Integrated analysis of programmed cell death ligand 1 expression reveals increased levels in high-grade glioma

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Abstract
Purpose Gliomas are the most frequent primary brain tumors of adults. Despite intensive research, there are still no targeted therapies available. Here, we performed an integrated analysis of glioma and programmed cell death ligand 1 (PD-L1) in 90 samples including 58 glioma and 32 control brain tissues.

Methods To identify PD-L1 expression in glioma, we performed immunohistochemical analysis of PD-L1 tumor proportion score (TPS) using the clinically valid PD-L1 22C3 antibody on 90 samples including controls and WHO grade I–IV gliomas.

Results We found that PD-L1 is highly expressed in a subfraction of glioma cells. Analysis of PD-L1 levels in different glioma subtypes revealed a strong intertumoral variation of PD-L1 protein. Furthermore, we correlated PD-L1 expression with molecular glioma hallmarks such as MGMT-promoter methylation, IDH1/2 mutations, TERT promoter mutations and LOH1p/19q.

Conclusion In summary, we found that PD-L1 is highly expressed in a subfraction of glioma, indicating PD-L1 as a potential new marker in glioma assessment opening up novel therapeutic approaches.

Keywords Glioma · Glioblastoma · Programmed cell death ligand 1 · PD-L1 · Molecularly targeted therapy

Introduction
Gliomas represent the most frequent primary brain tumors of adults (Louis et al. 2016a). According to the guidelines of the World Health Organization (WHO) for classification of brain tumors, gliomas are assigned to WHO-Grade I–IV tumors representing the degree of aggressiveness (Louis et al. 2016a).

While WHO-Grade I pilocytic astrocytomas (PA) are slow growing gliomas with a good prognosis, WHO-Grade IV glioblastomas (GBM) are highly malignant and diffusely infiltrating brain tumors with a very unfavorable outcome (Louis et al. 2016a). With a reported annual incidence of 3–4 cases per 100,000 population in the western world, GBMs are also the most frequently diagnosed brain tumors in adult patients (Louis et al. 2016a). The highly aggressive clinical behavior of GBMs is also reflected by the histological appearance: They show a high mitotic count, microvascular proliferation and necrosis (Louis et al. 2016a).

For advanced glioma stratification, the 2016 WHO Classification for Central Nervous System (CNS) Tumors integrated molecular genetic findings for advanced tumor classification: (Louis et al. 2016a). Key findings are mutations of the IDH1 and IDH2 (Isocitrate Dehydrogenase), H3F3A (Histone H3 Family 3A), HIST1H3B and HIST1H3C genes, TERT (Telomerase Reverse Transcriptase) promoter mutations as well as combined chromosomal losses of chromosome 1p and 19q (loss of heterozygosity, LOH) (Louis et al. 2016a). Integrating these molecular findings with histology, there is a severe advance in the prediction of patient outcome (Louis et al. 2007, 2016a, b).

With regard to therapeutic targets, the analysis of the O6-methylguanin–DNA–methyltransferase (MGMT) promoter is of crucial importance (Hegi et al. 2008, 2009; Kaina et al. 2007). The MGMT protein is associated with DNA
repair mechanisms, and epigenetic silencing of MGMT transcription by promoter hypermethylation compromises DNA repair mechanisms. Thus, a hypermethylated tumor promoter status has been found to associate with significantly improved survival in patients receiving combined and adjuvant radio-chemotherapy with temozolomide according to the EORTC/NCIC protocol (Hegi et al. 2005). Despite intensive research there is still no targeted therapy available and even by applying temozolomide, the patient outcome is still very unfavorable (Hegi et al. 2004, 2005, 2008, 2012; Hau et al. 2007).

Programmed Cell Death Ligand 1 (PD-L1) is a key player in triggering immune response in human cancers (Campesato et al. 2015; Gatalica et al. 2014; Ohaegbulam et al. 2015). Thereby, PD-L1 interacts with PD-1 (Programmed Cell Death 1) and inhibits immune response by induction of IL-10 (Interleukin) in monocytes (Said et al. 2010). In many tumors, there is an overexpression of PD-L1 that represents a druggable target (Sun et al. 2018; Honda et al. 2017; Kataoka and Ogawa 2016; Kataoka et al. 2016; Isaacsson Velho and Antonarakis 2018; Fan et al. 2019). In lung, breast, gastrointestinal and many other cancers with PD-L1 overexpression showed good response with PD-L1 inhibitors (Reck et al. 2016; Li et al. 2016; Fujita et al. 2015).

However, there is no reliable data available on PD-L1 in glioma with regard to morphological subtypes and genetic profiles. Here, we analyzed 58 glioma samples of WHO Grades I–IV and 32 control brain tissue specimens (16 frontal cortex and 16 frontal white matter samples). Furthermore, we performed integrated analysis of PD-L1 expression and molecular hallmarks of analyzed gliomas.

Molecular genetic characterization of gliomas

Molecular genetic analysis of glioma samples was performed as previously described (Kraus et al. 2020). In brief, DNA extraction for molecular pathological analysis was performed of microscopically identified representative tumor tissues with at least 90% of viable tumor cells applying the Maxwell system (Promega) according to the manufacturer’s instructions. IDH1 and IDH2 and BRAF hot spot mutations were analyzed applying the AmpliSeq for Illumina Cancer Hotspot Panel v2 (Illumina) on an Illumina MiniSeq next generation sequencing device (Illumina) according to the manufacturer’s protocols. Hot spot loci of TERT promoter, H3F3A, HIST1H3B and HIST1H3C genes were analyzed by Sanger sequencing as described previously (Kraus et al. 2020). MGMT promoter methylation was assessed by methylation specific PCR (MSP) and bisulfite sequencing (Kraus et al. 2015a, b). Assessment of 1p/19q status was performed by Fluorescence in situ hybridization (FISH) applying Zytolight 1p/1q and 19q/19p probe sets (Zytovision) following the manufacturer’s protocols. According to the guidelines of the current WHO classification, 1p/19q status was assessed in all IDH mutated glioma, since loss of 1p and 19q is only occurring in gliomas harboring IDH mutations (Louis et al. 2016a).

Immunohistochemical analysis

Routine immunohistochemistry performed on glioma samples included antibodies against GFAP, Ki67 and PHH3. PD-L1 expression was assessed applying the PD-L1 22C3 antibody (M3653 antibody kit, Dabo). Quantification of PD-L1 levels were performed by DH, TFJK and GH using the tumor proportion score (TPS) (Li et al. 2017; Neuman et al. 2016; Roge et al. 2017). All immunohistochemical stains were performed on a Ventana BenchMark Ultra device (Roche) according to the manufacturer’s protocols.

Materials and methods

Tissue collection

We analyzed 90 anonymized tissue samples including 58 glioma and 32 control brains samples. Gliomas were allocated to WHO Grades I to IV and an integrated molecular profiling was performed according to the 2016 WHO classification of CNS tumors (Louis et al. 2016a). All samples were formalin-fixed and paraffin-embedded (FFPE) and stored in the tissue collection of the University Institute of Pathology of the University Hospital Salzburg. Control samples included 16 frontal cortex and 16 frontal white matter samples of post-mortem brains that were formalin-fixed and paraffin-embedded and stored in the tissue collection of the University Institute of Pathology of the University Hospital Salzburg. Details on glioma and control samples including PD-L1 status can be found in Tables 1 and 2.
| ID  | Age [years] | Sex | Diagnosis           | WHO Grade | IDH1 | IDH2 | LOH 1p/19q | TERT   | MGMT | H3F3A | PD-L1 22C3 positive [%] |
|-----|-------------|-----|---------------------|-----------|------|------|------------|--------|------|-------|------------------------|
| T01 | 39          | f   | Pilocytic Astrocytoma | I         | wt   | wt   | n.a        | wt     | Unmethylated | n.a | 0 |
| T02 | 16          | m   | Pilocytic Astrocytoma | I         | wt   | wt   | n.a        | wt     | n.a | 0 |
| T03 | 39          | m   | Diffuse Astrocytoma  | II        | p.R132H | wt | wt | wt | Methylated | n.a | 0 |
| T04 | 47          | m   | Diffuse Astrocytoma  | II        | p.R132S | wt | wt | wt | Methylated | n.a | 0 |
| T05 | 76          | m   | Diffuse Astrocytoma  | II        | wt | wt | wt | C228T | Unmethylated | n.a | 0 |
| T06 | 39          | m   | Diffuse Astrocytoma  | II        | p.R132H | wt | wt | wt | Methylated | n.a | 1 |
| T07 | 63          | f   | Oligodendroglioma    | II        | p.R132H | wt | 1p/19q | C250T | Methylated | n.a | 0 |
| T08 | 54          | m   | Oligodendroglioma    | II        | p.R132H | wt | 1p/19q | C250T | Methylated | n.a | 0 |
| T09 | 42          | m   | Oligodendroglioma    | II        | p.R132H | n.a | 1p/19q | n.a | n.a | n.a | 0 |
| T10 | 37          | f   | Anaplastic Astrocytoma | III     | p.R132H | wt | wt | wt | Methylated | n.a | 1 |
| T11 | 67          | m   | Anaplastic Astrocytoma | III     | wt | wt | wt | C250T | Methylated | n.a | 7 |
| T12 | 46          | f   | Anaplastic Oligodendroglioma | III     | p.R132H | wt | 1p/19q | wt | Methylated | n.a | 45 |
| T13 | 32          | m   | Glioblastoma         | IV        | wt | wt | lp | C228T | Unmethylated | n.a | 88 |
| T14 | 72          | m   | Glioblastoma         | IV        | wt | wt | wt | C228T | Methylated | n.a | 0 |
| T15 | 65          | m   | Glioblastoma         | IV        | wt | wt | wt | C250T | Methylated | n.a | 5 |
| T16 | 77          | f   | Glioblastoma         | IV        | wt | wt | wt | C228T | Methylated | n.a | 95 |
| T17 | 79          | f   | Glioblastoma         | IV        | wt | wt | wt | C250T | Unmethylated | n.a | 0 |
| T18 | 28          | m   | Glioblastoma         | IV        | wt | wt | wt | Unmethylated | n.a | 40 |
| T19 | 52          | f   | Glioblastoma         | IV        | wt | wt | wt | C250T | Unmethylated | n.a | 100 |
| T20 | 44          | m   | Glioblastoma         | IV        | wt | wt | n.a | C250T | n.a | 2 |
| T21 | 78          | f   | Glioblastoma         | IV        | wt | wt | n.a | C228T | n.a | 34 |
| T22 | 45          | f   | Glioblastoma         | IV        | wt | wt | n.a | C228T | Methylated | wt | 70 |
| T23 | 77          | f   | Glioblastoma         | IV        | wt | wt | n.a | C228T | n.a | 90 |
| T24 | 50          | m   | Glioblastoma         | IV        | wt | wt | n.a | C250T | Methylated | n.a | 0 |
| T25 | 61          | m   | Glioblastoma         | IV        | wt | wt | n.a | wt | Unmethylated | n.a | 5 |
| T26 | 70          | f   | Glioblastoma         | IV        | wt | wt | n.a | C228T | Unmethylated | n.a | 0 |
| T27 | 69          | m   | Glioblastoma         | IV        | wt | wt | n.a | C250T | Unmethylated | n.a | 1 |
| T28 | 83          | m   | Glioblastoma         | IV        | wt | wt | n.a | C228T | Unmethylated | n.a | 0 |
| T29 | 51          | m   | Glioblastoma         | IV        | wt | wt | n.a | C250T | Methylated | n.a | 0 |
| T30 | 63          | f   | Glioblastoma         | IV        | wt | wt | n.a | C250T | Methylated | n.a | 0 |
| T31 | 66          | f   | Glioblastoma         | IV        | wt | wt | n.a | C228T | Methylated | n.a | 100 |
| T32 | 77          | m   | Glioblastoma         | IV        | wt | wt | n.a | C228T | Unmethylated | n.a | 0 |
| T33 | 58          | m   | Glioblastoma         | IV        | wt | wt | n.a | C228T | Unmethylated | wt | 0 |
| T34 | 56          | f   | Glioblastoma         | IV        | wt | wt | n.a | C228T | n.a | 0 |
| T35 | 76          | f   | Glioblastoma         | IV        | wt | wt | n.a | wt | methylated | n.a | 5 |
| T36 | 72          | f   | Glioblastoma         | IV        | wt | wt | n.a | C250T | n.a | 7 |
Table 1 (continued)

| ID  | Age [years] | Sex | Diagnosis          | WHO Grade | IDH1 | IDH2 | LOH 1p/19q | TERT     | MGMT | H3F3A  | PD-L1 22C3 positive [%] |
|-----|-------------|-----|--------------------|-----------|------|------|------------|----------|------|--------|------------------------|
| T37 | 25          | m   | Glioblastoma       | IV        | wt   | wt   | n.a        | wt       | Unmethylated | wt       | 32                     |
| T38 | 52          | m   | Glioblastoma       | IV        | wt   | wt   | 19q        | C228T    | Methylated   | n.a      | 76                     |
| T39 | 53          | m   | Glioblastoma       | IV        | wt   | wt   | wt         | C228T    | Unmethylated | n.a      | 90                     |
| T40 | 79          | f   | Glioblastoma       | IV        | wt   | wt   | wt         | wt       | Methylated   | n.a      | 55                     |
| T41 | 53          | m   | Glioblastoma       | IV        | wt   | wt   | wt         | C228T    | Unmethylated | n.a      | 15                     |
| T42 | 56          | m   | Glioblastoma       | IV        | wt   | wt   | wt         | wt       | Methylated   | n.a      | 100                    |
| T43 | 60          | m   | Glioblastoma       | IV        | wt   | wt   | n.a        | C250T    | Methylated   | n.a      | 42                     |
| T44 | 64          | m   | Glioblastoma       | IV        | wt   | wt   | wt         | C228T    | Methylated   | n.a      | 55                     |
| T45 | 66          | m   | Glioblastoma       | IV        | wt   | wt   | n.a        | C228T    | Methylated   | n.a      | 98                     |
| T46 | 82          | f   | Glioblastoma       | IV        | wt   | wt   | n.a        | C228T    | Unmethylated | n.a      | 0                      |
| T47 | 72          | m   | Glioblastoma       | IV        | wt   | wt   | n.a        | C228T    | Unmethylated | n.a      | 1                      |
| T48 | 87          | m   | Glioblastoma       | IV        | wt   | wt   | n.a        | C228T    | Unmethylated | n.a      | 28                     |
| T49 | 56          | m   | Glioblastoma       | IV        | wt   | wt   | 19q        | C228T    | Methylated   | n.a      | 0                      |
| T50 | 64          | m   | Glioblastoma       | IV        | wt   | wt   | 1p         | C250T    | n.a          | n.a      | 48                     |
| T51 | 59          | m   | Glioblastoma       | IV        | wt   | wt   | n.a        | C250T    | n.a          | n.a      | 90                     |
| T52 | 71          | m   | Glioblastoma       | IV        | wt   | wt   | wt         | wt       | Unmethylated | n.a      | 10                     |
| T53 | 50          | f   | Glioblastoma       | IV        | wt   | wt   | wt         | C228T    | Methylated   | n.a      | 0                      |
| T54 | 58          | m   | Glioblastoma       | IV        | wt   | wt   | 1p         | C250T    | Methylated   | n.a      | 24                     |
| T55 | 75          | f   | Glioblastoma       | IV        | wt   | wt   | 1p         | C250T    | Unmethylated | n.a      | 90                     |
| T56 | 41          | m   | Glioblastoma       | IV        | wt   | wt   | wt         | C250T    | Unmethylated | n.a      | 86                     |
| T57 | 39          | m   | Diffuse Midline Glioma | IV  | wt   | wt   | n.a       | C228T    | Unmethylated | K27M     | 2                      |
| T58 | 33          | f   | Diffuse Midline Glioma | IV  | wt   | wt   | n.a       | wt       | Methylated   | K27M     | 0                      |

Indicated are details on all 58 glioma samples

wt wild type, n.a. not available
Results

PD-L1 is expressed in human gliomas

To evaluate the significance of PD-L1 expression in gliomas, we used the PD-L1 22C3 antibody and performed immunohistochemical analysis in 90 tissue samples. These samples include 58 gliomas of WHO grades I, II, III and IV and 32 control brain samples including cortex and white matter regions. We found that there was no PD-L1 expression in control tissue, i.e., cortex (n = 16, Fig. 1a, b) and white matter (n = 16, Fig. 1c, d). In gliomas, we found uneven PD-L1 expression. Low grade gliomas consisting of WHO grade I pilocytic astrocytomas (n = 2, Fig. 1e, f) and WHO grade II diffuse gliomas (n = 7, Fig. 1g, h) did not show noteworthy PD-L1 expression. High grade gliomas consisting of WHO grade III anaplastic gliomas (n = 3, Fig. 1i, j) and WHO grade IV glioma (n = 46, Fig. 1k, l), showed intermediate to high PD-L1 expression. PD-L1 tumor proportion scores (TPS) of all 90 analyzed samples can be found in Fig. 1m.

PD-L1 is significantly overexpressed in high grade gliomas

A detailed analysis of PD-L1 expression in all 90 tissues specimen revealed significant overexpression of PD-L1 in glioma compared with healthy brain tissue: There was a statistically significant overexpression in glioma compared to cortex (p < 0.01, Fig. 2a) and white matter (p < 0.01, Fig. 2a). Analyzing PD-L1 expression and WHO grade confirmed high PD-L1 expression in high grade gliomas with a significant overexpression in WHO grade IV glioblastomas (p < 0.05, Fig. 2b). A detailed analysis of PD-L1 expression in glioma showed that 24% of all glioma showed TPS of ≥ 50%, 14% showed TPS of 25–50%, 10% showed TPS of 10–25%, 4% showed TPS of 5–10%, 10% showed TPS of 1–5% and 38% showed TPS of < 1% (Fig. 2c).

Integrated analysis of PD-L1 expression and molecular glioma hallmarks

Since gliomas show distinct molecular hallmarks, we next performed an integrated analysis of PD-L1 TPS and molecular genetic status: IDH mutation, TERT promoter mutation, MGMT promoter methylation and loss of heterozygosity of 1p and 19q (LOH 1p/19q). Interestingly, IDH wild-type glioma (n = 46) showed a significant higher expression of PD-L1 compared with IDH mutated gliomas (n = 8, p < 0.05, Fig. 3a). Due to the different biological backgrounds (Louis et al. 2016a) of pilocytic astrocytomas and H3F3A mutated diffuse midline gliomas, these samples were excluded from IDH analysis. In case of TERT promoter mutation, TERT mutated gliomas (n = 42) showed higher PD-L1 expression compared with TERT wild-type gliomas (n = 5, p > 0.05) (Fig. 3b). An analysis of loss of heterozygosity of 1p and 19q (LOH 1p/19q) showed higher PD-L1 expression in gliomas without LOH 1p/19q (n = 54) compared to LOH 1p/19q aberrant gliomas (n = 4, p > 0.05 (Fig. 3c)). Analysis of PD-L1 expression and MGMT promoter methylation showed higher PD-L1 expression in MGMT methylated glioma (n = 26) compared to MGMT unmethylated glioma (n = 23, p > 0.05) (Fig. 3d).

Table 2: Details on control samples

| ID  | Age [years] | Sex | Region            | PD-L1 22C3 positive [%] |
|-----|-------------|-----|-------------------|-------------------------|
| C01 | 95          | m   | Frontal Cortex    | 0                       |
| C02 | 56          | m   | Frontal Cortex    | 0                       |
| C03 | 62          | m   | Frontal Cortex    | 0                       |
| C04 | 65          | f   | Frontal Cortex    | 0                       |
| C05 | 92          | f   | Frontal Cortex    | 0                       |
| C06 | 75          | f   | Frontal Cortex    | 0                       |
| C07 | 75          | f   | Frontal Cortex    | 0                       |
| C08 | 87          | f   | Frontal Cortex    | 0                       |
| C09 | 54          | f   | Frontal Cortex    | 0                       |
| C10 | 67          | f   | Frontal Cortex    | 0                       |
| C11 | 79          | f   | Frontal Cortex    | 0                       |
| C12 | 69          | f   | Frontal Cortex    | 0                       |
| C13 | 89          | f   | Frontal Cortex    | 0                       |
| C14 | 52          | m   | Frontal Cortex    | 0                       |
| C15 | 59          | m   | Frontal Cortex    | 0                       |
| C16 | 54          | m   | Frontal Cortex    | 0                       |
| W01 | 95          | m   | Frontal White Matter | 0              |
| W02 | 56          | m   | Frontal White Matter | 1              |
| W03 | 62          | m   | Frontal White Matter | 0              |
| W04 | 65          | f   | Frontal White Matter | 0              |
| W05 | 92          | f   | Frontal White Matter | 0              |
| W06 | 75          | f   | Frontal White Matter | 0              |
| W07 | 75          | f   | Frontal White Matter | 0              |
| W08 | 87          | f   | Frontal White Matter | 0              |
| W09 | 54          | f   | Frontal White Matter | 0              |
| W10 | 67          | f   | Frontal White Matter | 0              |
| W11 | 79          | f   | Frontal White Matter | 0              |
| W12 | 69          | f   | Frontal White Matter | 0              |
| W13 | 89          | f   | Frontal White Matter | 0              |
| W14 | 52          | m   | Frontal White Matter | 0              |
| W15 | 59          | m   | Frontal White Matter | 0              |
| W16 | 54          | m   | Frontal White Matter | 1              |

Indicated are details on all 32 control samples.
Discussion

Despite intensive research, there are still no curative therapies available for GBM patients (Louis et al. 2016a). One milestone in glioblastoma therapy was the discovery of the connection between methylation of the MGMT promoter (Hegi et al. 2008, 2009; Kaina et al. 2007) and tumor response to chemotherapy using temozolomide in 2005 (Hegi et al. 2005). However, since then there have not been any significant advances in glioblastoma therapy.

In anti-tumor therapy, PD-L1 is already a key player in personalized medicine, since it represents a druggable target (Sun et al. 2018; Honda et al. 2017; Kataoka and Ogawa 2016; Kataoka et al. 2016; Isaacsson Velho and Antonarakis 2016).

**Fig. 1** PD-L1 expression in healthy brain tissue and glioma. Analysis of 90 tissue samples showed no PD-L1 expression in healthy cortex (a, b) and white matter regions (c, d). There was no noteworthy PD-L1 expression in low grade glioma, i.e., WHO grade I pilocytic astrocytoma (e, f) and diffuse astrocytoma (g, h). In high grade glioma there was an uneven PD-L1 expression with strong intertumoral heterogeneity in WHO grade III anaplastic astrocytoma (i, j) and glioblastoma (k, l). Distinct PD-L1 TPS scores of all 90 analyzed samples is presented in m. **CX** cortex, **WM** white matter, **PA** pilocytic astrocytoma, **DA** diffuse astrocytoma, **AA** anaplastic astrocytoma, **GBM** glioblastoma. a–l Scale bar: 100 µm
In many tumors, such as lung, breast, gastrointestinal PD-L1 inhibitors show great advances in patient treatment (Reck et al. 2016; Li et al. 2016; Fujita et al. 2015). Thereby, the expression profile of PD-L1 is assessed immunohistochemically.

Here, we assessed PD-L1 expression using the tumor proportion score (TPS), i.e., the percentage of PD-L1 positive tumor cells compared with all vital tumor cells (Li et al. 2017; Neuman et al. 2016; Roge et al. 2017) to assess PD-L1 expression in gliomas, and thus to evaluate the feasibility of PD-L1 inhibitors in highly aggressive brain tumors.

Our analysis of PD-L1 expression revealed that there are high PD-L1 expression levels in high grade glioma with a high interindividual variation (Fig. 1). While control cortex and white matter tissues showed mean PD-L1 TPS of 0%, gliomas showed significantly increased PD-L1 TPS with a mean of 28% in all 58 gliomas (Fig. 2a). A further subgroup analysis of different WHO grades showed that PD-L1 expression can be found predominantly in high grade gliomas with mean amounts of 18% positive tumor cells in WHO grade III gliomas and 34% positive tumor cell in WHO grade IV glioblastomas, respective (Fig. 2b).

Furthermore, we performed integrated analysis of molecular key hallmarks in glioma (IDH, TERT, MGMT methylation) and PD-L1 expression. Interestingly, we found significantly higher PD-L1 expression in IDH wild-type glioma (mean amounts of 32%) compared with IDH mutated gliomas (mean amounts of 6%, \( p < 0.05 \), Fig. 3a). In terms of TERT, we found higher PD-L1 expression in TERT mutated glioma (mean amounts of 32%) compared with TERT wild-type glioma (mean amounts of 20%, \( p > 0.05 \), Fig. 3b). An analysis of loss of heterozygosity of 1p and 19q (LOH 1p/19q) showed higher PD-L1 expression in gliomas without LOH 1p/19q (mean amounts of 30%) compared with LOH 1p/19q aberrant gliomas (mean amounts of 11%, \( p > 0.05 \), Fig. 3c). Analysis of MGMT promoter methylation showed higher PD-L1 expression in MGMT methylated glioma (mean

![Fig. 2 Statistical analysis of PD-L1 expression. Statistical analysis of PD-L1 expression showed significant overexpression of PD-L1 in glioma compared with healthy cortex (a) and white matter (b). Analysis of WHO grade I, II, III and IV glioma showed significant overexpression in high grade glioblastoma compared with low grade diffuse glioma. Analysis of individual TPS showed PD-L1 expression in glioma showed TPS of \( \geq 50\% \) in 27% of gliomas, TPS of 25–50% in 12% of gliomas, TPS of 10–25% in 10% of gliomas, TPS of 5–10% in 2% of gliomas, TPS of 1–5% in 9% of gliomas, and TPS of <1% in 40% of gliomas (c). a, b Indicated are mean and SEM. *\( p<0.05 \), **\( p<0.01 \)](image)

![Fig. 3 Integrated analysis of PD-L1 expression and molecular genetic hallmarks of glioma. Analysis of PD-L1 expression and IDH status showed higher expression of PD-L1 in IDH wild type compared to IDH R132H mutated glioma (a). In case of TERT promoter mutation there was higher PD-L1 expression in TERT C228T and C250T mutated glioma (b). Analysis of LOH 1p/19q showed higher expression of PD-L1 in glioma without LOH 1p/19q (c). Analysis of MGMT promoter methylation showed higher expression of PD-L1 in methylated glioma (d). Indicated are mean and SEM. m methylated, u unmethylated; n.s. not significant (\( p > 0.05 \), *\( p < 0.05 \))](image)
amounts of 30%) compared with MGMT unmethylated glioma (mean amounts of 26%, Fig. 3d).

Considering the gliomagenesis and aggressiveness of glioma, Louis et al. (2016a) these findings are of high therapeutic impact: while IDH mutation is a key pathway in gliomagenesis of WHO grade II and III gliomas and secondarily progressed WHO grade IV glioblastomas, IDH wild-type is a typical hallmark of primary WHO grade IV glioblastomas. Thus, the finding of high PD-L1 expression in IDH wild-type primary glioblastomas is of severe clinical importance opening new therapeutic approaches in therapy of highly aggressive glioblastoma. Vice versa to IDH mutations, TERT mutations are predominantly present in glioblastoma. Thus, the result of high PD-L1 expression in TERT mutated gliomas may also be of high clinical importance for therapy of highly aggressive glioblastomas.

Since the importance of PD-L1 has already been established as personalized medicine target in other tumor entities (Sun et al. 2018; Honda et al. 2017; Kataoka and Ogawa 2016; Kataoka et al. 2016; Isaacsson Velho and Antonarakis 2018; Fan et al. 2019; Reck et al. 2016; Li et al. 2016; Fujita et al. 2015) our findings in glioma may also open new therapeutic approaches in future brain tumor therapy. Thereby, our results are well in line with published data: Nduom et al. found that PD-L1 expression can be found in a subfraction of glioblastoma (Nduom et al. 2016). Thereby high PD-L1 expression is correlated with worse outcome (Nduom et al. 2016). Heiland et al. also report of high PD-L1 expression in glioblastoma with predominance of IDH wild-type glioblastomas (Heiland et al. 2017). Berghoff et al. analyzed PD-L1 expression and tumor infiltrating lymphocytes (TIL) in diffuse glioma and found that high PD-L1 expression and prominent TILs are predominantly present in IDH wild-type glioma compared with IDH mutant glioma (Berghoff et al. 2017). Hao et al. performed a meta-analysis of PD-L1 expression in glioblastoma and also confirmed that high PD-L1 expression can be found predominantly in glioblastoma with unfavorable outcome (Hao et al. 2020). This finding is well in accordance with our results demonstrating that highly aggressive IDH wild-type gliomas show higher PD-L1 expression. In contrast to previous studies, Nduom et al. (2016), Heiland et al. (2017), Hao et al. (2020) we performed PD-L1 expression using the widely accepted and clinically applicable PD-L1 22C3 clone (M3653 antibody kit, Dako) and the tumor proportion score (TPS). Thus, our approach using the PD-L1 22C3 antibody and TPS to evaluate PD-L1 expression opens the way for monoclonal antibody therapies such as pembrolizumab in a clinical setting (Illie et al. 2017). However, the significance of this study is limited due to the low number of cases in distinct subgroups of glioma, such as oligodendroglioma and diffuse midline glioma. Thus, further studies with an increased number of cases will be needed to validate these results. A further limitation of this study is that only a limited set of molecular parameters was assessed, e.g., there was no molecular assessment of the BRAF status in pilocytic astrocytomas. Furthermore, there is one case of diffuse astrocytoma with IDH wild-type status and TERT mutation included in this study. This is a very untypical genotype–phenotype combination and there should be further molecular assessment according to the cIMPACT guidelines (Louis et al. 2020; Gonzalez Castro and Wesseling 2021) including copy number profiling (CNP) to further characterize such cases and to assess, if the underlying biology is that of glioblastoma IDH wild type.

In summary, our findings demonstrate the significance of PD-L1 testing in glioma enabling new individualized strategies for molecularly targeted therapy in highly aggressive brain tumors.

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Data availability Details on data can be found in Tables 1 and 2, further information on the datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflicts of interest/Competing interests None declared.

Ethical approval All procedures performed in studies were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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