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Heterogeneity of cancer-Initiating cells within glioblastoma

Elena Binda[1,2], Alberto Visioli[1,2], Brent Reynolds[3], Angelo Luigi Vescovi[1,2,4]

[1]StemGen SpA, Piazza della Scienza 2, Building U3, 20126 Milan, Italy, [2]Department of Biosciences and Biotechnology, University of Milan Bicocca, Piazza della Scienza 2, Building U3, 20126 Milan, Italy, [3]The Evelyn F. and William L. McKnight Brain Institute, Program in Stem Cell Biology and Regenerative Medicine, The University of Florida, 100 S. Newell Drive, P.O. Box 100015-1, Gainesville, FL 32610-0015, [4]IRCCS Casa Sollievo della Sofferenza, Opera di San Pio da Pietralcina, Viale Cappuccini, 71013 San Giovanni Rotondo, FG, Italy

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1. ABSTRACT

Malignant gliomas, particularly glioblastoma multiforme (GBM), account for the majority of brain tumors. Their incidence is increasing world wide and they are incurable. Although a transient response to therapy is observed, tumor recurrence is inevitable and occurs within tissue that has received cytotoxic therapy. This suggests that a subpopulation of resistant cells is responsible for tumor regrowth. The treatment of GBMs represents a daunting challenge to clinicians due principally to the lack of effective therapeutic options. One explanation for this is the marked cellular and genetic heterogeneity within and across these types of tumors. Unravelling the cellular composition of gliomas and describing cell lineage relationships are essential for therapeutic breakthroughs. The recent proposal that a small percentage of cells with stem cells characteristics are responsible for tumor initiation and growth has sparked an interest in applying approaches used to study somatic stem cells toward an understanding of the cellular elements responsible for cancer progression and recurrence. To outline the relevance of these findings is the purpose of this review.

2. INTRODUCTION

Gliomas are the intrinsic tumors of the brain. The severity of primary brain tumors is due to the central role of the brain and the functional consequences of neuronal loss and parenchyma disruption. As reported by the American Cancer Society, approximately 13,100 Americans die from malignant brain tumors each year with most patients succumbing to their disease regardless of treatment (1). Malignant gliomas are incurable, particularly grade IV gliomas, named glioblastoma (GBM), which account for a third of all of the brain tumors (2-3). GBMs are highly heterogeneous tumors, which shows cellular and tissue dissimilarities, harboring a strong hemorragic component, broad necrotic areas, elevated mitotic index, high endothelial proliferation and the presence of a well-vascularized and diffuse tissue infiltration pattern. The latter is a critical feature of these tumors for, by the time a diagnosis is reached, the rapid and extensive dissemination of GBM cells within the brain precludes effective surgical resection. Moreover, most of the infiltrating cells are endowed with limited proliferative activity, which partially explains the inefficacy of chemo- and radiotherapy (4-5).

Therefore, disease recurrence occurs and the prognosis is discouraging (6-7-8). As tumors are composed of a heterogeneous population of cells, of which a small percentage appear able to initiate and sustain long-term tumor growth (i.e. the tumor-initiating cells, TICs), basing tumor classification on an analysis of this kind of cells may provide not only an additional classification criteria but also a potential prognostic and/or diagnostic tool for therapeutic development. Furthermore, while the TIC population is obviously important and likely the most relevant target for curing brain tumors, for most high grade gliomas the quiescent tumor stem cell population may play a secondary role relative to the active proliferating population (i.e. the progenitor or transient amplifying population), which likely determines disease progress and therapeutic failure. Hence, understanding, at the cellular level, the heterogeneity that exists in malignant gliomas and the contribution that each population makes to tumor progression is important to ultimately defining meaningful targets. Despite significant advances in neuroimaging, neurosurgical techniques, chemo- and radiation therapy, the prognosis for patients with malignant gliomas, particularly glioblastomas, remains dismal (9-10-11-12). The median survival after surgical resection alone is 6 months with only 7.5% of patients surviving 2 years post-operatively. Additional radiation therapy prolongs median survival to 9 months while systemic chemotherapy provides minimal survival benefits (13-14). These poor results suggest we have an insufficient understanding of the biology of GBM, the diversity of cellular components and the mechanisms that drive their growth (15). However, the advent of genomic and proteomic tools are providing the bases to obtain a deeper appreciation of the heterogeneity and molecular means that make GBMs resistant to conventional therapies. The development of new markers, the identification of specific molecular targets and the overall process of developing therapeutics for GBMs has been hampered by the lack of information as to the actual identity and nature of the normal cell type(s) that were hit by transformation and, more importantly, by the absence in a detailed understanding of the heterogeneity that exists within the tumor and a fundamental understanding of the cell types that are involved in tumor promotion. The recent proposal that a small percentage of cells with stem cell characteristics are responsible for tumor initiation and growth in blood and solid tissues cancers (16-17) has sparked an interest in applying approaches used to study somatic stem cells toward an understanding of the cellular elements responsible for cancer progression and resistance to treatment. While the validity of this hypotesis is still being tested, the advantage of studying single or defined population of cells, as opposed to large or less defined populations, will likely benefit not only our understanding of the contribution that different populations make towards tumor growth and resistance, but will also allow a more detailed and accurate mapping of tumor heterogeneity. These results will not only define the cellular populations to be targeted and outline the inter- and intra-tumor heterogeneity, but also identify the resistant population to be targeted with secondary lines of treatment.

3. CLASSIFICATION OF GLIOMAS

Brain tumors can vary in malignancy, but even so-called benign tumors are often lethal because of their infiltrating properties and their tendency to undergo malignant transformation over time. Tumors that start in the brain are called primary brain tumors, their name depending on where the tumor originated, its pattern of growth, and whether it is cancerous or not. Thus, adenomas arise from a gland (pituitary adenoma), blastomas are malignant tumors whose cells have undeveloped (embryonic) characteristics and gliomas are (benign or malignant) tumors that arise from the supportive tissue of the brain, namely macroglia. According to the Central Brain Tumor Registry of the United States, the incidence rate of all primary benign and malignant brain tumors is 14 cases per 100,000 person-years (5.7 per 100,000 person-years for benign tumors and 7.7 per 100,000 person-years for malignant tumors) (18). The most common types of gliomas can be classified in three main types: astrocytomas, oligodendrogliomas, and mixed oligoastrocytomas, which are usually distinguished by their histological features. On the basis of nuclear atypia, mitoses, microvascular proliferation, and necrosis the gliomas can be divided in low grade or World Health Organization (WHO) grades I and II, or high grade or malignant, WHO grades III and IV. In order of increasing anaplasia, types of astrocytomas usually include pilocytic astrocytoma (grade I), diffuse astrocytoma (grade II), anaplastic astrocytoma (grade III), and eventually glioblastoma (grade IV), which is the most frequent subtype. The WHO classification for oligodendrogliomas and mixed oligoastrocytomas includes two grades- low grade (grade II) and anaplastic (grade III). Incidence of oligodendrogliomas has been reported by several groups as having risen substantially from the classic figure of 5% up to 25-33% of all glial tumors, and that for astrocytomas having fallen concomitantly. This change suggests that some tumors previously classified as astrocytomas are now being labeled oligodendrogliomas or mixed oligoastrocytomas (6). In addition to their striking propensity to infiltrate throughout the brain, low-grade gliomas undergo malignant transformation over time.

3.1. Molecular markers

Due to the inherent heterogeneity and the promiscuous morphology of many brain tumors, morphological classification can often be difficult. Hence, there is growing consensus that molecular genetic analyses will soon become important to improve classification of various glioma subtypes (19-20-21). While this approach is a natural evolution based on the development of new technology, we would argue that its application, while a valuable addition to the currently used method, suffers from the same inherent flaw as morphological classification – namely categorization based on a population analysis. The majority of cells within the tumor population dominate the final analysis of histological and molecular characterization. Apart from morphological parameters, the classification and grading increasingly includes molecular markers (22-23), which have in recent years greatly contributed to the description of molecular oncogenic pathways such as the activation of oncogenes (mostly

growth factor pathways) or the loss of cell cycle control elements (mostly tumor suppressor genes like p53, the retinoblastoma gene Rb or PTEN). Genetic and cell signaling abnormalities accumulate, leading to the tranformation of somatic cells into glioma cells (24-25). The sequence of this accumulation is used to describe the two glioblastoma multiforme (GBM) subtypes: primary and secondary. Primary GBM is usually diagnosed in older patients (more than 50 years) and is more often associated with epidermal growth factor receptor (EGFR) amplification/mutations, loss of heterozygosity of chromosome 10q, deletion of the phosphatase and tensin homologue on chromosome 10 (PTEN), and p16 deletions (24-25-26). Secondary GBMs result from transformation of lower grade astrocytomas in younger patients. These tumors are characterized by p53 mutations, platelet-derived growth factor receptor (PDGFR) overexpression, p16 and retinoblastoma (Rb) abnormalities, and loss of heterozygosity of chromosome 10q. With the advent of powerful sequencing tools, a genomewide mutational analysis of glioma recently showed a high proportion of mutations in the isocitrate dehydrogynase I gene (IDH I) in grade II and GATA6, a member of the GATA family of transcriptional regulators which are also expressed in cells of the CNS (27). Methylation of the members of the GATA family is implied in non-glial oncology leading to the assumption that GATA6 may be an astrocytoma suppressor gene (27). Supporting the role not only of mutation but also of gene regulation, two transcription factors, C/Epbbeta and STAT3 have been recently identified as the master regultors for the acquisition of a mesenchymal phenotype during the evolution of gliomas (28).

4. SPECIFIC CELL BIOLOGY OF GLIOMA

As many solid tissue cancer, GBMs are composed of a heterogeneous population of cells. Despite decades of chemo- radio- and surgical therapy there have been no dramatic changes in the survival of GBM patients (29). These poor results suggets we have an insufficient understanding of the biology of GBM, the diversity of cellular components and the mechanisms that drive their growth. Until recently, it was generally accepted that the generation of new nerve cells in the CNS did not persist into adulhood (30). However it is now clear that there exists in the adult brain an endogenous population of precursor cells, which continue to divide throughout life and are capable of migrating and generating new neurons and glia, which then integrate into the existing functional circuitry (31-32). This resident stem cell population provides the basis for replacing those neurons lost as a result of normal processes, such as ageing, as well as in response to pathological insults including neurodegenerative disease or stroke (33). Significantly, the characteristics that define neural stem cells (NSCs), such as the ability to self-renew and to generate large numbers of progeny, in many ways reflect the behaviour of glioma cells (34). Progress in understanding the cell signaling and molecular pathogenesis of malignant glioma has lead to a surge in novel therapy development. For instance, the invasive properties of malignant tumors of the CNS, and in particular of high-grade gliomas, are of great clinical relevance since they dramatically contribute to the poor prognosis of these neoplastic diseases. Migration biology of glioma cells has for a long time been studied and what has emerged so far is that it is a highly complex cellular program with an ever-growing repertoire of molecules being added as genomic approaches are entering the field (35). Increasing efforts are being made to target the abnormal cell signals or molecular processes in malignant glioma. While it remains unclear if the molecular and functional characteristics of the GBM tumor-initiating cells is a reflection of their origin from mutated normal brain stem cells or, rather, emerge from the acquisition of stem-like features by more mature CNS cells following transformation, it is emergently clear that they and their immediate progeny are key players and targets in GBM physiology and therapy.

4.1. Molecular and signaling diversity of cells that contribute to GBM tumor growth

Many successful treatments for cancer have been developed based on a sound understanding of the biology and mechanisms that drive tumor growth (*i.e.* Gleevec, Herceptin) and improved outcomes for high-grade gliomas will likely be no different. GBMs demonstrate signal transduction dysregulation that leads to uncontrolled proliferation and invasion. The plethora of genetic changes leads to a number of notable alterations in signaling pathways that seem to be important for GBM proliferation, survival and invasion. For instance, greater than 80% of GBMs show vigorous AKT activation and mutated or loss of PTEN (36). Frequently, these tumors show amplification of receptor tyrosine kinase (RTK) activity due to receptor over-expression, amplification or mutation that leads to increased PI3K/AKT activity (15). The over-activation of multiple RTKs together with inactivation of tumor suppressor provides a rich and plentiful supply of options for a cell to choose for continued growth. The application of single RTK inhibitors have been disappointing clinically with only a small population of patients responding (0-15%) and limited 6-months progression-free survival (37). It is likely that the downstream activation of signaling pathways involved in proliferation and survival are determinated by the sum of multiple inputs that are represented by a multitude of deregulated pathways, receptors and ligands. Hence, application of single agents targeting individual points in the signaling cascade will be incapable of producing a substantial clinical effect. Stommel and colleagues recently reported on the co-activation of RTKs in cultured GBMs, expression of multiple phosphorylated RTKs on fresh primary cells and intratumoral heterogeneity based on co-expression of activated RTKs in individual dissociated cells. Their conclusion support the hypotesis that monotherapy for many solid tissue cancers is not likely to be successful but may be improved by targeting multiple RTKs (38). Growth factor pathways in gliomas that contribute to malignancy by using tyrosine kinases include epidermal growth factor (EGF), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), hepatocyte growth factor/scatter factor (HGF/SF), and insuline-like growth factor (IGF) (39). EGFR, VEGF and PDGF have been investigated as important targets for new therapies with

moderate success (15-40). In addition to growth factor receptors, the intracellular effectors of malignant glioma have provided new potential therapeutic targets. These molecules regulate multiple cellular functions such as proliferation, differentiation, invasion and protein trafficking, thereby making these molecules attractive targets. RAS-RAF-MEK-ERK pathway, for example, has been studied in great detail in malignant glioma. RAS often has increased activity in GBM, and several inhibitors of farnesyltransferase, a key upstream stop in RAS maturation (41) have been developed (39). Another regulator of cell growth and proliferation is PI3K, a serine/threonine kinase. It is activated in GBM by loss of PTEN, and affects AKT and mTOR downstream (39). New inhibitors of these complexes are being studied (42-43). Another serine/threonine kinase, protein kinase C (PKC), mediates cell proliferation as well as invasion and angiogenesis. This pathway has also been targeted in recent trials (44-45). However, targeting of these pathways had modest success. This phenomenon may be due to the heterogeneity of malignant gliomas and the need for targeting multiple pathways simultaneously (39-45).

5. CANCER STEM CELLS

5.1. Hematopoietic malignancies

The idea that not all cells within a cancer are able to re-initiate or propagate the tumor was first demonstrated experimentally in the early 1960's (46-47), and while the notion that a self-renewing, relatively quiescent stem-like cell was responsible for evading conventional treatment, was found in the cancer research literature (48-49-50-51-52), it was not until the 1990's that the idea began to take hold after two publications by John Dick's laboratory (53-54). The papers described the identification of an acute myeloid leukemia (AML) initiating cell based on cell surface antigen expression (CD34+ CD38-) and its ability to establish human leukemia in severe combined immunodeficient (SCID) mice. Like non-tumor, somatic stem cells, the leukemia-initiating cell (LIC) or leukemia stem cell, was present in low frequency, shared the CD34+ CD38- phenotype with hematopoietic stem cells, and exhibited *in vivo* self-renewal as assessed by serial transplantation. Recently, targeting of the transmembrane glycoprotein, CD44, which is nearly ubiquitously expressed on all AML blast cells, has demonstrated a marked reduction in tumor burden based on altering the fate and homing of the AML LIC (55). A second type of leukemia (chronic myeloid leukemia, CML) has also been shown to be driven by a stem-like cell. CML develops from an acquired dominant genetic mutation that leads to a constitutively active tyrosine kinase (BCR-ABL) that enhances cell survival and proliferation, leading to an overproduction of immature leukemic blast cells (56). A detailed understanding of the cellular and molecular basis of CML has led to the development of a specific tyrosine kinase inhibitor (TKI), which reverses auto-phosphorylation of BCR-ABL and is one of the more successful stories in translational medicine (Imatinib mesylate, Gleevec) (57). However, while Imatinib is able to suppress CML, it rarely is curative and relapse is inevitable with cessation or interruption of therapy (58). The reason that Imatinib is unable to eradicate all of the BCR-ABL cells is due to the primitive CML stem cells, which are refractory to TKI (59). Hence, for at least two types of leukemia, the concept of a cancer stem cell seems to be well established. A cell with a somatic stem cell phenotype (*i.e.* $CD34^+/CD38^-$) and functional characteristics (long-term self-renewal and slow-cycling) is responsible for driving tumor initiation, growth and plays a role in resistance to therapy.

5.2. CNS cancer stem cells

The presence of stem cells in cancer outside of the blood came initially in 2002, when it has been reported the existence of stem-like cells for the first time in solid tissue cancer, in this case in human cortical gliomas that were capable of forming clonal spheres in culture, exhibiting a multipotent differentiation potential and expressing a unique gene expression profile suggestive of deregulated cellular signaling (60). This revealed similarities (*i.e.* the ability to form neurospheres and exhibit multilineage differentiation, as well as expressing hallmark molecular markers of neural stem/progenitors cells including nestin and tenascin-C) between somatic neural stem cells and tumor cells, implying a possible relationship between the two populations. The follow year the Clarke laboratory published a prospective identification of a breast cancer stem cell where they demonstrated, based on the absence of lineage specific markers together with high expression of CD44 and low expression of CD24, that as few as 100 such cells were capable of forming tumors when injected into the mammary fat pads of NOD/SCID mice (61). This paper generated a great deal of attention and seemed to firmly establish the existence of solid tissue cancer stem cells. In the same year Hemmati *et al* reported on the isolation of cells from pediatric brain tumor with properties similar to somatic neural stem cells such as the ability to exhibit self-renewal, multipotent differentiation and the expression of genes found in neural and other stem cells such as CD133, Sox2, Bmi-1 and Musashi-1 (62). Singh *et al* reported on the identification of a brain tumor cancer stem cell that exhibited the ability to proliferate, self-renew and multi-lineage differentiation potential, importantly they have reported that cancer stem cell population was found exclusively in the CD133-immunoreactive population (63). Also, in 2004, the Vescovi group carried out a detailed analysis of the functional stem cell characteristics of adult human GBMs, including a description of their ability to exhibit extensive self-renewal *in vivo* via serial transplantation and multi-lineage differentiation potential. These cells recapitulated the histological features, invasion, microvascular proliferation and pseudo-pallisading necrosis that are characteristic of human GBMs (64-65). A finding that was supported by the expression profile and genotype of cultured human GBM tumor stem cells, revealing a similarity to the tumors from which the cells were obtained (66). These findings indicate that the *in vitro* defined brain tumor stem cells faithfully preserve the key *in vivo* features of human GBM (hGBM).

At this point, the cancer stem cell hypotesis, as it pertained to solid tissue cancers (*i.e.* breast and in particular

the brain), appeared to be fairly well established based the following criteria:

1.the existence of a low frequency population of cells that exhibit stem cell characteristics *in vitro* (*i.e.* neurosphere formation, self-renewal and multi-lineage differentiation potential)

2.the ability of cells derived from solid tissue cancers (*i.e.* human GBM) to exhibit stem cell characteristics *in vitro* and to initiate tumor formation *in vivo*, and

3.the identification of a selective putative cancer stem cell marker (CD133 for brain and CD44 high/CD24 low for the breast).

5.3. Modeling GBM using stem-like tumorigenic cells

As mentioned, recent advances in understanding cancer biology has extended to GBMs the basic concept that was initially established for other tumors (54-61-67-68), *i.e.* only a minor subset of cancer cells are actually endowed with tumor-initiating ability, can sustain neoplastic growth, perpetuate GBMs and support recurrence (34-60-62-63-64-69). Stem-like TICs from GBMs are rapidly proving an invaluable tool as key players and targets in GBM physiology and therapy. These cells can be isolated from human GBM surgery specimens, cloned and expanded in culture extensively, while retaining multipotency, self-renewal ability and the capacity to reproduce, down at the clonal level, a faithful phenocopy of the original disease, ones transplanted in the brain of immunodeficient mice (64-66). It is worth emphasizing that these typical GBM features were never observed in previous studies using common xenograft or allograft-based brain tumor models. This allows for the possibility to directly investigate the most critical functional properties of the cells that initiate and perpetuate GBMs *in vitro*. Most important, GBM tumor stem like cells can be used to generate hundreds of copies of the patient's own GBM in mice. In these, the main histological, cytological and architectural GBM features, including infiltration, migration and neovascular processes are well-recapitulated (64-66).

Due to the ability of brain GBM- stem-like tumor initiating cells to generate true phenocopies of the human disease over time, they now represent one of the most suitable models for studying GBM physiology *in vitro* and *in vivo*, to discover new and more specific molecules that selectively impinge on the TICs in this disorder and to study the cellular, molecular and genetic underpinnings of GBM development, expansion and recurrence (70).

5.4. Stem cell markers

The research into glioma stem like cells has been based on markers that identify the cells that are capable of tumor formation at a low cellular density and have a capacity for self renewal, clonogenity, spherical growth and differentiation into a broad range of cell types – properties that define the cell's stemness (71).

CD133 (also called prominin-1) a transmembrane glycoprotein with largly unknown function (72) has been used as the major marker together with nestin, an intermediate filament protein (73), Sox-2 (a transcription factor found to be expressed in multipotent stem cells of the neuroepithelium) (74) and Musashi (an RNA binding protein enriched in mammalian CNS stem cells required for asymmetric cell division) (75).

The concept at the heart of the term and definition of the cancer stem cell hypotesis, is that a TIC derived from human gliomas does exhibit somatic stem cell characteristics and that it is these stem cell characteristics that make it resistant to treatment. To this end, and based on the attractiveness of the idea that a single antigen (CD133) defines the entire tumor initiating population, several groups have looked at the resistence of the CD133-IR population to treatment. The first publication in this area by Bao *et al* (76), similar to the Singh *et al* publication, reported that the tumor-initiating population was found exclusively in the CD133+ fraction and that the CD133 cells survived ionizing radiation in greater proportions relative to cells that lacked CD133 expression and that this effect was due to the preferential activation of DNA damage checkpoint kinases (Chk1 and Chk2) together with an increased ability to repair radiation-induced DNA damage relative to the CD133 non expressing cells. While these results, and those of others (77-78) support the hypotesis that the tumor-initiating (CD133+) cells exhibit an increased resistance relative to non-tumor-initiating (CD133-) cells, it is important to note that the selectively of CD133+ cells for tumor-initiating has been challenged (79-80-81), even by the group that first reported its selectively for tumor formation (82). Therefore, while CD133 may well be expressed on TICs, its expression is likely not selective to this population nor does it define the entire tumor-initiating population (83). In addition, the relationship of CD133 to somatic NSCs is currently unclear as to what portion of the population it defines, its selectively, and more important, does it identify a population with stem cell features, and if so, then if these stem cell characteristics were expressed in tumor stem cells would it make them resistant to therapy.

A candidate solution came in May 2009 from the group of Howard Fine, which proposed that expression of the stage-specific embryonic antigen 1 (SSEA-1/CD15/LewisX (LEX), an antigen originally expressed on a somatic NSCs population (84-85), might be used to isolate and enrich for tumorigenic subpopulations in human glioblastoma, also devoid in CD133+ (86). In the same manner, other neural/progenitor cell antigens have been proposed as true candidate tumor-initiating cell markers for brain tumors: nestin, expressed by uncommitted neural precursor cells (73-87-88) and detected at high levels in high malignant tumors as GBM when compared to less anaplastic glial tumors (89-90-91), notch proteins, essentials for the maintenance of the neural stem cell (92-93-94) and overexpressed in TICs (95-96-97) or A2B5, a marker of all the glial-restricted precursor cells, shown to be present in a population of tumor-initiating cells that are CD133- and able to propagate tumors (80). Other markers have been identified in TICs that are transcription factors, oncogenes and cell cycle modulators. Sox2, a transcription

factor that appears to has a fundamental role for maintenance of the self-renewal capacity of NSCs also when they have acquired cancer properties (62-98) or Bmi-1, a proto-oncogene essential for the generation of self-renewing adult human SCs (99) and highly enriched in stem-like cells within brain tumors (62). OLIG-2, another transcription factor that promote proliferation of both neural progenitors and GBM-initiating cells (100-101). Maternal embryonic leucine zipper kinase (MELK), a cell cycle modulator, that is expressed by neural stem cells and found to be elevated in TICs (62-102). Recently, it has been also identified a glioma-initiating cell population in human GBM that expresses high levels of CD44, proposed as an enriched marker for tumor stem cells in several malignancies of haematopoietic and epithelial origin (61-103-104) but not extensively studied in glioma. This CD44 high glioma-initiating population confers poor prognosis in GBM patients (105-106) and is associated with glioma progression and invasion (107-108).

Of note, by avoiding the use of molecular markers, in February 2010 an alternative approach has been proposed for identifying glioma-initiating cells based purely on phenotypic qualities of this population. This method allows the isolation and enrichment of self-renewing and tumor-initiating glioma cells by exploiting their intrinsic and distinct morphology and autofluorescence ($FL1^{+}$) (109).

For all of these reasons, the rationale is quite clear for why gliomas, and particularly GBMs, require careful cellular, molecular and antigenic phenotypying, even following *in vitro* growth, expansion and enrichment for the clonogenic populations, if new approaches to isolate, identify, enrich and target the most important culprit in GBM tumorigenesis, the human stem-like tumor initiating cells, are to be devised.

6. THERAPEUTIC APPROACHES

6.1. Targeting glioma cancer stem cells

The immaturity of the neural stem cell field contrasts with the study of hematopoiesis, largely contributing to our relative lack of knowledge concerning the lineage relationships and molecular mechanisms that control adult neural cell genesis. This is not to say that we are totally control naive in this regard, however, comparatively speaking the NSC field is at a significant disadvantage, a disadvantage that is magnified when it comes to cellular heterogeneity in brain tumors. For instance, markers that have been successfully used to enrich for human stem cells (*i.e.* CD34+/CD38-) have translated into understanding and defining the malignant stem cell population. This type of translation has yet to take place in the CNS field, even though several methods for enriching for somatic neural stem cells have been proposed (84-110-111). Hence, the absence of maturity in the understanding of somatic NSCs biology and the transition of stem cell progeny into functional cells has stymied the study and characterization of the complexity of cellular interactions in human gliomas, making it difficult to develop targeted therapies when one does not know what the target is.

The poor prognosis of malignant glioma, especially GBM, has been attributed to the failure of treatment efficacy (*i.e.* molecular targeted therapies such as tyrosine kinase inhibitor, monoclonal antibodies) due to the cellular diversity and the limited therapeutic access to the tumor infiltrated brain (15-112-113). For instance, the discovery of O6-methylguanine-DNA methyltransferase (MGMT) status influencing treatment response to temozolomide (TMZ) highlights one of the major hurdles in the treatment of malignant glioma – tumor cellular and genetic heterogeneity (114). Cell surface protein expression and molecular defects vary dramatically among and within the tumors (115-116-117-118-119-120). This heterogeneity represents a significant therapeutic challenge and most likely complements the *de novo* and acquired resistance of these tumors, especially with monotherapy.

After the surgical resection of accessible neoplastic tissue (121), conventional strategies to control the heterogeneous cell populations that culminate into a human glioma have ranged from untargeted insults of ionizing radiation (XRT) or cytotoxic chemotherapy (*i.e.* TMZ) to targeted manipulation of the proliferation of a select population of cells within the tumor (4-122-123-124). An alternative approach to targeting the proliferating (and relatively quiescent stem) cells with cytotoxic therapy is to induce stem and progenitor cell differentiation, causing them to lose their stem and proliferative qualities. This regimen would make these tumors less aggressive and more sensitive to cytotoxic treatment (125). This approach has been confirmed using retinoic acid in hematologic malignancies for differentiation of leukemic stem cells (126-127). Use of all-*trans* retinoic acid (ATRA) with chemotherapy raised the complete remission rate of acute promyeocytic leukemia (APL) forcing leukemic stem cells to differentiate (128). Exploiting this model in the treatment of brain tumors, it has been recently demonstrated that a member of the bone morphogenetic proteins (BMPs), namely BMP4, which regulates neural stem cell activity within the adult brain niche, elicits its effects through the BMP receptors (65-129) by triggering intracellular Smad 1,5,8 phosphorylation. This drives a pro-differentiation cascade of events that leads to the depletion of the cancer stem cell pool in GBMs, both *in vitro* and *in vivo*. This result is similar to the outcome of exposing BMPs to somatic NSCs, where it induces their differentiation down an astrocyte pathway (130-131). This approach did not only identify BMP4 as a potential, powerful anti-cancer drug in GBM but established new candidate therapeutic concepts, in that tumor therapies might be effectively designed to target a specific cancer stem cell population inside a solid tumor and that such therapies may be tailored to enforce differentiation of these cells rather than killing, thereby depleting the tumor stem cell pool and tumorigenicity.

6.2. Other therapeutic considerations

Drug delivery to tumor tissue has also been a critical factor limiting therapy efficacy. In addition to new therapies, optimizing delivery is being studied to improve outcomes in patients with malignant glioma. As a matter of fact, the blood brain barrier (BBB) limits drug access to the

central nervous system based on chemical constitution and size (132-133). Additionally, BBB efflux proteins actively pump drugs from the CNS (134-135) and tumor factors such as dysfunctional tumor vasculature leading to relative hypoxia diminished cytotoxic therapy (136-137-138-139). These challenges have lead to interest in locoregional therapy to circumvent the BBB and avoid systemic toxicities (15). Convection Enhanced Delivery (CED) is the latest technique of direct intraparenchymal intervention being used to deliver novel therapies for patients with malignant glioma. By placing catheters in and around tumor tissue, the drug is infused over several days to achieve homogeneous delivery that extends several centimeters beyond the 3mm achieved with passive diffusion (140). The ultimate goal is to treat as large a volume as possible of the tumor infiltrated brain. Furthermore, this technique allows the design of therapeutics specific for the compartmental target and the application of effective concentrations in the anticipated treatment volume. Several human trials have demonstrated safety of CED. In particular, large molecules such as enzymes, antibodies proteins and even viruses, will remain in the extracellular space of the brain for long times until they are cleared. Gene therapy for malignant glioma has been tested with intratumoral injections of cells with the gene thymidine kinase of herpes simplex virus type 1 (HSV-TK). Results from phase I/II studies of HSV-TK gene therapy in recurrent GBM demontrated safety (141-142). More recently, the precise trial completed a phase III study of interleukin 13 bound to Pseudomonas exotoxin (IL13-PE) for GBM using CED (143).

While some protocols (37-65-144-145) have shown benefit clinically and demonstrate great promise pre-clinically, they are unlikely to be curative or to provide a substantial benefit comparable to the use of Gleevec for CML. In developing the next generation of targeted therapeutic molecules for treating CNS tumors a more comprehensive understanding of the cell types involved in driving tumor progression and which cells are targeted by a single treatment or combination treatment, will need to be achieved. These results will not only define the cellular populations to be targeted and outline the *inter*- and *intra*-tumor heterogeneity, but also identify the resistant populations to be targeted with secondary lines of treatment.

7. CONCLUSIONS AND PERSPECTIVE

The analysis of glial tumors and of the related cell lines has provided some prognostic markers as well as potential therapeutic targets as well as key mutational events characteristic for the individual tumor types thus refining classification and grading. In addition some clues were generated about the relationship between developmental glial lineages, tumor stem cells and subtypes of tumors, based on their gene expression patterns. The insight gained into glioma development and biology has not yet translated into much therapeutic progress, especially not effective targeted therapy mainly because of its heterogeneity, which represents a formative challenge to treatment particularly in the application of monotherapy, but also a barrier to any therapeutic regime due to acquired resistance.

A body of evidence now exists suggesting that brain tumors contain relatively rare subpopulation of transformed cells with stem cell-like properties and that this population may be resistant to conventional therapy based on the notion that conventional therapy targets the heterogeneous body of cancer cells in a relatively non-specific fashion and spares the tumor stem cells due to their unique properties (16-67-146). Targeting this population may be an important therapeutic strategy in treating patients with brain tumors (34-147). However, as attractive as this hypotesis sounds, it has been challenged and is still largely untested when it comes to solid tissue cancers (148-149). The hypotesis does not take into account the inability of current treatments to target the bulk of the tumor. Additionally, a convincing body of evidence does not exist that treatment resistance and recurrence are due to the relatively rare cancer stem cell population (150). Hence, while targeting the cancer stem cell may ultimately be important for an effective cure, one cannot dismiss the bulk of the proliferating cells within the tumor. The reamining key issues are therefore still the need for even more refined array based molecular analysis of appropriate, defined tumor entities serving as key comparators and the same for tumor derived stem-like cells and the yet undefined human neuro-glial stem cells or committed progenitors.

With the integration of onco- and neurobiological analyses, the next generation of targeted therapeutic molecules for treating CNS tumors will likely entail a more comprehensive understanding of the cell types involved in driving tumor progression and which cells are affected by a single treatment or combination treatment.

8. REFERENCES

1. Craig R. Miller, Arie Perry: Glioblastoma. *Arch Pathol Lab Med* 131, 397-406 (2007)

2. Julie M. Legler, Lynn A. Ries, Malcolm A. Smith, Joan L. Warren, Ellen F. Heineman, Richard S. Kaplan, Martha S. Linet: Cancer surveillance series [corrected]: brain and other central nervous system cancers: recent trends in incidence and mortality. *J Natl Cancer Inst* 1999 91, 1382-90 (1999)

3. Kenneth R. Hess, Kristine R. Broglio, Melissa L. Bondy: Adult glioma incidence trends in the United States, 1977-2000. *Cancer* 101, 2293-9 (2004)

4. Eric C. Burton, Michael D. Prados: Malignant gliomas. *Curr Treat Options Oncol* 1, 459-68 (2000).

5. Davide Schiffer, Paola Cavalla, Anna Dutto, Lucia Borsotti: Cell proliferation and invasion in malignant gliomas. *Anticancer Res* 17, 61-9 (1997)

6. Anthony Behin, Khe Hoang-Xuan, Antoine F. Carpentier, Jean-Yves Delattre: Primary brain tumors in adults. *Lancet* 361, 323-331 (2003)

7. Faith G. Davis, Sally Freels, James Grutsch, Suna Barlas, Steven Brem: Survival rates in patients with primary malignant brain tumors stratified by patient age and tumor histological type: an analysis based on Surveillance, Epidemiology, and End Results (SEER) data, 1973-1991. *J Neurosurg* 88, 1-10 (1998)

8. Dietmar Krex, Barbara Klink, Christian Hartmann, Andreas von Deimling, Torsten Pietsch, Matthias Simon, Michael Sabel, Joachim P. Steinbach, Oliver Heese, Guido Reifenberger, Michael Weller, Gabriele Schackert: Long-term survival with glioblastoma multiforme. *Brain* 130, 2596-606 (2007)

9. Adam S. Garden, Moshe H. Maor, W. K. Alfred Yung, Janet M. Bruner, Shiao Y. Woo, Richard P. Moser, Ya-Yen Lee: Outcome and patterns of failure following limited-volume irradiation for malignat astrocytomas. *Radiother Oncol* 20, 99-110 (1991)

10. Elizabeth A. Maher, Frank B. Furnari, Robert M. Bachoo, David H. Rowith, David. N. Louis, Webster K. Cavenee, Ronald A. DePinho: Malignant glioma: genetics and biology of a grave matter. *Genes Dev* 15,1311-33 (2001)

11. John W. Henson: Treatment of glioblastoma multiforme: a new standard. *Arch Neurol* 63, 337-41 (2006)

12. James G. Gurney, Nina Kadan-Lottock: Brain and other central nervous system tumors: rates, trends, and epidemiology. *Curr Opin Oncol* 13, 160-6 (2003)

13. Fred G. Barker, Susan M. Chang, Philip H. Gutin, Mary K. Malec, Michael W. McDermott, Michael. D. Prados, Charles B. Wilson: Survival and functional status after resection of recurrent glioblastoma multiforme. *Neurosurgery* 42, 709-20 (1998)

14. Dasarahally S. Mohan, John H. Suh, Jennifer L. Phan, Patrick A. Kupelian, Bruce H. Cohen, Gene H. Barnett: Outcome in elderly patients undergoing definitive surgery and radiation therapy for supratentorial glioblastoma multiforme at a tertiary care institution. *Int J Radiat Oncol Biol Phys* 42, 981-7 (1998)

15. David A. Reardon, Jeremy N. Rich, Henry S. Friedman, Darell D. Bigner: Recent advances in the treatment of malignant astrocytoma. *J. Clin Oncol* 24, 1253-65 (2006)

16. Brian J. Huntly, D. Gary Gilliand: Cancer biology: summing up cancer stem cells. *Nature* 435, 1169-70 (2005)

17. Michael F. Clarke, Margaret Fuller: Stem cells and cancer: two faces of eve. *Cell* 124, 1111-5 (2006)

18. Stephen W. Coons, Peter C. Johnson, Bernd W. Scheithauer, Allan J. Yates, Dennis K. Pearl: Improving diagnostic accuracy and interobserver concordance in the classification and grading of primary gliomas. *Cancer* 79, 1381-93 (1997)

19. Justin S. Smith, Robert B. Jenkins: Genetic alterations in adult diffuse glioma: occurrence, significance, and prognostic implications. *Front Biosci* 5, 213-31 (2000)

20. Eran Segal, Nir Friedman, Naftali Kaminski, Aviv Regey, Daphne Koller: From signatures to models: understanding cancer using microarrays. *Nat Genet* 37, S38-45 (2005)

21. Chand Khanna, Lee J. Helman: Molecular approaches in pediatric oncology. *Annu Rev Med* 57,83-97 (2006).

22. Markus J. Riemenschneider, Guido Reifenberger: Molecular neuropathology of gliomas. *Int J Mol Sci* 10, 184-212 (2009)

23. Erik P. Sulman, Marisol Guerrero, Ken Aldape: Beyond grade: molecular pathology of malignant gliomas. *Semin Radiat Oncol* 19, 142-9 (2009)

24. Frank B. Furnari, Tim Fenton, Robert M. Bachoo, Akitake Mukasa, Jayne M. Stommel, Alexander Stegh, William C. Hahn, Keith L. Ligon, David N. Louis, Cameron Brennan, Lynda Chin, Ronald A. DePinho, Webster K. Cavenee: Malignant astrocytic glioma: genetics, biology, and paths to treatment. *Genes Dev* 21, 2683-710 (2007)

25. Hiroko Ohgaki, Paul Kleihues: Genetic pathways to primary and secondary glioblastoma. *Am J Pathol* 170, 1445-53 (2007)

26. Patrick Y. Wen, Santosh Kesari: Malignant gliomas in adults. *N Engl J Med* 359, 492-507 (2008)

27. Deepak Kamnasaran, Baoping Qian, Cynthia Hawkins, William L. Stanford, Abhijit Guha: GATA6 is an astrocytoma tumor suppressor gene identified by gene trapping of mouse glioma model. *Proc Natl Acad Sci USA* 104, 8053-8 (2007)

28. Maria S. Carro, Wei K. Lim, Mariano J. Alvarez, Robert J. Bollo, Xudong Zhao, Evan Y. Snyder, Erik P. Sulman, Sandrine L. Anne, Fiona Doetsch, Howard Colman, Anna Lasorella, Ken Aldape, Andrea Califano, Antonio Iavarone: The transcriptional network for mesenchymal transformation of brain tumours. *Nature* 463, 318-25 (2010)

29. Lesley A. Stewart: Chemotherapy in adult high-grade glioma: a systematic review and meta-analysis of individual patient data from 12 randomised trials. *Lancet* 359, 1011-8 (2002)

30. Charles G. Gross: Neurogenesis in the adult brain: death of a dogma. *Nat Rev Neurosci* 1, 67-73 (2000)

31. Brent A. Reynolds, Samuel Weiss: Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. *Science* 255, 1707-10 (1992)

32. Fred H. Gage: Mammalian neural stem cells. *Science* 287, 1433-8 (2000)

33. Andreas Ardvidsson, Tove Collin, Deniz Kirik, Zaal Kokaia, Olle Lindvall: Neuronal replacement from endogenous precursors in the adult brain after stroke. *Nat Med* 8, 963-70 (2002)

34. Angelo L. Vescovi, Rossella Galli, Brent A. Reynolds: Brain tumor stem cells. *Nat Rev Cancer* 6, 425-36 (2006)

35. Tim Demuth, Jessica L. Rennert, Dominique B. Hoelzinger, Linsey. B. Reavie, Mitsutoshi Nakada, Christian. Beaudry, Satoko Nakada, Eric M. Anderson, Amanda N. Henrichs, Wendy S. McDonough, David Holz, Anna Joy, Richard Lin, Kuang H. Pan, Chih J. Lih, Stan N. Cohen, Michael E. Berens: Glioma cells on the run – the migratory transcriptome of 10 human glioma cell lines. *BMC Genomics* 2008 9, 54 (2008)

36. Jing Li, Clifford Yen, Danny Liaw, Katrina Podsypanina, Shikha Bose, Steven I. Wang, Janusz Puc, Christa Miliaresis, Linda Rodgers, Richard McCombie, Sandra H. Bigner, Beppino C. Giovanella, Michael Ittmann, Ben Tycko, Hanina Hibshoosh, Michael H. Wigler, Ramon Parsons: PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science* 275, 1943-7 (1997)

37. Ingo K. Mellinghoff, Maria Y. Wang, Igor Vivanco, Daphne A. Haas-Kogan, Shaojun Zhu, Ederlyn Q. Dia, Kan V. Lu, Koji Yoshimoto, Julie H. Huang, Dennis J. Chute, Bridget L. Riggs, Steve Horvath, Linda M. Liau, Webster K. Cavenee, P. Nagesh Rao, Rameen Beroukhim, Timothy C. Peck, Jeffrey C. Lee, William R. Sellers, David Stokoe, Michael Prados, Timothy F. Cloughesy, Charles L. Sawyers, Paul S. Mischel: Molecular determinants of the response of glioblastomas to EGFR kinase inhibitors. *N Engl J Med* 353, 2012-24 (2005)

38. Jayne M. Stommel, Alec C. Kimmelman, Haogiang Ying, Roustem Nabioullin, Aditya H. Ponugoti, Ruprecht Wiedemeyes, Alexander H. Stegh, James E. Bradner, Keith. L. Ligon, Cameron Brennan, Lynda Chin, Ronald A. DePinho: Coactivation of receptor tyrosine kinases affects the response of tumor cells to targeted therapies. *Science* 318, 287-90 (2007)

39. Sith Sathornsumetee, David A. Reardon, Annick Desjardins, Jennifer A. Quinn, James J. Vredenburgh, Jeremy N. Rich: Molecularly targeted therapy for malignant glioma. *Cancer* 110, 13-24 (2007)

40. Jeremy N. Rich, Darell D. Bigner: Development of novel targeted therapies in the treatment of malignant glioma. *Nat Rev Drug Discov* 3, 430-46 (2004)

41. Edward G. Shaw, Robin Rosdhal, Ralph B. D'Agostino Jr, James Lovato, Michelle J. Naughton, Michael E. Robbins, Stephen R. Rapp: Phase II study of donepezil in irradiated brain tumor patients: effect on cognitive function, mood, and quality of life. *J Clin Oncol* 24, 1415-20 (2006)

42. Hiroyuki Momota, Edward Nerio, Eric C. Holland: Perifosine inhibits multiple signaling pathways in glial progenitors and cooperates with temozolomide to arrest cell proliferation in gliomas *in vivo*. *Cancer Res* 65, 7429-35 (2005)

43. Evanthia Galanis, Jan C. Buckner, Matthew J. Maurer, Jeffrey I. Kreisberg, Karla Ballman, J. Boni, Joseph M. Peralba, Robert B. Jenkins, Shaker R. Dakhil, Roscoe F. Morton, Kurt A. Jaeckle, Bernd W. Scheithauer, Janet Dancey, Manuel Hidalgo, Daniel J. Walsh: Phase II trial of temsirolimus (CCI-779) in recurrent glioblastoma multiforme: a North Central Cancer Treatment Group Study. *J Clin Oncol* 23, 5294-304 (2005)

44. Alba A. Brandes, Mario Ermani, Sergio Turazzi, Elvira Scelzi, Franco Berti, Pietro Amistà, Antonino Rotilio, Claudio Licata, Mario V. Fiorentino: Procarbazine and high-dose tamoxifen as a second-line regimen in recurrent high-grade gliomas: a phase II study. *J Clin Oncol* 17, 645-50 (1999)

45. Alexander M. Spence, Richard A. Peterson, Jeffrey D. Scharnhorst, Daniel L. Silbergeld, Robert C. Rostomily: Phase II study of concurrent continuous Temozolomide (TMZ) and Tamoxifen (TMX) for recurrent malignant astrocytic gliomas. J Neurooncol 70, 91-5 (2004)

46. W. Robert Bruce, Henk Van Der Gaag: A quantitative assay for the number of murine lymphoma cells capable of proliferation *in vivo*. Nature 199,79-80 (1963)

47. Chester Southam, Alexander Brunschwig: Quantitative studies of autotransplantation of human cancer. Cancer 14, 461-463 (1961)

48. Larry M. Weisenthal, Marc E. Lippman: Clonogenic and nonclonogenic *in vitro* chemosensitivity assay. Cancer Treat Rep 69, 615-32 (1985)

49. Giancarlo Secchi: Cancer chemotherapy. Ann Ital Med Int 5, 288-95 (1990)

50. Douglas E. Mattox, Daniel D. Von Hoff: Culture of human head and neck cancer stem cells using soft agar. Arch Otolaryngol 106, 672-4 (1980)

51. Adi F. Gazdar, Desmond N. Carney, Harvie L. Sims, Alfreda Simmons: Heterotransplantation of small-cell carcinoma of the lung into nude mice: comparison of intracranial and subcutaneous routes. Int J Cancer 28, 777-83 (1981)

52. Ronald N. Buick, Lung J. Chang, Hans A. Messner, Joseph E. Curtis, Ernest A. McCulloch: Self-renewal capacity of leukemic blast progenitor cells. *Cancer Res* 41, 4849-52 (1981)

53. Tsvee Lapidot, Christian Sirard, Josef Vormoor, Barbara Murdoch, Trang Hoang, Julio Caceres-Cortes, Mark Minden, Bruce Paterson, Michael A. Caligiuri, John

E. Dick: A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. *Nature* 367, 645-8 (1994)

54. Dominique Bonnet, John E. Dick: Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat Med* 3, 730-7 (1997)

55. Liqing Jin, Kristin J. Hope, Qiongli Zhai, Florence Smadia-Joffe, John E. Dick: Targeting of CD44 eradicates human acute myeloid leukemic stem cells. *Nat Med* 12, 1167-74 (2006)

56. Edward Kavalerchik, Daniel Goff, Catriona. H. Jamieson: Chronic myeloid leukemia stem cells. *J Clin Oncol*. 26, 2911-5 (2008)

57. Brian J. Druker, Charles L. Sawyers, Hagop Kantarjian, Debra J. Resta, Sofia F. Reese, John M. Ford, Renaud Capdeville, Moshe Talpaz: Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. *N Engl J Med* 344, 1038-42 (2001)

58. Tim P. Huges, Jaspal Kaeda, Susan Branford, Zbigniew Rudzki, Andreas Hochhaus, Martee L. Hensley, Insa Gathmann, Ann E. Bolton, Iris C. Van Hoomissen, John M. Goldman, Jerald P. Radich: Frequency of major molecular responses to imatinib or interferon alfa plus cytarabine in newly diagnosed chronic myeloid leukemia. *N Engl J Med* 349, 1423-32 (2003)

59. David J. Barnes, Junio V. Melo: Primitive, quiescent and difficult to kill: the role of non-proliferating stem cells in chronic myeloid leukemia. *Cell Cycle* 5, 2862-6 (2006)

60. Tatyana N. Ignatova, Valery G. Kukekov, Eric D. Laywell, Oleg N. Suslov, Frank D. Vrionis, Dennis A. Steindler: Human cortical glial tumors contain neural stem-like cells expressing astroglial and neuronal markers *in vitro*. *Glia* 39, 193-206 (2006)

61. Muhammad Al-Hajj, Max S. Wicha, Adalberto Benito-Hernandez, Sean J. Morrison, Michael F. Clarke: Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci USA* 100, 3983-8 (2003)

62. Houman D. Hemmati, Ichiro Nakano, Jorge A. Lazareff, Michael Masterman-Smith, Daniel H. Geschwind, Marianne Bronner-Fraser, Harley I. Kornblum: Cancerous stem cells can arise from pediatric brain tumors. *Proc Natl Acad Sci USA* 100, 15178-83 (2003

63. Sheila K. Singh, Ian D. Clarke, Mizuhiko Terasaki, Victoria E. Bonn, Cynthia Hawkins, Jeremy Squire, Peter B. Dirks: Identifications of a cancer stem cell in human brain tumors. *Cancer Res* 63, 5821-8 (2003)

64. Rossella Galli, Elena Binda, Ugo Orfanelli, Barbara Cipelletti, Angela Gritti, Simona De Vitis, Roberta Fiocco, Chiara Foroni, Francesco Dimeco, Angelo L. Vescovi: Isolation and characterization of tumorigenic, stem-like neural precursors from human glioblastoma. *Cancer Res* 64, 7011-21 (2004)

65. Sara G. Piccirillo, Brent A. Reynolds, Nadia Zanetti, Giuseppe Lamorte, Elena Binda, Giovanni Broggi, Henry Brem, Alessandro Olivi, Francesco Dimeco, Angelo L. Vescovi: Bone morphogenetic proteins inhibit the tumorigenic potential of human brain tumor-initiating cells. *Nature* 444, 761-5 (2006)

66. Jeongwu Lee, Svetlana Kotliarova, Yuri Kotliarov, Aiguo Li, Qin Su, Nicholas M. Donin, Sandra Pastorino, Benjamin W. Purow, Neil Christopher, Wei Zhang. John K. Park, Howard A. Fine: Tumor stem cells derived from glioblastomas cultured in bFGF and EGF more closely mirror the phenotype and genotype of primary tumors than do serum-cultured cell lines. *Cancer Cell* 9, 391-403 (2006)

67. Tannishtha Reya, Sean J. Morrison, Michael F. Clarke, Irving L. Weissman: Stem cells, cancer, and cancer stem cells. Nature 414, 105- 11 (2001)

68. Costas G. Hadjipanayis, Erwin G. Van Meir: Tumor initiating cells in malignant gliomas: biology and implications for therapy. *J Mol Med* 87, 363-74 (2009)

69. Xiangpeng Yuan, James Curtin, Yizhi Xiong, Gentao Liu, Sebastian Waschsmann-Hogiu, Daniel L. Farkas, Keith L. Black, John S. Yu: Isolation of cancer stem cells from adult glioblastoma multiforme. Oncogene 23, 9392-400 (2004)

70. Sara G. Piccirillo, Angelo L. Vescovi: Brain tumor stem cells: possibilities of new therapeutic strategies. Expert Opin Biol Ther 7, 1129-35 (2007)

71. Sheila K. Singh, Cynthia Hawkins, Ian D. Clarke, Jeremy A. Squire, Jane Bayani, Takuichiro Hide, R. Mark Henkelman, Michael D. Cusimano, Peter B. Dirks: Identification of human brain tumor initiating cells. Nature 432, 396-401 (2004)

72. Dominique Boivin, David Labbé, Nicolas Fontaine, Sylvie Lamy, Edith Beaulieu, Denis Gingras, Richard Béliyeau: The stem cell marker CD133 (prominin-1) is phosphorylated on cytoplasmic tyrosine-828 and tyrosine-852 by Src and Fyn tyrosine kinases. Biochemestry 2009 48, 3998-4007 (2009)

73. Urban Lendahl, Lyle B. Zimmerman, Ronald D. McKay: CNS stem cells express a new class of intermediate filament protein. Cell 60, 585-95 (1990)

74. Mila Komitova, Peter S. Eriksson: Sox-2 is expressed by neural progenitors and astroglia in the adult rat brain. *Neurosci Lett* 369, 24-7 (2004)

75. Shin-ichi Sakakibara, Takao Imai, Kayoko Hamaguchi, Masataka Okabe, Jun Aruga, Kazunori Nakajima, Daisuke Yasutomi, Takashi Nagata, Yasuyuki Kurihara, Seiichi Uesugi, Takaki Miyata, Masaharu Ogawa, Katsuhiko

Mikoshiba, Hideyuki Okano: Mouse-Musashi-1, a neural RNA-binding protein highly enriched in the mammalian CNS stem cell. Dev Biol 176, 230-42 (1996)

76. Shideng Bao, Qiulian Wu, Roger E. McLendon, Yueling Hao, Qing Shi, Anita B. Hielmeland, Mark W. Dewhirst, Darell D. Bigner, Jeremy N. Rich: Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature 444, 756-60 (2006)

77. Ed R. Blazek, Jennifer L. Foutch, Guitta Maki: Daoy medulloblastoma cells that express CD133 are radioresistant relative to CD133-cells, and the CD133+ sector is enlarged by hypoxia. *Int J Radiat Oncol Biol Phys* 67, 1-5 (2007)

78. Gentao Liu, Xiangpeng Yuan, Zhaohui Zeng, Patrizia Tunici, Hiushan NG, Iman R. Abdulkadir, Lizhi Lu, Dwain Irvin, Keith L. Black, John S. Yu: Analysis of gene expression and chemoresistance of CD133+ cancer stem cells in glioblastoma. *Mol Cancer* 5, 67 (2006)

79. Jian Wang, Per Ø. Sakariassen, Oleg Tsinkalovsky, Heike Immervoll, Stig O. Bøe, Agnete Svendsen, Lars Prestegarden, Gro Røsland, Frits Thorsen, Linda Stuhr, Anders Molven, Rolf Bjerkvig, Per Ø. Enger: CD133 negative glioma cells form tumors in nude rats and give rise to CD133 positive cells. Int *J Cancer* 122, 761-8 (2008)

80. Alfred T. Ogden, Allen E. Waziri, Richard A. Lochhead, David Fusco, Kim Lopez, Jason A. Ellis, Joann Kang, Marcela Assanah, Guy M. McKhann, Michael B. Sisti, Paul C. McCormick, Peter Canoll, Jeffrey N. Bruce: Identification of A2B5+CD133- tumor-initiating cells in adult human gliomas. Neurosurgery 62, 505-14 (2008)

81. Dagmar Beier, Peter Hau, Martin Proescholdt, Annette Lohmeier, Jörg Wischhusen, Peter J. Oefner, Ludwig Aigner, Alexander Brawanski, Ulrich Bogdahn, Christoph P. Beier: CD133(+) and CD133(-) glioblastoma-derived cancer stem cells show differential growth characteristics and molecular profiles. Cancer Res 67, 4010-5 (2007)

82. Steven M. Pollard, Koichi Yoshikawa, Ian D. Clarke, Davide Danovi, Stefan Stricker, Roslin Russell, Jane Bavani, Renee Head, Marco Lee, Mark Bernstein, Jeremy A. Squire, Austin Smith, Peter B. Dirks: Glioma stem cell lines expanded in adherent culture have tumor-specific phenotypes and are suitable for chemical and genetic screens. *Cell Stem Cell* 4, 568-80 (2009)

83. Virginie Clément, Valérie Dutoit, Denis Marino, Pierre-Yves Dietrich, Ivan Radovanovic: Limits of CD133 as a marker of glioma self-renewing cells. Int J Cancer 125, 244-8 (2009)

84. Alexandra Capela, Sally Temple: LeX/ssea-1 is expressed by adult mouse CNS stem cells, identifying them as nonependymal. Neuron 35, 865-875 (2002)

85. Alexandra Capela, Sally Temple: LeX is expressed by principle progenitor cells in the embryonic nervous system, is secreted into their environment and binds Wnt-1. Dev Biol 291, 300-313 (2006)

86. Myung J. Son, Kevin Woolard, Do-Hyun Nam, Jeongwu Lee, Howard A. Fine: SSEA-1 is an enrichment marker for tumor-initiating cells in human glioblastoma. *Cell Stem Cell* 4, 440-52 (2009)

87. Lyle Zimmerman, Urban Lendahl, Miles Cunningham. Ron McKay, Brian Parr, Brian Gavin, Jeff Mann, Galya Vassileva and Andrew McMahon: Independent regulatory elements in the nestin gene direct transgene expression to neural stem cells or muscle precursors. *Neuron* 12, 11-24 (1994)

88. Jonas Dahlstrand, V. Peter Collins and Urban Lendahl: Expression of the class VI intermediate filament nestin in human central nervous system tumors. *Cancer Res* 52, 5334-41 (1992)

89. Per M. Almqvist, Richard Mah, Urban Lendahl, Bjorn Jacobsson, Glenda Hendson: Immunohistochemical Detection of Nestin in Pediatric Brain Tumors. *J Histochem Cytochem* 50, 147-58 (2002)

90. Tadej Strojnik, Gro Vatne Røsland, Per Oystein Sakariassen, Rajko Kavalar and Tamara Lah: Neural stem cell markers, nestin and musashi proteins, in the progression of human glioma: correlation of nestin with prognosis of patient survival. *Surg Neurol* 68, 133-43 (2007)

91. Sharon K. Thomas, Conrad A. Messam, Barbara A. Spengler, June L. Biedler and Robert A. Ross: Nestin Is a Potential Mediator of Malignancy in Human Neuroblastoma Cells. *J Biol Chem* 279, 27994-9 (2004)

92. Seiji Hitoshi, Tania Alexson, Vincent Tropepe, Dorit Donoviel, Andrew J. Elia, Jeffrey S. Nye, Ronald A. Conlon, Tak W. Mak, Alan Bernstein and Derek van der Kooy: Notch pathway molecules are essential for the maintenance, but not the generation, of mammalian neural stem cells. *Genes & Dev* 16, 846-858 (2002)

93. Seiji Hitoshi, Raewyn M. Seaberg, Cheryl Koscik, Tania Alexson, Susumu Kusunoki, Ichiro Kanazawa, Shoji Tsuji and Derek van der Kooy: Primitive neural stem cells from the mammalian epiblast differentiate to definitive neural stem cells under the control of Notch signaling. *Genes & Dev* 18, 1806-1811 (2004)

94. Spyros Artavanis-Tsakonas, Matthew D. Rand and Robert J. Lake: Notch Signaling: Cell Fate Control and Signal Integration in Development. *Science* 284, 770-6 (1999)

95. Anjali Shiras, Sivarajan T. Chettiar, Varsha Shepal, Ganeshkumar Rajendran, G. Rajendra Prasad, Padma Shastry: Spontaneous Transformation of Human Adult Nontumorigenic Stem Cells to Cancer Stem Cells Is Driven by Genomic

Instability in a Human Model of Glioblastoma. *Stem Cells* 25, 1478-89 (2007)

96. Xue-Ping Zhang, Gang Zheng, Lian Zou, Hui-Ling Liu, Li-Hong Hou, Peng Zho, Dan-Dan Yin, Qi-Jun Zheng, Liang Liang, Su-Zhen Zhang, Lei Feng, Li-Bo Yao, An-Gang Yang, Hua Han, Jing-Yuan Chen: Notch activation promotes cell proliferation and the formation of neural stem cell-like colonies in human glioma cells. *Mol Cell Biochem* 307, 101-8 (2008)

97. Xing Fan, William Matsui, Leila Khaki, Duncan Stearns. Jiong Chun, Yue-Ming Li and Charles G. EberhartExpand+: Notch Pathway Inhibition Depletes Stem-like Cells and Blocks Engraftment in Embryonal Brain Tumors. *Cancer Res* 66, 7445-52 (2006)

98. Rosaria Gangemi, Laura Paleari, Anna Maria Orengo, Alfredo Cesario, Leonardo Chessa, Silvano Ferrini. Patrizia Russo: Cancer stem cells: a new paradigm for understanding tumor growth and progression and drug resistance. *Curr Med Chem* 16, 1688-703 (2009)

99. In-kyung Park, Dalong Qian, Mark Kiel, Michael W. Becker, Michael Pihalja, Irving L. Weissman, Sean J. Morrison & Michael F. Clarke: Bmi-1 is required for maintenance of adult self-renewing haematopoietic stem cells. *Nature* 423, 302-5 (2003)

100. Keith L. Ligon, John A. Alberta, Alvin T. Kho, Jennifer Weiss, Mary R. Kwaan, Catherine L. Nutt, David N. Louis, Charles D. Stiles and David H. Rowitch: The oligodendroglial lineage marker OLIG2 is universally expressed in diffuse gliomas. J *Neuropathol Exp Neurol* 63, 499-509 (2004)

101. Keith L. Ligon, Emmanuelle Huillard, Shwetal Mehta, Santosh Kesari, Hongye Liu, John A. Alberta, Robert M. Bachoo, Michael Kane, David N. Louis, Ronald A. DePinho, David J. Anderson, Charles D. Stiles and David H. Rowitch: Olig2-regulated lineage-restricted pathway controls replication competence in neural stem cells and malignant glioma. *Neuron* 53, 503-17 (2007)

102. Ichiro Nakano, Michael Masterman-Smith, Kuniyasu Saigusa, Andres A. Paucar, Steve Horvath, Lorelei, Shoemaker, Momoko Watanabe, Alejandra Negro, Ruchi Bajpai, Amy Howes, Vincent Lelievre, James A. Waschek, Jorge A. Lazareff, William A. Freije, Linda M. Liau, Richard J. Gilbertson, Timothy F. Cloughesy, Daniel H. Geschwind, Stanley F. Nelson, Paul S. Mischel, Alexey V. Terskikh, Harley I. Kornblum: Maternal embryonic leucine zipper kinase is a key regulator of the proliferation of malignant brain tumors, including brain tumor stem cells. J Neurosci Res 86, 48-60 (2008)

103. Margot Zöller: CD44: can a cancer-initiating cell profit from an abundantly expressed molecule? Nat Rev Cancer 11, 254-67 (2011)

104. Piero Dalerba, Scott J. Dylla, In-Kyung Park, Rui Liu, Xinhao Wang, Robert W. Cho, Timothy Hoey, Austin Gurney, Emina H. Huang, Diane M. Simeone, Andrew A. Shelton, Giorgio Parmiani, Chiara Castelli and Michael F. Clarke: Phenotypic characterization of human colorectal cancer stem cells. Proc Natl Acad Sci USA 104, 10158-63 (2007)

105. Stella Maris Ranuncolo, Virginia Ladeda, Sergio Specterman, Mirta Varela, José Lastiri, Ana Morandi, Elena Matos, Elisa Bal De Kier Joffe. Lydia Puricelli, Maria Guadalupe Pallotta: CD44 expression in human gliomas. *J Surg Oncol* 79, 30-5 (2002)

106. Judit Anido, Andrea Sàez-Borderias, Alba Gonzalez-Juncà, Laura Rodon, Gerard Folch, Maria A. Carmona. Rosa M. Prieto-Sanchez, Ignasi Barba, Elena Martinez-Saez, Ludmilla Prudkin, Isabel Cuartas, Carolina Raventos, Francisco Martinez-Ricarte, M. Antonia Poca, David Garcia-Dorado, Michael M. Lahn, Jonathan M. Yingling, Jordi Rodon, Juan Sahuquillo, Josè Baselga and Joan Seoane: TGF-β Receptor Inhibitors Target the $CD44^{high}/Id1^{high}$ Glioma-Initiating Cell Population in Human Glioblastoma. *Cancer Cell* 18, 655-68 (2010)

107. Rachid Marhaba, Pamela Klingbeil. Tobias Nuebel. Irina Nazarenko, Markus W. Buechler, Margot Zoeller: CD44 and EpCAM: cancer-initiating cell markers. *Curr Mol Med* 8, 784-804 (2008)

108. Marzenna Wiranowska, Sharron Ladd, Sarice R. Smith and Paul E. Gottschall: CD44 adhesion molecule and neuro-glial proteoglycan NG2 as invasive markers of glioma. *Brain Cell Biol* 35, 159-72 (2006)

109. Virginie Clément, Denis Marino, Cristina Cudalbu, Marie-France Hamou, Vladimir Mlynarik, Nicolas de Tribolet, Pierre-Yves Dietrich, Rolf Gruetter, Monika E. Hegi and Ivan Radavanovic: Marker-indipendent identification of glioma-initiating cells. *Nat Methods* 7, 224-8 (2010)

110. Mina Kim, Cindi M. Morshead: Distinct populations of forebrain neural stem and progenitor cells can be isolated using side-population analysis. *J Neurosci* 23, 10703-9 (2003)

111. Rodney L. Rietze, Helen Valcanis, Gordon F. Brooker, Tim Thomas, Anne K. Voss, Perry F. Bartlett: Purification of a pluripotent neural stem cell from the adult mouse brain. *Nature* 412, 736-9 (2001)

112. Tzu-Ting Huang, Shawn M. Sarkaria, Timothy F. Cloughesy, Paul S. Mischel: Targeted therapy for malignat glioma patients: lessons learned and the road ahead. *Neurotherapeutics* 6, 500-12 (2009)

113. Florence Lefranc, Michal Rynkowski, Olivier DeWitte, Robert Kiss: Present and potential future adjuvant issues in high-grade astrocytic glioma treatment. *Adv Tech Stand Neurosurg* 34, 3-35 (2009)

114. Peter C. Burger, Flôres S. Vogel, Sonya B. Green, Thomas A. Strike: Glioblastoma multiforme and anaplastic astrocytoma. Pathologic criteria and prognostic implications. *Cancer* 56, 1106-11 (1985)

115. Shekar N. Kurpad, Henry S. Friedman, Gary E. Archer, Roger E. McLendon, William M. Petros, Herbert E. Fuchs, Al Guaspari, Darell D. Bigner: Intraarterial administration of melphalan for treatment of intracranial human glioma xenografts in athymic rats. *Cancer Res* 55, 3803-9 (1995)

116. William W. Vick, Carol J. Wikstrand, Dennis E. Bullard, John T. Kemshead, Hugh B. Coakham, Jeffrey Schlom, William W. Johnston, Darell D. Bigner, Sandra H. Bigner: The use of a panel of monoclonal antibodies in the evaluation of cytologic specimens from the central nervous system. *Acta Cytol* 31, 815-24 (1987)

117. Gheeyoung Choe, Steve Horvath, Timothy F. Cloughesy, Katherine Crosby, David Seligson, Aarno Palotie, Landon Inge, Bradley L. Smith, Charles L. Sawyesrs, Paul S. Mischel: Analysis of the phosphatidylinositol 3'-kinase signaling pathway in glioblastoma patients *in vivo*. *Cancer Res* 63, 2742-46 (2003)

118. Arnab Chakravarti, Gary Zhai, Yoshiyuki Suzuki, Sormeh Sarkesh, Peter M. Black, Alona Muzikansky, Jay S. Loeffler: The prognostic significance of phosphatidylinositol 3-kinase pathway activation in huma gliomas. *J Clin Oncol* 22, 1926-33 (2004)

119. Max Chaffanet, Christiane Chauvin, Michèle Lainé, François Berger, Monique Chédin, Nathalie Rost, Marie-France Nissou, Alim L. Benabid: EGF receptor amplification and expression in human brain tumours. *Eur J Cancer* 28, 11-7 (1992)

120. Timothy P. Fleming, Abha Saxena, W. Craig Clark, James T. Robertson, Edward H. Oldfield, Stuart A. Aaronson, Iqbal Unnisa Ali: Amplification and/or overexpression of platelet-derived growth factor receptors and epidermal growth factor receptor in human glial tumors. *Cancer Res* 52, 4550-3 (1992)

121. Robert C. Rostomily, G. Evren Keles, Mitchel S. Berger: Radical surgery in the management of low-grade and high-grade gliomas. *Baillieres Clin Neurol* 5, 345-69 (1996)

122. Michael D. Walker, Eben J. Alexander, William E. Hunt, Collin S. MacCarty, M. Stephen J. Mahaley, John J. Mealey, Horace A. Norrell, Guy Owens, Joseph Ransohoff, Charles B. Wilson, Edmund A. Gehan, Thomas A. Strike: Evaluation of BCNU and/or radiotherapy in the treatment of anaplastic gliomas. A cooperative clinical trial. *J Neurosurg* 49, 333-343 (1978)

123. Michael D. Walker, Sylvan B. Green, David P. Byar, Eben Alexander Jr, Ulrich Batzdorf, William H. Brooks, William E. Hunt, Collin S. MacCarty, M. Stephen Mahaley Jr, John Mealey Jr, Guy Owens, Joseph Ransohoff 2nd, James T. Robertson, William R. Shapiro, Kenneth R. Smith Jr, Charles B. Wilson, Thomas A. Strike: Randomized comparisons of radiotherapy and nitrosoureas for the treatment of malignant glioma after surgery. *N Engl J Med* 303, 1323-9 (1980)

124. Maria Geraldina Castro, Rachel Cowen, Ian K. Williamson, Alexandria David, Maximiliano J. Jimenez-Dalmaroni, Xiuqin Yuan, Alireza Bigliari, Julie C. Williams, Jianying Hu, Pedro R. Lowenstein: Current and future strategies for the treatment of malignant brain tumors. *Pharmacol Ther* 98, 71-108 (2003)

125. Peter B. Dirks: Cancer: stem cells and brain tumours. *Nature* 444, 687-8 (2006)

126. Nigel P. Mongan, Lorraine J. Gudas: Diverse actions of retinoid receptors in cancer prevention and treatment. *Differentiation* 75, 853-70 (2007)

127. Rihab Nasr, Marie-Claude Guillemin, Omar Ferhi, Hassan Soilihi, Laurent Peres, Caroline Berthier, Philippe Rousselot, Macarena Robledo-Sarmiento, Valérie Lallemand-Breitenbach, Bernard Gourmel, Dominique Vitoux, Pier Paolo Pandolfi, Cécile Rochette-Egly, Jun Zhu, Hugues de Thé: Eradication of acute promyelocytis leukemia-initiating cells through PML-RARA degradation. *Nat Med* 14, 1333-42 (2008)

128. Zhen-Yi Wang, Zhu Chen: Acute promyelocytic leukemia: from highly fatal to highly curable. *Blood* 111, 2505-15 (2008)

129. Jeongwu Lee, Myung J. Son, Kevin Woolard, Nicholas M. Donin, Aiguo Li, Chui H. Cheng, Svetlana Kotliarova, Yuri Kotliarov, Jennifer Walling, Susie Ahn, Misuk Kim, Mariam Totonchy, Thomas Cusack, Chibawanye Ene, Hilary Ma, Qin Su, Jean Claude Zenklusen, Wei Zhang, Dragan Maric and Howard A. Fine: Epigenetic-mediated dysfunction of the bone morphogenetic protein pathway inhibits differentiation of glioblastoma-initiating cells. *Cancer Cell* 13, 69-80 (2008)

130. Daniel A. Lim, Anthony D. Tramontin, Jose M. Trevejo, Daniel G. Herrera, Jose M. Garcia-Verdugo, Arturo Alvarez-Buylla: Noggin antagonizes BMP signaling to create a niche for adult neurogenesis. *Neuron* 28, 713-26 (2000)

131. Prithi Rajan, David M. Panchision, Laura F. Newell, Ronald D. McKay: BMPs signal alternately through a SMAD or FRAP-STAT pathway to regulate fate choice in CNS stem cells. *J Cell Biol* 161, 911-21 (2003)

132. Dennis R. Groothius: The blood-brain and blood-tumor barriers: a review of strategies for increasing drug delivery. *Neuro Oncol* 2, 45-59 (2000)

133. Edward A. Neuwelt: Mechanisms of disease: the blood-brain barrier. *Neurosurgery* 54, 131-40 (2004)

134. Pauline Breedveld, Jos H. Beijnen, Jan H. Schellens: Use of P-glycoprotein and BCRP inhibitors to improve oral bioavailability and CNS penetration of anticancer drug. *Trends Pharmacol Sci* 27, 17-24 (2006)

135. Simon Dai, Mary Lin Huang, Chang Y. Hsu, K. S. Clifford Chao: Inhibition of hypoxia inducible factor 1alpha causes oxygen-independent cytotoxicity and induces p53 independent apoptosis in glioblastoma cells. *Int J Radiat Oncol Biol Phys* 55, 1027-36 (2003)

136. Peter Vajkoczy, Michael D. Menger: Vascular microenvironment in gliomas. *Cancer Treat Res* 117, 249-62 (2004)

137. Karl H. Plate, Hans D. Mennel: Vascular morphology and angiogenesis in glial tumors. *Exp Toxicol Pathol* 47, 89-94 (1995)

138. Asao Hirano, Takanori Matsui: Vascular structures in brain tumors. *Hum Pathol* 6, 611-21 (1975)

139. Don M. Long: Capillary ultrastructure in human metastatic brain tumors. J *Neurosurg* 51, 53-8 (1979)

140. R. Hunt. Bobo, Douglas W. Laske, Aytac Akbasak, Paul F. Morrison, Robert L. Dedrick, Edward H. Oldfield: Convection-enhanced delivery of macromolecules in the brain. P*roc Natl Acad Sci USA* 91, 2076-80 (1994)

141. Luisa Barzon, Marco Pacenti, Elisa Franchin, Federico Colombo, Giorgio Palù: HSV-TK/IL-2 gene therapy for glioblastoma multiforme. *Methods Mol Biol* 542, 529-49 (2009)

142. Federico Colombo, Luisa Barzon, Elisa Franchin, Marco Pacenti, Vittore Pinna, Daniela Danieli, Mariano Zanusso, Giorgio Palù: Combined HSV-TK/IL-2 gene therapy in patients with recurrent glioblastoma multiforme: biological and clinical results. *Cancer Gene Ther* 12, 835-48 (2005)

143. Sandeep Kunwar, Michael D. Prados, Susan M. Chang, Mitchel S. Berger, Frederick F. Lang, Joseph M. Piepmejer, John H. Sampson, Zvi Ram, Philip H. Gutin, Robert D. Gibbons, Kenneth D. Aldape, David J. Croteau, Jeffrey W. Sherman, Raj K. Puri: Direct intracerebral delivery of cintredekin besudotox (IL13-PE38QQR) in recurrent malignant glioma: a report by the Cintredekin Besudotox Intraparenchymal Study Group. *J Clin Oncol* 25, 837-44 (2007)

144. Roger Stupp, Warren P. Mason, Martin J. van den Bent, Michael Weller, Barbara Fisher, Martin J. Taphoorn, Karl Belanger, Alba A. Brandes, Christine Marosi, Ulrich Bogdahn, Jürgen Curschmann, Robert C. Janzer, Samuel K. Ludwin, Thierry Gorlia, Anouk Allgeier, Denis Lacombe, J. Gregory Cairncross, Elizabeth Eisenhauer, René O. Mirimanoff: Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med* 352, 987-96 (2005)

145. Akio Soeda, Akihito Inagaki, Naoki Oka, Yuka Ikegame, Hitomi Aoki, Shin-ichi Yoshimura, Shigeru Nakashima, Takahiro Kunisada, Toru Iwama: Epidermal growth factor plays a crucial role in mitogenic regulation of human brain tumor stem cells. *J Biol Chem* 283, 10958-66 (2008)

146. Craig T. Jordan, Monica L. Guzman, Mark Noble: Cancer stem cells. *N Engl J Med* 355, 1253-61 (2006)

147. Jeremy. N. Rich, Shideng Bao: Chemotherapy and cancer stem cells. *Cell Stem Cell* 1, 353-5 (2007)

148. Gregor B. Adams, David T. Scadden: A niche opportunity for stem cell therapeutics. *Gene Ther* 15, 96-9 (2008)

149. Elsa Quintana, Mark Shackleton, Michael S. Sabel, Douglas R. Fullen, Timothy M. Johnson, Sean J. Morrison: Efficient tumour formation by single human melanoma cells. *Nature* 456, 593-8 (2008)

150. Mikhail V. Blagosklonny: Target for cancer therapy: proliferating cells or stem cells. *Leukemia* 20, 385-91 (2006)

Abbreviations: CNS: central nervous system; GBM: glioblastoma multiforme; TICs: tumor-initiating cells, WHO: World Health Organization; Rb: retinoblastoma; PTEN: phosphatase and tensin homolog; EGFR: epidermal growth factor receptor; PDGFR: platelet-derived growth factor receptor; IDH1: isocitrate dehydrogenase 1; GATA: GATA binding protein; C/Epbbeta: CCAAT-enhancer-binding protein beta; STAT3: signal transduction and transcription 3; NCSs: neural stem cells; AKT: murine thymoma viral oncogene; PI3K: phosphatidylinositol 3-kinase; RTK: receptor tyrosine kinase; EGF: epidermal growth factor; PDGF: platelet-derived growth factor; VEGF: vascular endothelial growth factor; HGF/SF: hepatocyte growth factor/scatter factor; IGF: insuline-like growth factor; RAS: rat sarcoma; RAF: rapidly accelerated fibrosarcoma; MEK: mitogen-activated ERK kinase; ERK: extracellular signal-regulated kinases; mTOR: mammalian target of rapamycin; PKC: protein kinase C; AML: acute myeloid leukemia; SCID mice: severe combined immunodeficient mice; LIC: leukemia initiating cell; CML: chronic myeloid leukemia; BCR-ABL: breakpoint cluster region and Abelson proto-oncogene fusion gene; TKI: tyrosine kinase inhibitor; Sox-2: sry-related HMG box-containing genes 2; Chk1-2: DNA damage checkpoint kinase 1-2; SCs: stem cells; Bmi-1: B-cell-specific Moloney murine leukemia virus integration site 1; OLIG-2: oligodendrocyte transcription factor 2; MELK: maternal embryonic leucine zipper kinase; MGMT: O6-methylguanine-DNA methyltransferase; TMZ: temozolomide; XRT: ioning radiation; ATRA: all-trans retinoic acid; APL: acute promyelocytic leukemia; BMPs: bone morphogenetic proteins; Smad: homologs in vertebrate of both the drosophila protein mothers against decapentaplegic (MAD) and the Caenorhabditis elegans protein SMA; BBB: blood brain barrier; CED: convection enhanced delivery; HSV-TK: thymidine kinase of herpes simplex virus type 1; IL13-PE: interleukin 13 bound to Pseudomonas exotoxin

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Send correspondence to: Angelo Luigi Vescovi, Piazza della Scienza 2, Building U3, 20126 Milan, Italy, Tel: 0039 02 64483351, Fax: 0039 02 64483314, E-mail: vescovi@tin.it