Bevacizumab in high grade glioma: Is there a subgroup that benefits?

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Abstract

Background: Prognosis for patients with original glioblastoma diagnosis remains poor. Antiangiogenic therapy with Bevacizumab in first line treatment did not lead to improvement in overall survival, while progression-free survival was prolonged by 3 to 4 months, in recent phase III studies. Bevacizumab therapy in glioblastomas is not successfully associated with any prognostic biological markers. Our study investigates the correlation between the use of bevacizumab and several clinical and molecular markers measured in gliomas everyday clinical practice.

Methods: We analyzed retrospectively 47 patients with high grade gliomas treated with bevacizumab in our medical oncology department. We examined the prognostic biomarkers used in clinical practice like IDH1 and IDH2 mutation status, EGFRvIII expression, MGMT promoter methylation status and BRAF V600E mutation status. We evaluated general patient characteristics, chemotherapy and overall survival.

Results: Our analysis revealed a trend towards improved overall survival in glioblastoma patients with poor prognosis and the use of bevacizumab. Overall survival in original glioblastoma diagnosis patients whose tumors carried unfavorable prognostic factors, such as EGFRvIII and unmethylated MGMT promoter, was similar to the survival of those with favorable prognostic factors. However, we were unable to identify a biomarker that was statistically associated with longer survival on bevacizumab.

Conclusions: Our study reports some hypothesis generating hints that bevacizumab is a treatment that seems to work better in patients whose tumors carry poor prognostic factors.

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Materials and methods

We performed a retrospective review of patients with malignant gliomas accrued between 2006 and 2015 and collected tissue samples (tumor cell content > 75% in all cases) from 47 adult patients. All patients with original glioblastoma diagnosis received temozolomide (tumor cell content > 75% in all cases) from 47 adult patients. All gliomas accrued between 2006 and 2015 and collected tissue samples

IDH1 mutation was detected either with immunohistochemistry or with PCR. Immunohistochemical staining was performed on 4 μm thick formalin-fixed, paraffin-embedded (FFPE) tissue sections with a Bench Mark Ultra immunostainer (Ventana Medical Systems, Tuscon, AZ, USA). Following deparaffinization and pretreatment with Ultra Cell Conditioner 1 (Ventana Medical Systems, Tuscon, AZ, USA), sections were incubated with anti-IDH1R132H antibody, clone H09 (Dianova, Hamburg, Germany). For chromogenic detection, the iView DAB Detection Kit (Ventana) was used. Strong cytoplasmic staining was scored as positive.

For IDH testing with PCR, exon 4 of IDH1 gene was amplified by PCR and mutation detection was carried out by sequencing analysis. PCR conditions and primer pairs for IDH1 & IDH2 genes were previously reported [29].

Sequencing was then performed (ABI Prism 3130 sequencer) and the sample’s DNA sequence was compared with reference sequences. In all samples negative for the presence of the R132H IDH1 mutation, exon 4 of IDH2 gene was tested.

For EGFRvIII detection via Real-time PCR, cDNA synthesis followed by Real Time PCR was carried out for the detection of EGFRvIII variant using SYBR green chemistry, on a RotorGene 6000 real-time analyzer (Qiagen), as described previously [30]. Each assay was performed in triplicate. Two housekeeping genes, β-actin and ABL, were used as reference genes. In the case of positive samples, DNA sequencing analysis was performed, to confirm the specificity of the obtained Real-time PCR products.

For EGFRvIII detection via Immunohistochemistry, FFPE specimens were sectioned at 4-5 microns and mounted on positively charged slides. Slides were air-dried and then deparaffinized and hydrated through a series of xylene, graded alcohol and water stations. Slides were placed in 3% hydrogen peroxide followed by heat induced epitope retrieval in a pressure chamber while submerged in citrate buffer. Protein block was applied to the slides followed by primary antibody (EGFRvIII) incubation. Staining was visualized with DAKO EnVision Rabbit HRP and DAKO DAB. Cut off for Negative was staining for < 10% of the cells and Positive ≥ 10%. EGFRvIII Immunohistochemistry testing was performed by Clarient Diagnostic Services, Inc. and funded by Cellidex Therapeutics, Inc.

The methylation pattern in the CpG island of MGMT was determined by chemical modification of unmethylated, but not methylated, cytosine to uracil, using the EpiTest Bisulfit Kit (Qiagen, Germany). A total of 10μl of bisulfite-treated DNA was carried on for PCR using specific primers for the modified methylated and the unmethylated DNA. PCR assays and primer pairs for MGMT gene were previously described [31].

BRAF exon 15 V600E mutation status was determined by PCR cycling and HRM analysis performed on the Rotor-Gene 6000" (Corbett Research, Mortlake, Australia). The intercalating dye used was SYTO 9 (Invitrogen Life Technologies, Carlsbad, CA, USA). PCR assays were performed as previously described. All HRM reactions were run in triplicate [32].

Sequencing analysis was performed whenever an aberrant melting profile was obtained (ABI Prism 3130 sequencer). The sample’s DNA sequence was compared with reference sequences for detection of V600E Braf mutation.

Statistical analysis

Categorical variables were presented as counts and corresponding percentages, while continuous variables are means, standard deviations and respective ranges. Possible associations among categorical variables, either markers or clinico-pathological / treatment characteristics, were examined by the use of Fisher’s exact test. The Kruskal-Wallis test was used in order to examine for associations among categorical and continuous variables (i.e., age, time to bevacizumab discontinuation).

Overall survival (OS) was measured from the date of diagnosis until death from any cause or date of last contact. Respectively, overall survival-bevacizumab (OS-bevacizumab) was measured from the date of bevacizumab initiation, while time to bevacizumab discontinuation from the date of treatment initiation until discontinuation. Time-to-event distributions were estimated using Kaplan-Meier method, while the log-rank test was used to assess differences. In addition, univariate and multivariate Cox regression analysis with line of treatment as a time-dependent covariate was performed in order to adjust for the lead time bias caused by the fact that only those patients who survive long enough have the chance to receive treatment with bevacizumab as 2nd or 3rd line.

All univariate tests were two-sided and significance level was set at 5%. The statistical analysis was performed using the SAS software (SAS for Windows, version 9.3, SAS Institute Inc., Cary, NC).

Results

In our analysis, we included 47 patients with primary brain tumors with median age 47.9 (from 21 to 69) years old. Original histologic diagnosis was glioblastoma multiforme in 35 cases while 12 patients had other glioma of low grade. 5 of the latter developed glioblastoma in subsequent biopsies. Radiotherapy was performed in every patient included. Maximal safe resection was achieved in 27 patients (57%), while partial resection or tumor biopsy was performed in 20 patients (43%) (Table 1).
Table 1. Patients Characteristics. 1: First Line Treatment, 2: Second Line Treatment, 3: Beyond Progression. A II: Astrocytoma Grade II, AA III: Anaplastic Astrocytoma Grade III, AOA III: Anaplastic Oligoastrocytoma Grade III, GBM: Glioblastoma, ODG II Oligodendroglioma Grade II, AO III: Anaplastic Oligodendroglioma Grade III.

| All Patients | N | % |
|-------------|---|---|
| Age         | Mean (SD) | 47.9 (12.2) |
|             | Min-Max   | 21-69 |
| Original Histological Diagnosis | | |
| AII         | 5 | 10.6 |
| AA III      | 19 | 40.4 |
| GBM         | 12 | 25.5 |
| ODG II      | 5 | 10.6 |
| AO III      | 1 | 2.1 |
| Second Histological Diagnosis | | |
| GBM         | 5 | 10.6 |
| AO III      | 2 | 4.2%

| Lines of Treatment | | |
| 1                | 4  | 8.6% |
| 1, 2             | 9  | 19.2% |
| 1, 2, 3          | 2  | 4.2% |
| 2                | 24 | 51% |
| 2, 3             | 3  | 6.4% |
| 3                | 6  | 12.8% |

| RT | | |
| Yes | 47 | 100% |

| Sex | | |
| Female | 17 | 36.2% |
| Male   | 30 | 63.8% |

| Type of Surgery | | |
| Biopsy/Subtotal Resection | 20 | 42.6% |
| Resection           | 27 | 57.4% |

IDH1 was mutated in 13 cases (27.6%). IDH2 was not mutated in any case studied and in 3 patient samples the material was not enough for IDH1 mutation screening.

MGMT promoter was found to be methylated in 30 cases (63.8%), unmethylated in 13 cases (27.7%) and 4 samples were not analyzed.

EGFRVIII expression was seen in 11 patients’ tumors (23.4%). This variant was not present in 35 cases (74.5%) and 1 sample was not analyzed.

BRAF V600E mutation was present in 2 samples (4.3%) and the rest 45 samples (95.7%) were wild type (Table 2).

Bevacizumab was prescribed as a monotherapy in 11 patients (23.4%) and in combination with other agents in 36 cases (76.6%). Bevacizumab was prescribed as a first line treatment with temozolomide after radiotherapy, in 15 patients (31%). Thirty-seven patients (78.7%) received bevacizumab as a second line treatment, in several combinations and as a third line treatment in 10 patients.

We also tried to investigate the statistical association of the biomarkers tested with the duration of therapy with bevacizumab. Patients received bevacizumab for 2 to 249 weeks. No statistically significant association between duration of therapy and biomarkers was found.

As expected, in our series overall survival was longer in patients with histology other than glioblastoma (median OS 221 vs. 127 weeks, log-rank p = 0.0043 and patients who had gross total resection as opposed to biopsy only or subtotal resection (median OS 154 vs. 93 weeks, p = 0.0381). IDH1 mutated patients and patients without EGFRVIII had longer overall survival (median OS 221 vs. 120 weeks, p = 0.0015 and median OS 151 vs. 120 weeks, p = 0.0372 respectively).

When we studied only the cases with original histology of glioblastoma, there was no statistical association of overall survival and any biomarker analyzed. The same was true for overall survival from the date of bevacizumab initiation. (Table 3, 4 and 5)

Associations among the biological markers studied did not reveal any statistically significant result.

Our analysis revealed a trend towards improved overall survival in glioblastoma patients with poor prognosis and the use of bevacizumab. Overall survival in original glioblastoma diagnosis patients whose tumors carried unfavorable prognostic factors, such as EGFRVIII and unmethylated MGMT promoter, was similar to the survival of those with favorable prognostic factors. Overall survival in EGFR VIII and wild type EGFR patients was 120 and 127 weeks respectively, while for unmethylated MGMT promoter and methylated MGMT promoter was 124 and 126 weeks respectively. This trend was not observed for IDH mutations (Table 4).

The line of first bevacizumab treatment was also tested as predictor of survival and in initial analysis it was found that patients receiving bevacizumab in the 3rd line for the first time had favorable OS although not statistically significant longer (Figure 1). In order to adjust for lead time bias, bevacizumab line of treatment was also used as a time-dependent factor in univariate and multivariate analysis. OS was measured from diagnosis date, and not from bevacizumab initiation date, since waiting time had to be taken into account. This time the 3rd line results were in contrast to the initial analysis. Patients who received bevacizumab as 2nd and/or 3rd line of treatment had a statistically significant increased risk of death compared to those who received it as a 1st line (2nd and 3rd vs. 1st, HR= 5.546 p < 0.001, 3rd vs. 1st HR = 4.549 p = 0.22, 2nd vs. 1st HR = 9.737 p = 0.0027, 3rd vs. 2nd HR = 0.92 p = 0.913). The findings regarding 3rd and/or 2nd line vs 1st line remained statistically significant even when bevacizumab treatment line was adjusted to patients’ molecular markers status and type of surgery (p = 0.0006) (Table 6).

Discussion

Though the published prospective randomized studies with bevacizumab have been negative, all of us treating patients with high grade glioma have seen some patients derive clinical benefit. The need to identify the subpopulation that benefits is imperative, so that appropriately enriched studies can be designed.

Table 2. Biomarkers Frequency.

| BIOMARKER | N | % |
|-----------|---|---|
| IDH1      | 31 | 66 |
| normal    | 2  | 4.3 |
| mut       | 13 | 27.7 |
| not done  | 3  | 6.4 |
| IDH2      | 42 | 89.4 |
| normal    | 5  | 10.6 |
| not done  | 5  | 10.6 |
| MGMT      | 13 | 27.7 |
| unmeth    | 30 | 63.8 |
| meth      | 4  | 8.5 |
| not done  | 4  | 8.5 |
| B-RAF     | 45 | 95.7 |
| normal    | 2  | 4.3 |
| mut       | 35 | 74.5 |
| EGFR VIII | 11 | 23.4 |
| not done  | 1  | 2.1 |
As expected, our study showed statistically significant association of overall survival with glioblastoma histology. In the upcoming WHO classification, histopathology is combined with molecular and genomic classification of glioblastomas, in an attempt to better characterize their biological and clinical behavior [1,33-35]. Median overall survival of glioblastoma patients in our series was 2.5 years, quite a bit longer from the overall survival of one year reported in the literature. Radical surgical resection of patients’ tumors in conjunction with a very

Table 3. Overall Survival.

| Variables          | N  | Min | Max  | Median | LL  | UL  | P-value |
|--------------------|----|-----|------|--------|-----|-----|---------|
| AAAll patients     | 47 | 32  | 599  | 129    | 98  | 160 |        |
| Age cut off at 50% | 23 | 40  | 483  | 98     | 62  | 129 | 0.004   |
| Low                | 24 | 32  | 599  | 174    | 127 | 222 |        |
| B-RAF Mut          | 2  | 129 | 292  | 210    | 129 | 292 | 0.7231  |
| Normal             | 45 | 32  | 599  | 127    | 98  | 160 |        |
| EGFR VIII Normal   | 35 | 52  | 599  | 151    | 114 | 186 | 0.0372  |
| viii variant       | 11 | 40  | 213  | 120    | 55  | 143 |        |
| Hist. Diagnosis    | 12 | 32  | 599  | 221    | 54  | .   |        |
| AA III-A II        | 35 | 51  | 417  | 127    | 91  | 146 |        |
| IDH1 Mut           | 13 | 32  | 599  | 221    | 120 | .   | 0.0015  |
| Normal             | 31 | 40  | 417  | 120    | 66  | 146 |        |
| IDH2 Normal        | 42 | 32  | 599  | 129    | 98  | 174 |        |
| MGMT Meth          | 30 | 52  | 483  | 129    | 93  | 186 | 0.7004  |
| Unmeth             | 13 | 40  | 599  | 154    | 55  | 179 |        |
| Hist. Diagnosis    | 12 | 32  | 599  | 221    | 54  | .   |        |
| AA III             | 3  | 40  | 120  | 54     | 40  | 120 |        |
| AOA III            | 1  | 483 | 483  | 483    | .   | .   |        |
| GMB                | 35 | 51  | 417  | 127    | 91  | 146 |        |
| ODG II             | 3  | 221 | 415  | .      | 221 | .   |        |
| AO III             | 3  | 32  | 175  | .      | 151 | .   |        |
| RT                 | 47 | 32  | 599  | 129    | 98  | 160 | .       |
| SEX                | 17 | 40  | 415  | 127    | 55  | 146 | 0.2575  |
| RT                 | 47 | 32  | 599  | 129    | 98  | 160 | .       |

Table 4. Overall Survival in GBM Patients.

| Variables          | N  | Min | Max  | Median | LL  | UL  | P-value |
|--------------------|----|-----|------|--------|-----|-----|---------|
| AAAll patients     | 35 | 51  | 417  | 127    | 91  | 146 |        |
| Age cut off at 50% | 19 | 51  | 186  | 98     | 62  | 127 | 0.0137  |
| Low                | 16 | 52  | 417  | 154    | 91  | 213 |        |
| B-RAF mut          | 2  | 129 | 292  | 210    | 129 | 292 | 0.3037  |
| Normal             | 33 | 51  | 417  | 126    | 89  | 146 |        |
| EGFR VIII normal   | 25 | 52  | 417  | 127    | 66  | 160 | 0.3946  |
| viii variant       | 10 | 51  | 213  | 120    | 55  | 143 |        |
| IDH1 mut           | 4  | 114 | 213  | 170    | 114 | 213 | 0.4569  |
| Normal             | 28 | 52  | 417  | 120    | 89  | 146 |        |
| IDH1 or IDH2 mut   | 4  | 114 | 213  | 170    | 114 | 213 | 0.5528  |
| Not done           | 3  | 51  | 100  | .      | .   | .   |        |
| IDH2 normal        | 30 | 52  | 417  | 126    | 89  | 154 |        |
| MGMT meth          | 23 | 52  | 417  | 126    | 91  | 174 | 0.252   |
| MGMT unmeth        | 9  | 51  | 179  | 124    | 55  | 160 |        |
| RT                 | 35 | 51  | 417  | 127    | 91  | 146 |        |
| SEX                | 14 | 51  | 222  | 127    | 55  | 146 | 0.2532  |
| RT                 | 35 | 51  | 417  | 127    | 91  | 146 |        |
| type of surgery    | 14 | 55  | 292  | 93     | 66  | 160 | 0.7053  |
| Resection          | 21 | 51  | 417  | 127    | 64  | 174 |        |
thorough and close monitoring and supportive care, are probably crucial determinants of this result. Reifenberger et al., investigated long-term survivors of glioblastoma with genome and transcriptome wide profiling, without finding any specific DNA copy number aberrations or expression signature. Besides the known molecular determinants like IDH mutations and MGMT methylation other factors, seem to be responsible for this long survival [35].

IDH1 mutated patients had longer survival (221 weeks) compared to normal IDH1 patients (120 weeks) (p value 0.0015). Favorable prognosis of IDH1 mutated patients is reported in several publications [13,14,35,36]. IDH1 mutation is not only a prognostic biomarker but also may be used as a target for immunotherapy [37].

Our study revealed a trend towards improved overall survival and the use of bevacizumab in glioblastoma patients with poor prognostic factors. For example, median overall survival since bevacizumab initiation in patients with EGFRvIII and unmethylated MGMT

| Variables          | N  | Min | Max | Median | LL  | UL  | P-value |
|--------------------|----|-----|-----|--------|-----|-----|---------|
| Age cut off at 50% | 23 | 3   | 113 | 50     | 35  | 56  | 0.0566  |
| IDH1 mut           | 13 | 0   | 254 | 47     | 20  | .   | .       |
| Hist. Diagnosis    | 12 | 0   | 254 | 64     | 39  | 76  | .       |
| Age cut off at 50% | 24 | 0   | 296 | 51     | 35  | 77  | .       |
| IDH1 mut           | 35 | 3   | 296 | 54     | 43  | 76  | .       |
| Hist. Diagnosis    | 12 | 0   | 254 | 51     | 35  | 93  | .       |
| Age cut off at 50% | 23 | 3   | 113 | 50     | 35  | 56  | 0.0566  |
| IDH1 mut           | 13 | 0   | 254 | 47     | 20  | .   | .       |
| Hist. Diagnosis    | 12 | 0   | 254 | 64     | 39  | 76  | .       |
| Age cut off at 50% | 24 | 0   | 296 | 51     | 35  | 77  | .       |
| IDH1 mut           | 35 | 3   | 296 | 54     | 43  | 76  | .       |
| Hist. Diagnosis    | 12 | 0   | 254 | 51     | 35  | 93  | .       |
| Age cut off at 50% | 23 | 3   | 113 | 50     | 35  | 56  | 0.0566  |
| IDH1 mut           | 13 | 0   | 254 | 47     | 20  | .   | .       |
| Hist. Diagnosis    | 12 | 0   | 254 | 64     | 39  | 76  | .       |
| Age cut off at 50% | 24 | 0   | 296 | 51     | 35  | 77  | .       |
| IDH1 mut           | 35 | 3   | 296 | 54     | 43  | 76  | .       |
| Hist. Diagnosis    | 12 | 0   | 254 | 51     | 35  | 93  | .       |
| Age cut off at 50% | 23 | 3   | 113 | 50     | 35  | 56  | 0.0566  |
| IDH1 mut           | 13 | 0   | 254 | 47     | 20  | .   | .       |
| Hist. Diagnosis    | 12 | 0   | 254 | 64     | 39  | 76  | .       |
| Age cut off at 50% | 24 | 0   | 296 | 51     | 35  | 77  | .       |
| IDH1 mut           | 35 | 3   | 296 | 54     | 43  | 76  | .       |
| Hist. Diagnosis    | 12 | 0   | 254 | 51     | 35  | 93  | .       |
| Age cut off at 50% | 23 | 3   | 113 | 50     | 35  | 56  | 0.0566  |
| IDH1 mut           | 13 | 0   | 254 | 47     | 20  | .   | .       |
| Hist. Diagnosis    | 12 | 0   | 254 | 64     | 39  | 76  | .       |
| Age cut off at 50% | 24 | 0   | 296 | 51     | 35  | 77  | .       |
| IDH1 mut           | 35 | 3   | 296 | 54     | 43  | 76  | .       |
| Hist. Diagnosis    | 12 | 0   | 254 | 51     | 35  | 93  | .       |
| Age cut off at 50% | 23 | 3   | 113 | 50     | 35  | 56  | 0.0566  |
| IDH1 mut           | 13 | 0   | 254 | 47     | 20  | .   | .       |
| Hist. Diagnosis    | 12 | 0   | 254 | 64     | 39  | 76  | .       |
| Age cut off at 50% | 24 | 0   | 296 | 51     | 35  | 77  | .       |
| IDH1 mut           | 35 | 3   | 296 | 54     | 43  | 76  | .       |
| Hist. Diagnosis    | 12 | 0   | 254 | 51     | 35  | 93  | .       |
| Age cut off at 50% | 23 | 3   | 113 | 50     | 35  | 56  | 0.0566  |
| IDH1 mut           | 13 | 0   | 254 | 47     | 20  | .   | .       |
| Hist. Diagnosis    | 12 | 0   | 254 | 64     | 39  | 76  | .       |
| Age cut off at 50% | 24 | 0   | 296 | 51     | 35  | 77  | .       |
| IDH1 mut           | 35 | 3   | 296 | 54     | 43  | 76  | .       |
| Hist. Diagnosis    | 12 | 0   | 254 | 51     | 35  | 93  | .       |
| Age cut off at 50% | 23 | 3   | 113 | 50     | 35  | 56  | 0.0566  |
| IDH1 mut           | 13 | 0   | 254 | 47     | 20  | .   | .       |
| Hist. Diagnosis    | 12 | 0   | 254 | 64     | 39  | 76  | .       |
| Age cut off at 50% | 24 | 0   | 296 | 51     | 35  | 77  | .       |
| IDH1 mut           | 35 | 3   | 296 | 54     | 43  | 76  | .       |
| Hist. Diagnosis    | 12 | 0   | 254 | 51     | 35  | 93  | .       |
| Age cut off at 50% | 23 | 3   | 113 | 50     | 35  | 56  | 0.0566  |
| IDH1 mut           | 13 | 0   | 254 | 47     | 20  | .   | .       |
| Hist. Diagnosis    | 12 | 0   | 254 | 64     | 39  | 76  | .       |
| Age cut off at 50% | 24 | 0   | 296 | 51     | 35  | 77  | .       |
| IDH1 mut           | 35 | 3   | 296 | 54     | 43  | 76  | .       |
| Hist. Diagnosis    | 12 | 0   | 254 | 51     | 35  | 93  | .       |
| Age cut off at 50% | 23 | 3   | 113 | 50     | 35  | 56  | 0.0566  |
| IDH1 mut           | 13 | 0   | 254 | 47     | 20  | .   | .       |
| Hist. Diagnosis    | 12 | 0   | 254 | 64     | 39  | 76  | .       |
| Age cut off at 50% | 24 | 0   | 296 | 51     | 35  | 77  | .       |
promoter was similar to those with favorable prognosis. This trend was not seen for IDH genes, probably because this mutation is crucial in gliomagenesis and its significance is thus unaffected.

Univariate analysis of the biomarkers tested as they relate to overall survival of patients treated with bevacizumab did not manage to reveal any statistically significant results therefore we did not proceed to multivariate analysis. Associations among the biological markers used in our study also did not yield any statistically significant result. Although the small number of patients in our study and its retrospective nature may be responsible for this result, other interpretations are also possible. One such factor may be intra-tumoral heterogeneity [38].

Bevacizumab treatment line was tested as predictor of survival and it was found that bevacizumab received in the 3rd line for the first time was associated with favorable OS although not with statistical significance. Taking into consideration that the time passed since bevacizumab initiation causes lead time bias, we further analyzed our data. Overall survival was measured from diagnosis date and not from bevacizumab initiation date, since waiting time had to be taken into account. Patients who received bevacizumab as a 2nd and/or 3rd line treatment demonstrated a considerably increased risk of earlier death, which indicates that higher line of treatment is associated with a shorter OS. This result remained statistically significant when adjusting for molecular parameters and surgery type.

Overall, we were unable to identify a marker that was associated with longer survival on bevacizumab. However, known poor prognosis parameters were not associated with worse outcome in our group. Despite the fact that our analysis has a small number of patients, and is thus more prone to random results, it is reasonable to assume that maybe this subgroup of glioblastoma patients that carry poor prognostic characteristics like IDH1 wild type, subtotal tumor resection and EGFRvIII expression may benefit from bevacizumab treatment.

The most likely explanation is that poor prognosis patients were more likely to get bevacizumab earlier, thus getting a comparative advantage, or a form of lead time advantage. This is shown by the fact that once line of therapy with bevacizumab is included in the assessment, the known poor prognosis parameters (such as EGFRvIII expression and wild type IDH) regain significance.

Whether the earlier initiation is in and of itself significant or it is the chance of getting bevacizumab in 2 or more lines, cannot be assessed since 1) most 11/15 patients that got bevacizumab in 1st line also got it in 2nd, 2) our numbers are too small and 3) there is no control group in the analysis, namely patients not treated with bevacizumab, therefore a causal relationship of this finding to bevacizumab per se cannot be proven.

Once again, we would like to reinforce that a plausible explanation for our findings may well be the retrospective nature of the study and small number of patients. An initial assumption that bevacizumab may benefit poor prognosis patients more, cannot be proven from this cohort. This is particularly the case since we have not included a non-bevacizumab treated cohort.

The assumption that earlier therapy with bevacizumab may in itself be more advantageous compared to therapy with subsequent lines is also not shown here due to the small numbers, and rather it was used to identify the lead time bias. Furthermore, this assumption is not supported by the published literature [6,7].

Both first and second line randomized control studies with bevacizumab in GBM have failed to show an advantage in the totality of the treated populations. However, all those involved in glioma patient care, have seen patients with impressive clinical responses. Therefore, the need to identify the subgroup that benefits is imperative, as regulatory constraints, are likely to deprive all patients from access to this agent.

The TCGA project subclassifies glioblastomas in 4 molecular types (classical, mesenchymal, neural and proneural) with specific genetic, epigenetic and transcriptional alterations [33]. Interestingly, Sandman et al., in their retrospective analysis of AVAglio raise the question whether molecular subtyping of glioblastoma tumors may reveal variants of this disease that may benefit with bevacizumab in first line [39]. The decision DX-GBM 9-gene assay separates patients with favorable outcome and proneural gene expression profile from those with poor prognosis expressing mesenchymal and angiogenesis genes, and was tested as a predictor for bevacizumab therapy in RTOG 0825 study [6,40]. This 9-Gene profile in combination with the existing clinical and molecular markers could be used to optimize therapeutic options for individual patients. Though, the application of the 9-Gene favorable predictive signature in patients with MGMT methylated glioblastomas treated with bevacizumab showed an adverse effect in survival, additional analysis of RTOG 0825 revealed a molecular signature of 43 genes and a 10-gene predictor of outcome for bevacizumab. The clinical application of this signature is under evaluation [41].

Conclusions

Our study implies that new treatment options in glioblastoma should be considered. There are a few hints that bevacizumab is a treatment that may work better in patients carrying poor prognostic factors. It is also obvious that the biomarkers tested here do not identify these glioblastoma patients adequately. New genetic analyses might reveal biological markers with direct relation to prognosis and treatment options.

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