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Review Article

Personalized and translational approach for malignant brain tumors in the era of precision medicine: the strategic contribution of an experienced neurosurgery laboratory in a modern neurosurgery and neuro-oncology department

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ABSTRACT

Personalized medicine (PM) aims to optimize patient management, taking into account the individual traits of each patient. The main purpose of PM is to obtain the best response, improving health care and lowering costs. Extending traditional approaches, PM introduces novel patient-specific paradigms from diagnosis to treatment, with greater precision. In neuro-oncology, the concept of PM is well established. Indeed, every neurosurgical intervention for brain tumors has always been highly personalized. In recent years, PM has been introduced in neuro-oncology also to design and prescribe specific therapies for the patient and the patient's tumor. The huge advances in basic and translational research in the fields of genetics, molecular and cellular biology, transcriptomics, proteomics, and metabolomics have led to the introduction of PM into clinical practice. The identification of a patient's individual variation map may allow to design selected therapeutic protocols that ensure successful outcomes and minimize harmful side effects. Thus, clinicians can switch from the "*one-size-fits-all*" approach to PM, ensuring better patient care and high safety margin. Here, we review emerging trends and the current literature about the development of PM in neuro-oncology, considering the positive impact of innovative advanced researches conducted by a neurosurgical laboratory.

Abbreviation: PM, Personalized Medicine; GBM, Glioblastoma; HGG, High Grade Glioma; LGG, Low Grade Glioma; TMZ, Temozolomide; GSCs, Glioblastoma Stem Cells; EGFR, Epidermal Growth Factor Receptor; VEGF, Vascular Endothelial Factor; MET/HGF, Mesenchymal Epithelial Transition/Hepatocyte Growth Factor Receptor; CAR-T, Chimeric antigen receptor T cell; TECs, Tumor Endothelial Cells; VWF, von Willebrand factor; PLTs, platelets; S1P, Sphingosine-1-phosphate; MRI, Magnetic Resonance Imaging

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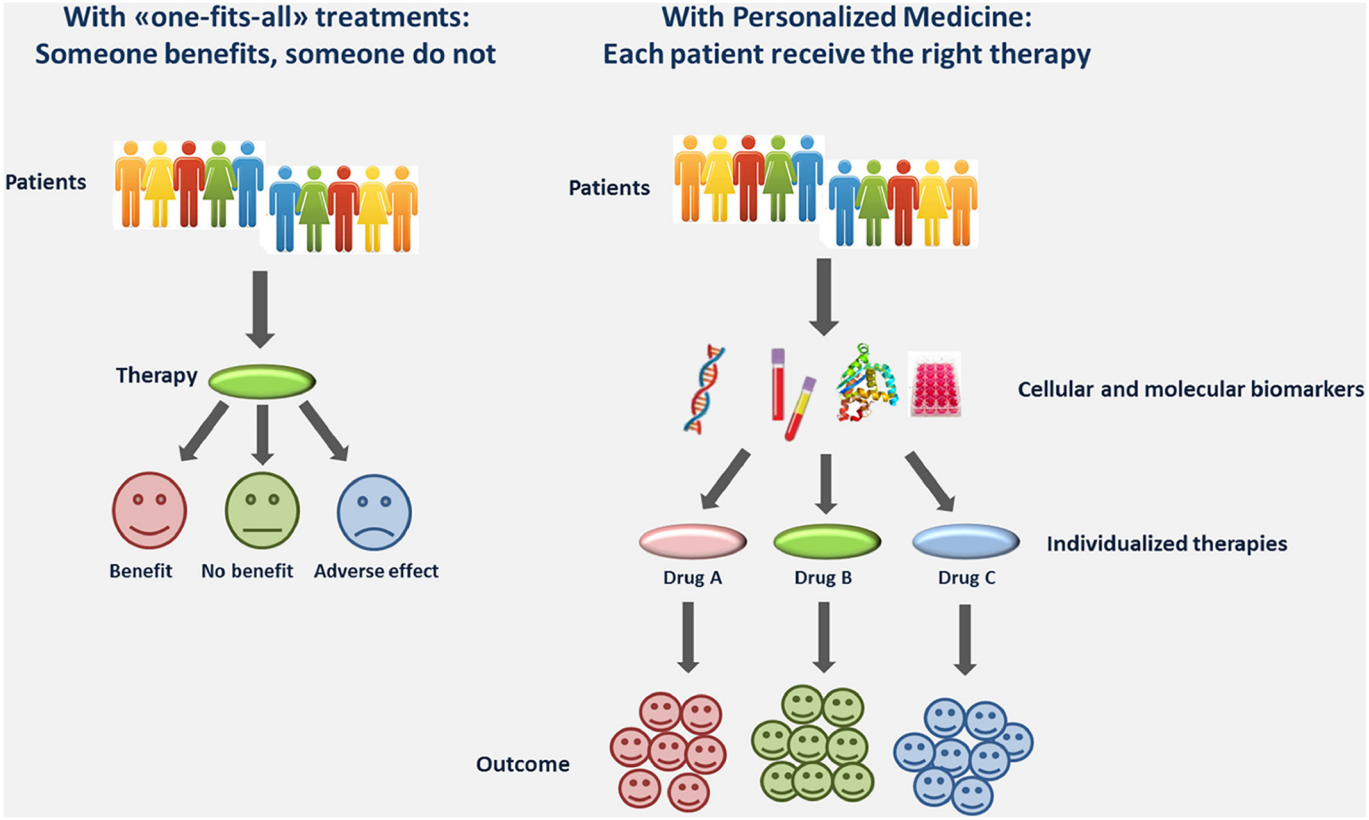


Fig. 1. Advantages of PM in cancer treatment. The "one-fits-all" treatment approach do not consider individual's susceptibility, eliciting different therapy response, from benefit to adverse effects. On the contrary, PM allows to examine patient's specific disease-related features, leading to favorable outcome.

1. Introduction

1.1. Personalized medicine in neuro-oncology

Personalized medicine (PM) is based on information about the tumor molecular profiling, based on gene and protein expression and environment, in order to prevent, diagnose and treat cancers. In oncology, PM has become a reality, through the definition of genetic alterations and the development of precise molecular diagnosis, which allow individual patient-specific tailored treatments. In neuro-oncology, PM can be of great importance to maximize the therapeutic efficacy, minimize unwanted adverse effects, and improve the clinical outcome in patients affected by brain tumors (Fig. 1). Indeed, the new advantages of data obtained by genomic, transcriptomic, epigenetic, proteomic, and imaging techniques, in parallel with clinical information, guarantee the possibility to identify patients' individual traits and to design a patient-specific map. Brain tumors harbor a portfolio of molecular and genetic alterations, as well as specific microenvironmental and plasmatic markers, which constitute a "signature". Thus, the application of PM from diagnosis to patient management has progressively attracted increased attention [1]. PM relies on a multidisciplinary and multimodal approach, through the coordination of surgeons, oncologists, radiation oncologists, geneticists and biologists, to optimize cancer outcomes by the combination of tailored therapeutic strategies as surgery, radiotherapy, chemotherapy, growth factor inhibitors, immunotherapy, and cell/gene therapies [2]. In this context, the results of tumor molecular profiling by high throughput screening assays together with circulating alterations may provide an instant snapshot of the patient, driving out novel biomarkers with prognostic and/or a predictive value and improving brain tumor taxonomy.

Indeed, unfortunately, despite the histopathologic classification and genetic analysis being accurate, tumor growth does not always reflect tumor grade. Indeed, brain tumors are characterized by a significant intra- and inter-tumor genetic and phenotypic heterogeneity, which results in a high variability in drug sensitivity/resistance from patient to patient. Thus, the main pillar of PM is tumor classification, stratification and patient clustering, in parallel with the identification of additional indicators, for strict prediction of the brain tumor status and prescription of the most effective therapeutic options.

2. Brain tumor overview

Brain tumors affect about 200,000 people worldwide every year [3], representing approximately 2% of cancer deaths [4]. The pie chart in Fig. 2 represents a schematic description of the distribution of brain tumors. The World Health Organization (WHO) specifies a grading system for brain tumors ranging from grade I, the least aggressive with best prognosis, to grade IV, the most malignant with worst prognosis. Grade I tumors are biologically benign and can be cured with surgical resection. Grade II, as diffuse oligodendrogliomas and astrocytomas, present slow growing rate, and they may follow long clinical courses, unlike infiltrating tumors. Grade III, as anaplastic oligodendrogliomas or astrocytomas, grow faster and more aggressively than grade II, exhibiting increased anaplasia and infiltration in neighboring tissues. Grade IV, glioblastomas (GBM), can develop directly, as primary tumor, or evolve from lower grade tumors, as secondary GBM. GBM exhibit more advanced features of malignancy, including vascular proliferation and necrosis, and they are recalcitrant to radio- and chemotherapy. Most malignant primary tumors are classified as gliomas, for their origin from glial cells or glial cell precursors. Notably, gliomas are also categorized as: low-grade gliomas (LGGs, WHO grade I, II) and high-grade gliomas (HGGs, WHO grade III, IV). In 2016, WHO updated the guidelines for cerebral neoplasms, and, for the first time, molecular parameters have been integrated into diagnostic procedure previously

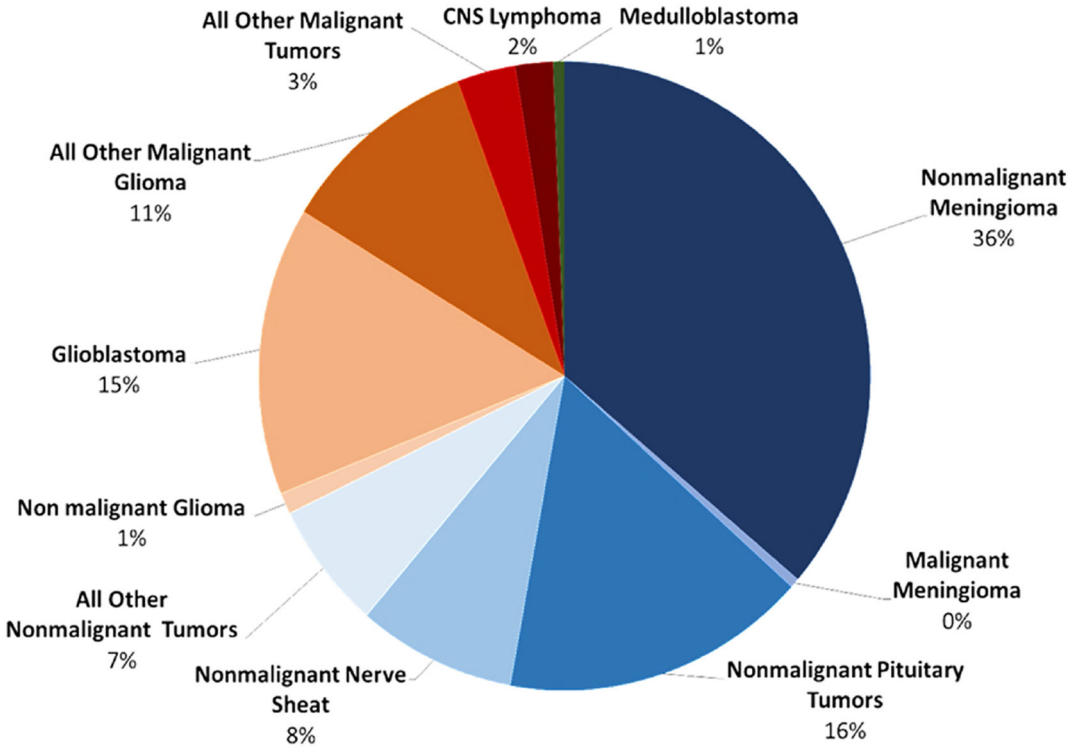


Fig. 2. Epidemiology of brain tumors. Every slice of the pie chart represents the frequency of a type of tumor.

based only on histopathological features [5]. This, in turn, facilitates a more accurate determination of prognosis and the development of targeted treatments, shifting the traditional diagnostic approach, primarily based on the microscopic features, to a molecularly oriented approach.

3. Glioblastoma: the biggest challenge

Among all primary human brain tumors, GBM is the most malignant and frequent (~70%), with a poor prognosis of about 14 months and a 5-year survival rate at 5% [6], representing an extreme therapeutic challenge. GBM is characterized by intense angiogenesis, invasion, cell infiltration, rapid progression, resistance to radio- and chemotherapies, with high frequency of relapse. Karnofsky Performance Status (KPS) > 70, an age at diagnosis < 50 years, O6-methylguanine DNA methyltransferase (MGMT) methylation (> 9%), gross/subtotal resection (> 90%), and the tumor being located in a non-eloquent area of the brain, represent the most favorable prognostic indicators [7]. The current standard protocol for the treatment of GBM consists in gross total resection, followed by radio- and chemotherapy with concomitant and adjuvant temozolomide (TMZ). GBM diagnosis is performed by computed tomography (CT or CAT scan) and magnetic resonance imaging (MRI), in parallel with histopathological evaluation. During the clinical routine, several molecular markers, including MGMT promoter methylation, isocitrate dehydrogenase (IDH) mutation, loss of heterozygosity (LOH) of chromosomes 1p and 19q, loss of heterozygosity 10q, amplification of epidermal growth factor receptor (EGFR) and its active mutant EGFR variant III (EGFRvIII), vascular endothelial growth factor (VEGF) overexpression, tumor suppressor protein (TP53) and phosphatase and tensin homolog (PTEN) mutation, are commonly tested [8]. Despite aggressive therapeutic approaches, most patients experience tumor recurrence in less than one year. This event may be caused by the extravasation of therapeutic agents through the weak blood-brain barrier (BBB), the reactivation of pro-survival pathways after therapies, and above all, the tumor biology. Indeed, it is widely recognized that the cellular components of GBM are highly heterogeneous with the presence of GBM stem cells (GSCs), which hierarchically drive tumor onset and progression. GSCs are considered tumor-initiating and propagating cells. GSCs are highly proliferative, pluripotent, genetically unstable with self-renewing ability. They are responsible for resistance to radio- and chemotherapies and for the high frequency of GBM recurrences, thanks to the production of a plethora of pro-survival molecules, which sustains resistance after treatments [9,10]. Moreover, intense neovascularization is a key malignant feature of GBM, with the formation of abnormal new blood vessels, presenting loss of vascular integrity and increased permeability. These abnormal blood vessels provide oxygen and nutrient to tumor mass, promoting its growth, progression and infiltration in the surrounding tissues. The in-depth characterization of the molecular and cellular components of GBM, as well as the identification of different tumor subtypes, has prompted the development of novel anti-GBM therapies targeting specific alterations as small molecular inhibitors of growth factors and their receptors, antibody-based drug conjugates, and, more recently, inhibitors blocking the immune checkpoints [11], as discussed below.

4. Advances in GBM treatment

The wide heterogeneity of GBMs and, consequently, the large differential patient response to treatments, highlight the great importance of PM in neuro-oncology. Indeed, it is widely recognized that GBM resistance to current therapies is particularly due to a subpopulation of tumorigenic stem-like cells, known as GBM stem cells (GSCs), which hierarchically drive the tumor onset and progression. GSCs have self-renewing properties, are pluripotent, highly proliferative, and genetically unstable. Because of their critical heterogeneity, studying them at cellular and molecular levels represent an important challenge to identify specific targets for individual patients. Indeed, the discovery of the key mechanisms driving cancer progression and resistance, allow the identification of novel suitable targets and the implementation of countless anti-cancer compounds. Molecular target therapies, immunotherapies, CAR-T cell therapy represent a valid example of translation of basic research into clinical practice. Importantly, the

synergy between researchers and clinicians allows conducting a more rational drug design, thus optimizing patient management. Indeed, the multidisciplinary approach succeeded in introducing in clinic, innovative-targeted anti-cancer strategies, such as molecular target therapies, blocking pro-survival and pro-angiogenic factors, immunotherapy, CAR-T cell therapy, and lastly, electric fields.

4.1. Molecular target therapy

Gliomas included in WHO grade IV classification are histologically characterized by necrosis and microvascular proliferation as distinctive features. According to cIMPACT (the Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy), peculiar genetic alterations as epidermal growth factor receptor (EGFR) gene amplification, telomerase reverse transcriptase (TERT) promoter mutation, together with whole chromosome 10 loss and whole chromosome 7 gain (−10/+7) can be considered as molecular markers for the diagnosis of IDH-wildtype GBM, even in absence of histological features. Many high throughput genomic, transcriptomic and epigenomic studies reported the existence of up to seven GBM molecular subtypes characterized by mutational and expression profiles, as well as different DNA methylation patterns [12–14]. Among them, the receptor tyrosine kinase (RTK) 1 and 2 subgroups and the mesenchymal subgroup are the most common [14]. The distinction of molecular subtypes may help to evaluate the specific therapeutic vulnerabilities, being relevant in terms of treatment strategy. The benefit arising from current standard of care, including radiotherapy with concomitant and adjuvant TMZ chemotherapy, is limited to patients whose tumors present aberrant CpG methylation of the promoter region of the O6-methylguanine DNA methyltransferase (MGMT) gene. For this reason, MGMT promoter methylation is actually considered as a predictive biomarker for TMZ efficacy, for newly diagnosed GBM [15]. A recent study established a novel assay to profile GBM cancer models, as microtumors and spheroids, compared to patient-derived orthotopic xenografts (PDX), considered as the best preclinical model. Using Nanostring technologies, it was to possible to perform targeted gene expression to characterize several GBM phenotypes and to correlate molecular signature with therapeutic response to various agents [16]. The results of this investigation reported genes differentially expressed between drug responders and non-responders advancing the concept of PM potential efficacy. Most of clinical trials targeting GBM focus on oncopromoter signaling, susceptibility to apoptosis and cell cycle control.

4.1.1. Epidermal growth factor receptor (EGFR)

EGFR is certainly one of the most prominent oncogenes in IDH-wildtype GBM. Amplification events involving chromosomes 7 (EGFR/MET/CDK6) occurs in 57.4% of primary GBM patients compared to 8% of secondary GBM patients and are associated with high levels of EGFR protein [17]. A peculiar deletion mutation, known as EGFRvIII, is present in 25% of tumors and it is considered a promising neoantigen [18]. Approaches targeting EGFR or EGFRvIII, as vaccine and rindopepimut, determined a survival increase when combined with bevacizumab in recurrent GBM (NCT01498328), but no effect were reported in newly diagnosed GBM (ACT IV) [19]. The ACT IV trial also demonstrated that the EGFRvIII expression is unstable, since a marked loss of expression was recorded in both trial groups. Erlotinib (Tarceva®, Genentech Inc), an EGFR inhibitor, is able to compete with ATP and reversibly binds the intracellular tyrosine kinase domain of both EGFR or EGFRvIII, thus inhibiting receptor phosphorylation and downstream signaling [20]. In preclinical studies, erlotinib showed to exert promising antitumoral effects against GBM, reducing cell viability of GBM stem cells through the inhibition of the MAPK signaling pathway. The clinical efficacy of erlotinib was evaluated by Raizer et al. in patients with recurrent GBM and non-progressive GBM in a phase II trial [21]. Unfortunately, the study did not report any significant improvement of clinical outcome in both experimental groups. In the same year, Yung et al. reported a significant increase of medial OS and 6-months PFS in recurrent GBM patients, but the study was discontinued for insufficient number of responses [22].

In addition, also Cetuximab (CTX, Erbitux, Bristol-Myers Squibb), which targets EGFR-expressing tumors, was considered as a promising drug. It was reported that CTX is able to induce apoptosis, cell cycle arrest, and inhibition of invasion, metastasis dissemination, and angiogenesis [23]. A phase I trial of super-selective intraarterial cerebral infusion of CTX for treatment of relapsed/refractory GBM and anaplastic astrocytoma was performed in 2010 but, unfortunately, no result has been posted yet [NCT01238237]. Afterwards, Blesa reported the efficacy of CTX in co-administration with BEV. The case report showed that a patient with relapsed GBM presented a durable complete remission with a complete radiologic response after 20 months, when combination of CTX and BEV, in a third-line setting, was administered. The authors suggested that the co-treatment showed a dual mechanism of action: i) the targeting of GSCs by the two antibodies and ii) the potential recruitment of the immune system to directly trigger a response against the GSCs [24].

Very recently, the efficacy of CTX in combination with alpha-cyano-4-hydroxycinnamic acid (CHC) drug encapsulated into polymeric nanoparticles based on poly(lactic-*co*-glycolic acid) and chitosan was reported. Ferreira and colleagues demonstrated that, when administered to GBM cellular models, CTX seemed to enhance therapeutic efficacy. Although the work was conducted in *in vitro*, the results seem to be promising in targeting EGFR positive GBM by CTX and nanoformulations [25].

4.1.2. Vascular endothelial growth factor (VEGF)

VEGF is the best characterized pro-angiogenic factor in cancer, of which it promotes angiogenesis, endothelial cell proliferation and survival. Further, VEGF is known to increase blood vessel permeability and loss of integrity, also recruiting precursor cells from bone marrow [26]. Bevacizumab, Avastin®, a monoclonal antibody against human VEGF, was quickly approved by the US Food and Drug Administration (FDA) in 2009 as a single agent for patients with GBM with progressive disease following prior therapy, after the promising results reporting increased response rates and 6-month more progression-free survival (PFS), compared to controls [27]. Despite the high initial radiographic response, bevacizumab effects proved to be transitory and most GBM patients recurred after a median of 3–5 months [28]. More recently, bevacizumab was administered in co-administration with Lomustine (CCNU) but results, in terms of overall survival (OS), were not very encouraging [29]. On the other hand, Sorafenib and Sunitinib, multi-tyrosine kinase inhibitors targeting VEGF receptors (VEGFRs) and other non-endothelial receptors, such as Platelet Derived Growth Factor (CD140) receptor and CD117, have been tested, without showing any effect in terms of OS increase [30,31]. This failure may be explained by the fact that GBM is able to activate collateral pathways, bypassing the effect of administered drugs by endothelial cell proliferation, survival and neoangiogenesis. Unfortunately, the delicate mechanisms enhancing these alternative strategies of tumor vascularization and GSC differentiation are still unclear [32].

4.1.3. Mesenchymal epithelial transition/hepatocyte growth factor receptor (MET/HGF)

MET/HGF is a proto-oncogene, coding for the hepatocyte growth factor receptor, which has been reported to have a key role in the proliferation, growth, angiogenesis, invasiveness, and migration of glioma cells, also in response to hypoxia, angiogenesis inhibition and irradiation. Immunohistochemical staining revealed high expression of MET in tumor cells, peri-necrotic areas and blood vessels of glioma tissues, with significant correlation with OS and PFS in GBM patients [33,34]. Genetic analysis reported also a gain in MET gene in 47% of primary GBM and 44% of secondary GBM [35], indicating that this alteration may be important in the pathogenesis of both GBM subtypes.

Moreover, MET alteration have been reported during the progression of low-grade-gliomas to secondary GBM [36]. A humanized monoclonal antibody anti-HGF, YYB-101, have been reported to inhibit tumor growth both *in vitro* and in orthotopic mouse models of GBM, through the down-regulation of cellular signaling effectors as p-MET, p-FAK, MMP2 and Ki-67 [37,38]. In another recent study the combination of YYB-101 and TMZ in GBM xenografts was reported to suppress tumor growth and increase OS, compared to single treatment. A Phase I study started in 2015 (NCT02499224) to assess the safety, tolerability, and pharmacokinetics of YYB101 in advanced solid tumor patients, refractory to standard therapy, but no results have been actually posted. Onartuzumab, a single arm monoclonal anti-MET antibody, showed to inhibit GBM growth in preclinical tests [39], but in a phase II clinical trial for recurrent GBM, ornartuzumab administered in combination with bevacizumab did not show clinical benefits [40]. Cabozantinib (XL184), a tyrosine kinase inhibitor of MET, VEGFR2 and AXL, proved to have efficacy in xenograft models, exerting anti-proliferative, anti-invasive and anti-angiogenic activity [41]. Furthermore, in a preclinical study in mice orthotopic xenograft, cabozantinib showed to inhibit tumor growth and invasion, thus prolonging mice survival [42]. In a Phase II clinical trial evaluating the response rate and the 6-months PFS of patients affected by recurrent and progressive GBM and treated with XL184, only modest clinical efficacy has been reported (NCT00704288) [43]. No phase III clinical trials have been conducted on this agent for GBM in the last 5 years.

4.1.4. TERT promoter mutation

Telomerase reverse transcriptase (TERT) gene is located on chromosome 5p15.33 and it encodes for the catalytic subunit of telomerase, an enzyme which adds nucleotides to telomeres [44]. The activity of telomerase is relatively low in differentiated cells, allowing cell apoptosis and senescence. TERT expression increase is due to twopoint mutations substitute a cytosine for a thymidine at position 228 (C228T) and 250 (C250T) of the TERT gene promoter (pTERT). pTERT mutation are reported in about 90% of human tumors, being considered a key element for cancer onset and progression [45]. In IDH-wildtype GBM, pTERT mutations are the most common molecular alterations and the increase of TERT expression supports the immortalization of tumor cells [46]. Notwithstanding, pTERT mutations have not yet been targeted with pharmacological treatments, but eribulin, an inhibitor of tubulin polymerization, has been proved to inhibit also TERT activity in GBM models, prompting its clinical testing [47]. A very recent phase I/II clinical trial, actually recruiting, aims to evaluate UCPVax treatment in GBM. UCPVax is a therapeutic anti-cancer vaccine based on the telomerase-derived helper peptides designed to induce strong TH1 CD4 T cell responses in cancer patients (NCT02818426).

4.1.5. p53 pathway

The tumor-suppressor gene p53 (TP53) is one of the most studied gene in GBM pathogenesis and progression. TP53 is mutated in 30–50% of GBMs and this mutation is responsible for malignancy and tumorigenicity, due to the loss of the transcription function of wild-type TP53. The key function of TP53 is to arrest cell cycle in G0/G1 phase and to initiate apoptosis in response to genotoxic stimuli. Therefore, mutant TP53 may decrease apoptosis, increase cell growth and survival and resistance to chemotherapy [48]. Because of these reasons, drugs restoring p53 functions by conformation refolding have been largely studied, but unsuccessful results have been reported. In this context, a recruiting phase I clinical trial is actually testing the side effects and the best dose of MDM2 inhibitor KRT-232 for patients with newly diagnosed or recurrent GBM harboring unmethylated MGMT promoters and wild-type TP53, given in combination with standard radiation following surgery (NCT03107780) [49]. Furthermore, the efficiency of tumor cells transduction with adenovirus p53 delivered stereotactically to patients with recurrent GBM, is under investigation in a Phase I clinical trial (Gene Therapy in Treating Patients with Recurrent or Progressive Brain Tumors NCT00004080). Furthermore, another Phase II trial was developed with the potential to restore wild type function of p53. The experimental protocol consisted in intravenously administration of SGT-53, a cationic liposome encapsulating a normal human wild type p53 DNA sequence in a plasmid backbone. SGT-53 was administered in combination with oral temozolomide to determine efficacy and safety in patients with confirmed GBM who have proven tumor recurrence or progression (NCT02340156, Phase II Study of Combined Temozolomide and SGT-53 for Treatment of Recurrent Glioblastoma). The study is currently under investigation and no results have been posted.

4.2. Immunotherapy

The CNS is traditionally considered an immune-privileged organ, due to the absence of a lymphatic drainage system and the presence of the BBB, which guides the diffusion of molecules and cells [50]. However, paradoxically, brain tumors have the capacity to elicit potent antitumor immune responses and, recently, this phenomenon has been explained by the discovery of a CNS lymphatic system. Indeed, in animal models bearing intracranial tumors, it has been reported that tumor-antigens can be drained from the cerebrospinal fluid into the cervical lymph nodes to stimulate specific T-cells [51]. As a consequence, in the last few years, immunotherapy is quickly becoming a column in GBM therapy. Indeed, immunotherapy has the power to induce, enhance or suppress immune responses to destroy cancer cells. Immunotherapeutic strategies may consist in active immunotherapy, using immune stimulants, cellular vaccines or tumor vaccines, and passive immunotherapy, which consists in transfering effector immune cells into patients, thus inducing anti-cancer effects [52].

4.2.1. Cancer vaccines

Cancer vaccines are designed to induce an immune response against the tumor. Vaccine therapies for GBM treatment include the direct exposure to tumor antigens, as glioma-associated peptides or DNA in combination with immune-stimulating molecules and patient-derived antigen presenting cells (APC), such as dendritic cells (DC). Among anti-tumor immunotherapies, tumor vaccines and T-cell therapies rely on the enhancement of tumor-specific T-cells to seek and destroy cancer cells. To be safe and effective, a tumor vaccine must target an antigen specifically expressed in tumor cells and not in normal cells, thus called tumor-specific antigen (TSA). A good example of this primary requirement is the epidermal growth factor receptor variant III (EGFRvIII). The EGFRvIII mutation has been reported in about 25–30% of GBM and it has been considered an independent negative prognostic factor [53]. Promising results arose from a phase II clinical trial of a 13-amino acid EGFRvIII peptide vaccine, *Rindopepimut*™, a conjugated EGFRvIII-specific peptide (also known as CDX-110 and PEPvIII), by Celldex therapeutics. Conjugated to adjuvant, the administration of rindopepimut resulted in an increased OS, correlated with the extent of induced tumor immunity [54,55]. These results led to an international phase III, ACT IV, clinical trial in newly diagnosed GBM patients with EGFRvIII mutation. Unfortunately, this trial did not achieve the expected results and no significant difference was observed in OS for patients with GBM in the rindopepimut group plus TMZ when compared to the control group (TMZ alone) [56].

Another promising single antigen vaccine is SurVaxM, a mimic peptide of survivin conjugated to Keyhole limpet hemocyanin (KLH), used as vaccination adjuvant.

Survivin is a member of the inhibitor of apoptosis proteins (IAP) family. Survivin is strongly expressed in a majority of tumors [57], whereas it is absent in normal differentiated tissues [58]. Survivin inhibits apoptosis, regulates cell-cycle progression and induces chromosomal instability [59]. Expression of survivin has also been associated with poor prognosis and chemotherapy resistance [60,61] and a recent study reported that the expression of survivin is associated with tumor grade, suggesting that it could be used as a novel prognostic factor in

gliomas [62]. Thanks to whole-genome RNA sequencing, it was observed that patients with high expression of survivin had shorter OS times than those with low expression [63].

A multi-center phase II trial have been conducted for patients with newly diagnosed GBM treated with adjuvant TMZ and survivin-targeted immunization. Study results reported that SurVaxM is safe and that, compared to historical matched controls, the addition of SurVaxM improved 6-months PFS and 12-months OS; moreover, patients with poor prognostic factors (unmethylated MGMT, higher survivin levels) treated with SurVaxM achieved better survival than expected [64].

On the other hand, customized vaccines represent a promising area of clinical research. Producing customized vaccines requires a small volume of tumor tissue to be used as a constitutive part; as a consequence, only patients with resectable tumors are eligible. A great example is DCVax®-L, developed by Northwest Biotherapeutics, which uses the whole lysate tumor to pulse patient-derived DCs. A phase III trial has been designed to determine the efficacy and the impact on disease progression and survival time, as well as safety, on patient with GBM, treated with surgical resection, radiation and TMZ (NCT00045968). Over 10 years after the diagnosis, some of patients recruited for the phase I trial of the vaccine were still alive, but no result of phase III study has been posted yet; however, recent reports described a median OS of 23.1 months for all partecipants (90% of whom received the DC-Vax-L treatment due to crossover design) [65].

4.2.2. Checkpoint inhibitors

Another promising immunotherapeutic strategy consists in immune checkpoint molecules responsible for maintaining self-tolerance and preventing autoimmune reactions. Checkpoint inhibition has revolutionized treatment of several advanced malignancies providing hope for cancer treatment. In this context, the most highly investigated molecules are Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4), involved in early T-cell activation, and programmed cell death protein 1 (PD-1), which inhibits T-cells at later stage [66]. Recent studies reported that inhibition of CTLA-4 and PD-1 induce tumor regression, promoting long-term survival in glioma mouse models [67,68]. The first FDA-approved immune checkpoint inhibitor has been *Ipilimumab*, a humanized CTLA-4 antibody, which improved OS in a phase III clinical trial for metastatic melanoma patients, of which only 2% showed a complete response [69]. On the other hand, the inhibition of the signaling pathway PD-1/programmed cell death protein ligand 1 (PDL1), has shown promising results. In a completed phase II clinical trial the researchers have demonstrated the effectiveness of pembrolizumab (MK-3475), an anti-PD-1 antibody, administered alone or in combination with bevacizumab for the treatment of recurrent GBM. The results reported that pembrolizumab is well tolerated +/− bevacizumab, but has limited monotherapy activity for recurrent GBM. Furthermore, the antitumor activity of pembrolizumab plus bevacizumab was comparable to historical bevacizumab monotherapy data (NCT02337491). Another PDL1 inhibitor is durvalumab (MEDI4736), a human high-affinity monoclonal antibody that blocks PD-L1 by binding to PD-1 and CD-80, already used in the treatment of non-small cell lung cancer [70]. An ongoing phase II multicenter, non-randomized study of durvalumab for GBM patients aims to assess the clinical efficacy measured by the OS rate at 12 months and PFS at 6 months. The study concluded that durvalumab was well tolerated when combined with RT and seemed to have efficacy among patients with new unmethylated GBM (NCT02336165). Finally, an active randomized phase III clinical trial is comparing the efficacy and safety of nivolumab, a monoclonal antibody anti-PD1, administered alone *versus* bevacizumab in patients diagnosed with recurrent GBM, and to evaluate the safety and tolerability of Nivolumab administered alone or in combination with Ipilimumab in patients with different lines of GBM therapy (NCT02017717). to these days, no result has been posted.

4.3. CAR-T cell therapy

The genetic engineering of T-cells to express chimeric antigen receptors (CARs) directed against tumor specific antigens has opened the door to a new era of personalized cancer therapy. As the achievements of CAR-T cell therapy in hematological cancers confirmed their potential to elicit a durable remission, this technology has been recently introduced also in patients with solid cancers, including GBM [71]. Typically, the CAR-T therapy is based on the collection of patient-T-cells or immune cells, which are genetically engineered to recognize specifical tumor antigens. Targeting with CAR-T cells has the advantages to allow the active passage of T-cells toward tumor sites in which immune cells can kill cancer cells, sparing normal cells and preventing side effects [71]. Because of the limited availability of targetable TSAs in GBM, which guarantee the foster of healthy tissues, researchers are actually focused on the EGFRvIII. Treating 10 recurrent GBM patients with autologous EGFRvIII-CAR T-cells, in a single intravenous infusion, O'Rourke and colleagues reported that no patient manifested toxicities or cytokine release syndrome, demonstrating that systemic infusion of EGFRvIII-CAR T-cells is safe and feasible. Unfortunately, except for one patient with stable residual disease for over 18 months, no objective radiographic response has been observed. Interestingly, the scientists described a transient but significant expansion of the CAR T-cells, as well as a promising infiltration in tumor location. They ultimately observed a decrease of EGFRvIII-expressing tumor cells and an overexpression of immune inhibitory molecules, such as PD-L1 and indoleamine-2,3-deoxygenase 1 (IDO1) [72]. Other trials with CAR-T cells targeting IL13Ra2, Her2/CMV have been recently conducted and results reported objective radiologic responses, however addressing essential question as tumor microenvironment remodeling and T-cell trafficking in CNS. Each study provided evidence that the selection of multiple target antigens in each patient is an important step in creating combinatorial therapy to approach address tumor heterogeneity and achieve a greater curative potential [73].

Despite innumerable steps forward, PM in immunotherapy for neuro-oncology patients is presently in early stages, and further effort has yet to be done in order to determine its therapeutic value.

4.4. NOVO TTF

A promising, relatively new, non-invasive technique for GBM patients is the tumor-treating field technology (TTF). The OPTUNE™ system, or NOVOTTF-100A, is an FDA-approved novel anti-mitotic device that delivers continuous alternating electric fields to disrupt tumor cell division, causing cancer cell death in the treatment of primary and recurrent GBM. Optune is indicated for patients aged > 22 years, with histologically confirmed supratentorial GBM. The Optune system is composed of four transducer arrays, a field-generator that delivers pre-set electric fields (200 kHz) and with a minimum field intensity of 1.0 V/cm [74]. The field generator delivers fields through the insulated transducer arrays, which are applied to the shaved scalp of the patient. A randomized trial of TTF administered from the initiation of maintenance TMZ reported superior PFS and OS compared with TMZ alone [75]. Despite this treatment being safe, usually well tolerated (except for local skin reactions) and despite its acceptance by patients, relatives and healthcare professionals being good, in most parts of Europe finding funds for its cost remains a critically debated issue.

Therefore, despite the promising studies and clinical trials in progress for GBM patients, the successes are still limited and the remaining work is hard. Indeed, up to now, no monoclonal antibodies, targeted drugs, immunotherapeutic strategies or combinations of chemotherapeutic agents have been proved more effective than the current standard therapy based on surgery, TMZ, and radiotherapy. In an effort to define novel therapeutic drugs against new molecular targets, basic, translational, and clinical neuro-oncological research should be

implemented to deepen knowledge regarding all aspects related to the tumor, with the aim to define a new direction for GBM therapy.

5. The strategic contribution of an experienced neurosurgery laboratory in neuro-oncology

As advanced by Harvey Cushing at the beginning of '900, "laboratory experiments and clinical experience must go hand in hand to develop innovative approaches to therapy". An experimental laboratory can be considered an innovative tool to connect advanced researches with clinical practice, staying at the forefront of brain tumor battle. Laboratory mission relies on the in-depth investigation of the cellular and molecular mechanisms involved in brain tumor onset and progression, with the main aim to identify innovative and individualized treatments. Faithful to the creed "care is better where advanced research is done", research should be conducted on basic, clinical and translational levels, from bench to bedside, filling the translational gap in brain cancer research.

5.1. Patient-centered screening for personalized disease management: the presence of Neurosurgery Unit at Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico

The need to improve patient's OS, PFS and quality of life prompts both clinicians and researchers to design patient-specific personalized therapies, as well as to identify novel and helpful early and easy-accessible diagnostic and prognostic biomarkers. In order to achieve this aim, the synergy between clinic and research is mandatory.

At Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico in Milan (Italy), the Center of Reference for Oncological Neurosurgery closely collaborates with an experienced Laboratory of Experimental Neurosurgery and Cell Therapy, to set-up diagnostic, therapeutic and care paths starting from patient's entrance to the hospital (Fig. 3).

The Center of Reference for Oncological Neurosurgery of Fondazione Policlinico hosts the "Associazione Amici della Clinica Neurochirurgica", a no-profit association in which volunteer staff made of doctors, researchers, neurosurgeons, neuropsychologists, nurses and palliative care professionals work closely together, also with patients and their family to improve patient's quality of life. Since 2005, the Neurosurgery Unit of Ospedale Maggiore Policlinico has been enrolled for neurosurgical procedure overall $n = 628$ grade IV patients, $n = 85$ grade III glioma patients and $n = 71$ grade II glioma patients. The demographic and genetic characteristics of patients tested by the laboratory are resumed in Table 1. Since 2011, a dedicated neuropsychologist before and after surgery, and every three months during neuro-oncological and neuro-radiological follow-up, has cognitively evaluated patients in our department. Patients with tumors in eloquent areas (for example involving the language networks) were cognitively studied to consider the possibility of awake-surgery procedure. Patients eligible for the awake craniotomy were tested also during surgery with neurophysiological monitoring. Preservation of higher cognitive functions after surgery is one of the goals to achieve; as a matter of fact, permanent deficits in cognitive functions impact quality of life [76–78]. In addition, the neuropsychological evaluation allows us to monitor changes in cognitive status and, when a decline is observed, closer clinical, radiological, and neuro-oncological follow-up are administered [79]. Furthermore, also in neuropsychological field, the synergy between clinic and research is one of our objectives. We implemented and published several researches regarding neuropsychologic tests and standardizations of cognitive tools, or about neurocognitive functions in patients affected by brain tumors (LGG, HGG, and meningiomas) [77–84], and/or hydrocephalus. The Laboratory of Experimental Neurosurgery and Cell Therapy is a great example of the benefits of having a research laboratory closely linked physically and operationally to the Department of Neurosurgery and Neuro-oncology.

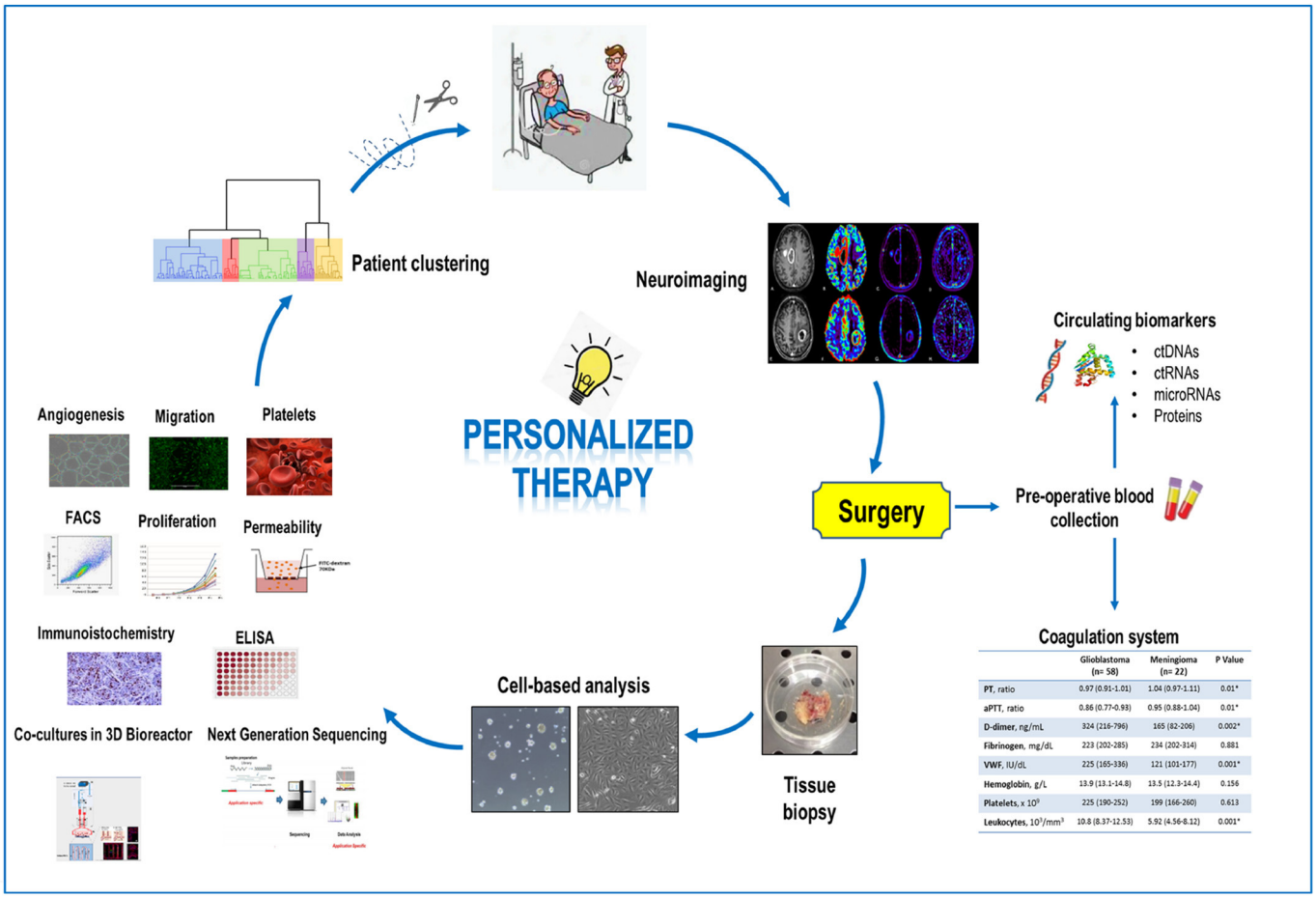
5.2. The experience of the laboratory of experimental neurosurgery and cell therapy laboratory

The Laboratory fosters emphasis on translational research in neuro-oncology and, specifically, on detecting novel tissue and circulating biomarkers as well as testing chemotherapeutic agents targeting brain tumor pathologic mechanisms. The translational approach aims to promote "patient-centered therapies" by offering the possibility of: i) performing sensitivity/resistance screening for different drugs; ii) investigating tissue and blood prognostic and disease-related markers; iii) analyzing the metabolism of onco-modulating mediators; iv) combining different parameters to design a "patient-specific map" through the integration of "omic" data, such as genome, transcriptome, epigenome, proteome and metabolome, with cellular and clinical parameters. Examples of the research studies carried out by the Laboratory will be discussed in the following paragraphs.

5.2.1. Specific molecular signature of GBM and GSCs to define new potential targetable alterations

Starting from the evidence that the resistance of GBM to current therapies is particularly due to a subpopulation of tumorigenic stem-like cells, known as GSCs, we focused our research activity in isolating, characterizing and investigating molecular, cellular, and metabolomic features of GSCs.

GSCs, also known as tumor-initiating cells, are multipotent cells, with self-renewing ability and the capacity to establish and maintain GBM tumors [85]. GSCs, in culture conditions, form free-floating neurospheres, preserving many of the fundamental characteristics of the parent tumor, including cell proliferation, heterogeneity, and the ability of forming heterogeneous tumors when transplanted in animal models, thus being able to recreate the parent tumor cell's composition and invasiveness [86]. GSC's identification is based on the expression of specific antigens on cell membrane; the largely studied cell surface markers are: CD133, CD15/SSEA, CD44, or A2B5 for GSC isolation [87–89]. Not only a single marker, but a panel of cell membrane antigens, as shown above, is able to define a universal GSC population. The identity of GSCs is still unresolved and which markers better define a stem cell phenotype is controversy [85]. Moreover, GSCs express a plethora of molecules that promote cell survival [90,91]. In this context, a deeper understanding of the phenotype and molecular characterization of GSCs represents a critical step to investigate GSC stemness, plasticity, resistance to therapy and maybe advantageous in developing effective therapeutic strategies. Thus, gaining a deeper understanding of the underlying molecular processes that drive cancer stem cell maintenance, plasticity, and resiliency will enhance our ability to selectively target and ablate these tumor-initiating and -propagating populations. To this purpose, we analyzed the genetic signature of primary GSCs and their matched GBM tumor masses [92], using a comparative genomic hybridization array (aCGH). Interestingly, in both GSCs and matched GBM, we detected copy number alterations (CNAs), such as the chromosome 7 polysomy, encompassing both EGFR and MET genes, a fundamental event driving GBM tumourigenesis, chromosome 10 monosomy, encompassing the PTEN locus and the focal deletion of chromosome 9p21 surrounding CDKN2A [93]. From a genomic perspective, the typical genetic features of primary GBMs were markedly concordant between GBM/GSC couples, confirming the high similarity between the tumor mass and its respective GSC population. Regarding TERT mutations, the 80% of GBMs and matched GSCs were mutated in the promoter region. Of note, TERT mutations have a prognostic role in gliomas, being a negative prognostic factor in primary GBM without MGMT methylation [94]. Furthermore, aCGH revealed a strong genomic instability both in GBM and matched GSCs, even showing a high intra-patient similarity and a marked inter-patient variability. The elevated concordance between GSCs and the primary tumor masses corroborates the use of the aforementioned as reliable *in vitro* and *in vivo* models of GBM; on the other hand, the dissimilarities



Coagulation system	Glioblastoma (n= 58)	Meningioma (n= 22)	P Value
PT, ratio	0.97 (0.91-1.01)	1.04 (0.97-1.11)	0.01*
aPTT, ratio	0.86 (0.77-0.93)	0.95 (0.88-1.04)	0.01*
D-dimer, ng/mL	324 (216-796)	165 (82-206)	0.002*
Fibrinogen, mg/dL	223 (202-285)	234 (202-314)	0.881
VWF, IU/dL	225 (165-336)	121 (101-177)	0.001*
Hemoglobin, g/L	13.9 (13.1-14.8)	13.5 (12.3-14.4)	0.156
Platelets, x 10^3	225 (190-252)	199 (166-260)	0.613
Leukocytes, $10^3/mm^3$	10.8 (8.37-12.53)	5.92 (4.56-8.12)	0.001*

Fig. 3. Workflow of patient management in the Neurosurgery Unit of Fondazione Policlinico in Milan. With the approach "from bench to bedside", and *vice versa*, clinicians and researchers adopt a multidisciplinary strategy, examining MRI-data, circulating biomarkers and molecular and cellular features of brain tumors. The main aim is to cluster patients and provide a personalized treatment for each patient.

Table 1
Clinical and molecular features of brain tumor patient cohort.

	Grade IV	Grade III	Grade II
Patients, n°	132	15	28
Sex, m/f (%)	81/51 (61/39)	9/6 (60/40)	20/8 (71/29)
Age, years	61.8(53–72)	53.3 (44–64)	45 (35–57)
Age male, years	59.6 (52–69)	53.1 (43–53)	44 (35–53)
Age female, years	65.2(59–76)	53.6 (44–67)	47 (40–59)
KPS, %	77.4 (70–90)	82 (70–90)	84.4 (70–100)
OS, months	11.31 (4–15)	21 (6–30)	26.6 (13–38)
Ki-67, %	33 (20–40)	19.5 (8–28)	7.7 (3 – 11)
MGMT, %	22.5 (4–39,2)	28.2 (5–43)	25.6 (15–36)
Patients with MGMT > 9%	69 (52)	9 (60)	22 (79)
Patients with IDHmut,	19 (14)	5 (33)	13 (46)
Patients with LOH	20 (19)	5 (33)	8 (29)

Data are presented as median (IQR) or number (%). Grade IV: GBM; Grade III: Anaplastic oligodendrogliomas, anaplastic astrocytomas, anaplastic oligoastrocytomas; Grade II: Diffuse oligodendrogliomas, diffuse astrocytomas, and diffuse oligoastrocytomas. KPS: Karnofsky performance status; OS: overall survival; IQR: interquartile range. A value of MGMT promoter methylation > 9% is considered a favorable prognostic indicator, associated with a better response to treatment.

could be useful to understand clonal evolution of the tumor and to test new targeted treatments. GO-term enrichment analysis pointed out impairments in pathways essential for the development and progression of cancer, such as pathway related to angiogenesis, as well as to the immune system regulation. This last point is particularly interesting, considering the promising advances in immunotherapies.

5.2.2. Cell-based analyses to define specific patient signature

GSC microenvironment is regulated by a sensitive crosstalk between different molecules, including lipid mediators [95,96]. Among them, sphingosine-1-phosphate (S1P) has largely emerged as a key bioactive factor, favoring invasion, growth, angiogenesis and therapy resistance in different tumors, including GBM [97,98]. S1P is produced during sphingolipid metabolism *via* the conversion of ceramide (Cer) to sphingosine (Sph), through the phosphorylation by sphingosine kinases 1–2 (SphK1–2) [99,100]. The majority of the signaling functions of S1P have been attributed to its activation, at low nanomolar potency, of a family of five G protein-coupled receptors, S1PR1–5 [89]. Research publications reporting the involvement of S1P in GBM are increasing since the 1990s. It has been reported that human GBM cell lines responded mitogenically to nanomolar concentrations of S1P and express mRNA encoding the S1PR1/S1PR2/S1PR3, resulting in increased cell proliferation, motility and invasiveness [101,102]. Elevated levels of S1P were found in GBM tissues [103] and the expression of SphK1 was reported to correlate with overall survival in GBM patients [104]. Interestingly, a study conducted on GBM cell lines in hypoxic conditions, revealed that hypoxia-inducible factor (HIF)-1α and -2α are able to increase SphK1 mRNA levels, protein expression, and enzyme activity, followed by intracellular S1P production and S1P release [105]. Quint et al. investigated the role of SphK1, SphK2, and of S1PRs in primary, secondary, and recurrent GBM tissue samples. Authors showed that SphK1 and S1PR were overexpressed as much as 44-fold compared to normal brain tissue, whereas, with a 25-fold increase, SphK2 was higher in primary tumors [106]. Previous report demonstrated that GSCs, isolated from a glioblastoma cell line, express S1PRs and that S1P, when

administered to the cell culture, promotes GSC migration and invasiveness [107]. Furthermore, Mora and colleagues reported that the inhibition of SphK1 decreases cell proliferation, triggers neurosphere dissociation, and promotes cell death in GSCs [108]. In this context, we also demonstrated a pivotal role for S1P in proliferation, survival, and migration of GSCs [109]. In addition, we proved that fast-proliferating GSCs export a 10-fold higher amount of S1P in the extracellular milieu, through the rapid degradation of newly synthesized Cer, suggesting that S1P is able to critically modulate GBM microenvironment toward a malignant phenotype [109]. Prompted by these findings, we also demonstrated that S1P may be considered a multi-compartmental target for glioma therapy, acting also on GBM angiogenesis.

Therefore, based on our previous studies on endothelial cell features and functionality [110,111], we developed a cell platform for the isolation and the phenotypic, molecular and functional characterization of primary tumor endothelial cells (TECs). By this approach we developed a model as a "disease in a dish", to reproduce tumor vasculature, biologically as close as possible to the *in vivo* condition. By this tool, it is possible to screen patient-derived TECs at cellular and molecular level and test different anti-cancer compounds, adding a piece to the puzzle of the different grade-related angiogenic features of human brain cancers [112]. Furthermore, our experimental tool revealed that TECs isolated from different patients respond differently to the same therapy, as well as differently to diverse therapies, supporting the concept of PM for GBM [113]. Our research on the vascular compartment revealed that the tumor endothelium is able to interact with GBM cells through a bi-directional communication in which several mediators are important. Among these molecules, S1P plays a pivotal role. Indeed, the co-culture between endothelial cells and GBM cells promoted the expression, activity, and plasma membrane enrichment of SphK2, leading to increased cellular levels of S1P, and its secretion. Further, in the extracellular milieu, S1P stimulated GBM cell proliferation, as well as tumor endothelial cell migration and angiogenesis *via* expression of S1PR1 and S1PR3, the principal S1PRs involved in cell growth and invasiveness. [114]. All in all, these results suggested an important role for S1P, as potential marker for clinical prognosis and a potential target for cancer therapy. In this context, one of the most studied approach was to investigate the effect of the inhibition of SphK activity and the factors that regulate the balance of the sphingolipid rheostat. It has been reported that SK1-I, a sphingosine analogue and competitive inhibitor of SphK1, attenuated glioblastoma growth and/or proliferation in cell lines and xenograft models [115]. Two SphK inhibitors, D,L-threodihydrosphingosine (safingol) and *N*,*N*,*N*-trimethylsphingosine, have been investigated for a number of years as possible anticancer agents [116–118]. While these agents did not appear to have significant anti-tumoral activity alone, there was evidence that they might potentiate the anticancer effects of known chemotherapy drugs [119]. More recently, FTY720 (Fingolimod, Gilenya), attracted a lot of attention. FTY720 is a sphingosine analogue drug derived from myriocin, which has been successfully used to therapeutically target sphingolipid signaling for the treatment of patients affected by multiple sclerosis. The pro-drug FTY720 is phosphorylated to P-FTY720 by SphKs, becoming a structural analogue of S1P and a functional antagonist for S1PR1 and thus promoting tumoral suppression *via* S1PR-dependent [120] or receptor-independent [121] mechanisms. Among its actions, P-FTY720 is a superagonist of the S1PR on lymphocytes and can prevent these cells from leaving the lymph node [116,122]. A key property of FTY720 is that it is lipophilic and crosses the BBB, which appears of relevance for making it an attractive candidate for GBM therapy. However, FTY720 affects multiple other enzymes that can alter ceramide/S1P balance, including SMase, ceramide synthase, acid ceramidase, S1P lyase and S1P phosphatases [123–125]. FTY720 has emerged as a potential effective drug for several cancers, causing suppression of tumor growth, as well as induction of apoptosis in multiple tumor cell types [126], including GBM cells and stem cells [109,127,128]. Recently, it has been reported that FTY720 suppresses the Nrf2/ARE pathway in human GBM cell lines, in which constitutive Nrf2 activation enhances cell survival and resistance to anti-cancer drugs, sensitizing also cells to TMZ [129]. Despite the promising results of numerous experimental studies conducted both *in vitro* and *in vivo* on GBM models by targeting sphingolipids metabolism and signaling, to this day only one clinical trial has been conducted in patients affected by GBM with FTY720. Investigators proposed the use of FDA approved for multiple sclerosis fingolimod prior to the initiation of radiation (NCT02490930). In this phase I trial, whose recruitment status has been completed in September 2017, five patients affected by GBM were treated with Fingolimod 1 week prior to the initiation of concurrent radiation and temozolomide and was discontinued immediately upon completion of the six weeks of therapy. So far no result has been posted.

5.2.3. Circulating biomarkers as new tool to develop anti-cancer multitarget therapies

In last few decades, the detection of plasmatic biomarkers, measurable by minimally invasive techniques, such as liquid biopsy, has increasingly gained attention for the possibility to identify novel molecules, which reflect disease biology. Taking into account the great potential of new targets to serve as diagnostic and prognostic markers in patients with brain tumor, we investigated the correlation between the preoperative plasmatic level of von Willebrand factor (VWF) and patient survival. VWF is a multimeric glycoprotein that participates in coagulation process and hemostasis, carrying the coagulation factor VIII in plasma, and being considered as a negative modulator of angiogenesis [130]. Currently, VWF is considered a potential circulating biomarker for tumor angiogenesis in different cancer subtypes. Starting from these premises, we measured plasmatic VWF antigen (VWF:Ag) levels in a cohort of 57 patients with confirmed diagnosis of GBM and 23 with meningiomas (MNGs), enrolled as controls. Our results revealed that GBM patients showed a median level of VWF:Ag significantly higher than in those with MNGs. The 1-year OS was significantly shorter in GBM patients with VWF:Ag levels > 200 IU/dL, which reported a 3-fold higher risk of death [131]. According to this evidence, VWF:Ag may be considered a prognostic circulating biomarker, to be associated with current disease-related markers. The prognostic role of VWF has been reported in several types of cancer, in which high VWF levels are associated with disease stage, tumor size, residual disease after surgery and presence of metastases [130].

5.2.4. The significant contribution of the coagulation system to GBM malignancy

Our previous findings have driven the idea that brain tumors, and GBM in particular, are not only local tumors, but systemic pathologies in which blood circulation is involved in a self-sustaining cycle [130–132]. In this context, we investigated circulating biomarkers as biochemical parameters and platelets, as non-tumor circulating cells. Focusing on the coagulative system, interestingly we found that GBM patients showed an hypercoagulable profile, in terms of pre-operative blood parameters such as partial activated thromboplastin time (aPTT), prothrombin time (PT), fibrinogen (FB), D-Dimer (DD), von Willebrand factor (VWF), platelet counts (PLTs), white blood cells, and hemoglobin levels. More specifically, we reported that hypercoagulable patients presented reduced OS compared to non-hypercoagulable patients [132]. Our data suggest that an altered coagulation profile could be a useful clinical tool, in association with routine tests, such as methylation of MGMT promoter and IDH mutation status, to predict the OS of brain tumor patients. Furthermore, the hypercoagulable status may be considered in the decision of an antithrombotic prophylaxis, acting as prognostic factor of the disease. The coagulative phenotype may allow classification and stratification of patients, leading to an effective personalization of the treatment regimens. In parallel, we investigated the role of platelets, since they participate to the hemostasis and appear to contribute to the intense and dysregulated angiogenesis of solid tumors [133]. Indeed, platelets are considered functional carriers and

reservoirs of proangiogenic factors that can be secreted upon specific physiological and pathological stimulation [134,135]. Because in GBM blood vessels are dysregulated and often damaged, we hypothesize that the pathological exposure of VWF may induce abnormal platelet adhesion and aggregation. Accordingly, we investigated the content and the effect of platelet releasate on GBM endothelial cells. We showed, for the first time, that GBM platelets released higher amount of VEGF compared to healthy subjects, negatively correlating with OS in our patient cohort [136]. Furthermore, we recently reported another evidence of the "tumor-education" mechanism, by which PLTs of GBM patients express and carry pro-tumor molecules. Indeed, GBM-PLTs showed higher expression of VEGFR-1, VEGFR-2, VWF, S1P, S1PR1, SphK1, and SPNS and increased concentrations of VEGF and its receptors VEGFR1 and VEGFR2, VWF, and S1P were found in GBM-PLT-releasate with respect to HD-PLTs. Finally, GBM-PLT-releasate showed a pro-angiogenic effect on TECs, increasing the vascular network's complexity [137]. Our results sustain the contribution of PLTs to GBM aggressiveness, advancing the potential of an anti-PLT therapy and the usefulness of PLT cargo as predictive and monitoring biomarkers. Finally, PLTs may be used as easily accessible, non-invasive biomarkers to predict the response to therapy and monitor tumor progression and eventual relapses *via* a PM strategy.

5.2.5. The importance of a well-defined neuroimaging protocol to predict prognosis of patients affected by GBM

In neuroradiology, dynamic-contrast enhanced magnetic resonance imaging and dynamic-susceptibility contrast (DCE-MRI and DSC-MRI) are high spatial resolution techniques used to estimate tumor vasculature, by the measurement of perfusion and permeability in terms of blood flow (BF), blood volume (BV), volume transfer constant (K^{trans}), flux rate constant (K_{ep}), plasma volume and extravascular extracellular volume fractions (Vp and Ve, respectively). The correlation between tumor vascularity and tumor BV was confirmed over 15 years ago. Notably, in physiologic condition, when the BBB is intact, K^{trans} and K_{ep} are unmeasurable by MRI techniques [138].

In GBM, neoangiogenesis gives rise to an increased number of vessels, a high proportion of which are immature with increased endothelial permeability. This process facilitates the bidirectional transfer of gadolinium chelate contrast agents between plasma and the extracellular space. So, when the BBB is intact, K^{trans} and K_{ep} are effectively zero (or unmeasurable by MRI techniques) irrespective of blood flow. With loss of BBB integrity, K^{trans} and K_{ep} are associated with the leakiness of the vessels and the total surface of the leaky vessels.

These parameters show strong discriminative power in distinguishing LGG and HGG [138] and predicting prognosis [139–141], and we reported a role for K^{trans} and K_{ep}, together with VWF plasma level, as prognostic indicators of postoperative survival of patients with GBM.

Indeed, after having divided patients in two groups, according to the plasmatic level of VWF:Ag, we found that both K^{trans} and K_{ep} were significantly higher in high-VWF:Ag patients than in those with low-VWF:Ag. Further, patients with high-VWF:Ag and high-K_{ep} experienced a 1-year OS shorter than patients with low-VWF:Ag and low-K_{ep} [142]. Currently, the prognostic value of DCE-MRI in GBM patients has not been well established. K^{trans} is considered a synonym for tissue permeability and blood flow, being influenced by cerebral blood flow and vascular permeability, thus it is known to predict glioma grading [143]. Similarly, K_{ep} is strictly related to vascular permeability and aggressive tumors, and it is considered a more robust parameter than K^{trans}, because it is not so dependent on the T1 values of the tissue or Ve [144]. Although preliminary, these results suggest a promising role for MRI and biochemical parameters such as K^{trans}, K_{ep} and VWF, in predicting GBM patient OS, addressing the urgent need in neuro-oncology for accessible, minimally invasive and reproducible markers to optimize neuroimaging during diagnosis and follow-up.

5.3. The benefit of the comprehensive strategy for patient management

Due to short-life expectancy of GBM patients, there is an urgent need to develop modern and advanced approaches to counteract these harmful diseases. The achievement of new knowledge, in parallel with the introduction of new technologies have improved GBM management, although much remains to be done. The synergy between research and medical assistance could be the right key in the era of PM, in order to make competitive innovation by bringing back biomedical research at the service of the person and the society. The concept of PM driven by patient clustering and molecular stratification of brain tumors is appealing and scientifically sound. The laboratory should work not only to study and develop the most effective experimental protocols for the individual patient, but also participating in the discussion of clinical cases and expressing opinions about the therapeutic options available, case-by-case. According to the most current international recommendations, in fact, all patients with brain cancer should be offered the opportunity to be enrolled in the most advanced and suitable clinical trials. In order to personalize care, even the assistance to the individual patient, during the course of the disease, deserves special attention. Indeed, brain malignant tumors represent a complex pathology due to the severity of neurological symptoms, as well as for the social and economic burden. To this aim, a patient-centered care should be characterized by the integration of assistance and research by cutting-edge technologies in the diagnosis and treatment of brain tumors. The current need is to define a set of Integrated Care Pathways (ICP), in order to guarantee an integrated multidisciplinary approach with a high clinical-assistance impact and a targeted allocation of resources, from the diagnostic suspicion to the end of life. The ICP are tools that allow outlining, with respect to a pathology, the best possible path within an organization and between organizations for the care of the patient and his family, reducing costs for the National Health Service. Beyond all, the dearth of anti-cancer compounds available today to treat brain tumors represents the major limitation of our ability to treat patients with a personalized approach. From this, it is mandatory the presence of a well-structured Neuro-oncology Unity together with an Experimental Neurosurgery Laboratory that may combine the innovative and advance research with the real-world experience of clinicians. Indeed, we believe that the fight against GBM starts in the operating room, of course, but that it must then continue with determination also by looking for the most intimate molecular and cellular mechanisms that are the basis of its development.

6. Conclusion

The optimal pathway for the treatment of nervous system cancer outlines seven critical steps in the diagnostic-therapeutic care pathway, consisting in: i) prevention and early identification; ii) presentation, initial investigations and consultancy; iii) diagnosis, evaluation and treatment planning; iv) therapeutic strategies; v) treatment after initial therapy and hospitalization; vi) management of recurrent or progressive disease; vii) care at the end of life [145].

Although the seven steps appear as a linear path, in clinical practice, the treatment of the patient depends on particular factors, such as the type of tumor, diagnosis modality and timing, prognosis, management, personal decisions and physiological response to treatment. Key principles such as appropriate person-centered care, safe and quality care, multidisciplinary care, coordination of care and research are essential to this optimal and humanized path for the treatment of brain tumors.

Author contributions

RC, LG, SEN and GM contributed to conception, design, data collection and writing of the manuscript. MC, BZ, GC, NLV, CG, AR, SL, LR and ML contributed to data collection and writing of the manuscript. All authors read and approved the final manuscript.

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Declaration of Competing Interest

The authors declare the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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