GLIOBLASTOMA

LIQUID BIOPSY IN HIGH GRADE BRAIN TUMORS.

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Adult and pediatric high-grade brain tumors are lethal malignancies characterized by infiltrative behavior (preventing complete surgical resection), resistance to standard radio- and chemo-therapy, and genetic and/or phenotypic heterogeneity (largely responsible for the failure of targeted therapies). The recently elucidated genetic landscape of gliomas allowed the identification of genetic alterations behaving as “entity-defining biomarkers” (such as IDH mutations) essential for glioma sub-classification, and/or “predictive biomarkers” exploitable to recognize patients eligible for innovative targeted or immunological therapies. Recently, in many tumor types, analysis of tumor-derived fragmented DNA circulating (ctDNA) in the blood (“liquid biopsy”) has shown the potential to conveniently replace the genetic analysis of tissue samples. However, in case of brain tumors, blood liquid biopsy is ineffective as the tumor DNA release is hindered by the blood-brain barrier. As an alternative, liquid biopsy of the cerebrospinal fluid (CSF) has been proposed, and shown to be feasible in principle. We thus intended to set-up CSF biopsy through systematic comparison of genetic lesions detectable in tumor tissues and in the corresponding CSFs. From 29 adult patients with high-grade gliomas, we derived tumor and constitutive DNA, together with CSF and plasma ctDNA. The analysis of tumor DNA showed that 26 samples harbor at least one trackable genetic lesion to infer the presence of tumor DNA in plasma and CSF ctDNA. To date ctDNAs from 21 patients have been studied. In plasma ctDNA no tumor genetic alterations could be detected, while, in 13/21 CSF ctDNAs, tumor-specific DNA alterations (mutations, amplification, deletions and MGMT methylation) were found, supporting the conclusion that CSF is a reliable source of tumor DNA. Further analyses are aimed to assess whether the fragmented CSF ctDNA is amenable to NGS analysis of a comprehensive panel of genetic alterations, to complement the diagnosis and longitudinal monitoring of high-grade brain tumor patients.

GLIOBLASTOMA

PROLONGED SURVIVAL OF A PATIENT WITH HYPERMUTATED GLIOBLASTOMA TREATED BY NIVOLUMAB.

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Different approaches of immunotherapy are under scrutiny in the treatment of glioblastoma, the most malignant brain tumor. Inhibition of immune "checkpoints", like PD-1, provided important progress in the treatment of melanoma and other cancers. Here we report on a patient with recurrent glioblastoma treated with the anti-PD1 antibody nivolumab. The patient is 37 years, female: her father was diagnosed with gastric cancer, the mother and her brother with uterine cancer and gastric and intestinal carcinoma, respectively. After her first surgery on July 2013 for frontal glioblastoma, she was treated with radiotherapy and temozolomide. Fourteen months later she had second surgery and on January 2015 she started immunotherapy with nivolumab that is still ongoing as there is no sign of progression at MRI. However she recently had surgery for a colon adenoma with high-grade dysplasia. Increased mutational load may favor anti-PD1 immunotherapy. To investigate this, total DNA was isolated from peripheral blood and FFPE tissue from first and second surgery and exome sequencing performed in collaboration with BGI (www.genomics.cn). We identified germinal mutations of MSH2 (p.Arg359Ser) and MSH6 (p.Gln1155Arg), both associated with decreased expression of protein, confirmed on both tumor by immunochemistry. The gene products of MSH2 and MSH6 are involved in mismatch repair and found mutated in Lynch syndrome, a condition associated with increased cancer risk. Notably, while glioblastoma have usually around 50 somatic mutations, first and second tumors shared 12.431 sequence changes, while 113 were specific of the first one and 1,683 of the second one. Considering the median survival of glioblastomas (14.6 months) and the absence of prognostic positive factors (MGMT not methylated/IDH-1 wild type), this patient survival is clearly beyond the expectations. The data strongly suggest that tumor hypermutations, in the context of a Lynch syndrome, are important drivers of this clinical response.

GLIOBLASTOMA

GENETIC FINGERPRINT OF RECURRENT GLIOBLASTOMA REVEALS GENOMIC ALTERATIONS IN MMR GENES.

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BACKGROUND:
Glioblastoma (GBM) is the most common primary brain tumor in adults and the Stupp protocol represents the current standard of treatment. However, the tumor invariably relapses, suggesting marked intra-tumor genetic heterogeneity enabling rapid adaptation to therapy. Therefore, deeper characterization of recurrent GBM (rGBM) might contribute to better understand mechanisms behind tumor progression and therapy resistance.

MATERIALS & METHODS:
GBM samples (n=57) have been collected from adult patients who underwent two surgical interventions (at diagnosis and recurrence) and were treated with the Stupp protocol. Expression of mismatch repair (MMR) proteins (MLH1; PMS2; MSH2; MSH6) was evaluated by IHC, followed by exome sequencing (100x) of 3 pairs showing loss of MSH6 reactivity. MSI was assessed by fluorescent PCR and capillary electrophoresis using a panel of 5 polyA mononucleotide markers.

RESULTS:
According to IHC, 15 out of 52 rGBM samples (28.8%) lacked expression of MMR proteins. In particular 12 out of 15 samples (80%) show partial or total reduction of MSH6 expression. Conversely, all GBM samples at diagnosis but two (96.4%) stained positive for the 4 MMR markers. Consistent with IHC data, exome sequencing disclosed lack of variants in MMR genes in primary samples, whereas matched recurrent tumor samples lacking MSH6 expression carried single or multiple somatic mutations in the abovementioned genes and notably shared a splicng variant in MSH6. Importantly, MSH6- specimens were characterized by an hypermutated profile. Notably, MMR deficiency was associated with significant telomere shortening. Strikingly, in our study population lack of MMR protein expression was not associated with microsatellite instability.

CONCLUSIONS:
Our study shows that IHC staining of rGBM for MMR markers is a valuable tool to identify alterations in MMR genes associated with high mutation burden. This subset of patients could represent eligible candidates for drugs targeting immune checkpoint inhibitors.

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Abstract

GLIOBLASTOMA

REGOMA: A RANDOMIZED, MULTICENTER, CONTROLLED OPEN-LABEL PHASE II CLINICAL TRIAL EVALUATING REGORAFENIB ACTIVITY IN RELAPSED GLIOBLASTOMA PATIENTS.

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BACKGROUND:
There is no established treatment regimen for recurrent glioblastoma (rGBM). GBMs have high expression of pro-angiogenic factors and activation of multiple signaling pathways in the tumor microenvironment, including the receptor tyrosine kinases, VEGFR, FGFR, and PDGFR. Regorafenib (REG), an oral multikinase inhibitor, inhibits these angiogenic kinases and oncogenic kinases KIT, RET, and B-RAF.

METHODS:
The primary aim of this trial was to assess REG activity in prolonging overall survival (OS) in patients (PTS) with rGBM after surgery and Stupp regimen (α = 0.2, 1-sided; α =0.2). Secondary objectives were disease control rate (DCR), safety, progression free survival (PFS), quality of life (QoL) and analysis of angiogenic and metabolic tissue biomarkers as possible predictors of response to REG. Eligible PTS with histologically confirmed GBM, ECOG PS 0-1, and documented disease progression were randomized 1:1 to REG 160 mg/day or lomustine (LOM) 110 mg/m2 until disease progression or unacceptable toxicity. Tumor response was evaluated by gadolinium brain MRI every 8 weeks according to the RANO criteria.

RESULTS:
119 PTS were enrolled (n=59 REG; n=60 LOM) and stratified for surgery at recurrence. MGMT was methylated in 47.5% and 44.1% of REG and LOM PTS, respectively. 27 PTS (22.7%) had surgery at recurrence. Median OS was 6.5 months (m) (95% CI 5.6-12.0) for REG and 5.5m (95% CI 4.7-8.0) for LOM (HR=0.64; 80% CI 0.47-0.87; p=0.028, 1-sided log-rank test). DCR (CR+PR+SD) was 44.8% and 21.1% (p=0.009) for REG and LOM, respectively. The 6-month PFS rates were 15.5% and 8.3% for REG and LOM (HR=0.69; 95% CI 0.47-1.01; p=0.051). Grade ≥3 adverse events were reported in 56% and 40% of PTS who received REG and LOM.

CONCLUSIONS:
REG significantly improved OS and DCR in rGBM compared to LOM. REG administration was feasible and safe. QoL and biomarker analyses are ongoing.

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Abstract

GLIOBLASTOMA

TRANSLATIONAL IMPACT OF GLIOBLASTOMA STEM CELLS IN PREDICTING TUMOR RESPONSE TO CHEMO-RADIOTHERAPY.

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BACKGROUND:
Glioblastoma (GBM) is a highly heterogeneous tumor. Glioma stem cells (GSCs) are a small fraction of GBM cells able to self-renew and sustain tumor growth. GSCs are extremely resistant to chemo-radiation and thus are responsible for recurrences. GSC research has increased our knowledge on GBM biology but had a scarce clinical impact. In the present study we explored the translational power of GSCs analysis.

METHODS:
We analyzed a cohort of 176 GBMs treated with surgery and chemo-radiotherapy with temozolomide (TMZ). Immediately after surgery, specimens were mechanically dissociated and cultured in serum-free medium supplemented with growth factors for GSC generation as floating tumorspheres. The effect of radiation (6 to 60 Gy) and TMZ (3.9 to 1000 μM) on GSCs was then related with patients' survival. We defined LD50 as the radiation dose necessary to kill 50% of GSCs, and IC50 as the half-maximal inhibitory concentration of TMZ.

RESULTS:
Of 176 GBMs, 52 (29.5%) generated GSC cultures. GSCs generation was an independent negative prognosticator ($p<0.0001$ and $p=0.0010$ for OS and PFS, respectively). Growth rate and clonogenicity of GSCs predicted poor OS. GSCs were highly chemo/radioresistant; TMZ resistance was stronger in GSCs with high clonogenicity and fast growth ($p<0.02$). Shorter PFS was associated with LD50>12 Gy of matched GSCs ($p=0.0484$). A direct relationship was found between GSCs sensitivity to TMZ and survival ($p=0.0167$ and $p=0.0436$ for OS and PFS, respectively). GSCs with TMZ IC50>180 μM derived from GBMs with worse OS and PFS ($p=0.0039$ and $p=0.0022$, respectively). Importantly, TMZ IC50<50 μM (approximating plasma level during Stupp) identified GBMs with longer OS ($p=0.0020$) and PFS ($p=0.0016$).

CONCLUSIONS:
Analysis of GSCs holds translational relevance by predicting the response of parent tumors to radiation and, particularly, to TMZ. We suggest a combined treatment strategy for GBM, targeting both the fast-growing progenitors and the slow-growing, highly clonogenic GSCs.

GLIOBLASTOMA

MOLECULAR CHARACTERIZATION OF GLIOBLASTOMA-DERIVED ENDOTHELIAL CELLS AS TOOL FOR DISSECTING THE MOLECULAR MECHANISMS UNDERLYING GBM-ASSOCIATED NEOVASCULARIZATION AND TO IDENTIFY NOVEL THERAPEUTIC TARGETS.

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Glioblastoma Multiforme (GBM) is the most common and aggressive primary malignant brain tumor in adults. Angiogenesis represents a crucial process in tumor maintenance and progression, since GBM is characterized by robust neovascularization. GBM aggressiveness has also been ascribed to a subpopulation of cells with stem-like features, Glioblastoma Stem-like Cells (GSCs). GSCs are able to trans-differentiate into functional endothelial-like cells, contributing to GBM vasculature. In the attempt to find druggable signaling pathways in GBM, we assessed the effect of a commercial kinase inhibitor library (Selleckchem), containing 349 compounds approved for clinical trials or therapy, able to counteract most cancer-related pathways. We screened four representative patient-derived GSC lines cultivated either in stem cell medium or differentiated in endothelial conditions (GSC-derived endothelial-like cells, GdECs). Among the few compounds active at submicromolar concentrations, Elesclomol showed the most antiproliferative effect on both GSCs and GdECs. It has been demonstrated that Elesclomol induces apoptosis in cancer cells through the rapid generation of reactive oxygen species (ROS), inducing a transcriptional gene profile characteristic of oxidative stress response. Although numerous studies have shown that miRNAs play a role in several diseases by regulating oxidative stress, this field is still at the beginning. We analyzed the miRNA profile (Agilent Technologies) of the 4 GSC lines cultivated in stem cell medium or in endothelial conditions in order to identify miRNAs with potential relevant functions in GdEC survival. This analysis revealed a signature of three miRNAs, miR-4516, miR-1281 and miR-1825, clearly discriminating GSCs or GdECs. Gene Set Enrichment Analysis of the three miRNA targets revealed modulation of genes associated with pathways involved in angiogenesis, hypoxia, ROS metabolism. Currently, we are performing reverse-phase protein array analysis to identify pathway activation patterns correlated with the response of GSCs and GdECs to Elesclomol to subsequently explore and associate it to miRNA expression profile.

GLIOBLASTOMA

ORTHOGONAL ARRAYS OF PARTICLES ALTER CYTOSKELETON AND CELL INVASION DYNAMICS IN GBM AND GLIOMA CELLS.

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Changes in cell shape and in cytoskeletal dynamics are critically involved in cell adhesion, migration, invasion and the overall tumour development. Fluid homeostasis plays a pivotal role in actin dynamics and cytoskeletal rearrangements. This balance is maintained by a water channel protein, Aquaporin-4 (AQP4) in the brain. To analyse the biological role of AQP4 in cytoskeletal dynamics, a total of 6 cell lines were transfected with two major AQP4 isoforms: AQP4-M1 and the Orthogonal Arrays of Particle (OAP) forming isoform, AQP4-M23. Immunofluorescence analysis demonstrated that the expression of AQP4-OAPs triggers cell shape changes only in U87 (GBM) and C6 (glioma) cells. U87 and C6 cells adopted a stellate morphology with the appearance of cytoplasmic protrusions and an increase in the actin polymerization grade. OAP expressing U87 showed irregular shape and a higher F-actin to total actin ratio (50±5%) compared to AQP4-M1 expressing U87 (30±5%). We next examined invasion ability using 3d scaffold and boyden chamber assays and apoptosis using FITC-Annexin V staining. Results demonstrated that AQP4-OAPs expression in U87 cells leads to apoptosis and decreased invasion ability. Several AQP4-OAP mutants at C-terminus tail and extracellular loops were therefore generated to investigate the OAP sequence responsible for morphological changes. Results showed that the C-terminus tail is involved in the actin dynamics and two prolines (254 and 296) are determinant for cytoskeletal remodeling. All together these results indicate that AQP4-OAPs play a key role in actin cytoskeleton reorganization in turn involved in cell dynamics which are common mechanisms in brain cancer.

GLIOBLASTOMA

CLINICAL SIGNIFICANCE OF PLASMA EVS IN GLIOBLASTOMA PATIENTS.

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Glioblastoma (GBM) is the most common primary brain tumor. Despite aggressive treatment (surgery, chemo- and radiotherapy), the prognosis is dismal (median overall survival: 14m). Actually, early diagnosis and treatment monitoring represent major unmet needs. Extracellular vesicles (EVs) are potentially optimal biomarkers owing to high stability and their presence in blood and cerebrospinal fluid. These features, combined to their genetic and proteomic composition that mirrors the intratumoral environment, highlight EV applicability as blood-based biomarkers for disease diagnosis and therapeutic monitoring. In this effort, we collected plasma samples from GBM patients before and after surgery, isolated plasma EVs and analyzed their concentration in order to explore their diagnostic potential. A significant EV enrichment was detected in the plasma of GBM patients, indicating the ability of circulating EVs to distinguish healthy control and patients harboring other CNS malignancies from GBM patients. Meanwhile, we found that GBM surgical removal was accompanied by a significant drop in the amount of circulating EVs, thus indicating the tumor mass as the major donor of circulating EVs in GBM patients. In agreement with the data on human plasma EVs, the increased EV concentration in mice harboring GBM highlighted the clinical value of circulating EVs for the early detection of GBM. The analyses of EV protein cargo by mass spectrometry revealed a specific signature, which included members of the complement/coagulation cascade and regulators of iron metabolism, that reflected tumor-dependent modifications essential to propagate and form tumors and that allowed the distinction of GBM patients from healthy controls. The reduction/disappearance of these proteins from plasma EVs after GBM surgical removal may reflect the therapeutic intervention. Thus, our work highlights the clinical utility of circulating EVs per se as potential biomarker for GBM and suggest how EV protein cargo may provide information about response to therapies and tumor progression.

GLIOBLASTOMA

FUNCTIONAL DIVERSITY AND CO-OPERATIVITY OF SUBCLONAL POPULATIONS IN PGBM AND DIPG.

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Paediatric glioblastoma pGBM) and diffuse intrinsic pontine glioma (DIPG) are aggressive glial tumours affecting children and young adults with a dismal prognosis. Despite advances in understanding their unique biological drivers, a major challenge to improve patient outcomes is their extensive intratumoral heterogeneity.
To explore the mechanisms underlying the pGBM and DIPG complex subclonal architecture we have developed a methodology for the isolation and propagation of genotypically and phenotypically distinct populations pf pGBM/DIPG cells. Single cell-derived colonis were grown under stem cell conditions and expanded in two-dimensional (2D, adherent on laminin) and three-dimensional (3D, neurosphere) culture, applicable to biopsy, resection and autopsy samples. Colonies harbour overlapping but distinct mutational spectra, displayed stem cell-like characteristics, differential growth rate, as well as significantly differing capacities for 3D invasion into matrigel and 3D migration onto ECM proteins (e.g. fibronectin, tenascin-C) in vitro. Using this approach we isolated and characterised rare subpopulations, an exemplar of which is represented by a single cell-derived neurosphere harbouring an inactivating mutation in the H4K20 histone methyltransferase SUV420H1, present in <1% of cells derived from the autopsy of a DIPG patient. This subclone has loss of H4K20me2 and when compared to its natural isogenic SUV420H1 wild-type (WT) clone, it displayed increased invasion into matrigel and migration onto different ECM as well as an increased infiltrative growth in vivo. RNAseq data revealed overexpression of chemokines (Co-culture of SUV CXCL2, CCL2) and integrins. Co-culture of SUV420H1 mutant and WT cells showed the 2 different populations frequently co-localised to form an interconnected network suggestive of co-operative mechanisms in movement across the matrix.
Glioma subclones, even those present at low frequencies, may exert a profound influence on the tumour mass as a whole. Although severly limiting the effectiveness of existing treatments, understanding and disrupting such interclonal communication provides novel avenues for future therapeutic development.

GLIOBLASTOMA

IDENTIFICATION OF CIRCULATING PROGNOSTIC OR PREDICTIVE MARKERS IN PATIENTS WITH GLIOBLASTOMA.

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BACKGROUND:

Glioblastomas are highly malignant brain tumors with a poor prognosis. Despite aggressive surgical resection, chemotherapy, and radiation, median survival of patients remains about 14 months. Lack of strategies for effective therapeutic monitoring remains a barrier in the management of glioblastoma patients as radiological examinations are not enough sensitive and often do not permit to distinguish between progression disease and necrosis. Minimally invasive biomarkers that reliably reflect disease status are needed. Moreover an alternative source of derived tumor material could be useful to test predictive markers of response and prognosis when it is not possible to perform a biopsy.

HYPOTHESIS:

Glioblastoma cells release microvescicles as exosomes containing miRNAs, mRNAs, DNA and proteins. Cancer derived microvescicles serve as a means of delivery of genetic information to recipient cells in the tumor microenvironment. The evaluation of exosome cargo will help to study the natural history of the tumor and to identify prognostic or predictive markers. Cerebrospinal fluid (CSF) and serum has being emerged as a promising biomarker platform.

PROPOSAL:

IRST IRCCS proposes to conduct a ACC network prospective project on Glioblastoma patients within the Glioblastoma WG. At time of surgery a sample of cerebrospinal fluid and of blood will be collected. Exosomes will be extracted from liquid biopsies and mirnoma and exoma will be investigated in both circulating samples and compared with those of matched specimens.

EXPECTED OUTCOMES:

The multicentric prospective study will provide a sufficient enrolment to obtain robust statistical data. The comparative approach will permit to validate the reliability of exosome cargo in CSF and serum. The comparison with matched cancer tissue will give information on the natural history of glioblastoma. The final expected outcome is a panel of circulating markers to evaluate the prognosis and prediction to therapy of glioblastoma patients.

GLIOBLASTOMA

WNT5A DRIVES STATE TRANSITION TO AN INVASIVE PHENOTYPE IN HUMAN GLIOBLASTOMA STEM-LIKE CELLS.

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Numerous pathways underlie brain invasion by tumors, a critical element underpinning recurrence and lethality in human glioblastomas (hGBMs). The identification of the master factors that elicit these pathways globally, driving invasion altogether, eludes us. We report that high expression levels of non-canonical Wnt5a characterize the most invasive gliomas, epitomize dismal prognosis and discriminate the most infiltrating mesenchymal hGBMs from proneural and classical ones. Exacerbated Wnt5a define mesenchymal hGBM cells (Wnt5aHigh) possessing prototypical invasive phenotypes and tumor-promoting stem-like cells characteristics (TPCs) but not their Wnt5aLow siblings. While inhibition of Wnt5a suppresses infiltration in mesenchymal hGBM TPCs, administration or over-expression of Wnt5a elicits the opposite effects, switching on infiltrative "mesenchymal-like" molecular programs in poorly motile, classical hGBM TPCs and Wnt5aLow mesenchymal TPCs, ex vivo and intracranially. Anti-Wnt5a antibody or antagonist Wnt5a peptide block invasion and increase survival in clinically relevant intracranial hGBM models. Wnt5a emerges as a master regulator in gliomatous invasion, endowing hGBM TPCs with archetypal, infiltratory transcriptional and functional profiles, providing a unique target to tackle brain invasion by hGBM cancer stem cells.

GLIOBLASTOMA

LONG TERM SURVIVAL IN GLIOBLASTOMA (GBM-LTS) PATIENTS: WHY SO DIFFERENT? PLANNING OF A COLLABORATIVE STUDY ADDRESSING THE GENETIC AND METABOLIC SIGNATURE, HOST-DERIVED AND CLINICAL FACTORS OF GBM-LTS.

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BACKGROUND AND RATIONALE:

Glioblastoma (GBM) is a rare, rapidly fatal tumor, and only 6% reached 5-year survival (Long Term Survival, LTS). The clinical and molecular determinants of extended survival remain uncertain. At Besta, a monocentric study on GBM-LTS was just approved (CE 5.4.2017). The incoming interest of GBM-LTS, the cooperative reality that ACC (Alleanza Contro il Cancro) offers and our early data about LTS bring us to expand the patient population and to extend molecular and metabolic analysis.

SPECIFIC AIMS:

Characterization of histopathological and molecular profile in GBM-LTS, including host immune profile. In the prospective phase: tumoral metabolism, quality of life and neurocognitive assessment. Working plan. The project includes a retrospective and prospective phase in GBM-LTS, defined as primary GBM, IDH wild-type, surviving longer than 5 years.

METHODS:

All samples will be centralized for histopathological review. Planned analysis include: immunohistochemical study of immune-related and metabolic markers; genetic analysis using large NGS panels or exome sequencing; immune infiltration by immunohistochemistry. Prospective phase: in vivo tumoral metabolism profile addressed by imaging techniques (MR/PET 18F-FET and MRS); extensive clinical assessment including neurocognitive function and quality of life. Novelty of the proposal. We plan to address a large cohort of GBM-LTS patients, expanding the research on several and integrative aspects, including somatic mutations, chromosome abnormality duplication, DNA methylation, copy number variation, as well as host-immune contribution. The prospective phase will address topics not yet studied in such patients, including tumor metabolic alterations and neurocognitive/quality of life evaluation.

PROJECT FEASIBILITY AND MANAGEMENT:

Project coordinator will be the Besta Institute. The study will be open to ACC centers involved in GBM research willing to participate. Neuropathological study and genomic-morphologic analysis will be centralized. Imaging evaluation and neurocognitive examination will be performed at IOV, Padua and Besta, Milan. All data will be recorded in a dedicated database.