Trends in Malignant Glioma Monoclonal Antibody Therapy

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Abstract: Although new passive and active immunotherapy methods are emerging, unconjugated monoclonal antibodies remain the only kind of biological preparations approved for high-grade glioma therapy in clinical practice. In this review, we combine clinical and experimental data discussion. As antiangiogenic therapy is the standard of care for recurrent glioblastoma multiforme (GBM), we analyze major clinical trials and possible therapeutic combinations of bevacizumab, the most common monoclonal antibody to vascular endothelial growth factor (VEGF). Another humanized antibody to gain recognition in GBM is epidermal growth factor (EGFR) antagonist nimotuzumab. Other antigens (VEGF receptor, platelet-derived growth factor receptor, hepatocyte growth factor and c-Met system) showed significance in gliomas and were used to create monoclonal antibodies applied in different malignant tumors. We assess the role of genetic markers (isocitrate dehydrogenase, O6-methylguanine-DNA methyltransferase) in GBM treatment outcome prediction. Besides antibodies studied in clinical trials, we focus on perspective targets and briefly list other means of passive immunotherapy.

Keywords: Angiogenesis, bevacizumab, glioma, monoclonal antibodies, nimotuzumab, passive immunotherapy.

1. INTRODUCTION

Gliomas make up almost 80% of malignant tumors, among them glioblastoma multiforme (GBM) constitutes about 45% [1]. Low standard chemotherapeutic and neuro-radiological approach effectiveness due to intensive invasive tumor growth makes sense of searching for effective anti-glioma medications, including those based on antibodies to glioma-associated antigens [2].

Application of antibodies for brain tumor therapy is mainly explained by their unique biological properties: highly specific affinity of complementarity-determining regions, and avidity – general antigen binding stability. Tumor immunotherapy gained a new impulse after an epochal discovery of hybridoma technology by G.Köhler and C.Milstein in 1975 [3]. The breakthrough hybridoma method made possible to harvest B-lymphocytes from the spleen of immunized mice, immortalize those cells and produce almost unlimited amounts of antibodies, which are specific only to a single epitope of an antigen [4]. Although this technology could afford gaining highly purified murine monoclonal antibodies, using them for therapy is complicated by anti-mouse antibodies generation, which abrogates specific therapeutic agent functions [5]. The main keys to this problem include producing chimeric, humanized or fully human antibodies by substitution of murine genetic sequences for human ones [6].

Nevertheless, according to Gedeon et al., [7] even applying fully human antibodies has several limitations. For example, a monoclonal antibody Fc fragment may bind to FcyRIIb-receptors on macrophage or lymphocyte surface and thus a therapeutic agent function is inhibited [8]. Moreover, IgG1 is able to bind non-effector cells, e.g. platelets [9], reducing a general antibody effect. All antibodies introduced into the bloodstream
face the problem of permeating the blood-brain barrier (BBB). It is a consequence of big molecular mass and bulky antibody molecule size, as well as of endothelial and choroid plexus neonatal receptor FcRN, which was proved responsible for antibody secretion from interstitial fluid back into bloodstream in a rat model [10].

Complications mentioned above force the investigators to design new approaches to antibody-based therapy of central neural system diseases and glial tumors in particular. In order to avoid these complications a new technology of combining different antibody fragments appeared. The sum of these fragments has a lower molecular mass than an antibody but provides required affinity and avidity. Among such combinations it is necessary to list single-chain variable fragments (scFv), which are produced by peptide-linker heavy chain (VH) and light chain (VL) variable domain fusion [11]; antigen-binding fragments (Fabs), bispecific antibodies (fused antigen-specific moieties of different specificity) [12], bispecific scFvs (bi-scFvs) [13]. It is also possible to cotranslate a VH and VL of different antibodies and vice versa and combine two products yielding a bispecific diabody [14].

Therapeutic monoclonal antibodies gained suffixes according to international nomenclature – “omab” for murine antibodies, “ximab” for chimeric antibodies, “zumab” for humanized antibodies and “umab” for fully human antibodies [15].

2. APPLICATION OF MONOCLONAL ANTIBODIES

2.1. VEGF Antagonists

As GBM is characterized by a high vasculatization degree, one of the alternative therapeutic methods is angiogenesis inhibition. Glioma endotheliocytes express high levels of vascular endothelial growth factor (VEGF-A), which correlates with the tumor grade [16]. VEGF-A belongs to a glycoprotein family, which also includes VEGF-B, VEGF-C, VEGF-D and placental growth factor. VEGF specifically binds to receptors (VEGFR-1, VEGFR-2, VEGFR-3), activating signaling cascades leading to blood and lymphatic vessel endotheliocytes proliferation and stimulating vascular invasion [17]. Bevacizumab (molecular mass 149 kDa) is a humanized IgG1 monoclonal antibody specific to all isoforms of VEGF-A [18]. According to clinical study results mentioned in a review of Narita et al. [19], single-agent bevacizumab effectiveness was proved for recurrent GBM [20] [21]. Han et al. performed a meta-analysis including 91 clinical trials of different therapeutic agents in GBM, which showed a strong correlation between progression-free survival (PFS) and overall survival (OS). The latter fact gives an opportunity to substitute OS by PFS, which results to earlier data assessment [22]. Initially bevacizumab and irinotecan combination effectiveness for radiographic and clinical outcomes was proved by Stark-Vance in 2005 [23]. Later the combination was thoroughly studied by Vredenburgh et al., [24], Friedman et al. (BRAIN study) [25] and Kreisl et al. [26] (Table 1). Although PFS and OS were quite similar among all three studies, BRAIN was the only to include a parallel control group treated with bevacizumab alone (patients treated by Kreisl et al. received single-agent bevacizumab before tumor progression). The latter fact is critical to assess the bevacizumab effectiveness. As for BRAIN, we can say that only PFS improved with irinotecan addition, which is though very important, given the severity of GBM. FDA approval of bevacizumab for recurrent GBM was gained on the basis of BRAIN trial [25] and a study conducted by Kreisl et al. [26]. The European Medicines Agency denied bevacizumab registration because two studies listed above did not contain a bevacizumab-non receiving patient control group [27].

Reardon et al. studied a possibility of adding etoposide as an alternative pair to bevacizumab in treatment of recurrent GBM (27 patients) and grade III gliomas (32 patients). Median OS was 11.6 and 15.8 months and 6-month PFS was achieved in 44.4% and 40.6% respectively [28]. Another trial performed by Reardon et al. (25 patients) tested a regimen of bevacizumab, carboplatin and irinotecan in patients with GBM who progressed on single-agent bevacizumab. Median OS and PFS were 5.8 months and 2.3 months, 6-month PFS was reached in 16% of all cases [29]. Desjardins et al. proposed a therapeutic combination of low-dose temozolomide and bevacizumab (32 patients). Median OS and PFS were 9.25 months and 3.95 months, respectively, 6-month OS was 62.5%, 6-month PFS was 18.8% [30]. Quant et al. studied advantages of replacing a chemotherapeutic bevacizumab pair after reveal-
ing progression of GBM (54 patients). The authors’ hope was to potentiate the tumor sensitivity to bevacizumab after chemotherapy replacement, but they concluded that a progressing malignant glioma did not react to any of the combinations [31]. These results coincide with data obtained by Norden et al. who used irinotecan, carboplatin and carmustine as second agents after the malignant glioma progression onset (55 patients) granted chemotherapeutic agent change effectiveness only in single cases [32]. In combination with sorafenib in recurrent GBM (54 patients) bevacizumab did not show outcome improvement (median OS and PFS were 5.6 and 2.9 months, 6-month PFS was 20.4%) [33], as well as with temsirolimus (13 patients, median OS was 3.75 months, median PFS was 2 months) [34].

In the light of bevacizumab studies, the nitrosourea application for recurrent GBM has gained a new impulse. Vaccaro et al. have been studying the combination bevacizumab and fotemustine regimen in a heterogenic population of patients with recurrent GBM, as well as with lower grade, albeit anaplastic recurrent gliomas (26 patients in total). The median PFS for patients with GBM 3 months (4 months for patients with anaplastic gliomas). 6-months PFS in the whole population was 23.1%, the median OS was 6 months, although the last two scores were not differentiated by the glioma histotype [35]. Soffietti et al. conducted a trial to test a bevacizumab and fotemustine regimen in a group of patients suffering from recurrent GBM (n=54). As the median PFS was 5.2 months; the 6-months PFS was 42.6% and the median OS was 9.1 months, the authors have concluded a failure to prove a combination success over existing regimens (either bevacizumab alone or in combination) [36]. That implies not the bevacizumab and fotemustine ineffectiveness per se, but lack of breakthrough achievements against a ground of previous studies. A double-agent regimen applied to grade III glioma resulted in PFS of 5 months and median OS of 8.6 months [37].

Intra-arterial injection of bevacizumab was proved effective for increasing its local concentration in the peritumoral zone. Boockvar et al. proved doses up to 15 mg/kg to be safe [38]. Burkhardt et al. studied 14 patients with recurrent GBM, who received bevacizumab intra-arterially after pharmacological BBB disruption. In this study median OS (8.8 months) was lower than median PFS (10 months), because 4 patients died before the beginning of neurovisualization-detectable progression [39].

Several patients, who received bevacizumab, had a neurological improvement, and for 30-70% patients it was possible to reduce corticosteroid doses [20, 40]. According to Bähr et al., bevacizumab induced a kind of magnetic resonance imaging (MRI) lesions (T1 hypointensive, diffusion-restricted), which correlated with survival benefit in patients with recurrent GBM [41]. According to MRI data, 35% of recurrent GBM were characterized by progression of predominantly non-enhancing lesions after bevacizumab discontinuation related to tumor progression [42]. Seystahl and Weller showed that even decreasing of contrast enhancement cannot be regarded as a criterion of tumor reversion, because it can also be a consequence of BBB restoration [43, 44]. In several cases bevacizumab discontinuation can lead to a rebound effect result-

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### Table 1. Results of bevacizumab and irinotecan combination clinical studies.

| Clinical Study Data | Number of Patients | Progression-free Survival, Months | Overall Survival, Months | 6-month Progression-Free Survival, % |
|---------------------|--------------------|----------------------------------|--------------------------|-------------------------------------|
| Vredenburgh et al., 2007 [24] | 35 | 6.0 | 10.5 | 46.0 |
| Friedman et al., 2009 single-agent bevacizumab [25] | 85 | 4.2 | 9.2 | 42.6 |
| Friedman et al., 2009 bevacizumab + irinotecan [25] | 82 | 5.6 | 8.7 | 50.3 |
| Kreisl et al., 2009 [26] | 48 | 4.0 | 7.75 | 29.0 |
ing in tumor reversion back to its pre-treatment size or even in increasing of contrast enhancement lesion diameter up to 50% [45]. Clark et al. showed that survival of iteratively operated patients treated with bevacizumab was lower than that of patients who did not receive antiangiogenic therapy [46]. An abrupt bevacizumab discontinuation can also provoke brain oedema, which is why a gradual dose decrease is recommended [19].

The history of bevacizumab in newly diagnosed GBM is younger. Lai et al. studied a triple therapeutic combination of radiotherapy, temozolomide and bevacizumab in newly diagnosed GBM (70 patients). Median OS and PFS were 19.6 months and 13.6 months, respectively (the figures for a double combination without bevacizumab in European Organization for Research and Treatment of Cancer (EORTC) and National Cancer Institute of Canada (EORTC-NCIC) study were 14.6 and 6.9 months, respectively) [47, 48]. Chinot et al. refer to an independent study, where bevacizumab-containing 3-component therapy twice improved OS of patients with newly diagnosed GBM [49]. Nevertheless, larger ones do not confirm the data of two trials mentioned above. Gilbert et al. conducted a double-blind randomized placebo-controlled clinical bevacizumab trial (Radiation Therapy Oncology Group 0825, 621 patient), which was introduced intravenously as a temozolomide co-therapeutic agent and radiotherapy for newly diagnosed GBM. According to the study results, no significant difference in OS between bevacizumab receiving group and placebo group (15.7 and 16.1 months, respectively) was detected. PFS was greater in bevacizumab group (10.7 months vs. 7.3 months in placebo group), although it was also characterized by complications such as arterial hypertension, thromboembolism, neutropenia, cognitive deterioration and decrease in life quality [50]. As for the last point, the bevacizumab influence on life quality is not fully understood. Chinot et al. showed in AVAglio trial (911 patients) that although bevacizumab as a third component (besides temozolomide and radiotherapy) did not increase life quality, it was sustained on a constant level for a longer period than in placebo group. On the other hand, PFS in AVAglio study was practically the same as in RTOG 0825 (10.6 months in bevacizumab group vs. 6.2 months in placebo group) [51].

The latest study to be reported, the BELOB multicentre phase II clinical trial (148 patients), conducted by Taal et al. [52] concerns recurrent GBM. The authors divided all patients into 3 groups receiving bevacizumab alone, lomustine alone and a combination of both medications. Assuming the historical BRAIN trial results as a sample [25], the survival rate of 9 months was taken as a reference. This decision fully coincides with the FDA ground to approve bevacizumab for recurrent GBM and OS/PFS standard of bevacizumab combination effectiveness outlined by Soffietti et al. [36]. 9-month OS was 38% in the bevacizumab group, 43% in the lomustine group, 63% in the bevacizumab plus lomustine group. The median OS was 8 months in the bevacizumab group, as well as in the lomustine group, 12 months in the combination group. 6-month PFS was 16% in the bevacizumab group, 13% in the lomustine group, 42% in the combination group. Unlike earlier trials, BELOB showed particularly modest 6-month PFS on single-agent bevacizumab therapy. Van den Bent et al. suggest that the more a medication is used in the general population (“a less selected recurrent GBM population”) [52], the worse outcomes in comparison to the initial trials are. They also believe that the trial results do not recommend the single-agent bevacizumab for recurrent GBM studies continuation. Hence they assume that only a combination of bevacizumab and lomustine deserves further investigation in a phase III study [53, 54]. Such a study has been conducted by the EORTC, the estimate completion date is September 2015 [55]. As for newly diagnosed GBM, H.S.Friedman underlines the role of the FDA review of the AVAglio and RTOG 0825 trials to come, which can shed light on the single-agent bevacizumab problem [56].

In BELOB trial context, Taal et al. found out that isocitrate dehydrogenase (IDH) wild-type tumors were associated with median OS of 9 months, whereas IDH mutant GBM patients had 20 monthsmedian OS, although the number of patients with IDH mutant tumors was quite small to draw significant outcomes from (nevertheless, this question deserves further exploration) [52].

In most studies bevacizumab was used in doses of 10 mg/kg every 2 weeks, but according to Wong et al., who conducted a meta-analysis of trials published in 2005-2009, there was no differ-
ence between 5 mg/kg and 10-15 mg/kg doses [57].

2.2. VEGFR and PDGFR Antagonists

The VEGF/VEGFR intracellular signaling pathway can be blocked on the transmembrane receptor level. Ramucirumab, a monoclonal antibody to VEGFR-2, has successfully undergone phase III clinical trial as a therapeutic agent for gastric cancer and gastro-esophageal junction carcinoma [58]. Korchagina et al. consider selective VEGFR-2 inhibition, supported by hypoxia-inducible factor 1 and placental growth factor suppression, as a perspective means of glioma targeted therapy [59]. According to Ikeda et al., VEGFR-1 has a much weaker tyrosine kinase activity [60] and can abrogate VEGF angiogenetic effect exerted via VEGFR-2, acting as a natural angiogenesis inhibitor [43].

Glioma-associated angiogenesis stimulation is triggered not only by VEGFR, but also by other growth factors, for example, platelet-derived growth factor (PDGF) and its receptors (PDGFRα and PDGFRβ), belonging to a receptor tyrosine kinase family [61]. IMC-3G3 (olaratumab) is a fully human antibody (IgG1), which specifically binds to PDGFRα but does not react with PDGFRβ [62]. IMC-3G3 showed ability to inhibit tumor growth by 65% in a murine model of U118 GBM xenotransplanted cells. Phase I clinical trial revealed that IMC-3G3 is well-tolerated and does not cause specific adverse effects [63]. A ramucirumab and IMC-3G3 for recurrent GBM clinical trial was conducted, but there are no results yet [64].

2.3. Antagonists of Hepatocyte Growth Factor and its c-Met Receptor

Scatter factor/hepatocyte growth factor (SF/HGF) and its tyrosine kinase receptor c-Met are hyperexpressed in many tumors including glial ones, and HGF/c-Met expression levels correlate with tumor aggressiveness [65]. Cao et al. have obtained anti-HGF antibodies, but later it was determined that three epitopes on HGF surface should be blocked in order to fully inhibit its signaling via c-Met [66]. Burgess et al. solved this problem, designing 5 fully human monoclonal antibodies IgG2 to an epitope located in HGF β-chain (presumably, positions 507-585). These antibodies, injected into herotopic U87 and U118 glioma xenotransplants in mice, inhibited tumor growth and stimulated apoptosis [67]. The preparation of monoclonal antibodies to HGF (AMG 102, rilotumumab) sensitized subcutaneously transplanted U87 cells to radiation in a murine model [68]. In a similar experiment, AMG 102 increased temozolomide and docetaxel effectiveness [69] and reduced isotope accumulation in positron emission tomography [70]. Effectivity and tolerability of AMG 102 were studied in phase II clinical trial published in 2011 [71]. AMG 102 was introduced systemically to 58 patients with recurrent GBM (previously in doses of 10 and 20 mg/kg). Median OS was 6.5 months in the first cohort and 5.4 months in the second one. PFS was 4.1 weeks and 4.3 weeks, respectively. Median time since initial diagnosis was 16 and 14 months respectively. Forty-eight percent of patient have undergone anti-angiogenic treatment before enrollment. One patient with low-grade glioma was enrolled into the study by a protocol violation. There was also one patent, whose tumor was considered to have “other” histological type.

Mitra et al. showed benefits for nude mice with orthotopically transplanted U87 GBM treated with ficlatuzumab, a monoclonal antibody targeting HGF, and temozolomide (in 8 of 10 mice the course of GBM wasn’t accompanied by clinical symptoms, as opposed to single-agent ficlatuzumab, where only 1 of 9 mice survived) [72].

New perspective of antibody therapy was revealed as Martens et al. obtained one-armed antibodies to c-Met (onartuzumab, OA5D5). Injection of onartuzumab into orthotopically transplanted U87 GBM (HGF and c-Met positive) in a murine model resulted in tumor growth inhibition by 95% (decrease in proliferation by 75%, vascular density by 90%, apoptosis induction by 60%). G55 xenotransplants (c-Met positive and HGF-negative) did not react to onartuzumab [73]. This fact is a proof of natural c-Met agonist essential role in therapeutic function of the onartuzumab. Onartuzumab pharmacokinetics was studied in phase I and II clinical trials regarding recurrent non-small cell lung cancer [74].

Noteworthy, Navis et al. described a tumor-promoting mutant MET receptor localized cytoplasmatically in 6% of all high-grade gliomas. The auto-enhancing activity and transmembrane
domain lack makes the antigen inaccessible for the antibodies, rendering all antibody-based efforts to silence it ineffective [75].

2.4. Antagonists of EGFR and its Mutant Variant EGFRvIII

Monoclonal antibodies to epidermal growth factor receptor (EGFR) extracellular domain include chimeric (cetuximab), humanized (nimotuzumab) and fully human (panitumumab). ESMO approved cetuximab and panitumumab for metastatic RAS-wild type colorectal cancer treatment [76]. In phase II clinical trial of cetuximab monotherapy in patients with recurrent GBM (n=55) median OS was 5 months, but it did not correlate with EGFR amplification, as well as response to therapy. PFS was 1.9 months, 6-months OS and PFS were 37.9% and 7.3%, respectively [77]. Blesa et al. reported a case, where a combination of cetuximab and bevacizumab resulted in 20-month delay of pontine GBM [78]. A cetuximab, bevacizumab and irinotecan triple therapy did not prove to be more effective than bevacizumab and irinotecan combination [79]. Yi et al. showed synergism of cetuximab and DC101 (murine monoclonal antibodies to VEGFR-2) in HM55-BGIV-101 GBM cells transplanted to mice. The first medication exerted its activity through satellite tumor inhibition; the second one decreased the main tumor volume [80].

One of the earliest nimotuzumab and radiotherapy combination studies in newly diagnosed high-grade gliomas was performed by Ramos et al. [81], where patients with newly diagnosed GBM (16 patients), anaplastic astrocytoma (AA) (12 patients) and anaplastic oligodendroglioma (1 patient) were enrolled after debulking surgery. The median OS in patients with GBM was 17.47 months (not reached in AA population). Another study, conducted by Solomon et al. [82], included nimotuzumab plus radiotherapy or radiotherapy plus placebo groups of patients (n=70) with newly diagnosed high-grade gliomas, where debulkment was not an essential criterion for patient selection. The median OS was 17.76 months and 12.63 months in nimotuzumab and placebo cohorts, respectively (integrative data for tumors of all histological groups, GBM and anaplastic astrocytoma). For newly diagnosed GBM, median OS was 8.4 months (nimotuzumab cohort) vs. 8.36 months (placebo cohort). Data provided by Solomon et al. in 2014 (35 patients), showed median OS gain of nimotuzumab and radiotherapy in GBM and AA (12.4 months and 27.0 months, respectively vs. 8.0 and 12.2 months on single radiotherapy) [83].

Westphal et al. conducted a phase III clinical trial in patients with newly diagnosed GBM (142 patients), combining nimotuzumab application, surgical treatment, radiotherapy and temozolomide. Although median OS did not differ significantly (22.3 months in nimotuzumab-receiving cohort vs. 19.6 months in control group), a significant increase in patient survival was detected in EGFR positive and methylated O6-methylguanine-DNA methyltransferase (MGMT) groups [84].

Chinese researchers obtained noteworthy results for nimotuzumab in both newly diagnosed and recurrent malignant gliomas [85]. The only point to underline is that the results were not differentiated according to the exact tumor grade (III or IV). That is why it is quite difficult to refer the data to a specific histotype. Hong et al. performed a placebo-controlled phase I/II clinical study (41 patients) where nimotuzumab was combined with radiochemotherapy. The median OS in the study and control groups were 16.5 and 10.5 months, respectively (significant difference), but the 1-year survival rate difference did not show clinical significance [86].

Of particular concern is nimotuzumab application in recurrent malignant gliomas. In 2015 Yang et al. have reviewed their clinical study published in 2011 in Chinese (14 patients), where nimotuzumab was combined with chemotherapy [85]. The median PFS was 4 months and 6-month PFS was 30.6%. The effect of nimotuzumab was assessed as “moderate”, albeit the medication was well-tolerated [87]. Chong et al. have tried nimotuzumab and rapamycin combination in temozolomide-resistant cell lines, which could act as a model of recurrent GBM. The combination was effective in Asian patient-derived human glioma cell lines expressing wild-type EGFR, Caucasian patient-derived human glioma cell lines expressing either wild-type EGFR or EGFRvIII and U87MG cell line. Of particular interest is the EGFR-null Gli36 cell line, where nimotuzumab and rapamy-
cin were also effective (the exact mechanism is unclear) [88].

Results of nimotuzumab in children and adolescents suffering primarily from diffuse intrinsic pontine glioma (DIPG) remain controversial, showing results varying from modest [89] to encouraging [90]. The nimotuzumab, vinorelbine and radiotherapy combination in DIPG is of particular interest and needs further studies [91].

EGFRVIII mutation is caused by 801 base-containing deletion, which results in loss on extracellular part of the receptor and its constitutive activity [7, 92]. Monoclonal antibodies to extracellular domain of EGFRVIII (mAb 806 and their humanized analogues ABT 806) are capable of decreasing the mutant receptor autophosphorylation. They react with tumors, which express EGFRVIII or amplify and overexpress EGFR, but not with normal tissues [93]. mAb 806 potentiated the sensitivity of glioma xenotransplants to radiotherapy [94]. Wang et al. obtained CH12 antibodies to exon-4-deleted EGFR gene product. In vivo CH12 inhibited growth and metastases of such a mutant EGFR expressing U87 GBM more selectively than cetuximab [95].

Gedeon et al. have designed a bi-scFv to EGFRVIII and a T-cell coreceptor CD3. The bi-scFv enhanced the T-cell antitumoral response [7]. An in vivo experiment showed that systemic bi-scFv introduction into orthotopically transplanted EGFRVIII-expressing U87 glioma resulted in a complete tumor regression in 75% of the mice population but was not effective in case of EGFRVIII-negative gliomas [7, 13].

During the World Federation of Neuro-Oncology 4th Quadrennial Meeting (21-2 4th November 2013) the Phase II study (ReACT) interim results of bevacizumab and rindopepimut, a peptide vaccine with EGFRVIII epitope in recurrent GBM, were reported [96]. Several interim trial results were also announced by Celldex Therapeutics, Inc. on the 14th of November 2014 [97]. These two announcements differ in terms of OS and 6-month PFS. The OS in the combination group (bevacizumab-naïve patients) was 12 months (for both data), whereas in the bevacizumab group this rate reached 7.9 months in 2013 (8.8 month in 2014). The median PFS was 3.7 and 2.0 months in 2013, and, according to the last update, 6-month PFS was achieved in 27% and 11%, respectively. As for bevacizumab resistant patients treated with bevacizumab and rindopepimut, the median OS was 5.6 months in 2013 (5.1 months in 2014); 6-month and 2-month PFS in 2013 were 8% and 29%, respectively. According to ClinicalTrials.gov (NCT01498328), the estimate primary completion date of the study is June 2015 [98].

3. DISCUSSION

Fine, describing clinical trials of bevacizumab in newly diagnosed GBM, marks that although there is an increase in PFS in RTOG 0825 [48] and AVAglio [104], the median OS is practically same as in control group. The author explains this fact by potential bevacizumab ability to lower vascular permeability in GBM and thus to hinder lesions progression according to MRI [105]. That is why the most widely spread antibody-based medication in GBM application expediency, at any rate in newly diagnosed GBM, was challenged.

As for recurrent GBM, we find it noteworthy not only to assess median OS and PFS, but also to search for a correlation between median OS after monoclonal antibody treatment enrollment and median time from initial diagnosis. Unfortunately, several bevacizumab clinical trials have failed to provide information about the latter criterion. We have found six studies where authors have listed median time from initial diagnosis among other data (Fig. 1). Although this number is too small to draw outcomes from, we tested these figures for a correlation, but did not find significant one (R=0.57; p=0.086). Nevertheless, even such a modest trial selection may suffice, as correlation between PFS and OS among these studies is more manifest (R=0.68, p=0.04), which coincides with much more detailed and circumstantial results obtained by Han et al. [22]. We deliberately included not only bevacizumab-naïve patients concerning studies into the analysis. Patients in the study conducted by Reardon et al. (2011) have received bevacizumab either at recurrence (52%) or after the initial diagnosis (48%) [29]. Desjardins et al. (2012) have enrolled 12% bevacizumab-pretreated patients [30].

The crucial problem to solve is why several patients with recurrent GBM respond to bevacizumab, while others fail to do it. The origin of such a predisposition is still not discovered. Ac-
According to H.S. Friedman, the difference between the bevacizumab effect in newly diagnosed and recurrent GBM has a lot to deal with the patterns of progression, which is predominantly angiogenic [99] or invasive, respectively [32, 56]. This statement is also proved by the BELOB outcomes, where 20 patients showed median OS data similar to that in AVAglio study, suggesting a role of patient selection [53]. More to the mentioned above, it is necessary to underline the growing importance of IDH mutation and MGMT methylation status in patients entering clinical trials, as some of the phenotypes are connected with a higher survival. We think that a trend is emerging to assess genetic profile of patients to be subjected to anti-glioma treatment. In particular, results obtained by Lai et al. show that bevacizumab treatment in newly diagnosed GBM is more efficient in patients with methylated MGMT than in ones with unmethylated MGMT previously gaining benefit on temozolomide [47]. In RTOG 0825 study, Gilbert et al. suggested prognostic value of MGMT methylation status per se, which did not differ in bevacizumab and control groups. The median OS and PFS in MGMT methylated tumors were 23.2 months and 14.1 months, compared to 14.3 months and 8.2 months, respectively, in unmethylated MGMT tumors. In BELOB trial [52], 7 of 8 IDH-mutant (and prognostically favorable) patients also had a methylated MGMT promoter, which scatters the role of IDH mutation in tumor response prediction and demands further clinical trials. In a study performed by Westphal et al., unmethylated MGMT phenotype patients treated with nimotuzumab showed OS improvement [84]. We assume that MGMT methylated phenotype is prognostically positive, regardless of antibody-based treatment, but in case of less favored unmethylated MGMT phenotype application of nimotuzumab is preferred (Fig. 2).

4. PERSPECTIV TARGETS FOR PASSIVE IMMUNOTHERAPY

Unconjugated antibody therapy has several alternative as application of immunotoxins, radio-
immunotherapy and conjugates of antibodies to nanoparticles. Noteworthy, not only “canonical” antigens are used to generate antibodies. According to De Bonis et al., radioimmunotherapy targets include, besides EGFRvIII, tenascin-C (an extracellular matrix protein), podoplanin (a transmembrane glycoprotein and thrombocyte aggregation factor), extra-domain B of fibronectin, αvβ3 integrin and H1 histone [100]. Chandramohan et al. have reviewed immunotoxins to EGFR, EGFRvIII, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), human transferrin receptor, α2-interleukin (IL) 13, glycoprotein nonmetastatic melanoma protein B, high molecular weight melanoma-associated antigen, folate receptor β, podoplanin [101-103]. Piao et al. have designed an immunotoxin based on scFv to 3′-isoLM1 and 3′,6′-isoLD1 gangliosides [104]. Wang et al. have obtained an scFv-based immunotoxin to multidrug resistance protein 3 [105].

We should separately shortly review antigens, which present a potential for future glioma immunotherapy. Among these it is necessary to list cytokines, growth factors and their receptors, for example, neuropilin-1 (a VEGF signaling coreceptor) [106], Tie2 angiopoietin receptor [107], transforming growth factor β [108], mature brain-derived growth factor [109], monocyte chemoattractant protein-1 [110], CD25 (an IL2 receptor)
[111], IL6 receptor [112], CXC chemokine receptor type 4 [113]. Of particular interest are semaphorins, which exhibit their functions via neuropilins and plexins. Initially, they were described as axon guidance molecules in central nervous system, but later their functions were revealed to be localized far beyond the brain [114]. Class 2 and 3 semaphorins are secreted proteins, while semaphorins 1,4,5,6 are transmembrane proteins. Nowadays, the most well described in gliomagenesis semaphorins belong to class 3, but even their role remains controversial, as this subfamily is quite heterogenic [115]. More to the mentioned above, in general tumorogenesis conception, contrary to that in gliomas, neuropilin-1 and semaphorin 3 associated pathways are thought to stimulate vessel maturation and thus increase tumor perfusion, abrogating tumor-associated hypoxia and suppressing tumor growth [114, 116].

Some experts believe in success of death-domain associated receptor-targeting therapy (DR5 [117], CD95 [12]). Several authors suggest a role of stem-cell markers in glioma progression (CD133 [118], stage-specific embryonic antigen-4 [119]). There is also a rationale in targeting molecules responding for cell-substrate interactions (vascular cell adhesion molecule 1 [120], β1 integrin [121], CD44 – a galuronan receptor [122], neuron-glial antigen 2 (NG2) [123]). CD47, an integrin-associated protein, is upregulated in more than 90% of autopsy GBM specimens, according to results of Stanford Stem Cell Biology and Regenerative Medicine [96, 124]. Higgins et al. have constructed a Mab-Zap immunotoxin (targeting moiety chemically conjugated to saporin, a ribosome-blocking protein) to NG2 and ganglioside GD3A, which proved to be more effective than targeting either of these antigens [125].

Intracellular signaling pathway mediating molecules are also perspective targets in high-grade glioma therapy. Selective inhibition of some signaling molecules can result in tumor growth arrest. These are src-homology 3-domain GRB2-like 1 [126], G-protein coupled receptor-associated sorting protein 1 [127], receptor tyrosine kinase EphA3 [128], receptor tyrosine kinase Mer [129], receptor tyrosine phosphatase β/C, FERM-domain of Pyk2 tyrosine kinase [130] (Pyk2 promotes the post anti-VEGF treatment C6 glioma cell invasion [131]). Phosphatidylinositol in complex with β2-glycoprotein I [132] and calcium-binding protein S100A4 [133] also deserve attention.

Chitinase-like protein YKL-40 (with no enzymatic activity) is also a potential antigen for targeted therapy [134]. Relative YKL-39 is also highly expressed in gliomas, but it acts in opposite direction, inhibiting tumor cells proliferation [135].

Noteworthy, targeting co-stimulation and co-inhibition T-lymphocyte molecules also present a perspective in glioma treatment. These are CD137 – a receptor from the tumor necrosis factor superfamily [136], the immune checkpoint ligand programmed death-1 (PD-L1) and cytotoxic T-lymphocyte antigen 4 (CTLA-4). Wainwright et al. showed that inhibition of PD-L1, CTLA-4 (the first two with monoclonal antibodies) and tryptophan-catabolizing enzyme indoleamine 2,3-dioxygenase (IDO) (the latter with D-1-methyltryptophan, a low-molecular inhibitor) was followed by tumor Treg depletion and almost 100% survival of mice with orthotopically transplanted GL261 glioma cell line, but only in presence of CD4+ and CD8+ T-cells [137]. The authors have also found surprising that PD-L1/CTLA-4 blocking agents administered at day 14 after the tumor implantation showed almost the same survival rate in mice as the triple-agent therapy. Moreover, they insist on reassessing existing treatment patterns, because temozolomide may cause lymphopenia and thus abrogate the tumor-suppressing immune response [138]. A clinical trial is ongoing to outline benefits of combining CTLA-4 and PD-L1 blockade by ipilimumab and nivolumab in patients with recurrent GBM [139]. Agonistic CD40 monoclonal antibodies in combination with cyclooxygenase-2 inhibitor celecoxib enhance CD4+ and CD8+-dependent anti-glioma immunity [140].

Yusubalieva et al. have demonstrated therapeutic effect of monoclonal antibodies to second extracellular loop of connexin-43, which appeared to inhibit the size of C6 glioma xenotransplants in a rat model and prolong the life span of the animals [141]. The antibodies were also used in combination with temozolomide and radiotherapy in the same model. The authors report that radiotherapy potentiated the effect of anti-connexin-43 antibodies but it was abrogated by temozolomide [142].
These results present an evidence that connexin-43 can be considered as a perspective targeting molecule for treatment of GBM.

We assume that future glioma passive immunotherapy will be based on more potent cytotoxic abilities of targeted medications. This is why plain antibodies should serve as vectors to introduce cytotoxic moieties into tumors. Hence, the search for new therapeutic targets for high-grade gliomas is going on further. We hope that these researches, as well as ones revealing genuine glioma etiology and pathogenesis will create a background for significant progress of tumor treatment.

CONFLICT OF INTEREST

I. Chekhonin declared no potential conflict of interest. O. Gurina declared no potential conflict of interest.

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I. Chekhonin and O. Gurina contributed equally to this work.

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