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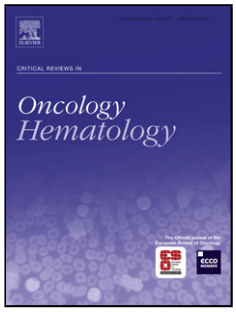


Anaplastic Astrocytoma: state of the art and future directions

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# Anaplastic Astrocytoma: state of the art and future directions

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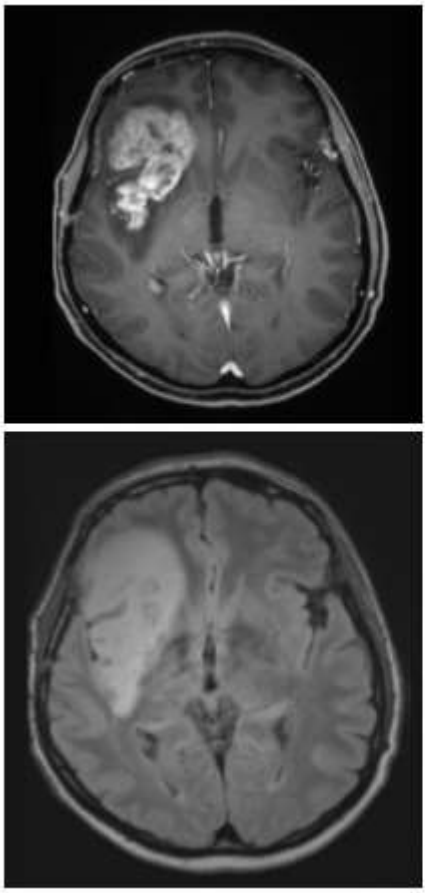
## Graphical abstract






**Abstract**

Anaplastic Astrocytoma(AA) is a malignant, diffusely infiltrating, primary brain tumor. According to the WHO 2016 classification of central-nervous-system tumors, AA has been described as a glial tumor with no co-deletion of 1p/19q, and is divided into IDHmutated tumor, characterized by better prognosis, and IDHwild-type form, with worse prognosis. The standard of care is maximal safe resection followed by radiotherapy and chemotherapy with temozolomide. Several efforts have been made to evaluate, according to molecular selection, which is the best post-surgical treatment. At recurrence, the treatment remains challenging and some trials are ongoing to evaluate new potential drugs,alone or in combination with chemotherapy. We perfomed a description of the status of the art on diagnosis, molecular characteristics and treatment of AA. In particular, we focused our details on

new drugs; indeed, a deeper knowledge of the molecular characteristics of gliomas could lead to to development of active personalized treatments according with precision medicine.

Keywords: anaplastic astrocytoma, new drugs, glioma

**Introduction**

Anaplastic Astrocytoma (AA - World Health Organization grade III) is a diuffusely infiltrating, malignat primary brain tumor arising from the neoplastic transformation of astrocytic cells, that usually evolves into (World Health Organization grade IV). The median age of onset is 41 years[1], although *IDH* mutated AA may occur at an earlier age[2]. AA affect males slightly more than females and represents 6-7 % of all gliomas and 1.7% of all tumors with 5-year survival rate of 30% and median OS of 3 years. The WHO 2016 classification[3] marked a revolution in the diagnostic and prognostic approach to gliomas, with the consideration of molecular characteristics in addition to the morphological aspects of the tumor. AA has both *IDH* wild-type and *IDH*-mutant variants, and unlike the anaplastic oligodendroglioma, without *1p/19q* codeletion. *IDH* wt gliomas usually have a more aggressive trend although the morphological characteristics are quite similar to IDH mut glioma. Furthermore, as shown by Christians et al [4], patients with IDHwt AA and patients with IDHwt GBM receiving the same treatment demonstrated a comparable prognosis. In particular, in the absence of IDH mutations, the histopathological grading criteria (necrosis and vascular proliferation) lost their prognostic significance; conversely, in the cases with IDH mutations, the presence of necrosis and vascular proliferation remained an important prognostic factor. In this type of tumor (*IDH*wt glioma), other molecular alterations are often present, such as *EGFR* amplification and *TERT* promoter mutation[5,6] , which makes them much more similar to glioblastoma from the molecular point of view. The backbone treatment of anaplastic astrocytoma remains, when possible, the maximal safe surgical

resection[7]; a second surgery could be considered in cases of incomplete resection at first surgery or in case of recurrent disease that is liable to new surgery. Radiotherapy and chemotherapy usually represent the post-surgical treatment of anaplastic astrocytoma as demonstrated by several trials that we will explore in detail below. In this review, we will analyze the clinical and molecular characteristics of AA with particular attention to treatment, based on the most recent literature; we will also evaluate future perspectives and ongoing trials that involve new drugs and different therapeutic approaches.

**Histology and Molecular Features**

AA often have heterogeneous morphological characteristics that can make histological diagnosis difficult; it is classified, according to WHO classification of CNS tumors[3], as a grade III anaplastic glioma. The main morphological features that characterize anaplastic astrocytoma are: increased cellularity (greater than diffuse grade II astrocytoma), MIB-1 labeling index of 5-10%[8] and possible tumor areas with poor cellularity but with a high percentage of mitoses that are still considered anaplastic, nuclear pleomorphism and atypia, presence of glial markers, absence of neuronal markers and absence of necrosis and vascular proliferation, the latter normally present in glioblastoma[9]. A diagnosis based only on the morphological characteristics may have several limitations because it is affected by high intra-operator and inter-operator variability with significant differences in terms of outcome in patients with the same histological diagnosis[10]. Thanks to the recent introduction of molecular markers for CNS tumors[3], the diagnosis of glioma is now based on molecular characteristics, with the possibility of obtaining more information, not only in prognostic terms but also in terms of predictive response to cancer treatments. No specific molecular alteration identifies AA but the 2016 WHO classification distinguishes these diagnoses into isocitrate dehydrogenase wild-type (*IDH*wt) and in *IDH* mutant (IDHmut)[3] with very different clinical and prognostic characteristics; these alterations seem to have a fundamental role in the pathogenesis of several

anaplastic astrocytomas[11,12]. *IDH* enzymes are involved in metabolic conversion of isocitrate to alfa-ketoglutarate, a very important metabolite of the Krebs cycle, using *NAD+* as a cofactor for alfa-ketoglutarate synthesis and *NADPH* for a reversible reaction[12]. *IDH* mutations usually involve an arginine residue causing a gain-of-function of the enzymes converting alpha-ketoglutarate into the catabolite D-2-hydroxyglutrate (2-HG)[13–15] but, at present, the role of this metabolite is not yet completely clear. A paper by Reiter-Brennan et al [16], explains the role of 2-HG in gliomagenesis . The oncometabolite 2HG can interfere with both glioma metabolism and vascularization; moreover, 2HG can affect epigenetic mechanisms leading to tumorigenesis and alteration of immune system activity. However, evidence in the literature shows a possible correlation between alteration of *IDH* enzymes and global methylation of CpG island (including *MGMT* promoter), with consequent chemotherapy sensitivity of *IDH*mut AA, and a correlation between low levels of *NADPH* in *IDH*mut cells and increased sensitivity to radiation therapy[17]. Furthermore, it would seem that the *IDH*mut status induces a defective homologous recombination with probable greater sensitivity to poly ADP ribose polymerase inhibitors (PARPi)[18,19]. The 2016 WHO classification of central nervous system tumors distinguishes the diagnosis of AA, as we have already mentioned, into two broad categories, mutated *IDH* forms (*IDH*mut) and wild-type forms (*IDH*wt)[3], both with absence of the *1p/19q* codeletion, an alteration that characterizes oligodendroglial histology. AA *IDH*mut is usually characterized by the presence of missense mutations of *IDH1* codon 132 or *IDH2* codon 172 with *ATRX* loss. If the immunohistochemical analysis of mutated forms of *IDH1* (R132H) is negative and the sequencing of codon 132 of *IDH1* and codon 172 of *IDH2* are negative for mutation, we can define these forms as *IDH*wt. *IDH*wt AA is rathe rare and often presents molecular and genetic characteristics typical of glioblastoma[20,21]. The *IDH*wt forms usually have a more aggressive clinical course than the *IDH*mut tumors with non-optimal response to conventional treatments and a biological behaviour similar to glioblastoma[22]: mutations of *PTEN* and *EGFR* genes are often found, with the possibility of having a loss of heterozygosity of chromosome 10q, polisomy of chromosome 7 and *TERTp* mutations, which make these subtypes much more similar to glioblastoma[21,23,24] .

*MGMT* is a crucial protein for genome stability that repairs the occurring mutagenic DNA alterations and prevents errors during DNA replication and transcription. About 80% of *IDH*mut secondary high grade gliomas (including anaplastic astrocytoma) report a *MGMT* promoter methylation[25]; unlike glioblastoma where the methylation of *MGMT* promoter is correlated with better survival, in anaplastic astrocytoma the prognostic and predictive role of the *MGMT* methylation status is still uncertain.

**Imaging and Clinical Presentation**

The gold standard for diagnosis, management and to monitor treatment response remains brain MRI with gadolinium contrast. Usually, AA appears as a T1-weighted hypointense and T2-weighetd hyperintense mass with surrounding edema and possible enhancing nodular areas although one-third of AA do not show enhancing areas[26]. Contrary to oligodendroglioma histology, AA does not present calcifications (more visible in computed tomography scans than MRI), it has a homogeneous signal intensity in T2-weighted brain MRI sequences, has a well definable margin, and does not usually invade the cerebral cortex[27]. The perfusion MRI sequences have high sensitivity to distinguish low-grade forms compared to high-grade forms of astrocytoma with higher blood volume in high-grade astrocytoma[28]; MR spettroscopy, on the other hand, is inferior to perfusion MRI for grading astroctyoma[29]. Functional molecular imaging such as Positron Emission Tomography (PET) can be considered helpful in the management of glioma patients because can provide additional insight beyond MRI into biology and treatment response as well as it could be useful for noninvasive grading, differential diagnosis, delineation of tumor extent, surgical and radiotherapy treatment planning, post-treatment follow-up and prognosis. Several tracers are used but amino acid transport such as ([11C-methyl]-methionine (11C-MET), O-(2-[18-F]-fluoroethyl)-L-tyrosine (18F-FET) and 3,4-dihydroxy-6-[18F]-fluoro-L-phenylalanine (18F-FDOPA), reported higher accuracy in primary diagnosis, different diagnosis and tumor grading (grade II Vs grade III-IV)[30]. Compared to low-grade gliomas,

in which the average age of incidence is between the second and fourth decade, the high-grade glioma diagnosis usually occurs at a slightly more advanced age. The clinical presentation is variable, based on the location of the disease and is characterized by focal or generalized neurological deficits, headaches, visual and sensory impairment, speech disorders, loss of strength and gait disturbances; in anaplastic astrocytoma, seizures are less frequent than in low-grade gliomas. In addition with molecular characteristics, several factors are considered important for prognosis in these patients, such as age, performance status, type of surgery, size and location of the tumor[31].

## Treatment

### -*Surgery*

The first curative approach in the treatment of AA, when possible and regardless of the mutational status of *IDH* gene, remains a maximal safe surgical resection[7]. When that is not feasible, a biopsy of the glial lesion can be performed to have material available for a correct histological and molecular definition, which can guide the subsequent post-surgical treatment. The data deriving from prospective, randomized trials comparing surgical resection Vs biopsy alone are scarce. Most of these are uncontrolled trials with selection bias, making the evaluation of such contributions difficult; despite the limitations described, the best survival data are among those who underwent a radical or subtotal surgery[31]. These data have been confirmed by several retrospective studies that analyzed the role of complete resection in patients with *IDH*mut gliomas: in these patients, even minimal residual disease can have a negative impact on survival[32,33]. The surgical resection offers direct decompression of brain structures restoring its functionality and preventing symptoms progression. At the time of progression / recurrence, a second look surgery could be considered in selected cases, in particular for patients with a symptomatic mass, good performance status and when disease is in a non-eloquent brain area; survival benefit of second surgery has yet to be confirmed.

**Post-Surgical Treatment**

Since most of the past and ongoing clinical trials evaluating the best adjuvant treatment for AA were designed and, in some cases, conducted before the introduction of the 2016 WHO classification of central nervous system (CNS) tumours[3], some of the available data are difficult to extrapolate. Surgery alone cannot be considered curative in the treatment of AA and, therefore, post-surgical treatment must always be considered. To overcome this limitation, post-hoc analyses were performed for several studies, which had the endpoint to evaluating the outcome of treated patients, stratified by molecular characteristics. Radiation therapy remains among the post-surgical standards of care for anaplastic glioma, as shown by several prospective, but outdated, clinical trials[34–37]. Normally, radiotherapy is administered on gadolinium-enhancing areas and on hyperintense T-2weighted region / FLAIR peritumoral surrounding tissue, adding a 1-2 cm margin of treatment (CTV: Clinical Target Volume) plus a 0.3-0.5 cm margin to create the PTV (Planning Target Volume)[7].Usually, the total radiotherapy dosage is 59.4Gy, with fractions of 1.8Gy, for 5 days a week. Post-surgical chemotherapy also has significant importance and has been compared with radiotherapy as an initial treatment of anaplastic gliomas in several randomized studies. The NOA-04 trial[38],a prospective randomized phase III study that enrolled anaplastic glioma patients randomized to receive traditional radiotherapy (RT) or chemotherapy with a PCV regimen (procarbazine, lomustine and vincristine) or Temozolomide as initial treatment. In fact, the study showed that the chemotherapy treatment, with deferred radiotherapy, turns out to be equivalent to radiotherapy treatment itself, not obtaining significant differences in terms of progression free survival (PFS) among the treatment arms. In NOA-04, PFS, time to treatment failure (TTF) and overall survival (OS) were longer in patients with oligodendroglial histology compared with AA Subsequently, a long-term analysis[39] of this trial confirmed the important role of the *IDH* mutational status regardless of the treatment arm for PFS. The *MGMT* promoter methylation was associated with improved PFS in chemotherapy arms, only in the subgroup of *IDH*wt patients. The PCV schedule was also evaluated in two different studies,

RTOG 9402 and EORTC 26951, initially developed to evaluate this chemotherapeutic approach in patients diagnosed with oligodendroglioma[40,41]; however, in both studies, patients without *1p/19q* co-deletion were also enrolled. In these trials, the addition of PCV to conventional adjuvant radiotherapy, did not result in a statistically significant advantage in terms of OS in patients without *1p / 19q* co-deletion, although it showed a trend of benefit from the addition of PCV (EORTC study: HR=0.83; 95% CI 0.62 to 1.10, p=0.18; RTOG study: HR=0.85, 95% CI 0.58 to 1.23, p=0.39). As known, the PCV scheme is associated with an important toxicity profile and the rate of early treatment discontinuation due to toxicity was approximately 40%[41,42]and, in the EORTC 26951 study, two thirds of patients did not receive the fifth and sixth planned therapy cycles. For this reason, the use of temozolomide, an alkylating drug with a much better manageable toxicity profile than the PCV scheme, can be considered a valid alternative, also considering that the results of the NOA-04 study suggest that the efficacy of temozolomide is comparable to PCV scheme in a *1p / 19q* non-coledeted population. The first study for the evaluation of anaplastic gliomas without *1p19q* co-deletion was the CATNON trial (EORTC 26053–22054)[43]. In this study, which had a 2x2 factorial design, the effectiveness of temozolomide as a concomitant and adjuvant treatment in addition to radiotherapy was assessed; patients were randomized into four arms: radiotherapy alone, radiotherapy followed temozolomide, concurrent radio-chemotherapy with temozolomide and concomitant radio-chemotherapy followed temozolomide. The first pre-planned interim analysis, published in October 2017, reported an OS advantage in the study population receiving adjuvant chemotherapeutic treatment with temozolomide (median OS: not reached vs 41.1 months; HR 0.67, 95 % CI 0.51 to 0.88) with a 5-year survival increase from 44% (95% CI: 36.3-51.6) to 56%(95%CI: 47.2-63.8)[43]. However, at the time, the follow-up of this analysis was still immature, with 70% of patients treated still alive and with only 46% having had disease progression. More recently, during the Annual Meeting of the American Society of Clinical Oncology (ASCO) 2019 in Chicago and the congress of European Association of Neuro-Oncology (EANO) 2019, the results of the second interim analysis of this study were presented, with a broader follow-up and with particular attention to the efficacy

evaluation of the of the concomitant radio-chemotherapy treatment[22]. Analyzing all patients, the combination of temozolomide and radiotherapy did not demonstrate a statistically significant increase of OS compared to radiotherapy alone (5-year OS rate of 53% versus 50%, respectively; HR: 0.93, 95% CI: 0.75-1.14; p= 0.464). However, IDH and MGMT status resulted important predictors of temozolomide efficacy. Indeed, in IDH mutated patients the 5-year OS rate was 76% and 68% (HR 0.63, 95% CI: 0.43-0.91; p= 0.012) in case of concomitant treatment or radiotherapy alone, respectively. Conversely, in IDHwt patients, concomitant chemoradiotherapy did not show any advantage in terms of OS (17.1 months in chemoradiotherapy arm vs 20.6 months in RT alone arm; HR: 1.16, 95% CI: 0.83-1.63; p= 0.380).

Regarding maintenance temozolomide, the 5-year OS rate was increased only in patients with mutated IDH: 83% when maintenance temozolomide was used vs 60% in the radiotherapy alone arm (HR: 0.46, 95% CI: 0.32-0.67; p= <0.0001). Conversely, in the IDHwt population, the addition of maintenance TMZ showed no significant advantage of survival (median OS of 19.4 months in maintenance TMZ arm Vs 17.5 months without maintenance TMZ (HR 1.03, 95% CI: 0.73-1.44; p= 0.881).

Although the authors presented preliminary data on the predictive role of MGMT methylation status, concomitant chemoradiotherapy and subsequent maintenance temozolomide seems to increase overall survival in MGMT methylated patients only; in particular, patients with MGMT methylated AA receiving concomitant treatment showed a median OS of 116.6 months vs 74.3 months in patients treated with radiotherapy alone (HR 0.66, 95%CI: 0.48-0.90; p=0.009); the benefit of concomitant therapy was not demonstrated in AA patients with unmethylated MGMT: median OS was 23.3 months in concurrent TMZ arm vs 29.3 months in radiotherapy alone, HR 1.02, 95%CI: 0.72-1.45; p= 0.914). The same difference was observed with maintenance temozolomide: in methylated population the 5yr-OS rate was 72% in maintenance arm vs 53% in no maintenance arm (HR 0.55, 95% CI: 0.40-0.75; p<0.0001). Conversely, no significant difference in terms of OS was

demonstrated in the unmethylated population: median OS was 29.3 months in maintenance TMZ arm was vs 22.3 months in the other group (HR: 0.75, 95% CI: 0.53-1.08; p=0.119). In conclusion, AA patients with IDHmut can benefit from concurrent and adjuvant TMZ. A longer follow up is needed to better understand the predictive role of MGMT methylation status.

**Elderly and poor performance status patients**

The adjuvant treatment of elderly and poor performance status AA patients should be discussed separately. The aforementioned patients generally have a reduced tolerance to cancer treatments; moreover, elderly patients often present unfavorable molecular characteristics which result in a poor response to treatment themselves. Several studies have evaluated the best approach for those patients. The NOA-08, a non-inferiority trial, compared a standard radiotherapy treatment (54-60 Gy) versus dose-dense temozolomide chemotherapy in a population of patients with AA (17 patients in temozolomide arm and 23 patients in radiotherapy arm) or glioblastoma aged > 65 years[44(p08)].The study reported a comparable outcome in the two treatment arms, suggesting that temozolomide could be a therapeutic option for the elderly or those with poor performance status populations, particularly in case of MGMT methylated tumors. The long-term analysis of the NOA-08[45(p08)] confirmed the non-inferiority of temozolomide compared to radiotherapy in the treatment of elderly patients with WHO grade III and IV gliomas, with an mOS of 8.2 months in temozolomide arm vs 9.4 months in radiotherapy group (HR 0.93, 95%CI 0.76-1.15); median Event Free Survival (EFS) was 3.4 months for temozolomide and 4.6 months for radiotherapy (HR 1.02, 95%CI 0.83-1-25). MGMT promoter methylation resulted a strong predictive biomarker for temozolomide efficacy: patients with MGMT promoter methylation had longer OS and EFS when treated with temozolomide than with radiotherapy (HR 0.44, 95%CI 0.27-0.70, p<0.001 for OS // HR 0.46, 95%CI 0.26-0.73, p=0.001 for EFS).

**Treatment at recurrence/progression**

Disease recurrence/progression is a frequent occurrence in anaplastic astrocytoma and the therapeutic alternatives for this condition are currently very limited. A second surgical resection could be considered if the patients is very symptomatic due to mass effect, when relapse/progression is not found in eloquent brain areas, and when the patient maintains good general clinical conditions. Surgery could result beneficial for symptoms but a significant survival advantage is still uncertain; however, the possibility of obtaining histological progression material could help the clinician to evaluate a possible evolution towards glioblastoma as well as obtaining material suitable for molecular investigations regarding possible precision therapies. A systemic therapy approach could include a rechallenge with temozolomide or switching to drugs such as nitrosoureas[7]. The addition of the antiangiogenic drug bevacizumab to temozolomide as a treatment for recurrence of anaplastic astrocytoma has not been shown to lead to an advantage either in terms of progression free survival (PFS) or overall survival (OS)[46]. Eflornithine showed interesting results in recurrent anaplastic gliomas. This drug is a specific and irreversible inhibitor of ornithine decarboxylase (ODC), an enzyme responsible for the catalysis of ornithine to putrescine, a critical step for the biosynthesis of polyamines, which are in turn indispensable for cell division and cell differentiation[47]. Eflornithine has been studied for years in different pathological conditions such as African trypanosomiasis and hirsutism. Its oral bioavailability is about 80% and can be taken in multiple daily administrations with limited side effects, such as a gastrointestinal toxicity and, in low percentage of patients, a decline in sensorineural hearing; when Eflornithine is taken with a chemotherapeutic agent, it could increase the myelotoxicity rate[48]. Due to its activity on cell growth, this drug has been evaluated as a potential treatment in different types of haematological malignancies such as leukemia, and many solid tumors such breast cancer, colon cancer, lung cancer and melanoma[49]. Eflornithine has also been evaluated in the treatment of brain cancer, particularly in gliomas, with various administration and dosage patterns as well as a single agent or in combination with several chemotherapy drugs such as mitoguazone (MGBG), nitrosoureas (BCNU) or with PCV scheme[50–54]. In a rather dated study by

Levin et al, Eflornithine was tested in association with mitoguazone or as single agent in 121 patients with brain tumors (about 70 anaplastic gliomas) demonstrating good antitumor activity; in particular, 44 patients with anaplastic glioma were treated with eflornithine alone reporting Disease Control Rate (DCR) of 45 % (4 patients with partial response, 9 patients with "minor" response and 7 patients with stable disease) with a median time to progression of 49 weeks[54]. In a more recent randomized phase III trial, Eflornithine was evaluated in combination with the PCV scheme versus PCV alone in 242 patients with anaplastic gliomas; in this study, 78.1% of evaluable patients in the combination arm and 69.3% of evaluable patients in the control arm had a diagnosis of AA[53].The addition of Eflornithine to the PCV scheme resulted in a not statistically but clinically significant advantage in terms of median mPFS with 56.2 months with the combination therapy compared to 22.2 months with PVC alone (p=0.18)in anaplastic astrocytoma histology (71.1m Vs 37.5m for PCV-EflornithineVs PCV in all study population). Median OS was 71.2 months in combination arm Vs 46 months in PCV arm (p= 0.12) in AA group (75.8 months in combination arm Vs 61.1 in PCV arm in all study population, p= 0.12); a statistically significant advantage in terms of survival was noted in the first 2 years of the study (HR, 0.53; p=0.02), which however was not confirmed after two years (HR 1.07; p=0.83)[53].Another randomized, phase III trial (STELLAR - NCT02796261) is currently enrolling patients with recurrent or progressed AA after receiving radiation therapy and chemotherapy with temozolomide; the trial will evaluate Eflornithine in combination with lomustine compared with lomustine alone. No data are currently available on the efficacy of this combination in the STELLAR trial, which appears to be very promising.

**Precision Medicine and Future Perspectives**

A more in-depth knowledge of the molecular characteristics of cancer (including gliomas) has led to the development of personalized treatments and that aim to improve the outcome of patients with poor therapeutic alternatives. The BRAF V600E mutation has been identified in different types of

gliomas, especially in pleomorphic xantoastrocytoma, which has a high rate of mutations (38% and 100%); the BRAF V600E mutation is much less frequent in high-grade gliomas, including glioblastoma (<3%)[55–57]. Some studies have evaluated the possibility of using BRAF V600E kinase inhibitors in patients with anaplastic gliomas, obtaining in some cases, interesting results. A basket trial evaluating vemurafenib (BRAF V600E kinase inhibitors) activity in patients with recurrent gliomas reported a high response rate in anaplastic pleomorphic xantoastrocytoma (43%), whereas in patients with AA and glioblastoma, the response rate was decidedly lower (9%). Median PFS for all patients was 5.5 months (95%CI, 3.7-9.6 months) with median PFS of 5.7 months (95%CI, 3.0 months – not reached[NR]), 5.3 months (95%CI, 1.8-12.9 months) and 3.7 months (95%CI, 2.0-13.6 months) for pleomorphic xantoastrocytoma, malignant diffuse gliomas and other cohorts respectively[58]. Median OS was 28.2 months (95%CI, 9.6-40.1 months) in all the study population with median OS duration not reached[NR] (95%CI, 5.0 months-NR), 11.9 months (95%CI, 8.3-40.1 months) and 28.2 months (95%CI, 12.8-31.6 months) for pleomorphic xantoastrocytoma, malignant diffuse gliomas and other cohorts respectively; a single patient with diagnosis of pleomorphic xantoastrocytoma showed the longest treatment duration (39.1 months)[58]. A further study evaluated the association between dabrafenib (BRAF V600E kinase inhibitor) and trametinib (MEK kinase inhibitor) as treatment for relapsed high-grade gliomas. The interim analysis showed a response rate of 22% and 29% in grade III and grade IV respectively[59]; another trial evaluating BRAF/MEK inhibitors (encorafenib / binimetinib) is currently ongoing (NCT03973918). An additional line of research for treatment of brain tumors concerns the possibility of targeting solid neoplasm shaving TRK fusions; this molecular alterationis an oncogenic driver of different cancers, including some CNS tumors. Larotrectinib, an oral, selective TRK inhibitor that crosses the blood-brain barrier[60], approved by FDA for the treatment of all TRK fusion cancers[61]. Drilon et al.[62] conducted a study that evaluated larotrectinib in patients with non-primary CNS solid tumors (lung cancer and thyroid cancer) or primary CNS tumors harbouring TRK fusion (3 gliomas, 2 glioblastoma, 1 astrocytoma and 3 NOS): in the 9 patients with primary CNS tumors, disease control rate was 100% with one case

of partial response ( -55% tumor shrinkage), 7 cases of stable disease and one case in which response was not assessable. Another similar drug, with a broader spectrum of action, is Entrectinib, an oral, selective, tyrosine kinase inhibitor for the treatment of NTRK, ROS1 and ALK fusion-positive solid tumors with good brain-blood barrier penetration. At ASCO 2019, data from a phase I/Ib were presented by Robinson[63]; this study has enrolled pediatric and adolescent (up to to 21 years) patients with relapsed or refractory solid tumors (including primary CNS tumors), with or without target molecular aberrations in NTRK1/2/3, ROS1 and ALK genes; six patients with primary CNS tumors were treated (5 with high-grade glioma and 1 with CNS embryonal tumor) with recommended dose of 550 mg/m$^2$ daily. All high-grade glioma patients with gene fusions had a response: one achieved a complete response ( with fusion of ETV6-NTRK3), 3 patients achieved partial response (with fusions of TPR-NTRK1, EEF1G-ROS1 and EML1-NTRK2), 1 achieved an unconfirmed partial response (with fusion of GOPC-ROS1) and one patient had yet to be evaluated at the time of analysis (fusion of KANK1-NTRK2)[63] These data have opened up an interesting scenario, emphasizing the importance of extensive molecular diagnostic in this subgroup of patients. In fact, a basket trial is active (NCT02568267) and is currently in recruiting phase, for evaluate the activity of entrectinib for the treatment of patients with solid tumors (also with primary brain tumors) harboring NTRK1/2/3, ROS1 and ALK gene rearrangements (fusions). Chromosomal translocations of fibroblast growth factor receptor and transforming acid coiled-coil gene (FGFR-TACC gene fusions) were discovered in glioblastoma and in several types of solid tumors. FGFR-TACC fusions seem to be events that arise at the beginning of tumorigenesis. Erdafitinib, a tyrosine kinase inhibitor of FGFR1–4 was analyzed in a multicenter phase I trial. In this study, patients with advanced or refractory solid tumors[64], including 13 glioblastoma patients, were enrolled; the results showed a good tolerability profile and clear antitumor activity in selected FGFR-fusion patients. A phase II clinical trial evaluating patients with advanced tumors and alteration of FGFR gene is currently ongoing (RAGNAR trial – NCT04083976). In consideration of the importance of the *IDH 1* and *IDH2* mutations in the onset and development of gliomas, studies are underway that are considering the

possibility of using selective inhibitors of *IDH1* and *IDH2* for the treatment of these types of brain cancers; in addition to this approach, some vaccines against the mutated form of *IDH 1* and *2* have also been studied. The first selective *IDH1 R132H / R132C* mutant inhibitor was AG-5198, which demonstrated, both in vitro and in vivo, the ability to promote differentiation in astrocytes and oligodendrocytes and promote the differentiation of glioma cells[65].Two other inhibitors of the mutated form of *IDH* are Ivosidenib (AG-120, anti-IDH1) and Enasidenib (AG-221, anti IDH 2); these two selective and reversible inhibitors have been studied in several early-phase studies. Enasidenib (AG-221) received FDA approval in 2017 for the treatment of acute myelocyticleukemia (AML) as a first cancer metabolismdrug[66]. AG-221 has been evaluated in all solid tumors harboring the *IDH 2* mutation, including gliomas, within a study of which no definitive results are currently available (NCT02273739). Because the *IDH1* mutation is more frequent than that of *IDH 2*, several studies have been carried out to evaluate the tolerability and safety, as well as an initial analysis of efficacy of AG-120 (ant-*IDH1*) in several solid tumors that have this type of mutation, including gliomas (NCT02073994); final results are currently not available. Among clinical trials evaluating *IDH1* peptide vaccine, a german phase I study (NCT02454634) was the first-in-human, multicenter, phase I study. This trial enrolled 33 patients with newly diagnosis of WHO grade III and IV astrocytoma with *IDH1 R132H* mutation (65% with diagnosis of AA); after chemoradiotherapy treatment, vaccinations with *IDH1 R132H* peptide were administered subcutaneously for eight times, for a period of 32 weeks, together with temozolomide maintenance. The study met its primary endpoints demonstrating safety and immunogenicity of mutation-specific *IDH1 R132H* peptide vaccine. Noteworthy, 4/32 patients (12.5%) showed progressive disease and all other patients (28/32, 87.5%) had stable disease according to RANO criteria[67].

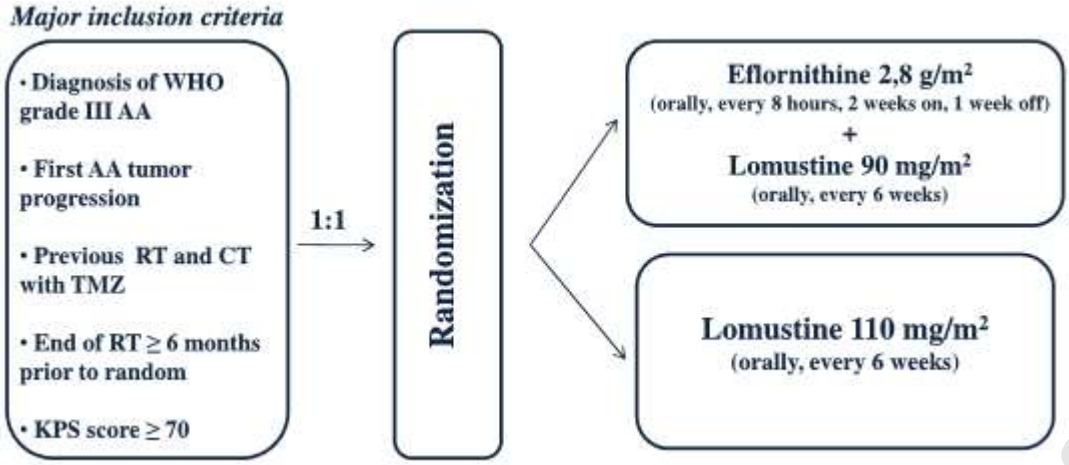



Figure 1: Stellar study. (AA: anaplastic astrocytoma; RT: radiotherapy; CT: chemotherapy; KPS: Karnofsky performance status; TMZ: temozolomide)

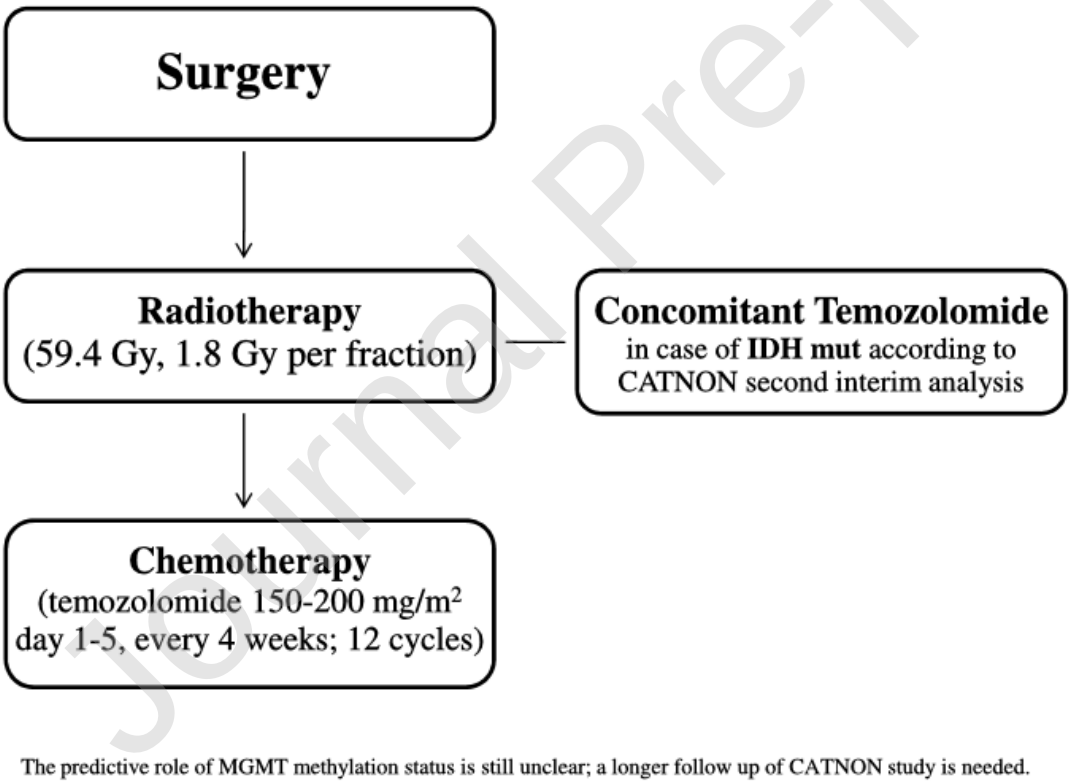



Figure 2: Treatment algorithm of anaplastic astrocytoma (wt: wild type; mut: mutated; *IDH*: isocitrate dehydrogenase; *MGMT*: $O^6$methylguanine DNA methyltransferase)

*: data referred to all gliomas

Table 1: Interesting new drugs for anaplastic astroctyoma treatment (NA: not applicable)

| **Drug** | **Mechanism of action** | **Trial** | **Design** | **Response Rate** | **Disease Control Rate** | **mOS (months)** | **mPFS (months)** | **% of Anaplastic Astrocytoma** |
|---|---|---|---|---|---|---|---|---|
| **Eflornithine**[53] (DFMO) | Ornithine Decarboxylase Inhibitor | Phase III | DFMO + PCV Vs PCV | 8.8%* | 90.4% | 71.2 | 56.2 | 78.1% (combination arm) |
| **Vemurafenib**[58] | *BRAF* Inhibitor | Phase II | Vemurafenib | 25%* | 66.7%* | 28.2* | 5.5* | 21% |
| **Dabrafenib + Trametinib**[59] | *BRAF* Inhibitor + *MEK* Inhibitor | Phase II | Dabrafenib+Trametinib | 26%* | NA | 11.7* | 1.9* | NA |
| **Larotrectinib**[62] | *TRK* inhibitor | Phase I | Larotrectinib | 11%* | 100%* | NA | NA | NA |
| **Entrectinib**[63] | *TRK, ROS1, ALK* fusion inhibitor | Phase I/Ib | Entrectinib | 100% * | 100* | NA | NA | NA |
| **IDH1R132H peptide vaccine**[67] | Vaccination with *IDH1R132H* peptide | Phase I | IDH1R132H peptide vaccine | 0% | 87.5%* | NA | NA | 65% |

## Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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**Matteo Fassan MD, PhD:** Molecular Pathologist and Professor at Padua University, expert in the molecular characterization of tumors

**Vittorina Zagonel MD:** Medical Oncologist, Director of Oncology 1 of Veneto Institute of Oncology-IRCCS in Padua, expert in the management of patients with tumor and caregivers

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