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"CLINICAL AND BIOLOGICAL ROLE OF ADJUVANT DENDRITIC CELLS VACCINATION IN NEWLY GLIOBLASTOMA PATIENTS"

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A Silvia,

“Dovremmo considerare perso ogni giorno

in cui non abbiamo danzato.

E dovremmo chiamare falsa ogni verità

che non sia stata accompagnata da una risata”.

F. Nietzsche

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ABSTRACT

Background. Glioblastoma (GBM) is the most common primary tumor of central nervous system and it has a poor prognosis. Standard first line treatment, which includes surgery followed by adjuvant radio-chemotherapy, produces only modest benefits to survival. The interest for immunotherapy in this field derives from the development of new drugs and effective therapies as immune-check points inhibitors, adoptive T-cell approaches or dendritic cell (DC) based vaccines or a combinations of these. GBM is described as a typical “immune-deserted” cancer exhibiting a number of systemic and environmental immunosuppressive factors. Considering the role of microenvironment, and above all the lower tumor load and depletion of immunosuppressive cells in GBM, our hypothesis is that DC vaccine may induce an immune response.

Main aims and study design. The main aim of this project is to study the role of immune system in GBM, including identification of potential prognostic and predictive markers of outcome and response to dendritic cell vaccine. Firstly, we performed a retrospective analysis on blood samples. Then, we analyzed the immuno-component in tissues samples of enrolled patients; and compared that with blood results. Then, the last part of the project is based on a prospective clinical trial on patients enrolled in DC-based vaccination produced at IRST Cell Factory and actually used for patients with melanoma and other tumors. The enrollment is still ongoing.

Expected results. The project will i) develop an immune-panel of prognostic and predictive markers to help clinicians to improve the therapeutic strategy

for GBM patients; ii) provide preliminary results on the effectiveness of immunotherapy on GBM patients.

INTRODUCTION

Brain tumors make up approximately 1% of all cancers and are responsible for 2% of cancer-related deaths [1]. The annual mortality by age in the USA is 4.25/100,000 people. During 2013, about 23,000 new cases of primary brain tumors originating from the central nervous system were estimated in the USA [2-3] with an incidence among adults over 20 years of 27/100,000 people [4]. Survival in adult patients has not significantly increased over the past fifty years and is 5%. In the European Union cancer registries an incidence of 3-5 cases/100,000 inhabitants/year is reported without significant variations between the various European countries and is confirmed as the cause of 2% of all cancer deaths [5]. The distribution curve of the incidence by age shows a peak between 0-4 years, a plateau up to 25 years and a progressive increase after 65 years. Considering all age groups, meningioma is the most frequent tumor (35%) followed by gliomas (29%). In adults, the most frequent histological type is meningioma followed by gliomas and in particular glioblastoma whose incidence shows a peak between the 5th and 6th decades [6].

In young people up to the age of 20, gliomas account for 50% and the most frequent histological type is pilocytic astrocytoma (20%) followed by a non-glial tumor, medulloblastoma (15%). In general, young age, good performance status and low histological grade are favorable prognostic factors for primary brain tumors. Less significant prognostic factors are long duration of symptoms, absence of neurological symptoms, cerebral localization, small tumor size and radical intervention [7]. There is a higher

incidence in males (5.2 versus 3.5/100,000 people per year in males and females, respectively), in part this difference is due to a higher incidence of meningiomas in females [8]. Many studies have documented the increase in incidence in industrialized countries especially in the elderly population with no clear difference by gender, ethnicity and geographical location. The increase in incidence is partly due to the use of more accurate imaging methods (CT and cerebral MRI) for diagnosis and partly to the attention to the care of elderly patients with a greater focus on neurological problems [9 -10]. Of all tumors, glioblastoma multiforme (GBM) accounts for 20% and is the most aggressive primary brain neoplasm. It accounts for 50% of gliomas [11]. It is highly aggressive and diffusely infiltrating with a tendency to cross the midline and invade the contralateral hemisphere. It is characterized by having two ways to develop as a primary or secondary glioblastoma [12]. The Italian incidence for each subtype is fully in line with the European incidence estimates based on the RARECAREnet database [13]. The proportion of NOS cancer cases in the AIRTUM database (Fig. 1) in the study period (2000-2010) is estimated at 37%, with a differential distribution across age, ranging from 20% in the 0-24-year age group to 52% in the over 65 age group [14]. One and 5-year relative survival (RS) of CNS tumours is 55% and 21%, respectively. However, these results are largely affected by astrocytic tumours, which are the most common among these tumours and those with the worst survival (49% and 13% at 1 and 5 years, respectively). There is a striking difference in relative survival between each of the other CNS tumours and astrocytomas; namely, 5-year RS is 76% for ependymal

tumours and 56%-57% for all other histotypes. The poor prognosis of astrocytomas is at least partially explained by the high proportion (64%) of WHO grade IV tumours in this group. On the contrary, ependymal tumours and oligodendrogliomas have a high proportion of WHO grade II tumours (82% and 71%, respectively); moreover oligodendrogliomas have a higher proportion of WHO grade III tumours compared to ependymal tumours, contributing to the estimated difference in survival between these two histotypes. Primary GBM is more frequent in the elderly population (90%), while the secondary one represents the evolution of a lower grade glial neoplasia and typically occurs in younger patients (about 45 years). The transformation of a low-grade tumor to a high-grade one is attributed to molecular alterations such as the inactivation of cell cycle checkpoints, the inactivation of tumor suppressors and marked angiogenesis [15].

Fig 1. AIRTUM, rapporto dei tumori rari in Italia 2015

INCIDENCE

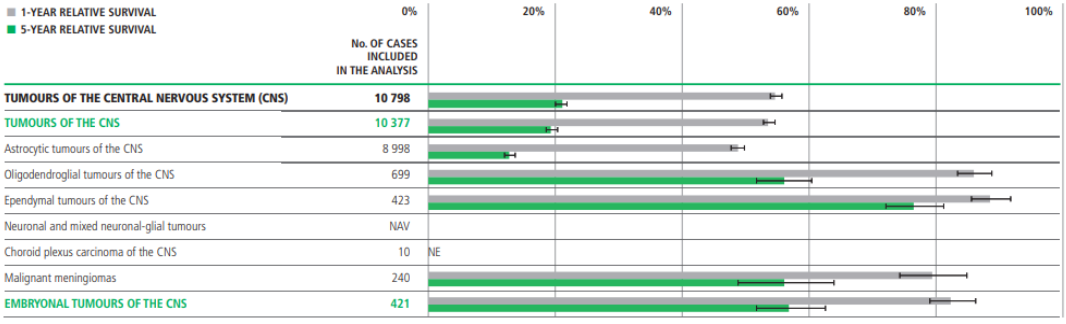
TUMORUS OF THE CENTRAL NERVOUS SYSTEM. Crude incidence (rate per 100,000/year) and 95% confidence interval (95% CI), observed cases and proportion of rare cancers on all (common + rare) cancers by site. Rates with 95% CI by sex and age. Estimated new cases at 2015 in Italy.

	AIRTUM POOL (period of diagnosis 2000-2010)														ITALY
					SEX				AGE						
					MALE		FEMALE		0-54 yrs		55-64 yrs		65+ yrs		
	RATE	95% CI	OBSERVED CASES (No.)	RARE CANCERS BY SITE (%)	RATE	95% CI	RATE	95% CI	RATE	95% CI	RATE	95% CI	RATE	95% CI	ESTIMATED NEW CASES 2015
TUMOUR OF THE CENTRAL CENTRAL NERVOUS SYSTEM (CNS)	**5.89**	**5.79-6.00**	**13 071**	**100%**	**6.99**	**6.84-7.15**	**4.87**	**4.74-4.99**	**3.17**	**3.08-3.26**	**11.64**	**11.24-12.05**	**11.67**	**11.36-12.00**	**3 725**
TUMOURS OF THE CNS	5.67	5.57-5.77	12 566	NA	6.70	6.54-6.85	4.70	4.58-4.83	2.86	2.77-2.94	11.58	11.18-11.99	11.62	11.30-11.94	3 588
Astrocytic tumours of the CNS	4.92	4.83-5.01	10 904		5.89	5.74-6.03	4.01	3.90-4.13	2.24	2.17-2.32	10.42	10.04-10.81	10.67	10.37-10.98	3 125
Oligodendroglial tumours of the CNS	0.38	0.35-0.40	836		0.43	0.40-0.48	0.32	0.29-0.36	0.33	0.30-0.36	0.64	0.55-0.74	0.39	0.33-0.45	231
Ependymal tumours of the CNS	0.23	0.21-0.25	504		0.24	0.21-0.27	0.21	0.19-0.24	0.23	0.21-0.26	0.29	0.23-0.36	0.18	0.14-0.22	139
Neuronal and mixed neuronal-glial tumours	NAV	NAV	NAV		NAV	–	NAV	–	NAV	–	NAV	–	NAV	–	NAV
Choroid plexus carcinoma of the CNS	<0.01	0.00-0.01	13		NE	–	NE	–	NE	–	NE	–	NE	–	4
Malignant meningiomas	0.13	0.12-0.15	299		0.12	0.10-0.14	0.15	0.13-0.17	0.05	0.04-0.06	0.22	0.17-0.29	0.37	0.32-0.43	86
EMBRYONAL TUMOURS OF THE CNS	0.23	0.21-0.25	505	NA	0.30	0.26-0.33	0.16	0.14-0.19	0.31	0.28-0.34	0.06	0.03-0.10	0.05	0.03-0.08	137

NE: not estimable because 15 or less incident cases were observed **NAV:** not available **NA:** not applicable

SURVIVAL

TUMOURS OF THE CENTRAL NERVOUS SYSTEM. One and 5-year relative survival. Error bars are 95% confidence interval. Cohort approach (complete analysis), period of diagnosis 2000-2008.



NE: not estimable because 30 or less incident cases were observed **NAV:** not available

2021 WHO CLASSIFICATION OF CNS TUMORS

Gliomas are primary tumors that develop within the brain parenchyma. The term "glioma" derives from the fact that the cellular histology resembles normal glial cells (astrocytes, oligodendrocytes and ependymal cells). The fifth edition of the WHO Classification of Tumors of the Central Nervous System (CNS) was published in 2021 [16] introducing major changes that advance the role of molecular diagnostics in CNS tumor classification. At the same time, it remains wedded to other established approaches to tumor diagnosis such as histology and immunohistochemistry. In doing so, the fifth edition establishes some different approaches to both CNS tumor nomenclature and grading and it emphasizes the importance of integrated diagnoses and layered reports. New tumor types and subtypes are introduced, some based on novel diagnostic technologies such as DNA methylome profiling. The major general and specific changes are summarized in Fig. 2. [17]

Fig.2. Comparison between 2021 and 2016 WHO classifications of glioma.

Histology	WHO 2016	Grade	WHO 2021	Grade
Oligodendroglioma	Oligodendroglioma, **IDH**-mutant and **1p/19q**-codeleted	WHO grade II	Oligodendroglioma, **IDH**-mutant and **1p/19q**-codeleted	CNS WHO grade 2
Anaplastic oligodendroglioma	Anaplastic oligodendroglioma, **IDH**-mutant and **1p/19q**-codeleted	WHO grade III	Oligodendroglioma, **IDH**-mutant and **1p/19q**-codeleted	CNS WHO grade 3
Diffuse astrocytoma	Diffuse astrocytoma, IDH-wildtype or **IDH**-mutant	WHO grade II	Astrocytoma, **IDH**-mutant	CNS WHO grade 2
Anaplastic astrocytoma	Anaplastic astrocytoma, IDH-wildtype or **IDH**-mutant	WHO grade III	Astrocytoma, **IDH**-mutant	CNS WHO grade 3
Glioblastoma	Glioblastoma, IDH-wildtype or **IDH**-mutant	WHO grade IV	Glioblastoma, IDH-wildtype	CNS WHO grade 4
Astrocytoma			Astrocytoma, **IDH**-mutant and *CDKN2A/B* homozygous deletion	CNS WHO grade 4
Astrocytoma			Glioblastoma, IDH-wildtype & *TERT* **promoter** mutation, *EGFR* amplification, or gain/loss of **chromosome 7/10**	CNS WHO grade 4

MOLECULAR PROGNOSTIC FACTORS

As with other neoplasms, even in malignant brain tumors, WHO III and IV, an attempt is being implemented to identify molecular factors that could have a prognostic and predictive role of outcome and chemosensitivity. Therefore, a detailed molecular characterization is being added to the traditional histopathological definition.

MGMT

The first studies on brain tumor molecular markers focused on the enzyme O6-methylguanine-DNA methyltransferase (MGMT). The MGMT gene is located at the 10q26 locus and encodes a protein of the DNA repair system, O6-methylguanine-DNA methyltransferase, which removes potentially mutagenic alkyl groups from the O-6 position of the guanine bases, an important site of alkylation [18]. The areas affected by this epigenetic alteration are the CpG (cytidine phosphate guanosine) sites. DNA repair consumes the MGMT protein, the level of which must be restored to ensure cellular homeostasis. A high level of MGMT activity in the tumor cell antagonizes the effect of the alkylating agent, temozolomide (TMZ). Methylation is present in about 40% of patients in the various studies and was investigated mostly by methylation specific-PCR. There has been interest in investigating the role of MGMT methylation in newly diagnosed glioblastoma patients who are candidates for combined radio-chemotherapy treatment with TMZ, demonstrating that it is associated with a prolongation of survival and therefore the prognostic role of methylation [19].

ISOCITRATE DEHYDROGENASE 1/2

Mutations in the isocitrate dehydrogenase 1 (IDH1) or IDH2 genes, which are early events in tumor development of low-grade gliomas, can be used as molecular markers to distinguish between primary and secondary GBMs [20]. The IDH1 and IDH2 genes encode isocitrate dehydrogenase 1 and 2 enzymes, respectively. IDH1 is an enzyme involved in the citric acid cycle, catalyzing the carboxylation of isocitrate to alpha-ketoglutarate. This gene codes for a carboxylase which converts isocitrate into alpha-ketoglutarate with production of NADPH; the latter reduces glutathione which acts as an antioxidant in cellular metabolism. The mutated form of the enzyme leads to the formation of a substrate - 2 hydroxyglutarate-it representing an oncometabolite capable of modifying the methylation state of DNA [21]. Literature data reported that the IDH- mutated tumors had distinct genetic and clinical characteristics, generally arising in young patients and with a better prognosis patients with wild type cancer for IDH. This reason is the key for the switch IDH-mutant GBM to as IDH-mutant astrocytoma in the new WHO classification and at the same time a tumor is defined GBM only in case of IDH wild type tumor [22].

EPIDERMAL GROWTH FACTOR RECEPTOR

Genomic profiling has detected epidermal growth factor receptor (EGFR) gene alterations in more than half of GBMs. Major genetic events include amplification and mutation. EGFR is a transmembrane tyrosine kinase receptor. The identified gene mutation is EGFRvIII [23] characterized by deletion of exons 2 to 7 which leads to the synthesis of a truncated protein in

the extracellular component with activity consistently independent of the ligand [24]. Amplification is present in 33% of high-grade tumours, 20% of WHO grade III tumours, and is more frequent in primary than in low-grade transformed tumours. This amplification leads to tumor growth characterized by vascular invasion and is a possible cause of resistance to combined radio-chemotherapy [25-26]. A number of studies have assessed targeted intervention of EGFR in GBM using strategies such as antibodies, small-molecule tyrosine kinase inhibitors (TKIs), and vaccines; however, therapeutic benefit has not been achieved.

TREATMENT IN GLIOBLASTOMA

To date, the prognosis of patients with GBM remains unfavorable: in fact, both the use of chemotherapy and knowledge of the methylation status of O6-methylguanine-DNA-methyltransferase (MGMT) have not moved the median survival beyond 18 months. Surgery represents the only potentially curative modality and the experience of the surgeon plays a significant role in terms of improving survival [27]. The resection should be as extensive as possible with regard to the site and the patient's clinical condition [28].

COMBINED RADIO-CHEMIO POST-SURGICAL APPROACH

Postoperative radiotherapy has a significant role in high-grade brain tumors prolonging median survival up to about 12 months [29]. The efficacy emerged in randomized trials as early as the 1970s and 1980s [30]. The adequate dose provides for a delivery of 60 Gy in 30 daily fractions of 2 Gy each for a total of six weeks of treatment to be started within 4-6 weeks of surgery.

The same studies had shown how the addition of chemotherapy to adjuvant

radiotherapy was able to postively influence the outcome of patients . TMZ is an oral alkylating agent recommended in combined treatment with radiotherapy. There are no direct comparison studies between nitrosureas and TMZ but the magnitude of benefit in separate trials was greater with the alkylating agent [31], furthermore TMZ is associated with a better toxicity profile than nitrosureas. The efficacy of combined treatment with TMZ was demonstrated by the EORTC study [32] in which 573 newly diagnosed glioblastoma patients were randomized to receive post-operative radiotherapy alone (60 Gy in 30 fr) or the same RT concurrent with TMZ 75 mg /m2 daily for up to 49 days followed by six cycles of TMZ 150-200 mg/m2 for 5 days every 28 up to 6 cycles [57]. After a 5-year follow-up, an increase in progression free survival was observed in the experimental arm with combined treatment respect to control arm (11,2% vs 1,8% at 2 years and 4,1% vs 1,3% at 5 years, HR 0·56, 95% CI 0·47–0·66; p<0·0001). This study resulted in the approval of this treatment as a standard in operated patients. In a representative subgroup of 206 patients, MGMT promoter methylation status was the strongest prognostic factor for survival demonstrating in methylated a two-year survival of 49% vs 24% in the experimental arm compared to pts treated with RT alone (p=0·001). The methylation of MGMT therefore it would have a prognostic role and in addition a predictive value of response to TMZ even if other molecular factors that may interfere with the outcome are still being investigated. In elderly patients it is necessary to evaluate which is the best therapeutic approach and where the general clinical conditions allow it, surgery must always be performed because it

improves control of the symptom [33]. For postoperative treatment, the recent NOA-08 and NORDIC studies demonstrated that the choice can be made among the Stupp regimen, TMZ alone, or radiotherapy alone [34, 35]. Even for patients >65 years of age, the presence of MGMT methylation is confirmed as a favorable prognostic and predictive value for chemotherapy and this element would guide the choice towards chemotherapy (if methylation is present) or radiotherapy (if it is not present).

ROLE OF INFLAMMATION IN CANCER

The Hallmarks of Cancer, presented by Hanahan in 2011, were proposed the functional capabilities acquired by human cells when they change from normal to neoplastic states. Hanahan suggested that two additional mechanisms are involved in the pathogenesis of all cancers. One is based on the capability to reprogram the cellular metabolism to support neoplastic proliferation [36]. The second allows tumour cells to evade immunological control, in particular by T and B lymphocytes, macrophages, and natural killer cells. The eight hallmarks currently comprise the acquired capabilities for sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing/accessing vasculature, activating invasion and metastasis, reprogramming cellular metabolism, and avoiding immune destruction. The innate immune cells designed to fight infections and cancer can instead result in tumor-promoting consequences of inflammatory responses. The inflammatory step promotes a cellular microenvironment that allows the expansion of genomic aberrations and the initiation of carcinogenesis [37]. As the authors explained, the acute

inflammation is a self-limiting process and an important component of the immune system with therapeutic significance, but if the inflammatory responses was inadequate it would lead to various chronic diseases, including cancer [38]. The chronic inflammation in microenvironment interferes with the immune system constituents with cancer progression. In normal conditions, immune cells, including macrophages,granulocytes, mast cells, dendritic cells (DCs), innate lymphocytes, and natural killer (NK) cells fight against pathogens. Under stress conditions, macrophages and mast cells secrete matrix-remodeling proteins, cytokines and chemokines, which activate local stroma cells to lead circulating leukocytes into damaged tissue (acute inflammation), to eliminate the pathogens [39]. However, when these processes are not resolved could lead to chronic inflammation of the tumour tissue[40].

In several studies the role of tumor microenvironment (TME) gained a lot of interest: in fact the presence of T- lymphocyte subsets correlates with favorable prognosis in some cancers. Furthermore, a high density of CD3+T cells in the TME often associated with CD8+T cells correlates with better progression free survival in ovarian cancer and it is also associated with better prognosis in breast, melanoma, pancreatic and renal cell carcinoma [41]. Inflammation results in change levels of local and circulating neutrophils, lymphocytes and monocytes. These serum markers change their levels and they could be used as markers for host inflammation in many solid tumors including prostate, colon, hepatic and lung [42-46].

TUMOR MICROENVIRONMENT IN GLIOBLASTOMA

Comparing to the other cancers, CNS tumors has a low tumor-infiltrating lymphocytes (TILs) and other immune cell types[47]. This “cold tumor” phenotype is associated with poor responses to immune therapies such as immune checkpoint inhibitors[48]. In brain tumors, T cell can be induced by vaccination, but TIL numbers can remain low, and those cells present an exhausted phenotype. The reduced quantity and limited activity of T cells in CNS tumors is largely owing to the unique immune environment of the brain[49]. Due to the risk of potential damage the CNS may have evolved to be an environment in which both inflammatory and adaptive immune responses are tightly regulated. This regulation involves a variety of immunosuppressive mechanisms [50]. In response to inflammation brain stromal cells produce high levels of the immunosuppressive cytokines transforming growth factor β (TGFβ) and IL-10 to maintain homeostasis.. Glioma cells produce large amounts of indolamine 2,3- dioxygenase (IDO), which both stimulates the accumulation of regulatory T (Treg) cells and suppresses T cell activity by depleting tryptophan from the microenvironment[51]. Both microglia and tumor-infiltrating myeloid cells produce high levels of arginase, which inhibits T cell proliferation and function through the depletion of tissue arginine levels. The strategy of inhibiting specific immunosuppressive mediators (IDO, TGFb, kinase-inhibitors) in patients with brain tumors has not shown promise to date, due to the penetration through blood barrier brain The major cellular component of this microenvironment is tumor-associated macrophages (TAMs), which can

comprise up to 30% of the tumor mass[52]. The vast majority of brain tumor TAMs appear to recruit from circulating monocytes,and only the 15% from microglia. TAMs are believed to promote tumor growth, and TAM numbers correlate with tumor grade and progression. Comparing to regulatory T (Treg) cells, TAMs are a strong predictor of survival for patients with GBM . Mechanistically, TAMs could secret growth factors, cytokines and chemokines to remodel the GBM TME, which enables the tumor cells to proliferate and invasion, survive and promote angiogenesis. Accordingly, targeting these tumor supportive TAMs represents a novel promising treatment strategy to improve the prognosis of GBM patients [53].

TAMs are classified as M1 or M2 polarized cells that are relative with pro-inflammatory/antitumor or anti-inflammatory/pro-tumor property respectively. Interestingly, these two polarized states can convert from each other. TAMs behave the M1 phenotype and exert anti-tumor activities in the early stage of tumor development. The polarization of TAMs from M1 to M2 phenotype is associated with the tumor progression [54].

PART II

BACKGROUND

As already explained, it' s clear that the prognosis of GBM patients remains poor with a 5-year survival of 5% [55]. Traditional chemotherapy has little success, while TMZ is approved, the majority of tumors are MGMT unmethylated and unresponsive to this drug. The failure of current therapy for GBM has prompted researchers to look for novel approaches minimizing harm to health cells. [56-57] Past decades knew a renewed interest in immunotherapy of cancer due to new drugs and effective therapies like immune-checkpoints inhibitors or adoptive T-cell approaches or dendritic cell-based vaccines or combinations of these. Dendritic cells (Dcs) are the most potent professional antigen presenting cells that express both MHC 1 and 2 molecules and are the most efficient stimulus of new T- and B-cell responses. Due to their function of linkage between innate and adaptive immune response, DCs have become a promising way to generate a specific immune response against various cancers [58-60]. DC vaccines have been clinically investigated in a vast range of malignancies including prostate cancer, melanoma, renal cell carcinoma and even glioma [61]. Since 2001, we have treated more than 80 advanced melanoma patients with a tumor lysate loaded autologous DC vaccine, obtaining a clinical benefit of 54.1% without meaningful treatment-related toxicity. Patients developing antitumor immunity after vaccination have a better clinical course, but only two thirds of patients are immune responsive[62]. In these latter patients, DC vaccination induced a significant increase of CD8+ TILs and in general exerts an

important role in sustaining or de novo inducing a T cell inflamed TME[63]. The toxicity profile was very favorable with no grade 3-4 side effects correlated to treatment and only one grade 3 and one grade 4 tromboembolism not treatment-related. In addition, preliminary data obtained in the PD-L1 negative subset of this series indicates that the treatment induces PD-L1 expression in tumor cells in almost all cases. Regarding HGGs (High-grade gliomas) multiple phase I/II trials have been reported; close to 500 patients with GBM have been treated with DC vaccination in more than 38 studies and all documented feasibility and safety [64-66]. Even if the objective response rate was only 15.6% two metanalysis published in 2014 and some controlled studies indicated improved survival (OS) and progression free survival (PFS) with DC vaccination in HGGs patients [67-68]. In 16 non-randomized studies the median OS of newly diagnosed GBM patients ranged from 11.0 to 38.4 months. Moreover, a systematic review by Wang X. of 171 studies confirmed an advantage for DC vaccination in terms of OS and PFS without severe adverse events (Ads) and despite of cycles, doses and route of administration [69]. A recent phase III trial Liau et al compared OS between GBM treated after surgery with DC vaccine plus standard theray and without vaccine. They observed a longer OS in arm treated with DC vaccine [70]. GBM is configured as a typical “immune-deserted” cancer exhibiting a number of systemic and environmental immunosuppressive factors, a scarce immune infiltrate characterized by a paucity of Tcells, a massive recruitment of immunosuppressive cells, a low tumor mutational load (TML) with a consequent low neoantigen burden and

low immunogenicity [71-72]. DC vaccination can be easily integrated into first-line therapy and there is a rational for thus integration:

- After resection/radio-chemotherapy patients are in a state of minimal residual disease which is probably beneficial for immunotherapy because of the lower tumor load and depletion of immunosuppressive cells
- TMZ may reduce regulatory T cell
- The lymphocyte compartment recovering after chemotherapy appears to be beneficial for the induction of anti-tumor responses
- Dying tumor cells after radio-chemotherapy may act as danger signal and boost an effective antitumor immune response.

AIMS

The main aim of this project is to study the role of immune system in GBM. In particular we would like to create a tool consisting of potential prognostic and predictive markers from blood and tumor tissue related to outcome of OS and PFS. This panel could be used also to evaluate the response to DC vaccine. Before to clinical trial with DC vaccine, we studied the relation between inflammation, TME and tumor cells. We performed a retrospective analysis on blood samples of GBM population. Then, we analyzed the immuno-component in tissues samples of enrolled patients and compared hematological markers with immuno tissues. Finally, we conducted a prospective clinical trial on GBM patients enrolled in DC-based vaccination produced at IRST Cell Factory to study not only the efficacy and tolerance combining with standard therapy but also the biological effect of DC vaccine. The enrollment is still ongoing.

RETROSPECTIVE STUDY

OBJECTIVES

- To evaluate the prognostic role of inflammatory blood markers in a cohort of GBM patients receiving a concomitant radio-chemotherapy after surgery to improve the understanding on the systemic inflammation.
- To evaluate the prognostic role of immune infiltrate markers as indicators of tumor microenvironment's state in archival tumor tissues of patients at first diagnosis for GBM underwent to surgery and their relation with blood markers

MATERIALS AND METHODS

This retrospective multicentric study included a case series of patients with a histological diagnosis of GBM referred to the Rare Tumors Center (IRST-Meldola) and Oncology Unit of Rimini between January 2008-2019. All patients had undergone neurosurgery and radio-chemotherapy followed by chemo and were treated with steroid therapy pre and post surgery. We recorded clinical and molecular data about MGMT methylation status, surgery and radio-chemotherapy. NLR and PLR were computed as the ratio of the absolute neutrophil count and absolute platelet count by the absolute lymphocyte count respectively. Systemic Inflammatory Index (SII) was calculated as platelet ×neutrophil/lymphocyte count. The blood markers were evaluated: before neurosurgery, before radio-chemotherapy and at the end of Stupp regimen. Friedman’s test and Bonferroni post-hoc comparison were

used to test the differences over time. Time-dependent receiver operating characteristic (ROC) curve was used to evaluate the capability of each blood marker to classify the patients as alive/death or progressive disease/not and the area under the ROC curve (AUC) was calculated. An optimal cut-point value according to the highest difference between true-positive and false-positive predictions was obtained. Overall survival (OS) was defined as time from the date of start concomitant radio-chemotherapy to the date of death from any cause; progression-free survival (PFS) was computed from date of start concomitant radio-chemotherapy therapy to the date of disease progression or death from any cause, whichever came first. PFS and OS were reported as median values with 95% confidence interval (95%CI). Survival curves were estimated using the Kaplan–Meier method (two-sided 95%CIs) and compared with the log-rank test. Estimated HRs with 95% CI were calculated using univariate and multivariate Cox proportional hazard models. Furthermore, we selected a cohort of 31 pts from our population numerically balanced for pre-surgical SII-high and pre-surgical SII-low, to study their immune infiltrate through the archival formalin-fixed paraffin embedded (FFPE) tissue specimens. Statistical analyses were carried out with Stata software 15.1/SE for Windows, StataCorpLLC, College Station, TX, USA). Time dependent ROC curves were performed using timeROC and survivalROC packages in R software (version 4.2.0). MGMT promoter methylation status was performed on formalin fixed paraffin embedded samples by pyrosequencing technology using a commercially available kit.

IMMUNOHISTOCHEMISTRY ANALYSIS

Surgical specimens of GBM embedded in paraffin were sliced with a rotating microtome (Leica Biosystems, Wetzlar, Germany) and 3-µM-thick sections were mounted on positive-charged microslides (Thermo Fisher Scientific, Waltman, MA, USA). Immunohistochemistry was performed using the VENTANA Benchmark Ultra (Ventana Medical Systems Inc, Tucson, AZ, USA). The following antibodies (Ab) were used against CD3, CD4, CD8, CD20, CD45, CD68, CD163, CD66b and PDL-1 (Tab.1). IHC staining was evaluated when cellularity was sufficient for evaluation. Expression levels were classified according to a Score ranging from 0 to 4 (0=no expression; 1=1-25%; 2=26-50%, 3=51-74%; 4= 75%-100%). The tissue distribution and intensity of each Ab staining was recorded to evaluate biomarker positivity in two-tumoral area: Vascular/perivascular (V) and diffuse in tumour parenchyma (D). Percentage of infiltrating immune system cell was calculated by the rate of absolute number of positive staining cells/total number of cells multiplied by 100. The whole process was supervised by two institute pathologists.

RESULTS

INFLAMMATION MARKERS

Ninety-five patients were considered in this retrospective study: 61 male (64.2%) and 34 female (35.8%) were included and median age was 61 years (range: 37-77), as shown in table 1. Sixty-seven patients (72.8%) had a MGMT ≤30% and were defined as unmethylated and twenty-five (27.2%) were methylated (MGMT≥30%), while three patients had an unknown status.

In table 2, descriptive statistics were reported for all the blood markers that increased significantly among presurgery and pre chemotherapy, as well as among pre chemotherapy and the end of treatment; an exception was made for PLR (p-value 0.570) that had similar values among pre chemotherapy and the end of treatment. ROC curves were used to select an optimal cut-off value for different blood markers (pre-surgery SII, NLR, PLR, pre-chemo SII, NLR, PLR) related to the OS and PFS. We considered both the inflammatory index at pre-surgery and pre-chemotherapy time. AUC value was discriminant especially for pre-chemo-SII at 480 (supplementary table 1). Median overall survival for overall case series was 12.6 months (95%CI: 11.3-16.3). Patients aged <60 years showed better OS respect to patients ≥61 with a median OS of 15.6 months (95%CI:11.3-22.1) VS 11.9 months (95%CI:9.5-14.9,p-value 0.045); methylated patients had a better median OS statistically significant (p-value 0.020). Pre-chemotherapy SII <480 was related to a better OS (median 17.7 months, 95%CI: 12.6-22.2 VS 11.3 months, 95%CI:9.1-12.9 p-value 0.014). Pre-chemo NLR and PLR value didn't show a prognostic role (Table 3). Patients with PLR pre-surgery values <31 had a better OS respect to patients ≥31 (median 14.9 months, 95%CI: 11.8-19.7 VS 8.9 months, 95%CI:5.5-12.2 p-value 0.010).

Multivariable model (table 4) was carried out including age and MGMT, because statistically significant in univariable. Younger age, methylation, low value of prechemo-SII and presurgery-PLR were confirmend as prognostic parameters of OS.

Median progression-free survival for overall series was 6.7 months (95%CI:

5.5-8.8) (table 5). Patients with higher MGMT methylation value had a better median survival (12.2 vs 5.9 months methylated vs unmethylated patients, 95%CI:4.7-7.5, p-value<0.001).Pre-chemotherapy SII <480 was associated to a better PFS: 10.7 VS 5.7 months p-values p.0.04) with a possible prognostic role.NLR and PLR pre-chemo value didn't show a statistically significant prognostic role in PFS. In multivariable model (table 6) not all the variables statistically significant in univariable analysis were included due to collinearity among presurgery and prechemotherapy SII**:** only MGMT maintain an independent prognostic role with a lower risk of death for methylated patients (HR:0.40, 95%CI:0.24-0.66).

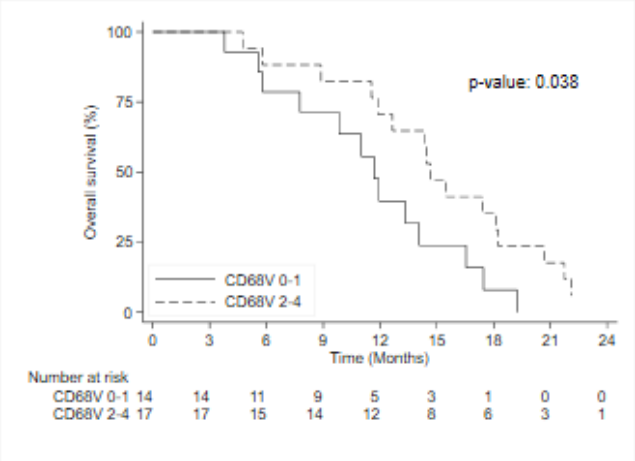
TISSUE IMMUNE-RELATED MARKERS

Evaluation of TILs and TAMs, as previously reported, through their distribution and intensity (score 0-4) as well as their presence in perivascular area (V) and parenchimal tumor (D) was performed on whole slides of 31 resected GBM tissues by IHC. Regarding the immune cells distribution, we considered the CD8/CD163 ratio. Our limit was to work with score (0-4) so we didn't estimate numerically the details. We observed M2 macrophages CD163+ more frequent than lymphocytes according to literature data, in which the necrotic tissue usually is highly infiltrated by macrophages. We have excluded from the analysis PDL-1 and B-lymphocyte subtype marker CD20, because all analyzed tissues were negative for PDL1 and only two were positive for CD20. We focused on the macrophage and monocyte CD68+ and CD66b+ neutrophils that were attracted to the tumor by cytokine during inflammation. Of note, when we correlate the expression level with the

overall survival, we found that CD68-V and CD66b-V showed a statistically significant prognostic role reporting a p-value of 0.038 and 0.029 respectively (Fig 3a, c). CD68-V showed a prognostic role for PFS (p-value 0.027, fig. 3b), while CD66b-V expressions did not (p-value 0.079, fig. 3d). The expression levels of CD3, CD4, CD8, CD45 and CD163 were not associated with OS and PFS. None of the tissue markers tested correlated with SII pre-surgery as marker of inflammation at diagnosis.

Fig.3 a,b Relation between immunomarker of macrophages and overall survival and progression free survival

a)



b)

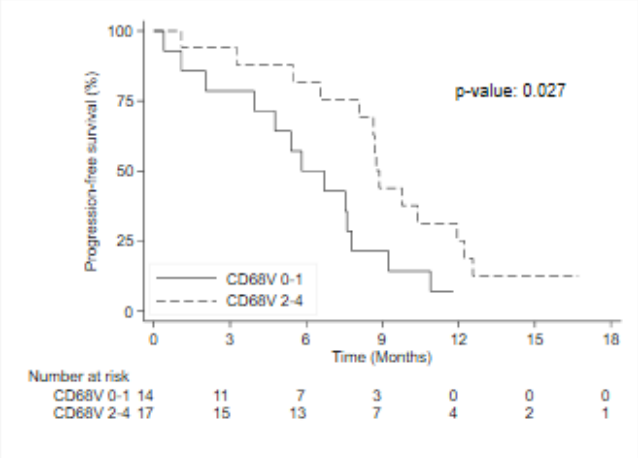
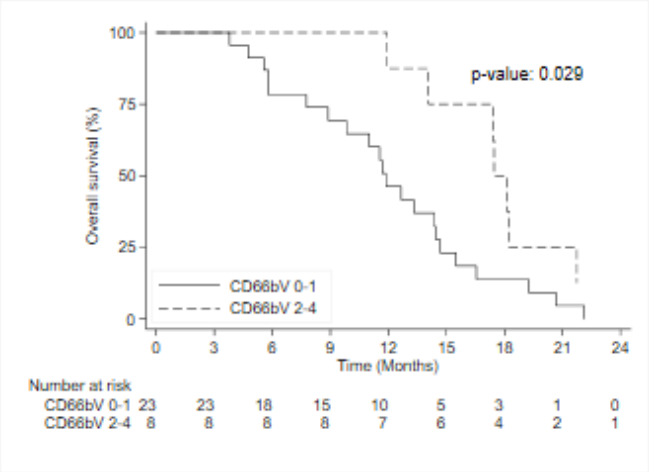
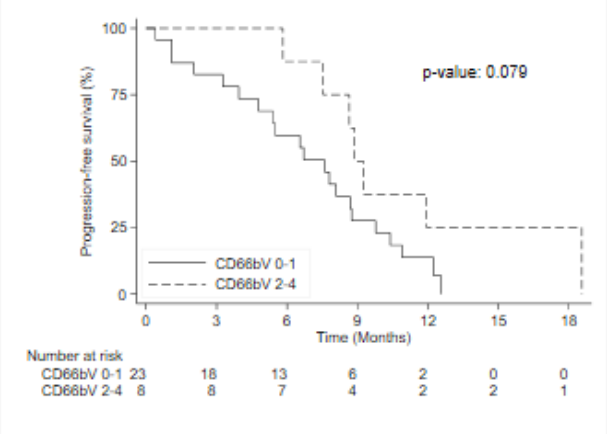


Fig.3 c,d Relation between immunomarker of neutrophils and overall survival and progression free survival

c)



d)



PROSPECTIVE STUDY

OBJECTIVES

Primary End Points

- To assess clinical activity and safety of the vaccination with autologous DCs in GBM patients enrolled in CombiGVax trial after surgery:

  - Progression free survival (PFS), measured as the proportion of patients without progression of disease at three months from leukapheresis.

  - Proportion of patients experienced grade 3 or higher adverse events related to the study treatment

- The evaluation of immune response in vivo, as well as the analysis of immunological efficacy and the efficacy in terms of overall survival.

Secondary End Points

- To assess the Immune response in vivo (Evaluation of the prognostic role of a positive DTH test after at least four vaccine administrations); the clinical outcome (Overall survival) and the Immunological efficacy (ability to enhance the proportion of circulating immune effectors specific for tumor antigens; evaluation of the persistence of an anti-tumor immune response; determination of plasma levels of a panel of inflammatory cytokines and proangiogenic factors; evaluation of the prognostic and predictive role of tumor antigen expression in tumor tissue; analysis of the prognostic and predictive role of immune cells in the peripheral blood and in the tumor microenvironment).

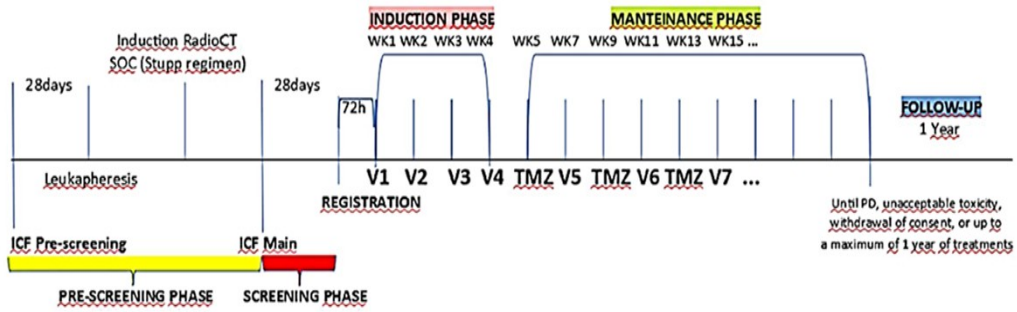
MATERIALS AND METHODS

The study is conducted on patients with GBM, surgically operated, with ≤ 5 ml residual tumor volume treated according to the Stupp regimen. After signing the informed consent form the patient access the procedures to obtain sufficient leukapheretic material for the dendritic cell vaccine (DCv) manufacturing and will perform the standard radiochemotherapy treatment (Stupp regimen). The DCv is produced according GMP procedures in the IRST Cell factory, which is authorized by AIFA for cell therapy production. The “autologous DC loaded with autologous tumor homogenate” is an Advanced Therapy Medicinal Product consisting of DC obtained by in vitro differentiation of peripheral blood monocytes, isolated by leukapheresis from each patient, with IL-4 and GM-CSF. Immature DC such obtained are then loaded with a homogenate of tumor tissue obtained from the same patient, matured with a cytokine cocktail containing IL1b, PGE2, IL6, and TNF-a (“maturation cocktail”). Pulsed mature dendritic cells (mDC) are collected at day 9, washed, counted, tested for quality control (vitality, purity, phenotype markers, sterility, endotoxin, mycoplasma) frozen in aliquots (at least 13×106 cells/aliquot) and stored in nitrogen vapours. The aliquots are thawed and packed in two insulin syringes for administration to the patient (10x106 total DC). The syringes are filled and closed in Class A area and report the product identity. The content of each syringe is administered intradermally with 5 injections in sites close to inguinal or axillary lymph node stations that had not been site of previous surgical exeresis, preferentially alternating injection sites in consecutive vaccine administrations. The vaccine

inoculation will start at the end of concomitant radio-chemotherapy with concurrent TMZ (75mg/m2/Day, 6 weeks). Vaccine cycles will be divided in: Induction phase between the end of radio-chemotherapy and the adjuvant TMZ and Maintenance phase during adjuvant TMZ (150-200mg/M2/day, days 1-5 of 28-day cycle, six or twelve cycles) (Fig.4).

Simon's two-stage design (Simon, 1989) will be used for the sample size calculation. A planned interim analysis will be done after the recruitment of the first 9 evaluable patients for toxicity and for efficacy. If study will not be stopped due to lack of safety or efficacy, a total of 28 evaluable patients will be enrolled for the trial.

Fig. 4 Protocol treatment scheme: Induction phase and Maintenance phase.



Evaluation of toxicity: The patients will be evaluable for toxicity if they perfomed at least one DC vaccination during the induction phase.

Evaluation of response: Time to events (PFS and OS) will be calculated with the Kaplan-Meier method and the analysis was performed on the eligible population. For the primary objective, the proportion of patients without

progression at three months from leukapheresis date will be evaluated. The proportion of patients experiencing vaccine-related grade ≥ 3 AEs during the treatment will be inferred by means of the two-sided Clopper Pearson, or a more appropriate one, 95% confidence interval. Descriptive statistics will be used to assess the extent of the secondary endpoints.

Tumour assessment: brain MRI (iRANO CRITERIA) after induction phase and every 2 cycles of study treatment during maintenance phase. The neuroradiologist will distinguish between real progression of GBM from radionecrosis and pseudo progression by evaluating the 5 ROIs of the perfusion study and correlate them with RANO imaging criteria.

RESULTS INTERIM ANALYSIS

The study was activated on 02/02/2021, and the first patient was enrolled on 27/07/2021. At 05/12/2022, the accrual had 9 patients consisting of 5 male (55.6%) and 4 female (44.4%) and median age was 58.2 years (range: 54.3-69.5), as shown in table 7.

The DCVax was well tolerated. Of 9 pts, no serious adverse events were registered. There were 1 case of neutropenia (grade 4), 1 case of asthenia (grade 3) and 2 cases of thrombocytopenia (grade 2 and 3) related to temozolomide not to the experimental drug. Grade 1-2 toxicities were mostly due to local skin reactions after DCVax inoculation.There was no evidence of any auto-immune reactions or cytokine storm among patients who received DCVax (Table 8). Regarding the analysis of the PFS, at 30/nov/2022, 8 of 9 patients were still alive with a median OS from surgery of 11 months. None of

the patients who discontinued the study due to progression started a second line of treatment. Only one patient died for to deterioration of general conditions for cancer.

DISCUSSION

The prognosis of GBM is poor with a median survival of around 12-14 months and a 5-year survival of less than 5%, even if the pts received Stupp regimen treatment after surgery [1]. In recent years, beyond the molecular factors MGMT and IDH1/2, the hematologic markers of inflammation and immune components in tissue have been the focus of attention in oncology as potential prognostic factors [19-22]. Recent studies have considered GBM to be correlated to chronic inflammation but they didn't considered both components (blood and FFPE) in the same study. Moreover, the blood biomarkers represent an attractive candidate due as it is not an invasive procedure. Starting from this, we conceived this retrospective work combining both analyses. Our series includes 95 patients affected by GBM referred to the Osteoncology and Rare Tumors Center (IRST-Meldola) and Oncology Unit of Rimini. All patients had undergone neurosurgery and radiochemotherapy following by chemo (TMZ). We described the outcomes in terms of OS and PFS of patients according of clinical and molecular data (MGMT). From this first analysis the following emerged: mOS and mPFS of our series are in line with the data reported in the literature. In particular, the relation between the younger age and the presence of highly MGMT methylation were reported as significant positive prognostic role . Among the

objectives of this study there is also the analysis of haematologic markers of inflammation and SII at pre-surgery and pre-treatment time. Inflammatory cells direct the interaction between tumor cells and TME. The first study on inflammatory markers in glioma by Zadora et al concluded that pre surgery NLR values correlate with grade of glioma [73]. In 2018, two studies were published contrasting in results. Yersal et al, considered 80 pts calculating NLR and PLR values pre-chemo. They showed that NLR<4 related to better OS but not statistically significant and no prognostic role of PLR was found. They concluded that these markers were not helpful to predict the prognosis in GBM [74]. Liang et a, used SII to perform differential diagnosis between high and low grade glioma; in particular they observed that the extent of neutrophil infiltration was positively correlated with the grade and number of platelets was linked with tumor progression [75]. In our series, presurgery PLR≥31 had a negative statistically significant impact on OS due to the role of platelet on tumor cell behavior (through TGFb and NF-kB pathways to induce an invasive phenotype). In contrast to their work, we didn't observed the NLR prognostic role both at presurgery and prechemo time. As shown in table 2, our patients had median presurgery-NLR value lower than prechemo (0.76 vs 2.5) probably due to the lymphocytes cells which could try to inhibit cancer development before surgery. Kaya et al in 2017, in a retrospective study, analysed the prognostic significance of the NLR and the PLR as indicators of systemic inflammatory response (SIR, calculated by combination of NLR and PLR) in GBM. They confirmed that OS was significantly correlated with SIR based on NLR count prior to treatment [76].

Our data suggested a different trend of blood markers over time with an important increase in SII, NLR and PLR from presurgery to prechemo samples due to inflammation induced by surgery; later only SII and NLR continued to raise. Applying SIR to our cases, we didn't observe a significant impact (p-value 0.406) but considering prechemo-SII≥480, we confirmed its poor prognostic factor for both OS and PFS which were significantly shorter in these patients (p-value 0.014; p-value 0.004 respectively). Regarding TME in GBM, some studies have already cited the immune suppressive component due to the blood derived macrophages and T-cells exhaustion both causes to GBM treatment and recurrence. All authors agree on the importance to investigate the immunomodulatory mechanisms involved in GBM TME to develop a future immunotherapeutic strategies. In 2020 Koshkaki et al, had published the first study about a different immuno component inside tumor core (TC) comparing to perivascular area (PTA) in nine GBM patients [77]. These cases were enriched in immunosuppressive M2 macrophage (CD163+) in both TC and PTA. The T-cells CD3+ were prevalent in the TC but lower than CD163+ so they explained the suppressive effect of TAM on T cells above all in periphery of TC. Finally, they tested the number of PD1 positive cells which were higher in TC than PTA contributing to TAM immunosuppressive inside TC and this is probably a cause of failure of anti PD1 therapies. In our 31 cases evaluated by IHC, we tested tumor core (D) and perivascular area (V). TIL subpopulation was constituted by a higher number of T-lymphocyte (CD3+) than B-lymphocyte (CD20) and T-lymphocyte CD8+ were the most prevalent . We analyzed the

ratio CD8+/CD163+ highlighting the higher presence of M2-macrophage than T cells both in D and V as reported in literature. When we verified a possible prognostic role of each component, CD68 expressed in vascular area has a positive significant impact on OS and PFS. This relation could be partially explained because TAM do not raise only from peripheral blood, but also from resident microglia. They are the first help to maintain brain homeostasis, but also important to protect brain through their proinflammatory property. Macrophages distributed with a high density in perivascular area where they were ready to migrate from blood vessels inside tumor tissue probably giving a positive impact in outcome. A second explanation, could be the presence of necrosis, above all in large tumor, because necrotic tissue is highly infiltrated by TAM. The role of neutrophils in gliomas has not been sufficiently studied. Fossati G et al. reported that neutrophil infiltration into tumours was significantly correlated with glioma grade and they provide a link between inflammation and progression [78]. In contrast, other studies showed that neutrophils can directly exert important antineoplastic activity as the evidence in other tumours [79]. Most studies shared that TAM can cooperate with CD66b+ to suppress the immuno TME in GBM. Also this evidence, we showed high density in CD66b+ in vascular area probably due to overproduction by tumor cells of the growth factors involved in the recruitment from blood and we had a significant relation with both OS and PFS. In contrast with literature, we did not obtain positivity for PDL1 but it could be a consequence of having old histological material (more than 5 years), or for the limited specificity of the utilized anti-PDL1 clone (SP142)

[80]. Probably due to the small sample size, in our patients there was not a relation between each infiltrate component and pre-surgery SII obtained from their blood. The last part of this PhD project, we conducted a prospective trial enrolling GBM patients in CombiGVax trial to test association of standard therapy with DC vaccine. In January 2023, Liau et al published a phase 3 trial about GBM pts, to compare OS between Stupp regimen vs association with DC vaccine [70]. They enrolled 331 patients and concluded demonstrating a statistically significant longer OS for pts received the combination therapy. Their primary end point was OS but also they planned an exploratory analyses on biomarkers and immunogenicity that may correlate with OS and repsonse to DC vaccine. Our trial is based on Simon's two-stage design so we reached the number of patients needed for the first step evaluation about toxicity and activity: in the first 9 patients, data about toxicity is similar to phase 3 trial with a good safety profile. The toxicities of grade ≥3 are related to TMZ treatment. The secondary endpoints will evaluate the immunological efficacy, similar to our retrospective scheme, through the evaluation of i) circulating immune effectors specific for tumor antigens; ii) plasmatic inflammatory cytokines and proangiogenic factors; iii) prognostic role of the tumor antigen expression in tumor tissue and iv) the prognostic and predictive role of immune cells in the peripheral blood and in the tumor microenvironment.

CONCLUSIONS

The main aim of this PhD-thesis wanted to investigate the role of the immune system in GBM. In particular, the retrospective study confirmed that age and MGMT methylation are still relevant prognostic factors in the choice of treatment. Our data confirmed the role of inflammation in GBM, especially of SII, derived from a combination of value like NLR and PLR as predictive value of response to Stupp regimen. Despite having a smaller cohort of tissues, the study on immuno infiltrate showed a different expression of a panel of immuno markers but with statistically significant value in OS and PFS for macrophages and neutrophils in vascular area. This difference is further confirmation that GBM is a heterogeneous disease in the tumor core and the perivascular area, so it will be important to create the future studies testing target therapy for the different component of TME. A limit of our data, is certainly that markers were tested by IHC on histological tissue older than 5 years, but they still offer an ideas on the role of TME and how to project the new immunotherapies. Finally, the preliminary data of CombiGVax trial and in particular, the lack of relevant toxicity deserved to be confirmed with an expansion of the case series, thus continuing the enrollment until a total of 28 patients. In the future, we will share the data about efficacy and the secondary endpoint based on immuno activity.

APPENDIX

Table 1. Patients characteristics (n=95)

Patients characteristics	N (%)
Gender	
Male	61 (64.2)
Female	34 (35.8)
Age at diagnosis	
Median (range)	61 (37-77)
MGMT (2)	
Unmethylated (0-29%)	67 (72.8)
Methylated (>=30%)	25 (27.2)
Unknown	3
Subsite of disease	
Occipitale	11 (11.9)
Frontale	32 (34.8)
Temporale	24 (26.1)
Parietale	19 (20.7)
Multifocale	4 (4.4)
Mesencefalo	2 (2.2)
Unknown	3
Surgery	
Gross total removal	35 (37.2)
No gross total removal	59 (62.8)
Unknown –No surgery?	1
PS (ECOG)	
0	35 (36.8)
1	52 (54.8)
2	6 (8.4)
N Temodal cycles	
None	17 (17.9)
1-6	53 (55.8)
>6	25 (26.3)

Table 2: Variation of blood markers over time

Blood markers	Pre surgery value (1) Median (iqr range)	Pre chemo value (2) Median (iqr range)	Post treatment value (3) Median (iqr range)	p-value from Friedman's test		
					2 vs 1	3 vs 2
	N=95	N=95	N=70			
SII	153.7 (126.6-192.0)	604.7 (396.9-1042.1)	576.3 (288.1-1133.3)	<0.001	<0.001	<0.001
NLR	0.76 (0.65-0.82)	2.5 (1.7-3.8)	3.7 (2.5-5.8)	<0.001	<0.001	<0.001
PLR	20.3 (14.1-30.3)	105.3 (75.0-163.6)	119.9 (85.1-171.8)	<0.001	<0.001	0.570

Table 3: Univariable analysis for overall survival

Variables	N° pts	N. deaths	Median OS (95%CI)	p- value (log-rank test)
All pts	95	84	12.6 (11.3-16.3)	-
Gender				
Male	61	55	12.5 (10.5-15.6)	0.190
Female	34	29	16.3 (9.4-23.1)	
Age at therapy start				
<60 years	44	38	15.6 (11.3-22.1)	0.045
≥60 years	51	46	11.9 (9.5-14.9)	
MGMT (30%)				
Unmethylated (0-29%)	67	61	12.2 (10.3-15.6)	0.020
Methylated (>=30%)	25	20	19.7 (11.3-37.4)	
Surgery				
Gross total removal	35	30	13.6 (10.3-19.7)	0.853
No gross total removal	59	53	12.6 (11.1-16.7)	
PS (ECOG)				
0	35	31	15.9 (11.3-21.3)	0.314
>0	60	53	12.2 (9.5-14.9)	
SII **presurgery** value				
SII<146.6	40	35	16.3 (12.9-19.8)	0.109
SII≥146.6	55	49	10.6 (9.1-12.2)	
SII **prechemo** value				
SII <480	36	31	17.7 (12.6-22.2)	0.014
SII ≥480	59	53	11.3 (9.1-12.9)	
NLR **presurgery** value				
NLR <0.87	80	70	14.3 (11.8-17.7)	0.542
NLR ≥0.87	15	14	9.7 (8.0-11.8)	
NLR **prechemo** value				
< 2.2	42	36	14.0 (11.3-20.6)	0.075
≥ 2.2	53	48	11.9 (9.1-15.6)	
PLR **presurgery** value				
< 31	75	65	14.9 (11.8-19.7)	0.010
≥ 31	20	19	8.9 (5.5-12.2)	
PLR **prechemo** value				
< 110	51	47	15.0 (11.3-19.8)	0.306
≥ 110	44	37	11.8 (8.0-15.5)	
Combination of NLR and PLR				
NLR ≥5 or PLR ≥150	32	28	12.6 (6.5-18.5)	0.406
NLR <5 and PLR <150	63	56	12.9 (11.1-17.7)	

NE → not estimable from statistical package

Table 4: Univariable and multivariable models for overall survival

Characteristics	Overall Survival		
	HR from univariable model (95%CI)	p-value	HR from multivariable model (95%CI)
Age at therapy start			
<60 years	1.00 (referent)	0.047	1.00 (referent)
≥60 years	1.56 (0.99-2.43)		1.85 (1.13-3.02)
MGMT			
0-29%	1.00 (referent)		1.00 (referent)
≥30%	0.54 (0.32-0.91)	0.023	0.51 (0.30-1.67)
Prechemo SII			
<480	1.00 (referent)		1.00 (referent)
≥480	1.74 (1.11-2.74)	0.015	1.76 (1.10-2.81)
Presurgery PLR			
< 31	1.00 (referent)		1.00 (referent)
≥ 31	1.99 (1.17-3.40)	0.012	1.83 (1.04-3.20)

Table 5: Univariable analysis for progression-free survival

Variables	N° pts	N. PD	Median PFS (95%CI)	p- value (log-rank test)
All pts	95	93	6.7 (5.5-8.8)	-
Gender				
Male	61	60	7.4 (5.5-9.4)	0.261
Female	34	33	5.9 (3.9-11.7)	
Age at therapy start				
<60 years	44	43	8.8 (5.8-11.1)	0.158
≥60 years	51	50	5.7 (4.4-8.7)	
MGMT (30%)				
Unmethylated (0-29%)	67	67	5.9 (4.8-7.4)	<0.001
Methylated (>=30%)	25	23	12.2 (9.5-20.4)	
Surgery				
Gross total removal	35	34	7.3 (5.1-10.5)	0.603
No gross total removal	59	58	6.7 (5.7-9.2)	
PS				
0	35	35	8.7 (5.5-10.7)	0.735
>0	60	58	6.5 (5.2-9.2)	
N Temodal cycles				
None	17	17	3.0 (2.1-4.9)	
1-6	53	52	6.0 (5.4-7.4)	<0.001
>6	25	24	15.4 (11.9-20.4)	
SII **presurgery** value				
SII<146.6	44	43	10.4 (7.5-12.2)	0.045
SII≥146.6	51	50	5.7 (4.4-6.7)	
SII **prechemo** value				
SII <480	36	35	10.7 (8.7-15.4)	0.004
SII ≥480	59	58	5.7 (4.9-6.7)	
NLR **presurgery** value				
< 0.87	80	78	6.7 (5.5-9.4)	0.931
≥ 0.87	15	15	5.7 (2.7-11.1)	
NLR **prechemo** value				
< 2.2	42	40	9.2 (5.7-11.8)	0.023
≥ 2.2	53	53	5.9 (5.1-7.4)	
PLR **presurgery** value				
< 31	75	73	8.1 (5.8-10.4)	0.198
≥ 31	20	20	5.1 (3.4-8.8)	
PLR **prechemo** value				
< 110	51	51	10.5 (6.5-12.0)	0.046
≥ 110	44	42	5.5 (4.3-6.7)	
Systemic inflammatory response from prechemo values (**prechemo**)				

Variables	N° pts	N. PD	Median PFS (95%CI)	p- value (log-rank test)
NLR ≥5 or PLR ≥150	32	2	5.5 (3.4-6.7)	0.064
NLR <5 and PLR <150	63	1	8.8 (6.1-10.9)	

Table 6: Univariable and multivariable models for progression-free survival

Characteristics	Progression-free Survival		
	HR from univariable model (95%CI)	p-value	HR from multivariable model (95%CI)
MGMT (30%)			
Unmethylated (0-29%)	1.00 (referent)		1.00 (referent)
Methylated (>=30%)	0.40 (0.25-0.66)	<0.001	0.40 (0.24-0.66)
SII presurgery value			
SII<146.6	1.00 (referent)		
SII≥146.6	1.52 (1.01-2.32)	0.048	
Pre chemo SII			
<480	1.00 (referent)		1.00 (referent)
≥480	1.86 (1.20-2.88)	0.005	1.94 (0.92-4.09)
Pre chemo NLR			
< 2.2	1.00 (referent)		1.00 (referent)
≥ 2.2	1.61 (1.06-2.46)	0.025	0.84 (0.43-1.64)
Pre chemo PLR			
< 110	1.00		1.00
≥ 110	1.52 (1.01-2.31)	0.048	1.07 (0.61-1.89)

Table 7: Patient characteristics of first 9 patients enrolled in Combi-GVax study

Variable	Overall n= 9 (%)
Age	
Median (range)	58.2 (54.3-69.5)
Sex	
Male	5 (55.6)
Female	4 (44.4)
Tumor site of disease	
Frontal lobe	3 (33.1)
Fronto-parietal lobe	1 (11.1)
Occipital lobe	1 (11.1)
Parietal lobe	1 (11.1)
Temporal lobe	3 (33.1)
Laterality	
Right	5 (55.6)
Left	4 (44.4)
MGMT	
0-9%	4 (44.4)
10-29%	3 (33.3)
≥30%	2 (22.3)
IDH1 (IHC)	
Not mutated	2 (22.3)
Wild type	7 (77.7)
IDH1 Molecular test	
Not mutated	3
Not done	6
IDH2 Molecular test	
Not mutated	2

Not done	7
Ki67 value	
10	1 (11.1)
25	5 (55.6)
30	2 (22.2)
40	1 (11.1)

Table 8: Targeted AEs reported among patients with at least 1 vaccine administrations and 30 days of follow-up on first 9 patient of Combi-Gvax study

AE	N° of patients (%)		
	G1	G2	G3
Asthenia	2	0	1
Fatigue	1		
Local reaction at vaccine	3	0	0
Nausea	1	1	0
Nervous system disorder	1	0	0
Neutropenia	2	0	0
Pain, specify	1	0	0
Pruritus, spec if gen	3	1	0
Redness in site of injection	1	0	0
Skin, specify	1	0	0
Thrombocytopenia	0	1	1
Constipation	1	0	0
Hypokalemia	0	1	0
Insomnia	2	0	0

*Maximum grade consolidates the reports *of a given type of AE* for a patient over time by taking the maximum across time;

Supplementary table 1: AUC, Sensitivity and specificity values for different blood markers at 3 and 12 months

Inflammatory index	Outcome	3 months AUC (95%CI)	12 months AUC (95%CI)	Proposed cut off	3 months Sens (%)	12 months Sens (%)	12 months Spec (%)
Pre surgery SII	OS	70.65 (41.67-99.63)	68.67 (57.89-79.45)	146.3	66.7	76.7	58.0
	PFS	65.19 (52.3-78.07)	65.83 (53.29-78.36)	146.3	81.3	65.7	60.0
Pre surgery NLR	OS	68.84 (47.85-89.83)	56.02 (44.21-67.84)	0.87	33.3	25.6	92.0
	PFS	54.51 (39.38-69.64)	59.14 (45.93-72.35)	0.87	25.0	21.4	88.0
Pre surgery PLR	OS	51.09 (24.27-77.90)	56.49 (44.61-68.38)	31	0.0	30.1	88.0
	PFS	50.24 (36.14-64.33)	48.74 (36.29-61.19)	31	18.8	24.3	88.0
Pre chemotherapy SII	OS	80.07 (64.85-95.30)	60.21 (48.66-71.76)	480	100.0	76.7	52.0
	PFS	57.20 (40.53-73.87)	63.54 (51.03-76.05)	480	68.8	71.4	64.0
Pre chemotherapy NLR	OS	67.75 (54.60-80.91)	56.46 (44.81-68.10)	2.2	100.0	60.4	50.0
	PFS	55.3 (403.82-69.78)	59.9 (48.16-71.79)	2.2	68.8	61.4	60.0
Pre chemotherapy PLR	OS	73.91 (49.15-98.68)	56.56 (44.76-68.39)	110	66.7	53.3	62.0
	PFS	59.7 (44.78-74.68)	62.23 (49.66-74.80)	110	62.5	54.3	76.00.00

BIBLIOGRAPHY

1.Ostrom QT, Bauchet L, Davis FG et al. The epidemiology of glioma in adults: a "state of the science" review. Neuro Oncol. 2014 May 19.

2.Siegel R. Cancer Statistics, 2013. A Cancer Journal of Clinicians 2013; 63:11–30.

3.Kohler BA, Ward E, McCarthy BJ, et al. Annual report to the nation on the status of cancer, 1975-2007, featuring tumors of the brain and other nervous system. J Natl Cancer Inst 2011; 103:714.

4.Wrensch M, Minn Y, Chew T, et al. Epidemiology of primary brain tumors: current concepts and review of the literature. Neuro Oncol 2002; 4:278

5.Crocetti E, Trama A, Stiller C et al. Epidemiology of glial and non glial brain tumours in Europe. EJC 2012

6.Ferlay J, Parkin DM, Steliarova-Foucher E. Estimates of cancer incidence and mortality in Europe in 2008. Eur J Cancer 2010; 46: 765–781.

7.Wen PY, Fine HA, Black PM, et al. High-grade astrocytomas. Neurol Clin 1995; 13:875

8.Up to date, version 2013.

9.Radhakrishnan K, Mokri B, Parisi JE, et al. The trends in incidence of primary brain tumors in the population of Rochester, Minnesota. Ann Neurol 1995; 37:67

10.Polednak AP. Interpretation of secular increases in incidence rates for primary brain cancer in Connecticut adults, 1965-1988. Neuroepidemiology 1996; 15:51.

11.CBTRUS Report, 2004-2005

12.Ohgaki H, Kleihues P. The definition of primary and secondary glioblastoma. Clin Cancer Res. 2013 Feb 15:19 (4):764-72.

13.www.rarecarenet.eu

14.https://www.registri-tumori.it/cms/pubblicazioni/i-tumori-italia-rapporto-2015-i-tumori-rari-italia

15.Ohgaki H, Kleihues P. Genetic Pathways to Primary and Secondary Glioblastoma. Am J Pathol. May 2007; 170(5): 1445–1453.

16.David N Louis et al. The 2021 WHO Classification of Tumors of the Central Nervous System: a summary. Neuro Oncol 2021 Aug 2;23(8):1231-1251

17.L Eric Huang. Impact of CDKN2A/B Homozygous Deletion on the Prognosis and Biology of IDH-Mutant Glioma. Biomedicines 2022 Jan 24;10(2):246

18.Gerson SL. MGMT: its role in cancer aetiology and cancer therapeutics. Nat Rev Cancer.2004 Apr;4 (4):296-307.

19.Esteller M, Garcia-Foncillas J, Andion E et al. Inactivation of the DNA-repair gene MGMT and the clinical response of gliomas to alkylating agents. N Engl J Med.2000 Nov 9; 343(19):1350-4

20.Adam Cohen, MD1, Sheri Holmen, PhD2, and Howard Colman. IDH1 and IDH2 Mutations in Gliomas.Curr Neurol Neurosci Rep. 2013 May; 13(5): 345.

21.Petr Ježek. 2-Hydroxyglutarate in Cancer Cells. Antioxidants & redox signaling Volume 33, Number 13, 2020.
22.Sue Han1 , Yang Liu1 , Sabrina J. Cai1 et al. IDH mutation in glioma: molecular mechanisms and potential therapeutic targets. British Journal of Cancer (2020) 122:1580–1589
23.Wikstrand CJ, McLendon RE, Friedman AH: Cell surface localization and density of the tumor-associated variant of the epidermal growth factor receptor, EGFRvIII. Cancer Res 1997;57: 4130– 40.
24.Ekstrand AJ, Longo N, Hamid ML, et al. Functional characterization of an EGF receptor with a truncated extracellular domain expressed in glioblastomas with EGFR gene amplification. Oncogene 1994;9:2313–20.
25.Heimberger AB: Prognostic effect of epidermal growth factor receptor andEGFRvIII in glioblastoma multiforme patients. Clin Cancer Res 2005, 1462–1466
26.Shinojima N, Tada K, Shiraishi S,et al.Prognostic value of epidermal growth factor receptor in patients with glioblastoma multiforme. Cancer Res. 2003 Oct 15;63 (20):6962-70.
27.Sanai N: An extent of resection threshold for newly diagnosed glioblastomas; J Neurosurg. 2011 Jul; 115 (1):3-8.
28.Wood JR: The prognostic importance of tumor size in malignant gliomas: a computed tomographic scan study by the Brain Tumor Cooperative Group, JCO Jul 10, 2008:3387-3394.
29.Andersen AP: Postoperative irradiation of glioblastomas. Results in a randomized series. Acta Radiol Oncol Radiat Phys Biol. 1978; 17 (6):475.
30.Walker MD, Green SB, Byar DP, et al. Strike TA. Randomized comparisons of radiotherapy and nitrosoureas for the treatment of malignant glioma after surgery. N Engl J Med. 1980; 303 (23):1323
31.Stewart LA. Chemotherapy in adult high-grade glioma: a systematic review and meta-analysis of individual patient data from 12 randomised trials: Lancet. 2002; 359 (9311):1011.
32.R.Stupp et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. Lancet Oncol. 2009 May;10(5):459-66.
33.Ewelt C. Glioblastoma multiforme of the elderly: the prognostic effect of resection on survival. J Neurooncol. 2011 Jul; 103 (3):611-8
34.Wick W, Platten M, Meisner C, Felsberg J,et al; NOA-08 Study Group of Neuro-oncology Working Group (NOA) of German Cancer Society. Temozolomide chemotherapy alone versus radiotherapy alone for malignant astrocytoma in the elderly: the NOA-08 randomised, phase 3 trial. Lancet Oncol. 2012 Jul;13(7):707-15.

35.Annika Malmström,Bjørn Henning Grønberg, Christine Marosi et al. Temozolomide versus standard 6-week radiotherapy versus hypofractionated radiotherapy in patients older than 60 years with glioblastoma: the Nordic randomised, phase 3 trial. Lancet Oncol. 2012 Sep;13(9):916-26

36.Hanahan D., Weinberg R.A. Hallmarks of cancer: The next generation. Cell. 2011;144:646–674.

37.Hanahan D. Hallmarks of Cancer: New Dimensions. Cancer Discov. 2022 Jan;12(1):31-46.

38.Bharat B Aggarwal 1, R V Vijayalekshmi, Bokyung Sung. Targeting inflammatory pathways for prevention and therapy of cancer: short-term friend, long-term foe. Clin Cancer Res. 2009 Jan 15;15(2):425-30

39.L.M. Coussens, L. Zitvogel, A.K. Palucka. Neutralizing Tumor-Promoting Chronic Inflammation: A Magic Bullet? Science 2013 Jan 18;339(6117):286-91

40.Mahin Khatami. .Inflammation, aging, and cancer: tumoricidal versus tumorigenesis of immunity: a

41.Zhen Zhang et al. T Cell Dysfunction and Exhaustion in Cancer. Front Cell Dev Biol. 2020 Feb 11;8:17.

42.Sharma G, Jain SK, Sinha VD. Peripheral Inflammatory Blood Markers in Diagnosis of Glioma and IDH Status. J Neurosci Rural Pract. 2021 Jan;12(1):88-94

43.You J, Zhu G Q, Xie L et al. Preoperative platelet to lymphocyte ratio is a valuable prognostic biomarker in patients with colorectal cancer. Oncotarget. 2016;7(18):25516–25527.

44.Sato H, Tsubosa Y, Kawano T. Correlation between the pretherapeutic neutrophil to lymphocyte ratio and the pathologic response to neoadjuvant chemotherapy in patients with advanced esophageal cancer. World J Surg. 2012;36(03):617–622. [PubMed] [Google Scholar]

45.Xue T C, Zhang L, Xie X Y et al. Prognostic significance of the neutrophil-to-lymphocyte ratio in primary liver cancer: a meta-analysis. PLoS One. 2014;9(05):e96072.

46.Cedrés S, Torrejon D, Martínez A et al. Neutrophil to lymphocyte ratio (NLR) as an indicator of poor prognosis in stage IV non-small cell lung cancer. Clin Transl Oncol. 2012;14(11):864–869.

47.De Felice F, Musio D, Cassese R, Gravina GL, Tombolini V.New Approaches in Glioblastoma Multiforme: The Potential Role of Immune- check Point Inhibitors.Curr Cancer Drug Targets. 2017;17(3):282-289.

48.Kurz SC1, Wen PY2. Quo Vadis-Do Immunotherapies Have a Role in Glioblastoma? Curr Treat Options Neurol. 2018 Apr 18;20(5):14.

49. Sampson JH, Gunn MD, Fecci PE, Ashley DM. Brain immunology and immunotherapy in brain tumours.Nat Rev Cancer. 2020;20(1):12-25. doi:10.1038/s41568-019-0224-7

50.Ransohoff RM, Brown MA. Innate immunity in the central nervous system. J Clin Invest. 2012;122(4):1164-1171. doi:10.1172/JCI58644

51.Hosseinalizadeh H, Mahmoodpour M, Samadani AA, Roudkenar MH. The immunosuppressive role of indoleamine 2, 3-dioxygenase in glioblastoma: mechanism of action and immunotherapeutic strategies. Med Oncol. 2022;39(9):130. 2022 Jun 18.

52.Vinogradov S, Warren G, Wei X. Macrophages associated with tumors as potential targets and therapeutic intermediates. Nanomedicine (Lond). 2014;9(5):695-707. doi:10.2217/nnm.14.13

53.Jun Wei. The controversial role of microglia in malignant gliomas. Clin Dev Immunol. 2013;2013:285246

54.Wang G, Zhong K, Wang Z, et al. Tumor-associated microglia and macrophages in glioblastoma: From basic insights to therapeutic opportunities. Front Immunol. 2022;13:964898. Published 2022 Jul 27.

55.Ostrom QT, Bauchet L, Davis FG, Deltour I, Fisher JL, Langer CE, Pekmezci M, Schwartzbaum JA, Turner MC, Walsh KM, Wrensch MR, Barnholtz-Sloan JS. The epidemiology of glioma in adults: a "state of the science" review. Neuro Oncol. 2014 Jul;16(7):896-913

56.Stupp R, Hegi ME, Mason WP, van den Bent MJ, et al,; European Organisation for Research and Treatment of Cancer Brain Tumour and Radiation Oncology Groups; National Cancer Institute of Canada Clinical Trials Group. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. Lancet Oncol. 2009 May;10(5):459-66. doi: 10.1016/S1470-2045(09)70025- 7.

57.Stupp R, Taillibert S, Kanner AA, et al. Maintenance Therapy With Tumor-Treating Fields Plus Temozolomide vs Temozolomide Alone for Glioblastoma: A Randomized Clinical Trial JAMA. 2015 Dec 15;314(23):2535-43

58.Palucka K, Banchereau J.Cancer immunotherapy via dendritic cells. Nat Rev Cancer. 2012 Mar 22;12(4):265-77.

59.Palucka K, Banchereau J Dendritic-cell-based therapeutic cancer vaccines. Immunity. 2013 Jul 25;39(1):38-48

60.Huber A, Dammeijer F, Aerts JGJV, Vroman H.Current State of Dendritic Cell-Based Immunotherapy: Opportunities for in vitro Antigen Loading of Different DC Subsets? Front Immunol. 2018 Dec 3;9:2804.

61.Rodríguez Pérez Á, Campillo-Davo D, Van Tendeloo VFI, Benítez-Ribas D. Cellular immunotherapy: a clinical state-of-the-art of a new paradigm for cancer treatment. Clin Transl Oncol. 2020 Apr 7.

62.De Rosa F, Ridolfi L, Fiammenghi L,et al. Dendritic cell vaccination for metastatic melanoma: a 14-year monoinstitutional experience. Melanoma Res. 2017 Aug;27(4):351-357.

63.Bulgarelli J, Tazzari M, Granato et al. Dendritic Cell Vaccination in Metastatic Melanoma Turns "NonT Cell Inflamed" Into "T-Cell Inflamed" Tumors. Front Immunol. 2019 Oct 9;10:2353

64.Polyzoidis S, Tuazon J, Brazil L et al. Active dendritic cell immunotherapy for glioblastoma: Current status and challenges. Br J Neurosurg. 2015 Apr;29(2):197-205 11.

65.Liau LM, Ashkan K, Tran DD, Campian JL et al First results on survival from a large Phase 3 clinical trial of an autologous dendritic cell vaccine in newly diagnosed glioblastoma. J Transl Med. 2018 May 29;16(1):142

66.Everson RG, Antonios JP, Liau LM.Cell-Based Immunotherapy of Gliomas.Prog Neurol Surg. 2018;32:90-100

67.Cao JX, Zhang XY et al. Clinical efficacy of tumor antigen pulsed DC treatment for high-grade glioma patients: evidence from a metaanalysis. PLoS One. 2014;9:e107173.

68.Eagles ME1, Nassiri F2,3, Badhiwala JH et al.Dendritic cell vaccines for high-grade gliomas.Ther Clin Risk Manag. 2018 Jul 26;14:1299-1313

69.Wang X, Zhao HY, Zhang FC,et al.Dendritic cell-based vaccine for the treatment of malignant glioma: a systematic review.Cancer Invest. 2014 Nov;32(9):451-7

70.Linda M Liau et al. Association of Autologous Tumor Lysate-Loaded Dendritic Cell Vaccination With Extension of Survival Among Patients With Newly Diagnosed and Recurrent Glioblastoma A Phase 3 Prospective Externally Controlled Cohort Trial. JAMA Oncol 2023 Jan 1;9(1):112-121.

71.Dunn GP, Fecci PE, Curry WT Cancer immunoediting in malignant glioma. Neurosurgery. 2012 Aug;71(2):201-22

72.Medikonda R, Dunn G, Rahman M et al. A review of glioblastoma immunotherapy.J Neurooncol. 2020 Apr 6.

73.Zadora P, Dabrowski W, Czarko K, et al. Preoperative neutrophil-lymphocyte count ratio helps predict the grade of glial tumor - A pilot study. Neurol Neurochir Pol 2015; 49(1):41–44)

74.Yersal Ö, Odabaşi E, Özdemir Ö, Kemal Y. Prognostic significance of pre-treatment neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio in patients with glioblastoma. Mol Clin Oncol 2018 Oct;9(4):453-458

75.Liang R, Chen N, Li M, Wang X, Mao Q, Liu Y. Significance of systemic immune-inflammation index in the differential diagnosis of high- and low-grade gliomas. Clin Neurol Neurosurg. 2018 Jan;164:50-52. doi: 10.1016/j.clineuro.2017.11.011. Epub 2017 Nov 15. PMID: 29175722.

76.Kaya V, Yıldırım M, Yazıcı G, et al. Prognostic Significance of Indicators of Systemic Inflammatory Responses in Glioblastoma Patients. Asian Pac J Cancer Prev. 2017;18(12):3287-3291. Published 2017 Dec 29. doi:10.22034/APJCP.2017.18.12.3287

77. Rahimi Koshkaki H, Minasi S, Ugolini A, et al. Immunohistochemical Characterization of Immune Infiltrate in Tumor Microenvironment of Glioblastoma.J 76. Pers Med. 2020;10(3):112. Published 2020 Sep 3. doi:10.3390/jpm10030112
78. Fossati G, Ricevuti G, Edwards SW, Walker C, Dalton A, Rossi ML. Neutrophil Infiltration Into Human Gliomas. Acta Neuropathol (1999) 98(4):349–54)
79. Oberg HH, Wesch D, Kalyan S, Kabelitz D. Regulatory Interactions Between Neutrophils, Tumor Cells and T Cells. Front Immunol. 2019 Jul 18;10:1690. doi: 10.3389/fimmu.2019.01690. PMID: 31379875; PMCID: PMC6657370
80. Ruo Qiao Chen. The Prognostic and Therapeutic Value of PD-L1 in Glioma. Front Pharmacol. 2019 Jan 9;9:1503.