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

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1. Introduction

- **Primary central nervous system lymphoma (PCNSL)** is an aggressive extranodal non-Hodgkin lymphoma exclusively confined to the brain, leptomeninges, eyes, or spinal cord.
- **Clinical outcomes** in PCNSL remain **significantly poorer** compared to systemic DLBCL despite genetic and histological similarities.
- There is an **ongoing need for prognostic biomarkers** in PCNSL in order to associate disease phenotypes with **clinical outcomes**.
- In systemic diffuse large B cell lymphoma (DLBCL), **double expression of MYC and BCL2 (double expressor, DE)** is associated with **inferior clinical outcomes**[1]. However, the prognostic impact of DE status in PCNSL has not been conclusively demonstrated.

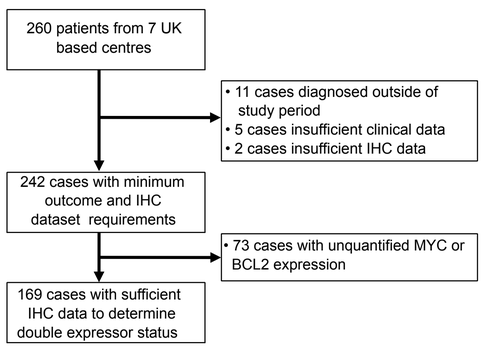
2. Aims & Methods

1. **Determine if DE status is associated with poorer clinical outcomes in PCNSL**
   - Assembly of a **large, retrospective clinical dataset** comprising 242 PCNSL patients treated over a 5-year period from 7 large, UK based treatment centers.
   - Capture of c**linical outcomes**, **treatment parameters** and diagnostic **immunohistochemistry (IHC)** assessment of **BCL2 & MYC expression**.
   - **Univariate** and **multivariate analyses** to determine associations with **progression-free** and **overall survival**.

2. **Determine the differential disease biology underpinning the aggressive clinical phenotype associated with DE-PCNSL**
   - Whole exome sequencing (WES) of n=99 PCNSL samples from 3 cohorts (16 samples from the TIER clinical trial[2] (**TIER Cohort**); 75 samples obtained from Semmelweis university, Budapest (**Budapest Cohort**); 11 samples obtained from St. Bart's hospital, London (**Barts Cohort**).
   - Integration with an existing WES dataset of 41 PCNSL cases obtained from the DNA databank of Japan (**DDBJ cohort**)[3].

3. Double expressor status is associated with adverse clinical outcomes

Study Consort diagram

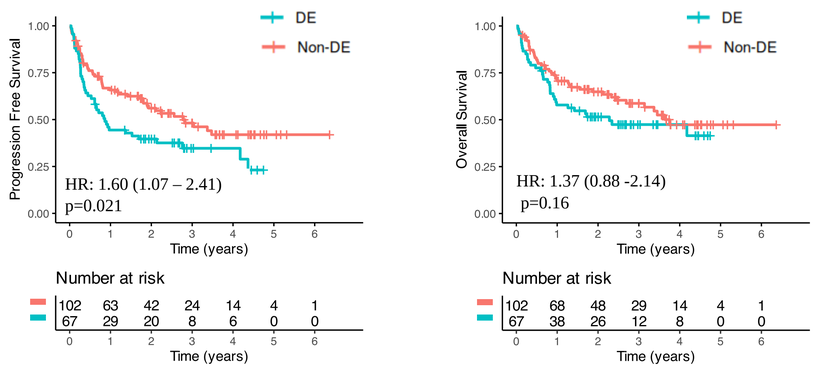


Study Inclusion criteria

- Biopsy proven PCSNL diagnosed between **1st May 2015** and **31st May 2020** from one of seven UK based tertiary treating centres.
- Either **MATRix** or a "less-intensive" high dose (>3g/m^2) methotrexate (**HD-MTX**) containing induction regimen.
- Available histopathology report including i**mmunohistochemistry (IHC)** and (where available) **florescence *in situ* hybridisation** (**FISH**).

	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
Overall response rate	172 (74)	9	133 (79)	5	39 (61)	4	**0.01**

- **40% of PCNSL cases** are **double expressors**, a higher rate than in seen in systemic DLBCL cohor



- **Double Expressor** status is associated with **significantly shorter progression free survival (PFS)** (median 0.86 years vs. 2.77 years, p=0.021), but not with shorter overall survival (OS).

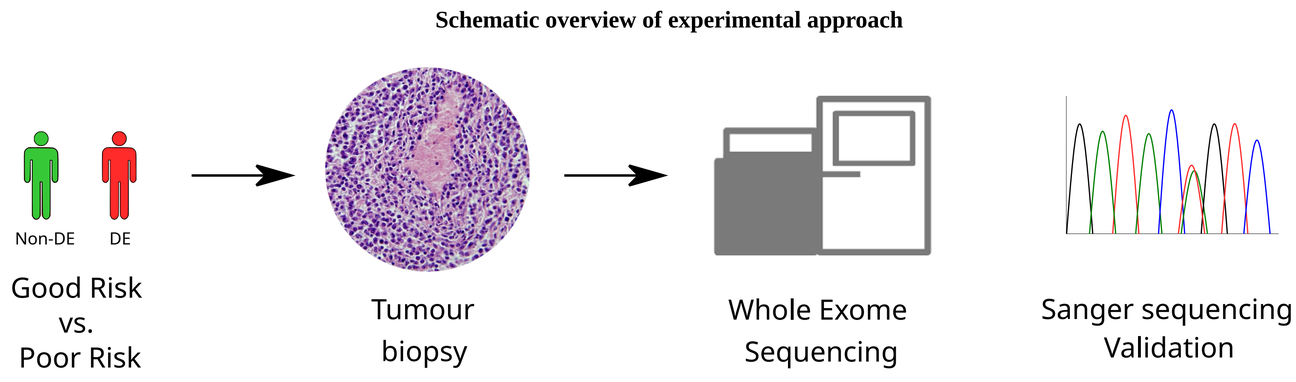
	Entire Cohort (n=169)				MATRix Cohort (n=116)			
	OS		PFS		OS		PFS	
	HR	p	HR	p	HR	p	HR	p
Double Expressor	1.46 (0.92 - 2.33)	0.11	1.75 (1.15 - 2.66)	<0.01	1.76 (0.93 - 3.33)	0.08	1.91 (1.08 - 3.36)	0.03
Age > 60	0.81 (0.46 - 1.43)	0.47	0.97 (0.57 - 1.66)	0.92	1.24 (0.63 - 2.42)	0.53	1.23 (0.67 - 2.24)	0.51
MATRix	0.74 (0.44 - 1.26)	0.27	0.69 (0.42 - 1.13)	0.14	-	-	-	-
PS ≤ 1	0.93 (0.57 - 1.5)	0.76	0.8 (0.51 - 1.25)	0.32	0.86 (0.43 - 1.69)	0.66	0.76 (0.41 - 1.42)	0.39
ASCT	0.16 (0.08 - 0.32)	<0.01	0.28 (0.16 - 0.49)	<0.01	0.13 (0.06 - 0.28)	<0.01	0.26 (0.14 - 0.48)	<0.01

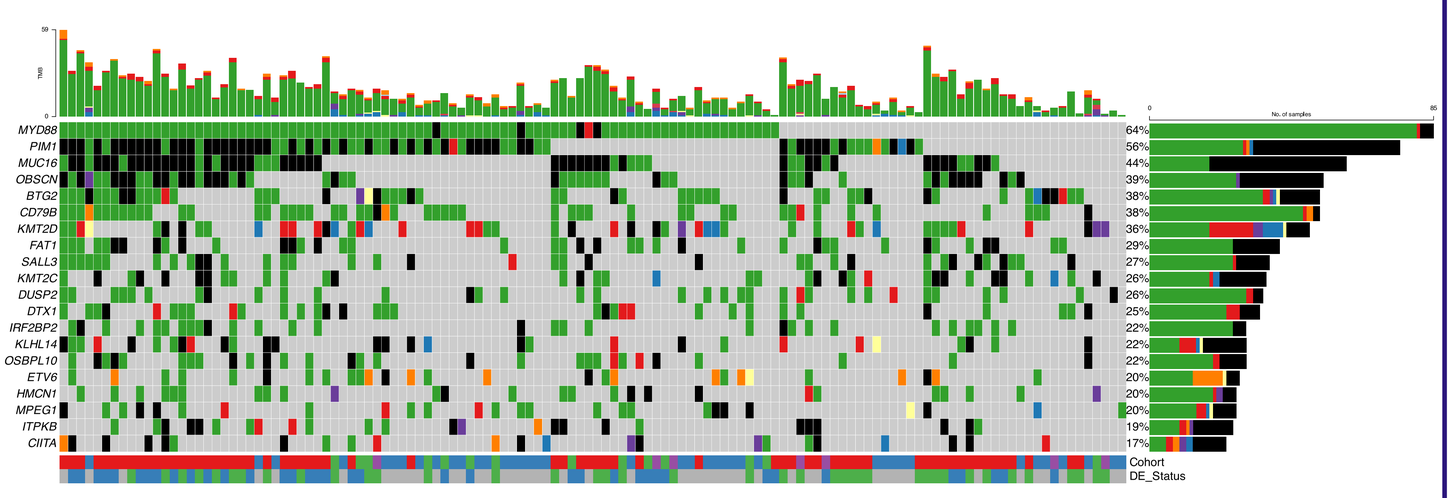
- DE status is **significantly associated with shorter PFS** in both in the **entire cohort** and in the subgroup of patients treated with **intensive MATRix induction chemoimmunotherapy**.

HR: Hazard ratio, PS: Performance score; ASCT: Autologous stem cell transplant; MATRix: Methotrexate, Cytarabine, Thiotepa, Rituximab; WBRT: Whole brain radiotherapy

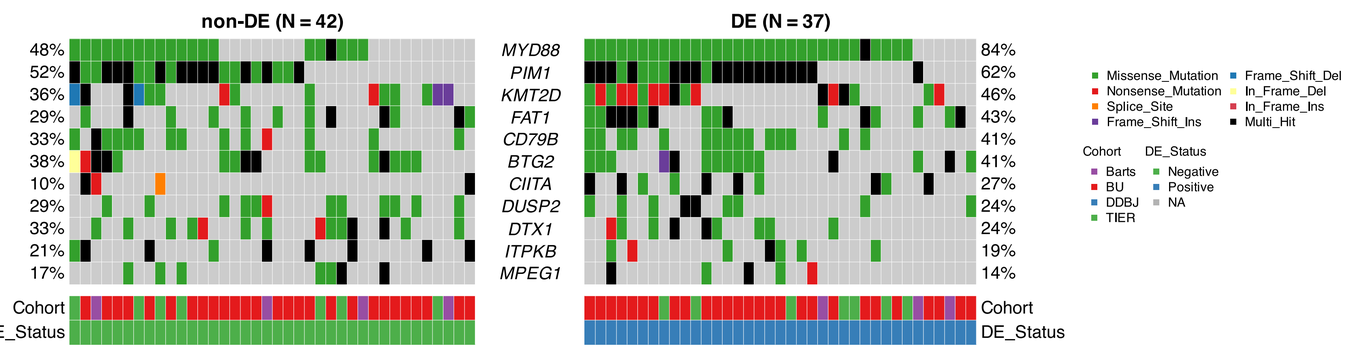
4. Determining the mutational signatures of Double expressor PCNSL tumours

Schematic overview of experimental approach





- Similar patterns of recurrent mutations as seen in previously published data series[5] with **high rates *MYD88, PIM1* and *CD79B*** mutations.
- Recurrent **nonsense *KMT2D* mutations** and ***ETV6* splice site** variants



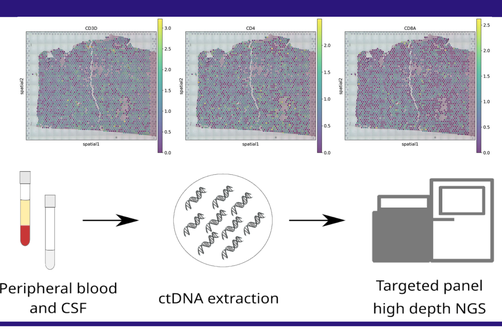
- Significantly **higher rates of *MYD88* L265P** mutation in DE- compared to non-DE PCNSL.
- Significantly **higher rates of C*IITA*** mutation in DE- compared to non-DE PCNSL.
- **Similar rates of mutation in *PIM1* and *CD79B*** between DE- and non-DE PCNSL.

5. Conclusions

- **Double expression of MYC and BCL-2 (DE-PCNSL)** occurs in **40% of cases** of PCNSL, a higher rate than in systemic DLBCL (20-30%).
- **DE-PCNSL** is associated with shorter **progression-free survival** even in patients treated with **intensive** MATRix chemotherapy.
- **DE-PCNSL** cases harbor significantly **higher rates** of ***MYD88* L265P and *CIITA* gene mutations** than non-DE-PCNSL cases. These biological differences may, in part, **underpin the poorer clinical outcomes in DE- compared to non-DE PCNSL**.

6. Ongoing Work

- Determine the differential **recurrent copy number aberrations** in DE and non-DE PCNSL cases.
- Bulk RNA-seq **transcriptional profiling** of a subset of cases to determine differential transcriptional programs between DE and non-DE cases.
- Spatial transcriptomic analysis of a subset of PCNSL tumour biopsies to characterise distinct **tumor and immune micro-environmental factors** in DE and non-DE PCNSL cases.
- Explore the utility of **detectable biomarkers** of DE status and aggressive disease in paired, sequential **CSF and plasma liquid biopsy** compartments.



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