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Impact of MYC and BCL2 double expression on outcomes in Primary CNS Lymphoma: a UK multicenter analysis

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Abstract:

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Authorship Statement

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Data Sharing Statement

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Primary diffuse large B-cell lymphoma of the central nervous system (PCNSL) is a rare aggressive extranodal B-cell lymphoma[1,2]. Despite intensive protocols such as MATRix (methotrexate, cytarabine, thiotepa, rituximab) and consolidative thiotepa-based autologous stem cell transplant (ASCT), refractory and relapsed disease remains a major clinical problem and limited prognostic tools are available[3–5]. Although PCNSL shares biological similarities with systemic *de novo* DLBCL, the prognosis remains significantly worse with a median overall survival of 2 years and only one third of patients are alive at 5 years[6]. The underlying drivers for the poorer survival in PCNSL remain unclear but likely reflect biological differences in disease biology, challenges in treatment delivery and a high burden of comorbidity at presentation. Although a number of clinical risk scores are available[7–9], these were developed prior to the adoption of intensive chemotherapy regimens such as MATRix and do not inform treatment decisions in contemporary clinical practice[10].

In systemic DLBCL, double expression of MYC and BCL2 (double expressor, DE) are associated with inferior clinical outcomes but the prognostic impact of DE status in PCNSL has not been conclusively defined[11]. We evaluated the prognostic value of BCL2 and MYC expression in a cohort of newly-diagnosed PCNSL patients contemporarily treated and demonstrate that DE status is associated with adverse clinical outcomes.

We retrospectively collected data from patients with histologically-confirmed PCNSL diagnosed consecutively between 1st May 2015 and 31st May 2020, treated with high-dose methotrexate-based (HD-MTX) induction from 7 UK referral centers. Exclusion criteria included evidence of systemic disease at diagnosis, post-mortem diagnosis and treatment with non high-dose methotrexate ($3.5g/m^2$) containing induction chemotherapy. Data were retrieved from local health records according to a standardized data collection proforma capturing baseline patient characteristics, treatment details and timings, diagnostic immunohistochemistry (IHC) assessment of BCL2, BCL6, MYC, CD10 and MUM1 as well as receipt and type of consolidation treatment. Positive expression of MYC and BCL2 was assessed in accordance with WHO reporting criteria (MYC: nuclear stain positive in >40% of cells; BCL2: cytoplasmic stain positive in >50% of cells)[12]. Staining was performed as per each institution's standard operating procedure. DE status was determined centrally and defined as IHC positivity (by WHO reporting criteria) for both MYC and BCL2.

Outcomes included end of treatment response rates, progression free- (PFS) and overall (OS) survival. Cox regression for PFS and OS were used to determine baseline factors associated with response and survival. Patients were retrospectively categorized into two groups according to induction treatment received. A significant *p*-value was defined as ≤ 0.05. All patient data were

anonymized at source and treated according to the principles of the declaration of Helsinki and the UK Data Protection Act (1998).

Data from 260 patients were collected. Of these, 18 were excluded (11 diagnosed outside of the study period; 5 with insufficiently annotated clinical outcome data; 2 with no diagnostic IHC data available) and in total, 242 patients were included for analysis (Supp. Figure 1). Key baseline patient characteristics and IHC data are summarized in Table 1. Median age was 65 years (IQR 56-71), 60% were male and 64% of patients had an ECOG performance status (PS) of 0-1. One hundred and seventy-four (72%) patients received treatment with MATRix chemoimmunotherapy and 68 (28%) patients received treatment with other non-MATRix, HD-MTX containing induction regimen. Patients who received MATRix chemotherapy (MATRix subgroup) were younger (median age 62 vs. 73 years, $p< 0.01$), had better baseline ECOG PS (PS $\leq$ 1: 75% vs. 37%, $p< 0.01$) and higher rates of consolidation with BCNU-thiotepa ASCT (58% vs. 7%, $p< 0.01$). Median follow up was 3.0 years (IQR 2.0 -4.2 years) during which 34% (MATRix subgroup: 28%; non-MATRix subgroup: 50%) of patients relapsed and 45% (MATRix subgroup: 36%; non-MATRix subgroup: 66%) died.

Across the entire cohort, the overall response rate (ORR) following induction treatment was 74% with a 2-year PFS of 52% and 2-year OS 60%. In the MATRix-treated subgroup, ORR was 79% with 2-year PFS and OS of 60% and 69% respectively. The non-MATRix treated subgroup ORR was 61% with 2-year PFS and OS of 30% and 37% respectively. In total, 30% and 21% of cases in the entire cohort had incomplete BCL2 or MYC expression data respectively, due to missing IHC expression data or not being reported in line with WHO criteria. In the entire cohort, 88% of cases were BCL6 positive, 59% were BCL2 positive and 60% were MYC positive, in keeping with previously reported rates[13,14].

BCL6 positivity has been shown to be associated with favorable outcomes[15]. In this study, BCL6 negativity by IHC (12% of cases) was associated with significantly shorter PFS and OS in the entire cohort. However, a significant association of BCL6 negativity with receipt of non-MATRix induction therapy and inferior baseline performance status confounds this association.

DE status was evaluable in 69% of patients (169/242). Clinical outcomes were comparable between DE-evaluable and DE-unevaluable (due to missing data) cases in the entire cohort (2-year PFS 49% vs 57% p=0.28 and 2-year OS 59% vs. 61% p=0.58 respectively) and between the two treatment subgroups. Among DE-evaluable cases (n=169 patients), 40% of patients had DE PCNSL, higher than estimates of DE prevalence in systemic DLBCL which range between 20 and 30%[12]. DE positive cases were not significantly associated with older age at diagnosis (p=0.99, median 65 years (IQR 53.5 - 71 years) vs. median 65, (IQR 57.25 - 71 years), poorer baseline PS (P=0.44,

ECOG PS ≤1 58% vs. 59%) or less intensive induction treatment (p=0.77, MATRix receipt 69% vs. 69%) compared to DE-negative cases.

Clinical parameters associated with significantly longer PFS or OS included PS ≤1, receipt of MATRix induction chemotherapy and receipt of ASCT consolidation. Neither BCL2 nor MYC expression positivity alone were independently associated with shorter PFS or OS in the entire cohort (Supp. Fig. 2). However DE status was associated with shorter PFS in the entire cohort compared to non-DE patients (median 0.86 years vs. 2.77 years; p=0.021), but not shorter OS (Figure 1A and B). In a subgroup analysis restricted to patients treated with MATRix chemotherapy (n=116 patients), there was a trend towards inferior PFS and OS, although this did not reach significance in a univariable analysis (Figure 1C and D).

In a multivariable Cox regression model accounting for age, baseline PS, induction treatment received, and DE status (Supp. Table 1), there was an independent association of DE status with shorter PFS for the entire cohort (n=163) (HR 1.78, 95% CI: 1.17 - 2.71; p<0.01). To investigate whether the association between DE status and shorter PFS was treatment dependent, we performed a further multivariate analysis with an additional interaction term between MATRix induction treatment and DE status. This term had no significant effect on progression free survival (p=0.57) indicating that patients with DE-PCNSL have inferior outcomes irrespective of type of induction treatment received. A trend towards an association between DE status and OS was noted but this did not reach significance in either a univariable or multivariable analysis.

In conclusion, we demonstrate that double expression of MYC and BCL2 is associated with poorer clinical outcomes in a large, real-world, contemporary cohort of PCNSL patients primarily treated with intensive induction therapy. Our data add clarity to the limited prior work in this area (including studies in single-center, non-contemporary cohorts and studies using alternative IHC thresholds for MYC and BCL2 positivity) where the prognostic relevance was unclear[13,15–17]. These findings have potential value in risk stratifying patients and guiding decisions around consolidation following induction chemotherapy. Validation in a prospective series is warranted and more work to understand the disease biology of DE-PCNSL is needed in order to identify biologically rational therapies for this higher risk cohort.

Disclosures

JS: AbbVie, Astra-zeneca

TAE: Roche, Gilead/Kite, Janssen, Abbvie, AstraZeneca, Loxo Oncology, Beigene, Incyte, Secura Bio, Autolus

PM: Gilead/Kite, Incyte, Janssen, Abbvie, AstraZeneca, Beigene, Celgene/BMS, Epizyme, Roche, Takeda

SC: AbbVie, Adicet Bio, Atara Biotherapeutics, Gilead/Kite, Novartis, Orion Pharma, Pierre Fabre, Roche, Takeda

KC: BeiGene, Roche, Celgene/BMS, Takeda, Gilead/Kite, Incyte, Atara, Janssen

CPF: Roche, BeiGene, Gilead/Kite, Incyte, Janssen, Roche, Takeda, Abbvie, AstraZeneca, Atarabio, Celgene/BMS

Contributions

EP, KC, CPF and JO conceived the study and design. EP, EC, JD, CP, DH, RP, TO, NT, EJ, PG, SC, PM, JS, TAE and NMC collected clinical data. AA, SP, TM and MC provided pathological data and interpretation. AA and AK provided statistical support. EP and JO analyzed the data and wrote the first draft of the manuscript. All authors approved the final version of the manuscript.

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References

1. Villano JL, Koshy M, Shaikh H, Dolecek TA, McCarthy BJ. Age, gender, and racial differences in incidence and survival in primary CNS lymphoma. Br J Cancer 2011;105(9):1414–1418.

2. Martinez-Calle N, Poynton E, Alchawaf A, et al. Outcomes of older patients with primary central nervous system lymphoma treated in routine clinical practice in the UK: methotrexate dose intensity correlates with response and survival. Br J Haematol 2020;190(3):394–404.

3. Ahn Y, Ahn HJ, Yoon DH, et al. Primary central nervous system lymphoma: a new prognostic model for patients with diffuse large B-cell histology. Blood Res 2017;52(4):285.

4. Ferreri AJM, Cwynarski K, Pulczynski E, et al. Chemoimmunotherapy with methotrexate, cytarabine, thiotepa, and rituximab (MATRix regimen) in patients with primary CNS lymphoma: results of the first randomisation of the International Extranodal Lymphoma Study Group-32 (IELSG32) phase 2 trial. Lancet Haematol 2016;3(5):e217-227.

5. Ferreri AJM, Cwynarski K, Pulczynski E, et al. Whole-brain radiotherapy or autologous stem-cell transplantation as consolidation strategies after high-dose methotrexate-based chemoimmunotherapy in patients with primary CNS lymphoma: results of the second randomisation of the International Extranodal Lymphoma Study Group-32 phase 2 trial. Lancet Haematol 2017;4(11):e510–e523.

6. Houillier C, Soussain C, Ghesquières H, et al. Management and outcome of primary CNS lymphoma in the modern era: An LOC network study. Neurology 2020;94(10):e1027–e1039.

7. Ferreri AJM, Blay J-Y, Reni M, et al. Prognostic scoring system for primary CNS lymphomas: the International Extranodal Lymphoma Study Group experience. J Clin Oncol Off J Am Soc Clin Oncol 2003;21(2):266–272.

8. Bessell EM, Graus F, Lopez-Guillermo A, et al. Primary non-Hodgkin's lymphoma of the CNS treated with CHOD/BVAM or BVAM chemotherapy before radiotherapy: long-term survival and prognostic factors. Int J Radiat Oncol Biol Phys 2004;59(2):501–508.

9. Abrey LE, Ben-Porat L, Panageas KS, et al. Primary Central Nervous System Lymphoma: The Memorial Sloan-Kettering Cancer Center Prognostic Model. J Clin Oncol 2006;24(36):5711–5715.

10. Jahr G, Broi MD, Holte H, Beiske K, Meling TR. Evaluation of Memorial Sloan-Kettering Cancer Center and International Extranodal Lymphoma Study Group prognostic scoring systems to predict Overall Survival in intracranial Primary CNS lymphoma. Brain Behav 2018;8(3):e00928.

11. Sarkozy C, Traverse-Glehen A, Coiffier B. Double-hit and double-protein-expression lymphomas: aggressive and refractory lymphomas. Lancet Oncol 2015;16(15):e555–e567.

12. Riedell PA, Smith SM. Double hit and double expressors in lymphoma: Definition and treatment. Cancer 2018;124(24):4622–4632.

13. Villa D, Tan KL, Steidl C, et al. Molecular features of a large cohort of primary central nervous system lymphoma using tissue microarray. Blood Adv 2019;3(23):3953–3961.

14. Son S-M, Ha S-Y, Yoo H-Y, et al. Prognostic impact of MYC protein expression in central nervous system diffuse large B-cell lymphoma: comparison with MYC rearrangement and MYC mRNA expression. Mod Pathol 2017;30(1):4–14.

15. Lossos C, Bayraktar S, Weinzierl E, et al. LMO2 and BCL6 are associated with improved survival in primary central nervous system lymphoma. Br J Haematol 2014;165(5):640–648.

16. Kim S, Nam SJ, Kwon D, et al. MYC and BCL2 overexpression is associated with a higher class of Memorial Sloan-Kettering Cancer Center prognostic model and poor clinical outcome in primary diffuse large B-cell lymphoma of the central nervous system. BMC Cancer 2016;16363.

17. Hatzl S, Posch F, Deutsch A, et al. Immunohistochemistry for c-myc and bcl-2 overexpression improves risk stratification in primary central nervous system lymphoma. Hematol Oncol 2020;38(3):277—283.

Figure Legends

Figure 1: Association of double expression (DE) of MYC (>40%) and BCL2 (>50%) with PFS and OS in the entire cohort (A and B) and in the MATRix treated subgroup (C and D).

Table 1. Baseline clinical characteristics and surface marker expression in the entire cohort and both the MATRix treated and non-MATRix treated subgroups. p-values refer to chi-squared testing MATRix vs. Non-MATRix treated subgroup (ASCT: Autologous stem cell transplantation; WBRT: Whole brain radiotherapy).

	Entire Cohort (n=242)		**MATRix (n=174)**		**Non-MATRix (n=68)**		
	N (%)	**Missing**	**N (%)**	**Missing**	**N (%)**	**Missing**	**p**
Male	144 (60)	-	108 (62)	-	36 (53)	-	0.24
Age >60	154 (64)	-	95 (55)	-	59 (87)	-	**<0.01**
Median Age	65 range (23-84)		62 (range 23-77)		73 range (34-84)		-
PS ≤ 1	146 (64)	14	121 (75)	13	25 (37)	1	**<0.01**
ASCT	104 (44)	3	99 (58)	2	5 (7)	1	**<0.01**
WBRT	22 (9)	2	20 (12)	2	2 (3)	-	**<0.01**
MYC Positive (>40%)	119 (60)	44	89 (62)	31	30 (55)	13	-
BCL2 Positive (>50%)	126 (59)	28	82 (54)	21	44 (72)	7	-
BCL6 Positive	199 (88)	15	153 (93)	10	46 (73)	5	**<0.01**
CD10 Positive	61 (27)	14	42 (25)	8	19 (31)	6	0.65
MUM1 Positive	216 (94)	12	154 (94)	10	62 (97)	4	0.71
Double Expressor	67 (40)	73	46 (40)	58	21 (40)	15	1.00

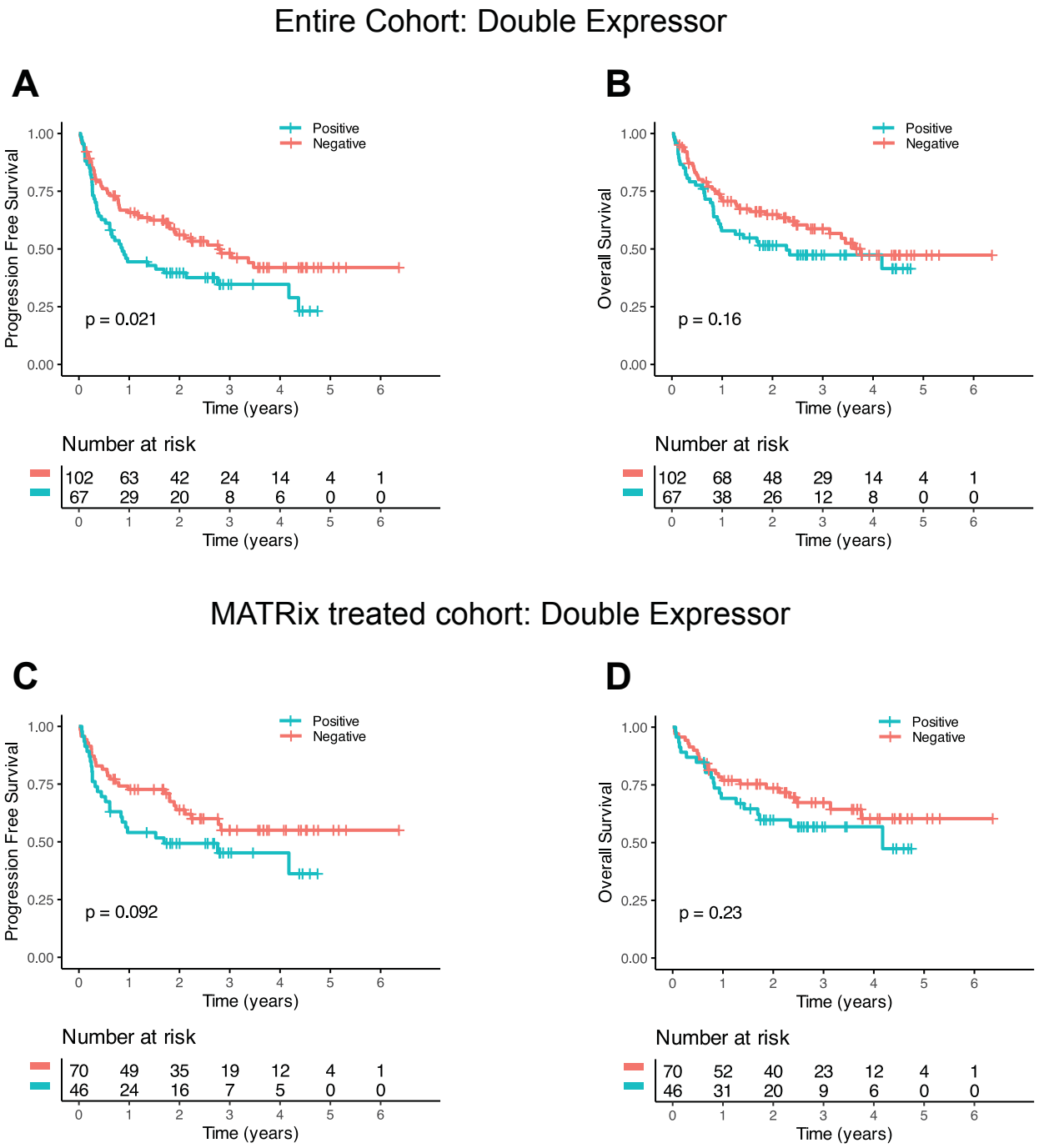

Figure 1: Association of double expression (DE) of MYC (>40%) and BCL2 (>50%) with PFS and OS in the entire cohort (A and B) and in the MATRix treated subgroup (C and D).