A Prospective Study of
Mediastinal Gray Zone Lymphoma

Wyndham H. Wilson¹, M.D., Ph.D., Stefania Pittaluga², M.D., Ph.D.
Alina Nicolae², M.D., Ph.D., Kevin Camphausen³, M.D., Margaret Shovlin¹, R.N.,
Seth M. Steinberg⁴, Ph.D., Mark Roschewski¹, M.D., Louis M. Staudt¹, M.D., Ph.D.,
Elaine S. Jaffe², M.D., and Kieron Dunleavy¹, M.D.,

¹Lymphoid Malignancy Branch, National Cancer Institute, Bethesda, MD
²Laboratory of Pathology, National Cancer Institute, Bethesda, MD
³Radiation Oncology Branch, National Cancer Institute, Bethesda, MD
⁴Center for Cancer Research, National Cancer Institute, Bethesda, MD

Address Reprint Requests to: Wyndham H. Wilson, M.D., Ph.D., Lymphoid Malignancy Branch, Center for Cancer Research, National Cancer Institute, Building 10, Room 4N/115, 9000 Rockville Pike, Bethesda, MD 20892. wilsonw@mail.nih.gov

Running head: Mediastinal Gray Zone Lymphomas
Key Points: Treatment of mediastinal gray zone lymphomas; Pathobiological spectrum of mediastinal aggressive lymphomas

ABSTRACT

Mediastinal B-cell lymphomas present in the mediastinum and are most frequent in young patients. Nodular sclerosis Hodgkin lymphoma (NSHL) and primary mediastinal B-cell lymphoma (PMBL) are the common types, whereas mediastinal gray zone lymphoma (MGZL) is extremely rare and has pathological features intermediate between NSHL and PMBL. The indeterminate pathobiology of MGZL has led to uncertainty regarding therapeutic strategy, and its clinical characteristics and treatment have not been characterized. We conducted a prospective study of infusional dose-adjusted etoposide, doxorubicin and cyclophosphamide with vincristine, prednisone and rituximab (DA-EPOCH-R) and filgrastim in untreated MGZL. We analyzed biomarkers of outcome and compared their clinical and biological characteristics to PMBL. Twenty-four MGZL patients had a median (range) age of 33 (14-59) years and 46% had mediastinal masses ≥ 10 cm. At 59 months median follow-up, the event-free survival and overall survival were 62% and 74%, respectively. The serum absolute lymphocyte count, the presence of tumor infiltrating dendritic cells, CD15 expression on the malignant cells and tumor morphology were biomarkers of outcome in MGZL. Compared with PMBL, MGZL patients were more likely to be male, express CD15, have lower expression of CD20 and to have a worse outcome. DA-EPOCH-R alone is effective in MGZL. The trial was registered at ClinicalTrials.Gov (NCT00001337).
INTRODUCTION

Mediastinal B-cell lymphomas present in the mediastinum of young patients and are mostly represented by nodular sclerosis Hodgkin lymphoma (NSHL) and primary mediastinal B-cell lymphoma (PMBL)\(^1\)-\(^3\). The most recent World Health Organization (WHO) classification of lymphoid tissues recognized mediastinal gray zone lymphoma (MGZL), a rare lymphoma with features intermediate between PMBL and NSHL, as a new pathological entity\(^3\)-\(^5\). Historically, these cases were often included in series of Hodgkin-like anaplastic large cell lymphoma, which was a heterogeneous group\(^6\)-\(^8\).

The clinical characteristics and treatment of MGZL have yet to be defined due to its recent identification and rarity. NSHL and PMBL have both overlapping and distinct clinical features, raising the question of where MGZL lies within the pathological and clinical spectrum of mediastinal B-cell lymphomas. Therapeutically, the Hodgkin-like pathological features of MGZL suggest they should be treated like Hodgkin lymphoma, whereas the strong expression of the CD20 B-cell protein by most MGZL, a feature of PMBL but not NSHL, suggests they should be treated with rituximab-based regimens like PMBL.

As a group, mediastinal B-cell lymphomas are hypothesized to derive from a thymic B-cell\(^2\),\(^4\),\(^7\),\(^9\). A significant proportion of PMBL and NSHL cases have amplification of the JAK2/PDL2 locus, which has also been reported in MGZL\(^9\). PMBL and NSHL also have overlapping gene expression profiles, indicating that they lie along a pathobiological continuum\(^2\). MGZL has been described as the “missing link” between NSHL and PMBL\(^4\). We undertook a study of untreated MGZL to describe its clinical outcome with the infusional dose-adjusted immuno-chemotherapy regimen, DA-EPOCH-R, an effective treatment for PMBL, and to describe its biological characteristics\(^10\).
PATIENTS AND METHODS

Study Design and Treatment

Twenty-four patients with untreated MGZL were prospectively enrolled between November 1999 and February 2013 on a study of DA-EPOCH-R at the National Cancer Institute. Due to the recent recognition of MGZL as a distinct entity by our center in 2004, we amended our prospective study of DA-EPOCH-R in PMBL to add a separate cohort for MGZL. All but three patients were enrolled after this date. Objectives included event-free and overall survival, and immunohistochemical analysis. Pathology was confirmed by SP or ESJ according to WHO criteria. Eligibility included all disease stages and performance status, negative test for Human immunodeficiency virus and pregnancy, and adequate organ function unless due to involvement by lymphoma. Evaluation included standard blood tests, whole body computed tomography (CT) and bone marrow biopsy. DA-EPOCH-R was administered as previously described. Disease sites were evaluated by CT scan after cycle 4 and 6 using standard response criteria. The Institutional Review Board (IRB) approved the study and all patients provided consent in accordance with the Declaration of Helsinki. The trial was registered at ClinicalTrials.Gov (NCT00001337).

Patients began on dose level one of DA-EPOCH-R (rituximab 375 mg/m² day 1; doxorubicin 10 mg/m²/day, etoposide 50 mg/m²/day, and vincristine 0.4 mg/m²/day (no cap) continuous infusion days 1, 2, 3, 4 (96-hour total); cyclophosphamide 750 mg/m² 30 minute infusion day 5; and prednisone 120 mg/m² divided twice daily on days 1, 2, 3, 4, 5 as previously described. Patients received filgrastim 300 µg on day 6 and continued until the ANC ≥ 5000/µl past the nadir. Dose-adjustments were based on the neutrophil nadir, which was monitored with twice weekly complete blood counts, and were made in 20% increments. Dose adjustments above
the starting dose level applied to etoposide, doxorubicin and cyclophosphamide, and adjustments below the starting dose level only applied to cyclophosphamide. Doses were increased above the previous cycle if the nadir ANC $\geq 500/\mu l$, and only reduced below the previous cycle if the ANC $< 500/\mu l$ on $\geq 3$ measurements or the nadir platelet $< 25,000/\mu l$. Patients received 2 cycles beyond CR or stable changes for 6 to 8 cycles. Patients with $> 1$ extranodal site and elevated LDH received intrathecal methotrexate 12 mg on day 1 and 5 of cycle 3 to 6. Bactrim® DS was administered 3 days/week.

**FDG-PET-CT Scan Evaluation**

To assess if there was disease at the end of treatment, patients with a residual mediastinal mass underwent 18-fluorodeoxyglucose positron emission tomography-computerized tomography (FDG-PET) scans. One patient who was treated early in the series received a gallium scan. Patients with standard uptake values (SUV) above the mediastinal blood pool received repeat scans at approximately 6-week intervals until they normalized or stabilized. Patients with significantly worsening FDG-PET abnormalities underwent a biopsy in most cases to assess the presence of disease. FDG-PET scans were not repeated in patients with negative post-treatment scans. The FDG-PET scans were scored according to the 5-point Deauville scoring system with a score of 1-3 and 4-5 considered negative and positive, respectively, for disease$^{15,16}$. Patients diagnosed with disease after DA-EPOCH-R received radiotherapy and were classified as events.

**Immunohistochemical Analysis**

Immunohistochemistry (IHC) was performed on formalin-fixed, paraffin-embedded tissue sections and included CD20, CD3, CD79a, PAX-5, OCT-2, Bob-1, BCL-6, CD68 and Dendritic
Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin (DC-SIGN). For DC-SIGN, tissue sections underwent deparaffinization and rehydration followed by antigen retrieval using low pH Dako Target retrieval solution (Dako, Carpinteria, California) in a microwave. The slides were incubated with primary antibody (1:100 dilution) for 2 hours. As detection system “I-View DAB detection kit” (Ventana Medical Systems, Tucson, Arizona) was used. The other IHC stains were performed as previously described. Positive controls were run with each set of slides and showed appropriate staining patterns.

CD20 and CD15 immuno-reactivity in the malignant cells was recorded as 0 (no positive cells observed), 1+ (1-25%), 2+ (26-50%), 3+ (51-75%) and 4+ (76-100%)(Figure 2A). DC-SIGN and CD68 stains on infiltrating bystander cells were scored as 0 (no positive cells observed), 1+ (1-25%), 2+ (26-50%) and 3+ (>50%)(Figure 2B). We also determined that at least 5% of infiltrating bystander cells expressed CD68 in all MGZL, a cutoff shown to be prognostic in HL. CD30 and BCL-6 immuno-reactivity was not quantitated and scored as positive for CD30 if there was any staining on the malignant cells or scored as positive for BCL-6 if there was staining on > 10% of the malignant cells. All slides were independently reviewed and the scores agreed upon joint re-review (SP and AN).

Comparison to Primary Mediastinal B-cell Lymphoma

We compared the MGZL cases to a cohort of untreated patients with PMBL who were prospectively treated with DA-EPOCH-R. Patient eligibility was identical for both groups except patients with PMBL were required to have a mediastinal mass at least 5 cm. Treatment and follow-up were identical in both groups. The clinical outcome of patients with PMBL was
previously published whereas the immunohistochemical endpoints for PMBL represent new information\textsuperscript{10}.

\textbf{Statistical Analysis}

Overall (OS) survival was calculated from on-study date until death or last follow-up and event-free survival (EFS) was calculated from on-study date until progression, radiotherapy, death or last follow-up. The probabilities of OS and EFS were determined by the Kaplan-Meier method\textsuperscript{18}. The significance of the difference between pairs of Kaplan-Meier curves was determined by an asymptotic or exact log-rank test as appropriate. In general, p-values are unadjusted for multiple comparisons due to being pre-specified or exploratory. However, when patients were analyzed initially by grouped marker values and the results suggested that a preferred, dichotomous division in the groupings would indicate a better prognostic association with the outcome, the resulting p-values were adjusted by multiplying by the implicit number of such comparisons performed to identify the final grouping. The individual prognostic factors and the score of the international prognostic index and international prognostic score were analyzed for prognostic significance. The prognostic significance of maximum mediastinal tumor size was dichotomized into < and $\geq$ 10 cm groups. All p-values are two-tailed. The median potential follow-up was calculated between on-study date and date of analysis.

\textbf{RESULTS}

\textbf{Patient Characteristics and Tumor Pathology}

Twenty-four patients were enrolled (Table 1). The median (range) patient age was 33 years (14-59) and 63\% were male. Forty-six percent of patients had a mediastinal mass over 10
cm and 50% had an elevated lactate dehydrogenase (LDH). A minority of patients had extranodal involvement or pleural or pericardial effusions. Most patients had low peripheral blood absolute lymphocyte counts (ALC) with a median of 0.88 cells/µl (range: 0.3-2.88) (Normal > 1.18 cells/µl).

All patients had histological and/or phenotypic features intermediate between PMBL and NSHL. Usually, the tumors have a predominant morphology, which was either PMBL-like in 33% (8/24), or more frequently Hodgkin-like in 63% (15/24) of cases, and one case was classified as composite NSHL and PMBL (Table 1). While the neoplastic cells from all cases showed some degree of CD20 expression, 71% had strong-diffuse staining on all the malignant cells (Table 1). As shown in Table 1, the neoplastic cells in 86% of tested cases also expressed the germinal center transcription factor, BCL-6. CD30 and CD15, which are typically expressed by the neoplastic cells in NSHL, were expressed by 100% and 54% of the MGZL cases, respectively (Figure 2B). In most cases, the malignant cells showed robust CD30 staining, while CD15 intensity was variable.

**Patient Outcome**

All patients responded with 19 complete and 5 partial remissions. Nine patients were diagnosed with active disease at a median (range) of 3 months (range: 1-6) after completing therapy. Presence of disease was diagnosed by biopsy in seven cases and by progressive disease on scans in two cases. Based on PET-CT evaluation showing disease limited to the mediastinal area, all nine patients received involved field salvage radiotherapy and four are in continuous remission at 3, 73, 91 and 128 months. At the median follow-up of 59 months (range 7-142), the EFS and OS was 62% and 74%, respectively (Figure 1A, 1B).
Twenty-one patients with residual mediastinal masses underwent post-treatment PET-CT scans Table 2). Maximum standard uptake values below or above the mediastinal blood pool were present in the residual mediastinal masses of 11 and 10 patients, corresponding to Deauville scores of 1-2 versus 3-5, respectively. When considering a Deauville score of 1-3 as negative and 4-5 as positive, FDG-PET had a sensitivity and specificity of 63% and 100%, respectively, and a positive and negative predictive value of 100% and 81%.

Toxicity was assessed on all 145 cycles of DA-EPOCH-R. The ANC pharmacodynamic target of < 500 cells/mm$^3$ was achieved on 53% of cycles, with infrequent ANC < 100 cells/mm$^3$ (9%) and thrombocytopenia < 25,000/mm$^3$ (2%) Fever and neutropenia occurred on 12% of cycles. Grade 3 or higher non-hematopoietic toxicities including ileus and neurosensory occurred in < 4% of patients and were similar to prior reports$^{10}$. There were no treatment related deaths.

**Clinical and Molecular Prognostic Markers**

The overlapping features of MGZL with PMBL and NSHL suggest they may share prognostic features. Due to the small sample size and absence of a validation cohort, our analyses are exploratory and hypothesis generating. We first analyzed the clinical International Prognostic Index, developed for DLBCL, and the International Prognostic Score, developed for Hodgkin lymphoma, as well as tumor mass size, and found no associations with EFS or OS in MGZL$^{19,20}$. Based on our finding of reduced peripheral blood lymphocytes in MGZL, which is prognostic in Hodgkin lymphoma, we analyzed the effect on EFS and OS. We assessed the outcome of patients with ALC above and below the median of 880 cells/$\mu$l and observed a
plateau in EFS of 83% and 42% (p=0.038), respectively, and OS of 100% and 52% (p=0.028) (Figure 1C and 1D).

The frequent expression of CD15 and CD30 by MGZL, commonly expressed on Reed-Sternberg cells, raised the hypothesis that immunohistochemical biomarkers for Hodgkin lymphoma may be prognostic in MGZL. Based on recent data that CD68 positive tumor-associated macrophages are biomarkers of poor survival in Hodgkin lymphoma, we analyzed CD68 expression in MGZL. Employing the 5% immunohistochemical expression cut-off that was prognostic in Hodgkin’s lymphoma, all MGZL cases contained CD68 positive tumor-associated macrophages (Table 1). To further assess if CD68 positive tumor-associated macrophages are predictive in MGZL, based upon limited data available, we divided the cases into those with 1+, 2+ and 3+ scores and explored the optimum cut off-point. This analysis showed that with a division of 1-2+ (n=11) versus 3+ (n=5), the EFS was 73% and 20% (p=0.063), respectively, and the OS was 82% and 50% (p=0.29) at 3 years, suggesting a trend that like Hodgkin lymphoma, CD68 positive tumor-associated macrophages are adverse in MGZL. Due to the limited number of cases, these results need to be assessed in a larger series.

Based on a gene expression analysis of MGZL, PMBL and NSHL, we identified a “dendritic cell” gene expression signature that distinguished MGZL and NSHL from PMBL. This signature includes CD209, which encodes the Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin (DC-SIGN), a marker of dendritic cells and activated macrophages. Interestingly, DC-SIGN gene expression was significantly associated with poor survival in NSHL, suggesting it may be a biomarker of macrophage activity in MGZL. To investigate this hypothesis, we performed immunohistochemistry and observed DC-SIGN positive tumor associated-dendritic/activated macrophage cells ≥ 1+ in 53% of our MGZL cases.
(Table 1; Figure 2). Cases with and without DC-SIGN-positive tumor infiltrating dendritic/activated macrophage cells showed an EFS of 67% and 40% (p=0.18), respectively, and an OS of 100% and 52% (p=0.0025) at 3-years, indicating these infiltrating cells are associated with a worse outcome in MGZL (Figure 1E and 1F).

The association of a Hodgkin-like microenvironment and poor outcome in MGZL raised the hypothesis that a predominant HL-like morphology and/or phenotype would also be associated with a worse outcome. To address this question, we first looked at the association between the predominant morphology and EFS and OS. Contrary to our hypothesis, there was a significant trend between the presence of a predominant PMBL-like morphology and lower EFS (p=0.025) but a weaker trend with respect to OS (p=0.21). Additionally, the intensity of CD20 staining on the malignant MGZL cells was not associated with outcome. In contrast, CD15 staining was associated with outcome. To provide a quantitative estimate of CD15 staining, cases were scored in quartiles. We analyzed two cut-off points for CD15 staining and outcome and identified a preferred cutoff between 0-2 versus 3-4, which showed an EFS of 74% and 38% (p=0.078 unadjusted; p=0.16 adjusted), respectively, and an OS of 93% and 38% (p=0.016 unadjusted; p=0.032 adjusted) at 3-years, indicating that robust expression of CD15 identifies a poor prognosis group in MGZL (Figure 1G and 1H). Notably there was no difference in the intensity or frequency of CD15 staining between PMBL-like and Hodgkin-like MGZL, indicating that the prognostic effect of CD15 was unrelated to the predominant morphology.

**Comparison of MGZL and PMBL**

Due to the high malignant cell expression of CD20 MGZL, suggesting it is more similar to an aggressive B-cell lymphoma, we were interested in comparing MGZL to PMBL patients...
enrolled on the same DA-EPOCH-R protocol, albeit in a separate cohort (Table 1)\textsuperscript{10}. Interestingly, the MGZL patients were significantly less likely to have an elevated LDH, extranodal disease or pleural or pericardial effusions and more likely to be male, clinical characteristics more frequent in massive mediastinal NSHL\textsuperscript{23}. Additionally, MGZL cases were significantly more likely to express CD30 and CD15, phenotypic hallmarks of NSHL, but commonly had strong-diffuse CD20 expression, which is not found in NSHL. Clinically, patients with MGZL had a significantly worse EFS and OS compared to our recently published series of 51 patients with PMBL who received identical treatment\textsuperscript{10}. With a median follow-up of 5-years, patients with MGZL compared to PMBL achieved a significantly lower EFS (62\% versus 93\%; p=0.0005) and OS (74\% versus 97\%; p=0.0012), respectively (Figure 3A and 3B). Expectedly, due to the low rate of treatment failure in PMBL, none of the biomarkers of outcome in MGZL including ALC were prognostic in PMBL.

**DISCUSSION**

We present the first prospective study of MGZL and describe its clinical and immunophenotypic characteristics and treatment outcome. MGZL shares clinical characteristics with PMBL and NSHL including young age and bulky mediastinal masses, but more like mediastinal NSHL, it has a male predominance, and a lower frequency of elevated LDH, extranodal disease and effusions\textsuperscript{10,23}. Clinically, 62\% of patients achieved continuous complete remissions with DA-EPOCH-R, indicating it is an effective treatment. Furthermore, among the 9 patients who failed to achieve a durable remission, 4 (44\%) were salvaged with involved field radiation alone and are in continuous remission. The remaining 5 patients were aggressively treated with salvage chemotherapy and/or allogeneic transplant and all died of disease,
highlighting the chemo-resistance of this subgroup. This contrasts with our findings in PMBL where the EFS and OS were 93% and 97%, respectively, at 60 months with DA-EPOCH-R$^{10}$.

The occurrence of treatment failure in one third of MGZL patients and the curative potential of salvage radiotherapy makes early identification of treatment failure important. FDG-PET provided excellent specificity but only a sensitivity of 63%. Clinical prognostic factors such as IPI and IPS and mass size were not predictive. However, the ALC and the presence of DC-SIGN positive dendritic/activated macrophage cells were relatively robust biomarkers of clinical outcome where pretreatment ALC above the median and the absence of DC-SIGN positive cells in the tumor biopsies were associated with a 100% survival. Notably, pretreatment ALC and tumor infiltrating macrophages are prognostic biomarkers in classical Hodgkin lymphoma, consistent with a biological connection between MGZL and NSHL and a shared pathobiology$^{17,19}$.

The MGZL malignant cells expressed CD15 in over half of cases and all expressed CD30, which are characteristically expressed on Reed-Sternberg cells$^3$. Interestingly, the robust expression of CD15 (scores 3-4) on the malignant cells was associated with a worse outcome, suggesting the resistant tumors may be more Hodgkin-like. This, however, was not the case. Tumors with a predominant PMBL-like morphology had a worse outcome but a similar level of CD15 expression as tumors with a predominant HL-like morphology, consistent with the intermediate histopathology of MGZL. Most MGZL cases also expressed the B-lineage-specific proteins, CD20 and BCL6, which is unlike HL, where loss of the B-cell program is a fundamental biological feature$^{24}$. While our results show that MGZL lies along a morphologic and immunophenotypic continuum between NSHL and PMBL, the clinical outcomes indicate that biological characteristics associated with Hodgkin lymphoma, including low ALC, tumor-
associated dendritic/macrophage cells and CD15 expression were associated with a worse outcome.

While our results are promising compared to standard HL-based chemotherapy plus radiation in bulky NSHL, in which over 30% of patients progress, we cannot rigorously determine if DA-EPOCH-R is optimal treatment for MGZL given the limited number of cases and absent a randomized study design. To help address the question of therapeutic strategy, it is useful to review the treatment outcome of Hodgkin-like anaplastic large cell lymphoma (HL-ALCL), which historically included some cases that might be considered MGZL today. Zinzani et al reported the largest prospective series of HL-ALCL, but that series specifically excluded tumors with B-cell markers. In that study, patients were randomized to receive either MACOP-B or ABVD to assess if these “borderline” tumors should be treated as aggressive high-grade non-Hodgkin lymphomas or Hodgkin lymphomas; 85% of patients had a mediastinal mass and patients with bulky tumors received consolidation radiation therapy. The relapse-free survival was 94% and 92% at 32 months for the two arms, respectively, indicating both treatment approaches were equally effective when combined with radiation therapy in most cases. While this series likely did not include cases we would classify as MGZL, as CD20 was an exclusion criterion, this study provides cogent evidence that histologically “borderline” tumors are not advantaged by the use of HL-based treatments.

A recent abstract from Evens et al suggests that gray zone lymphomas (GZL) have a relatively poor outcome compared to other aggressive B-cell lymphomas. In this study of 96 patients, 44% had mediastinal masses, suggesting a diagnosis of MGZL, while 56% had non-MGZL, which is not the same disease. At a median follow-up of approximately 2-years, the progression-free (PFS) and survival were 41% and 84%, respectively for all patients, and the
outcome was similar in patients with MGZL and non-MGZL, as well as in patients who received non-HL-based or HL-based regimens. These studies are consistent with our results that show MGZL has a poorer outcome, which is likely due to higher drug resistance and not to the use of a non-HL-based treatment. Nonetheless, DA-EPOCH-R alone produced durable remissions in most patients indicating it is an effective treatment for these relatively resistant lymphomas.

AUTHORSHIP

Wyndham H. Wilson conceived, conducted, analyzed and wrote study and manuscript. Stefania Pittaluga conceived, analyzed and helped write the manuscript. Alina Nicolae conceived, analyzed and helped write the manuscript. Kevin Camphausen treated, analyzed and helped write the manuscript. Margaret Shovlin analyzed and helped write the manuscript. Seth M. Steinberg analyzed and helped write the manuscript. Mark Roschewski analyzed and helped write the manuscript. Louis M. Staudt conceived, analyzed and helped write the manuscript. Elaine S. Jaffe conceived, analyzed and helped write the manuscript. Kieron Dunleavy treated, analyzed and helped write the manuscript. No authors have any conflict of interests to disclose.
REFERENCES

Table 1. Clinical and Pathological Characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>MGZL</th>
<th>PMBL</th>
<th>p2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Patients</td>
<td>24</td>
<td>51</td>
<td></td>
</tr>
<tr>
<td>Male Gender</td>
<td>15 (63%)</td>
<td>21 (41%)</td>
<td>0.14</td>
</tr>
<tr>
<td>Age years median (range)</td>
<td>33 (14-59)</td>
<td>30 (19-52)</td>
<td>0.61</td>
</tr>
<tr>
<td>Bulky Tumor ≥ 10 cm [range]</td>
<td>11 (46%) [1.3-20]</td>
<td>33 (65%) [5-18]</td>
<td>0.14</td>
</tr>
<tr>
<td>Stage IV disease</td>
<td>3 (13%)</td>
<td>15 (29%)</td>
<td>0.15</td>
</tr>
<tr>
<td>LDH &gt; Normal</td>
<td>12 (50%)</td>
<td>40 (78%)</td>
<td>0.017</td>
</tr>
<tr>
<td>Extranodal site</td>
<td>6 (25%)</td>
<td>27 (53%)</td>
<td>0.027</td>
</tr>
<tr>
<td>Pleural or pericardial effusion</td>
<td>5 (21%)</td>
<td>28 (55%)</td>
<td>0.006</td>
</tr>
<tr>
<td>ALC cells/µl median (range)</td>
<td>0.88 (0.3-2.88)</td>
<td>1.01 (0.24-2.15)</td>
<td>0.78</td>
</tr>
<tr>
<td>Predominant Tumor Morphology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PMBL-Like</td>
<td>8 (33%)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>NSHL-Like</td>
<td>15 (63%)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Composite (PMBL and NSHL)</td>
<td>1 (4%)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Immunohistochemistry*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD20+ (malignant cells)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st – 4th quartiles</td>
<td>24/24 (100%)</td>
<td>51/51 (100%)</td>
<td>1.0</td>
</tr>
<tr>
<td>≥ 4th quartile</td>
<td>17/24 (71%)</td>
<td>51/51 (100%)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>CD30+ (malignant cells)</td>
<td>24/24(100%)</td>
<td>30/44 (68%)</td>
<td>0.001</td>
</tr>
<tr>
<td>CD15+ (malignant cells)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 1st quartile</td>
<td>13/24 (54%)</td>
<td>2/34 (6%)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>BCL-6+ (malignant cells)</td>
<td>13/15 (86%)</td>
<td>33/37 (89%)</td>
<td>1.0</td>
</tr>
<tr>
<td>DC-SIGN+ (bystander cells)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 1+ staining</td>
<td>10/19 (53%)</td>
<td>12/35 (34%)</td>
<td>0.25</td>
</tr>
<tr>
<td>CD68+ (bystander cells)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 5% staining</td>
<td>16/16 (100%)</td>
<td>26/28 (93%)</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Abbreviations: LDH-Lactate dehydrogenase; ALC-Absolute lymphocyte count. DC-SIGN-Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin.
Table 2. End of Treatment FDG-PET-CT

<table>
<thead>
<tr>
<th>Variables</th>
<th>SUV&lt;sub&gt;max&lt;/sub&gt; ≤ Mediastinal Blood Pool (N=11)</th>
<th>SUV&lt;sub&gt;max&lt;/sub&gt; &gt; Mediastinal Blood Pool (N=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Uptake</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deauville Score</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Disease absent</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>Disease documented</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

**Outcome Measures**

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive Predictive Value</th>
<th>Negative Predictive Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>63%</td>
<td>100%</td>
<td>100%</td>
<td>81%</td>
</tr>
</tbody>
</table>

Abbreviations: SUV<sub>max</sub>-maximal standard uptake value. Deauville score 1-3 and 4-5 interpreted as negative and positive, respectively.
FIGURE LEGENDS

Figure 1. Kaplan-Meier Plots of Event-Free and Overall Survival of MGZL. Twenty-four patients were prospectively treated with DA-EPOCH-R. Patients were followed for a median of 59 (range 7-142) months and results are presented at 3-years. A. Overall event-free survival is 62% (95% Confidence Intervals (CI): 42% to 79%). B. Overall survival is 74% (95% CI: 51% to 89%). C. Event-free survival of patients with absolute lymphocyte counts (ALC) > and < the median (880 cells/μl) are 83% (95% CI: 55% to 95%) and 42% (95% CI: 19% to 68%), respectively (p=0.038). D. Overall survival of patients with ALC ≥ and < the median (880 cells/μl) is 100% (95% CI: 74% to 100%) and 52% (95% CI: 24% to 78%), respectively (p=0.028). E. Event-free survival of patients without or with tumor infiltrating DC-SIGN+ cells is 67% (95% CI: 35% to 88%) and 40% (95% CI: 17% to 69%), respectively (p=0.18). F. Overall survival of patients without or with tumor infiltrating DC-SIGN+ cells is 100% (95% CI: 66% to 100%) and 52% (95% CI: 5% to 68%), respectively. (p=0.0025). G. Event-free survival of patients with CD15 scores of 0-2 versus 3-4 is 74% (95% CI: 50% to 90%) and 38% (95% CI: 14% to 69%), respectively (p=0.16). H. Overall survival of patients with CD15 scores of 0-2 versus 3-4 is 93% (95% CI: 70% to 99%) and 38% (95% CI: 12% to 74%), respectively. (p=0.032).

Figure 2. MGZL immunohistochemistry photomicrographs. A. CD15 positive biopsies showing low (1+) and high (4+) staining of malignant cells. B. DC-SIGN positive biopsies showing low (1+) and high (3+) staining of infiltrating dendritic/macrophage cells.
Figure 3. Kaplan-Meier Plots of Event-Free and Overall Survival of MGZL and PMBL. A. Event-free survival is 62% (95% Confidence Intervals (CI): 42% to 79%) for MGZL (blue curve) compared to 93% (95% Confidence Interval (CI): 81% to 98%) for PMBL (red curve) at 5-years (p=0.0005). B. Overall survival is 74% (95% CI: 51% to 89%) for MGZL (blue curve) compared to 97% (95% CI: 83% to 99%) for PMBL (red curve) at 5-years (p=0.0012).
Figure 1

A. Event-Free Survival (All patients)

B. Overall Survival (All patients)

C. Event-Free Survival (ALC)

D. Overall Survival (ALC)

E. Event-Free Survival (DC-SIGN)

F. Overall Survival (DC-SIGN)

G. Event-Free Survival (CD15)

H. Overall Survival (CD15)
Figure 2

A. CD15 Malignant Cell Staining

B. DC-SIGN Dendritic Cell Staining
Figure 3

A. Event-Free Survival (MGZL and PMBL)

B. Overall Survival (MGZL and PMBL)

p=0.0005

p=0.0012
A prospective study of mediastinal gray zone lymphoma

Wyndham H. Wilson, Stefania Pittaluga, Alina Nicolae, Kevin Camphausen, Margaret Shovlin, Seth M. Steinberg, Mark Roschewski, Louis M. Staudt, Elaine S. Jaffe and Kieron Dunleavy

Advance online articles have been peer reviewed and accepted for publication but have not yet appeared in the paper journal (edited, typeset versions may be posted when available prior to final publication). Advance online articles are citable and establish publication priority; they are indexed by PubMed from initial publication. Citations to Advance online articles must include digital object identifier (DOIs) and date of initial publication.