Neuro-Oncology Advances

7(1), vdaf037, 2025 | https://doi.org/10.1093/noajnl/vdaf037 | Advance Access date 20 February 2025



# Blood group O attributes to prolonged progression-free survival, overall survival, and 5-year survival in isocitrate dehydrogenase-wildtype glioblastoma patients with MGMT promoter methylation

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**Abstract**

**Background**. Glioblastoma (GBM) remains incurable despite multimodal therapeutic approaches. Here, we assessed the relevance of ABO blood groups for progression-free survival (PFS), overall survival (OS), and long-term survival in a large cohort of isocitrate dehydrogenase (IDH)-wildtype (wt) GBM patients.

**Methods**. Consecutive GBM patients (2009-2020) at a large tertiary brain tumor center were included, and clinical data were retrospectively abstracted. We dichotomized patients into those with blood group O and those with a Non-O blood group. Patient, tumor, and treatment characteristics were compared between these groups. Association with outcomes was assessed in univariable and multivariable settings via log-rank tests and Cox proportional hazards regressions, respectively.

**Results**. Five hundred fifty-four GBM IDH-wt had available ABO information. There were no substantial differences in patient, tumor, or treatment characteristics between group O and group Non-O. In contrast, blood group O patients showed increased PFS, OS, and 5-year survivals in both univariable and multivariable analyses. Differences were strongly pronounced in patients with MGMT promoter methylated tumors receiving standardized radiochemotherapy (OS blood group O 24.6 months [95%CI 17.8-31.4] vs Non-O 17.7 months [14.1-21.3], $P = .015$ log-rank analysis, hazard ratio 0.70 [95%CI 0.53-0.94]), but not apparent in MGMT promoter unmethylated tumors and in patients without (standardized) adjuvant therapy.

**Conclusion**. Blood group O status in conjunction with MGMT promoter methylation (including weak methylation) is an independent favorable prognostic marker in GBM IDH-wt patients receiving standardized radiochemotherapy. This finding is unprecedented, suggesting a linkage between the downregulation of a DNA repair protein and the absence of a functional blood cell surface glycosyltransferase.

## Key Points

- In glioblastoma with MGMT promoter methylation, blood group O facilitates prolonged survival.
- The impact of blood group O is best visible in patients with lowest tumor burden following standardized therapy.

## Importance of the study

Rare in literature, blood group systems were investigated as potential prognostic factors for overall survival (OS) and progression-free survival (PFS) in glioblastoma (GBM) patients. Blood group 0 was indicated to be modestly beneficial regarding OS compared with Non-0 individuals, without taking MGMT promoter methylation and isocitrate dehydrogenase (IDH) mutation status into account.

Data provide compelling evidence that IDH-wt GBM patients with MGMT promoter methylation undergoing standardized radiochemotherapy have a longer PFS and OS, translating into a higher 5-year survival rate if their ABO blood group status is 0.

The study's observation is of high clinical relevance, particularly in enhancing personalized neuro-oncological treatment strategies, supporting precise risk-benefit analyses in disease management and giving justified hope to patients with blood group 0. It may also define a new field of medical research, "blood group-drug interplay," aiming for further basic research in GBM, as well as in other (tumor) entities and treatments in general.

Glioblastoma (GBM) is the most common malignant primary brain tumor in adults. The annual age-adjusted incidence rate is 3.27 per 100.000 person-years in the USA,[1] with a higher rate in US-Whites. The German rate is 4.3 per 100.000 inhabitants.[2] Prognosis remains poor despite exceptional research efforts. According to the most recent official United States statistical report, the median overall survival (OS) for all consecutive GBM patients in the USA is 8 months.[1] The most recent German report documents a 10-month median OS rate.[3] Thus, the therapeutic objective remains predominantly palliative, and prognostic factors are gaining increased relevance.

Basic research has identified the methylation status of the O-6-methylguanine-DNA-methyltransferase (MGMT) promoter as an important and independent predictive factor in GBM patients undergoing treatment with alkylating chemotherapy such as temozolomide (TMZ).[4–8] Mutations in the isocitrate dehydrogenase (IDH) gene are also decisive prognostic markers as GBM patients with IDH mutations have a substantially higher OS.[9] Even though these mutations are rare (5%-7%),[10,11] they prompted a key renewal of the World Health Organization (WHO) Classification of Tumors of the Central Nervous System: IDH-mutant WHO grade IV gliomas are no longer considered glioblastomas but astrocytomas according to the most recent fifth edition of the WHO classification.[12] Nevertheless, the majority of grade 4 gliomas are IDH-wildtype (wt) GBM and the focus of this study. They have a poor outcome at large.[9] However, a wide range of survival spans is observed in multimodally treated patients.

Blood group systems, especially the ABO blood group system, have been reported to impact incidence and prognosis in various tumors. For instance, in gastric and pancreatic cancer, solid data confirm a protective role of blood group O and an adverse effect of blood group A in developing cancer.[13,14] Furthermore, the Rhesus blood group system impacts OS in nonsquamous cell lung carcinoma.[15] Far less data are available regarding associations between ABO blood group systems and OS and progression-free survival (PFS), respectively, with mainly inconsistent and conflicting conclusions.[16]

In the past, potential correlations between GBM as well as glioma in general, and blood group systems were examined. The majority of these studies, primarily in the 1950s, 1960s,[17,18] and in the last decade,[19–23] focused on the impact of ABO on the incidence of GBM. Since these studies were conducted at different times with varying glioma classifications and included various high-grade tumor entities and ethnic backgrounds, the conclusions remain inconsistent and unclear.

In contrast, and only thrice in literature, blood group systems were investigated as potential prognostic factors for OS and PFS in GBM patients.[19–21] The most recent study from Turkey delivered the most detailed GBM patients' characteristics so far and indicated blood group O to be modestly beneficial regarding OS compared with Non-O individuals,[21] while the remaining 2 studies disagreed.[19,20] However, molecular pathology was unknown in all investigations, that is, MGMT methylation and IDH mutation status were not available.

The aim of this study is to determine the impact of the ABO blood group system on OS and PFS in IDH-wt GBM patients in a large cohort. For the first time, we demonstrate the fundamental prognostic influence of blood group O on GBM patients with MGMT promoter methylation when treated with alkylating agents in a multimodal treatment setting. This condition translates into a markedly higher 5-year OS in blood group O GBM patients.

## Methods

### Study population

This retrospective study includes all 716 consecutive patients diagnosed with GBM between January 2009 and December 2020, who were treated at the Department of Neurosurgery, Münster University Hospital at any stage of their condition. The cohort includes individuals of predominantly European descent: primarily Germans, few Dutch, few Polish, and sporadic other European nationalities (all together ~98%), with the remaining being of Near Eastern descent. The histological criteria of GBM comply with the respective valid WHO classification at the time of diagnosis.[12,24,25] To adapt to the 2021 WHO Classification of Tumors of the Central Nervous System, tumors with IDH 1/2 mutations or patients with missing IDH status were excluded. We further excluded secondary GBM since these

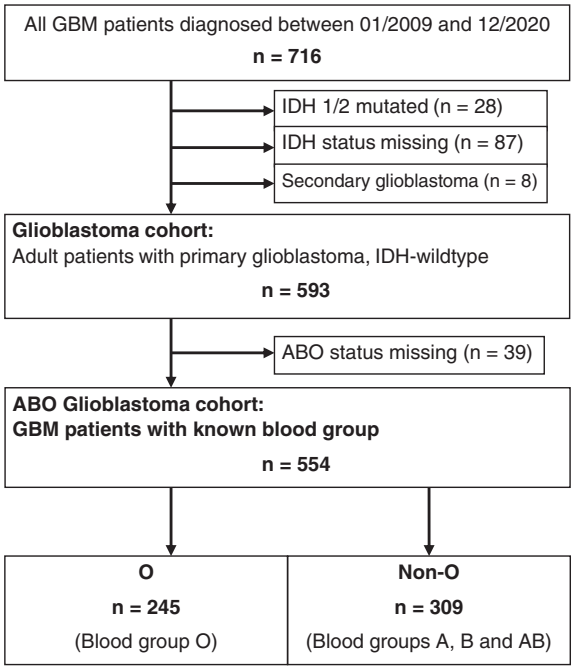



**Figure 1.** Glioblastoma cohort. All glioblastoma (GBM) patients diagnosed between January 2009 and December 2020 who were treated at the Department of Neurosurgery, Münster University Hospital provide the basis of this comprehensive database ($n$ = 716). Exclusion criteria include IDH 1/2 mutation, missing IDH data, and secondary GBM. A total of 554 patients remain for final analysis, categorized into blood group 0 patients (0) and blood group Non-0 patients (Non-0).

patients had already undergone first-line therapy due to the prior diagnosis of low-grade glioma. Thus, 593 primary GBM patients with an IDH-wt status remain for analysis (Figure 1).

Clinical data were collected in a strictly pseudonymous form. Based on preoperative contrast-enhanced T1-weighted MRI images, the number of lesions was captured, and the tumor localization according to the ICD-10 categorization for brain tumors (C71) was assessed. Intraoperative data were abstracted from the surgical report. If patients underwent a biopsy first and had a surgical tumor resection as a second surgical procedure, they would be classified as having undergone surgical resection. The extent of resection was grouped into "biopsy" (<5% of enhancing tumor removed), "partial/ incomplete resection" (5%-95% removed), and "complete/ gross total resection" (>95% removed) in line with recent studies.[26] This parameter was determined by radiological reports evaluating the postoperative MRI images (performed within 72 hours postoperatively). Adjuvant therapy data were collected in cooperation with the Department of Radiation Oncology. Only patients who fulfilled the criteria of adjuvant treatment according to Stupp et al.[7] or Herrlinger et al.[27] were included under the category of "standardized adjuvant radiochemotherapy."

Histopathological reports, including molecular analysis, were obtained from the Department of Neuropathology: MGMT-methylation analysis was uniformly performed according to Beier et al.,[28] with weak MGMT methylated tumors being classified as MGMT promoter methylated. IDH point mutation analysis included immunohistochemistry (IDH 1 p.R132H) and Sanger sequencing (IDH1/IDH2). Blood group assessment was performed as part of clinical preoperative routine. Based on cellular mechanisms, the ABO blood group system is dichotomized: Blood group O codes for a nonfunctioning or nonpresent glycosyltransferase versus Non-O, which summarizes blood groups A, B, and AB, all of which possess alleles coding for functioning glycosyltransferases.[29]

The date of diagnosis is defined as the date on which the sample was taken from the patient's brain that subsequently led to a positive pathology report and the diagnosis of glioblastoma. Date of death data was obtained from the "Westdeutsche Tumorzentrum," with the last query conducted on May 23, 2024.

Overall survival is defined as the time span between the date of diagnosis and the date of death of any cause or censoring at the date of last follow-up. Progression-free survival is defined as the time span between the date of diagnosis and the date of progression or censoring at the date of last follow-up. Tumor progression is defined as the image-based local tumor regrowth or the occurrence of new lesions according to the RANO criteria[30] and was determined in an interdisciplinary tumor board. Here, the date of imaging first demonstrating regrowth is considered the date of progression. Imaging data obtained in the period prior to this guideline were retrospectively analyzed.[30] If no tumor progression was observed prior to death, the date of death equals the date of progression.

## Ethical approval

This study was approved by the according Ethics Committee (Münster University and Medical Association Westphalia-Lippe, reference number 2021-685-f-S). The entire data acquisition and data processing were conducted in accordance with the ethical standards of the 1964 Helsinki Declaration, its later amendments, and comparable conventions and codes of conduct.

## Statistical analysis

The study population was characterized by descriptive statistical analyses of means and standard deviations (normal distribution), median, and frequencies (nonnormal distribution), respectively. Potential associations between categorical variables were analyzed via Fisher's exact test or Chi-square test. In subgroups with an expected count of less than 5, Fisher-Freeman-Halton's exact test would be used instead (ie, certain tumor localizations). Continuous variables were compared via Mann–Whitney-U test, as not all variables follow a normal distribution according to the Shapiro–Wilk test (ie, age).

Analyses of OS and PFS were conducted via log-rank tests and visualized via Kaplan–Meier plots for each individual subgroup. For multivariable analysis, Cox proportional hazards methods were applied to identify independent prognostic factors on PFS and OS. For 5-year

**Table 1.** Baseline characteristics. All GBM patients (A) split by extent of resection: (B) biopsy cohort (*n* = 102, 17.2%) and (C) resection cohort (*n* = 491, 82.8%), including complete resection (*n* = 210, 42.8%), partial resection (*n* = 225, 45.8%), and not determined (*n* = 56, 11.4%). *P*-values indicate comparative analysis of (B) biopsy cohort with (C) resection cohort.

| | A—All GBM patients | | B—Biopsy | | C—Resection | | P |
|---|---|---|---|---|---|---|---|
| | *n* = 593 | %[a] | *n* = 102 | %[a] | *n* = 491 | %[a] | |
| Age (y)[b] | 64.5 (19.6-89.1) | | 67.7 (19.6-89.1) | | 63.9 (22.8-87.6) | | **.005** |
| BMI (kg/m²)[b] | 25.8 (13.8-58.0) | | 25.7 (18.6-42.3) | | 25.8 (13.8-58.0) | | .832 |
| Sex[c] | | | | | | | .509 |
| Male | 349 | 58.9 | 57 | 55.9 | 292 | 59.5 | |
| Female | 244 | 41.1 | 45 | 44.1 | 199 | 40.5 | |
| Tumor localization (ICD-10) [d,e] | | | | | | | **<.001** |
| Frontal lobe | 94 | 15.9 | 5 | 4.9 | 89 | 18.1 | |
| Temporal lobe | 105 | 17.7 | 3 | 2.9 | 102 | 20.8 | |
| Parietal lobe | 52 | 8.8 | 6 | 5.9 | 46 | 9.4 | |
| Occipital lobe | 22 | 3.7 | 2 | 2.0 | 20 | 4.1 | |
| Cerebral ventricle | 4 | 0.7 | 1 | 1.0 | 3 | 0.6 | |
| Cerebellum | 3 | 0.5 | 0 | 0.0 | 3 | 0.6 | |
| Brain stem | 5 | 0.8 | 5 | 4.9 | 0 | 0.0 | |
| Overlapping lesion[f] | 287 | 48.4 | 66 | 64.7 | 221 | 45.0 | |
| Cerebrum, others[g] | 21 | 3.5 | 14 | 13.7 | 7 | 1.4 | |
| Number of lesions[c] | | | | | | | **<.001** |
| Singular | 429 | 75.5 | 54 | 56.8 | 375 | 79.3 | |
| Multiple | 139 | 24.5 | 41 | 43.2 | 98 | 20.7 | |
| Hemisphere[c] | | | | | | | **<.001** |
| Unihemispheric | 486 | 82.0 | 61 | 59.8 | 425 | 86.6 | |
| Bihemispheric | 107 | 18.0 | 41 | 40.2 | 66 | 13.4 | |
| Use of 5-ALA[c] | | | | | | | **<.001** |
| Yes | 435 | 77.1 | 3 | 3.0 | 432 | 93.1 | |
| No | 129 | 22.9 | 97 | 97.0 | 32 | 6.9 | |
| Anaesthesia awareness[c] | | | | | | | **<.001** |
| Yes | 121 | 21.4 | 5 | 5.0 | 116 | 24.9 | |
| No | 444 | 78.6 | 95 | 95.0 | 349 | 75.1 | |
| Adjuvant therapy[i] | | | | | | | **<.001** |
| No adjuvant therapy | 57 | 9.6 | 23 | 22.5 | 34 | 60.9 | |
| Standardized radiochemotherapy[j] | 446 | 75.2 | 51 | 50.0 | 395 | 80.5 | |
| Other and unknown | 90 | 15.2 | 28 | 27.5 | 62 | 12.6 | |
| ABO[d] | | | | | | | .436 |
| O | 245 | 44.2 | 35 | 40.7 | 210 | 44.9 | |
| A | 228 | 41.2 | 36 | 41.9 | 192 | 41.0 | |
| B | 59 | 10.6 | 13 | 15.1 | 46 | 9.8 | |
| AB | 22 | 4.0 | 2 | 2.3 | 20 | 4.3 | |
| RhD[c] | | | | | | | .754 |
| dd (neg) | 91 | 16.5 | 15 | 17.4 | 76 | 16.3 | |
| D.(pos) | 461 | 83.5 | 71 | 82.6 | 390 | 83.7 | |
| MGMT promoter[c] | | | | | | | .265 |
| Methylated[h] | 316 | 54.1 | 57 | 59.4 | 259 | 53.1 | |
| Unmethylated | 268 | 45.9 | 39 | 40.6 | 229 | 46.9 | |
| ATRX expression[c] | | | | | | | 1.000 |
| Yes | 427 | 96.2 | 74 | 96.1 | 353 | 96.2 | |

**Table 1.** Continued

| | A—All GBM patients | | B—Biopsy | | C—Resection | | P |
|---|---|---|---|---|---|---|---|
| | *n* = 593 | %[a] | *n* = 102 | %[a] | *n* = 491 | %[a] | |
| No (loss of ATRX) | 17 | 3.8 | 3 | 3.8 | 14 | 3.8 | |
| Ki67[b] | 24 (0-89) | | 24 (0-89) | | 23 (4-70) | | .218 |
| Median PFS (months)[k] | 6.6 (6.0-7.3) | | 3.6 (2.7-4.4) | | 7.4 (6.6-8.2) | | **<.001** |
| Median OS (months)[k] | 11.8 (10.4-13.2) | | 4.2 (3.3-5.1) | | 13.7 (12.3-15.2) | | **<.001** |
| 5-y long-term survivors[c] | 29 | 4.9 | 0 | 0.0 | 29 | 5.9 | **.005** |
| Alive patients[c,l] | 22 | 3.7 | 0 | 0.0 | 22 | 4.5 | **.021** |
| Median observation time of alive patients (months)[b] | 72.5 (40.5-135.1) | | N.A.[m] | | 72.5 (40.5-135.1) | | N.A |

[a]In percent of nonmissing values.
[b]Median (range), Mann–Whitney-U-test.
[c]Fisher's exact test.
[d]Fisher-Freeman-Halton exact test.
[e]Adapted at the International Statistical Classification of Diseases and Related Health Problems 10th revision C71 malignant neoplasm of brain.
[f]Any lesion overlapping at least 2 lobes, including bifrontal lesions.
[g]Cerebrum except lobes and ventricles.
[h]Including the weak methylated status (61/316 tumors [19.3%] are classified as "weakly methylated").
[i]Chi-square test.
[j]Therapy either with temozolomide (TMZ) or TMZ/Lomustin (CCNU) combination and radiotherapy up to acumulative dose of 60 Gy in 2 Gy fractions.
[k]Months since diagnosis, (95% confidence interval), Log Rank test.
[l]Patients who were alive at the last query on 23 May 2024; patients lost to follow-up before the last query was not included.
[m]No patient remained alive at last to follow-up.

survival analysis, logistic regression was applied. Forward stepwise variable selection was used for both model calculations (inclusion criterion: *P*-value in Likelihood-Ratio test < 0.05, exclusion criterion *P* > .10). Confidence intervals were set at 95%. A two-sided *P*-value < .05 was considered to be clinically relevant. An adjustment for multiplicity was not determined and could not be calculated, and therefore, the term "statistically significant" should not be applied. All analyses were performed using SPSS Statistics version 29.0.0 0 (IBM, Armonk, NY, USA).

# Results

## Study population

Baseline characteristics of the GBM IDH-wt cohort (*n* = 593, Figure 1) are detailed in Table 1. At the time of primary diagnosis, most GBMs were single lesions (75.5%) affecting only one side of the brain (82.0%). Nevertheless, almost half of all tumors involved more than 1 lobe (48.4%). In 54.1% of all tumor tissues, MGMT promoter was methylated. Three-quarters of all patients (75.2%) underwent standardized adjuvant radiochemotherapy up to a cumulative dose of 60 Gy in 2 Gy fractions. Median OS in all 593 patients was 11.8 months (95% CI: 10.4-13.2), and median PFS was 6.6 months (95% CI: 6.0-7.3).

Since the ABO blood group status was missing in 39 cases, 554 individuals remained for blood group analysis. Of these, 245 patients (44.2%) had ABO blood group O, and the remaining 309 (55.8%) were categorized as blood group Non-O (A = 41.1%, B = 10.6%, AB = 4.0%) (Table 1, Figure 1).

Baseline patient characteristics did not differ between blood group O and blood group Non-O except for PFS and survival (Supplementary Table S1). The study cohort's blood group distribution is representative when compared with a very large German collective[31] (*n* = 624,161 individuals, *P* = .455). The same applies for the Rhesus blood group system (*P* = .650, Supplementary Table S2).

## Univariable analysis

First, well-established prognostic factors were tested for associations with OS in the entire IDH-wt cohort (*n* = 593) to ensure comparability with other large GBM cohorts. As expected, strong associations were found for extent of resection, therapy regimen, MGMT promoter methylation, age at diagnosis, and ECOG score (Log Rank *P* < .001 for all comparisons; Supplementary Figure S1).

Within the entire ABO cohort (*n* = 554), patients with blood group O compared with patients with blood group Non-O had both a slightly longer PFS (7.1 months [95% CI: 6.2-8.1] vs 6.8 months [95% CI: 5.8-7.7], *P* = .014, Supplementary Figure S2A) as well as OS (12.9 months [95% CI: 10.3-15.5] vs 12.0 months [95% CI: 10.2-13.8], *P* = .112; Supplementary Figure S2B).

However, when analyzed in more detail, PFS and OS in patients with MGMT promoter unmethylated tumors undergoing standardized adjuvant radiochemotherapy are virtually identical for both blood group O and blood group Non-O, respectively (Figure 2A and B).

Contrarily, the impact of blood group O was by far stronger in the subgroup of patients with MGMT promoter methylated tumors who received standardized adjuvant

radiochemotherapy, for both PFS and OS (PFS blood group O: 14.6 months [95% CI: 11.9-17.2] vs blood group Non-O: 9.6 months [95% CI: 7.7-11.5], $P < .001$; OS: 24.6 months [95% CI: 17.8-31.4] vs 17.7 months [95% CI: 14.1-21.3], $P = .015$; Figure 2C and D).

Moreover, the impact of blood group O on PFS and OS in patients with MGMT promoter methylated tumors was most prominent in patients with a complete resection with a single enhancing lesion followed by standardized radiochemotherapy (PFS: 22.0 months [95% CI: 17.6-26.3] vs 13.5 months [95% CI: 6.0-21.1], $P = .013$; OS: 37.2 months [95% CI: 20.7-53.6) vs 20.4 months [95% CI, 15.7-25.2], $P < .001$; Figure 2E and F).

The observation that patients with MGMT promoter methylated (but not unmethylated) tumors benefit from blood group O could also be clearly seen in the Forest plot analysis (Figure 3). Of further note, the course of MGMT promoter weakly methylated GBM patients is distinct from unmethylated patients (Supplementary Figure S3), and their PFS and OS is almost as favorable as MGMT methylated patients. This is of high clinical importance with respect to the selection of neuro-oncological treatment strategies. Likewise, patients with blood group O and being MGMT promoter weakly methylated show a trend toward prolonged survival, which is obvious in fully methylated patients (Supplementary Figure S4).

The beneficial prognostic effect of blood group O on PFS and OS was visible in patients with MGMT promoter methylated tumors, regardless of whether these patients were treated by standard temozolomide chemotherapy[7] or a combination protocol with the addition of lomustine[27] (Supplementary Figure S5). However, due to the much smaller sample size of patients treated with lomustine-temozolomide combination therapy,[27] the result with this regimen requires verification in a larger sample size.

### Multivariable survival analysis for PFS and OS

Cox Proportional Hazards Models confirmed the independent prognostic benefit of blood group O in patients with MGMT promoter methylation receiving standardized adjuvant radiochemotherapy for both PFS and OS (Table 2). Included variables were age, sex (male vs female), hemisphere (unihemispheric vs bihemispheric), lesions (singular vs multiple), extent of resection (biopsy vs incomplete resection vs complete resection), and ABO blood group (O vs Non-O). For PFS, a younger age, unihemisperic, or singular lesions, a complete resection and blood group O independently favored survival. Only sex does not take an influence on survival. Similar results were found for OS: all above named parameters except sex and the number of lesions independently favor survival.

As anticipated by Kaplan–Meier estimates, the effect of blood group O on increased survival was even stronger in PFS ($P = .002$) than in OS ($P = .049$).

### Long-term survival rates

The overall observed 5-year OS rate is 4.9% (GBM cohort $n = 593$, Table 1) and 5.2% (ABO GBM cohort $n = 554$, Supplementary Table S1), respectively. There are major differences among subgroups: The 5-year survival probability is mainly driven by MGMT promoter methylated tumor patients treated with standardized radiochemotherapy with blood group O, as illustrated in Figure 4: 12.7% estimated, 11.9% observed. In contrast, blood group O did not affect survival in GBM without MGMT promoter methylation (estimated 5-year OS 1.9%, observed 2.8%), and in blood group Non-O, 5-year OS rate do not differ among patients with and without MGMT promotor methylation (Figure 4). In addition, only 1 patient with nonstandardized treatment survived beyond 5 years (0.8%, data not shown).

A multivariable logistic regression was applied to focus on 5-year survival, including the same parameters as described above regarding the multivariate analysis for PFS and OS. Age, singular lesions, MGMT promoter methylation, and blood group O were strong independent factors for 5-year survival (Supplementary Table S3).

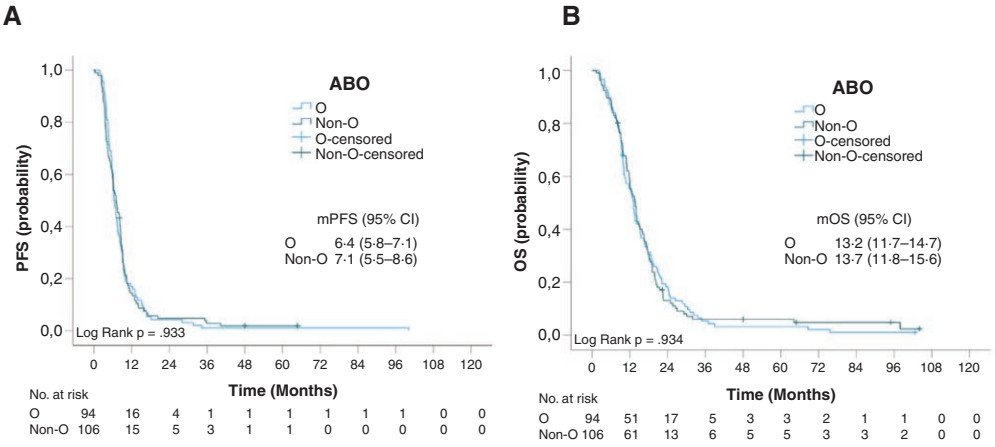
It remains to be seen whether elevated 5-year survival rates may translate into higher 10-year survival rates; currently (May 2024), observed 10-year survival is 0.65% (estimated: 3.0%) in blood Non-O, and 0.82% (estimated: 2.3%) in blood group O, respectively. Once GBM patients have reached 5-year survival, neither MGMT promoter methylation nor ABO blood group seem to impact further survival (Supplementary Figure S6).

## Discussion

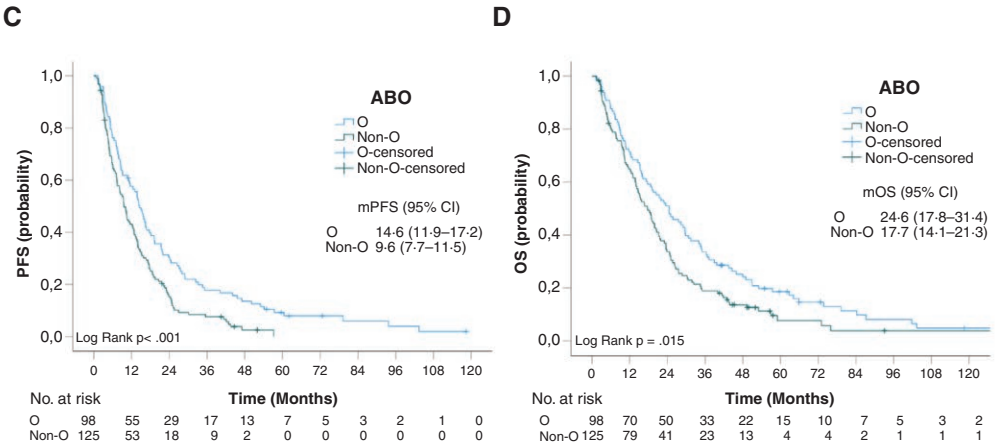
This study demonstrates for the first time a fundamental impact of blood group O on the outcome of IDH-wt GBM patients with MGMT promoter methylation treated with standardized radiochemotherapy. This observation is valid for both OS and PFS in a large Central European, predominantly German cohort, being "randomized at birth" regarding blood group status. In MGMT promoter methylated patients receiving standardized therapy, the impact of blood group O will manifest early. In the absence of standardized therapy, prognosis is very limited in general (Supplementary Figure S1B), and neither blood group nor MGMT methylation has an impact on OS (data on file).

Examinations of potential interactions between the ABO blood group system and GBM patients have been conducted several times previously, primarily focusing on the impact on GBM incidence. Briefly, the majority of studies report no correlations between ABO and GBM incidence,[19–21] alinging with our investigations. Regarding blood group and OS, only 1 study in a medium-sized Turkish GBM cohort reports that patients with blood group O have prolonged OS. Although molecular markers such as MGMT and IDH, as well as PFS, were not considered, the authors hypothesized that this missing information "had no influence on these results."[21] In contrast, leveraging a much larger cohort with detailed patient and tumor characteristics, we highlight that MGMT promoter methylation is, in fact, of the highest relevance mechanistically. Notably, the Turkish population has a different blood group distribution compared with Central Europe (Supplementary Table S4).[21] Therefore, the favorable influence of blood group O on overall survival does not depend on the blood group distribution of different ethnic groups.

**MGMT promoter unmethylated: patients with standardized radiochemotherapy**




**MGMT promoter methylated: patients with standardized radiochemotherapy**




**MGMT promoter methylated: complete resection of a singular lesion & standardized radiochemotherapy**

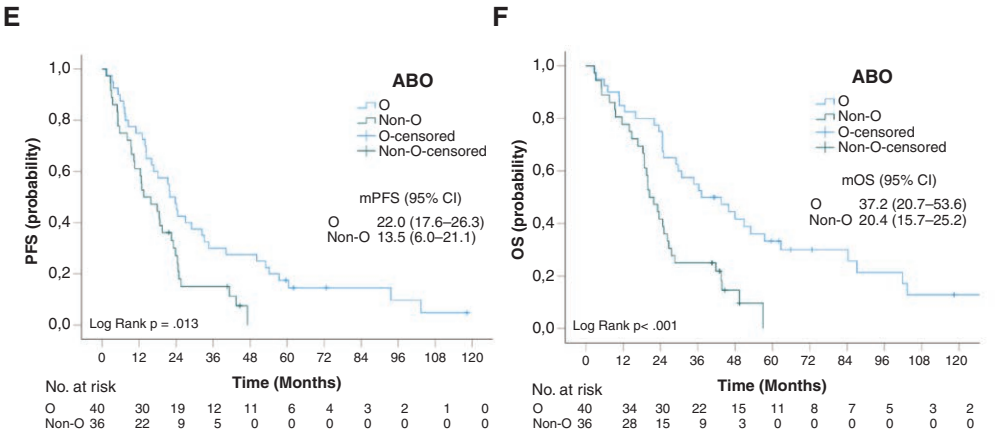



**Figure 2.** Patients with standardized radiochemotherapy: PFS and OS in response to ABO blood group. (A, B) MGMT promoter unmethylated patients ($n$ = 200) receiving standardized adjuvant radiochemotherapy regarding PFS (A) and OS (B). (C, D) MGMT promoter methylated patients ($n$ = 223) receiving standardized adjuvant radiochemotherapy. Blood group O has a significant better survival regarding PFS (C) and OS (D). The corresponding Cox regression is displayed in Table 2; Hazard ratio Figure 3, fourth row from the bottom. (E, F) In a subgroup of MGMT promoter methylated patients (singular lesion and complete resection only, $n$ = 76), the outcome is most favorable. The corresponding Hazard Ratio is displayed in Figure 3, bottom row.

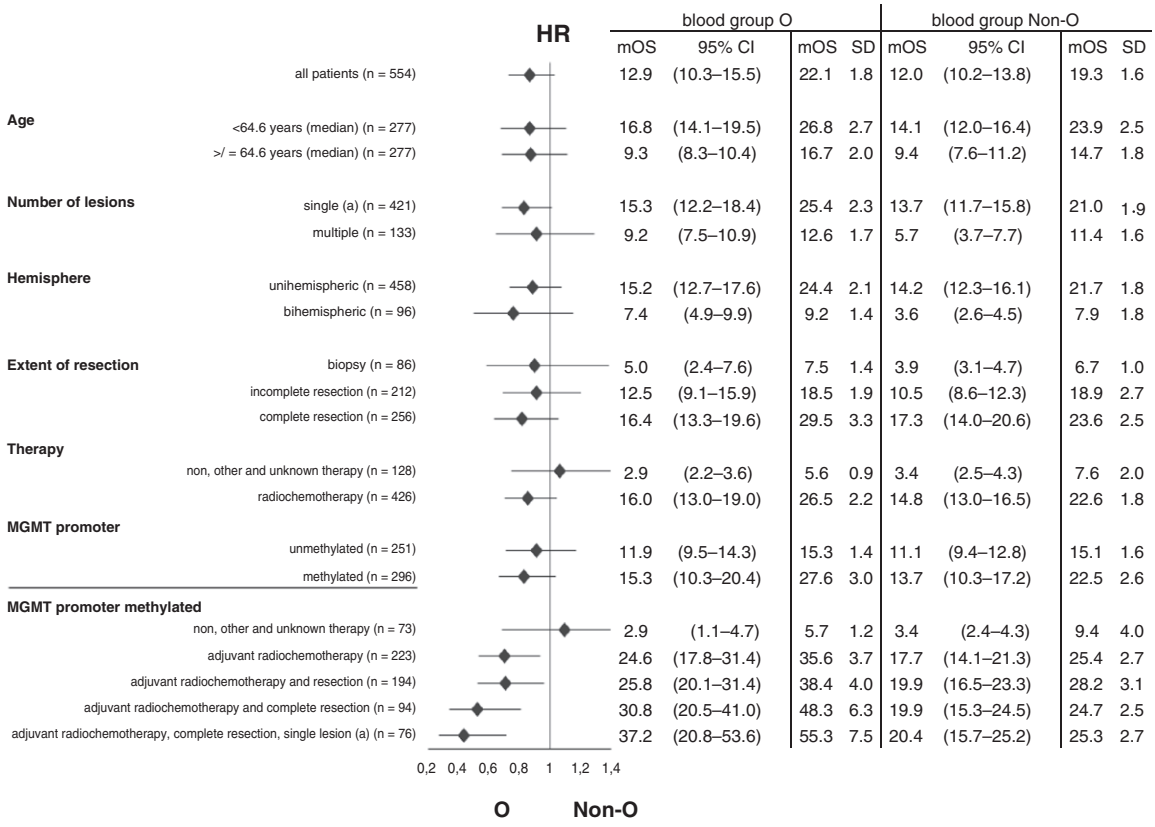



**Figure 3.** Forest plot of OS according to blood group status. Displayed are Hazard Ratios on OS and two-sided 95% CI of selected subgroups. Calculated values for median OS and mean OS are in months. Blood group O only favors MGMT promotor methylated patients with standardized treatment. [a]Including unknown cases (n = 17)

Importantly, our cohort's main descriptors are comparable with other Central European data. Compared with the German Cancer Registry GBM data (for the period 2011-2014, $n = 14{,}370$),[3] the median age and sex distribution of our GBM IDH-wt cohort are virtually identical (data not shown). In our certified, large tertiary brain tumor center, OS is somewhat longer than reported in the registry (11.8 months [95% CI: 10.4-13.2], Table 1, vs 10.0 months [95% CI: 10.0-10.0]), despite omitting all IDH-mutated cases (with better prognosis) from our cohort (which were not excluded in the pan-German registry).[3] Comparing our IDH-wt GBM cohort with IDH-wt GBM data from another center of excellence (ie, Zürich University Hospital, period 2005-2014, $n = 341$),[6] both cohorts are similar regarding central characteristics such as age (Zürich 66 years vs Münster 65 years), sex distribution (Zürich 63% males vs Münster 59% males), and OS rates (Zürich 10.9 months [95% CI: 9.1-12.7] vs Münster 11.8 months [95% CI: 10.4-13.2]).

Due to the practice-changing introduction of therapeutic regimes with alkylating agents,[7,27] longer-term survival in GBM has come to the forefront in the last decade.[6,32] Most recent data define long-term survival in GBM as 5-year survival, as described by Hertler et al.,[6] analyzing a large multinational GBM long-term survival cohort. Importantly, their long-term survivor patient characteristics and ours are well comparable (Supplementary Table S5). To the best of our knowledge, no real-world data on observed or estimated 5-year OS in IDH-wt GBM has been systematically reported so far, and even Hertler et al.[6] do not address this parameter. Including all consecutive GBM IDH-wt patients presenting at our center over the period of 12 years, the observed 5-year OS rate is 4.9% (GBM cohort $n = 593$, Table 1) and 5.2% (ABO GBM cohort $n = 554$, Supplementary Table S1), respectively. The markedly different 5-year OS regarding MGMT promoter methylation and ABO blood group O for patients treated with standardized radiochemotherapy is very similar for observed numbers and estimated figures (Figure 4), as should be expected if the follow-up period is sufficiently long.

Four treatment-independent factors independently contribute to observed 5-year OS: younger age, singular lesions, MGMT promoter methylated tumors, and blood group O, both for the entire cohort and the subgroup of patients being treated with standardized radiochemotherapy (Supplementary Table S3). Hence, for GBM 5-year survivors, we introduce ABO blood group as a new independent prognostic factor and confirm younger age as "most established" independent factor.

**Table 2.** Cox proportional Hazards Model for PFS (A) and OS (B) in patients with MGMT promoter methylated tumors and receiving standardized adjuvant radiochemotherapy ($n$ = 223). The corresponding Kaplan–Meier analysis for both MGMT promoter methylated and unmethylated tumors is displayed in Figure 2. Of note, in case of MGMT promoter unmethylated tumors treated with standardized radiochemotherapy ($n$ = 200), blood group O is not an independent prognostic factor for either PFS or OS (data not shown).

| | PFS | | OS | |
|---|---|---|---|---|
| | **HR (95%) CI)[a]** | ***P*** | **HR (95%) CI)[a]** | ***P*** |
| Age | 1.02 (1.00-1.03) | **.024** | 1.03 (1.02-1.05) | **<.001** |
| Sex | N.A. | .507 | N.A. | .459 |
| Hemisphere | | **.005** | | **.001** |
| Unihemispheric[b] | N.A. | N.A. | N.A. | N.A. |
| Bihemispheric | 2.06 (1.29-3.31) | .005 | 2.32 (1.42-3.79) | .001 |
| Lesions | | **.006** | N.A. | .106 |
| Singular[b,c] | N.A. | N.A. | N.A. | N.A. |
| Multiple | 1.68 (1.18-2.40) | .006 | N.A. | N.A. |
| Extent of resection | | **<.001** | | **<.001** |
| Biopsy[b] | N.A. | N.A. | N.A. | N.A. |
| Incomplete resection | 0.64 (0.39-1.03) | .067 | 0.45 (0.28-0.74) | .001 |
| Complete resection | 0.41 (0.25-0.68) | <.001 | 0.32 (0.19-0.52) | <.001 |
| ABO | | **.002** | | **.049** |
| Non-O[b] | N.A. | N.A. | N.A. | N.A. |
| O | 0.63 (0.48-0.84) | .002 | 0.75 (0.56-0.9) | .049 |

[a]Hazard Ratio (95 % CI), HR < 1 indicates improved survival.
[b]Reference parameter.
[c]Including unknown cases.

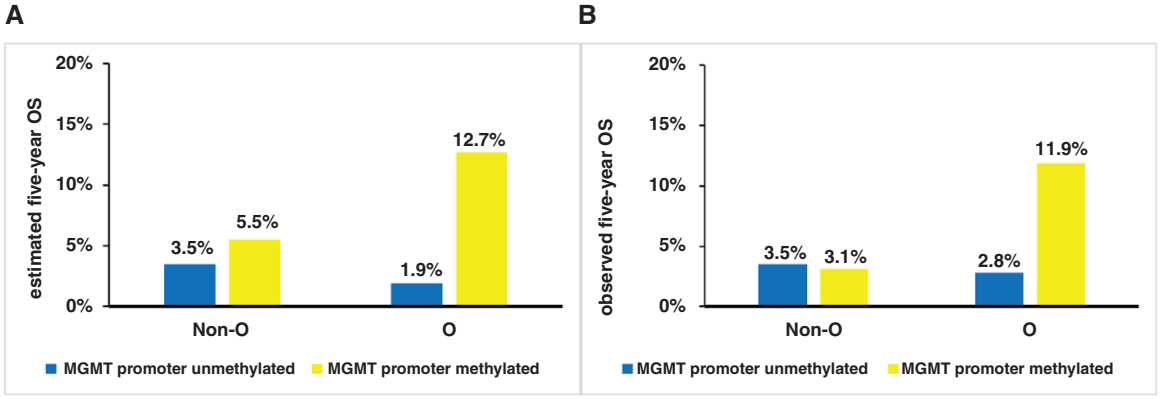



**Figure 4.** Estimated 5-year OS (A) and observed 5-year OS (B) for patients with standardized adjuvant radiochemotherapy ($n$ = 426). Of note, only one patient who received different treatment lived up to 5 years (compare Supplementary Figure S1B).

## Potential mechanism

MGMT is a DNA-repairing enzyme[33] that can be silenced by methylation of its promoter region. Normally, DNA damage caused by alkylating agents such as TMZ is repaired by MGMT. Conversely, if its promoter region is methylated, this DNA damage will remain unrepaired, thereby increasing the therapeutic effect.[4]

There are 4 possible phenotypic blood groups in the ABO system: A, B, AB, and O.[29,34] While the "A" and "B" alleles are both dominant, the "O" allele is inherited recessively. Biochemically, all 3 alleles code for glycosyltransferases that catalyze the addition of terminal sugar molecules to the "H antigen." In patients with blood group O, no functioning glycosyltransferase exists, so an unchanged H-determinant remains. Conversely, the blood groups' A, B, and AB have functioning glycosyltransferases that only differ in their substrate specificity. Hence, the dichotomization of blood groups into "O" vs "Non-O differentiates" between "nonfunctioning" vs "functioning glycosyltransferases to the H-antigen."[34]

As MGMT promoter methylation is a positive predictive factor for patients undergoing therapy with alkylating agents, but performs worse with functioning glycosyltransferases, an interplay between the ABO blood group system and the increased effectiveness of chemotherapy in patients with MGMT promoter methylated tumors must exist. This link has not been discovered yet; however, one might speculate on several interactions that are theoretical and purely speculative at this stage.

First, although MGMT and the glycosyltransferases group system neither share the same gene locus nor the same chromosome,[34,35] there might be an impact or even an interaction. Potentially, the "O" allele might be disproportionately inherited with apoptosis-favoring genes. This so-called linkage disequilibrium[22,36] could also occur in reverse: the "A" or "B" alleles are disproportionately linked to oncogenes.

Second, glycosyltransferases themselves might participate in unknown cellular pathways that initiate or disrupt apoptosis. For instance, "Non-O" functioning glycosyltransferases could disrupt pro-apoptotic processes by adding sugar molecules to other enzymes. Hence, in patients with blood group O, these processes might be enhanced, that is, the lack of glycosyltransferases in blood group O facilitates higher compartmental TMZ concentration. Although this hypothesis is as speculative as others, at least in mouse and rat cancer models, specific sugar molecules transferring enzymes are linked to higher resistance towards apoptosis and higher cell mobility.[37,38]

Third, the products of glycosyltransferases on the cell surface might alter apoptosis. Rosemann et al. hypothesize the participation of glycosyltransferases in cell–cell adhesion.[39] The precondition of active, extracellular soluble glycosyltransferases produced in tissues other than blood cells was proven by Yoshida et al.[40] For example, blood group A and B antigens are associated with increased cellular mobility, and blood group A antigen showed increased resistance toward apoptosis in rats.[34] Moreover, there are antigens that closely resemble the "A" antigen. The Forssman antigen is structurally identical to the "A" antigen determinant and is expressed in stomach and colon cancer.[41] Allouh et al. therefore hypothesize an immunoescaping impact of surface blood group antigens: "A"-tumor cells are not identified as such and remain hidden.[23] In the context of higher apoptosis in tumors with MGMT promoter methylation treated with alkylating agents, immune surveillance mechanisms may be critical for long-term survival. Even though this theory and the other mentioned hypotheses are appealing, they are purely speculative with no solid evidence in humans so far. Furthermore, adding a PD-L1 blocker to standard radiochemotherapy has produced unfavorable results,[42] undercutting the concept of "unmasking" GBM cells as a key therapeutic approach.

### Limitations and strengths

Several general limitations and strengths are present in this study. First, the study's design is retrospective, with missing data in some parameters, even for ABO status. However, a prospective, randomized design, as the only valid standard in clinical trials, is just not feasible due to the ABO status being determined at birth. Second, data were collected in a single-center setting. However, our tertiary cancer care center was the very first certified brain tumor center in Germany being certified since the onset of this trial, and all consecutive cases in the neurosurgical clinic over a period of 12 years were captured. While most patients are locoregional, at least one-fourth are referrals on a national and international basis. Moreover, approximately half of adjuvant therapies were performed outside the university hospital due to patients' preferences and needs, representing a "multi-institutional" specialized patient care. Third, as GBM is rare, the collection of large datasets demands a long period, during which therapeutic regimens evolve over time and histopathological and molecular WHO definitions of GBM continuously change.[12,24,25] Fortunately, the latest GBM definition and criteria could be applied to all (older) GBM cases, since detailed molecular pathological investigations have been standard in our center prior to certification and are available for most patients (Figure 1).

## Conclusion

For the first time, this study identifies blood group O as an independent favorable prognostic factor in IDH-wt GBM patients in conjunction with MGMT methylation and standardized radiochemotherapy with alkylating agents. The advantage is even greater when combined with other favorable prognostic factors such as single tumor localizations and (complete) tumor resection: the impact of blood group O is best visible in patients with lowest tumor burden following standardized therapy. In contrast, blood group O has no impact on survival in MGMT promoter unmethylated patients, despite standardized treatment. Taken together, our results are of high clinical relevance for GBM patients and therapists, as they represent an additional piece to the puzzle in advancing personalized oncological treatments for this devastating disease.

The observation that beneficial cancer therapy response and survival benefit, respectively, are linked to blood group O in conjunction with a downregulated DNA repair gene by promoter methylation is unprecedented. The mechanism of this new class of blood group-drug interplay may be complex and is not yet understood to unravel this interaction; fundamental research is required in GBM as well as in other tumor entities and treatments in general. We hypothesize the identification of further favorable blood group-specific drug efficiencies in defined molecular conditions.

## Supplementary Material

Supplementary material is available online at *Neuro-Oncology Advances* (https://academic.oup.com/noa).

## Keywords:

ABO blood group | glioblastoma | long-term survivors | MGMT promoter methylation | prognosis.

## Funding

R.W., C.T., H.T.E., and W.S. are endowed by the Medical Faculty of the University of Münster.

## Acknowledgments

Many thanks to Heike Duhme, cancer registry of the "Westdeutsches Tumorzentrum" (WTZ) Essen-Münster, Münster facility, University Hospital Münster, for her consistently swift and thorough survival status updates.
We are also very grateful for the continuous support from the Münster University Hospital Central Nervous System Association (Förderverein Zentrales Nervensystem e.V.), represented by Alice Kraul and Hansdetlef Wassmann, MD, PhD.
We acknowledge support from the Open Access Publication Fund of the University of Münster.
The manuscript is part of M.K.'s work for her doctoral thesis.

## Conflict of interest statement

The author's do not report potential conflicts of interest related to the current manuscript. The Münster University Hospital Central Nervous System Association (Förderverein Zentrales Nervensystem e.V.) supports database management and may cover possible article processing fees.

## Authorship statement

Conceptualization, design of study, methodology and supervision: R.W., M.K., W.S., and D.W.;
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Investigation and data acquisition: M.K., J.J.-E., F.M.T., B.O.T., C.T., R.W., and D.W.;
Data base validation: R.W., M.K., D.W.;
Formal analysis, visualization, and first draft: M.K. and R.W.;
Consolidated analysis: M.K., R.W., J.J.-E., F.M.T., and D.W.;
Supporting conduct of study and assistance of various tasks including data curation: all authors;
Review and editing on the various versions of the manuscript and approval of the final draft: all authors.

## Data availability

All original data from this manuscript will be made available upon reasonable request.

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