover, silencing FGFR1 also led to a significant decrease in vitro and in vivo of HIF1 $\alpha$  expression. All of these results highly suggest interest in combining FGFR1 inhibitor with radiotherapy in clinical trials for patients with glioblastoma<sup>22</sup>.

Having demonstrated the involvement of these channels in the radiation resistance of glioblastomas, we continued our work by studying the signaling upstream of RhoB and FGF-2. We focused particularly on  $\alpha$ v $\beta$ 3 and  $\alpha$ v $\beta$ 5 integrins, known to be highly expressed in glioblastomas and endothelial cells<sup>4</sup>.

## Pathway of $\alpha$ v $\beta$ 3 and $\alpha$ v $\beta$ 5 Integrins

Integrins are cell surface heterodimers composed of several types of noncovalently associated  $\alpha$  and  $\beta$  chains<sup>23</sup>. Integrin ligands are the components of the extracellular matrix which can transmit a cell-to-cell or a cell-to-matrix signal. These integrins control not only angiogenesis, but also cell survival signals. In addition, it has been shown in glioblastoma models that irradiation delivered at doses of 3 Gy induced migration of tumor cells associated with increased expression of  $\alpha$ v $\beta$ 3 integrin<sup>24</sup>. One of our issues

was to study the involvement of these receptors in the radiation resistance of glioblastoma cells.

## Integrins Control Intracellular Radioresistance via RhoB, Survivin, and Integrin Linked Kinase

We have shown that upstream of RhoB, irradiation could activate integrins  $\alpha$ v $\beta$ 3 and  $\alpha$ v $\beta$ 5, which control radioresistance of several glioblastoma cell lines by inhibiting radiation-induced mitotic cell death. This activation is controlled by integrin linked kinase (ILK) and passes through the activation of RhoB in its GTP form<sup>25</sup>. More recently, we showed that irradiation activates HIF1 $\alpha$  expression via ILK, which in turn inhibits radiation-induced mitotic cell death via survivin.

Furthermore, treating U87 cells with the specific survivin inhibitor YM155 significantly increased the percentage of giant multinucleated cells, centrosomal overduplication, and thus U87 cell radiosensitivity<sup>26</sup>, underlining the importance of the integrin/HIF1 $\alpha$  pathway in the modulation of the radiosensitivity of glioblastoma.

Furthermore, we have shown that pharmacological inhibition of these integrins by a specific inhibitor, cilengitide, before radiotherapy can induce a significant radiosensitization of radioresistant glioblastoma cell lines. These results demonstrate a crosstalk via RhoB between the signaling pathways of both angiogenic factors FGF-2 and  $\alpha$ v $\beta$ 3 and  $\alpha$ v $\beta$ 5, leading to the decrease of GBM radiosensitivity.

## Involvement of Integrins in the Regulation of Microenvironment

We then explored the involvement of integrins in the regulation of the hypoxia pathway. We first showed that hypoxia induced the inhibition of HIF1 $\alpha$  degradation through RhoB and GSK3 $\beta$  and then that upstream of RhoB,  $\alpha$ v $\beta$ 3 and  $\alpha$ v $\beta$ 5 integrins controlled HIF1 $\alpha$  expression through focal adhesion kinase (FAK) in 2 different glioblastoma cell lines, U87 and SF763<sup>14,25</sup>. Moreover, silencing this pathway in vivo in glioma cells of established xenografts using short interfering RNA-directed FAK or  $\alpha$ v $\beta$ 3 integrin led to a significant oxygenation and a vascular

normalization obtained in 4 days of inhibition, demonstrating the involvement of these receptors in glioblastoma radioresistance, by modulating intracellular radiosensitivity as well as tumor microenvironment<sup>25</sup>.

## Integrin Inhibitor Trials

Due to the encouraging preclinical results of the association of cilengitide with radiotherapy, several studies have been developed. Thus, a phase I-IIa trial on the association of cilengitide (i.v. 500 mg twice a week) with radiotherapy and temozolomide provided promising data on tolerance and efficacy, with median survival of 16, 1 months, favorably compared with historical data of 14.6 months for patients treated with temozolomide-based chemoradiation only. In addition, a gain of progression-free survival and overall survival rates was observed for patients whose MGMT (O<sup>6</sup>-methylguanine-DNA methyltransferase) promoter was methylated<sup>27</sup>.

One explication could be a better penetration of temozolomide into the tumor, through normalization of vascularization induced by  $\alpha v \beta 3$  inhibition, as our lab results suggested.

Based on these preliminary data, a multicenter randomized phase III trial was recently conducted among methylated GBM patients, to assess the added value of cilengitide combined with standard treatment. This study did not show a survival benefit in favor of cilengitide, but the injection regimen of this agent twice a week was probably not the most appropriate to highlight a significant difference<sup>28</sup>.

In addition, we confirmed the importance of the expression of these factors as independently prognostic of overall survival in glioblastoma 20 as well as radio- and chemosensitivity surrogate markers in tumors other than glioblastoma. Indeed, we have identified that coexpression of FGF-2 and  $\alpha v \beta 3$  integrins in the tumors of stage III non-small-cell lung carcinoma (NSCLC) patients treated with exclusive radiochemotherapy was associated with worse local control, suggesting that inhibition of  $\alpha v \beta 3$  integrin could induce a radiosensitization of such tumors<sup>29</sup>. These results allowed us to design an ongoing clinical trial which tests the association of the specific  $\alpha v \beta 3 / \alpha v \beta 5$  integrin inhibitor cilengitide given continuously with radiochemotherapy and then at a dose of 2000 mg twice a week in association with chemotherapy in patients with stage III NSCLC. Cilengitide given continuously with radiochemotherapy was well tolerated and showed encouraging clinical results, suggesting that targeting  $\alpha v \beta 3$  integrin continuously during radiochemotherapy in NSCLC is a promising approach to treat this disease<sup>30</sup>.

Moreover, we also showed that  $\alpha v \beta 5$ -FAK-GSK3 $\beta$  expression profile was predictive of chemotherapy sensitivity in high-grade osteosarcomas<sup>31</sup>, suggesting the key role of this pathway in modulating radio- and chemotherapy tumor sensitivity.

## Radioresistance and Tumor Stem Cells

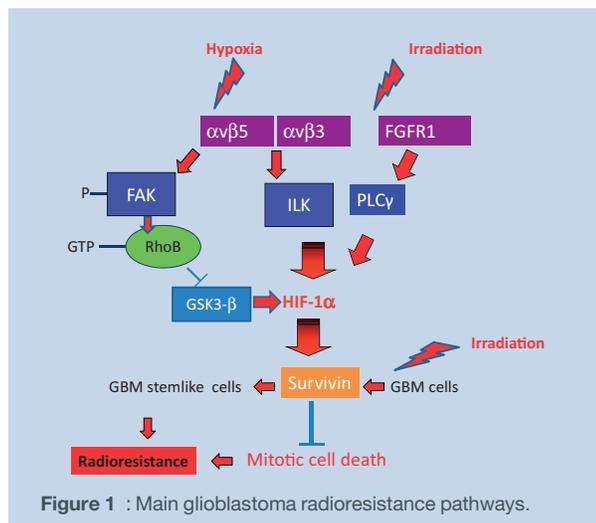
Tumor stem cells are known to contribute to glioma radioresistance, particularly through preferential activation of the DNA damage checkpoint response and increase in DNA repair capacity<sup>32</sup>. These stem cells are present in niches and require the proximity of vessels to survive, and in particular FGF-2, reinforcing the principle that inhibition of FGF-2 and its pathways would achieve radiosensitization of glioblastomas. Moreover, hypoxia has been shown to induce GBM plasticity and to induce a reprogramming from GBM to the GBM stemlike phenotype<sup>33</sup>.

In this way, one of the hypotheses that could explain the phenomenon of resistance to radiotherapy is the presence of these tumor stem cells within or outside the tumor burden; moreover, irradiation could induce a reprogramming or dedifferentiation process, thus leading to emergence of radioresistant stemlike GBM cells. We very recently published that clinically relevant doses of irradiation induce a reprogramming process and that survivin is a mediator of the pathway leading to this phenomenon<sup>34</sup>. We are currently deciphering the pathway that induces this phenomenon with the aim of detecting new inhibition targets in combination with radiotherapy in the treatment of glioblastoma. Moreover, having identified an imaging technique that could identify the site of subsequent relapse<sup>35</sup>, we set up a parallel ongoing biological study to determine through guided biopsies according to metabolic imaging whether such predictive sites of relapse are enriched in stem cells.

## Metabolic and Functional Imaging

Next to radiobiological considerations, our team is also strongly involved in the field of GBM advanced imaging, in attempts to better assess the radioresistant features of such tumors and to improve local control by adapting ballistic radiotherapy. GBM is an infiltrating and heterogeneous brain tumor characterized by high cellular proliferation, high cellular density, and active angiogenesis associated with areas of necrosis. Therefore, one promising strategy to circumvent GBM cell radioresistance consists in delivering a heterogeneous irradiation dose within the target volume, with focal increases in dose targeted at radioresistant clusters defined by metabolic/functional imaging. This *dose-painting* approach was first described by Ling in 2000 and brings new hope to improve outcomes of GBM patients<sup>36</sup>.

The main data on this field involved <sup>1</sup>H magnetic resonance spectroscopic imaging (MRSI) as a way to identify such aggressive clusters. Laprie et al<sup>35</sup> thus showed that



among patients from our phase I-II prospective clinical trial combining tipifamib with radiotherapy previously mentioned, a choline/N-acetyl-aspartate ratio up to 2 (CNR2) on pre-radiotherapy MR scan can identify the site of subsequent relapse with positive predictive values of 75% and 80%, depending on the location of CNR2 voxels within T1-weighted contrast-enhancing regions or T2-weighted abnormal regions, respectively. In light of these results, the authors worked on the integration of MRSI data into the treatment planning system. Indeed, MRSI images do not conform to DICOM standards and therefore cannot be automatically coregistered with the planning CT scans. After presenting a robust integration method of metabolic maps into the treatment planning system, the authors established (through dosimetric study) that a dose escalation targeted on metabolic abnormalities in a simultaneous integrated boost-intensity modulation radiotherapy (SIB-IMRT) approach was reasonably feasible in terms of dose to organs at risk<sup>37</sup>. All of these results have given rise to an ongoing multicenter prospective phase III clinical trial (NCT01507506), promoted by Institut Claudius Regaud/Institut Universitaire du Cancer de Toulouse-Oncopôle, assessing the benefit of a SIB-IMRT boost up to 72 Gy guided by MRSI compared with a standard dose of 60 Gy, both with temozolomide.

Other data on MRSI have recently also yielded strong results on the lactate/N-acetyl-aspartate ratio (LNR). Indeed, we showed that pre-radiotherapy LNR >0.4 voxels was significantly predictive of subsequent local recurrence, with a positive predictive value of 71%<sup>38</sup>.

Regarding perfusion and/or diffusion imaging, Ken et al<sup>19</sup> also prospectively evaluated the evolution of perfusion data within tumor following radiotherapy combined with tipifamib. All in all, a voxel-wise analysis of pre- and post-treatment relative cerebral blood volume from T2\* dynamic-susceptibility contrast MRI data allowed us to demonstrate a tumor perfusion normalization following the treatment. These

results come to confirm our preclinical data<sup>13,16</sup>. Other prospective trials are ongoing to assess the value of perfusion and diffusion imaging for treatment planning.

## Conclusion and Perspectives

The complexity of biological pathways controlling intrinsic tumor radiosensitivity and their microenvironment could explain the failed association of targeted therapies with radiotherapy. Indeed, the specific inhibition of a pathway may be the cause of an upregulation of another pathway involved in the radioresistance. Researchers have first to prove the concept of the efficacy of inhibiting a target in the laboratory, and they should critically interpret the results before moving into definitive large-scale phase III studies. Misuse of drugs can lead to their ineffectiveness, and metabolic imaging should be extensively used and added to early trial data to better understand the right schedule of administration of targeted drugs and radiotherapy. A lot of drugs have been explored over the past decade. Inhibition of angiogenesis and hypoxia remain prime treatment targets. Other tumor escape pathways could be caused by the inhibition of the immune system. Immune cells can traverse the blood-brain barrier and effect vigorous immune responses in the brain. Radiotherapy can cause immunogenic tumor cell death resulting in cross-priming of tumor-specific T cells, acting as an in situ tumor vaccine<sup>39</sup>. However, radiotherapy alone rarely induces effective antitumor immunity resulting in systemic tumor rejection. Local radiation therapy combined with immunotherapy can potentially synergize and produce an increased therapeutically effective antitumor response in local relapses of glioblastoma<sup>40</sup>. Our research will also focus on immunotherapy through a close collaboration between clinicians and biologists to try to improve outcome of patients with glioblastoma.

Conflict of interest statement. E. Cohen-Jonathan Moyal served as a member of an advisory board for Merck KGaA.

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# Development of a Questionnaire to Measure Instrumental Activities of Daily Living (IADL) in Patients with Brain Tumors

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## Background

In the last decade, patients' functioning and well-being have become increasingly important in clinical trials with primary or metastatic brain tumor patients. Most clinical trials now include health-related quality of life (HRQoL) and/or cognitive functioning as secondary outcomes, in addition to the more traditional outcome measures like progression-free and overall survival and tumor response assessed by neuroimaging<sup>1</sup>.

HRQoL and cognitive functioning give valuable insight into the patient's physical, cognitive, role, emotional, and social functioning, as well as into symptoms induced by the disease and the treatments<sup>2</sup>, and the patient's level of (dis)functioning on different cognitive domains,<sup>3,4</sup> respectively. However, these outcome measures are not easily translated to how patients actually function in their daily lives. Therefore, an additional Patient Reported Outcome (PRO) measure is needed to assess a patient's ability to perform activities of daily living (ADL).

There are 2 categories of ADL, the basic activities of daily living (BADL) and the instrumental activities of daily living (IADL). BADL include basic skills such as feeding, bathing, and dressing. IADL, on the other hand, include skills required for autonomous functioning like food preparation, the ability to handle finances, the ability to independently travel by car or public transportation, and the more modern skills, such as the ability to use a computer or smartphone. The characteristic cognitive decline in brain tumor patients is presumed to negatively impact their abilities to perform IADL in particular. This is because IADL involve higher-order activities "with little automated skills for which multiple cognitive processes are necessary"<sup>5</sup>.

The new proxy-based Amsterdam IADL Questionnaire<sup>®</sup> for early dementia patients was recently developed based on the input from patients, proxies, and health care professionals, and validation revealed good psychometric properties.<sup>6</sup> To our knowledge, no such questionnaire currently exists for brain tumor patients. However, early dementia patients and brain tumor patients both exhibit cognitive decline during the course of the disease and might experience similar problems with IADL. Therefore, a pilot study was conducted to determine if and how applicable the Amsterdam IADL Questionnaire<sup>®</sup> was for brain tumor patients.

## Methods

In the pilot study, primary brain tumor patients, their proxies, and health care professionals evaluated all items of the original Amsterdam IADL Questionnaire<sup>®</sup>. In step 1, patients and proxies ( $N = 20$ ) had to indicate for each item in the Amsterdam IADL Questionnaire<sup>®</sup> if they (i) recognized a problem with the IADL and (ii) if the question

was clearly formulated. Health care professionals ( $N = 6$ ) had to answer an additional question: (iii) if they considered the activity to be an IADL. In step 2, in-depth interviews were held with the same health care professionals and a new group of patients and proxies ( $N = 12$ ), to identify new IADL problems not covered by the Amsterdam IADL Questionnaire<sup>®</sup>. In step 3, a new group of patients and proxies ( $N = 12$ ) were requested to vocalize their thoughts on the items generated from the previous steps to identify any residual ambiguities or repetitiveness during a cognitive debriefing.

## Results

Although almost all Amsterdam IADL Questionnaire<sup>®</sup> items were considered as IADL, health care professionals indicated that just 63% of the items were considered affected in brain tumor patients. Remarkably, brain tumor patients and their proxies recognized problems with only 20% and 21% of the items, respectively. Moreover, patients and proxies considered most questions clearly formulated (93% and 86%, respectively), unlike the health care professionals, who suggested rephrasing for 51% of the items. The in-depth interviews generated several new issues, including items related to work, social interactions and activities, and raising (grand)children. The cognitive debriefing resulted in several questions being altered, merged, clarified with examples, rephrased, or omitted. Finally, the pilot study resulted in a provisional list of 37 items measuring IADL relevant to brain tumor patients.

## Conclusion

This pilot study revealed that IADL problems between early dementia patients and brain tumor patients only partially overlap. Many items in the Amsterdam IADL Questionnaire<sup>®</sup> were not relevant to brain tumor patients, and some relevant IADL issues were missing. Therefore, the need for an IADL questionnaire specifically for brain tumor patients still exists. This pilot study was the first step in the development of a valid and reliable IADL questionnaire for brain tumor patients. Further development of this IADL questionnaire is currently in progress. In the future, this questionnaire may be a beneficial additional outcome measure in both clinical practice and clinical trials.

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# A pro/con debate on the first-line treatment of patients with high-grade gliomas

## Interview with Roger Stupp and Michael Prados

We intended initiating a pro/con debate on the first-line treatment of patients with high-grade gliomas with an unmethylated O<sup>6</sup>-DNA methylguanine-methyltransferase (*MGMT*) promoter. Roger Stupp was invited to give a “con” statement to the actual standard of care and Michael Prados to argue “pro.” The answers are unexpected and pathbreaking.

**Christine Marosi, MD, for the Journal:**  
Do you see arguments for still treating all patients with newly diagnosed glioblastoma in the first line with concomitant and adjuvant temozolomide?

Roger Stupp, MD: This question has been out there since our initial report on the combination of temozolomide (TMZ) and radiotherapy (Stupp et al, *NEJM* 2005) and Monika Hegi and coworkers’ analysis of the limited benefit (if any) of TMZ in patients without an *MGMT* promoter methylation (Hegi et al, *NEJM* 2005). This is now over 10 years. Since then, a substantial body of additional data has been accumulated. Multiple studies have confirmed the predictive value of *MGMT* status in glioblastoma. And *MGMT* remains the most important prognostic factor for outcome. In other glioma subtypes, however, the predictive value is of lesser importance while its prognostic impact remains. Two studies in elderly glioblastoma multiforme (GBM) patients compared exclusive radiotherapy with TMZ chemotherapy alone (Wick et al, *Lancet Oncol* 2012; Malmstrom et al, *Lancet Oncol* 2012). In both trials patients fared best when they were treated with the modality adapted to their molecular profile—that is, methylated patients survived longer when they were treated with TMZ upfront, while patients with an unmethylated promoter in the tumor had a substantially inferior

survival when they did not receive radiotherapy.

The only not-very-convincing reasons not to use this now fully established molecular marker are the lack of better alternative treatments for patients without *MGMT* promoter methylation and doubts on the technical validity of the test result.

Michael Prados, MD: I do not feel all patients with newly diagnosed glioblastoma should be treated with concurrent and adjuvant TMZ. The available clinical data do not support a “clinically meaningful benefit” in those patients with promoter nonmethylated *MGMT* status and should not require us to use this medication in all patients. While it does seem clear that a benefit exists for the use of TMZ in *MGMT* promoter methylated patients, the benefit in *MGMT* nonmethylated patients is much less clear. Median overall survival in the prospective randomized phase III trial reported by Stupp et al for nonmethylated patients was 12.6 months for those patients treated with concurrent and adjuvant TMZ compared with 11.8 months for patients treated with radiation only, a difference of only 1 month (1,2). The study did not stratify by *MGMT* promoter methylation status, which was unknown for a number of cases, and the survival benefit was based upon a subset, post-hoc analysis. The actual number of patients with

known methylation status was small in both cohorts in the longer-term follow-up studies (60 and 54 patients, respectively). While the 2-year and beyond survival rates were different (favoring the combination treatment) in the promoter nonmethylated group, the reason for those differences cannot be accounted for entirely based upon the use of TMZ. The Radiation Therapy Oncology Group trial 0525, which tried to capitalize upon TMZ use (standard vs dose-dense TMZ) showed no difference in median overall survival for promoter nonmethylated patients (3). In a later review of the outcomes data for this trial, the survival advantage for patients with *MGMT* gene promoter methylation was substantial in the first 2 years but even in this group lessened thereafter (4). Thus even in the favorable methylation-positive group, the impact over time was less important with the use of TMZ (dose-dense or standard), suggesting that other biomarkers are likely accounting for the difference. Indeed, the progression-free survival benefit of dose-dense over standard-dose TMZ occurred only in the first 6 months (hazard ratio: 0.70; 95% CI: 0.58–0.86;  $P < .001$ ) and diminished thereafter. Conclusions about the benefit of TMZ on the longer-term outcomes beyond the median outcomes are uncertain at best and, to this investigator, unlikely to be specific to this agent.

Additional trials in elderly or poor-prognosis cases also support the notion that the use of TMZ is not beneficial in the non-methylated patient group. As just one example, the German Glioma Network, a prospective observational study of 233 elderly GBM patients >70 years of age, does support *MGMT* as a predictive marker for chemotherapeutic response. Again, however, the median overall survival significantly improved (by 5.3 mo) by adding chemotherapy to radiation only in methylated patients, but the improvement of 1.6 months in the unmethylated patients was not significant, with the author's recommendation to treat those patients with radiotherapy only (5).

Other biomarkers of outcome (prognostic biomarkers) may soon become more reliable to account for overall survival differences—including outcomes beyond the median—than the singular use of promoter methylation of *MGMT*. A recent study published in the *New England Journal of Medicine* supports the use of different biomarkers for survival, including isocitrate dehydrogenase mutation, telomerase reverse transcriptase promoter mutation, and 1p/19q deletion status (6). While none has yet been validated as a predictive biomarker, these will likely become important stratification factors over time and will add or possibly diminish the role of *MGMT* promoter methylation as a prognostic biomarker, and possibly impact its role as a predictive biomarker, particularly in promoter nonmethylated cases.

Mandating the use of TMZ as a standard of care in promoter nonmethylated patients also complicates the investigations of other single agents in this poor prognostic patient group. For instance, the GLARIUS trial, an open-label prospective randomized phase II study of irinotecan plus bevacizumab and radiation versus chemoradiation with TMZ in newly diagnosed *MGMT* promoter nonmethylated GBM, found a significantly prolonged median progression-free survival of 9.7 months in the experimental arm versus 5.99 months in the standard TMZ arm (7). No difference was seen in overall survival because of a high crossover treatment with bevacizumab in the TMZ-treated group. However, the median overall survival of 16.6 months in the experimental arm suggests that it is reasonable to omit TMZ in treatment of newly diagnosed unmethylated glioblastoma.

Given the evidence to date, the consensus of the US National Cancer Institute supported the Brain Malignancy Steering Committee Clinical Trials Planning

Workshop (Report from the Targeted Therapies Working Group) and strongly urged clinical trial development of new agents in the *MGMT* promoter nonmethylated patient group, omitting the use of concurrent and adjuvant TMZ for many of the reasons described above, including a lack of a clinically meaningful benefit that outweighs the potential toxicity of TMZ (8).

#### **Christine Marosi: Which test for *MGMT* promoter methylation do you rely on?**

Roger Stupp: There is no one single best test, and as with any method there are always advantages and potential pitfalls. Still, the one test that has been validated with outcome is based on methylation-specific PCR, marketed by MDxHealth, and licensed by LabCorp. Their methodology has been validated and correlated with outcome through many different datasets. It also allowed establishment of a clinically meaningful cutoff aiming to err on the side of calling a sample methylated in order not to withhold potentially effective TMZ treatment from a patient. There are other methods to assess the extent of gene promoter methylation; however, these methods lack the extensive clinical correlation.

Michael Prados: We currently use Sanger sequencing of bisulfite-treated DNA. The method assesses 17 cytosine-phosphate-guanine (CpG) islands with a score of 0–17, with 1 or greater being positive. The range of the methylation index (1–17) does help to support the relative nature of the methylation status of the tumor. A specific range has still to be validated, however, in terms of TMZ response or lack of response, particularly within the context of any multivariate analysis of impact of TMZ in overall outcomes, including progression-free and overall survival. Other methodologies—such as methylation bead-chip arrays (Illumina Human Methylation Bead-Chip 450K), methylation-specific PCR, protein expression, and CpG island methylation phenotype—are also used to assess methylation status. Specific methylation sites are likely not as predictive as the “total” amount of promoter methylation present, supporting the use of a methylation index score as potentially more predictive. A cutoff of greater than 30% to 40% of assessed sites being methylated is likely more predictive than less than 25%, but again, prospective assessment in larger randomized clinical trials is lacking. This has become an issue about the inconsistency in *MGMT* status “calls” across clinical trials.

#### **Christine Marosi: Do we need further studies to prove that TMZ and other alkylating agents do not show antitumor activity in unmethylated GBM?**

Roger Stupp: In my opinion, clearly not. If there is any effect, the effect is marginal and not clinically meaningful. I would rather participate in a clinical trial evaluating a novel compound than undergo treatment, albeit “well tolerated,” with minimal if any activity. TMZ has some potential toxicity. Patients sometimes realize the associated fatigue only when they discontinue TMZ. Myelosuppression is individually unpredictable, and the reduced bone marrow reserve may only be noted later when second-line chemotherapy is prescribed.

We at the European Organisation for Research and Treatment of Cancer and others have been conducting studies focusing on *MGMT* unmethylated tumors. While temsirolimus and cilengitide failed to improve outcome when added to radiotherapy in newly diagnosed glioblastoma, there was no evidence that these patients fared worse without concomitant and adjuvant TMZ [Wick et al, *Clin Cancer Res* 2016, in press; Nabors, *Neuro Oncol* 2015; Hegi & Stupp, Editorial, *Neuro Oncol* 2015].

I strongly believe we need to move on rather than waste time and effort on perfectionist proof and wanting to establish the single best methodology. What we need is a reliable, reproducible, and validated test. And in our quest to better treatments, we need to get rid of therapies that do not bring sufficient benefit.

Michael Prados: I do feel that additional studies to support the use of other strategies in newly diagnosed nonmethylated GBM are appropriate, either omitting the use of TMZ or at least considering randomized trials, perhaps limited to phase II as proof of concept, similar to the GLARIUS trial, before we consider proceeding to larger randomized phase III trials. Consistency in promoter methylation “calls” will be important, as well as appropriate stratification based upon promoter methylation status. The use of other prognostic biomarkers to correlate both outcome and response would be very helpful, controlling for other known patient factors. However, one could also make a strong argument to proceed to other trial designs *without* using TMZ as a “control” given the minimal impact on overall survival. The use of historical controls may be reasonable in this context and would allow novel strategies without the confounding impact of TMZ toxicity when combined with new agents.



# Indian Society of Neuro-Oncology: Challenges and Opportunities

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Although primary neoplasms of the nervous system comprise only a small proportion (1%–2%) of all tumors, they are the leading cause of disability and death from cancer in children and young adults worldwide. The diverse landscape of neuro-oncology in terms of demographics, presentation, histology, treatment, and outcomes provides unique challenges and opportunities to clinicians, scientists, and researchers alike for striving toward improving diagnosis, prognosis, and therapy. In addition, India's sociocultural diversity, vast geographic location, relative lack of resources, financial constraints, and consequent non-uniformity of care pose considerable challenges in modern neuro-oncology practice.

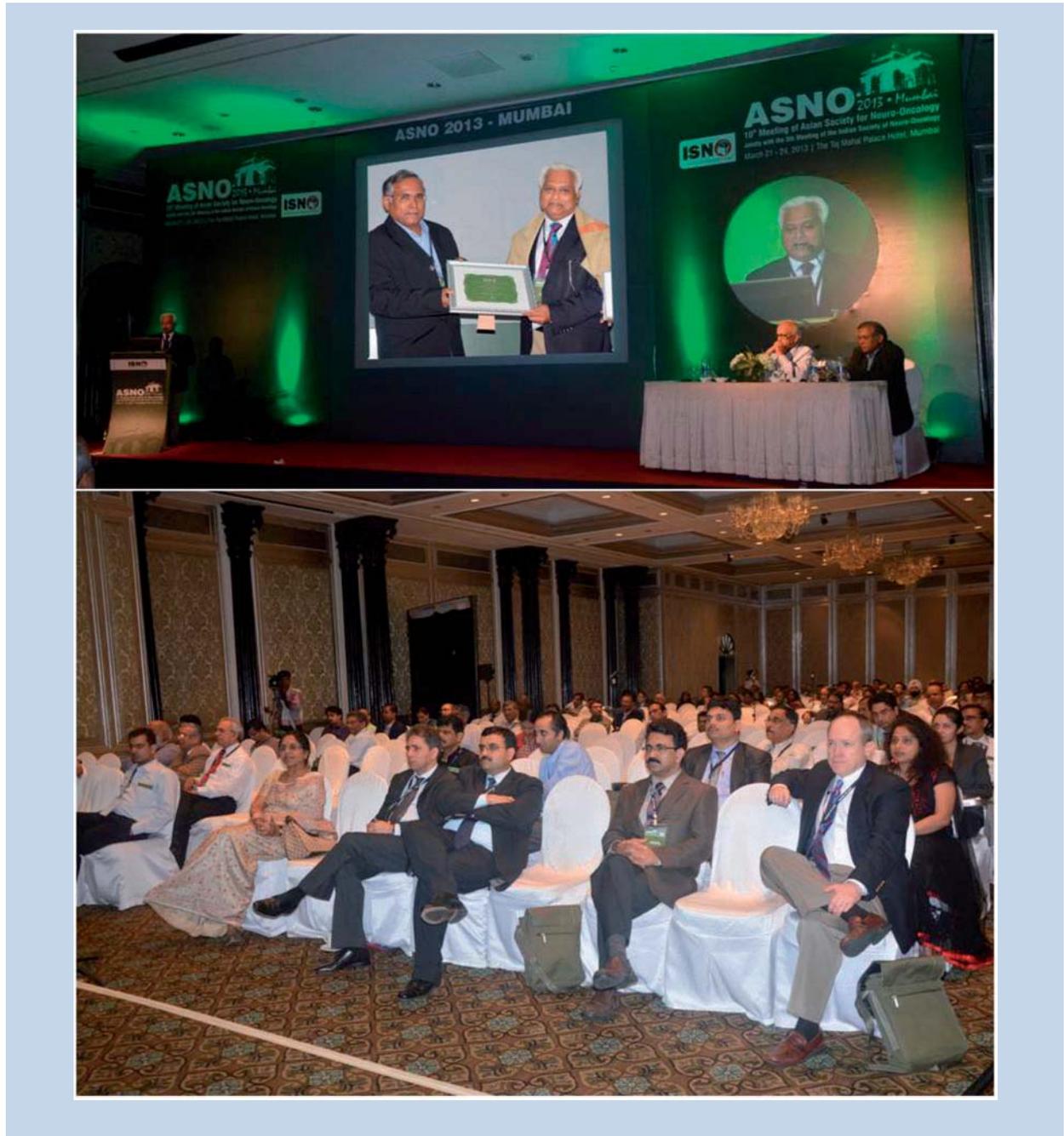
In a bid to harness and bring together the otherwise exceptional expertise existent within the country, the Indian Society of Neuro-Oncology (ISNO) was formally launched during the Annual Evidence Based Management meeting in 2008, held at Tata Memorial Centre, Mumbai, with "Evidence-Based Management in Neuro-Oncology" as its major theme. Over the years, ISNO has not only established itself as the premier academic forum for promotion and advancement of scientific knowledge and research in the field of neuro-oncology within India, but has also liaised with other preeminent societies to mark its presence in the international arena. Membership in the Asian Society for Neuro-Oncology (ASNO) and the World Federation of Neuro-Oncology Societies (WFNOS) was a natural corollary to this recognition, with the Xth Annual Meeting of ASNO being hosted in Mumbai in 2013, along with the Vth Annual Meeting of ISNO (Fig. 1). The allocation of a separate special "ISNO session" at the 2015 Annual Meeting of the Society for Neuro-Oncology (SNO) in San Antonio, Texas, USA, is a testimony to its fast-growing reputation and willingness for international cooperation and collaboration.

ISNO epitomizes multidisciplinary approaches and care, which is largely reflected in its diversified membership (presently comprising of 301 life members and 26 associate members) and includes representation from all major stakeholders relevant to neuro-oncology, such as neurosurgery, radiation oncology, neuro-oncology, neuro-pathology, basic neuro-sciences, pediatric oncology, medical oncology, neuro-radiology, neurology, psychiatry, and rehabilitation specialists. Since inception, ISNO has been holding its annual meeting (ISNOCON) every year in the spring (March–April) in different parts of the country, choosing a theme or topic for every conference. One of the highlights of ISNOCON is the "Ab Guha Oration," in loving memory of the late professor Abhijit Guha, clinician-scientist par excellence, who was instrumental in setting up the society, providing it mentorship and guidance for several years, and who continues to inspire us even today. In keeping with the high standards, this Oration has been delivered every year by the doyens of neuro-oncology, including Dr P. N. Tandon, Dr M.R.S.

Rao, Dr Andreas von Deimling, Dr Michael Brada, Dr Roger Stupp, Dr Martin Taphoorn, and Dr Hugues Duffau (2009–2015, respectively). Dr Patrick Wen, current editor of *Neuro-Oncology*, is the Ab Guha Orator for the forthcoming ISNOCON, scheduled to be held April 1–3, 2016 at Hyderabad. More details about the Society and activities are available through the website ([www.isno.in](http://www.isno.in)).

Another interesting and sought-after event at ISNOCON is the "Don't Miss It Session," wherein leading experts critically appraise and summarize some of the landmark studies presented/published in the preceding year that have either changed practice or improved our fundamental understanding. Under the dynamic leadership of its founding office bearers, ISNO has also taken up the challenge of generating "consensus recommendations and guidelines" for the management of "common brain tumors" such that there is uniformity of care throughout the country. Medulloblastoma, the poster boy of contemporary pediatric neuro-oncology, was chosen as the first prototype cancer for which an expert panel was constituted for drafting the consensus guidelines (first presented at ISNOCON 2015). Following widespread discussion and dissemination through various meetings and incorporation of suggestions from all quarters, the final version will soon be ratified and approved to be published as an ISNO Position Paper in a leading Indian journal. This year's guidelines will be focused on the new WHO classification of CNS tumors, which we hope will be simple and pragmatically applicable in common brain tumors for pathologists and for clinicians as well.

In an attempt to promote good science and academic competitiveness, ISNO now offers annual awards and training fellowships in basic, translational, and clinical neuro-oncology categories for both students and faculty. One such award is the Annual Award for Outstanding Contribution to Neuro-Oncology, which is conferred upon an individual or group of individuals for significant and seminal work done within the country with potential for international acclaim. To inculcate and foster the spirit of mutual collaboration, ISNO has been the driving force behind the collection and pooling of data (demographics, clinico-pathologic characteristics, outcomes) from major academic centers within the country resulting in several peer-reviewed publications. The next logical step is to help launch and coordinate prospective multicenter trials on questions relevant to the Indian context. One such trial on high-risk embryonal CNS tumors using upfront molecular risk stratification is in advanced stages of planning that could be opened to other major academic centers in the near future. A prospective randomized controlled trial on biomarker-based optimization of duration of adjuvant temozolomide in patients with newly diagnosed glioblastoma is also being considered for multicenter collaboration.



Finally, ISNO strives to bring about uniformity in the delivery of care within such a varied socioeconomic landscape and carries out CME programs in various parts of the country on a regular basis. Several gaps, including delivering optimal management, palliative and hospice care,

and ancillary support to our patients and families, are very much in our mind. We hope that with national and international cooperation, some of these challenges will be met in the next few years.

# Nurses Corner



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Welcome to the new Nursing Section of the newly re-vamped WFNOS magazine. I thought I would use this first edition to introduce myself, inform you of our new and exciting changes to the Nursing/AHP agenda for the forthcoming EANO conference in Mannheim (in October 2016), and ask for your participation and future ideas.

I took over the role of EANO lead nurse from Hanneke Zwinkels (Netherlands) following the Turin conference in 2014. Hanneke had very successfully ensured that the Nursing agenda was full of inspirational talks and topics of interest. At the last conference there were over 80 registered Nurses/AHPs from all over Europe and we hope this number is set to rise over the coming years.

Although originally from Sweden, I currently live and work in the United Kingdom. I have been in my present role of Neuro Oncology Clinical Nurse Specialist at Addenbrooke's Hospital, Cambridge, since 2009 and have a special interest in continuing professional development.

For the first time ever, EANO is proud to support the pre-symposium educational day specifically aimed at Nurses/AHPs on Thursday, October 13! This is made possible thanks to our UK-based sponsors, The Brain Tumour Charity, which has fully supported this event, making it FREE for all Nurses/AHPs who have registered for the whole EANO symposium. Others are welcome to attend at a nominal fee of €50.00.

The pre-symposium day will be dedicated to the management of low-grade and insular gliomas and will include exciting teaching sessions with a 3D neuro-anatomy course and topics of debate around the new molecular data and what these mean to our tumor grading system and our

patients' treatments and pathways. We will also explore how to manage personality changes, seizure management, and rehabilitation needs, to name but a few. We sincerely hope you will take this opportunity to join us for this exciting new venture, which has been fully endorsed by EONS (European Oncology Nursing Society).

We all work daily within an evidence-based practice, but do you know how to interpret this evidence? Do you know your Kaplan-Meier curves from your significant *P* intervals? Have you ever written an article, or is getting published something that feels like a myriad of pitfalls and obstacles? Would you like to learn how we work in other parts of Europe and what the areas of best practice are in other countries? In which case we hope you will enjoy our Nursing Day on Friday, October 14, which covers all of these aspects and more, with two specific workshops and a multitude of Nursing/AHP speakers lined up.

We also have two prizes to give away, which means the person YOU vote as the best Nurse/AHP presenter AND the person you felt had the best Nurse/AHP poster presentation will get free attendance to EANO Stockholm 2018, courtesy of the UK's Brainstrust Charity. And don't forget to log in to the EANO homepage Nurses section to take advantage of our Nurse/AHP Travel grants applicable for anyone submitting an abstract, or if you are from a less developed country – another EANO nursing first – you may be eligible for free attendance!

I hope to welcome you all to a fun-filled yet educational day where we can learn from one another, share best practice, and strengthen our passion for both our chosen profession and our patients. Welcome to EANO Heidelberg/Mannheim 2016!

# Brain Tumor Patient Advocacy—Who Needs It?



## **Kathy Oliver**

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It's been hailed as a “revolution,” a “crucial navigating tool for the medical maze,” and a “new modality” in cancer care.

However you describe it, patient advocacy—whether carried out locally, regionally, nationally, or internationally—is crucial in helping to ensure that patients' views are heard and acted upon. It's vital that people with cancer are not discriminated against or excluded in any way from obtaining optimal care. Advocates can play an important role in achieving the best journey possible for patients and caregivers.

In the brain tumor community, the lack of effective treatments, the fact that brain tumors are responsible for an average of over 20 years of life lost per patient, and the substantial economic stress for which these rare tumors are responsible give patient advocates plenty to do [1].

Whether it's campaigning for more government research funding (in the UK, for example, less than 1% of national cancer research funding is on brain tumors), providing much-needed support and information to patients and families, or campaigning for access to new (and usually costly) therapies, patient advocacy can deliver real change [2].

Brain tumor patient advocates also offer the opportunity for a new type of partnership paradigm to researchers, clinicians, and others involved in the field of neuro-oncology.

First, advocates can help identify the unanswered questions that new research should address. The Neuro-Oncology James Lind Alliance (JLA) Priority Setting Partnership (PSP) in the United Kingdom, for example, was set up to identify and promote the clinical research questions of greatest importance to people with brain or spinal cord tumors. Bringing together patients, patient advocates, carers, and clinicians, the initiative established the “top 10” uncertainties relating to brain tumor prevention, diagnosis, treatment, rehabilitation, and palliative care [3].

Second, patient advocates can engage with academia and industry to help design—even at the very earliest stages—clinical studies which are relevant, have the most appropriate endpoints, and are meaningful to patients. The vast range of firsthand knowledge which patient advocates bring to research can also lead to better recruitment strategies; findings which are more patient relevant; wider dissemination of those findings; and greatly improved information materials and informed consent documents.

Many of us are accidental advocates who came into this field as the result of a brain tumor diagnosis of our own or an experience with the diagnosis of a loved one. But really effective patient advocates don't just spring into existence fully formed. We need high-level training, just as any other professional does. Happily, we are now seeing the advent of comprehensive, sophisticated courses to specifically educate patient advocates about medicine research and development so we can contribute knowledgeably and confidently to, for example, the design of clinical studies [4].

Third, patient advocates can liaise with regulators and health technology assessment bodies to highlight the patient perspective and make sure that patient and caregiver voices are heard at the very highest level when it comes to medicine approvals and appropriate reimbursement decisions.

So who needs patient advocacy?

We all do.

As everyone is a potential patient, we all need someone fighting in our corner, supporting our rights and wishes, and providing a strong collective voice. Having opened the doors to researchers, regulators, health technology assessors, medical institutions, industry, and policymakers, patient advocates now have seats at some of the most important and influential tables in the medical and regulatory arenas. Although much remains to be done, we, as advocates, are working together with other stakeholders toward the same goal: to find the elusive cure for this devastating disease and improve patients' and caregivers' quality of life along the way.

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Targeting *IDH1R132H*  
in WHO grade III and IV  
*IDH1R132H*-mutated  
gliomas by a peptide  
vaccine: a first-in-man  
phase I safety,  
tolerability, and  
immunogenicity  
multicenter trial  
(NOA-16)

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Based on own preclinical data using a novel syngeneic humanized mouse model and patient immunogenicity studies, an immunogenic neoepitope was identified comprising the *IDH1R132H* mutation, frequently occurring in grade II and grade III astrocytomas. *IDH1R132H* is a disease-defining early mutation in these tumors and is present in all tumor cells, as evidenced by routine immunohistochemistry using a mutation-specific antibody. Based on this preclinical work, a peptide vaccine was designed that is capable of inducing a mutation-specific CD4+ T-cell response and of controlling the growth of syngeneic *IDH1R132H*-mutated tumors in animal models. "Targeting *IDH1R132H* in WHO Grade III and IV *IDH1R132H*-Mutated Gliomas by a Peptide Vaccine" (EudraCT No. 2014-000503-27, ClinicalTrials.gov Identifier NCT02454634) is a first-in-man phase I safety, tolerability, and immunogenicity multicenter trial in adults with non-1p/19q codeleted, *IDH1R132H*-mutated astrocytoma not previously treated with radio- or chemotherapy. Michael Platten, professor in neurology at the University of Heidelberg, Germany, National Center for Tumor Diseases, is the leading investigator for the trial. Sponsored by the German Consortium of Translational Cancer Research and conducted within the

Neuro-oncology Working Group (NOA) of the German Cancer Society, the study comprises 3 cohorts, each receiving 8 vaccines over a period of 24 weeks: (i) patients receiving primary temozolomide chemotherapy, (ii) patients receiving primary radiation therapy, and (iii) patients receiving combined radiochemotherapy. Key eligibility criteria include histologically confirmed diagnosis of an *IDH1R132H*-mutated glioma of World Health Organization (WHO) grade III or IV, absence of chromosomal 1p/19q codeletion in the tumor tissue, and loss of alpha thalassemia/mental retardation syndrome X-linked expression in the tumor tissue. Key outcome parameters will be safety and immunogenicity. The target population will be 39 patients. The results of this trial and its correlative studies will provide insight into the safety and immunogenicity of the *IDH1R132H* peptide vaccine and may indicate signs of biological efficacy. The trial started enrollment in Q3 2015 at 8 sites in Germany: Medical Centers of Heidelberg University, Munich University (LMU), Freiburg University, Tübingen University, Frankfurt University, Dresden University, University Düsseldorf/Essen, and Berlin University (Charité). For further details and site-specific contact information, Dr. Platten can be contacted at michael.platten@med.uni-heidelberg.de.



# Hotspots in Neuro-Oncology 2016

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## Long-term outcome of patients with spinal myxopapillary ependymoma: treatment results from the MD Anderson Cancer Center and institutions from the Rare Cancer Network

Weber et al, *Neuro-Oncology* 2015;17(4):588–595.

Myxopapillary ependymoma (MPE) is classified as a World Health Organization (WHO) grade I tumor and is believed to be a slowly growing neoplasia, thus with a good prognosis. However, the outcome of MPE is not universally favorable for all MPE patients.

The authors have assessed the outcome and patterns of failure of spinal MPE merging the 2 largest databases so far published (from the MD Anderson Cancer Center and the Rare Cancer Network) and expanding with new cases. The medical records of 183 MPE patients were retrospectively reviewed.

Treatment failure was observed in 58 (31.7%) patients. Local failure, distant spinal relapse, and brain failure were observed in 49 (26.8%), 17 (9.3%), and 11 (6.0%) patients, respectively. The estimated 10-year progression-free survival (PFS) was 61.2%. Age (<36 or ≥ 36 y), treatment modality (surgery alone vs surgery + radiotherapy), and extent of surgery (gross total resection vs subtotal resection and biopsy) were prognostic factors for local control and PFS on univariate analysis.

More in detail, the 10-year PFS was ~40% for younger patients compared with 85% for older patients. Second, adjuvant radiotherapy (RT) increased 10-year PFS from <40% to 70% in patients receiving this modality compared with those who received surgery alone (more frequently incomplete). The estimated 10-year overall survival (OS) was 92.4%.

The present series is the largest MPE study with a long follow-up period, and the treatment strategy and techniques have been fairly uniform among centers. Overall, this study suggests that adjuvant RT should be employed in patients with subtotal resection and/or piecemeal

resection, and thus could be particularly applicable in younger patients. Interestingly, this study has shown that despite a substantial recurrence rate, the OS of MPE patients is indeed good, and serious late toxicities from RT cannot be expected. Future studies should include a central pathology review in order to avoid the risk of including non-MPE histologies. Furthermore, prospective studies are needed to better understand the characteristics of distant spinal and/or brain leptomeningeal relapse by employing pre- and posttreatment MRI and CSF examinations.

## Phosphohistone H3: implications for clinical practice and risk assessment in meningioma

Duregon et al, *Neuro-Oncology* 2015;17(5):663–669.

Current WHO guidelines for meningioma grading are predominantly based on mitotic counts per 10 high-power microscope fields (HPF), assessed on hematoxylin and eosin (H&E) stained slides. Phosphorylation of histone H3 (pHH3) is a mitosis-specific event. Antibodies against pHH3 have been developed and used for mitotic figure detection and prognostic determination in various solid tumors with good results.

In this study, Duregon and coworkers investigated 70 meningiomas (15 grade I, 40 grade II, 15 grade III) for H&E and pHH3-positive mitoses counted per 10 HPF. They evaluated the intra- and interobserver reproducibility among 4 pathologists with different levels of experience in neuropathology, and defined pHH3 mitotic cutoff corresponding to the current WHO-defined H&E mitotic cutoff. H&E and pHH3 mitotic rates were highly correlated (Pearson's  $r = 0.92$ ,  $P < .0001$ ). Phosphohistone H3 mitotic counts had both a good mean interobserver correlation ( $R_m = 0.83$ ) and a good interclass correlation (0.78), which were higher than H&E mitotic indices ( $R_m = 0.77$ , interclass correlation = 0.71). After further stratification of meningiomas according to WHO grade, pHH3 performed better in terms of interobserver concordance compared with H&E, and pHH3-specific cutoff values for discriminating the 3 meningioma groups were proposed. Moreover, there were significant differences (for both H&E and pHH3) between the pathologist dedicated to

neuro-oncology diagnoses and one of the 2 more junior pathologists.

The authors of this study were the first to perform in meningiomas a thorough, long-awaited analysis of agreement for H&E and pHH3-defined mitotic counts among different pathologists and to demonstrate the advantages of using anti-pHH3 antibodies for mitotic figure detection. Two questions remain to be answered in future studies. First, what is the best pHH3 mitotic cutoff for recurrence risk stratification in meningiomas? Second, what would be more advantageous to report by the pathologist: a mitotic count per unit area (eg, 10 HPF) or a mitotic index (number of pHH3-positive mitotic figures per 1000 tumor cells)?

## Changes in the EGFR amplification and EGFRvIII expression between paired primary and recurrent glioblastomas

*van den Bent et al, Neuro-Oncology 2015;17(7):935–941.*

The efficacy of novel targeted therapies is often tested at the time of tumor recurrence. However, for glioblastoma multiforme (GBM) patients, surgical resection at recurrence is performed only in a minority of patients, and molecular data are predominantly derived from the initial tumor. Molecular data of the initial tumor for patient selection into personalized medicine trials at recurrence should therefore be used only when the specific genetic change is retained in the recurrent tumor.

In this study, the authors investigated whether amplification of epidermal growth factor receptor (EGFR) and expression of the most common mutation in GBM, such as EGFR variant III (vIII), are retained at tumor recurrence. EGFR amplification status (dichotomized to either non-amplified or amplified) was determined in 55 primary–recurrent tumor pairs. Of these, EGFR amplification was present in 40/55 (73%) samples at first diagnosis. EGFRvIII expression was detected in 17/35 (49%) primary tumors with EGFR amplification. Overall, EGFR amplification status remained identical in most tumor pairs (46/55, 84%), while qualitatively EGFRvIII status (present or absent) remained similar between primary and recurrent

tumor in 33/42 (79%) samples. Loss of EGFRvIII expression appeared to be a consequence of a clonal selection of the tumor and a result of epigenetic regulation.

In conclusion, the relative stability of EGFR amplification indicates that molecular data obtained in the primary tumor can be used to predict the EGFR status of the recurrent tumor. Conversely, care should be taken in extrapolating EGFRvIII expression from the primary tumor to the recurrent tumor: to repeat the biopsy should be considered in trials on recurrent GBM targeting EGFRvIII mutations.

## Programmed death ligand 1 expression and tumor infiltrating lymphocytes in glioblastoma

*Berghoff et al, Neuro-Oncology 2015;17(8):1064–1075*

Programmed cell death 1 (PD-1), which can be activated by its ligand (PD-L1), is a receptor with negative immunoregulator properties that inhibits T-cell response against tumor cells. Immune checkpoint inhibitors targeting PD-1 or PD-L1 have shown activity in metastatic melanoma and advanced non-small-cell lung cancer, and the expression of PD-L1 has been suggested to be a biomarker of response. Thus far, no data are available on the expression of PD-L1 in glioblastomas.

Berghoff et al performed immunohistochemistry for PD-1, PD-L1, and several lymphocyte antigens on 135 glioblastoma samples. PD-L1 gene expression was analyzed in 446 cases from The Cancer Genome Atlas. Diffuse/fibrillary PD-L1 expression of variable extent was observed in 103 of 117 (88%) newly diagnosed and 13 of 18 (72.2%) recurrent glioblastoma specimens. PD-L1 gene expression levels were low in the proneuronal and glioma CpG island methylator phenotype glioblastoma subtypes, while being higher in the mesenchymal subtype. No significant differences in PD-L1 expression or tumor lymphocyte density between newly diagnosed and recurrent glioblastoma were observed. However, neither PD-1 nor PD-L1 gene expression was correlated with outcome.

Clinical trials are needed to clarify whether PD-L1 expression patterns or molecular glioblastoma subtypes will correlate with response to specific inhibitors.

## Multicenter imaging outcomes study of The Cancer Genome Atlas glioblastoma patient cohort: imaging predictors of overall and progression-free survival

Wangaryattawanich et al, *Neuro-Oncology* 2015;17(11):1525–1537.

Multiple studies focusing on imaging correlates of survival in glioblastoma multiforme (GBM) patients have been published. However, no quantitative or volume-based parameters have been analyzed thus far. In this regard, quantitative imaging metrics could increase the accuracy and reproducibility of tumor assessments. Overall, the prognostic and predictive role of imaging characteristics in GBM has remained largely unmined.

The purpose of this study was to determine the significance of both quantitative and qualitative preoperative imaging variables with regard to survival and outcomes (overall survival [OS] and progression-free survival). The authors retrospectively identified 94 untreated GBM patients from The Cancer Imaging Archive who had pre-treatment MRI and corresponding patients' clinical information and outcomes in The Cancer Genome Atlas (TCGA). Qualitative imaging assessment was based on the Visually Accessible Rembrandt Images feature-set criteria. Volumetric parameters were obtained of the specific tumor components: contrast enhancement, necrosis, and edema/invasion. Univariate analysis demonstrated 10 imaging features and 2 clinical variables to be significantly associated with OS. Multivariate Cox regression analysis showed that tumor-enhancing volume ( $P=.03$ ) and eloquent brain involvement ( $P<.001$ ) were independent prognostic indicators of OS. The patients who had an enhancing tumor volume lower than  $3500 \text{ mm}^3$  had longer OS benefit of 5.1 months compared with the patients who had a higher enhancing tumor volume ( $P=.013$ ). In the multivariate Cox analysis of the volumetric features, an edema/invasion (nonenhancing component) volume of more than  $85\,000 \text{ mm}^3$  and the proportion of enhancing tumor were significantly correlated with higher mortality ( $P=.004$  and  $.003$ , respectively).

This is the first study investigating the contribution to survival of quantitative volumetric (true 3D volumes)

parameters, including specific cutoff values. Importantly, the study identified the cutoff values of the volume-based imaging parameters (ie, enhancing tumor, necrosis, edema/tumor invasion) to predict individual outcome in GBM patients. A limitation of the study is its retrospective design, using a preexisting database and images acquired from different scanners from various hospitals involved in data collection by TCGA. In conclusion, preoperative quantitative MRI parameters have a significant prognostic role, thus making them potentially useful for patient stratification and endpoint biomarkers in clinical trials.

## Long-term results of carmustine wafer implantation for newly diagnosed glioblastomas: a control propensity-matched analysis of a French multicenter cohort

Pallud et al, *Neuro-Oncology* 2015;17(12):1609–1619

The standard of care for newly diagnosed glioblastoma multiforme (GBM) is maximal safe surgical resection followed by chemoradiation. The rationale underlying the addition of carmustine (BCNU) wafers is twofold: to fill the gap between surgery and the start of chemoradiation by maintaining an alkylating treatment pressure on residual tumor cells, and to deplete with a nitrosourea the activity of O<sup>6</sup>-DNA methylguanine-methyltransferase, thereby enhancing the efficacy of subsequent temozolomide. Only a small number of studies have given information on this combination.

The authors have reported a large multicenter retrospective study aiming to assess the safety and efficacy of standard chemoradiation with or without prior carmustine wafer implantation at first surgery in patients with newly diagnosed GBM. The cohort with chemoradiation alone included 433 patients, while the carmustine cohort included 345 patients, the study being a propensity-matched cohort design. The median progression-free survival was 12 months in the implantation group and 10.0 months in the standard group, and the median overall survival was 20.4 months and 18 months, respectively. Carmustine wafer implantation was independently associated with longer progression-free survival in patients

with subtotal/total resection, whereas the difference in overall survival was not statistically significant. There were 2 problems associated with the addition of carmustine wafers: the difficulty of assessing intraoperatively the extent of resection (25% of partial removals in the implantation group), and the increase in postoperative infections, even if not negatively impacting the subsequent chemoradiation.

A major problem for neuro-oncologists in accepting carmustine wafer implantation is that this procedure is considered an exclusion criterion for clinical trials on new drugs, due to the potential confounding factors of changes on MRI being difficult to interpret. Anyway, the application of carmustine wafers in clinical practice should be restricted to patients undergoing a total resection.