

## Interim PET after 4 Cycles Predicts Outcome in Histomolecularly Confirmed Primary Mediastinal B-Cell Lymphoma

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### Abstract:

The GAINED study (NCT01659099) was a randomized phase 3 trial comparing obinutuzumab (G) to rituximab (R) plus ACVBP or CHOP14 induction, followed by PET-guided consolidation. This post-hoc analysis aimed to detail the outcomes of primary mediastinal B-cell lymphoma (PMBL) patients, verified through expert pathological review and the use of gene-expression profiling (GEP) and Next-Generation sequencing. Of the centrally reviewed 620 patients, 138 (22.3%) confirmed PMBL cases were analyzed. Baseline characteristics included a median age of 33.5 years, 63.8% female, 55.1% stage III-IV, 90.6% elevated LDH, 87.6% ECOG 0-1, 62.3% extranodal involvement, 52.6% aaIPI 2-3, and 53.6% Bulk (>10cm). Induction regimens were R/G-CHOP14 (56.9%) and R/G-ACVBP (43.1%). Post-induction treatments, based on interim PET (iPET) results, included: standard consolidation chemotherapy (59.8%) if  $\Delta\text{SUVM}_{\text{max}} > 66\%$  after cycle 2 and  $> 70\%$  after cycle 4 (PET2-/4-), intensive treatment and autologous transplantation (26.8%) if PET2+/4-, and salvage therapy (13.4%) if PET4+ ( $\Delta\text{SUVM}_{\text{max}} \leq 70\%$ ). Among patients with GEP data (n=107), 38 (35.5%) were PDL1high/PDL2high. Key somatic mutations data (n=87) included SOCS1 (70.1%), B2M (56.3%), STAT6 (49.4%), TNFAIP3 (47.1%), GNA13 (39.1%), CIITA (37.9%), CD58 (36.8%), and TP53 (29.9%). After a median follow-up of 39.5 months, 2-year PFS and OS rates were 86.2% and 93.2%, respectively. In a multivariate model including Bulk, aaIPI, and  $\Delta\text{SUVM}_{\text{max}}$  PET2/PET4, only Bulk and  $\Delta\text{SUVM}_{\text{max}}$  PET4  $\leq 70\%$  were associated with shorter PFS (HR 4.39 [1.28-15.11] and 4.95 [1.71-14.3], respectively), while none were associated with OS. The  $\Delta\text{SUVM}_{\text{max}}$ -based interim PET4 response emerged as the strongest predictor of patient outcomes in this selected clinical trial population.

**Conflict of interest:** COI declared - see note

**COI notes:** V.C. has received honoraria from Abbvie, Astra Zeneca, Bristol-Myers Squibb, Ideogen, Janssen, Kiowa Kirin, Kite Gilead, Novartis, Pfizer, Sanofi, Takeda. R.H. has received honoraria from Kite/Gilead, Novartis, Bristol-Myers Squibb/Celgene, Incyte, Janssen, MSD, Takeda, Amgen, Abbvie, and Roche; and is a member on an entity's Board of Directors or advisory committees of Kite/Gilead, Novartis, Bristol-Myers Squibb/Celgene, Tessa Therapeutics, Abbvie and Roche. W.B. has received travel grants from Leo Pharma, Roche and Takeda; and honoraria from Leo Pharma and BMS. All other authors have no conflict of interest to disclose.

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**Author contributions and disclosures:** V.C. designed and supervised the present work, collected, analyzed, interpreted the data, and wrote the manuscript. S.L.G designed the GAINED trial in collaboration with J.-P.J., C.H., and E.I., conducted the study, analyzed the results, enrolled and treated patients. H.G., L.O., F.M., H.T., V.R., R.H., C.T., H.M., F.C., K.B., G.L.D, L.M.F, R.N., P.F., D.S., G.C., C.B., W.B. and C.R. enrolled and treated patients. T.J.M., A.M., and J.B. performed the expert pathological review and interpreted the data. F.D. conducted the NGS experiments, interpreted the biological data, and participated in the integrated histomolecular diagnosis process. P.R. carried out and analyzed the GEP experiments and contributed to the integrated histomolecular diagnosis process. P.B.D., F.K.B., C.B.M., S.K., and E.I. centrally reviewed the PET exams. P.B.D., S.K., and E.I. conducted the TMTV and TLG assessments. M.H.E and L.C. performed the statistical analysis. F.J., T.J.M. and T.F designed and supervised the present work, analyzed, and interpreted the data, and edited the manuscript. All authors approved the manuscript.

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**Agreement to Share Publication-Related Data and Data Sharing Statement:** The sequencing data will be made publicly available on the EGA (European Genome-Phenome Archive, <https://ega-archive.org/>) website prior to the publication of this manuscript. For access to other original data, please contact [dpo@lysarc.org](mailto:dpo@lysarc.org). They will respond to your request in compliance with applicable data protection regulations, data security requirements, the principle of data minimization, and the relevant contractual provisions.

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**Data sharing statement**

The sequencing data is publicly available on the EGA (European Genome-Phenome Archive, <https://ega-archive.org/>) website:

EGAP50000000485 corresponds to the Data Policy Access, related to the corresponding Data Access Committee (DAC).

EGAC50000000540 is the Data Access Committee (DAC) accession number related to the dataset on the EGA platform.

For access to other original data, please contact [dpo@lysarc.org](mailto:dpo@lysarc.org). They will respond to your request in compliance with applicable data protection regulations, data security requirements, the principle of data minimization, and the relevant contractual provisions.

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#### **PRIOR PRESENTATION**

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## **Key points**

- Favorable outcomes were observed with dose-dense induction and PET-driven consolidation, with 2-year PFS and OS rates of 86.2% and 93.2%.
- The interim PET response, based on  $\Delta\text{SUV}_{\text{max}}$  at PET4 (threshold:  $\leq 70\%$  vs  $> 70\%$ ), emerged as the most robust predictor of outcomes.

## **Abstract**

The GAINED study (NCT01659099) was a randomized phase 3 trial comparing obinutuzumab (G) to rituximab (R) plus ACVBP or CHOP14 induction, followed by PET-guided consolidation. This post-hoc analysis aimed to detail the outcomes of primary mediastinal B-cell lymphoma (PMBL) patients, verified through expert pathological review and the use of gene-expression profiling (GEP) and Next-Generation sequencing. Of the centrally reviewed 620 patients, 138 (22.3%) confirmed PMBL cases were analyzed. Baseline characteristics included a median age of 33.5 years, 63.8% female, 55.1% stage III-IV, 90.6% elevated LDH, 87.6% ECOG 0-1, 62.3% extranodal involvement, 52.6% aaIPI 2-3, and 53.6% Bulk (>10cm). Induction regimens were R/G-CHOP14 (56.9%) and R/G-ACVBP (43.1%). Post-induction treatments, based on interim PET (iPET) results, included: standard consolidation chemotherapy (59.8%) if  $\Delta\text{SUV}_{\text{max}} > 66\%$  after cycle 2 and  $> 70\%$  after cycle 4 (PET2-/4-), intensive treatment and autologous transplantation (26.8%) if PET2+/4-, and salvage therapy (13.4%) if PET4+ ( $\Delta\text{SUV}_{\text{max}} \leq 70\%$ ). Among patients with GEP data (n=107), 38 (35.5%) were  $\text{PDL1}^{\text{high}}/\text{PDL2}^{\text{high}}$ . Key somatic mutations data (n=87) included *SOCS1* (70.1%), *B2M* (56.3%), *STAT6* (49.4%), *TNFAIP3* (47.1%), *GNAI3* (39.1%), *CIITA* (37.9%), *CD58* (36.8%), and *TP53* (29.9%). After a median follow-up of 39.5 months, 2-year PFS and OS rates were 86.2% and 93.2%, respectively. In a multivariate model including Bulk, aaIPI, and  $\Delta\text{SUV}_{\text{max}}$  PET2/PET4, only Bulk and  $\Delta\text{SUV}_{\text{max}}$  PET4  $\leq 70\%$  were associated with shorter PFS (HR 4.39 [1.28-15.11] and 4.95 [1.71-14.3], respectively), while none were associated with OS. The  $\Delta\text{SUV}_{\text{max}}$ -based interim PET4 response emerged as the strongest predictor of patient outcomes in this selected clinical trial population.

## **Introduction:**

The latest World Health Organization (WHO) and International Consensus (ICC) classifications defined Primary mediastinal B-cell lymphoma (PMBL) as a distinct entity of mature aggressive large B-cell lymphoma (LBCL) of supposed thymic B-cell origin that mostly locates in the anterior mediastinum<sup>1,2</sup>. Patients with PMBL are poorly represented in clinical trials for several reasons: (i) Diagnosis often occurs in emergency settings because of severe clinical presentations, including symptoms related to tumor mass, with 30-40% of patients developing superior vena cava syndrome<sup>3</sup>; (ii) the mediastinum, a challenging anatomical site to biopsy, frequently yields samples with suboptimal quality or quantity for definitive histopathological diagnosis<sup>4-6</sup> (iii) PMBL exhibits a unique biological complexity, characterized by an immune escape profile more akin to classical Hodgkin Lymphoma (cHL) and driven by specific genetic and molecular alterations that set it apart from other LBCL<sup>7-9</sup>; (iv) despite being a subtype of LBCL, PMBL shows an atypical favorable prognosis in first-line treatment, with 5-year survival rates exceeding 80%<sup>9-11</sup>. Molecular classifiers can distinguish PMBL from LBCL using ribonucleic acid (RNA) extracted from formalin-fixed paraffin-embedded (FFPE) tissue samples<sup>9,12,13</sup>. Next-generation sequencing (NGS)-based studies have further clarified the genetic landscape of PMBL, identifying recurrent somatic mutations (*SOCS1*, *STAT6*, *ITPKB*, *IGLL5*, *CIITA*, *TNFAIP3*, *ARID1A*, *CD58*, *IRF4*, *B2M*, *GNAI3*, *NFKBIE*, *XPO1*, *IL4R*), which can aid pathologists in diagnosing PMBL<sup>4,5,12,14-16</sup>. Whole-exome sequencing and NGS serve as valuable tools for risk stratification, with recent findings suggesting that mutations in *DUSP2* and *CD58* may correlate with patients outcomes and guide treatment decisions<sup>16</sup>. Furthermore, using gene expression profiling (GEP), the ‘PMBL LYSA’ study recently identified a subset (23-30%) of PMBL patients with high expression of both *PDL1* and *PDL2* genes who exhibit poor responses to first-line standard immunochemotherapy with anthracyclines and anti-CD20 monoclonal antibody (mAb)<sup>4</sup>. This real-life study, like many others in PMBL patients, was conducted retrospectively, carrying an

1 inherent risk of selection and sampling bias. In addition, due to the rarity of the disease and  
2 limited availability of biological samples, cases are often collected over extended periods,  
3 with variations in treatments and therapeutic response assessments across time and  
4 institutions. This can create challenges in generalizing the findings. Notably, in the ‘PMBL  
5 LYSA’ study, no blood samples were available at diagnosis to assess correlations between  
6 *PDL1/PDL2* gene expression and soluble PDL1 protein (sPDL1) levels — a promising  
7 disease biomarker in classical Hodgkin lymphoma<sup>17–19</sup> and LBCL<sup>20</sup>.

8 The GAINED study (NCT01659099) was a randomized phase 3 trial comparing  
9 Obinutuzumab (G) with Rituximab (R), both combined with standard chemotherapy. No  
10 significant difference in progression-free survival (PFS) or overall survival (OS) rates was  
11 observed between the R or G treatment arms<sup>21</sup>. Eligible patients were young (18-60 years), fit  
12 for high-dose chemotherapy intensification followed by autologous stem cell transplant  
13 (ASCT), and diagnosed with CD20+ diffuse large B-cell lymphoma (DLBCL) according to  
14 the 2008 WHO classification<sup>22</sup>. At that time, PMBL was often considered a subtype of  
15 DLBCL; however, PMBL cases were later confirmed via centralized histopathological review,  
16 integrating GEP and NGS for diagnosis<sup>23</sup>.

17 The present *post-hoc* analysis aimed to characterize the clinical, imaging, and biological  
18 features of this patient population and evaluate treatment responses, by performing  
19 correlations between patient outcomes and GEP, NGS, radiomics parameters, cell-free DNA  
20 (cfDNA) and sPDL1 data in a well-annotated, prospective cohort of PMBL enrolled in a  
21 multicenter phase III trial.

## Methods

### Patients, procedures and data collection

Details of the GAINED study design have been published previously<sup>21</sup>. It was an open-label multicenter randomized study conducted by the Lymphoma Study Association (LYSA) in 99 centers in Belgium and France. Eligible patients were 18 to 60 years old with newly diagnosed untreated histologically proven CD20+ DLBCL (2008 WHO classification), aaIPI  $\geq 1$ , at least 1 hypermetabolic lesion at baseline positron emission tomography (PET), eligibility for ASCT, and those who had a life expectancy of  $\geq 3$  months. Study design is shown in Supplemental Figure 1. The treatment plan was divided into two phases: induction and consolidation. The induction phase included four cycles of doxorubicin, cyclophosphamide, and prednisone, combined with either vindesine and bleomycin (ACVBP) or vincristine (CHOP14), administered on a 14-day schedule. Treatment responses during the induction phase were evaluated using PET after the second and fourth cycles (PET2/PET4), with  $\Delta$ SUVmax thresholds of 66% for PET2 and 70% for PET4<sup>24</sup>. Patients then received a PET-driven consolidation with either R/G-CHOP14 x4 or the conventional post-ACVBP consolidation strategy if PET2 and PET4 were negative, or therapeutic intensification followed by ASCT if PET2+/PET4-, or salvage treatment if PET2+/PET4+. PET at the end of treatment (EoT) was not performed (response evaluated by computed tomography scan).

The study protocol received approval from the appropriate local or national ethics committees in compliance with the legal requirements of each country. The research was conducted in line with the principles outlined in the Declaration of Helsinki. All patients provided written informed consent prior to their enrollment in the study. The data cutoff date for the present analyses was the same as for the final analysis of the GAINED study: December 1, 2017.

### GEP

A 137-gene expression assay combining reverse transcriptase multiplex ligation-dependent probe amplification (RT-MLPA) and high-throughput sequencing was applied as previously described (LymphoSign, Genexpath, Rouen, France)<sup>4,13</sup>.

#### **Expert pathologic review**

The process of FFPE review was organized and coordinated by the LYSA pathology platform (Henri Mondor University Hospital, Creteil, France). The histopathologic review of all cases with available material was performed by three expert hematopathologists and one molecular biologist following the diagnostic criteria established by WHO and ICC classifications<sup>1,2,25</sup>.

The final diagnosis of PMBL in this study was established by combining histological findings with available GEP and NGS data, resulting in a reconciled, integrated histomolecular diagnosis<sup>23</sup> (Supplemental Methods).

#### **NGS**

Targeted DNA sequencing was performed on DNA extracted from FFPE samples using a custom “Lymphopanel” to identify mutations in 70 genes related to lymphomagenesis. Library preparation, exome capture, sequencing, and analysis were carried out by IntegraGen SA (Evry, France), with sequencing done on an Illumina HiSeq4000 platform. Bioinformatic analysis, including variant calling and annotation, was performed using established tools such as MuTect and VEP (Supplemental Table 1, Supplemental Methods).

#### **Baseline cell-free DNA**

Plasma samples were collected at the time of randomization using BD™ P100® tubes (BD Diagnostics, Le Pont-De-Claix, France). These samples were shipped at room temperature to the core laboratory in Rennes (France) within 24 hours, then centrifuged and stored at -80°C. The concentration of cfDNA was measured based on DNA fragments within the 90- to 320-bp range. A high cfDNA concentration was defined as >55ng/mL, following previous studies<sup>26</sup>.

## Soluble PDL1

Baseline sPDL1 levels were measured using an enzyme-linked immunosorbent assay (PDCD1LG1 ELISA kit, USCN Life Science, Wuhan, China), following the manufacturer's instructions. The assay had a minimum detectable concentration of 0.057 ng/ml and a quantifiable range starting at 0.156 ng/ml. A threshold of 1.52 ng/ml was used to classify patients with elevated baseline sPDL1 levels, as defined in previous research<sup>20</sup>.

## Centralized PET review

Details of PET review process were previously described (Supplemental Methods)<sup>21,24</sup>. In addition, as a *post-hoc* analysis, whole-body PET volumes were semi-automatically segmented using the total metabolic tumor volume (TMTV) protocol from LIFEx<sup>27</sup>. Above the fixed SUV detection threshold of 4, connected regions were extracted and were individually pruned by three experienced physicians (PBD, SK, EI) to assess baseline TMTV and total lesion glycolysis (TLG), as previously described<sup>28–30</sup>. We previously observed that patients with a baseline TMTV  $\geq 360$  cm<sup>3</sup> had poorer outcomes<sup>4,11</sup>.

## Statistical analysis

Variables were summarized by numbers and percentages for categorical data and by the mean and standard deviation (SD) and median and quartiles for quantitative data. The tests for the comparisons of the different subpopulations were not corrected for multiple testing. Categorical variables were compared using Fisher or Chi square tests and quantitative variables using Kruskal-Wallis or Wilcoxon tests. Survival curves with 95% confidence intervals (CIs) were generated using the Kaplan–Meier method. Difference in survival was assessed by log-rank test and hazard rates with their 95% CIs calculated from Cox proportional hazard regression models. Univariate Cox models were built using clinical interest variables. An FDR correction for multiple testing were applied separately by type of

1 data (clinical, biological and radiomic) on univariate Cox models. The variables with a  
2 significant effect after FDR correction or well known to impact PFS (aaIPI) were retained for  
3 building the multivariate Cox model. However, TMTV was not retained because of its strong  
4 correlation with bulk. For OS, the multivariate analysis (MVA) was constructed using the  
5 same variables as in the PFS model to ensure consistency. All statistical analyses were  
6 performed using SAS (version 9.4 or higher) and R (version 4.3.1).

## **Results**

### **Patients disposition and characteristics**

From September 2012 to July 2015, 670 patients were enrolled in the GAINED study and randomized to receive either rituximab (n = 334) or obinutuzumab (n = 336). Overall, 620 (92.5%) cases underwent centralized histopathological review and molecular profiling through GEP and/or NGS when available. Of these, 138 patients (22.3%) were identified as having PMBL (Figure 1). These 138 PMBL patients, referred to as the "PMBL set" were included for further analysis. Baseline characteristics of this cohort included: median age: 33.5 years (range: 18-60 years), female: 63.8%, ECOG 0-1: 87.6%, stage III-IV: 55.1%, elevated Lactate Dehydrogenase (LDH): 90.6%, extranodal involvement: 62.3%, aaIPI 2-3: 52.6%, Bulk (mediastinal mass >10cm): 53.6%, median TMTV: 388.6 cm<sup>3</sup>, TMTV ≥360 cm<sup>3</sup>: 53%, and median TLG: 4440.2 g (Table 1). Bulk was strongly associated with higher TMTV and TLG (p<0.001, Supplemental Table 2). Indeed, 70% of patients with TMTV ≥360 cm<sup>3</sup> also had Bulk >10 cm.

Induction regimens were R-CHOP14 (31%), G-CHOP14 (26%), G-ACVBP (23%), R-ACVBP (20%) (Supplemental Figure 2). We did not observe any significant differences in patient characteristics nor protocol consolidation treatment between those who received CHOP-14 or ACVBP-based induction (Supplemental Table 3).

### **Biological features of the PMBL population**

Supplemental Figure 3 summarizes the number of patients with available GEP, NGS, sPDL1 and cfDNA data.

Among the 107 PMBL patients with available GEP data, 38 patients (35.5%) were identified as  $PDL1^{high}/PDL2^{high}$ , i.e. expression of  $PDL1 > 0.402$  and  $PDL2 > 9.147$ , as previously described<sup>4</sup>. A trend towards more frequent extranodal involvement was noted in the  $PDL1^{high}/PDL2^{high}$  group (73.7%) compared to the non- $PDL1^{high}/PDL2^{high}$  group (55.1%) ( $p=0.065$ ). In addition, the IPI score was significantly higher in the  $PDL1^{high}/PDL2^{high}$  group, with 54.1% of these patients having an IPI of 3 or higher, compared to the non- $PDL1^{high}/PDL2^{high}$  group (23.2%,  $p=0.002$ ). Finally, pleural and/or pericardial involvement were significantly more common in the  $PDL1^{high}/PDL2^{high}$  group (42.1%) compared to the non- $PDL1^{high}/PDL2^{high}$  group (15.9%) ( $p=0.005$ ), suggesting a more aggressive disease in this subgroup (Supplemental Table 4). We also observed a higher proportion of *PIMI* and *SETD1B* mutations in the  $PDL1^{high}/PDL2^{high}$  group, (Supplemental Figure 4). This finding suggests that patients with elevated *PDL1* and *PDL2* expression may exhibit distinct genetic alterations that could influence the disease's pathobiology —specifically, the *PIMI* gene, known for its role in promoting cell proliferation and survival<sup>31</sup>, along with *SETD1B*, a chromatin modifier involved in apoptosis resistance and BCL2 independence<sup>32</sup>. In addition, the tumor mutational burden (TMB), defined as the number of somatic mutations per megabase of analyzed genomic sequence, was significantly higher in the  $PDL1^{high}/PDL2^{high}$  group (Supplemental Figure 5,  $p<0.01$ ). Furthermore, among the four immune-escape associated genes tested, we observed higher VAFs for *CD58* and *CIITA* in the  $PDL1^{high}/PDL2^{high}$  group ( $p<0.05$ ), (Supplemental Figure 5).

sPDL1 level and cfDNA level were classified as high in 45 (52.3%) and 7 (16%) patients, respectively (Supplemental table 5). Of note, no correlation between sPDL1 and  $PDL1/PDL2$  gene expression was observed (Supplemental Figure 6).

With a mean read depth of 255 X and a panel size of 0.453 Gigabase (Gb), NGS mutational analysis ( $n = 87$ ) highlights recurrent somatic mutations in key PMBL-associated genes:

*SOCS1* (70.1%), *B2M* (56.3%), *STAT6* (49.4%), *TNFAIP3* (47.1%), *GNAI3* (39.1%), *CIITA* (37.9%) and *CD58* (36.8%, Figure 2, Supplemental Table 6-7). Mutations were identified in all coding regions of the panel except for the *MIR17HG* gene, with a median of 24 variants per case. Additionally, pathogenic *TP53* variants were identified in 29.9% of patients. Early or driver mutations, such as those affecting *SOCS1*, *TNFAIP3*, *STAT6*, *CIITA*, *CD58*, and *B2M* were observed with high median variant allele frequencies (mVAF, ~30%), supporting their role in the early pathogenesis of PMBL. In contrast, mutations in genes like *BCL2*, which displayed a lower mVAF (6.9%), are considered late or subclonal mutations (Supplemental Table 8).

### **Interim PET response rates and post-induction treatment**

PET-2 and -4 were performed in 129 (93.5%) and 128 (92.8%) patients, respectively. As shown in Table 2 and Figure 3, 77 patients (60.2%) had  $\Delta\text{SUV}_{\text{max}}$  PET2 >66%, of whom 76 (98.7%) received the planned immunochemotherapy. Thirty-four patients (26.5%) had  $\Delta\text{SUV}_{\text{max}}$  PET2  $\leq$ 66% and  $\Delta\text{SUV}_{\text{max}}$  PET4 >70% and all of them received the planned consolidation therapy followed by ASCT. Seventeen patients (13.3%) had  $\Delta\text{SUV}_{\text{max}}$  PET4  $\leq$ 70%, and all received salvage chemotherapy (Supplemental Table 9). In detail, these patients were categorized as disease progression in 5 patients (29.4%) and insufficient response, as determined by physician judgment based on PET4 (partial metabolic response or no metabolic response), in 12 patients (70.6%). Salvage chemotherapy regimens included R-DHAX-like therapies (rituximab, dexamethasone, high-dose cytarabine, and a platinum salt: oxaliplatin, cisplatin, or carboplatin) in 14 patients (82%) and high-dose methotrexate in 3 patients (18%). Mediastinal radiotherapy was performed in 3 patients (18%), and 12 patients (71%) underwent second-line consolidation with ASCT.

When evaluating iPET responses across the induction regimens, no significant differences were observed. PET2 and PET4 negativity rates were slightly higher in the ACVBP subgroups but did not reach statistical significance, and response rates according to Cheson 2007 criteria<sup>33</sup> were similar across all subgroups (Supplemental table 10). Notably, Deauville Score (DS) was missing in 108 cases (78.3%) at PET2 and 127 cases (92%) at PET4 because the protocol recommended using  $\Delta$ SUVmax to assess treatment response, meaning this variable was mostly not collected. No significant association was observed between baseline TMTV and PET2/PET4 responses (Supplemental Table 11).

PET responses and proportion of salvage therapy rates were analyzed according to *PDL1/PDL2* gene expression status. No statistically significant differences were observed across the analyzed characteristics (Supplemental Table 4 and 12).

### Patients' outcomes

After a median follow-up of 39.5 months, 2-year and 4-year PFS rates were 86.2% [95%CI:79.2-91%] and 83.9% [76.5-89.1%], respectively. The 2-year and 4-year OS rates were, respectively, at 93.2% [87.3-96.4%] and 90.8% [84.3-94.7%] (Figure 4). Of the 138 patients, 12 (8.7%) deaths were recorded. Causes of death included lymphoma in 7 patients (58.3%), toxicity of study treatment (including related cancers) in 3 patients (25%), concurrent illness in 1 patient, and other reasons in 1 patient (Supplemental Table 13). Only one patient experienced a relapse in the central nervous system.

A stratified analysis of patients based on iPET results revealed notable differences in PFS. Patients with PET4+ had significantly worse PFS compared to patients with PET2-/4- and PET2+/4- ( $p<0.001$ ). Because of consolidation ASCT, the difference between PET2-/4- and PET2+/4- was not statistically significant ( $p=0.193$ , Table 3). The 2-year PFS rates were

93.5% for PET2-/4- patients, 82.4% for PET2+/4- patients, and 58.8% for PET4+ patients (Figure 5).

The differences in OS were not statistically significant with 2-year OS rates of 96% for PET2-/4-, 90.9% for PET2+/4- and 85.6% for PET4+ patients ( $p=0.123$ , Figure 5).

## Univariate and multivariate analysis

Univariate analysis highlighted that Bulk >10cm (HR 4.37 [95%CI: 1.48-12.92]), TMTV  $\geq 360$  cm<sup>3</sup> (HR 3.91 [1.31-11.7]), stage III-IV (HR 4.06 [1.38-12.01]) and  $\Delta$ SUVmax  $\leq 70\%$  (PET4+, 6.6 [2.3-18.77]) were associated with shorter PFS, whereas  $\Delta$ SUVmax PET4 (continuous variable) was associated with longer PFS (HR 0.26 [0.15-0.43], for an increase of a SD). Additionally, stage III-IV (HR 9.9 [1.28-76.57]) and TMTV  $\geq 360$  cm<sup>3</sup> (HR 8.57 [1.09-67.62]) were linked to shorter OS, whereas  $\Delta$ SUVmax PET4 (continuous variable) was associated with longer OS (HR=0.36 [0.21-0.62], for an increase of a SD). None of the other factors assessed (induction regimen, anti-CD20 mAb, cfDNA and sPDL1 levels,  $PDL1^{high}/PDL2^{high}$  status, *B2M*, *CD58*, *TP53* alterations, TLG) were significantly associated with outcomes (Table 3 and 4).

Finally, in a MVA including Bulk, aaIPI (which includes stage), and  $\Delta$ SUVmax PET2/PET4, only Bulk and  $\Delta$ SUVmax PET4  $\leq 70\%$  were associated with shorter PFS (HR 4.39 [1.28-15.11] and 4.95 [1.71-14.3], respectively), while none were associated with OS (Table 3 and 4).

## **Discussion**

We conducted a *post-hoc* analysis of a large international phase III trial to focus on the outcome of newly diagnosed PMBL patients treated with R/G-CHOP14 or R/G-ACVBP induction and iPET-driven consolidation strategies without radiotherapy. PMBL was a common diagnosis (~22%) among the centrally reviewed cases in the GAINED study. Due to the primary focus on DLBCL in the GAINED study, a potential selection bias cannot be excluded as some centers may have omitted PMBL patients. Compared to previous PMBL reports, this may partly explain the higher proportion of stage IV and extranodal involvement observed in our cohort<sup>10,11,34–36</sup>. This entity exhibited markedly different biology and clinical course, further supporting the need for precise diagnosis and distinction of PMBL from other LBCL subtypes, as is now clearly established in international classifications<sup>1,2</sup>. We emphasize the importance of an integrated diagnostic approach that combines expert histological analysis with at least one molecular technique, such as GEP or NGS, to achieve a precise diagnosis of PMBL. This approach is essential because it directly influences treatment decisions: PMBL patients benefit from dose-dense regimens<sup>10,37</sup>, unlike DLBCL patients<sup>38–40</sup>. In this study, we utilized the LymphoSign GEP panel alongside NGS to aid pathologists in distinguishing PMBL from other lymphoma subtypes, forming an integrated histo-molecular diagnostic procedure<sup>23</sup>. Adopting this strategy into routine practice could significantly improve patient outcomes by ensuring accurate diagnoses and appropriate therapeutic approaches.

Overall, PFS and OS were favorable in this PMBL population (2-year PFS: 86.2%, 2-year OS: 93.2%) and consistent with other published studies, reinforcing the excellent outcomes of patients treated with frontline dose-dense immunochemotherapy without radiotherapy<sup>10,11,37,41</sup>. In treatment subgroups, obinutuzumab demonstrated efficacy comparable to rituximab, as previously observed in DLBCL<sup>21,42</sup>. Similarly, no significant difference in outcomes was found between CHOP14 and ACVBP induction regimens, aligning with findings from a

1 pooled analysis of five clinical trials conducted by LYSA<sup>41</sup> and our previous earlier  
2 retrospective study, which analyzed real-world data from a large non-trial population<sup>11</sup>. The  
3 rate of consolidation with ASCT was twice as high in the PMBL population (26.5%)  
4 compared to the overall GAINED study population<sup>21</sup>, reflecting a greater frequency of  
5 positive iPET in PMBL, even when  $\Delta\text{SUV}_{\text{max}}$  was used for interpretation.

6  $\Delta\text{SUV}_{\text{max}}$  PET2 >66% and  $\Delta\text{SUV}_{\text{max}}$  PET4 >70% were achieved in 62% and 87% of  
7 patients with iPET data, respectively -higher than expected for a PMBL population. In  
8 comparison, previous studies reported complete metabolic response (CMR) rates (based on  
9 DS) ranging from 10%-47.8% at PET2<sup>11,43</sup> and 52.8%-73.2% at PET4<sup>11,44</sup>. Notably, PET4  
10 response ( $\Delta\text{SUV}_{\text{max}} \leq 70\%$ ) emerged as the strongest dynamic predictor of shorter PFS (HR  
11 6.6 [2.3-18.77]), regardless of the treatment group.

12 The lack of prognostic impact for aaIPI in MVA may be attributed to limited statistical power,  
13 the small sample size, and the low number of events. Additionally, the use of ASCT may have  
14 mitigated the influence of baseline prognostic factors. Bulk (tumor mass >10 cm) emerged as  
15 a strong baseline prognostic marker -a finding not previously reported in other recent  
16 studies<sup>11,45-49</sup>. This may indicate increased chemotherapy resistance in these patients,  
17 potentially due to anatomical characteristics of large mediastinal tumors, where the substantial  
18 volume and dense, fibrotic, and compartmentalized structure may impair the penetration and  
19 distribution of chemotherapeutic agents, including those in ASCT conditioning regimens.  
20 Such limited drug diffusion could impact local pharmacodynamics, especially in patients not  
21 exposed to radiotherapy, as suggested by preclinical models of solid tumors<sup>50-53</sup>. We did not  
22 include TMTV  $\geq 360 \text{ cm}^3$  in the MVA because the model lacked sufficient power to estimate  
23 both parameters (TMTV  $\geq 360 \text{ cm}^3$  and Bulk >10 cm), which are highly correlated. Indeed,  
24 70% of PMBL patients with TMTV  $\geq 360 \text{ cm}^3$  also had Bulk >10 cm. Given the limited  
25 sample size, the model could not simultaneously estimate the effects of these two variables.

Therefore, we retained Bulk in the MVA, as it had higher HR in the univariate analysis. Other studies have proposed alternative thresholds, such as 25% or 41% of SUVmax or fixed SUV  $\geq 2.5$  methods<sup>11,21,24,54–57</sup>. However, in our series, the correlation between the 41% of SUVmax and fixed SUV4 segmentation approaches was approximately 90% (data not shown), resulting in comparable outcomes. Moreover, recent data presented as an abstract at the ASH 2024 conference further support the use of an SUV $\geq 4$  threshold for volumetric measurements in PMBL, reporting a median TMTV value of 396 ml, which is very close to that of our cohort<sup>58</sup>. Further research is needed to evaluate whether Bulk >10 cm could serve as a surrogate marker for more advanced tumor volume or metabolic distribution tools, such as metabolic heterogeneity or TLG<sup>30,49,59</sup>.

A limitation of our study is that DS was largely unrecorded, as the protocol recommended using  $\Delta$ SUVmax to evaluate treatment response. This omission prevents analysis of DS predictive value on iPET, as has been conducted in other studies<sup>11,43,44</sup>. Furthermore, EoT CMR was not assessed in the GAINED trial. By contrast, in our earlier retrospective study, we reported similar ~86% CMR rates across the R-ACVBP and R-CHOP14 groups<sup>11</sup>. Consequently, we are unable to directly compare our results with those of the IELSG37 trial, which assessed the omission of radiotherapy in patients achieving CMR at EoT<sup>35</sup>.

Regarding biological features, the mutational profile identified in this study perfectly aligns with the existing literature on PMBL. We observed recurrent mutations in genes such as *SOCS1* (70.1%), *B2M* (56.3%), *STAT6* (49.4%), *TNFAIP3* (47.1%), *GNAI3* (39.1%), *CIITA* (37.9%), and *CD58* (36.8%), all of which are well-established genetic alterations associated with PMBL. In addition, we observed a higher TMB in the *PDL1*<sup>high</sup>/*PDL2*<sup>high</sup> group, a feature known to correlate with responses to checkpoint inhibitors<sup>60–62</sup>. These findings further support the characterization of PMBL as having a distinct genetic landscape compared to other LBCL, as described in previous genomic studies<sup>5,15,16</sup>.

Patients with  $PDL1^{high}/PDL2^{high}$  status exhibited worse clinical characteristics (e.g., elevated LDH, higher aaPI scores, ECOG  $\geq 2$ ), and a higher incidence of PET4+ (23.5% vs 9.5%), correlating with a trend toward poorer outcomes (PFS: HR=2.27, OS: HR=3.87), consistent with but not as pronounced as findings from our previous report<sup>4</sup>. Approximately 25% of PMBL patients underwent ASCT, a higher rate than in the overall GAINED trial population (~15%)<sup>21</sup> and slightly higher than in our previously retrospective study (22%)<sup>11</sup>. This may have influenced the lack of significant associations between baseline factors and outcomes. We acknowledge that interpreting the predictive capacity of iPET results is limited by our study design, where treatment was adapted based on iPET findings. The iPET-guided strategy aimed to reduce PFS/OS differences between risk groups defined by iPET. As observed in the GAINED study, no significant difference in PFS was reported between PET2- and PET2+ patients among PET4- patients, suggesting that ASCT may mitigate the risk in PET2+ patients. However, no PFS improvement was observed in PET4+ patients, even when treated with salvage therapy. These findings suggest that PET4 results remain a prognostic factor for identifying high-risk patients, despite treatment adaptations in our study. In addition, growing evidence supports the use of iPET as a predictive tool in other LBCL. For instance, a study by Sun et al. demonstrated that iPET using  $\Delta SUV_{max}$  was predictive of PFS (HR = 20.4) and OS (HR = 12.7) in non-GCB DLBCL<sup>63</sup>. Additionally, a study by Johansson et al. investigated iPET in DLBCL using  $\Delta SUV_{max}$  in a MVA integrating baseline clinical factors and the time-dependent variable iPET. The authors observed that iPET positivity was associated with adverse outcomes (HR = 6.1 for time to progression and HR = 5.5 for OS)<sup>64</sup>. Furthermore, the small group of patients with GEP data and the PET-driven consolidation - where PET4+ patients received salvage therapy- may explain the limited prognostic impact of  $PDL1^{high}/PDL2^{high}$  status, a feature previously shown to be strongly unfavorable in non-PET-driven trial settings. It is plausible that  $PDL1^{high}/PDL2^{high}$  patients represent those with highly

1 symptomatic disease and/or high tumor burden at diagnosis, which could preclude them from  
2 waiting for phase III trial enrollment. Indeed, in our prior study, *PDL1*<sup>high</sup>/*PDL2*<sup>high</sup> patients  
3 had higher proportion of elevated LDH (94.6% vs. 77.1%) and greater baseline TMTV (358.5  
4 vs. 255.7 cm<sup>3</sup>) compared to other patients<sup>4</sup>. Consequently, prognostic factors identified in  
5 real-world settings may not be fully replicated in controlled clinical trial environments. The  
6 lack of statistical significance in this analysis may also stem from the limited sample size and  
7 the non-random distribution of patients with available GEP data. Similarly, the small number  
8 of patients with NGS data limits the ability to draw robust conclusions regarding the  
9 prognostic significance of specific genetic alterations, such as *CD58*, *TP53* or *B2M* mutations,  
10 which have been previously reported as unfavorable features in other studies<sup>4,16,65</sup>.

11 Finally, regarding the broader implications of our findings, it is worth noting that ASCT is no  
12 longer recommended as a first-line consolidation treatment for DLBCL and has never been a  
13 standard for PMBL<sup>66–68</sup>. The PET2/PET4-guided strategy, hindered by a high rate of PET2-  
14 positivity (~38%, according to  $\Delta\text{SUV}_{\text{max}} \leq 66\%$ ), does not reliably discriminate patients at  
15 risk of progression or relapse. A PET4-only strategy could offer greater utility, enabling the  
16 identification of slow responders or progressive patients—whose prognosis is notably  
17 worse—and guiding them toward salvage therapies including for instance radiotherapy, anti-  
18 PD1 immunotherapy anti-PD1 and/or CAR-T cell treatments in cases of PET4 positivity  
19 ( $\Delta\text{SUV}_{\text{max}} \leq 70\%$ ). These approaches have demonstrated promising results for PMBL  
20 patients in several recent studies<sup>69–73</sup>, and this strategy warrants further exploration in future  
21 clinical trials targeting high-risk PET4-positive populations.

22 In conclusion, this analysis underscores the strong representation, distinct characteristics and  
23 excellent outcomes of PMBL patients in the GAINED trial. It highlights the importance of  
24 expert histopathological and molecular characterization for accurate diagnosis, which is  
25 crucial for ensuring these patients receive appropriate dose-dense treatments. Genetics were

1 not associated with outcomes within the context of a PET-driven strategy while iPET  
2 response, particularly  $\Delta\text{SUV}_{\text{max}}$  PET4, emerged as a key predictor of outcomes in this  
3 carefully selected clinical trial population, where consolidation treatments were tailored based  
4 on iPET results.

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## Authorship contribution

V.C. designed and supervised the present work, collected, analyzed, interpreted the data, and wrote the manuscript.

S.L.G designed the GAINED trial in collaboration with J.-P.J., C.H., and E.I., conducted the study, analyzed the results, enrolled and treated patients.

H.G., L.O., F.M., H.T., V.R., R.H., C.T., H.M., F.C., K.B., G.L.D, L.M.F, R.N., P.F., D.S., G.C., C.B., W.B. and C.R. enrolled and treated patients.

T.J.M., A.M., and J.B. performed the expert pathological review and interpreted the data.

F.D. conducted the NGS experiments, interpreted the biological data, and participated in the integrated histomolecular diagnosis process.

P.R. carried out and analyzed the GEP experiments and contributed to the integrated histomolecular diagnosis process.

P.B.D., F.K.B., C.B.M., S.K., and E.I. centrally reviewed the PET exams.

1 P.B.D., S.K., and E.I. conducted the TMTV and TLG assessments.

2 M.H.E and L.C. performed the statistical analysis.

3 F.J., T.J.M. and T.F designed and supervised the present work, analyzed, and interpreted the  
4 data, and edited the manuscript.

5 All authors approved the manuscript.

### 8 **Conflict-of-interest disclosure**

9 V.C. has received honoraria from Abbvie, Astra Zeneca, Bristol-Myers Squibb, Ideogen,  
10 Janssen, Kiowa Kirin, Kite Gilead, Novartis, Pfizer, Sanofi, Takeda.

11 R.H. has received honoraria from Kite/Gilead, Novartis, Bristol-Myers Squibb/Celgene,  
12 Incyte, Janssen, MSD, Takeda, Amgen, Abbvie, and Roche; and is a member on an entity's  
13 Board of Directors or advisory committees of Kite/Gilead, Novartis, Bristol-Myers  
14 Squibb/Celgene, Tessa Therapeutics, Abbvie and Roche.

15 W.B. has received travel grants from Leo Pharma, Roche and Takeda; and honoraria from Leo  
16 Pharma and BMS.

17 All other authors have no conflict of interest to disclose.

## **Figures legends**

### **Figure 1: Population selection from the GAINED cohort**

**Figure 2: Oncoprint showing the distribution of genomic alterations in PMBL patients from the GAINED cohort with available targeted next-generation sequencing (NGS, n=87), clustered based on the *PDL1/PDL2* gene expression status (GEP).**

*Abbreviations: G: Obinutuzumab; R: Rituximab; ACVBP: Obinutuzumab, doxorubicine, cyclophosphamide, vindesine, bleomycine, prednisone; CHOP14: Obinutuzumab, doxorubicine, cyclophosphamide, vincristine, prednisone delivered every 14 days; DLBCL EBV+; diffuse large B-cell lymphoma EBV positive; DLBCL NOS: diffuse large B-cell lymphoma not otherwise specified; sPDL1: baseline soluble PDL1 level; cfDNA: baseline cell-free DNA level*

### **Figure 3: Treatments received according to interim PET responses**

*\*One patient died before treatment start ; \*\* In patients with missing PET2 and/or PET4 data, consolidation treatments data were also missing; \*\*\*One patient experienced treatment discontinuation after 3 cycles of R-ACVBP (toxicity). Switch for R-CHOP (n=2 cycles) then etoposide-ifosfamide (2 cycles).*

*ASCT: autologous stem cell transplantation; PET: positron emission tomography*

### **Figure 4: Progression-free survival (A) and overall survival (B) in the PMBL set**

**Figure 5: Progression-free survival (A) and overall survival (B) according to interim PET2 and PET4 response in the PMBL set**

## **Tables**

**Table 1: Patients' characteristics at baseline**

|                                                                     | <b>PMBL set</b>        |         |
|---------------------------------------------------------------------|------------------------|---------|
|                                                                     | N=138                  |         |
| <b>Age at randomization (years)</b>                                 |                        |         |
| N                                                                   | 138                    |         |
| Mean (SD)                                                           | 34.1 (9.98)            |         |
| Median                                                              | 33.5                   |         |
| Q1 ; Q3                                                             | 26 ; 40                |         |
| Min ; Max                                                           | 18 ; 60                |         |
| <b>Female sex</b>                                                   | 88                     | (63.8%) |
| <b>Elevated LDH (&gt; Upper Limit)</b>                              | 125                    | (90.6%) |
| <b>Performance Status (ECOG) 0 - 1</b>                              | 120                    | (87.6%) |
| Missing                                                             | 1                      | -       |
| <b>Ann Arbor Stage in class</b>                                     |                        |         |
| I-II                                                                | 62                     | (44.9%) |
| III-IV                                                              | 76                     | (55.1%) |
| <b>Patients with at least one extra-nodal involvement</b>           | 86                     | (62.3%) |
| <b>aaIPI in class</b>                                               |                        |         |
| 0-1                                                                 | 65                     | (47.4%) |
| 2-3                                                                 | 72                     | (52.6%) |
| Missing                                                             | 1                      | -       |
| <b>IPI</b>                                                          |                        |         |
| 1                                                                   | 58                     | (42.3%) |
| 2                                                                   | 31                     | (22.6%) |
| 3                                                                   | 43                     | (31.4%) |
| 4                                                                   | 5                      | (3.6%)  |
| Missing                                                             | 1                      | -       |
| <b>IPI in class</b>                                                 |                        |         |
| 0-2                                                                 | 89                     | (65%)   |
| 3-5                                                                 | 48                     | (35%)   |
| Missing                                                             | 1                      | -       |
| <b>CNS IPI score in class</b>                                       |                        |         |
| 0-1                                                                 | 58                     | (42.3%) |
| 2-3                                                                 | 66                     | (48.2%) |
| 4-6                                                                 | 13                     | (9.5%)  |
| Missing                                                             | 1                      | -       |
| <b>Presence of B symptoms</b>                                       | 56                     | (40.6%) |
| <b>Bulk : mediastinal mass maximal diameter &gt; 10 cm</b>          | 74                     | (53.6%) |
| <b>Pleura and/or pericardium involvement</b>                        | 37                     | (26.8%) |
| <b>Presence of an anterior mediastinal mass</b>                     | 137                    | (99.3%) |
| <b>Baseline total metabolic tumor volume (TMTV, cm<sup>3</sup>)</b> |                        |         |
| N                                                                   | 132                    |         |
| Missing                                                             | 6                      |         |
| Mean (SD)                                                           | 453.4273 (292.91971)   |         |
| Median                                                              | 388.6400               |         |
| Q1 ; Q3                                                             | 233.720 ; 611.080      |         |
| Min ; Max                                                           | 20.150 ; 1551.280      |         |
| <b>TMTV ≥ 360 cm<sup>3</sup></b>                                    | 70                     | (53%)   |
| <b>Total lesion glycolysis (TLG, g)</b>                             |                        |         |
| N                                                                   | 132                    |         |
| Missing                                                             | 6                      |         |
| Mean (SD)                                                           | 5038.5966 (3599.43252) |         |
| Median                                                              | 4440.2150              |         |
| Q1 ; Q3                                                             | 2486.945 ; 6897.395    |         |
| Min ; Max                                                           | 103.500 ; 16571.940    |         |

*Abbreviations: GCB: germinal center B-cell ; non-GC: non germinal center B-cell. IPI: international prognostic index; CNS: central nervous system; LDH: lactate deshydrogenase*

**Table 2: Interim PET (iPET) results**

|                                                                          | PMBL set         |         |
|--------------------------------------------------------------------------|------------------|---------|
|                                                                          | N=138            |         |
| <b><math>\Delta</math>SUVmax - PET2</b>                                  |                  |         |
| N                                                                        | 130              |         |
| Missing                                                                  | 8                |         |
| Mean (SD)                                                                | 77.504 (14.9323) |         |
| Median                                                                   | 80.240           |         |
| Q1 ; Q3                                                                  | 74.88 ; 86.93    |         |
| Min ; Max                                                                | 4.96 ; 96.35     |         |
| <b>PET2 Response</b>                                                     |                  |         |
| $\Delta$ SUVmax PET2 >66%                                                | 80               | (62%)   |
| $\Delta$ SUVmax PET2 ≤66%                                                | 49               | (38%)   |
| Missing                                                                  | 9                |         |
| <b><math>\Delta</math>SUVmax - PET4</b>                                  |                  |         |
| N                                                                        | 128              |         |
| Missing                                                                  | 10               |         |
| Mean (SD)                                                                | 84.042 (8.9557)  |         |
| Median                                                                   | 85.85            |         |
| Q1 ; Q3                                                                  | 81.18 ; 89.44    |         |
| Min ; Max                                                                | 28.75 ; 96.06    |         |
| <b>PET4 Response</b>                                                     |                  |         |
| $\Delta$ SUVmax PET4 >70%                                                | 111              | (86.7%) |
| $\Delta$ SUVmax PET4 ≤70%                                                | 17               | (13.3%) |
| Missing                                                                  | 10               |         |
| <b>PET2/PET4 response</b>                                                |                  |         |
| $\Delta$ SUVmax PET2 >66% (PET2-)                                        | 77               | (60.2%) |
| $\Delta$ SUVmax PET2 ≤66% and $\Delta$ SUVmax PET4 >70%<br>(PET2+/PET4-) | 34               | (26.5%) |
| $\Delta$ SUVmax PET4 ≤70% (PET4+)                                        | 17               | (13.3%) |
| Missing                                                                  | 10               |         |

*SUVmax: maximum standardized uptake value; SD: standard deviation; PET2: positron emission tomography after 2 cycles of treatment; PET4: positron emission tomography after 4 cycles of treatment;*

**Table 3: Univariate and multivariate analyses of prognostic factors associated with progression-free survival (PFS)**

| Univariate analysis      |                                                                                |           |                                   |                                   |             |             | Multivariate analysis |                                   |                                   |         |
|--------------------------|--------------------------------------------------------------------------------|-----------|-----------------------------------|-----------------------------------|-------------|-------------|-----------------------|-----------------------------------|-----------------------------------|---------|
| Variable                 | Modality                                                                       | HR        | 95% Lower Confidence Limit for HR | 95% Upper Confidence Limit for HR | Raw p-value | FDR p-value | HR                    | 95% Lower Confidence Limit for HR | 95% Upper Confidence Limit for HR | p-value |
| $\Delta$ SUVmax PET2     | Continuous                                                                     | 0.785     | 0.548                             | 1.124                             | 0.2285      | 0.228       |                       |                                   |                                   |         |
| $\Delta$ SUVmax PET4     | Continuous                                                                     | 0.256     | 0.151                             | 0.434                             | <0.0001     | <0.0001     |                       |                                   |                                   |         |
| $\Delta$ SUVmax (cutoff) | $\Delta$ SUVmax PET2 $\leq$ 66% (PET2-)                                        | Reference | -                                 | -                                 | 0.0041      | 0.012       | Reference             | -                                 | -                                 | -       |
|                          | $\Delta$ SUVmax PET2 $\leq$ 66% and $\Delta$ SUVmax PET4 $>$ 70% (PET2+/PET4-) | 2.065     | 0.694                             | 6.146                             | 0.1925      | -           | 2.309                 | 0.771                             | 6.913                             | 0.1348  |
|                          | $\Delta$ SUVmax $\leq$ 70% (PET4+)                                             | 6.566     | 2.298                             | 18.767                            | 0.0004      | -           | 4.952                 | 1.714                             | 14.305                            | 0.0031  |
| Bulk                     | $> 10$ cm                                                                      | 4.37      | 1.479                             | 12.916                            | 0.0024      | 0.02        | 4.388                 | 1.275                             | 15.106                            | 0.019   |
| Ann Arbor Stage          | III-IV                                                                         | 4.064     | 1.375                             | 12.012                            | 0.004       | 0.02        |                       |                                   |                                   |         |
| aaIPI                    | 2-3                                                                            | 2.653     | 1.038                             | 6.782                             | 0.0308      | 0.103       | 2.303                 | 0.817                             | 6.495                             | 0.1148  |
| TMTV*                    | Continuous                                                                     | 1.267     | 0.996                             | 1.61                              | 0.0724      | 0.109       |                       |                                   |                                   |         |
| TMTV cutoff              | $\geq 360$ cm <sup>3</sup>                                                     | 3.91      | 1.307                             | 11.699                            | 0.0064      | 0.013       |                       |                                   |                                   |         |

|                                       |                                                |       |       |        |        |       |  |  |  |  |
|---------------------------------------|------------------------------------------------|-------|-------|--------|--------|-------|--|--|--|--|
| TLG**                                 | Continuous                                     | 1.078 | 0.971 | 1.196  | 0.1815 | 0.218 |  |  |  |  |
| ECOG                                  | 2-4                                            | 2.878 | 1.125 | 7.363  | 0.044  | 0.11  |  |  |  |  |
| Pleura and/or pericardium involvement | Absence                                        | 0.502 | 0.214 | 1.174  | 0.1227 | 0.245 |  |  |  |  |
| B symptoms                            | Presence                                       | 1.853 | 0.801 | 4.29   | 0.1491 | 0.248 |  |  |  |  |
| Induction chemotherapy                | CHOP14                                         | 1.609 | 0.649 | 3.986  | 0.2937 | 0.42  |  |  |  |  |
| Monoclonal antibody                   | Rituximab                                      | 1.125 | 0.486 | 2.604  | 0.7826 | 0.783 |  |  |  |  |
| Age                                   | Continuous                                     | 1.145 | 0.759 | 1.729  | 0.5226 | 0.621 |  |  |  |  |
| LDH level                             | Elevated                                       | 0.683 | 0.202 | 2.31   | 0.5586 | 0.621 |  |  |  |  |
| Baseline cfDNA level                  | Continuous                                     | 2.502 | 0.291 | 21.525 | 0.4595 | 0.715 |  |  |  |  |
| cfDNA cutoff                          | cfDNA low                                      | 0.689 | 0.143 | 3.319  | 0.6543 | 0.727 |  |  |  |  |
| sPDL1                                 | Continuous                                     | 0.694 | 0.34  | 1.419  | 0.2493 | 0.715 |  |  |  |  |
| sPDL1 cutoff                          | sPDL1 low                                      | 2.058 | 0.69  | 6.143  | 0.1848 | 0.715 |  |  |  |  |
| <i>PDL1/PDL2</i> gene expression      | <i>PDL1<sup>high</sup>/PDL2<sup>high</sup></i> | 2.274 | 0.877 | 5.897  | 0.0929 | 0.715 |  |  |  |  |
| <i>TP53</i>                           | mutated                                        | 0.703 | 0.193 | 2.553  | 0.5806 | 0.726 |  |  |  |  |
| <i>CD58</i>                           | mutated                                        | 1.023 | 0.335 | 3.128  | 0.9679 | 0.968 |  |  |  |  |
| <i>B2M</i>                            | mutated                                        | 0.688 | 0.231 | 2.048  | 0.5007 | 0.715 |  |  |  |  |

*\*The TMTV effect corresponds to an increase of 200 cm<sup>3</sup>. We did not include TMTV  $\geq 360$  cm<sup>3</sup> in the multivariate analysis because the model lacked sufficient power to estimate both parameters (TMTV  $\geq 360$  cm<sup>3</sup> and Bulk > 10 cm), which are highly correlated. Indeed, 70% of PMBL*

patients with  $TMTV \geq 360 \text{ cm}^3$  also had  $Bulk > 10 \text{ cm}$ . Given the limited sample size, the model could not simultaneously estimate the effects of these two variables. Therefore, we retained  $Bulk > 10 \text{ cm}$  in the multivariate analysis, as it had higher HR in the univariate analysis.

**\*\*The TLG effect corresponds to an increase of 1000.**

*TMTV: total metabolic tumor volume. TLG: total lesion glycolysis. sPDL1: soluble PDL1; cfDNA: cell-free DNA; LDH: lactate deshydrogenase; aaIPI: age-adjusted international prognostic index; SUVmax: maximum standardized uptake value;*

**Table 4: Univariate and multivariable analyses of prognostic factors associated with overall survival (OS)**

| Univariate analysis |                                                       |           |                                   |                                   |             |             | Multivariable analysis |                                   |                                   |         |
|---------------------|-------------------------------------------------------|-----------|-----------------------------------|-----------------------------------|-------------|-------------|------------------------|-----------------------------------|-----------------------------------|---------|
| Variable            | Modality                                              | HR        | 95% Lower Confidence Limit for HR | 95% Upper Confidence Limit for HR | Raw p-value | FDR p-value | HR                     | 95% Lower Confidence Limit for HR | 95% Upper Confidence Limit for HR | p-value |
| ΔSUVmax PET2        | Continuous                                            | 0.736     | 0.471                             | 1.15                              | 0.2304      | 0.346       |                        |                                   |                                   |         |
| ΔSUVmax PET4        | Continuous                                            | 0.361     | 0.211                             | 0.616                             | 0.0024      | 0.014       |                        |                                   |                                   |         |
| ΔSUVmax (cutoff)    | ΔSUVmax PET2 ≤66%                                     | Reference | -                                 | -                                 | 0.1635      | 0.327       | Reference              | -                                 | -                                 | -       |
|                     | ΔSUVmax PET2 ≤66% and ΔSUVmax PET4 >70% (PET2+/PET4-) | 2.376     | 0.594                             | 9.500                             | 0.2211      | -           | 3.008                  | 0.737                             | 12.285                            | 0.125   |
|                     | ΔSUVmax ≤70% (PET4+)                                  | 4.217     | 0.943                             | 18.860                            | 0.0597      | -           | 3.175                  | 0.7                               | 14.395                            | 0.1342  |
| Bulk                | > 10 cm                                               | 2.875     | 0.778                             | 10.624                            | 0.0893      | 0.179       | 1.908                  | 0.496                             | 7.341                             | 0.3476  |
| Ann Arbor Stage     | III-IV                                                | 9.885     | 1.276                             | 76.572                            | 0.0032      | 0.032       |                        |                                   |                                   |         |
| aaIPI               | 2-3                                                   | 4.885     | 1.07                              | 22.297                            | 0.0176      | 0.059       | 4.292                  | 0.885                             | 20.809                            | 0.0705  |
| TMTV*               | Continuous                                            | 1.161     | 0.803                             | 1.677                             | 0.4483      | 0.501       |                        |                                   |                                   |         |
| TMTV cutoff         | ≥ 360 cm <sup>3</sup>                                 | 8.566     | 1.085                             | 67.618                            | 0.0083      | 0.025       |                        |                                   |                                   |         |
| TLG**               | Continuous                                            | 1.056     | 0.906                             | 1.231                             | 0.5012      | 0.501       |                        |                                   |                                   |         |

|                                       |                                                |       |       |        |        |       |  |  |  |  |
|---------------------------------------|------------------------------------------------|-------|-------|--------|--------|-------|--|--|--|--|
| ECOG                                  | 2-4                                            | 2.477 | 0.67  | 9.155  | 0.2106 | 0.351 |  |  |  |  |
| Pleura and/or pericardium involvement | Absence                                        | 0.341 | 0.11  | 1.058  | 0.0686 | 0.172 |  |  |  |  |
| B symptoms                            | Presence                                       | 4.541 | 1.23  | 16.775 | 0.0132 | 0.059 |  |  |  |  |
| Induction chemotherapy                | CHOP14                                         | 1.368 | 0.4   | 4.674  | 0.6126 | 0.613 |  |  |  |  |
| Monoclonal antibody                   | Rituximab                                      | 0.665 | 0.211 | 2.097  | 0.4833 | 0.537 |  |  |  |  |
| Age                                   | Continuous                                     | 1.251 | 0.722 | 2.168  | 0.4303 | 0.537 |  |  |  |  |
| LDH level                             | Elevated                                       | 0.518 | 0.114 | 2.365  | 0.4308 | 0.537 |  |  |  |  |
| Baseline cfDNA level                  | Continuous                                     | 3.047 | 0.316 | 29.339 | 0.3975 | 0.497 |  |  |  |  |
| cfDNA cutoff                          | cfDNA low                                      | 0.447 | 0.082 | 2.445  | 0.379  | 0.497 |  |  |  |  |
| sPDL1                                 | Continuous                                     | 0.425 | 0.13  | 1.388  | 0.0776 | 0.259 |  |  |  |  |
| sPDL1 cutoff                          | sPDL1 low                                      | 2.705 | 0.7   | 10.462 | 0.1299 | 0.325 |  |  |  |  |
| <i>PDL1/PDL2</i> gene expression      | <i>PDL1<sup>high</sup>/PDL2<sup>high</sup></i> | 3.873 | 0.968 | 15.498 | 0.0465 | 0.249 |  |  |  |  |
| <i>TP53</i>                           | mutated                                        | 0.477 | 0.056 | 4.08   | 0.4646 | 0.516 |  |  |  |  |
| <i>CD58</i>                           | mutated                                        | 0.825 | 0.151 | 4.506  | 0.8227 | 0.823 |  |  |  |  |
| <i>B2M</i>                            | mutated                                        | 0.402 | 0.074 | 2.194  | 0.2757 | 0.459 |  |  |  |  |

\*The TMTV effect corresponds to an increase of 200 cm<sup>3</sup>.

\*\*The TLG effect corresponds to an increase of 1000.

TMTV: total metabolic tumor volume. TLG: total lesion glycolysis. sPDL1: soluble PDL1; cfDNA: cell-free DNA; LDH: lactate deshydrogenase; aaIPI: age-adjusted international prognostic index; SUVmax: maximum standardized uptake value;

Figure 1

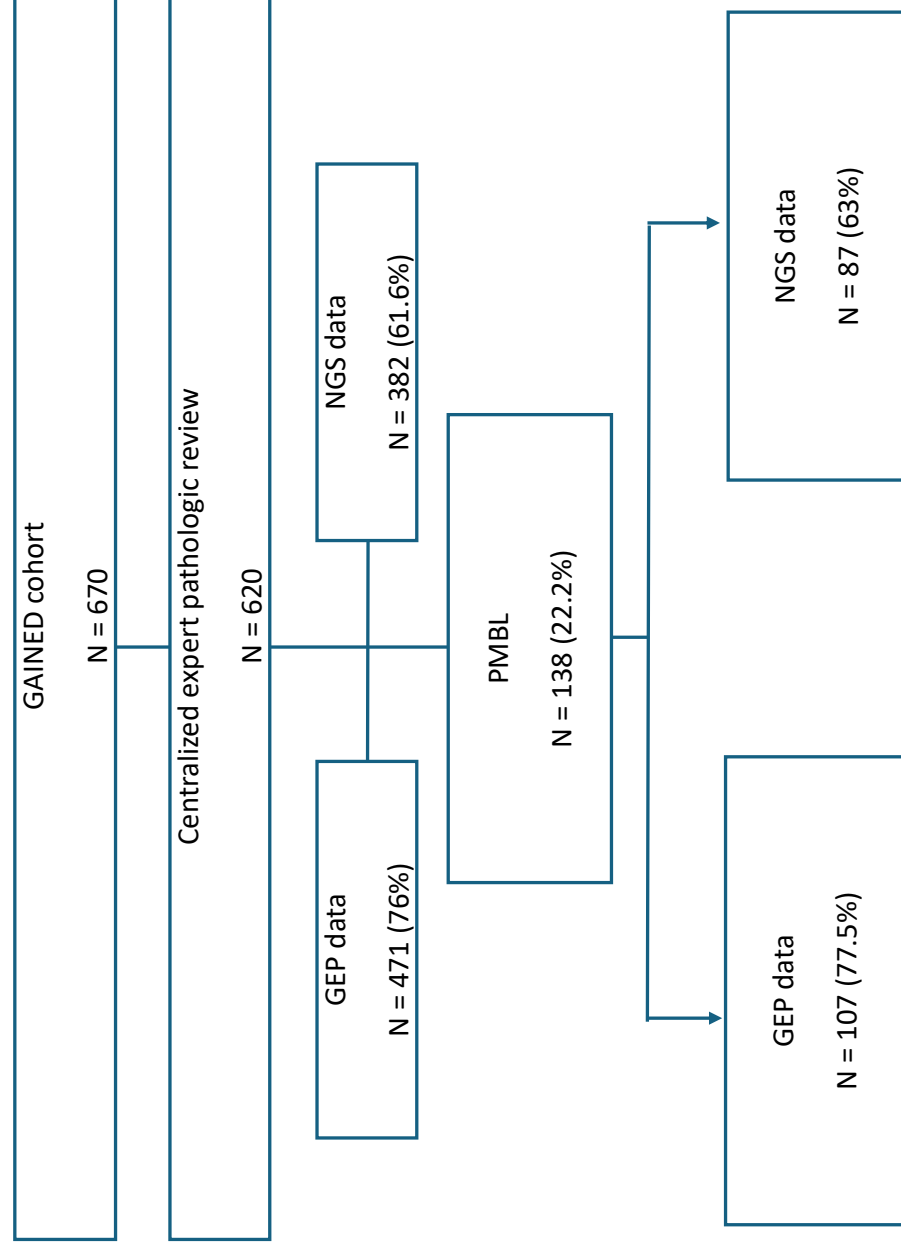


Figure 2

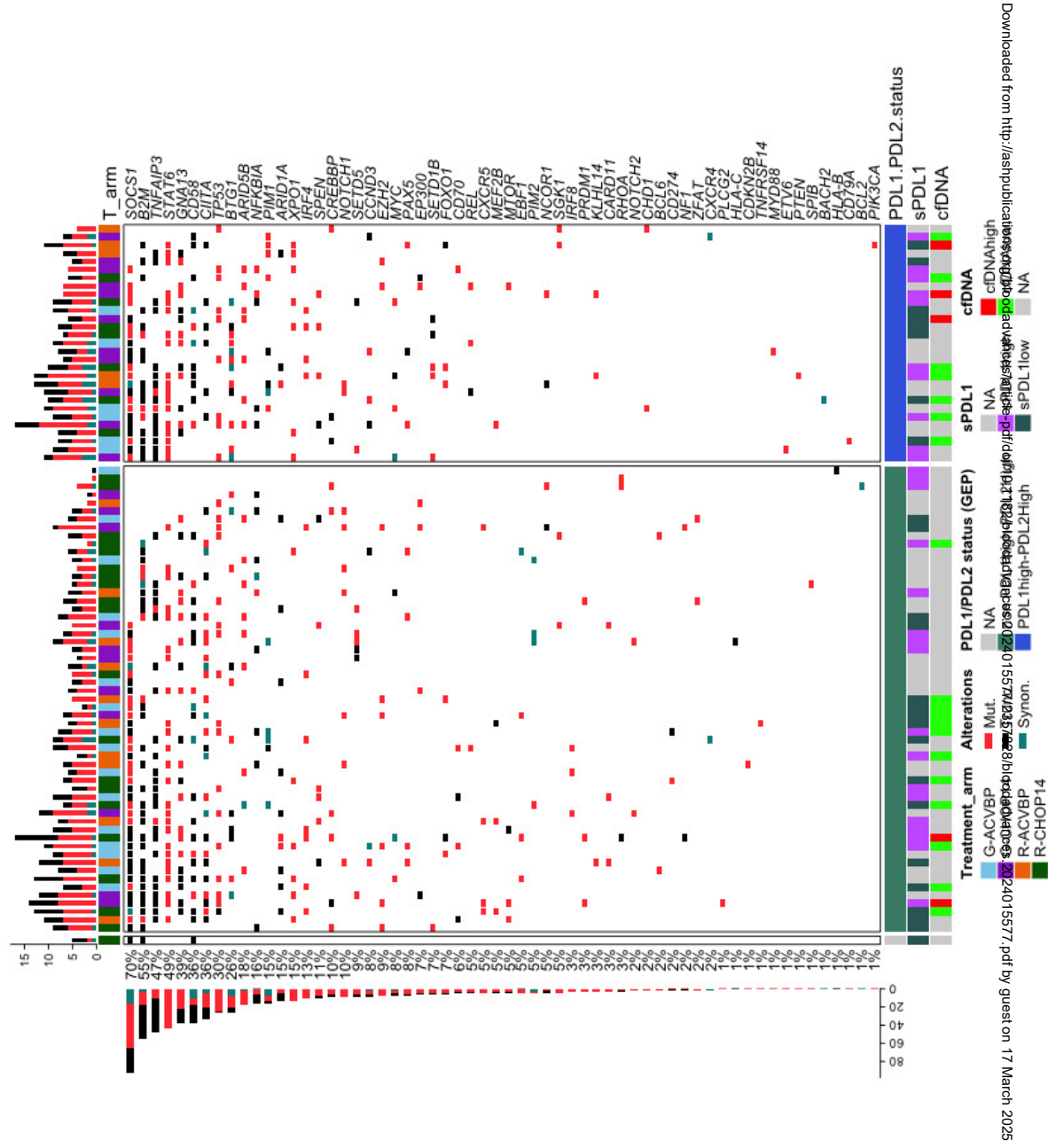
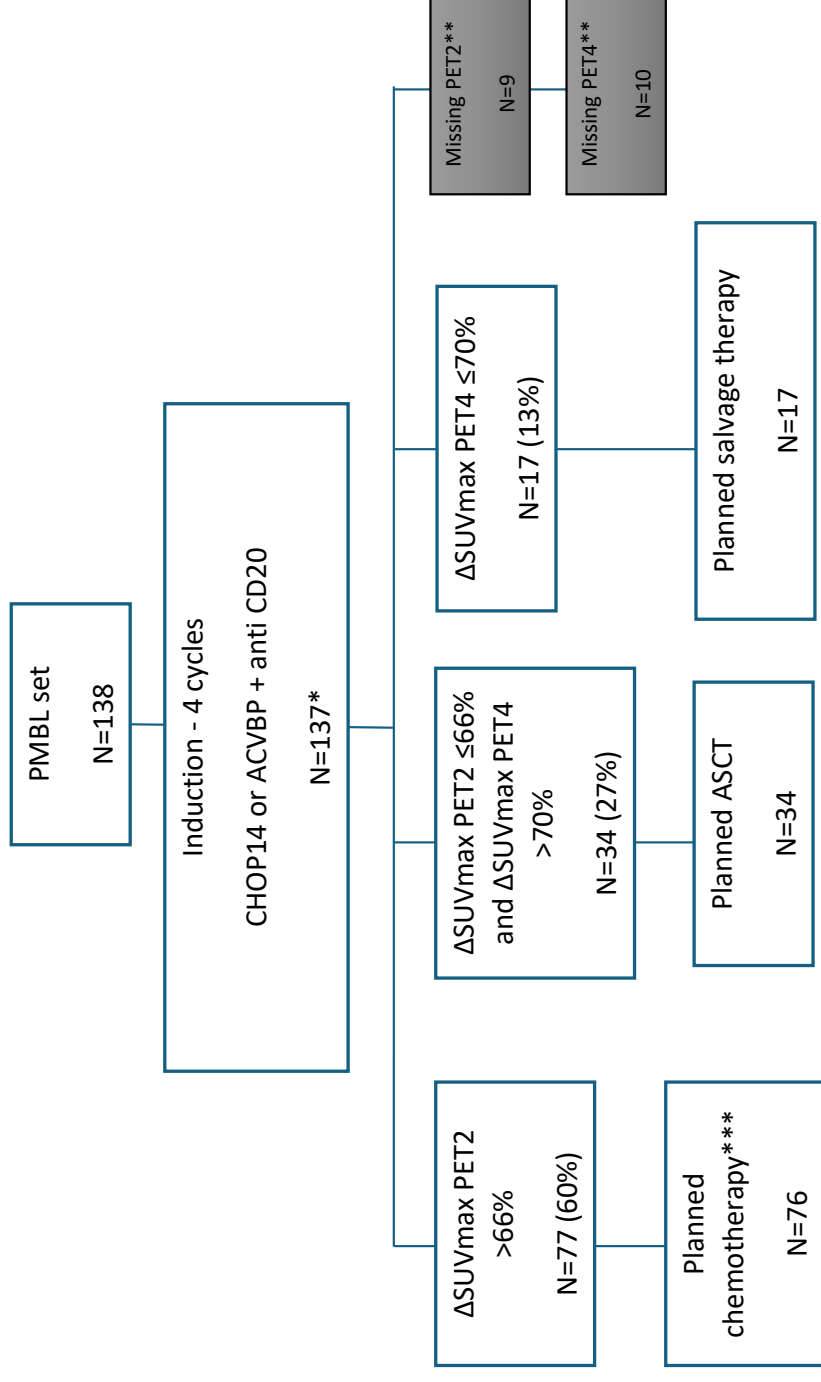


Figure 2

Figure 3



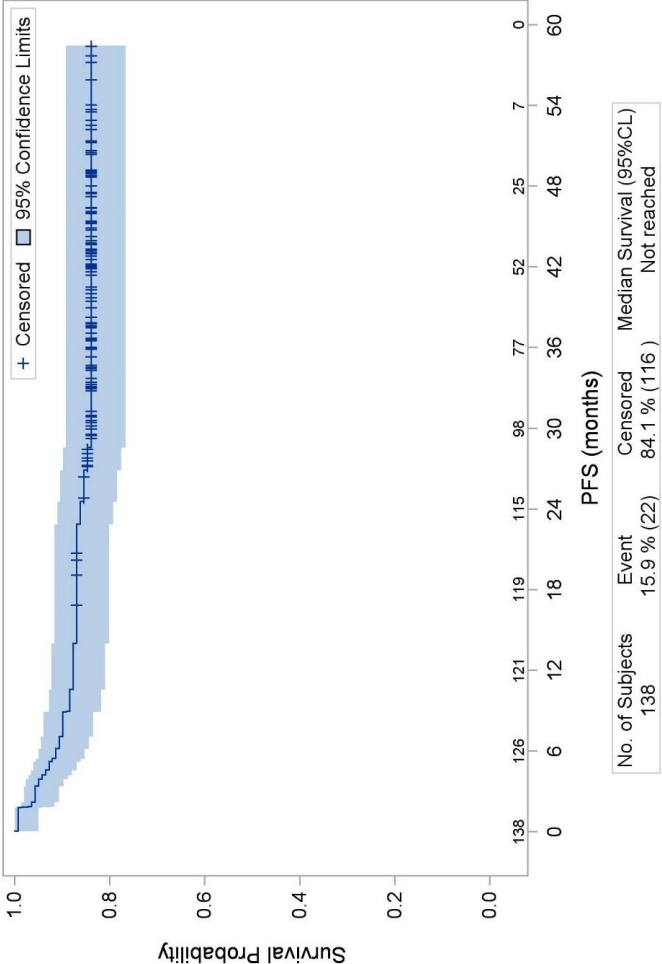
\*One patient died before treatment start; \*\* In patients with missing PET2 and/or PET4 data, consolidation treatments data were also missing; \*\*\*One patient experienced treatment discontinuation after 3 cycles of R-ACVBP (toxicity). Switch for R-CHOP (n=2 cycles) then etoposide-ifosfamide (2 cycles).

ASCT: autologous stem cell transplantation; PET: positron emission tomography

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Figure 4

A



B

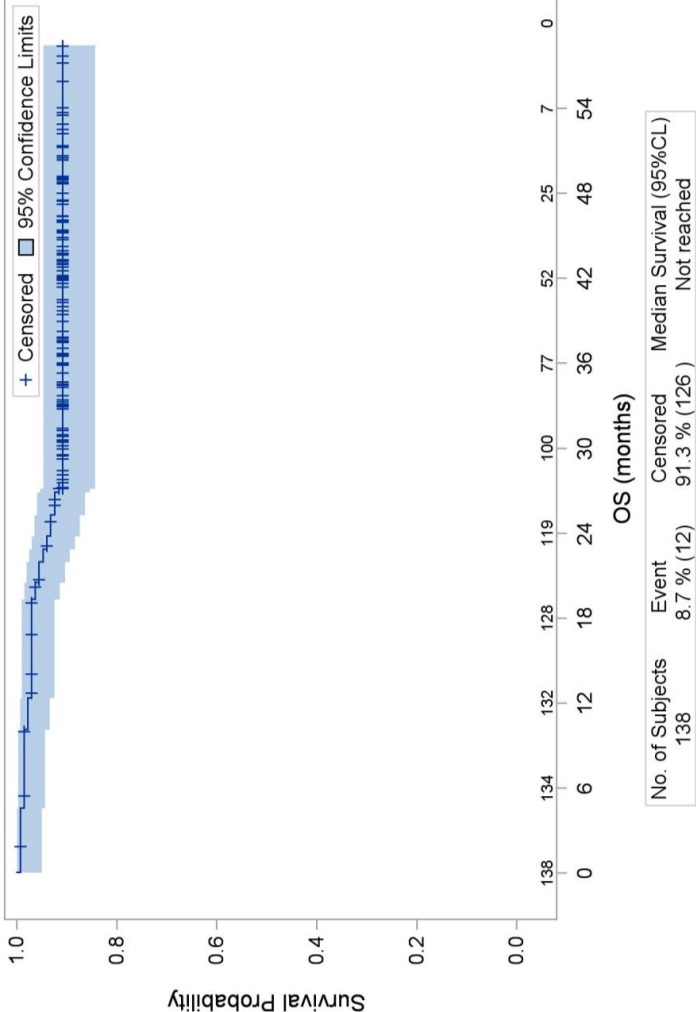
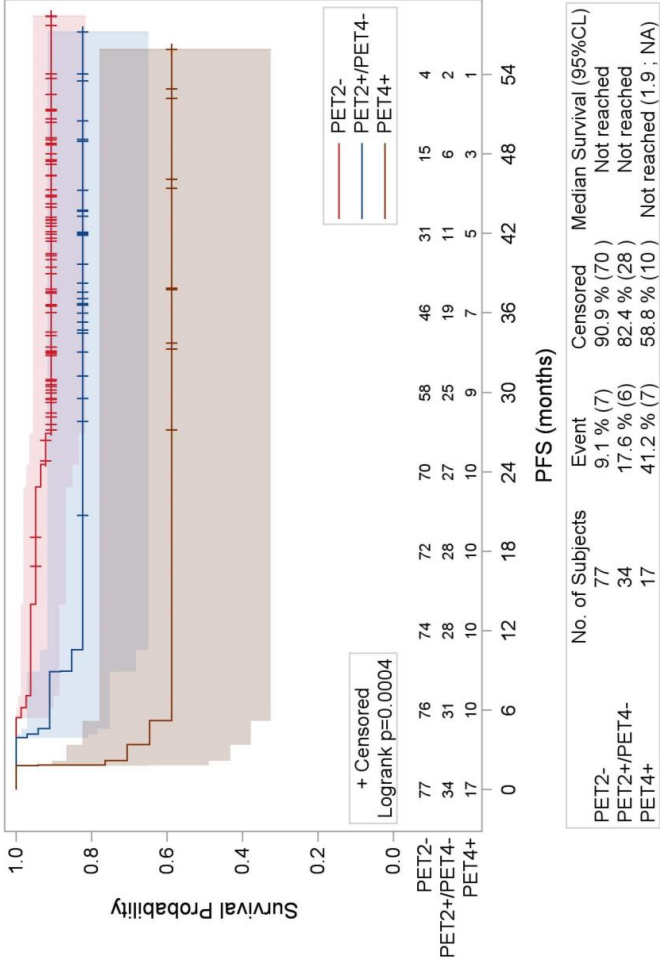


Figure 4

Figure 5

A



B

