Improving the outcomes of patients with lymphoproliferative disorders

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Abstract

Lymphoproliferative disorders (LPD) collectively form the commonest category of haematologic malignancy in Australia. Most patients are not cured and improvements in treatments are urgently needed. Within this thesis I have taken two broad approaches to this problem.

Optimising standard therapies

1. Using PET-CT in identify patients at risk of early disease relapse

The identification of patients at highest risk of failing existing therapies is a rational starting point to improve outcomes. Using clinical datasets from Peter MacCallum Cancer Centre, I studied the ability of PET-CT to identify patients at risk of disease relapse. Theoretically, early detection of relapse when tumour burden may increase the probability of successful salvage therapy and ultimate cure. Therefore I reviewed the outcomes and method of detecting relapse patterns in patients with de novo DLBCL and transformed indolent lymphoma. No clear benefit from a surveillance strategy was demonstrated, meaning that patients can be spared the anxiety and radiation of scanning. I also explored the role of PET-CT in primary mediastinal B-cell lymphoma and found negative end of treatment PET-CT was predictive of excellent progression-free survival, but positive scans require histologic confirmation prior to escalation of therapy.

2. Central nervous system (CNS) relapse in aggressive NHL

This complication is typically rapidly fatal and identifying patients at increased risk In the first study I analysed a group of high-risk patients with DLBCL and found that the addition of high dose systemic methotrexate and/or cytarabine was associated with lower rates of CNS relapse compared with intrathecal prophylaxis alone. This finding highlights the benefits obtaining when a customised therapeutic approach is used. In a second study, by collaborating through a large, international multicentre network I collated a large series of patients with mantle cell lymphoma who developed CNS...
Abstract

relapse. Within this, I identified blastoid histology, high mantle cell lymphoma international prognostic index, raised serum lactate dehydrogenase and poor performance status as risk factors for CNS involvement.

Developing new therapies

The second half of this thesis focuses on the development of novel therapeutic strategies.

3. NMP and anti-CD20 monoclonal antibodies in lymphoma

N-methyl-2-pyrrolidone (NMP) shares biologic properties with the established anti-cancer immune modulating drug lenalidomide, which is active with rituximab in lymphoma. I have shown that NMP has in vitro enhancement of rituximab-mediated induction of antibody dependent cellular cytotoxicity on lymphoma cell lines. The promising pre-clinical activity of this combination will be assessed in future clinical trials.

4. Rational clinical trial design using immunotherapies

Finally, I designed three early phase clinical trial protocols using immunotherapies: 1) oral NMP in myeloma, 2) intra-tumoral α-galactosceramide and CpG and 3) ISCOMAB and rituximab, in indolent B-cell lymphoma. These clinical trial protocols combine correlative scientific with clinical endpoints and are now ready for activation.
Declaration

This is to certify that:

1. This thesis constitutes my original work towards to DMedSc except where indicated in the Preface;
2. Due acknowledgment has been made in the text to all other material used
3. The thesis is less than 100,000 words in length exclusive of tables, figures and bibliography.

Signature..........................................................................

Chan Yoon Cheah
Preface

The work presented in this thesis is my own, with the following exceptions.

Dr Paul Neeson and Prof David Ritchie (co-heads, Haematology Immunology Translational Research Laboratory, Peter MacCallum Cancer Centre) assisted with experimental design in results chapter III: Investigation of NMP in combination with anti-CD20 monoclonal antibodies on NHL cell lines.

Prof John Seymour assisted in the design of the clinical studies reported in PET-CT in DLBCL and CNS relapse in aggressive NHL.

This work in thesis is substantially drawn from published works (listed below) but sections have been modified for continuity and flow. These are appended in their complete published form in the Appendix (page 216).

Publications from work presented in this thesis

The majority of work presented in my thesis has undergone peer review, and to date has resulted in the five original, first author publications. I acknowledge the contributions of co-authors in detail below.


Co-author contributions
Drs Herbert, O’Rourke, Kennedy, George, Gilbertson, Tan, Fedele, Opat, Prince, Ritchie, Dickinson, Burbury, Carney, Harrison, Tam, Wolf, Januszewicz and Seymour contributed patients and data, comprising in totality 30% of the work.


**Co-author contributions**
Dr George designed the case report form and assisted with data collection. Drs Gine, Chiapella, Kluin-Nelemans, Jurczak, Mosurska, Mocikova, Klener, Salek, Walewski, Symczyk, Smolej, Auer, Ritchie, Arcaini, Williams, Dreyling and Seymour contributed patients and data, comprising in totality 30% of the work.

3. Limited role for surveillance PET-CT scanning in patients with diffuse large B-cell lymphoma in complete metabolic remission following primary therapy.  
*Br J Cancer* 2013 Jul 23;109(2):312-7

**Co-author contributions**
A/Prof Hofman and Prof Seymour performed blinded PET reviews. Drs Herbert, Prince, Ritchie, Dickinson, Burbury, Harrison, Tam, Wolf, Hicks, Januszewicz and Seymour contributed patients and data, comprising in totality 20% of the work.

4. Surveillance PET-CT scanning in patients with transformed indolent lymphoma in complete metabolic remission is of limited benefit.  
*Ann Hematol* Epub March 5, 2014

**Co-author contributions**
A/Prof Hofman and Prof Seymour performed blinded PET reviews. Drs Herbert, Prince, Ritchie, Dickinson, Burbury, Harrison, Tam, Wolf, Hicks, Januszewicz and Seymour contributed patients and data, comprising in totality 20% of the work.

5. The utility and limitations of PET-CT in patients with primary mediastinal B-cell lymphoma: a single centre experience and literature review.  
*Leuk Lymphoma* epub 14 April 2014
Co-author contributions

A/Prof Hofman performed blinded PET reviews. Drs Herbert, Prince, Ritchie, Dickinson, Burbury, Harrison, Tam, Wolf, Hicks, Januszewicz and Seymour contributed patients and data, comprising in totality 20% of the work.
Other publications related to thesis content

These papers comprise review articles, commentaries or non-first author original research related to the central thesis topic.

   Cheah CY, Seymour JF, Dickinson M.
   *Int J Hematol Oncol*, in press (accepted 6 March 2014)

2. Adding weight to a sinking ship: more reasons not to perform routine surveillance imaging in patients with diffuse large B-cell lymphoma in remission.
   Cheah CY, Seymour JF.
   *Leuk Lymph* 2014 Epub Jan 10.

3. Primary testicular lymphoma.
   Cheah CY, With A, Seymour JF.
   *Blood* 2014 Jan 23;123:486-493

4. Breast Implant-associated Anaplastic Large-cell Lymphoma: Long Term Follow-up Analysis of 60 Cases Suggests that Disease Cure can be Achieved in Most Patients.
   *J Clin Oncol* 2014 Jan 10;32(2):114-20

5. Is there still a need for specific central nervous system directed prophylaxis for diffuse large B-cell lymphoma in the rituximab era?
   Cheah CY, Seymour JF

6. Rituximab for the treatment of follicular lymphoma [review].
   Cheah CY, Lingaratnam S, Seymour JF.
   *Future Oncol* 2013 Sep;9(9):1283-98

Cheah CY, Seymour JF.

Leuk Lymphoma 2013 September; 54(9): 1859-1861
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Professor John Seymour, for providing me with a backstage, all-access pass into the world of academic haematology, patient refinement of dozens of my manuscripts and his (ongoing) masterclass “How to Get Your Work Published.”

To my son James, for teaching me how to be a child again and reminding me of what is most important in life.

To Kylie, light of my life, for making it all worthwhile.
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Chapter 1. Literature Review

Introduction to lymphoproliferative disorders

Lymphoproliferative disorders (LPD) are collectively amongst the most common blood cancers, with approximately 5,300 new diagnoses in Australia in 2010.\textsuperscript{1} Most common amongst these are non-Hodgkin lymphomas (NHL) of which there were 4,462 new cases in 2010. For reasons that are unclear, the age-standardised incidence rates are increasing (Figure 1).

\textit{Figure 1 Incidence of NHL in Australia from 1982 – 2009. Abbreviations: ASI: age-standardised incidence.}\textsuperscript{1}

Classification of lymphoproliferative disorders

The classification of the lymphoid neoplasms has evolved over time from a morphologic classification system (Rappaport, Kiel) into the current World Health Organisation classification, which incorporates molecular, immunophenotypic, cytogenetic, morphologic and clinical features.\textsuperscript{2} Lymphoproliferative disorders can broadly be classified according to their normal haematopoietic counterpart (B-cell
malignancies, T and NK-cell malignancies or Hodgkin lymphoma) and the point of maturation of the cells (precursor or mature). The majority of my thesis focuses on the group of mature B-cell malignancies, with an emphasis on the two most common histologic subtypes of NHL, diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma (FL). It is important to note that even within histologic subtypes, there exists considerable heterogeneity in disease biology, clinical behaviour and outcome. To illustrate this, the group of entities considered within the umbrella term DLBCL includes subtypes with disparate clinical presentations and outcomes (Table 1).
Literature Review

Table 1 Subtypes of diffuse large B-cell lymphoma. Abbreviations: DLBCL = diffuse large B-cell lymphoma, NOS = not otherwise specified, EBV = Epstein-Barr virus; PMBL = primary mediastinal B-cell lymphoma; CNS = central nervous system.

<table>
<thead>
<tr>
<th>Subtypes of Diffuse Large B-Cell Lymphoma</th>
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<tr>
<td>Diffuse large B-cell lymphoma (DLBCL), NOS</td>
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<tr>
<td>T-cell/histiocyte rich large B-cell lymphoma</td>
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<td>EBV+ DLBCL of the elderly</td>
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<td>DLBCL with a predominant extranodal location</td>
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<td>Primary mediastinal (thymic) large B cell lymphoma (PMBL)</td>
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<tr>
<td>Intravascular large B-cell lymphoma</td>
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<tr>
<td>Primary cutaneous DLBCL, leg type</td>
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<tr>
<td>Primary DLBCL of CNS</td>
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<tr>
<td>Lymphomatoid granulomatosis</td>
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<td>Large-cell lymphomas of terminally differentiated B-cells</td>
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<td>ALK positive large B-cell lymphoma Plasmablastic lymphoma</td>
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<tr>
<td>Primary effusion lymphoma</td>
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<tr>
<td>DLBCL associated with chronic inflammation</td>
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<tr>
<td>B-cell neoplasms with features intermediated between DLBCL and other lymphoid</td>
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<tr>
<td>tumours</td>
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<tr>
<td>B-cell lymphoma, unclassifiable, with features intermediate between diffuse</td>
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<tr>
<td>and Large B-cell lymphoma and Burkitt lymphoma</td>
</tr>
<tr>
<td>B-cell lymphoma, unclassifiable, with features intermediate between diffuse</td>
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<tr>
<td>and Large B-cell lymphoma and classical Hodgkin lymphoma</td>
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Furthermore, within DLBCL, not otherwise specified (NOS) distinctions can be made on the basis of gene expression profiling with two distinct signatures, the activated B-cell (ABC) and germinal centre B-cell (GCB) phenotypes. One of the challenges for clinicians treated patients with lymphoma is incorporating this wealth of information into a tailored management strategy to suit the risk profile of each individual patient, as opposed to adopting a simplistic “one-size-fits-all” approach. In my thesis I have addressed this by analysing trying to identify patients at highest risk of disease failure either through systemic or CNS relapse.
Epidemiology and risk factors

DLBCL is mostly a disease of the elderly, with median age of diagnosis in the seventh decade. It comprises 25-30% of adult NHL and the causes of are largely unknown. It should be noted that DLBCL may occur de novo, i.e. without antecedent history of indolent NHL or may be a manifestation of histologic transformation. Histologic transformation may occur from a variety of low grade histologies including FL, marginal zone lymphoma (MZL), nodular lymphocyte predominant Hodgkin lymphoma or small lymphocytic lymphoma (SLL)/chronic lymphocytic leukaemia (CLL). Such patients were traditionally thought to have an aggressive disease course with inferior outcomes compared with de novo DLBCL, however the natural history of this condition may be changing in the era of chemo-immunotherapy. Various chemical agents including pesticides and fertilizers have been suggested as potential aetiologic agents. Acquired and congenital immunodeficiency states have both been implicated. Viruses have been implicated in the pathogenesis of NHL, with a pathogenic link suggested for patients with hepatitis C virus. Patients with the human immunodeficiency virus (HIV) are at substantially increased risk of developing a variety of lymphoproliferative disorders, with lymphomas in HIV-infected patients more commonly presenting with extranodal primary sites, including the testis. HIV-associated lymphomas include Burkitt lymphoma, primary CNS lymphoma, plasmablastic lymphoma, primary effusion lymphoma and a polymorphic “post-transplant” lymphoproliferative disorder (PTLD). Iatrogenic immunodeficiency (classically related to either immunosuppression following solid organ transplantation or chronic high dose steroid exposure is a particularly strong risk factor for PTLD. The risk of lymphoma is increased by 50-120% in solid organ transplant recipients compared with the general population. Large epidemiologic studies suggest increased risk of NHL amongst patients with autoimmune disease. A meta-analysis of 20 studies found that patients with Sjogren syndrome, systemic lupus erythematosus and rheumatoid arthritis were associated with increased risk of NHL; the standardised incidence ratios were 18.8, 7.4 and 3.9 respectively. Finally, germline variation in
DNA repair genes (particularly the non-homologous end joining pathway) been implicated in lymphomagenesis.  

**Diagnosis and staging of lymphoma**

Because of the array of histologic subtypes possible, patients suspected to have lymphoma should undergo excisional lymph node biopsy whenever safe and feasible to do so. Fine needle aspiration, although often sufficient to distinguish non-lymphoid tumours from lymphoma is inadequate for confidently identifying lymphoma subtype or excluding lymphoma (particular Hodgkin lymphoma) if inflammatory cells are seen. If excisional biopsy is not possible, CT guided core biopsy (with fresh samples for immunophenotyping as well as formalin fixed tissue) is an acceptable alternative.

In addition to physical examination, routine staging procedures for patients with NHL are $^{18}$F-FDG-PET-CT (PET-CT) in histologic subtypes which are FDG avid (discussed on page 10). In indolent forms of NHL, which are only variably FDG avid, computed tomography (CT) with contrast of the neck to pelvis is sufficient. The need for bone marrow biopsy in patients with Hodgkin lymphoma in particular has been challenged in a number of recent studies with undergoing staging with PET-CT. In DLBCL, although some studies have also suggested that marrow biopsy adds little additional benefit to PET-CT staged patients discordant marrow histology cannot be detected reliably by PET, and the presence of this finding has been associated with greater risk of subsequent relapse with low grade lymphoma. An adequate sample (>20mm) increases yield and obviates the need to perform bilateral biopsies. Thus apart from Hodgkin lymphoma, bone marrow biopsy as part of staging remains an essential component of staging. Patients at high risk of CNS involvement (discussed in detail on page 14) or those with symptoms or signs suggestive of CNS lymphoma should undergo careful staging.

Lymphomas are staged according the Ann Arbor system with Cotswold modification (Table 2). This system was originally designed for Hodgkin lymphoma, and has been adapted for NHL. Compared with Hodgkin lymphoma, NHL has a greater propensity to involve extranodal sites which raises some difficulties. For example, primary
extranodal lymphomas often have their own specific staging systems (e.g. Lugano staging system for gastrointestinal lymphoma\textsuperscript{19} or the modified ISCL/EORTC Revisions to the TNMB Classification for cutaneous T-cell lymphoma\textsuperscript{20}). In addition to the number and location of involved sites, the presence or absence of extranodal sites (designated “E”), constitutional symptoms (designated “B”) and bulk (designated “X”) are all incorporated.

*Table 2 Modified Ann Arbor criteria for staging of lymphoma*

<table>
<thead>
<tr>
<th>stage</th>
<th>Ann Arbor criteria</th>
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<tbody>
<tr>
<td>I</td>
<td>Lymphoma involving a single lymph node region or single extranodal organ or site (IE)</td>
</tr>
<tr>
<td>II</td>
<td>Two or more involved lymph node regions on the same side of the diaphragm or with localized involvement of an extralymphatic organ or site (IIE)</td>
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<tr>
<td>III</td>
<td>Lymph node involvement on both sides of the diaphragm, or with localized involvement of an extralymphatic organ or site (IIIE) or spleen (IIIS) or both (IIIES)</td>
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<tr>
<td>IV</td>
<td>Diffuse or disseminated involvement of one or more extralymphatic organs (e.g. liver, bone marrow, lung) with or without associated lymph node involvement</td>
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It can be argued that in contrast to Hodgkin lymphoma, for various reasons, stage in isolation is a poor prognostic tool in the majority of patients with NHL. For instance in FL only 20-30% of patients are stage I/II, whilst the remaining 60-70% are stage III/IV.\textsuperscript{21} There is no functional distinction between stage III and stage IV, as the management is common to both. In mantle cell lymphoma, the large majority of patients have involvement of bone marrow, peripheral blood or gastrointestinal tract if sufficiently sensitive techniques are used\textsuperscript{22} therefore stage has little prognostic implication. Supporting this, those studies which have addressed the small population of patients with apparently “limited stage” MCL have found that outcomes are similar to patients with advanced stage.\textsuperscript{23}
Prognostic Indices

Given the limited ability of stage in isolation to predict outcome, attempts have been made to develop prognostic indices with greater discriminatory power. Prognostic indices are important tools for informing discussion with patients and their families, selecting treatments and designing clinical trials.

In aggressive NHL the most influential such index was developed by the collaboration of 16 institutions and collaborative groups in the United States, Canada and Europe (termed the “International Prognostic Index (IPI”).24 This analysis pooled outcomes from 3273 patients with aggressive NHL treated on prospective phase II/III protocols with multi-agent chemotherapy protocols (without rituximab) between 1982-7. Five variables (age >60, elevated serum LDH, multiple extranodal sites, stage III/IV and ECOG performance status ≥2) were identified by multivariate analysis as independently prognostic of outcome. When each factor was assigned one point, patients could be stratified into four risk groups with divergent outcomes. The power of this index is its relative simplicity (being readily calculable using the digits of one hand) and consequently it remains widely used in clinical practice more than two decades later. However, as the IPI was derived in the era prior to the widespread use of rituximab, its discriminatory power was eroded by the general improvement in outcomes seen following the introduction of rituximab as part of standard induction therapy. In particular, in patients treated with R-CHOP the IPI divided patients into two prognostic groups rather than the original four. This led to the development of the revised IPI, which reallocated patients into three risk groups using the same five variables.25 The most recent refinement of this incorporated heavier weighting for advanced age and normalised LDH.26 The functional effect of this is to achieve greater discriminatory power, particularly for the patients with lowest (0-1 points) and highest (≥6 points) risk groups in which the 5-year OS in the external validation cohort was 96% and 39%, respectively. The various prognostic indices used in DLBCL are displayed in Table 3.
Although the original IPI has been shown to predict outcome in other forms of aggressive NHL, many histology specific prognostic indices have also been developed. These include histology specific prognostic models for follicular lymphoma (FLIPI\textsuperscript{27} and FLIPI\textsuperscript{28}); mantle cell lymphoma (MIPI\textsuperscript{29} and MIPI-B\textsuperscript{30}); splenic marginal zone lymphoma.\textsuperscript{31}
Table 3 Prognostic Indices in aggressive NHL/ DLBCL. Abbreviations: IPI: international prognostic index; R-IPI = revised IPI; NCCN = National Comprehensive Cancer Network; LDH = lactate dehydrogenase; ECOG = Eastern Cooperative Oncology Group *marrow, liver/GIT, CNS lung

<table>
<thead>
<tr>
<th>prognostic index</th>
<th>year</th>
<th>number of patients</th>
<th>treatment</th>
<th>variables</th>
<th>risk groups</th>
<th>outcome</th>
</tr>
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<tbody>
<tr>
<td>IPI²⁶</td>
<td>1993</td>
<td>3273</td>
<td>multi-agent anthracycline containing chemotherapy</td>
<td>age &gt;60 elevated serum LDH multiple extranodal sites stage III/IV ECOG performance status ≥2</td>
<td>low (0-1 points) low-int (2) high-int (3) high (4-5)</td>
<td>5-yr OS 73% 51% 43% 26%</td>
</tr>
<tr>
<td>age-adjusted IPI²⁴</td>
<td>1993</td>
<td>3273</td>
<td>multi-agent anthracycline containing chemotherapy</td>
<td>stage III/IV elevated LDH ECOG performance status ≥2</td>
<td>low (0) low-int (1) high-int (2) high (3)</td>
<td>5-yr OS 83% 69% 46% 32%</td>
</tr>
<tr>
<td>R-IPI²⁵</td>
<td>2007</td>
<td>365</td>
<td>R-CHOP</td>
<td>age &gt;60 elevated serum LDH multiple extranodal sites stage III/IV ECOG performance status ≥2</td>
<td>very good (0) good (1,2) poor (3-5)</td>
<td>4-yr OS 94% 79% 55%</td>
</tr>
<tr>
<td>NCCN-IPI²⁶</td>
<td>2014</td>
<td>2788 (1650 in training set, 1138 in validation cohort)</td>
<td>NCCN : not reported but presumably mostly R-CHOP BCCA: R-CHOP</td>
<td>age (points) &gt;40 - ≤60 (1) &gt;60 - ≤75 (2) &gt;75 (3) LDH, normalised 1-3 (1) &gt;3 (2) extranodal disease* (1) stage III/IV (1) ECOG performance status ≥2 (1)</td>
<td>low (0-1) low-int (2-3) high-int (4-5) high (&gt;6)</td>
<td>5-yr OS 96% 77% 56% 38%</td>
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PET-CT as a risk stratification tool in non-Hodgkin lymphoma

An introduction to PET in lymphoma

Positron emission tomography (PET) is a functional and metabolic imaging modality that detects positron emissions from radiotracers, which are administered to patients. $^{18}$F-fluorodeoxyglucose ($^{18}$F-FDG) are the combination of isotope and tracer most widely used; it exploits the differential glucose transport of malignant cells relative to normal cells. In the modern era, PET scanners are combined with computed tomography (CT) scanner allowing computerised overlay of the functional (PET) and anatomic (CT) imaging to form a co-registered image which precisely allows identification of the anatomic structure associated with abnormal FDG uptake (shown in Figure 2). $^{18}$F-FDG PET-CT is useful in many subtypes of lymphoma. Aggressive histologic subtypes such as Burkitt lymphoma, DLBCL and HL have with more rapid growth and glucose utilisation and are therefore nearly universally FDG avid, whilst more indolent subtypes such as SLL and MZL are FDG avid in 50-83% of cases. $^{32,33}$

The use of PET-CT in staging

PET-CT has superior ability to detect lymphomatous involvement than conventional CT due to 1) capacity to detect disease in lymph nodes of normal size and 2) greater ability to detect extranodal disease (e.g. bone marrow, spleen and liver). $^{34}$ PET-CT results in an alteration of management strategy in around 15% of patients although this figure varies considerably by disease type and pre-PET staging. $^{35-37}$ Furthermore, PET-CT is much more sensitive than bone marrow biopsy in the detection of marrow involvement in both HL $^{13}$and DLBCL $^{38}$, leading some experts to suggest bone marrow biopsy is no longer indicated in these histologic subtypes.

Figure 2 $^{18}$F-FDG PET-CT in lymphoma. A: Axial slices using conventional computed tomography; B - using positron emission tomography; C - co-registered image which facilitates accurate anatomic co-location of areas of abnormal FDG uptake (in this case in the spleen); D: whole body PET coronal image
Interim PET-CT as a predictor of outcome in PMBL

Primary mediastinal B-cell lymphoma (PMBL) is an uncommon, but biologically distinct histological subtype of DLBCL accounting for approximately 2% of cases of NHL and 5-10% of DLBCL. The disease commonly affects young females, with a median age of onset of 35 years. Clinicopathological correlation is particularly important with this subtype of DLBCL as morphologically differentiating this entity from classical nodular sclerosis HL and other subtypes of DLBCL can be difficult, however gene expression and molecular profiles are distinct. Most patients present with a bulky mediastinal mass, and thoracic outlet obstruction leading to superior vena cava syndrome is common. Direct infiltration of surrounding tissues such as the lung, pleura and
pericardium is often seen, and pleural or pericardial effusions are present in one third of cases.\textsuperscript{43} \textsuperscript{18}F- FDG PET-CT has been established as a staging procedure prior to commencement of treatment and provides prognostic information at the completion of treatment in DLBCL.\textsuperscript{44,45} Interim PET (iPET) scans (evaluated by $\Delta$SUVmax\textsuperscript{46,47}, 5-point scale\textsuperscript{48-50} or used to guide biopsy for histologic confirmation\textsuperscript{50}) have been investigated as a means of identifying patients with DLBCL and inferior outcome with mixed success to date.

There are compelling reasons to critically examine the role of PET in PMBL, separately from unselected DLBCL. The pathobiology of PMBL overlaps with that of DLBCL and Hodgkin lymphoma\textsuperscript{41,51,52}, and the intense inflammatory reaction seen in this disease may result in metabolic activity even in the absence of viable tumour. Moreover, PMBL has traditionally been regarded as an entity where consolidation radiotherapy following chemotherapy is frequently used.\textsuperscript{40,53} In view of its common occurrence in young females, and the attendant risk of secondary breast malignancies and late cardiovascular disease, there is considerable interest in exploring PET as a tool to facilitate selective avoidance of exposure to radiotherapy in this histology. The planning of such PET-based risk stratification treatment approaches requires a good understanding of the natural history of PET evolution in PMBL across different treatment timepoints. Finally, there is no common agreement on which method of PET interpretation is most predictive of outcomes in this histology. In order to address these questions, I analysed PET-CT results in patients with PMBL treated at PMCC (page 81).

**Surveillance PET-CT in de novo DLBCL**

Despite improvements in cure rates for patients with DLBCL, up to 40% develop disease relapse, mostly within 18 months from treatment.\textsuperscript{54} There is no consensus as to the most appropriate form of post-remission surveillance. Salvage chemotherapy and subsequent high-dose therapy (HDT) with autologous transplantation is potentially applicable for selected patients up to age 75 and can cure up to 40% of patients with relapsed disease.\textsuperscript{55,56} However, this approach is less likely to be
successful in those in whom relapse occurs early after primary therapy.\textsuperscript{57} The established prognostic factors for response to salvage including relapse stage, elevated serum lactate dehydrogenase (LDH), and bulk reflect tumour burden, suggesting early detection may increase the likelihood of cure.\textsuperscript{58}

Current guidelines recommend clinical review every 3-6 months after completion of therapy for 5 years and annually thereafter with computed tomography (CT) scans at 6 month intervals up to 2 years.\textsuperscript{59,60} Despite this, there is little evidence to support the use of CT with 83-89% of relapses being detected by symptoms despite surveillance scans.\textsuperscript{61-63} PET combined with computer tomography (PET-CT) has become the modality of choice for initial staging and end of treatment assessment in DLBCL.\textsuperscript{44,64} The improved sensitivity of PET-CT suggests advantages over CT in the detection of subclinical relapse. Few studies have examined the role of PET-CT surveillance in patients with DLBCL achieving remission after primary therapy.\textsuperscript{65-69} Liedtke et al found patients with subclinical relapse were more likely to have lower second line IPI (RR 4, 95%CI 0.58-27.6) with a non-significant trend toward survival benefit (actuarial 5 year survival of 54% v 43%; \(P=0.13\)).\textsuperscript{70} Therefore I did a study to evaluate the role of FDG PET-CT scans in the surveillance of patients achieving complete metabolic response (CMR) after primary therapy for DLBCL, and define a risk-adapted strategy for surveillance imaging (Surveillance PET in DLBCL, page 67).

**Surveillance PET-CT in transformed indolent lymphoma**

Histologic transformation from indolent to high-grade lymphoma is an important cause of treatment failure.\textsuperscript{3} Patients with transformed indolent lymphoma (TrIL) have long been considered to have an adverse prognosis\textsuperscript{71-74} although more recent data suggests that in the rituximab era outcomes may be improving, particularly in patients attaining complete response to therapy.\textsuperscript{75,76} In contrast to de novo DLBCL (in which most cases of disease relapse occur within two years of completion of therapy\textsuperscript{77}) in patients with TrIL, late disease relapse is more common. A series from MD Anderson Cancer Centre found 9/21 (43%) of patients with TrIL relapsed beyond two years.\textsuperscript{78} Similarly, a study from Lyon reported that in a cohort of patients with DLBCL, 13/54
(24%) of late relapses (defined as more than five years after completion of therapy) occurred in patients who had TrIL demonstrated at diagnosis. These relapses occurred at a median of 7.5 (range 5-20.5) years after initial treatment. This differing pattern of relapses suggests a customised approach to post-remission follow up may be warranted. In chemotherapy naïve patients, anthracycline containing chemotherapy regimens with rituximab (typically rituximab, cyclophosphamide, doxorubicin, vincristine and prednisolone (R-CHOP)) are considered standard of care. Current treatment guidelines do not offer explicit recommendations on optimal follow up and surveillance imaging of patients with TrIL. In particular, no studies to my knowledge have examined the role of surveillance imaging for patients with TrIL in complete metabolic remission (CMR) after therapy. In de novo DLBCL, I found surveillance 18F-FDG PET-CT scanning did not result in clinical benefit in patients with low- or intermediate-risk disease by IPI at any time, nor in patients with high-risk IPI beyond 18 months from completion of therapy. In subsequent chapter I examine the utility of surveillance PET-CT scans following immuno-chemotherapy for patients with TrIL achieving CMR (page 74).

Treatment principles for patients with lymphoma

In this section I will provide a brief overview of the treatment principles in DLBCL and FL in order to highlight some existing limitations and how these will be addressed. In general, patients with B-cell NHL who require treatment receive a combination of cytotoxic chemotherapy and the anti-CD20 mAb rituximab. The choice of chemotherapy backbone varies depends on histologic subtype, regional access to drugs, patient comorbidities and patient and physician preference. Even in the commonest NHL subtypes, DLBCL and FL some disagreement about the standard induction regimen exists.

DLBCL

Multiagent chemotherapy with CHOP remained the standard of care for patients with DLBCL for many years. Many attempts were made to improve on CHOP. The 2nd generation regimens were developed on the principle of increasing dose intensity by
adding non-cross resistant cytotoxic agents. An example of this was the addition of methotrexate to bleomycin, doxorubicin, cyclophosphamide, vincristine and dexamethasone (m-BACOD). Prior to the introduction of growth factors, the principle of alternating myelosuppressive with non-myelosuppressive therapy to maximise dose intensity led to the design of the 3rd generation MACOP-B regimen (methotrexate, doxorubicin, cyclophosphamide, vincristine and prednisolone).

However, despite early promise in phase II studies, the large phase III study found m-BACOD, MACOP-B and proMACE-CytaBOM did not improve efficacy over CHOP.

However, the French intergroup explored the more intensive regimen ABCVP (doxorubicin, cyclophosphamide, vindesine, bleomycin, prednisolone) in a randomized phase III study conducted pre-rituximab, patients with aggressive poor risk lymphoma were randomised to intensive chemotherapy with ACVBP or 8 cycles of CHOP. CR rates were similar, however despite patients randomized to ACVBP having a greater toxic death rate (13% v 7%, $P=0.014$) then 5-year EFS (39% v 29%, $P=0.005$) and 5-year OS (46% v 38%, $P=0.036$) were superior in the more intensively treated arm. Although provocative, the adoption of the more intensive ABCVP was overshadowed by the striking impact of rituximab. Also – number needed to treat to benefit 1 patient was unfavourable given increased early death rate – also at same time CHOP-14 then looked better than CHOP-21, and was more readily delivered. That was then the rationale for the R-CHOP 14 vs 21 studies

**Elderly patients**

In elderly patients, the addition of rituximab to CHOP chemotherapy has been consistently shown to improve OS by 10-15% without significant increase in toxicity in several large, prospective studies including over 2000 patients. This result was confirmed in the “real-world” experience reported by the BCCA. Numerous attempts to improve on R-CHOP have been made, without consistent demonstration of benefit. One of the most common variations is an increase in dose density from one treatment every three weeks (R-CHOP21) to every two weeks (R-CHOP14). These two regimens have been compared in two large, prospective, randomised studies (collectively enrolling 1680 patients) with neither showing superiority in terms of
response rates, PFS or OS.\textsuperscript{91,92} The British National Lymphoma Investigation (BLNI) study compared 6 cycles of R-CHOP14 plus 2 cycles of rituximab with 8 cycles of RCHOP21\textsuperscript{91} whilst the Groupe d’Etude des Lymphomes de l’Adulte (GELA) study used 8 cycles of R-CHOP14 with R-CHOP21.\textsuperscript{92} From a practical perspective although the overall treatment duration for R-CHOP14 is shorter, the toxicity is also greater. In particular, the increase in steroid density leads to greater risk of \textit{Pneumocystis jirovecii} pneumonia\textsuperscript{93} although this can be prevented using chemoprophylaxis with sulfamethoxazole/trimethoprim. Furthermore, in order to maintain dose intensity the use of granulocyte colony stimulating factors is needed, which significantly adds to the cost of the more dose intensive regimen. Using >6 cycles of R-CHOP14 does not appear to improve outcomes: in the Deutsche Studiengruppe für Hochmaligne Non-Hodgkin-Lymphome (DSHNHL) RICOVER60 study, patients receiving 8 cycles of R-CHOP14 did no better than those treated with 6 cycles of R-CHOP14 (+ 2 cycles of rituximab).\textsuperscript{89}

\textbf{Younger patients with low-risk disease (aaIPI 0-1)}

The Mabthera International Trial (MINT) was a cooperative effort of 18 study groups, in which 824 patients aged 18-60 with ≤1 IPI risk factor were randomised to receive 6 cycles of CHOP(like) chemotherapy ± rituximab.\textsuperscript{94} After a median follow up of 34 months, the R-chemo arm had superior 3-year EFS (79 v 59%, \textit{P}<0.0001) and OS (93 v 84%, \textit{P}=0.0001). Although previous studies suggested the addition of etoposide to CHOP (CHOEP21) improved results over CHOP21,\textsuperscript{95} in the MINT study the more intensive CHOP-like regimens, R-CHOEP21 and rituximab, methotrexate, doxorubicin, cyclophosphamide, vincristine, prednisolone, bleomycin (R-MACOPB) were not superior to R-CHOP21.\textsuperscript{94} The questions of dose intensity and value of radiation to areas of bulk in this population is being addressed in the UNFOLDER study, in which patients were randomised to either R-CHOP14 or 21 and radiation to areas of bulk (defined as ≥ 7.5cm). The non-radiation containing arms were terminated after an interim analysis found inferior 3-year EFS in the non-radiation containing arms. (Michael Pfrenschnuh, personal communication) Final analysis from this study will answer whether the 14-day schedule results in any improvement in outcome.
**Younger patients with high-risk disease (aaIPI 2-3)**

Treatment of young patients with high-risk disease remains one of the areas of persisting uncertainty in the treatment of patients with DLBCL. Up to one-third of this population fail treatment with R-CHOP. Numerous efforts have been made to improve on this with more intensive regimens and/or high-dose chemotherapy and autologous stem cell transplantation (ASCT). In the pre-rituximab era, a meta-analysis of 11 studies found no benefit in terms of EFS or OS for ASCT over chemotherapy alone.\(^9^6\)

The apparent superiority of ACVBP over CHOP led to the conduct of the LNH03-2B study, in which rituximab was added to each arm.\(^9^7\) This study randomised 379 younger patients (aged 18-59) with at least one adverse risk factor to either R-ACVBP or 8 cycles of R-CHOP in 21-day cycles. Although more toxic (febrile neutropenia in 38% vs 9%, and serious adverse events in 42% vs 15%) the R-ACVBP arm appeared to result in superior disease control. with 3-year PFS (87% vs 73%, \(P=0.0015\)) and OS (92% vs 84%, \(P=0.0071\)). However, in contrast to DSHNHL studies described above, radiotherapy was not used. Perhaps as a consequence, the 3-year EFS (67% vs 80%) and OS (84% v 90%) of R-CHOP21 was numerically lower in the French study compared with the MINT study (although the risk profile of patients was more favourable in MINT). In contrast, R-ACVBP in the GELA study returned similar results to R-CHOP21 in the MINT study. One possible interpretation of these data is that the greater intensity of R-ACVBP was able to overcome the omission of radiotherapy, however comparison between studies is potentially misleading. The generalisability of the ACVBP regimen outside Europe is limited by the lack of availability of vindesine, and larger studies confirming this benefit are required before firm conclusions can be drawn. Additionally, 92% of patients treated with R-ACVBP did not benefit from the intensified regimen; 8% died anyway, and 84% would have achieved good outcomes with R-CHOP ± ASCT at disease relapse. Thus the number of patients needed to treat with R-ACVBP to benefit one patient is approximately 12. Holte et al reported the results of a non-randomized phase II study in a similar patient group (DLBCL or grade 3 FL aged 18-65 with aaIPI 2-3).\(^9^8\) All 156 patients received treated with R-CHOEP at 14 days intervals, followed by a course of high dose cytarabine and methotrexate as
CNS prophylaxis. Amongst this young but high-risk patient cohort, the 3-year PFS and OS were 65% and 81% respectively.

The role of HDT in the rituximab era has been tested in several non-randomised phase II studies with 5-year PFS ranging from 34-77% and 5-year OS 43-81%. Several ongoing randomised phase III studies are being conducted to evaluate the comparative benefit of high dose consolidation in R-chemo treated patients.

Areas of unmet need for patients with lymphoma

Although the application of myeloablative doses of chemotherapy supported by autologous stem cell reinfusion (autologous stem cell transplantation) is able to cure a proportion of patients with DLBCL with chemo-sensitive relapse, the toxicity of is considerable and thus patients older than 70 years of age or those with other major organ dysfunction are generally considered ineligible. The outlook for such patients is particularly poor.

CNS relapse in aggressive NHL

Designing tailored management strategies for patients with aggressive NHL continues to increase in complexity. This is particularly true of determining which patients would benefit from receiving central nervous system (CNS)-directed prophylaxis, where the data available to stratify patients for their CNS risk is ever increasing.

The most important determinate of CNS risk in patients with NHL is the histologic subtype of their disease. CNS involvement in patients with clinically indolent NHL subtypes such as follicular lymphoma, marginal zone lymphoma, small lymphocytic lymphoma, and lymphoplasmacytic lymphoma is very rare in the absence of histologic transformation; CNS prophylaxis is not warranted. At the other end of the spectrum, patients with Burkitt lymphoma and lymphoblastic lymphoma have a risk of CNS involvement exceeding 30% and treatment protocols such as CODOX/M-IVAC or Hyper CVAD routinely incorporate CNS-penetrating doses of methotrexate and cytarabine and intrathecal chemotherapy. Patients with other aggressive subtypes of
NHL fall between these two extremes and deciding which of these patients warrant CNS prophylaxis poses difficulty. In the absence of improvements in systemic treatment over R-CHOP21, optimisation of CNS prophylaxis is possibly the single greatest opportunity to improve patient outcomes, as CNS relapse is a disastrous complication.

**Diffuse large B-cell lymphoma**

Despite the considerable number of studies attempting to define the risk factors for CNS relapse in patients with DLBCL, specific and sensitive criteria for identifying patients at increased risk of CNS recurrence remains challenging due to methodological limitations of the studies. Because of the difficulty in conducting prospective randomised studies, much of the data informing practice is derived from single centre, retrospective analyses in which patient composition, primary treatment strategy, and use of CNS-directed prophylaxis were variable.\(^{107-112}\) Two of the most frequently cited studies regarding this topic were performed in heterogeneous patient cohorts treated prior to the introduction of rituximab. Van Besien et al from the MDACC studied 605 patients treated in prospective clinical trials and identified elevated serum lactate dehydrogenase (LDH) and multiple extranodal sites of involvement as independent predictors of CNS relapse using multivariate analysis.\(^{113}\) Hollender et al reviewed 2514 consecutive patients with NHL, including 1220 with high-grade NHL (which included histologic subtypes including centroblastic, immunoblastic, high-grade B-cell, peripheral T-cell and high-grade unclassified). In addition the two risk factors identified by Van Besien (LDH and multiple extranodal sites) they found age >60, involvement of retroperitoneal nodes and serum albumin <35g/L to predict CNS relapse by Cox regression analysis and developed a 5-point score.\(^{105}\) A recent meta-analysis of CNS risk factors from seven studies (five retrospective,\(^{110,112,114-116}\) two prospective\(^{117,118}\)) in which patients were treated with CHOP±R identified stage III/IV (OR 2.2, 95% CI 1.6 – 3.1), IPI >1 (OR 2.6 (1.6 – 4.3)), ECOG PS >1 (OR 1.7 (1.2 – 2.3)), elevated LDH (OR 2.2 (1.5 – 3.2)) and involvement of the bone marrow (OR 2.8 (2.0 – 4.1)), or testes (OR 3.8 (2.0 – 8.0)) as factors independently associated with risk of CNS recurrence.\(^{119}\) Given that many of these
factors are included in the IPI, some investigators have identified IPI ≥3 as a predictor of CNS relapse\textsuperscript{120}. However, using this criterion on its own to select patients for CNS-directed prophylaxis is overly inclusive, losing specificity and would result in unnecessary treatment of many patients. For example, in the RICOVER-60 study in which elderly patients with DLBCL were treated with CHOP±R, 507 (42\%) patients had IPI ≥3 but only 30 (8\%) of these experienced CNS relapse.\textsuperscript{117} When only patients treated with R-CHOP were considered the presence of raised serum LDH, ECOG performance status >1 and multiple extranodal sites of involvement comprised 4.8\% of the total patients, in whom the actuarial 2-year rate of CNS relapse was 33\%.\textsuperscript{117} Building on this, the same group recently presented (in abstract form) a five factor “CNS-IPI” based on an analysis of 2164 patients treated in German prospective studies treated with R-CHOP including stage III/IV, age>60, raised serum LDH, ECOG performance status >1 and renal or adrenal involvement as components of their risk model.\textsuperscript{121} Low (score of 0-1, 51\% of patients), intermediate (score 2-3, 44\%) and high (score 4-5, 5\%) had 2-year actuarial CNS risks of 0.6\%, 4.1\% and 17.0\% respectively. This index appears promising, although it requires prospective validation in independent cohorts. Additionally, I argue that the ability to identify patients with CNS-relapse risk of ≥10\% (who warrant consideration of prophylaxis) with a high sensitivity should be a goal of a CNS predictive risk model. The exact threshold for use of prophylaxis will vary depending on patient related factors such as comorbidities, probability of systemic control and tolerance of prior chemotherapy. If one assumes a conservative hazard ratio for CNS relapse of 0.5 for patients treated with effective CNS-direct prophylaxis, for an event rate of 10\% the number of patients needed to treat to prevent one CNS relapse is 20. It is my belief that this constitutes a reasonable balance of benefit and risk given that CNS relapse is typically fatal and estimates of clinically significant renal impairment are approximately 9-11\%.\textsuperscript{122,123}

Specific anatomic sites of involvement

Many different specific anatomic sites have been proposed as risk factors for CNS relapse. Van Besien et al identified bone marrow, parenchymal organs and the pooled sites of skin/subcutaneous tissue and muscle as high-risk by univariate analysis, but
none individually retained significance by multivariate analysis.\textsuperscript{113} The relatively low number of any specific individual site likely limited the power of this analysis. Hollender et al found the presence infiltration of the lungs, pancreas, breast, or ovaries to be significant on univariate analysis but chose not to include them in the multivariate analysis because of low frequency.\textsuperscript{105} It becomes clear that one of the difficulties for the treating clinician in interpretation this data is lack of reproducibility of risk factors between series. The main reason for this is that even in large series of unselected patients with DLBCL, the low frequency of specific extranodal sites limits ability to detect difference in CNS risk. For example, not all series of unselected DLBCL identify testicular lymphoma as a risk factor, yet in most series consisting solely of patients with primary testicular lymphoma it is apparent that the risk of CNS relapse is significant.\textsuperscript{124}

**Kidney and adrenals**

Lymphomatous involvement of the renal tract\textsuperscript{110,112,121} and adrenal glands\textsuperscript{111} have been identified by multivariate analysis as risk factors for CNS involvement. The study by Van Besien et al reported an increased risk for adrenal involvement, however this extranodal site was pooled with kidney, which was likely to have influenced the finding.\textsuperscript{113}

**Sites with physical proximity to CNS**

*Craniofacial sites (orbits, paranasal sinuses, salivary glands, thyroid, nasopharyngeal)*

The majority of orbital lymphomas are of marginal zone histology, however even amongst case series of DLBCL of the orbit CNS involvement is reported in <2% of cases; thus CNS prophylaxis does not appear warranted.\textsuperscript{125} Several pre-rituximab series described increased risk of CNS relapses in DLBCL involving the paranasal sinuses.\textsuperscript{126,127} Given the relative rarity of lymphomatous infiltration at these sites, most series have grouped the different nasal sinuses together, making attempts to precisely dissect CNS risk for each individual sinus challenging.\textsuperscript{128,129} The Vancouver experience
is worth particular mention, as the crude incidence of CNS relapse was 2/5 (40%) prior to the introduction of routine IT MTX and 3/39 (8%) after.\textsuperscript{130} In their updated report, involvement of the paranasal sinus was no longer an independent risk factor, perhaps due to beneficial effect from IT methotrexate.\textsuperscript{112} However, a recent International Extranodal Lymphoma Study Group (IELSG) study examined 488 cases of head and neck lymphoma, of whom 53 involved the nasal cavity and paranasal sinuses.\textsuperscript{131} Despite infrequent use of CNS prophylaxis, only 3/53 (6%) cases of primary lymphoma involving the nasal cavity of paranasal sinuses experienced CNS relapse. The IELSG-23 study also surveyed Waldeyer’s ring, thyroid, palate and oral cavity, parotid and salivary glands and only 1 case of CNS relapse occurred across all sites, yielding an aggregate CNS relapse risk of <1%.\textsuperscript{131} Murawski et al performed a retrospective analysis of 284/3840 (7%) patients with craniofacial involvement (including the orbits, paranasal sinuses, nasal cavity, tongue and salivary glands) in German prospective DLBCL studies.\textsuperscript{132} They confirmed higher risk of CNS involvement in patients with craniofacial involvement treated prior to the introduction of rituximab (2-year risk 4.2 vs 2.8%, P=0.038), however after the addition of this rituximab this effect was no longer present. Although these data were presented in abstract form and must be interpreted with caution, when taken in combination with the IELSG data they suggest that craniofacial extranodal involvement alone may no longer be an indication for CNS prophylaxis in patients treated with full dose R-CHOP.

**Epidural DLBCL**

Several pre-rituximab studies have described high-grade NHL of the epidural space as an independent risk factor for CNS involvement, with estimated aggregated risk approximately 8%.\textsuperscript{125,133-140} These are confounded by the inclusion of patients with non-DLBCL histologies such as Burkitt lymphoma and their applicability to patients treated with R-chemo is uncertain. The Vancouver study included 13 patients with epidural involvement, of whom two experienced CNS relapse, but the authors did not find it to be an independent predictor of CNS risk.\textsuperscript{112} Regarding primary epidural lymphoma, a Rare Cancer Network study found 4/42 (10%) of patients not given IT prophylaxis developed CNS relapse compared with 0/10 who did receive such
prophylaxis - although formal statistical analysis was not provided, within the limits of a
non-randomised retrospective study with small numbers, constitutes another
suggestion of potential efficacy. 141

Large cell involvement of bone marrow

Bone marrow involvement with DLBCL was established in a number of early reports as an independent risk factor for CNS involvement113,142,143 and on the basis of these findings involvement at this site has been used in practice as a criterion for CNS prophylaxis. It should be noted that such patients are by definition stage IV and typically have other CNS risk factors such as raised serum LDH and multiple extranodal sites of disease. The previously mentioned meta-analysis of seven studies did report marrow involvement as an independent risk factor, however 50% of included patients did not receive rituximab.119 Several large series of patients predominantly treated with R-CHOP(like) chemotherapy have not found marrow involvement at diagnosis as an independent predictor of CNS risk despite low rates of IT prophylaxis in these studies.110,112,121,144 Thus it appears that in patients treated with R-CHOP although marrow involvement counts as an extranodal site, it should no longer be considered in isolation as an indication for CNS-prophylaxis in isolation.

Primary DLBCL of the testis

The best estimate of risk in primary DLBCL of the testis is drawn from the large IELSG study in which the 5- and 10-year risks of CNS involvement in the absence of prophylaxis were 19% and 34%, respectively.145 Other studies have confirmed a similarly elevated frequency of CNS relapse146,147 and also the unusual temporal pattern of ongoing risk of late CNS relapses. In the prospective phase II IELSG-10 study the addition of four doses of intrathecal methotrexate to six cycles of R-CHOP resulted in a 5-year cumulative CNS relapse rate of 6%, supporting a role for intrathecal methotrexate in this setting, however at the time of reporting median follow-up was short and concerns remain concerning ongoing risk.148 Consequently, the current IELSG protocol also incorporates two cycles of intravenous methotrexate
at a dose of 1.5g/m² as additional CNS prophylaxis (clinicaltrials.gov identifier: NCT00945724).

Primary DLBCL of the breast

The reported rates for CNS relapse in primary breast lymphoma range from 5-13%.149,151 A comprehensive recent review of DLBCL involving the breast considered 24 (and largely small, retrospective) studies with a cumulative crude CNS relapse rate of 8.8% among 980 cases.152 It would be useful to further stratify patients with breast lymphoma for risk, however such attempts are difficult given the rarity of this entity. Small series have suggested bilateral breast involvement153 and tumour size >5cm154 as potential risk factors, however formal statistical analysis is lacking. A multi-centre US collaboration recently reported on 76 cases of primary breast DLBCL, finding an overall CNS relapse rate of 16%. The differences in CNS relapse risk by stage (IE 12% vs IIE 22%) and stage modified IPI (0-1 13% v 2-4 22%), were not reported to be statistically significant.155 The data on impact of rituximab on CNS relapse in DLBCL of the breast are scarce and prospective studies in this subgroup are lacking. In studies by Aviles et al (n=32) and Caon et al, (n=12) no patients treated with R-chemo experienced CNS relapse.156,157 In contrast, 9/17 patients (53%) treated with R-chemo in the US study experienced CNS relapse.155 Therefore the balance of available evidence suggests that involvement of the breast does confer additional risk, and given the lack of convincing ability to identify a subgroup with higher risk, such patients thus warrant consideration of CNS-directed prophylaxis, particularly in the presence of one or more potential risk factors such as stage IIE, stage-modified IPI score >2, bilateral breast involvement or tumour size >5cm).

DLBCL involving the ovary

Although ovarian involvement in DLBCL is rare, one of the largest case series found 7/24 (29%) cases to have CNS involvement, two at diagnosis and five at relapse. Six of these were patients with stage IV DLBCL and secondary ovarian involvement, whilst only one had primary ovarian DLBCL.158
Other DLBCL subtypes

**PMBL**
Primary mediastinal B-cell lymphoma (PMBL) is a rare subtype of DLBCL typically involving young, female patients often associated with direct invasion of adjoining structures and frequent extranodal involvement at relapse. A recent review of published series yielded an estimate risk of CNS relapse of approximately 5% in patients treated prior to the availability of rituximab, compared to 2.2% in patients treated with R-CHOP.\(^{159}\) The frequency with which patients with PMBL have raised serum LDH and multiple extranodal sites of involvement appear to reduce their utility as predictors of CNS risk; a Greek analysis of 145 patients (which incorporated systemic failure as a competing risk) did not identify any clear predictive factors, with only leukocytosis approaching significance (\(P=0.06\)). The difficulty in identifying a high-risk subgroup is attributable to 1) the rarity of PMBL and 2) the low frequency of CNS relapse - and the overall risk appears sufficiently low as to not warrant routine use of CNS prophylaxis in the primary treatment of this subtype. No CNS relapses were reported in a recent series of 51 patients treated with DA-EPOCH-R.\(^{160}\) However, patients with relapsed or refractory disease who are being treated with curative intent should be considered at higher risk.

**DLBCL, leg type**
Although primary cutaneous DLBCL, leg type has an aggressive clinical course with frequent dissemination to extranodal sites, secondary CNS involvement appears rare with only a four cases reported to date.\(^{161}\) Of note, the few CNS relapses reported have occurred late in the disease course, up to four years after initial diagnosis.\(^{162,163}\) Thus routine CNS prophylaxis as a component of primary therapy is not warranted.

**Intravascular B-cell lymphoma (IVL)**
IVL is a rare, highly aggressive type form of DLBCL characterised by growth of neoplastic lymphocytes within the lumina of blood vessels.\(^{164}\) CNS involvement is common at diagnosis, ranging from 25-39% in the larger series.\(^{165,166}\) Ferreri et al noted that patients with IVL and CNS disease at diagnosis treated with CHOP or CVP
had dismal prognoses, whilst the single patients treated with high-dose IV methotrexate and ASCT achieved durable remission. Amongst patients without evidence of CNS disease at diagnosis, Shimada et al estimated the 3-year CNS relapse risk to be 25% and although the (varied and non-systematic) use of CNS prophylaxis did not clearly lower the risk in that study. An ongoing Japanese multicentre phase II study of R-CHOP and R-high-dose methotrexate is being conducted. (Japanese Clinical Trials Registry identifier: UMIN000005707).

**MYC/BCL2 positive “double-hit” lymphoma and B-cell lymphoma, unclassifiable with features between DLBCL and Burkitt lymphoma**

Lymphomas with chromosomal breakpoints at the MYC/8q24 locus in combination with another breakpoint, most commonly t(14;18)(q32,q21) involving BCL2 comprise are highly aggressive entities with poor outcome irrespective of current treatment strategy. MYC/BCL2 lymphomas comprise around 60% of all double-hit lymphomas when defined by FISH and 5% of DLBCL; such cases carry a substantial risk of CNS involvement. Studies defining cases using the presence of dual MYC and BCL2 translocations by FISH have described in aggregate 28/68 (48%) of cases bear evidence of CNS involvement at diagnosis. Savage et al identified 12/135 (9%) cases of MYC+ DLBCL by FISH, this lesion associated with a greatly increased risk of CNS relapse (HR = 8.0; 95% CI, 1.3-48.0). The cumulative incidence of CNS relapse in MYC+ cases was 2/12 (17%) compared with 4% in non-MYC rearranged cases. One of the MYC+ cases (with testicular involvement) developed CNS relapse despite IT methotrexate. Many of these cases fall under the WHO 2008 morphologic classification of “B-cell lymphoma, unclassifiable with features intermediate between DLBCL and Burkitt lymphoma”. As evidence is accumulating, careful evaluation of patients with MYC/BCL2 DLBCL for CNS disease at diagnosis is recommended. Furthermore, CNS prophylaxis for such patients should be considered regardless of stage.

**HIV associated lymphoma**

CNS involvement was reported in up to 40% of patients with HIV-associated lymphoma in historic series although, many patients 1) had CNS involvement at
diagnosis and 2) would likely be classified as either Burkitt lymphoma or primary CNS lymphoma using WHO 2008 criteria. It is difficult to ascertain the risk of CNS relapse in patients with HIV-associated DLBCL treated with rituximab containing chemotherapy regimens as the trials employed intrathecal chemotherapy as CNS prophylaxis in patients with Burkitt lymphoma or marrow involvement and did not explicitly report CNS relapse rates. However, up to 20% of HIV-associated DLBCL bear a MYC translocation and many cases display immunoblastic morphology, both risk factors for CNS risk in the HIV-negative patients with DLBCL. Primary effusion lymphoma is an aggressive, rare subtype of lymphoma caused by HHV-8 infection, typically occurs in patients with HIV. The risk of CNS involvement or relapse is difficult to assess due to its rarity, with only one CNS relapse identified in the two largest retrospective series of 11 and 28 patients. Although a few other case reports CNS involvement in primary effusion lymphoma exist, the overall risk is likely to be <5% and does not warrant specific prophylaxis. Plasmablastic lymphoma is another type of aggressive NHL associated with immunodeficiency. It can occur in the setting of HIV infection, where it typically affects the oral cavity; alternatively HIV-negative cases may occur as a post-transplant lymphoproliferative disorder or after steroid therapy, in which case there is tropism for extranodal sites other than the oral cavity. Despite this predilection for extranodal involvement, CNS relapse is rarely reported irrespective of HIV status. One case series reported a crude incidence of 4/5 (80%), however much larger series of 228 patients did not report similar patterns of relapse. Acknowledging the limitations of the evidence, the use of CNS prophylaxis in patients with HIV associated MYC+ DLBCL or immunoblastic DLBCL line should be considered.

**Histologic transformation of indolent lymphoma**

Histologic transformation from indolent NHL to DLBCL appears to confer an increased risk of CNS involvement above that of the original indolent lymphoma, although the exact magnitude is difficult to quantify as most published series do not specifically report rates of CNS relapse. The only series to our knowledge in which CNS relapse was specifically reported comes from Lyon, in which the incidence of CNS
involvement at time of transformation was 12-15%. This estimate must be interpreted in the context of a relatively small series of 60 patients, lack of rituximab exposure and high frequency of other CNS risk factors present. At this point in time, my opinion is that consideration of CNS prophylaxis in these patients should be based on the same factors as de novo DLBCL.

Other histologic subtypes

**Mantle cell lymphoma**

The risk of CNS involvement in mantle cell lymphoma has been estimated at 5-13%, though most existing series are derived from single centres, with small numbers of patients and wide confidence intervals. Studies have proposed risk factors including high mantle cell lymphoma international prognostic index (MIPI), raised serum LDH and ECOG performance status >1 blastoid histology (at initial diagnosis or relapse) stands out as most consistently identified risk factor. In order to better characterise the clinical characteristics and outcomes of patients with MCL and CNS involvement I performed a multicentre retrospective analysis of series in collaboration with 14 centres from the European Mantle Cell Lymphoma Network (CNS relapse in mantle cell lymphoma, page 58).

**Peripheral T-cell lymphoma (PTCL)**

There are fewer data to guide clinicians on the risk of CNS involvement in PTCL owing to the rarity of the disease. Pro and Perini reported the MD Anderson Cancer Centre experience, with a crude incidence of CNS relapse of 6/250 (2.4%) cases. Yi et al reported an analysis of 228 Korean patients with a variety of PTCL subtypes (including PTCL not otherwise specified (NOS), anaplastic large cell, angioimmunoblastic, hepatosplenic and enteropathy associated T-cell lymphomas but excluding peripheral NK/T cell lymphomas) and found a crude incidence of CNS of 8.8%. Most of these event occurred in patients with PTCL, NOS and ALCL and they were able to derive a two-factor risk model incorporating raised serum LDH and paranasal sinus involvement. The presence of zero (33% of patients), one (58%) and two (9%) risk factors predicted CNS risk of 1.3%, 10.6% and 23.8% respectively. Thus, patients
with neither risk factor do not warrant CNS-directed prophylaxis; patients with one or both factors warrant consideration of prophylaxis if the chosen induction chemotherapy regimen does not contain CNS-penetrating doses of cytarabine or methotrexate.

**Extranodal NK/T-cell lymphoma**

The largest series reporting CNS risk was also a Korean study of 208 patients largely treated with CHOP in which the cumulative CNS relapse risk was 5%. Although this disease frequently involves the paranasal space, this fact alone was not predictive of CNS risk. Patients with a NK-cell lymphoma prognostic index (NKPI) of III-IV (i.e. two or more of: B symptoms, raised serum LDH, lymph node involvement or stage III/IV) had a CNS risk of 20% and patients treated with CHOP warrant consideration of specific CNS-directed prophylaxis. Furthermore, the minority of patients with disease outside the upper aerodigestive tract appeared to have a greater risk of CNS relapse (HR 4.7 (1.5 – 14.5)). Patients treated with the SMILE chemotherapy regimen (with each cycle containing IV methotrexate 2g/m²) likely receive sufficient protection, as none of the CNS relapses in the Korean series occurred in patients so treated (though the number of patients treated with SMILE was not stated).

**Impact of rituximab**

Studies are mixed with regard to the impact of rituximab on CNS relapse risk in patients with DLBCL, with some studies showing a reduction and others showing no reduction. A meta-analysis of eight randomized studies including 4911 patients found a modest reduction from 5.7% in patients receiving chemotherapy alone to 4.7% in patients also treated with rituximab. It appears that CNS relapse in patients treated with rituximab containing chemotherapy regimens tends to be more commonly parenchymal rather than leptomeningeal, isolated to the CNS rather than with systemic therapy and occurs later after completion of therapy compared with patients not treated with rituximab.

**Optimum type, timing and delivery of CNS prophylaxis**
A detailed discussion of the role of flow cytometry and optimal timing, nature and delivery of CNS-directed prophylaxis is beyond the scope of this review, and this topic has been covered in detail in several recent reviews\textsuperscript{205,206} and the British Committee for Standards in Haematology recently published guidelines containing a thorough discussion of these issues.\textsuperscript{207} However, in brief intrathecal chemotherapy alone provides insufficient protection with numerous studies (albeit not designed specifically to answer the question) showing no impact on CNS relapse rates.\textsuperscript{110,112,117,208} Furthermore, most CNS relapses have a parenchymal component\textsuperscript{209} and the IT methotrexate is limited by both uneven distribution around the neuroaxis and inadequate penetration into deep parenchymal structures.

Liposomal cytarabine is a sustained release formulation of cytarabine designed for intrathecal administration.\textsuperscript{210} It has demonstrated high efficacy but considerable toxicity in the treatment of CNS involvement in acute lymphoblastic leukaemia and aggressive lymphoma.\textsuperscript{211} Retrospective studies describing the use of intrathecal liposomal cytarabine as CNS prophylaxis have demonstrated similar trends. In a Polish study, although apparently effective (no CNS relapses occurred after a median of 28 months of follow up) there was additional toxicity with grade 1-2 and 3-4 headache in 61% and 6% of patients.\textsuperscript{212} Of some concern, a Spanish group reported 4/14 (28%) patients treated with intrathecal liposomal cytarabine developed cauda-equina/conus medullaris syndrome, in the absence of structural lesions or lymphoma on CSF analysis.\textsuperscript{213}

Increasingly, CNS-penetrating doses of antimetabolites such as methotrexate and cytarabine are being used and there are several non-randomised studies\textsuperscript{98,122,214} as well as one randomised study\textsuperscript{215} supporting its efficacy in reducing CNS relapse. The prospective randomized Groupe d’Etude des Lymphomes de l’Adulte (GELA) study compared ACVBP with CHOP in patients with high-grade NHL, of whom 80% were DLBCL. Although CNS risk was not a stratification criteria the arms were even balanced for factors such as serum LDH, stage, aAIPI, proportion of patients with multiple extranodal sites and presence of B symptoms. The CHOP arm contained no CNS-directed prophylaxis, whilst the ACVBP arm contained four doses of IT and two
cycles of IV methotrexate (3g/m²). The CNS relapse rate was 8.3% in the CHOP arm and 2.8% in the ACVBP arm (P=0.004) suggesting the addition of IT and IV methotrexate was responsible.\textsuperscript{215} Ideally this hypothesis would be tested in an adequately powered, prospective study randomizing patients to R-CHOP + IT MTX ± high dose IV MTX and/or cytarabine, with the primary endpoint 2-year rate of CNS relapse. There are however, several practical difficulties with performing such a study. Many clinicians believe sufficient evidence exists to support the efficacy of high dose IV MTX ± cytarabine for CNS-directed prophylaxis and may be uncomfortable enrolling patients to a protocol with a chance of not receiving it. Secondly, because CNS relapse remains a rare complication, adequately powering a study is costly and difficult. Limiting the study to only high risk patients (with estimated CNS relapse risk of around 15%) would reduce the sample size needed, but such patients comprise <10% of DLBCL overall.\textsuperscript{121} Given the paucity of prospective randomised data, I performed a multi-centre retrospective analysis comparing three strategies of CNS prophylaxis in patients at high risk of this complication (CNS relapse in aggressive NHL, page 56).
The importance of immunotherapies in lymphoma

**Anti-CD20 monoclonal antibodies in B-cell lymphoma**

The development of monoclonal antibodies (mAbs) has proven the single greatest advance in the treatment of patients with B-cell NHL in the last 20 years. Eight-five percent of NHL are B-cell in origin, a fact which is relevant because most of these lymphomas express the B-cell lineage marker CD20. This surface marker was the first B-cell differentiation antigen identified. Despite this, its physiologic role remains uncertain, however it appears to be involved in B-cell activation and proliferation and Ca²⁺ transport across the plasma membrane. CD20 deficient mice display normal B-cell development and function, but abnormal B-cell receptor and CD19 induced calcium responses. Study of a patient with homozygous deletion of the CD20 gene (MS4A1) whose B-cells completely lacked surface CD20 and displayed normal B-cell development but markedly impaired T-cell independent antibody responses. The selective expression of CD20 on B-cells has facilitated the development of anti-CD20 mAbs as a form of lymphoma immunotherapy with outstanding success most exemplified by the development of chimeric anti-CD20 IgG1 mAb rituximab.

Anti-CD20 mAbs have several potential mechanisms of action, including antibody dependent cell mediated cytotoxicity (ADCC), complement dependent cytotoxicity (CDC), homotypic adhesion leading to programmed cell death and adaptive cellular immunity through induction of T-cell mediated anti-tumour responses. Which of these mechanisms is most important is difficult to determine as pre-clinical models have demonstrated contrasting results. At present, it appears that Fc-FcγR dependent mechanisms are particularly important for the in vivo efficacy of anti-CD20 mAbs when used as single agents. However the primary effector cells responsible for ADCC remain uncertain; there are data to suggest that neutrophils, macrophages and natural killer (NK) cells may all be important in vivo. The variable region of the antibody interacts with CD20 on the surface of the tumour cell, whilst the Fc portion
engages with Fcγ receptor IIIa (CD16) on effector cells. Correlative studies in patients treated with rituximab containing chemotherapy regimens suggests that rituximab may be able to overcome the adverse prognosis conferred by high tumour associated macrophage content which may mean that macrophages are critical effectors in this setting.

mechanisms of resistance to rituximab

Despite the success of rituximab, the disease develops resistance in some patients. Rituximab resistance is clinically defined by one of: 1) disease progression during rituximab therapy 2) the achievement of less than a partial response to a rituximab containing treatment regimen or 3) lymphoma relapse within six months of treatment with a rituximab containing regimen. In untreated patients with follicular lymphoma, approximately 27% of patients display primary resistance to rituximab monotherapy. When rituximab is combined with CHOP or bendamustine, around 10% of untreated patients have resistant disease. Considering the scope of the problem of rituximab resistance, the precise mechanisms remain incompletely described. However there are data regarding potential resistance through each of the mechanisms of action.

Resistance to CDC

Although CDC appears not to be a key mechanism of action for anti-tumour effect, expression of complement regulatory proteins has been demonstrated on rituximab resistant tumour cells lines. Alternatively, deficiency of complement proteins has been proposed as a mechanism by which CDC is impaired; in one study, rituximab resistant patients with CLL were infused with fresh frozen plasma (to replenish complement levels) prior to rituximab re-treatment, with objective responses. Similar resistance to ofatumumab induced CDC has been demonstrated in patients with CLL.

Resistance to ADCC

An elegant investigation showed that ligation of inhibitory killer immunoglobulin receptor (KIR) attenuates rituximab induced ADCC against B-cell lymphoma in vitro.
In another study, overexpression of human phosphatidylethanolamine binding protein 4 (hPEBP4) by lymphoma cells resulted in inhibition of rituximab induced ADCC, whilst silencing of hPEBP4 enhanced rituximab induced ADCC. Furthermore, genetic polymorphisms in FCGR3A (which codes for Fc\(\gamma\)IIIA) have been identified which influence the affinity of binding to human IgG\(_1\). However, the clinical implications of such genetic variation is mixed. Cartron et al found that patients with untreated FL in whom NK cells were homozygous for FCGR3A-158V (which has tighter binding to IgG\(_1\)) had superior response rates following rituximab monotherapy compared with patients who were homozygous for FCGR3A-158F. However the clinical impact of FCGR3A polymorphisms may vary depending on the type of lymphoma, the relative importance of ADCC and the ethnic origin of the patient.

**Resistance to apoptosis**

It has been shown that prolonged rituximab exposure leads to downregulation of pro-apoptotic Bcl-2 proteins including Bax, Bak thus leading to resistance to apoptosis.

**Loss of CD20 expression**

Downregulation of the CD20 epitope has been studied as another potential mechanism of rituximab resistance. Mutations or deletions of the CD20 gene are rarely reported; in one series of patients with DLBCL, 6% were reported to have mutations in the rituximab binding epitope of the CD20 gene (located on exon 5 of the MS4A1 gene). In a separate study, C-terminal mutations were found in 22% of patients with a variety of B-cell NHL, and only in those in whom disease progression occurred. A recently described phenomenon is ‘modulation’ of CD20 expression causing epitope loss and resistance. “Shaving” of CD20-antibody complexes by monocyte Fc\(\gamma\)R was proposed by. An alternative model is modulation via CD20 internalisation and lysosomal uptake which is non-phagocyte dependent. Further evidence suggests lymphoma cell lines exposed to rituximab develop resistance with reduced CD20 expression and a defect in CD20 transport from the Golgi apparatus to the cell membrane. A potential role for epigenetic downregulation of CD20 expression was suggested by an intriguing study in which primary CD20 negative
lymphoma cells sourced from a patient relapsing after R-CHOP was “re-programmed” to restore CD20 expression using a DNA methyltransferase inhibitor. 244

Clinical impact of rituximab

Rituximab has moderate single agent activity in indolent lymphomas, with an overall response rate (ORR) of 46-54% in phase II studies of relapsed and refractory indolent lymphoma.245-247 As a monotherapy in patients with untreated FL, it is a generally non-toxic treatment with reasonable response rate and some long term responders.248 The greatest impact of this agent however has been in combination with chemotherapy (so-called chemo-immunotherapy).

Pivotal phase III studies have established the substantial benefits in ORR, PFS and in some studies with long term follow-up, OS in patients with previously untreated CLL 249, DLBCL 87 and FL 250. In MCL, when R-CHOP is used without high dose consolidation, despite an impressive ORR of 96% in untreated patients, the PFS was a disappointing 16.6 months.251 To improve on this, an Italian multicentre study of 28 patients using standard chemotherapy induction followed by rituximab containing intensified therapy and ASCT achieved OS and EFS rates of 89% and 79% at 54 months.252 As a consequence, rituximab has become incorporated into standard of care in virtually all CD20 positive malignancies.

Another schedule in which rituximab has profoundly influenced management of patients with lymphoproliferative disorders has been post-induction maintenance. Although the concept of maintenance is not novel, the nature of rituximab (an immunotherapy, rather than cytotoxic) led investigators to explore prolonged and repeated dosing of rituximab in an attempt to prolong PFS. The initial studies conducted were in the relapsed setting. The German Low Grade Lymphoma Study group treated patients with relapsed FL or MCL with FCM±R; responders underwent a second randomisation to maintenance rituximab or observation alone.253 Patients randomised to maintenance rituximab achieved significantly longer response duration (not reached v 16 months, P=0.001). Van Oers et al reported the phase III intergroup study of CHOP±R with responders randomised to maintenance rituximab or
observation alone, with similar results: the median PFS from second randomization was 51.5 months versus 14.9 months with observation \((HR, 0.40; P < 0.001)\). The ECOG E1496 study enrolled patients with advanced staged indolent B-NHL, and used 6-8 cycles of CVP as a chemotherapy backbone. Patients achieving stable disease or better were then randomised to maintenance rituximab or observation. Rituximab administered in this setting improved the depth of response and PFS, with a non-significant trend toward improvement in OS. However, the lack of rituximab as part of induction was an obvious criticism of this study and a second, larger study was conducted to address this shortcoming. In the PRIMA study 1193 patients with advanced stage, previous untreated FL received investigators choice of chemoimmunotherapy induction (R-CHOP, R-CVP or R-FCM) followed randomisation to rituximab maintenance (given every 2 months for a total of 2 years) or observation. Rituximab improved the depth of response (unconfirmed CR (CRu) or greater, 71.5\% v 57.6\%, \(P<0.0001\)) and improved PFS (3-year PFS 74.9\% v 57.6\%, \(P<0.0001\)). At the most recent update with median follow up of 73 months from randomisation, the PFS benefit was sustained (6-year PFS 59.2\% v 42.7\%, \(P<0.0001\)). The PFS benefit of maintenance rituximab was seen irrespective of age, sex, FLIPI and type of chemotherapy induction by multivariate analysis. Receiving rituximab maintenance did not appear to adversely affect the response from salvage therapy (76\% v 79\%, \(P=NS\)). OS was excellent in both arms, and with current follow-up does not differ between the two arms (6-year OS 87.4\% v 88.7\%, \(P=NS\)). Thus there are robust data that the use of prolonged maintenance rituximab after chemoimmunotherapy provides sustained benefit in patients with advanced stage, previously untreated FL. It may be speculated that the most important mechanism of action in the maintenance setting is induction of adaptive cellular immunity as the bulk of the tumour is typically effectively killed by the completion of chemoimmunotherapy.

**Newer anti-CD20 mAbs**

Numerous attempts have been made to improve upon rituximab and overcome rituximab resistance. Second-generation antibodies such as veltuzumab, ocrelizumab, ofatumumab are fully humanised or are fully human to reduce
immunogenicity but retain an unchanged Fc component. The most promising of the next generation of anti-CD20 mAbs is obinutuzumab (previously known as GA101). This is a humanised type II IgG1 mAb. Type I mAbs (such as rituximab) are defined by their ability to localise CD20 into lipid rafts. Type II mAbs (obinutuzumab and tositumomab) in contrast do not localise into lipid rafts or activate complement. Obinutuzumab was glycoengineered to contain and nonfucosylated Fc fragments with higher affinity interaction with FcγR and therefore enhanced ADCC even in patients with high affinity polymorphisms. Furthermore it is able to potently evoke direct programmed cell death, and through higher affinity for CD16B can induced neutrophil induced phagocytosis of CLL cells in vitro. These substantial preclinical advantages appear to translate to greater clinical efficacy, with the promising results of the CLL11 study in which elderly patients with untreated CLL were randomised to obinutuzumab or rituximab in combination with chlorambucil vs chlorambucil monotherapy. Patients receiving obinutuzumab had a substantially improved PFS (HR 0.39, 95% CI 0.31 – 0.49, \( P < 0.0001 \)) and higher rate of minimal residual disease (MRD) negative remission (29.4% v 2.5%) compared with rituximab. At the time of writing, in addition to CLL11 three phase III clinical trials using obinutuzumab were in progress (Table 4).

Table 4 Currently active phase III clinical trials involving obinutuzumab.

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<td>GADOLIN</td>
<td>rituximab refractory indolent NHL</td>
<td>bendamustine±G</td>
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It is important to note that despite the promise shown by this agent thus far, the dosing schedule used in the above studies is 1000mg/m², compared with the 375mg/m² dose of rituximab used in NHL. Thus the relative equimolar clinical efficacy of the two antibodies remains untested.
Indolent lymphomas and the potential of immunologic control

The sensitivity of indolent B-NHL to immunological control is demonstrated by the prognostic impact of the presence of an autologous immune response to the iB-NHL at the time of diagnosis and the curative potential of allogeneic stem cell transplantation in which the newly engrafting, donor-derived T and NK cells eradicate lymphoma cells (the “graft versus lymphoma effect”). However, even using reduced intensity conditioning, allogeneic stem cell transplantation carries significant risk of morbidity and mortality. A combination of factors including demographics (most patients are elderly), frailty, or lack of a suitable stem cell donor, means that allograft is not available for most patients. There are, however, other ways to modify the immune system to treat iB-NHL. Others have shown that direct stimulation of iB-NHL tumor cells with TLR9 agonists promotes their antigen presenting capacity resulting in immune targeting with subsequent clinical responses. However, one of the most important advances in the treatment of iB-NHL has been targeting CD20 with mAbs such as rituximab. This agent has significant activity both alone and in combination with chemotherapy, and has been exploited recently in chemotherapy-free combination regimens with immuno-potentiating agents such as lenalidomide and pidilizumab. However, the use of chemo-immunotherapy remains non-curative and new and more potent treatments are urgently needed.

Lenalidomide has activity in lymphoma

The first immunomodulatory agent used clinically was thalidomide, an agent developed for the treatment of morning sickness and banned due to the subsequent development of birth defects. Thalidomide was subsequently found to have activity against multiple myeloma and attempts to find safer alternatives led to the development of the structural analogues lenalidomide and pomalidomide. Although the focus of most of the clinical investigation using these compounds has been in the treatment of multiple myeloma, both agents also display activity in lymphoma. The mechanisms of action of lenalidomide have been extensively investigated but remain incompletely understood. The immunomodulatory effects of lenalidomide
include CD4+ and CD8+ T-cell co-stimulation, Treg suppression, Th1 cytokine production and enhancement of NK-cell induced ADCC. Lenalidomide also has anti-angiogenic, anti-inflammatory properties, down regulates adhesion molecules, has anti-osteoclastic activity and direct anti-proliferative activity. Recent data has suggested that the E3 ligase protein cereblon is the molecular target for thalidomide and is responsible for its teratogenicity. There are accumulating data to suggest that cereblon may be the molecular target of lenalidomide and pomalidomide also. Preclinical data suggests that the preferential activity of lenalidomide against ABC-type DLBCL may be due to inhibition of IRF4 expression and the BCR-NFκB pathway in a cereblon dependent manner.

Lenalidomide and rituximab in lymphoma: preclinical data

Several studies have examined the preclinical activity of lenalidomide with or without rituximab in NHL. An early study in identified that in the Namalwa CSN.70 chromosome 5 deleted cell line, lenalidomide induced G0/G1 cell cycle arrest, inhibited Akt and Gab1 phosphorylation and inhibited the ability of Gab1 to associate with a receptor tyrosine kinase. Another study found lenalidomide exerted anti-proliferative effects on the LP-1 and U266 B-cell lymphoma cell lines and increased synergistic apoptosis on Namalwa cell lines in concert with valproate. A key piece of evidence that a functional immune system is required was provided by a study in which lenalidomide had modest impact on proliferation in a Raji (Burkitt lymphoma) cell line monoculture, with or without rituximab. However, treatment of peripheral blood mononuclear cells (PBMCs) with lenalidomide resulted in significant enhancement of K562 (human leukaemia) cell lines. NK-cell appeared to be critical for this effect, as following treatment with lenalidomide resulted marked upregulation the NK cell activation marked and adhesion molecule CD56 and a two-fold increase in NKT cells. This study also demonstrated synergistic apoptosis against Raji cell line when rituximab and lenalidomide were both present, hinting at the potential clinical efficacy of this combination. A group from Roswell Park Cancer Institute used a severe combined immunodeficient (SCID) mouse disseminated Raji xenograft model to evaluate the effects of rituximab and lenalidomide. Briefly, they found neither
lenalidomide nor pomalidomide decreased CD20 expression on NHL cell lines, but concurrent administration of pomalidomide (and to a lesser extent, lenalidomide) controlled lymphoma growth and prolonged survival in mice more than rituximab alone. In a subsequent experiment using the same xenograft model, the same group demonstrated that lenalidomide resulted in increased recruitment of NK cells to tumour sites, which was mediated by stimulation of dendritic cells and modification of the cytokine environment associated with an increase in monocyte chemotactic protein-1 (MCP-1), TNFα, interferon-γ (IFNγ) and had anti-angiogenic properties.  

Another group of investigators performed a series of experiments to further elucidate the mechanism by which lenalidomide enhanced rituximab ADCC against lymphoma. They showed that 1) in the presence of IL2 or IL12, and CD16 stimulation via immunoglobulin, lenalidomide stimulates IFNγ from NK cells at nanomolar concentrations, with a clear dose-response relationship. Furthermore, the pretreatment of purified NK cells (to ~85%) with lenalidomide resulted in synergistic ADCC of CD20 positive NHL cells lines treated with rituximab. This synergism was present for doses of lenalidomide in the range 0.1- 10μM (Figure 3) but did not occur in cells coated with a control mAb with a different epitope (trastuzumab) or on a CD20 negative cell line (Figure 3, panels H and I). This enhanced ADCC was associated with increased NK cell expression of IL-8, MCP-1, RANTES, IP-10 and GM-CSF as well as enhanced expression of FasL and production of granzyme B through a dose-dependent increase in pERK expression.
A group from MD Anderson Cancer Centre investigated the activity of lenalidomide and rituximab on mantle cell lymphoma (MCL) lines and found that the combination was able to enhance rituximab induced JNK and the mitochondrial apoptotic pathway and lenalidomide treated PBMCs augmented rituximab induced ADCC (Figure 4, left) but not after NK cell depletion (right). They further showed that in vivo MCL tumour growth in SCID mice was delayed, and survival improved in mice treated with the combination of lenalidomide and rituximab compared with either agent alone (Figure 5). These observations all suggest that the combination of lenalidomide and rituximab would have potent anti-lymphoma efficacy in patients, and led to clinical trials using these agents in combination.
Figure 4 LEN augments RTX-dependent NK cell-mediated cytotoxicity via increasing the expression of CD16 in CD56lowCD161 NK cells. Left: Cytotoxicity of whole PBMCs, and right: cytotoxicity of CD561 NK cell-depleted PBMCs against MINO cells. PBMCs were exposed to 1 mM of LEN (LEN, LEN1RTX) or equal volume of DMSO (Control, RTX) overnight. Then PBMCs were incubated with target cells in the presence or absence of 10 mg/mL of RTX (RTX, LEN1RTX) or human IgG (control, LEN) for 4 hr before 51Cr release was measured. With permission from 283.

Figure 5 LEN plus RTX inhibits the growth of MCL cells in SCID mice. (A) Tumor burden was measured as tumor volume. (B) Survival curve of tumor-bearing SCID mice.

Lenalidomide and rituximab: clinical trials in lymphoma

Results from a single centre phase II trial in 110 untreated patients with indolent NHL were recently reported.268 The lenalidomide dose was 20mg daily on days 1-21 and rituximab 375mg/m2 on day 1 of each 28-day cycle. The ORR was 90% with a CR rate of 64%. In the 46 evaluable patients with FL, the CR rate was 87%. These response
rates were high irrespective of tumour bulk and FLIPI score at study entry. The estimated 2-year PFS was 83% for all patients and toxicity was predominantly hematologic, with grade 3 neutropenia or greater observed in 40% of patients. 284

The MDACC group also conducted a phase I/II study in relapsed and refractory MCL, treating 52 patients. 285 After establish the maximum tolerated dose (MTD) of lenalidomide to be 20mg daily, of the 44 patients treated at the phase II dose, the ORR was 57% with CR rate 36%. The treatment was well tolerated and the median duration of response was an impressive 18.9 months. Although the combination appears to have some activity in relapsed and refractory DLBCL, the ORR is 28-35%, although most of these were CRs 286(Zinzani, 2011 #197). Two phase III studies using this chemotherapy-free induction regimen with standard chemo-immunotherapy are ongoing (Table 5).

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Due to the success of lenalidomide and rituximab in lymphoma, I explored the in vitro activity of a novel compound with IMiD-like properties N-Methyl-2-pyrrolidone (NMP) in combination with anti-CD20 mAbs against lymphoma.

**NMP mechanisms of action**

NMP has hitherto undescribed potent in vivo anti-MM efficacy (Figure 6 a, b, c, f) mediated by both immunomodulation (figure 1c-e). NMP is chemically distinct from the IMiDs and is neither a parent nor metabolite of these existing compounds. NMP is
a non-toxic, stable reagent, which is already in use as a pharmacological solvent in biomedical applications including as a delivery vehicle for a number of marketed pharmaceuticals. Pre-existing toxicity studies in humans following NMP exposure have demonstrated few adverse effects and reliable pharmacokinetics.

Figure 6 NMP has in-vivo anti-myeloma efficacy and demonstrates key immunological hallmarks of IMiD activity. (A) 4-7 mice bearing Vk*MYC MM tumours were treated with either Methylcellulose or NMP by gavage until either disease progression or day 50. Improved survival was observed in the NMP-treated mice, which progressed only on withdrawal of therapy (B) in vivo downregulation of TNFα in MM-bearing mice treated with daily 10%NMP solution by oral gavage (C) Human NK-cells treated with NMP or lenalidomide (LEN) or left untreated prior to cytotoxicity against K562 targets in a chromium release assay (D) Human T-cells were treated with anti-CD3 alone or co-stimulated with anti-CD3 in combination with NMP or lenalidomide (LEN) for 24h. Culture supernatants were assayed for IL-2 production. Data from Shortt et al, Cell Reports in press.

The transplantable Vk*MYC MM mouse model is monitored by serial serum electrophoresis and overall survival. Mice develop all of the hallmarks of MM in
addition to humoral immune paresis and dysregulated serum inflammatory cytokine networks, typical of those seen in patients with MM. The power of this model is the co-existence of MM in the context of a syngeneic immune system, making this the ideal mouse model to dissect the mechanism of action for IMiD-induced MM disease control in vivo. In this model we first identified that NMP prevented MM disease progression and improved survival (Figure 6a) and resulted in decrease inflammatory markers in tumor-bearing mice (Figure 6b). We subsequently showed that similar to the IMiD lenalidomide, NMP was able to promote human NK cell function (Figure 6c) and T cell co-stimulation (Figure 6d). Given the profound IMiD-like effects of NMP on both in vivo MM control and ex-vivo immunity, we next demonstrated that maximal NMP efficacy was only seen in the presence of functional immunity (C57BL/6 recipients) but not immunodeficient (Rag-/- cγ/-/-) recipients (Figure 7). Critically the efficacy of NMP was still maintained when the MM tumours in vivo demonstrated lenalidomide resistance (Figure 8).

Figure 7 NMP requires adaptive immunity to control MM. NMP responses were compared in immuno-competent C57BL/6 (NMP, n=8; SAL, n=7) vs immuno-deficient RAG-2-yc/- (RAG2;NMP, n=6; SAL, n=9) mice transplanted with the same Vk*MYC clone. No response to NMP was seen in the RAG-2-yc/-mice. Data presented as mean +/- SEM. NS, not statistically significant, *p <0.05.
Figure 8 NMP has more potent anti-MM activity than lenalidomide. A) NMP, but not lenalidomide, shows a fall in the myeloma-specific kappa-restricted paraprotein over a 28 day course of treatment in \( Vk^*MYC \) tumor bearing mice. \(* = p<0.05\)

Summary of the pre clinical rationale for the immunomodulatory activity of NMP

1. The novel IMiD-like compound NMP can overcome primary lenalidomide-resistance in pre-clinical testing of MM (Figure 6).
2. NMP is orally available, has an established safety profile in humans, and has been extensively used in the pharmaceutical industry as a drug solvent.
3. Functional host immunity is critical to mediate optimal anti-MM effects of NMP (Figure 7).
4. NMP produces in vitro immune responses similar to those observed with lenalidomide, yet has more potent MM control in vivo (Figure 8).

NMP preclinical studies, toxicity

Due to the widespread industrial and biomedical application as a solvent, toxicity and pharmacokinetic data already exists. It has low acute rodent toxicity with an LD\(_{50}\) of 3600-7700mg/kg, and the no-observed effect level in mice is 229-324mg/kg.\(^{291}\) No in vitro or in vivo genotoxicity in bacterial and mammalian tests, and no carcinogenic effects in rats after long-term exposures via inhalation or the diet.\(^{292}\) The major toxic effects are reproductive, but require high exposures; the no-effect level in rats for a decrease in pup body weight is 160 mg/kg/day in the diet.\(^{293}\) Healthy human
volunteers have received up to 100mg orally and doses of 300mg transdermally as a single dose with rapid absorption and no adverse effects.\textsuperscript{288,289} NMP undergoes biotransformation (predominantly to 5-hydroxy-N-methyl-pyrolidone and 2-hydroxy-N-methyl-succinimide) and these metabolites are excreted in urine.\textsuperscript{288} Small amounts (1-5%) are exhaled unchanged as NMP in the lungs or in the faeces. Based on published PK data, peak plasma concentrations following a single dermally applied dose of NMP (300mg) range between 5 and 40uM, with a urinary half-life of elimination of 3-4hrs. This plasma concentration is equivalent to immunomodulatory concentrations of NMP ex vivo observed at concentrations as low as 1 – 10uM.

**Rationale for combining NMP with anti-CD20 mAbs**

Because of the promising preclinical activity of NMP it was a logical step to explore the combination of NMP with rituximab in NHL cell lines.
Rational clinical trial design using immunotherapies in lymphoproliferative disorders

Introduction to immunotherapies

Immunotherapies are taking on increasing importance amongst the available arsenal of anti-cancer therapies. However, the successful implantation of the immune system to target cancer is not a new concept. The surgical oncologist William Coley pioneered the concept using a mixture of killed bacteria (Streptococcus pyogenes and Serratia maracens) which induced regression in some cancers. Despite scepticism from the rest of the medical profession, this treatment (dubbed “Coley’s vaccine or Coley’s toxins”) were used against a variety of cancers with mixed results. In the modern era, bacillus Calmette-Guérin (BCG) delivered intravesically to treat non-invasive bladder carcinoma was an early success of immunotherapy. Otherwise, advances in cancer care in the latter half of the 20th century were largely driven by the development of multi-agent combinations of cytotoxic drugs such as ABVD in Hodgkin lymphoma and CHOP in NHL. It was not until the successful development of rituximab that immunotherapies regained prominence.

Immunotherapies broadly include mAbs (e.g. rituximab, brentuximab) autologous cell transfer (e.g. chimeric antigen receptor T-cell therapy), cytokines (e.g. IFNγ, IL-2) and checkpoint blockade inhibitors (e.g. ipilimumab, pidilizumab) and of course allogeneic stem cell transplantation.

Differences between immunotherapies and conventional cytotoxics

Because of the different mechanism of action of immunotherapies compared with cytotoxic therapies the kinetics and biomarkers of response are often quite different. Immunotherapies typically produce a delayed onset but durable response rather than a rapid but transient response more typical of cytotoxic agents. Conventional measures of response mainly reliant on structural or anatomic changes may therefore not give the complete picture when considering the efficacy of immunotherapies,
which can cause tumour necrosis or inflammatory changes, infiltrates with lymphocytes or macrophages causing an initial increase in size of affected nodes, followed by durable disease control. In such a scenario, PFS loses its value as a surrogate marker for OS, the ultimate endpoint of interest in any anti-cancer treatment. An example illustrating this concept is the immunotherapy sipuleucel-T. In studies using this treatment, there was no significance difference in time to disease progression between treatment arms, however patients receiving the experimental arm derived an OS benefit.

Furthermore, there is conceptual appeal in introducing immunotherapies early in the disease course (Figure 9) to maximise the chance of efficacy. Counteracting this is the effect of tumour-related immunosuppression. Combinatorial strategies using immunotherapies either in multiple combinations, or with small molecule inhibitors or conventional cytotoxics may further enhance efficacy due to breaking “oncogene addiction” and allowing tumour clearance by T-cells. Furthermore, tumour cell destruction results in antigenic shedding, which further optimises antigen presentation to dendritic cells, thus contributing to anti-tumour immunity.

Figure 9 Depiction of the progressive decline in function of the immune system over time, providing a rationale for incorporating immunotherapies early in the disease course rather than in heavily pretreated patients with limited capacity to mount successful immune responses.

Taking these factors into account, a phase I/Ib immunotherapy trial has a subtly different focus to a phase I cytotoxic study. Although both studies are often primarily
Literature Review

Concerned with determination of a dose for phase II studies, in cancer immunotherapies, the optimum biologic dose (OBD – the dose beyond which further increases result in increased toxicity but not efficacy) may be well below the maximum tolerated dose (MTD).\textsuperscript{301} In conventional cytotoxic therapies seeking to maximise tumour cell log-kill, use of the MTD in phase II made sense; in immunotherapy trials such an approach is likely both unnecessary and inefficient. Thus a rational phase I clinical trial design in immunotherapy should serve not only to determine safety, but also as “proof of principle”, allowing insights into the immunology of response, potentially identifying biomarkers of response or failure and allowing greater patients selection for efficacy studies to maximise the likelihood of successfully developing the agent for clinical use. Although it would be ideal to perform serial biopsies of tumour to directly monitor patterns of response during the disease course, this is not always possible from a patient safety or logistic perspective. Clinicians treating lymphoid malignancies are perhaps fortunate in that the peripheral blood and bone marrow are frequently involved, and represent an opportunity to conduct correlative biologic and immunologic response assessments more readily than many solid tumours.

Correlative immunologic studies in phase I cancer immunotherapy studies can be critical in the successful development of these agents. Determining a reliable method of predicting clinical response (or failure), toxicity greatly improves the chances of success and registration following efficacy studies. One approach for achieving this by using an assay which is either 1) already widely available or 2) easily implemented. A highly successful recent example of latter is the parallel development of the HER2 immunohistochemical assay in conjunction with the anti-HER2 mAb trastuzumab.\textsuperscript{302}

The advantages of accelerated titration design

One of the disadvantages of the standard “3 by 3” trial design using immunotherapies is that many patients are treated at sub-therapeutic doses, and that as a result trials are slow to complete.\textsuperscript{303} To avoid this inefficient, and some would argue unethical process accelerated titration designs have been proposed. As immunotherapies tend to have favourable safety profile compared with cytotoxics, they provider ideal
scenarios in which to test accelerated titration design schedules. One particular trial design has appeal with the goal underlying their design to expose as few patients at sub-therapeutic dose levels.
General materials and methodology

PET-CT in DLBCL

The following methods were used in the three PET-CT studies are presented below. I conducted a retrospective review of patients with de novo DLBCL, PMBL or transformed indolent lymphoma who had undergone PET-CT scanning at the Peter MacCallum Cancer Centre. Data collection was compliant with the institutional ethics requirements.

For patients with DLBCL (de novo or TrIL) in the period analysed, departmental protocol recommended six-monthly PET-CT scans for patients in CMR, for the first two years, and then annually until five years after completion of therapy for patients in whom there existed intention to intervene if subclinical relapse was identified. In most cases this intervention consisted of intensive salvage chemotherapy following by autologous stem cell transplantation. Implementation was at the discretion of the treating physician. I included patients who had a confirmed diagnosis of de novo DLBCL treated at Peter MacCallum Cancer Centre between January 2002 and December 2009 (de novo) or 2012 (TrIL and PMBL) who had achieved CMR at the completion of primary therapy and underwent at least one surveillance PET-CT scan.

The definition of TrIL was a documented diagnosis of indolent lymphoma (including non-follicular histology) and a synchronous or subsequent diagnosis of DLBCL. For some exploratory analyses, patients were divided into two groups. Those in whom the diagnosis of histologic transformation was made concurrently with diagnosis of indolent lymphoma (either due to composite histology on a single biopsy, or two biopsies from different anatomic sites within one month with one showing indolent lymphoma and the other DLBCL) were termed “Group 1”. Those patients in whom diagnosis of histologic transformation was made greater than one month after diagnosis of indolent lymphoma were termed “Group 2”. Patients suspected to have transformed disease on clinical grounds (any one or more of the following features; rapid disproportionate growth of a single nodal area, rapidly rising serum lactate
dehydrogenase (LDH), new extranodal sites of disease, constitutional symptoms or hypercalcemia) were included, as their prognosis has been shown to be similar to biopsy-proven TrIL.71,74 Of these, only patients who achieved CMR after primary therapy who had ≥1 subsequent surveillance PET-CT were included in the final analysis.

For the PMBL study, PMBL was defined using clinical and histologic criteria treated at the Peter MacCallum Cancer Centre (PMCC) between January 2001 and December 2012. During this time, departmental protocol recommended PET-CT scans at baseline (bPET), an interim timepoint (after 2 - 4 cycles of chemotherapy, or iPET) and at the end of chemotherapy but prior to any planned radiotherapy (ePET). iPETs were typically performed on the day prior to the next scheduled chemotherapy. Subsequent PET-CT scans were performed if there was clinical suspicion of relapse or to follow up on areas of residual FDG avidity of uncertain interpretation.77 During this period, the significance of positive PET scans in PMBL was interpreted in the same way as DLBCL: where feasible and considered safe, FDG avid residual masses were biopsied; in cases without biopsy, salvage treatment (including chemotherapy, autologous stem cell transplantation and radiotherapy) were prescribed if PET positivity occurred in the original disease site, with no alternate diagnoses.

**Data collection**

For each patient, I collected baseline characteristics including sex, performance status, age, serum LDH, primary therapy, date and details of follow up PET-CT scans, and follow-up data including the date and site of relapse, type (subclinical or suspected), relapse IPI, biopsy histology (reactive, clow-grade or aggressive), second malignancies, cause and date of death.

In all three studies, the primary endpoints were determination of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of PET-CT for the detection of relapse. In the PMBL study, these values were calculated for both interim and
Fluorodeoxyglucose (FDG) PET-CTs were obtained on a dedicated PET/CT scanner (Discovery LS, GE Medical Systems, Milwaukee, USA; Discovery STE, GE Medical Systems, Milwaukee, USA or Biograph 64, Siemens Medical Solutions, Knoxville, USA) from the skull-base to upper-thigh level, unless there was suspicion or known disease outside this field-of-view. Patients were fasted for six hours before administration of 5 MBq/kg \(^{18}\)F-FDG, to a maximum of 400 MBq adapted for weight and imaged after a \(\geq 60\)-minute uptake phase.

**Interpretation of PET scans**

For all three studies, I reviewed PET reports and classified them as positive, negative or indeterminate for relapsed lymphoma. In generating the original PET report, the imaging specialist had access to prior investigation results, including the baseline and post-treatment FDG PET-CT studies. It should be noted that the time period covered by the study was mostly prior to the publication of both the International Harmonisation Project\(^{304}\) and the Deauville criteria\(^{305}\). A positive scan suggested relapsed lymphoma, with true positive results requiring either biopsy confirmation or unequivocal scan progression. A false positive scan was refuted by biopsy and/or follow up showing resolution of areas of increased FDG uptake. A negative scan was interpreted as negative for relapsed lymphoma: true negatives had no clinical relapse and false negatives manifest relapse within three months from the date of the scan. Cases in which uncertainty in the interpretation of the scan existed were referred to a review panel (which included one imaging specialist) and re-scored with majority opinion accepted. If no determination could be made scans were recorded as “indeterminate”. A “suspected relapse” was defined as relapse preceded by signs, symptoms or other clinical features (such as rising serum LDH). A “subclinical relapse” was defined as relapse detected without the above features, on the basis of imaging findings.

PET-CT scans were scored at time of initial reporting by visual analysis, using rules proposed by consensus criteria\(^{305}\). In order to control for changes in PET reporting style over time, blinded re-reading of PET scans was performed by an expert nuclear medicine physician. For the PMBL study, PET was interpreted using two criteria: 1)
visual analysis 2) 5-point scale (5PS).\textsuperscript{305} Change in SUVmax from baseline to post-treatment was explored but not presented because of missing data. For 5PS, residual activity with score $\geq 4$ was considered positive. For visual analysis, a similar threshold was applied but the volume and pattern of residual metabolic activity was also considered so that very small foci of residual uptake with score $\geq 4$ in the setting of bulky baseline abnormality was considered negative. In visual analysis, both iPET and ePET were categorized as complete metabolic response (CMR), partial metabolic response (PMR), stable disease (SD) or progressive disease (PD). CMR was defined by 5PS of 1-3. PMR was defined by reduction in intensity or extent of metabolic abnormality with 5PS 4-5, and PD as increased in intensity or extent of metabolic abnormality, or new sites of disease. If the response did not fit into either of these categories, the response would be defined as stable metabolic disease, although no patients met this criteria. For survival analysis purposes in the PMBL study, patients were compared using the outcomes of both methods.

**Statistical analysis**

Continuous variables are expressed as median and range and compared using the unpaired t-test. Non-normally distributed variables are expressed as median and range, and compared using Mann-Whitney U-test. Categorical variables are reported as percentages, and compared using Fisher’s exact test. Event-free survival, overall survival (OS) and time to relapse were determined using the method of Kaplan and Meier, with curve comparisons using log-rank analysis. A $P$-value $<0.05$ was considered significant.
CNS relapse in aggressive NHL

Both studies were retrospective, multi-centre analyses. The DLBCL study compared three different forms of CNS prophylaxis on the incidence of CNS relapse in patients with DLBCL judged at high risk of this complication. Patients were identified by searching institutional databases from 1996 to 2011 (to allow a minimum of two years of follow up) for patients with a confirmed histologic diagnosis of DLBCL by WHO criteria. Patients with DLBCL following histologic transformation of low-grade lymphoma and HIV-associated DLBCL were included, however patients with Burkitt or Burkitt-like lymphoma and patients with CNS involvement at diagnosis were excluded. Data collection was compliant with local Institutional Review Board requirements at each site. Patients were selected for CNS prophylaxis strategies by their primary managing haematologist if they fulfilled two or more of the following criteria: 1) multiple extranodal sites; 2) raised serum LDH; 3) or B-symptoms. Also, involvement of specific high-risk anatomical sites i.e. bone marrow (with large cell lymphoma), breast, testis, kidney, adrenal glands, paranasal sinus (based on data from the pre-rituximab era prior to the initiation of this policy), nasopharynx, liver and paravertebral was also considered an indication for CNS prophylaxis.

The features of patients by treatment groups in summarised in Table 6. In brief from 1991 - 2003 patients received CHOP and IT MTX (group 1). As previously described, our units adopted a policy of adding high-dose intravenous (IV) MTX at different times: PMCC and the Royal Brisbane and Women’s (RBWH) in 2003 and Monash Medical Centre (MMC) in 2007. This consisted of 1-3g/m² (tailored according to renal function) on Days 1 and 15 commenced two to four weeks after the completion of the CHOP-like regimen (group 2). Patients <65 years of age with age adjusted IPI of ≥2 were treated with dose intensive therapy containing anti-metabolites (Hyper-CVAD or CODOXM-IVAC, with rituximab after it became available – group 3). Patients also received IT MTX (12mg via lumbar puncture) with each cycle of chemotherapy, aiming for a total of six doses unless contraindicated, patients refused, or unacceptable toxicity. A summary of chemotherapy protocols used can be found in the appendix.
Materials and Methodology

Table 6 Comparison of treatment strategies for contributing centers. Abbreviations: aaIPI = age adjusted international prognostic index; R-HyperCVAD = rituximab, hyper fractionated cyclophosphamide, doxorubicin, vincristine, dexamethasone; R-MA = rituximab, high-dose methotrexate, high-dose cytarabine; CODOXM/IVAC = cyclophosphamide, vincristine, doxorubicin, methotrexate, ifosfamide, etoposide, cytarabine; R-MACOPB = rituximab, methotrexate, doxorubicin, cyclophosphamide, vincristine, prednisolone, bleomycin. R-CHOP = rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone; HD-MTX = high dose intravenous methotrexate. See Appendix for dosage and administration details.

<table>
<thead>
<tr>
<th></th>
<th>Royal Brisbane and Womens’ Hospital</th>
<th>Monash Medical Centre</th>
<th>Peter MacCallum Cancer Centre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>35</td>
<td>39</td>
<td>143</td>
</tr>
<tr>
<td>Primary chemotherapy (age &lt;60 + aaIPI 2,3)</td>
<td>R-Hyper-CVAD/R-MA</td>
<td>CODOXM/IVAC R-MACOPB</td>
<td>R-Hyper-CVAD/R-MA</td>
</tr>
<tr>
<td>Primary chemotherapy (all others)</td>
<td>R-CHOP</td>
<td>R-CHOP</td>
<td>R-CHOP</td>
</tr>
<tr>
<td>Year HD-MTX commenced</td>
<td>2003</td>
<td>2007</td>
<td>2003</td>
</tr>
</tbody>
</table>

CNS staging with lumbar puncture and cerebrospinal fluid (CSF) analysis for cytology, flow cytometry and biochemical analysis was typically performed at baseline, however baseline neuroimaging was typically only performed in the presence of clinical evidence suggesting CNS lymphoma or positive CSF cytology or flow cytometry. CNS involvement was defined by one or more of (1) histologically confirmed CNS involvement, (2) neuroimaging findings compatible with CNS involvement with lymphoma, in conjunction with consistent clinical presentation and the absence of other clinically feasible diagnosis, or (3) positive CSF (lymphoma cells detected by cytology and/or flow-cytometry).
CNS relapse in mantle cell lymphoma

I conducted a multicentre, retrospective case series through 14 sites within the European Mantle Cell Lymphoma Network (EMCLN). Each site fulfilled their individual institutional requirements for review of medical records. I requested sites report 1) the total number of MCL cases and 2) the number with CNS involvement diagnosed at any time during follow-up from their databases. I have included updated data from 15 previously reported cases (11 from reference\(^{308}\) and 4 reference\(^{194}\)), with the remaining 42 cases previously unreported. The initial diagnosis of MCL was based on published histological, immunophenotypic and molecular criteria.\(^2\) Although no centralised pathology review was performed, cyclin D1 expression by IHC and/or t(11;14) by FISH or conventional karyotype analysis was required for inclusion. Member sites of the EMCLN have considerable experience in the diagnosis and treatment of MCL, through numerous prior clinical\(^{309-311}\) and pathological\(^{312-314}\) studies. Staging required a minimum of CT scan of the body, physical examination and bone marrow biopsy. Due to the retrospective design, baseline CNS staging, PET-CT and endoscopy were not mandatory.

I collected the following data on MCL patients with CNS involvement:

**Baseline characteristics**

Demographics, date of diagnosis, stage, histological subtype, Ki-67 (IHC), total lymphocyte and leukocyte counts, B-symptoms, Eastern Cooperative group (ECOG) performance status, serum lactate dehydrogenase (LDH), \(\beta_2\)-microglobulin, bulk (defined as maximal nodal diameter >10 cm), number and sites of extranodal involvement. Mantle-cell Lymphoma International Prognostic Index (MIPI) was derived as published.\(^29\)

**Disease features at diagnosis of CNS involvement**

Date of proven CNS involvement, disease status (i.e. initial diagnosis, isolated CNS relapse, concurrent systemic relapse), neurological symptoms, cerebrospinal fluid (CSF) features, imaging findings, prior treatment.
Treatment for CNS disease and outcome

Chemotherapy regimen including use of intrathecal chemotherapy, radiotherapy, response to therapy (investigator determined according to standard criteria\textsuperscript{315}), outcome at last follow-up, date of relapse/death and cause of death. CNS involvement was defined by at least one of (1) histologically confirmed CNS involvement, (2) neuro-imaging findings compatible with CNS involvement with lymphoma, in conjunction with consistent clinical presentation and the absence of other clinically feasible diagnosis, or (3) positive CSF (lymphoma cells detected by cytology and/or flow-cytometry). Correction for peripheral blood contamination was performed according to the discretion of each individual laboratory: methods for diagnosing peripheral blood contamination included simultaneous analysis of peripheral blood by flow-cytometry, comparison of red-cell to white-cell ratio and presence of granulocyte clusters.

Statistical analysis

Continuous variables are expressed as median and range and compared using the Mann-Whitney U-test. Categorical variables are reported as proportions, and compared using Chi squared or Kruskal-Wallis tests, as appropriate. Progression-free survival (PFS), overall survival (OS) and time to CNS relapse were determined from date of diagnosis using the method of Kaplan and Meier,\textsuperscript{316} and compared using log-rank analysis. A PFS event was defined by any one of CNS or systemic relapse or death from any cause. Cumulative incidence of CNS relapse was calculated using the Kaplan-Meier method and competing risk regression analysis using Fine and Gray’s proportional hazard model.\textsuperscript{317} In this analysis, death without CNS relapse was defined as the competing risk. Statistical analysis was performed using STATA version 12.1 (Statacorp, College Station, TX) and any P-value \(<0.05\) was considered significant.
Materials and Methodology

NMP and anti-CD20 monoclonal antibodies in NHL

Introduction

This section outlines the general material and methods employed in the science section of the thesis.

Materials

The following materials were used during the experiments outlined.

Reagents

- Ficoll-paque was purchased from GE Healthcare Life sciences
- RPMI media 1640 500ml bottles was purchased from Gibco/Life technologies
- 2% human AB serum (obtained from healthy donors via the Australian Red Cross Blood Service (West Melbourne, Australia)
- Phosphate buffered saline (PBS) was manufactured in-house in the Cancer Immunology Research program, Peter MacCallum Cancer Centre, East Melbourne, VIC, Australia
- Bovine serum albumin was purchased from Life technologies
- MACS buffer was made from PBS (pH 7.2) with 0.5% bovine serum albumin (BSA) and 2mM EDTA
- FACS buffer was made from PBS with 1% BSA and 0.1% sodium azide
- MACS NK-cell negative isolation kit was purchased from Miltenyi Biotech, Bergisch Gladbach, Germany
- LS separation columns were purchased from Miltenyi Biotech, Bergisch Gladbach, Germany
- Healthy donor peripheral blood mononuclear cells, obtained fresh from healthy volunteer blood donors via the Australian Red Cross Blood Service (West Melbourne, Australia)
- targets (Raji and Daudi cell lines, obtained American Type Culture Collection, Manassas, VA)
• anti-CD20 mAbs
  o clinical grade rituximab (20mg/ml), obinutuzumab (50mg/ml) and trastuzumab (22mg/ml) obtained from Hofman-La Roche pharmaceuticals (Basel, Switzerland)
  o ofatumumab (100mg/ml) obtained from GlaxoSmithKline (Brentford, London, UK)
• Lenalidomide was purchased from Celgene Australia, Sydney, NSW, Australia
• NMP was purchased from Sigma-Aldrich Australia, Crows Nest, NSW, Australia

Protocol for processing Buffy pack to obtain bulk PBMCs

This protocol was obtained from Pasquale Petrone, Research Assistant, Peter MacCallum Cancer Centre.

Method

Discard all blood-contaminated waste into a separate bag before placing into waste bin (double bagged). In a clean tissue culture hood, I set up the Ficoll gradient by adding 15 ml Ficoll-paque into 4x50 ml Falcon tubes with filters. The tubes were spun at 1400 rpm for 10 mins. The end of tubing was cut off from the buffy coat bag, and the blood was squeezed into 2 x 50 ml Falcon tubes (25 ml into each). The blood was then diluted by adding 25 ml sterile PBS into each tube, mixed by inverting tubes. Then 25-30ml of diluted blood was pipetted into each Falcon tube containing the ficoll gradient, spun at 1000rpm for 10 mins. The plasma layer (approximately 10ml) was discarded before collecting the lymphocytes, which lay just above the filter. Remove approx.

The lymphocyte layer was then aspirated with a pipette and placed into 50 ml tubes, then topped with sterile PBS. The lymphocytes were then spun at 2000rpm for 15 mins, supernatant aspirated and cell pellet washed x3 in PBS (with spins at 1400 rpm for 10 mins between each wash step). The final step was resuspending cells in RPMI + 10% FCS and counting.
NK cell purification from PBMCs

In my experiments I used both bulk PBMCs and purified NK cells. To obtain purified NK cells, I used a commercial magnetic activated cell sorting (MACS) NK cell negative isolation kit. The protocol for magnetic separation was performed according to manufacturer’s instructions.

The first step was the preparation of MACS buffer, which was made from PBS (pH 7.2), 0.5% BDA, 2mM EDTA. The buffer was then degassed and ready for use. During all stages in magnetic labelling and separation cells were kept cold using pre-cooled suspensions. I typically selected the LS separation column, designed for up to $10^8$ cells. The PBMC cell suspension as centrifuged at 300G for 10min, the supernatant aspirated, and the pellet resuspend in 40μL buffer per $10^7$ cells. A 30μM pre-separation filter was used to remove clumps and debris, then 10μL of NK-cell antibody cocktail was added per $10^7$ cells. The suspension was then mixed well and incubated for 5 minutes at 4C. 30μL buffer per $10^7$ cells, then 20μL of NK-cell antibody cocktail per $10^7$ cells was added. The suspension was then mixed well and incubated for 10 minutes at 4C. The cells (up to $10^9$) were then resuspended cells in 500μL of buffer.

For the magnetic separation, the LS column was placed in on a MACS separation stand within a strong magnetic field. The column was prepared by rinsing with 3ml of buffer, and the cell suspension was applied onto the column. The flow through containing enriched NK cells was collected. To ensure maximum yield, the column was washed with unlabelled cells 3ml of MACS buffer. To evaluation of NK-cell purity, the aliquot was stained with antibodies (as below) and analyse by flow cytometry (FACS Canto II Loader cytometer).

Table 7  Experimental design for analysing NK cell purity after MACS isolation

<table>
<thead>
<tr>
<th>Tube</th>
<th>contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>comp controls</td>
<td>CD45 CC</td>
</tr>
<tr>
<td></td>
<td>CD3 CC</td>
</tr>
<tr>
<td></td>
<td>CD4 CC</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>CD8 CC</td>
<td>CD8-PECy7</td>
</tr>
<tr>
<td>CD14 CC</td>
<td>CD14-APC</td>
</tr>
<tr>
<td>CD19 CC</td>
<td>CD19-FITC</td>
</tr>
<tr>
<td>CD56 CC</td>
<td>CD56-PE</td>
</tr>
</tbody>
</table>

**Methods**

The aliquots from MACS experiment above were resuspended in 1mL FACS buffer in a labelled 10mL tube. To ensure accurate analysis, each FACS tube required 50uL cells (for 11 tubes, this represented 550uL cells). The cells were therefore resuspended in 600 uL. Antibody-fluorophore conjugates stored on rack on ice on bench top 1-2uL delivered to appropriate FACS tube using P10 pipette. The cells added to labelled FACS tubes, film added, rack wrapped in aluminium foil for subsequent analysis.

At FACS Canto Loader, a gating strategy was selected from template. The voltages were set to spread MFI from 0 -10^3 using comp controls, with live compensation adjustments made for all fluorophore combinations. The events were then captured both compensated and uncompensated, saved and exported to to FloJo (Treestar Inc, Ashland, OR) for analysis.
Materials and Methodology

Table 8 Antibody panel used for confirming NK cell purity after negative isolation using MACS kit

<table>
<thead>
<tr>
<th>FLurochrome</th>
<th>Fluorophore-Antibody</th>
<th>dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>FL1</td>
<td>CD19-FITC</td>
<td>1:100</td>
</tr>
<tr>
<td>FL2</td>
<td>CD56-PE</td>
<td>1:40</td>
</tr>
<tr>
<td>FL3</td>
<td>CD45-PerCP</td>
<td>1:100</td>
</tr>
<tr>
<td>FL4</td>
<td>CD8-PECy7</td>
<td>1:200</td>
</tr>
<tr>
<td>FL5</td>
<td>CD14-APC</td>
<td>1:25</td>
</tr>
<tr>
<td>FL6</td>
<td>CD4-AmCyan</td>
<td>1:400</td>
</tr>
<tr>
<td>FL7</td>
<td>CD3-PacBlue</td>
<td>1:400</td>
</tr>
</tbody>
</table>

ADCC assay

The protocol for ADCC is a modification of the method reported by Wu et al.282 Two forms of effectors were used: bulk PBMCs and purified NK cells. To prepare the effectors, either bulk PBMCs or MACS purified NK cells. The effectors were resuscitated in low dose IL-2 overnight in 12 well plates at 37C (5% CO2). The cells were then consolidated into one tube, resuspend in culture media at 1x10⁷/ml and counted. The effectors were then pipetted at varying E:T ratios (50 → 5:1) depending on the requirements of the specific experiment. To label the targets, 1x 10⁶ Raji and Daudi cells were washed in media and resuspend on ice in two separate 10ml Falcon tubes (in about 200µL of media). To this suspension ⁵¹Cr equivalent to 100uCi was added, and then incubated for 2 hours at 37C in 5% CO₂. Following labelling, the cells were spun at 1400rpm for 4 minutes to remove excess ⁵¹Cr and washed x 3 to ensure spontaneous ⁵¹Cr release was minimised. The concentration of the cells was adjusted to 1x10⁵/ml, and 10µL cell suspension (1x10⁴ cells) was added to each well. The next step was the addition of the various anti-CD20 mAb at 5µg/ml. This was achieved through the addition of 50µl of mAb solution to 50µl of Raji and Daudi cells, incubating for 37C for 30 min and washing off unbound antibody. Next, the targets were treated with bound antibody to effectors at the appropriate concentration for the experimental condition. The positive control was 50µl of 10% SDS (to achieve
complete cell lysis and Cr release); the negative control was 50μl of culture media, to estimate spontaneous release. The target-effector combination was then spun at 400G for 5 min to associate the cells, and incubated at 37C for 4 hours. At this point, the plate was spun 1400rpm for 4 minutes and 50μl of supernatant was harvest and counted using a gamma counter. Each experimental condition was carried out in triplicate, and the individual counts were recorded. Specific lysis was then calculated using the following formula: (measured – spontaneous)/(total – spontaneous) x 100.

**FACS analysis of Raji cell line treated with rituximab**

To investigate CD20 expression on Raji cells pre- and post-treatment with rituximab at 37C for 30 minutes, Raji cells harvested from culture (1x10^7/ml), washed in media, resuspended in 1mL FACS buffer in a labelled 10mL tube. In each FACS tube approximately 50ul (5e5) cells per tube was pipetted. The different experimental conditions included varying temperatures and times (37C for 30 min and 4C for 5 min) and with or without anti-CD20 mAb rituximab prior to incubation. Each antibody-fluorophore conjugates was stored on rack on ice on bench top, and 1-4uL delivered to appropriate FACS tube using P10 pipette as outlined below. The cells added to labelled FACS tubes, film added, rack wrapped in aluminium foil for later analysis.

<table>
<thead>
<tr>
<th>Fluorophore-Antibody</th>
<th>dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD20-PECy7</td>
<td>1:25</td>
</tr>
<tr>
<td>CD20 isotype (mouse anti-human IgG1-PECy7)</td>
<td>1:25</td>
</tr>
<tr>
<td>mouse anti-human IgG1-FITC</td>
<td>1:100</td>
</tr>
<tr>
<td>IgG1 isotype (anti-mouse IgG1-FITC)</td>
<td>1:25</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>tube</th>
<th>Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>comp controls</td>
<td>CD20 CC</td>
</tr>
<tr>
<td></td>
<td>IgG1 CC</td>
</tr>
<tr>
<td>isotype controls</td>
<td>CD20 isotype</td>
</tr>
<tr>
<td></td>
<td>IgG1 isotype</td>
</tr>
</tbody>
</table>
Rational clinical trial design in lymphoid malignancies

The methods for this section have been deliberately left blank, as all relevant work pertaining to this section is contained in the Results section.
Results I

The use of PET to identify patients with DLBCL at high risk of failure

Surveillance PET in DLBCL

I identified 116 patients with de novo DLBCL within the specified time period. Eighty-four were ineligible for the following reasons: histological transformation from a variety of indolent lymphoma subtypes (n=29), no surveillance PET-CT scans performed (predominantly patients aged over 70 or otherwise unfit for intensification, n=26), did not achieve CMR (n=14), end of treatment PET positive for another reason e.g. sarcoidosis or infection (n=7), palliative management only (n=5), had prior chemotherapy at another institution (n=3). Only two patients without surveillance PET scans relapsed within six months of completing therapy (3.2 and 5.4 months) only one of whom was a suitable candidate for autologous stem cell transplant. Of the cohort (n=116) analysed, the median was age 59 years (range 16 – 85), 54% were male and 51% had an elevated serum LDH. Eastern Cooperative Oncology Group (ECOG) performance status was ≤ 1 in 96% of patients, with <2 sites of extranodal involvement in 75% and baseline IPI[24] - determined using PET-CT and bone marrow biopsy was <3 in 77 (66%) and ≥3 in 37 (32%) of patients. In two patients baseline IPI could not be calculated due to missing data. Initial immunochemotherapy was R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine and prednisolone) in 110 (95%), while six (5%) received R-Hyper-CVAD (rituximab, hyper-fractionated cyclophosphamide, vincristine, doxorubicin and dexamethasone) alternating with high-dose methotrexate and cytarabine at the discretion of the treating clinician due to the presence of high risk features. Sixty-six patients (57%) received radiotherapy as consolidation for bulky or localised disease.

In 116 patients, 450 surveillance PET-CT scans were performed with a median of four scans per patient (range 1-10). At 1st January 2012, with a median of 53 (range 8 – 133) months follow-up from completion of therapy, 13 patients (11%) had relapsed and 97 (84%) remain relapse-free in ongoing complete remission (CR). Features
associated with relapse in these patients are displayed in Table 9. Of those who relapsed, eight died from progressive disease and five are in remission after salvage therapy. Six patients died from other causes: gastric cancer (n=2), pneumonia complicating oesophageal cancer (n=1), ruptured abdominal aortic aneurysm (n=1), metastatic squamous cell carcinoma (n=1) and cause unknown, whilst in clinical remission (n=1).

Table 9 Factors associated with relapse after achieving a complete remission at the end of therapy (univariate analysis). IPI = international prognostic index, LDH = lactate dehydrogenase, EN = extranodal, ECOG = Eastern Cooperative Oncology Group. PET IPI is calculated using the stage based on PET rather than contrast CT. Reproduced from 77, with permission.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Relapse</th>
<th>No relapse</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>median age (years)</strong></td>
<td>n=13</td>
<td>n=103</td>
<td></td>
</tr>
<tr>
<td></td>
<td>59</td>
<td>59</td>
<td>0.96</td>
</tr>
<tr>
<td><strong>PET stage 3/4 at diagnosis</strong></td>
<td>11 (84%)</td>
<td>35 (34%)</td>
<td>0.005</td>
</tr>
<tr>
<td><strong>PET IPI 3-5</strong></td>
<td>8 (62%)</td>
<td>29 (28%)</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>2+ EN sites</strong></td>
<td>7 (54%)</td>
<td>22 (21%)</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>ECOG &gt;1</strong></td>
<td>3 (23%)</td>
<td>1 (1%)</td>
<td>0.004</td>
</tr>
<tr>
<td><strong>median LDH (IU/L)</strong></td>
<td>634</td>
<td>514</td>
<td>0.21</td>
</tr>
</tbody>
</table>

Test performance of PET-CT surveillance scanning

There were 13 true positive scans, six false positives, no false negatives and 424 true negatives. The PPV was 68% and the NPV 100%. Of the seven indeterminate scans, six were shown by follow up to be negative for lymphoma and one was biopsy confirmed to be positive. If include indeterminate scans by scoring the former as false positives and the latter as false negatives respectively, test performance remained robust with revised sensitivity 95%, specificity 97%, PPV 60% and NPV 99%.
However, when considering patients with baseline IPI ≥3 (n=37) there were eight true positives, two false positives, no false negatives, 112 true negatives and two indeterminate scans. Whilst sensitivity, specificity and NPV (100%) were essentially unchanged, the PPV increased to 80%. In patients with baseline IPI <3 (n=77), there were five true positives, four false positives, no false negatives, 312 true negatives and five indeterminate scans. This resulted in a lower PPV (56%). Most relapses (and therefore true positive scans) occurred within the first 18 months. The number of scans needed to detect one subclinical relapse was analysed as a function of both baseline IPI (≥3 vs <3) as well as time following completion of primary therapy. Averaged over the first 18 months following completion of therapy, 92 scans were performed to detect one subclinical relapse in patients with baseline IPI <3, but only 22 scans in patients with baseline IPI ≥3 (86 scans to detect 4 subclinical relapses). Surveillance PET-CT had low yield after 18 months regardless of baseline IPI, with only one (clinically suspected) true positive result in a patient (baseline IPI 3) from a total of 170 scans (Table 10).
Table 10 Distribution of PET-CT results as a function of time elapsed from completion of primary chemotherapy for all patients. Reproduced from 77 with permission.

<table>
<thead>
<tr>
<th>months post treatment</th>
<th>0-6</th>
<th>6-12</th>
<th>12-18</th>
<th>18-24</th>
<th>24-36</th>
<th>36-48</th>
<th>48+</th>
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</thead>
<tbody>
<tr>
<td>indeterminate</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>false positives</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>true positives (suspected)</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>true positives (subclinical)</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>true negatives</td>
<td>91</td>
<td>96</td>
<td>68</td>
<td>50</td>
<td>66</td>
<td>31</td>
<td>22</td>
</tr>
<tr>
<td>total number of scans</td>
<td>99</td>
<td>105</td>
<td>74</td>
<td>51</td>
<td>68</td>
<td>31</td>
<td>22</td>
</tr>
</tbody>
</table>

Patterns of relapse

Two-thirds of relapses occurred within 18 months of completing chemotherapy and 85% within two years, with a median time to relapse of 12.8 months. The time distribution of surveillance PET-CT scans in the 13 relapsing patients is displayed in Figure 2.

Relapses were detected clinically in seven patients (54%) with examination findings \( n=4 \), fever \( n=2 \) or collapse \( n=1 \). Five (71%) suspected relapses occurred at sites which were previously uninvolved by DLBCL. PET-CT was concordant in all seven cases, with confirmatory biopsies including one case (intra-abdominal relapse) where PET directed the biopsy. In the remaining six cases, biopsy site was selected clinically. The remaining six relapses were subclinical, with three (50%) occurring at previously uninvolved sites. Four (67%) subclinical relapses detected by PET-CT would very likely
have been missed by CT alone as either nodal disease was <15mm (n=2) or relapse was extranodal (bony without structural abnormality n=2). There was no difference in overall survival between the two groups (P=0.73, Figure 10). Of six subclinical relapses, four had second line IPI <3 and two cases ≥3. Amongst seven suspected relapses, four had second line IPI <3, one case second line IPI was 3 and in two cases not evaluable due to serum LDH at relapse not being performed. There was no difference in second line IPI between the two groups. (P=1.00)

*Figure 10 Overall survival according to method of relapse detection. Reproduced from 77 with permission.*

Management of relapse

The median age of the 13 patients who relapsed (at the time of relapse) was 64 (range 21 to 82) years. All patients received salvage therapy, 11 with R-ICE (rituximab, ifosfamide, carboplatin and etoposide), one (who was 82) with R-CVP and one (who relapsed with follicular histology) with \(^{131}\)I-rituximab. Of the 11 patients receiving R-ICE, seven proceeded to cyclophosphamide, carmustine, etoposide (CBV) conditioned autologous stem cell transplant. The remaining four patients did not proceed to transplant because their disease was refractory (n=2) or they did not tolerate (n=2) salvage chemotherapy.
Six false positive scans for recurrent lymphoma occurred at a median of 9.0 (range 3.9 – 25.1) months following completion of treatment. Two false positives were in patients with baseline IPI ≥3 and four occurred in those with baseline IPI <3. The sites involved were the tonsils (n=2), a cervical lymph node (n=1), mediastinal nodes (n=2), and a peri-duodenal node (n=1). In all cases either biopsy (n=3) or clinical follow-up and resolution (n=3) demonstrated no recurrent lymphoma. There were seven indeterminate scans; two in patients with baseline IPI ≥3 and five in patients with baseline IPI <3. The sites involved were lung in the setting of a chest infection (n=1), tonsils (n=2), cervical (n=1), suboccipital (n=1), mediastinal (n=1) and inguinal nodes (n=1). In all but the final case (biopsy proven recurrent DLBCL) repeat scanning showed resolution of changes. In the terminology used by Zinzani, there were six ‘inconclusive negative’ and one ‘inconclusive positive’ scans. In 67% of false positive and indeterminate scans combined, the region of interpretative uncertainty was a nodal site involved on baseline PET-CT.

Second malignancies were detected by surveillance PET-CT in eight (7%) patients (Table 11). In addition, PET prompted colonoscopy and polypectomy in one patient. There were two false positive scans suggesting second malignancy, with PET-CT suggesting possible breast cancer in one patient (mammogram suggesting benign fibroadenoma) and colonic cancer in one patient (colonoscopy normal).
Table 11 Second malignancies detected by PET-CT during surveillance scanning. Abbreviations: M = male, F = female, SCC = squamous cell carcinoma. Reproduced from [77] with permission.

<table>
<thead>
<tr>
<th>Age</th>
<th>Sex</th>
<th>Second tumor</th>
<th>Months post treatment</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>M</td>
<td>Gastric (recurrent)</td>
<td>7</td>
<td>death (pyloric obstruction)</td>
</tr>
<tr>
<td>65</td>
<td>F</td>
<td>Hepatocellular</td>
<td>25</td>
<td>resection, alive in remission</td>
</tr>
<tr>
<td>70</td>
<td>M</td>
<td>SCC</td>
<td>30</td>
<td>palliative radiotherapy, death</td>
</tr>
<tr>
<td>62</td>
<td>M</td>
<td>oesophageal</td>
<td>30</td>
<td>resection, survived 28m</td>
</tr>
<tr>
<td>72</td>
<td>M</td>
<td>Prostate</td>
<td>6</td>
<td>alive, on anti-androgen Rx</td>
</tr>
<tr>
<td>63</td>
<td>M</td>
<td>SCC</td>
<td>13</td>
<td>T1N1 left piriform fossa, curative RT</td>
</tr>
<tr>
<td>57</td>
<td>F</td>
<td>Breast</td>
<td>5</td>
<td>mastectomy, alive in remission</td>
</tr>
<tr>
<td>81</td>
<td>F</td>
<td>Breast</td>
<td>6</td>
<td>lumpectomy/radiotherapy → remission, death cause unknown 50 months</td>
</tr>
</tbody>
</table>
Results

Surveillance PET in transformed indolent lymphoma

Ninety-eight patients with TrIL were identified of whom 43 were ineligible for the following reasons: no surveillance PET-CT scan (n=35), did not achieve CMR (n=7), insufficient follow up data available (n=1) leaving 55 available for analysis. Of these, 30 patients were in Group 1 (concurrent diagnosis of histologic transformation and indolent lymphoma) and 25 patients were in Group 2 (delayed diagnosis of transformation). The characteristics of these patients are summarised in Table 12; characteristics were similar between groups apart from more patients in group 1 receiving rituximab as part of their primary chemotherapy (93% v 64%, P=0.007).

Reproduced from 319, with permission.

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>entire group</th>
<th>Group 1 Evidence of transformed histology at time of initial diagnosis of lymphoma</th>
<th>Group 2 Initial diagnosis of indolent lymphoma with time interval to transformation</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>55</td>
<td>30</td>
<td>25</td>
<td>0.56</td>
</tr>
<tr>
<td>Median age (range), years</td>
<td>59 (35 – 83)</td>
<td>57 (37 – 72)</td>
<td>61 (35 – 83)</td>
<td></td>
</tr>
<tr>
<td>Female (%)</td>
<td>22 (40%)</td>
<td>15 (50%)</td>
<td>7 (28%)</td>
<td>0.10</td>
</tr>
<tr>
<td>Chemotherapy prior to transformation</td>
<td>N/A</td>
<td>17/25 (68%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indolent histology (data available n=55)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FL</td>
<td>46 (65%)</td>
<td>28 (93%)</td>
<td>18 (72%)</td>
<td>0.17</td>
</tr>
<tr>
<td>MALT</td>
<td>5 (9%)</td>
<td>2 (7%)</td>
<td>3 (12%)</td>
<td></td>
</tr>
<tr>
<td>CLL/SLL</td>
<td>3 (5%)</td>
<td></td>
<td>3 (12%)</td>
<td></td>
</tr>
<tr>
<td>other</td>
<td>1 (2%)</td>
<td></td>
<td>1 (4%)</td>
<td></td>
</tr>
<tr>
<td>IPI at transformation (n=52)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>low (0 – 1)</td>
<td>15 (27%)</td>
<td>9 (30%)</td>
<td>6 (26%)</td>
<td>0.24</td>
</tr>
<tr>
<td>intermediate (2 – 3)</td>
<td>31 (61%)</td>
<td>16 (56%)</td>
<td>15 (65%)</td>
<td></td>
</tr>
<tr>
<td>high (4 – 5)</td>
<td>6 (12%)</td>
<td>4 (14%)</td>
<td>2 (9%)</td>
<td></td>
</tr>
<tr>
<td>Median serum LDH:ULN (range) (n=51)</td>
<td>1.03 (0.6 – 4.7)</td>
<td>1.0 (0.6 – 4.7)</td>
<td>1.1 (0.7 – 2.2)</td>
<td>0.22</td>
</tr>
<tr>
<td>Extranodal sites (n=53)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-1</td>
<td>33 (62%)</td>
<td>19 (66%)</td>
<td>14 (58%)</td>
<td>0.35</td>
</tr>
<tr>
<td>≥2</td>
<td>20 (38%)</td>
<td>10 (33%)</td>
<td>10 (42%)</td>
<td></td>
</tr>
<tr>
<td>ECOG performance status (n=54)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-1</td>
<td>51 (95%)</td>
<td>29 (97%)</td>
<td>22 (92%)</td>
<td>0.14</td>
</tr>
<tr>
<td>≥2</td>
<td>3 (5%)</td>
<td>1 (3%)</td>
<td>2 (8%)</td>
<td></td>
</tr>
<tr>
<td>Stage (n=55)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-II</td>
<td>11 (20%)</td>
<td>7 (23%)</td>
<td>4 (13%)</td>
<td>0.42</td>
</tr>
</tbody>
</table>
### Results

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n=52)</th>
<th>Group 2 (n=55)</th>
<th>Group 3 (n=55)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>III-IV</td>
<td>44 (80%)</td>
<td>23 (77%)</td>
<td>21 (87%)</td>
<td></td>
</tr>
<tr>
<td>B symptoms (n=52)</td>
<td>12 (23%)</td>
<td>7 (24%)</td>
<td>5 (22%)</td>
<td>0.52</td>
</tr>
<tr>
<td>Stem cell transplantation after histologic transformation (n=55)</td>
<td>38 (69%)</td>
<td>21 (70%)</td>
<td>17 (68%)</td>
<td>0.87</td>
</tr>
<tr>
<td>Rituximab as part of initial therapy (n=55)</td>
<td>44 (80%)</td>
<td>28 (93%)</td>
<td>16 (64%)</td>
<td><strong>0.007</strong></td>
</tr>
<tr>
<td>Maintenance rituximab after histologic transformation (n=55)</td>
<td>12 (21%)</td>
<td>5 (17%)</td>
<td>7 (23%)</td>
<td>0.31</td>
</tr>
</tbody>
</table>

Fifty-three (96%) patients had biopsy proven DLBCL at time of transformation. After a median follow-up of 34 (range 3 – 191) months, the actuarial 3-year PFS for Groups 1 and 2 were 82% (95%CI 68 – 96%) and 70% (95%CI 52 – 88%) respectively. The actuarial 3-year OS were 89% (95%CI 78 – 99%) and 88% (75 – 99%) respectively. There was no significant difference in PFS (P=0.54, Figure 10) or OS (P=0.60) between the two groups.
Table 13 Progression Free Survival by timing of transformation. Group 1 = concurrent, Group 2 = delayed.

Other potential prognostic factors including IPI score at time of transformation, advanced stage, serum LDH at transformation and type of indolent histology were not shown to be predictive of PFS by univariate analysis. (data not shown)

In total 180 surveillance PET-CT scans were performed during the period analysed, 103 and 77 in groups 1 and 2 respectively. This equated to a median of 3 (range 1-10) scans per patient. The results of these scans as a function of time are displayed in Table 14 (Group 1) and
Table 15 (Group 2) respectively. In total, there were 153 true negatives, four false positives, one false negative, seven indeterminate and 15 true positives.

Table 14 Results of surveillance PET-CT scans, by time elapsed since completion of therapy for group 1 (patients with histologic transformation present at time of initial diagnosis of indolent lymphoma).

<table>
<thead>
<tr>
<th></th>
<th>0-6 mo</th>
<th>6-12 mo</th>
<th>12-18 mo</th>
<th>18-24 mo</th>
<th>24-36 mo</th>
<th>36-48 mo</th>
<th>48+ mo</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>True positives (subclinical)</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>True positives (suspected)</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>False positives</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>False negatives</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>True negatives</td>
<td>18</td>
<td>23</td>
<td>15</td>
<td>14</td>
<td>11</td>
<td>5</td>
<td>5</td>
<td>91</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>26</td>
<td>21</td>
<td>15</td>
<td>12</td>
<td>6</td>
<td>5</td>
<td>103</td>
</tr>
<tr>
<td>% PET scans detecting subclinical relapse</td>
<td>0%</td>
<td>4%</td>
<td>0%</td>
<td>7%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>
Table 15 Results of surveillance PET-CT scans, by time elapsed since completion of therapy for group 2 (patients with time delay between initial diagnosis of indolent lymphoma and histologic transformation).

<table>
<thead>
<tr>
<th>Time Interval (mo)</th>
<th>0-6 mo</th>
<th>6-12 mo</th>
<th>12-18 mo</th>
<th>18-24 mo</th>
<th>24-36 mo</th>
<th>36-48 mo</th>
<th>48+ mo</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>True positives (subclinical)</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>True positives (suspected)</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>False positives</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>False negatives</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>True negatives</td>
<td>16</td>
<td>15</td>
<td>10</td>
<td>7</td>
<td>9</td>
<td>5</td>
<td>0</td>
<td>62</td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
<td>18</td>
<td>14</td>
<td>8</td>
<td>10</td>
<td>8</td>
<td>0</td>
<td>77</td>
</tr>
</tbody>
</table>

Considering the five indeterminate scans which were not followed by relapse as false positives, and two indeterminate scans which were followed by relapse as false negatives, the overall specificity of PET-CT for detecting relapse was 94%, sensitivity 83%, positive predictive value 63% and negative predictive value 98%. Considering groups 1 and 2 separately resulted in similar test performance (Table 16).
Table 16 Comparison of surveillance PET-CT test performance between Group 1 (concurrent diagnosis of histologic transformation and indolent lymphoma), Group 2 (delayed diagnosis of histologic transformation) and the entire cohort.

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>entire cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>86%</td>
<td>90%</td>
<td>83%</td>
</tr>
<tr>
<td>Specificity</td>
<td>95%</td>
<td>93%</td>
<td>94%</td>
</tr>
<tr>
<td>positive predictive value</td>
<td>55%</td>
<td>64%</td>
<td>63%</td>
</tr>
<tr>
<td>negative predictive value</td>
<td>99%</td>
<td>98%</td>
<td>98%</td>
</tr>
</tbody>
</table>

Sixteen patients experienced relapsed lymphoma; seven were subclinical (three in group 1, four in group 2), and nine symptomatic (three in group 1, six in group 2). The symptomatic relapses were invariably with DLBCL and occurred at a median of 17 (range 2.8 – 41.8) months post therapy. The presenting symptoms were patient-detected lymphadenopathy (n=6), hypercalcemia (n=1), cranial nerve palsies in a patient with isolated CNS relapse (n=1), and shortness of breath in a patient who developed malignant pleural effusion (n=1). All patients were confirmed histologically by excisional biopsy (n=6), lumbar puncture (n=1) or analysis of pleural fluid (n=1). Eight of the nine were true positive scans; the patient presenting with shortness of breath due to malignant effusion had a false negative PET-CT scan at the time of relapse (although the pleural effusion was demonstrated by the CT component, the FDG avidity was considered physiological and the scan was reported as ongoing complete metabolic response of the lymphoma).

The seven subclinical relapses occurred a median of 9.8 (range 2.9 – 20.6) months following completion of therapy. In six cases, biopsy of the lesion with the highest FDG avidity showed follicular lymphoma. In one case, CT guided core biopsies of a right retrocrural node was non-diagnostic but the lymph node continued to enlarge on interval imaging and the patient was treated for presumed relapsed lymphoma with salvage chemotherapy, involved field radiotherapy, achieved CMR and remains in remission seven years later. Although the histology of relapsed lymphoma was not
proven to be follicular, given the durability of response this seems more likely. The patients with subclinical/low grade relapse had a trend towards better OS post relapse compared to those with symptomatic/high grade relapse (3-year OS 100% vs 56%, \(P=0.07\)).

The four false positive results occurred at 3, 8, 17 and 37 months post therapy, respectively. In all cases the abnormal \(^{18}\)F-FDG uptake was confined to the mediastinum and/or Waldeyer’s ring; two of these cases had symptoms of upper respiratory tract infection, one patient had recent Varicella zoster infection, and one patient had no symptoms to suggest infection. All received follow up scans around three months later, which showed resolution of prior abnormalities. The seven indeterminate scans showed increased FDG uptake in the cervical (n=2), tonsillar (n=2), axillary (n=1), para-aortic (n=1) and inguinal (n=1) regions. In five cases there was clinical suspicion of infective process to explain the FDG uptake; in one case (uptake isolated to an inguinal node) progressed in intensity and extent on a follow up scan six months later and was associated with increased multifocal nodal abnormalities in keeping with unequivocally relapsed disease (interpreted as a true positive scan). An excisional biopsy showed follicular lymphoma. In the other case low volume axillary lymphadenopathy was reported as potentially reactive, but on repeat scanning just over three months later there was persistent abnormal uptake. Excisional biopsy again revealed follicular lymphoma.

**Interim PET in PMBL**

I identified 28 patients with PMBL within the specified time period. The median age was 33 (range 18 – 69) years, 60% were female and 56% had an elevated serum LDH. ECOG performance status was ≤1 in 96% of patients. There were ≥2 sites of extranodal involvement in 33% of patients; the commonest sites were pleural effusion or lung parenchyma (29%), pericardium or pericardial effusion (14%) and bone (11%). Age adjusted International Prognostic Index (aaIPI) was low (0 points), low-intermediate (1 point), high-intermediate (2 points) and high (3 points) in 30%, 39, 17%
and 13% of cases respectively. Bulky disease (defined as maximum diameter ≥7.5cm) was present in 19 (68%) patients.

Initial therapy was R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine and prednisolone) Q14 in 15 (54%) and Q21 in 5 (18%); in total 20 (71%), CHOP Q14 without rituximab in one (3%), dose adjusted EPOCH-R (etoposide, prednisolone, doxorubicin, vincristine, rituximab160) in four (14%), Hyper-CVAD (hyper-fractionated cyclophosphamide, vincristine, doxorubicin and dexamethasone) alternating with high-dose methotrexate and cytarabine320 in two (7%) and Hyper-CVAD with rituximab in one patient (3%). Thus 25 patients (89%) received rituximab as part of their initial treatment and 3 (treated between 2001 and 2004 prior to reimbursement of rituximab in Australia) did not. All 20 patients treated with R-CHOP received 6 cycles, with the exception of two patients in whom therapy was escalated in response to iPET result after 3 cycles of R-CHOP. See “Patients with intermediate and positive ePET” below. Radiotherapy was typically planned prior to commencing chemotherapy, but in select cases the decision was made in response to positive ePET scan. Overall, radiotherapy was delivered to 17 (61%) of patients; 14/21 (67%) patients treated with CHOP+/−R, 1/4 (25%) patients treated with DA-EPOCH-R, and 2/3 (75%) patients treated with HyperCVAD+/−R.

After a median follow up of 2.6 (range 0.4 – 11) years, four patients have developed relapsed disease and two of these have died (both of disease progression). The actuarial 2-year PFS and OS rates are 86% and 94%, respectively. All patients but one (who developed respiratory distress and could not remain supine) had bPET, 23 (82%) had iPET (after two (n=8, 35%), three (n=13, 56%) and four (n=2, 9%) cycles respectively) and all patients ePET. A comparison of methods of interpreting PET scans is displayed in Table 17 (iPET) and Table 18(ePET). The most striking feature was the high NPV of ePET (95-100%) regardless of method of interpretation. The PPV of ePET was moderate; semi-quantitative visual analysis or 5-point scale identified a subgroup with inferior PFS (P<0.0001, Figure 11). Tumor bulk (dichotomized at 7.5cm) was not predictive of positive iPET or ePET (P=1.0 and 0.64 respectively). I performed
an exploratory analysis of PFS by iPET result and found irrespective of method of interpretation, positive iPET was not predictive of inferior PFS (data not shown) although NPV was high (86-100%).

Patients with negative ePET (5PS 1-2)

Nineteen patients were negative at ePET (5PS 1-2) following R-CHOP (n=16), DA-EPOCH-R (n=2) and Hyper-CVAD (n=1) respectively. Involved field radiotherapy was applied to 10/16 (62%) patients receiving R-CHOP, and the patient who received Hyper-CVAD, but not to the patients receiving DA-EPOCH-R. Eighteen remain alive in clinical remission and one patient developed CNS relapse two months after completing therapy, and subsequently died of progressive disease at 1.2 years from diagnosis. After a median follow-up of 4.5 (range 0.4 – 11) years after completion of therapy, the 4-year actuarial PFS rate of this group was 94% (95% CI 84 – 100%).

Patients with intermediate (5PS 3) and positive ePET (5PS 4,5)

Seven patients had ePET 5PS of 4-5, and two patients a score of 3. A detailed summary of their initial therapy and clinical course is provided in Table 19. Figure 12 depicts the sequence of PET-CT scans for the 53 year-old female listed in Table 19. Figure 13 depicts a “flow diagram” of patient outcome as a function of 5PS, and is complementary to the survival curves depicted in Figure 11.
**Results**

*Figure 11* Progression free survival by end of treatment response assessment. Abbreviations: PFS = progression free survival, PET = positron emission tomography, 5PS = 5 point score, PD = progressive disease, PMR = partial metabolic response, CMR = complete metabolic response.

![PFS by ePET 5PS](chart1.png)

- 1-2
- 3
- 4-5

\[ P = 0.039 \]

*Figure 12* Sequence of PET-CT images for a patient with primary mediastinal B-cell lymphoma at baseline (A); after 3 cycles of R-CHOP (B); after 6 cycles of R-CHOP (C) and after salvage therapy with R-ICE (D). This patient subsequently underwent autologous stem cell transplant.
and had post transplant PET-CT persistent (stable) FDG avidity in the mediastinal mass, which was similar to that seen in image D. Biopsy of this mass (2.5 months post transplant) demonstrated foamy macrophages and necrotic change but no viable lymphoma.
Results I

Figure 13 Outcome of patients by end of treatment PET (5 point score). Abbreviations: iPET = interim position emission tomography scan, ePET = end of treatment position emission tomography scan, RT = radiotherapy, ASCT = autologous stem cell transplantation.
**Table 17 Predictive value for relapses of different methods of interpreting interim and end of treatment PET-CT scan. Abbreviations: PET = positron emission tomography, PPV = positive predictive value**

<table>
<thead>
<tr>
<th>Method</th>
<th>Interim PET (iPET)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Relapse(s)</td>
<td>PPV</td>
<td>Negative</td>
<td>Relapse(s)</td>
</tr>
<tr>
<td>Visual analysis</td>
<td>7/23 (30%)</td>
<td>1</td>
<td>14%</td>
<td>16</td>
<td>2</td>
</tr>
<tr>
<td>5 point scale ≥ 4</td>
<td>8/23 (35%)</td>
<td>2</td>
<td>25%</td>
<td>15</td>
<td>2</td>
</tr>
</tbody>
</table>
Table 18 Predictive value for relapses of different methods of interpreting interim and end of treatment PET-CT scan. Abbreviations: PET = positron emission tomography, PPV = positive predictive value, \( \Delta \text{SUVmax} \) = change in maximum standardised uptake.

<table>
<thead>
<tr>
<th>Method</th>
<th>Post-treatment PET (ePET)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Visual analysis</td>
<td>7/28 (25%)</td>
</tr>
<tr>
<td>5 point scale ≥ 4</td>
<td>7/28 (25%)</td>
</tr>
</tbody>
</table>
Table 19 Patients with positive ePET by 5PS (≥4). Abbreviations: (R)-CHOP = (rituximab), cyclophosphamide, doxorubicin, vincristine and prednisolone; DA-EPOCH-R = dose adjusted, infusional etoposide, prednisolone, doxorubicin, vincristine with rituximab; Hyper-CVAD = hyper-fractionated cyclophosphamide, vincristine, doxorubicin and dexamethasone) alternating with high-dose methotrexate and cytarabine; (R)-ICE = (rituximab), ifosfamide, carboplatin, etoposide; iPET 5PS = interim positron emission tomography result 5-point score; ePET 5PS = end of treatment positron emission tomography result 5-point score; PR = partial response; CR = complete response; PD = progressive disease; ASCT = autologous stem cell transplant. * this patient achieved a conventional partial response at the mediastinal mass, but new renal medullary lesion.

<table>
<thead>
<tr>
<th>Age, sex</th>
<th>Initial therapy</th>
<th>iPET SPS</th>
<th>2nd line treatment</th>
<th>end of treatment response</th>
<th>further treatment</th>
<th>status at last follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chemotherapy</td>
<td>#</td>
<td></td>
<td>ePET SPS</td>
<td>CT response</td>
<td>biopsy</td>
</tr>
<tr>
<td>32F</td>
<td>R-CHOP</td>
<td>3</td>
<td>5</td>
<td>R-ICE</td>
<td>3</td>
<td>SD</td>
</tr>
<tr>
<td>18F</td>
<td>R-Hyper-CVAD</td>
<td>5</td>
<td>4</td>
<td>R-ICE</td>
<td>3</td>
<td>PR</td>
</tr>
<tr>
<td>53F</td>
<td>R-CHOP</td>
<td>6</td>
<td>4</td>
<td>R-ICE</td>
<td>4</td>
<td>PR</td>
</tr>
<tr>
<td>25F</td>
<td>DA-EPOCH-R</td>
<td>6</td>
<td>4</td>
<td>R-ICE</td>
<td>4</td>
<td>PR</td>
</tr>
<tr>
<td>28M</td>
<td>Hyper-CVAD</td>
<td>4</td>
<td>5</td>
<td>R-ICE</td>
<td>4</td>
<td>PR</td>
</tr>
<tr>
<td>35M</td>
<td>DA-EPOCH-R</td>
<td>6</td>
<td>4</td>
<td>Hyper-CVAD</td>
<td>4</td>
<td>PR</td>
</tr>
<tr>
<td>38M</td>
<td>CHOP</td>
<td>8</td>
<td>3</td>
<td>ICE</td>
<td>5</td>
<td>PD</td>
</tr>
<tr>
<td>26M</td>
<td>R-CHOP</td>
<td>8</td>
<td>not done</td>
<td>R-ICE</td>
<td>5</td>
<td>PR*</td>
</tr>
<tr>
<td>29F</td>
<td>R-CHOP</td>
<td>3</td>
<td>4</td>
<td>Hyper-CVAD</td>
<td>5</td>
<td>PD</td>
</tr>
</tbody>
</table>
Results II

Reducing CNS relapse in patients with aggressive NHL

CNS relapse in DLBCL

I identified 217 patients with DLBCL judged as high risk for CNS involvement by the stated criteria. Thirty-five patients (15%) were treated at RBWH, 39 (18%) at MMC and 143 (69%) at PMCC. Group 1 (reference) was drawn from PMCC and MMC, whilst all centres contributed cases to groups 2 and 3. The baseline characteristics of patients in the three groups are summarized in Table 20. Fewer patients in group 1 received rituximab given the timeframe of its availability within Australia, and as expected patients selected for intensive approaches (group 3) were younger, had higher risk disease features (higher normalised serum LDH, B-symptoms). The distribution of extranodal sites for each of the three groups was similar, with a non-significant trend toward greater frequency of epidural/paraspinal disease in group 1. The majority of patients (84%) underwent baseline CSF analysis; the remaining 16% had no clinical evidence of CNS involvement at baseline and the first available CSF cytology was negative. The proportion of patients without baseline CSF analysis was similar between the three groups.
Table 20 Baseline characteristics of patients in each group. Abbreviations: MTX = methotrexate; LDH = lactate dehydrogenase; ULN = upper limit of normal; ECOG PS = Eastern Cooperative Oncology Group Performance Status; IPI = international prognostic index; R-HyperCVAD = rituximab, hyper fractionated cyclophosphamide, doxorubicin, vincristine, dexamethasone; R-MTX-ara-c = rituximab, high dose methotrexate, high dose cytarabine; CODOXM/IVAC = cyclophosphamide, vincristine, doxorubicin, methotrexate, ifosfamide, etoposide, cytarabine; R-MACOPB = rituximab, methotrexate, doxorubicin, cyclophosphamide, vincristine, prednisolone, bleomycin; R-CHOP = rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone; MVP = methotrexate, vincristine and procarbazine with two cycles of high-dose cytarabine *one patient in group 1 received R-CHOPx2 followed by IVAC (ifosfamide, etoposide, cytarabine) x 2.

<table>
<thead>
<tr>
<th></th>
<th>group 1 CHOP intrathecal MTX</th>
<th>group 2 R-CHOP-like chemo + high dose IV MTX</th>
<th>group 3 HyperCVAD or CODOXM/IVAC +/- rituximab</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>49 (23%)</td>
<td>125 (58%)</td>
<td>43 (20%)</td>
<td></td>
</tr>
<tr>
<td>Centres contributing</td>
<td>PMCC, MMC</td>
<td>RBH, MMC, PMCC</td>
<td>RBH, MMC, PMCC</td>
<td></td>
</tr>
<tr>
<td>median age, years (range)</td>
<td>54.5 (19-84)</td>
<td>63 (23-84)</td>
<td>45 (16-74)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>male (%)</td>
<td>33/49 (67%)</td>
<td>81/125 (65%)</td>
<td>24/43 (57%)</td>
<td>0.78</td>
</tr>
<tr>
<td>stage III/IV (%)</td>
<td>35/48 (73%)</td>
<td>104/125 (84%)</td>
<td>38/43 (88%)</td>
<td>0.18</td>
</tr>
<tr>
<td>B symptoms</td>
<td>16/48 (33%)</td>
<td>43/106 (41%)</td>
<td>27/42 (64%)</td>
<td>0.007</td>
</tr>
<tr>
<td>median normalised serum LDH (range)</td>
<td>1.3 (0.3-6.0)</td>
<td>1.2 (0.3 – 11.4)</td>
<td>1.6 (0.7 – 25.7)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ECOG PS ≥2</td>
<td>12/48 (25%)</td>
<td>25/122 (20%)</td>
<td>12/42 (29%)</td>
<td>0.53</td>
</tr>
<tr>
<td>transformed histology</td>
<td>2/49 (4%)</td>
<td>17/124 (14%)</td>
<td>3/43 (7%)</td>
<td>0.15</td>
</tr>
<tr>
<td>IPI 3-5</td>
<td>23/48 (48%)</td>
<td>82/122 (67%)</td>
<td>27/42 (66%)</td>
<td>0.06</td>
</tr>
</tbody>
</table>
### Results II

<table>
<thead>
<tr>
<th>extranodal sites ≥2</th>
<th>23/49 (47%)</th>
<th>71/122 (58%)</th>
<th>20/43 (47%)</th>
<th>0.27</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>specific extranodal sites</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>bone marrow</td>
<td>17 (35%)</td>
<td>34 (27%)</td>
<td>15 (35%)</td>
<td>0.47</td>
</tr>
<tr>
<td>bone</td>
<td>15 (31%)</td>
<td>34 (27%)</td>
<td>16 (38%)</td>
<td>0.41</td>
</tr>
<tr>
<td>breast</td>
<td>2 (4%)</td>
<td>4 (3%)</td>
<td>0 (0%)</td>
<td>0.45</td>
</tr>
<tr>
<td>ovary</td>
<td>1 (2%)</td>
<td>2 (2%)</td>
<td>3 (7%)</td>
<td>0.16</td>
</tr>
<tr>
<td>testes</td>
<td>3 (6%)</td>
<td>8 (6%)</td>
<td>2 (5%)</td>
<td>0.94</td>
</tr>
<tr>
<td>renal</td>
<td>3 (6%)</td>
<td>8 (6%)</td>
<td>1 (2%)</td>
<td>0.60</td>
</tr>
<tr>
<td>hepatic</td>
<td>6 (12%)</td>
<td>21 (17%)</td>
<td>7 (16%)</td>
<td>0.75</td>
</tr>
<tr>
<td>paranasal sinuses</td>
<td>1 (2%)</td>
<td>7 (6%)</td>
<td>1 (2%)</td>
<td>0.47</td>
</tr>
<tr>
<td>nasopharynx</td>
<td>0 (0%)</td>
<td>3 (2%)</td>
<td>0 (0%)</td>
<td>0.33</td>
</tr>
<tr>
<td>bowel</td>
<td>1 (2%)</td>
<td>8 (6%)</td>
<td>1 (2%)</td>
<td>0.35</td>
</tr>
<tr>
<td>epidural/paraspinal</td>
<td>5 (10%)</td>
<td>7 (6%)</td>
<td>0 (0%)</td>
<td>0.09</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>chemotherapy</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>CHOP 31</td>
<td>RCHOP 11*</td>
<td>CHOP 3</td>
<td>Hyper CVAD 22</td>
<td></td>
</tr>
<tr>
<td>R-MACOPB 7</td>
<td></td>
<td>R-CHOP 122</td>
<td>R-Hyper CVAD 16</td>
<td></td>
</tr>
<tr>
<td>R-CODOXMIVAC 2</td>
<td></td>
<td></td>
<td>R-CODOXMIVAC 1</td>
<td></td>
</tr>
<tr>
<td>CODOXMIVAC 1</td>
<td></td>
<td></td>
<td>MVP 1</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>rituximab</th>
<th></th>
<th></th>
<th></th>
<th>&lt;0.0001</th>
</tr>
</thead>
<tbody>
<tr>
<td>18/49 (37%)</td>
<td>123/125 (98%)</td>
<td>18/43 (42%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| IT methotrexate (any) |             |             |             | 0.005   |
| median number doses (range) |             |             |             |         |
| 49/49 (100%)         | 84/104 (81%) | 28/33 (85%)  |             |         |
| 5 (1 - 6)            | 6 (0 - 7)   | 5 (0 - 6)   |             |         |
CNS prophylaxis

Figure 14 Cumulative incidence of CNS relapse using Kaplan-Meier method, by treatment group.

Outcomes

The median follow-up in the entire cohort was 3.4 (range 0.2 – 18.6) years. Amongst groups 1-3 the median follow-up was 5.8, 3.0 and 3.8 years respectively. During this time, 23 CNS relapses have occurred (12, 10 and 1 in groups 1-3 respectively). The median time to CNS relapse of 10.8 (range 4 – 109.6) months from initial diagnosis. The number and distribution of CNS relapses, 3-year cumulative incidence rates of CNS relapse and OS by treatment group are displayed in Table 21. The Kaplan Meier analysis of time to CNS relapse is displayed in Figure 14, whilst the cumulative incidence function of CNS relapse is shown in Figure 15. Briefly, the CNS relapse risk was highest in group 1 (P=0.006). Although the CNS relapse risk appeared numerically lower in group 3 compared with group 2, direct comparison between the two showed no statistically significant difference (P=0.16). The actuarial 3-year PFS rates were 65.5% (49.8 – 77.3%), 82.9% (74.7 – 88.6%) and 70.6% (53.9 – 82.2%) in Groups 1 – 3, respectively (P=0.051, Figure 3). Isolated CNS relapse (in the absence of systemic
relapse) occurred in 20/23 (87%) patients suffering CNS relapse, with the remaining 3 (12%) occurring in conjunction with systemic relapse. Of 17 patients with sufficient data, the distribution of CNS relapses was leptomeningeal alone in six (35%), parenchymal alone in nine (53%) and both in two (12%). The pattern of localisation did not differ between groups (P=0.16, Table 21). While recognising that all patients were considered to be at high risk for CNS relapse, I explored several potential risk factors identified in other studies. By univariate analysis, the only factor affecting CNS relapse risk was treatment group (Table 4). I also performed a multivariate analysis which included treatment group, use of rituximab, age>60, ECOG≥2, IPI ≥3, raised serum LDH, B symptoms, multiple extranodal sites and paraspinal disease - only treatment group impacted on CNS relapse. The hazard ratios (HR) for group 2 was 0.26 (95% CI 0.08 – 0.81, P=0.02) and group 3, 0.07 (0.01 – 0.55, P=0.01). Number of doses of IT MTX did not impact risk of CNS relapse (HR for 4 or more doses compared with 3 or less 0.84 (95% CI 0.29 – 2.40, P=0.75).

In group 2, 109 (81%) received both intended cycles of systemic high-dose MTX, with 25 (19%) receiving only one (for reasons described under “toxicity” below). For patients in group 2, the median length of inpatient admission to receive high-dose IV MTX was 4 (range 2-16) days per cycle. Compliance with planned IT MTX was high, with a median of 5,6 and 5 doses of IT MTX in groups 1-3 respectively. All patients in group 1 received at least one dose of IT MTX, compared with 81% and 85% of patients in groups 2 and 3 respectively (P=0.005).
Results II

Figure 15 Cumulative incidence of CNS relapse with death as a competing risk, using the method of Fine and Gray.\textsuperscript{317}

Table 21 Overall and CNS relapse free survival by group. Abbreviations: R-HyperCVAD = rituximab, hyper fractionated cyclophosphamide, doxorubicin, vincristine, dexamethasone; R-MTX-ara-c = rituximab, high dose methotrexate, high dose cytarabine; CODOXMIVAC = cyclophosphamide, vincristine, doxorubicin, methotrexate, ifosfamide, etoposide, cytarabine; R-CHOP = rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone.
Results II

<table>
<thead>
<tr>
<th></th>
<th>group 1</th>
<th>group 2</th>
<th>group 3</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>12</td>
<td>10</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Localisation</td>
<td></td>
<td></td>
<td></td>
<td>0.16</td>
</tr>
<tr>
<td>leptomeningal</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>parenchymal</td>
<td>4</td>
<td>5</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>both</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>unknown</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>3-year cumulative incidence of CNS relapse (95% CI)</td>
<td>18.4% (9.5 - 33.1%)</td>
<td>6.9% (3.5 - 13.4%)</td>
<td>2.3% (0.3 - 15.4%)</td>
<td>0.009</td>
</tr>
<tr>
<td>3-year overall survival</td>
<td>68.0% (52.4 - 79.3%)</td>
<td>85.9% (77.6 - 91.3%)</td>
<td>89.2% (73.7 - 95.8%)</td>
<td>0.029</td>
</tr>
</tbody>
</table>

Risk factors

I explored several potential risk factors identified in other studies from relapse for the purposed of potentially exploring subsets with the highest risk (acknowledging that this cohort was already high-risk by definition).\textsuperscript{113,115,120,321} By univariate analysis, the only factor affecting CNS relapse risk was treatment group (Table 22). I also performed a multivariate analysis which included treatment group, use of rituximab, age>60, ECOG≥2, IPI ≥3, raised serum LDH, B symptoms, multiple extranodal sites and paraspinal disease - only treatment group impacted on CNS relapse(Table 23). The hazard ratios (HR) for group 2 was 0.26 (95% CI 0.08 – 0.81, P=0.02) and group 3, 0.07 (0.01 – 0.55, P=0.01). Number of doses of IT MTX did not impact risk of CNS relapse (HR for 4 or more doses compared with 3 or less 0.84 (95% CI 0.29 – 2.40, P=0.75).

Table 22 Cox regression univariate analysis of risk factors for CNS relapse amongst patients selected for high risk of this complication.
### Risk factor (univariate)

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>HR (95%CI) Cox</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>age &gt;60</td>
<td>1.22 (0.53 - 2.82)</td>
<td>0.64</td>
</tr>
<tr>
<td>stage III/IV</td>
<td>1.49 (0.44 - 5.06)</td>
<td>0.50</td>
</tr>
<tr>
<td>histologic transformation</td>
<td>0.84 (0.20 - 3.62)</td>
<td>0.82</td>
</tr>
<tr>
<td>ECOG performance status ≥2</td>
<td>1.78 (0.75 - 4.28)</td>
<td>0.21</td>
</tr>
<tr>
<td>serum LDH &gt;ULN</td>
<td>1.28 (0.52 - 3.14)</td>
<td>0.59</td>
</tr>
<tr>
<td>multiple extranodal sites</td>
<td>1.50 (0.65 - 3.47)</td>
<td>0.34</td>
</tr>
<tr>
<td>IPI 3-5</td>
<td>2.38 (0.87 - 6.47)</td>
<td>0.068</td>
</tr>
<tr>
<td>B symptoms</td>
<td>0.71 (0.28 - 1.80)</td>
<td>0.47</td>
</tr>
<tr>
<td>paraspinal disease</td>
<td>1.38 (0.41 - 4.67)</td>
<td>0.61</td>
</tr>
<tr>
<td>group 1</td>
<td>N/A (reference)</td>
<td>-</td>
</tr>
<tr>
<td>group 2 (high dose IV MTX)</td>
<td>0.38 (0.16 - 0.91)</td>
<td>0.03</td>
</tr>
<tr>
<td>group 3 (high dose IV MTX/ara-c)</td>
<td>0.10 (0.01 - 0.76)</td>
<td>0.03</td>
</tr>
<tr>
<td>rituximab</td>
<td>1.21 (0.48 - 3.05)</td>
<td>0.69</td>
</tr>
<tr>
<td>number of doses of IT MTX (≥4 vs 0-3)</td>
<td>0.84 (0.29 - 2.40)</td>
<td>0.75</td>
</tr>
</tbody>
</table>

Table 23 Cox regression multivariate analysis of risk factors for CNS relapse amongst patients selected for high risk of this complication.

### Risk factor (multivariate)

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>HR (95%CI) Cox</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>group 2 (high dose IV MTX)</td>
<td>0.26 (0.08 - 0.81)</td>
<td>0.02</td>
</tr>
<tr>
<td>group 3 (high dose IV MTX/ara-c)</td>
<td>0.07 (0.01 - 0.55)</td>
<td>0.01</td>
</tr>
</tbody>
</table>
**Impact of rituximab**

In total 159/217 (73%) of patients received rituximab as part of induction therapy. Nearly all patients in group 2, but only 37% and 42% of patients in groups 1 and 3 received rituximab. However, use of rituximab had no impact on CNS relapse when all groups were considered collectively HR 0.62 (95%CI 0.27 – 1.44), \( P=0.27 \) and when considering the impact within groups 1 and 3 there was no difference in CNS relapse \( (P=0.28 \) and \( P=0.24 \) respectively, data not shown).

**Toxicity (described for patients in group 2 only)**

Despite routine urinary alkalinisation, the most frequent toxicity of systemic MTX was renal impairment of any grade, occurring in 70% of cycles overall, the majority (55%) grade 1 in severity. Most of these events were minor and transient elevations of serum creatinine without clinical impact. In two cases, grade 1 renal impairment was associated with delayed MTX clearance (defined as >5 days). Grade 2 renal impairment occurred in 14% of cycles and grades 3 and 4 were rare (<1%). All patients recovered renal function without need for haemodialysis. The second cycle was omitted in 20 cases due to renal impairment and delayed MTX clearance \( (n=8) \), grade 3+ alanine transaminase (ALT) elevation \( (n=3) \), CNS toxicity \( (n=1) \), sepsis \( (n=2) \) and reason not specified \( (n=4) \). Dose reductions for the second cycle occurred in 11/104 patients (10.6%) because of renal impairment \( (n=4) \), painful neuropathy \( (n=1) \), delayed clearance with normal renal function \( (n=1) \) and reason not documented \( (n=4) \). Asymptomatic elevation of ALT resolved in all cases spontaneously without complication.

**CNS relapse in mantle cell lymphoma**

In total, 1396 patients with MCL were screened, with 57 identified as having CNS involvement. Thirteen patients had CNS involvement at diagnosis and 44 at relapse. Thus the crude incidence of CNS involvement was 0.9% (95% CI 0.5 - 1.6%) at
diagnosis and 4.1% (95% CI; 3.2 – 5.2%) overall. The median follow up of the cohort with CNS relapse was 17 (range 0.2-170) months from initial diagnosis.

**Baseline characteristics (Table 24)**

Fifty-three (93%) had at least one site of extranodal involvement, with 61% having ≥2 sites; the most common of which at initial diagnosis were bone marrow (90%), blood (77%), liver (14%), and lung (11%). Ki-67 by IHC was performed in 27 patients (47%); 70% expressed Ki-67 in >30% of cells. MIPI score was evaluable in 72%; low, intermediate and high scores were seen in 10 (24%), six (15%) and 25 (61%) cases, respectively. In 37% of patients, relapses were isolated to the CNS and in 63% it was concurrent with systemic relapse.

I compared baseline features in patients with CNS involvement to a series of 105 consecutive patients with MCL treated at Peter MacCallum Cancer Centre (PMCC) from 01/1994 to 06/2012 without development of CNS involvement at most recent follow-up. These patients were used as a comparator because complete data from the remainder of the cohort were unavailable. Blastoid histology, B-symptoms, increased serum LDH, ECOG performance status ≥ 2 and high MIPI score were more frequent in the cohort with CNS involvement (Table 1).
Table 24 Baseline characteristics of patients with and without CNS involvement by mantle cell lymphoma. Patients without CNS involvement drawn from a single centre due to lack of availability of detailed data from other participating centres. Reproduced from Cheah et al.196, with permission.

<table>
<thead>
<tr>
<th>Baseline characteristics</th>
<th>CNS involved cohort (current study)</th>
<th>CNS not involved (PMCC)</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>57</td>
<td>105</td>
<td>–</td>
</tr>
<tr>
<td>Median age, years (range)</td>
<td>61 (38–82)</td>
<td>63 (30–85)</td>
<td>0.28</td>
</tr>
<tr>
<td>Male</td>
<td>70%</td>
<td>73 (70%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Blastoid histology</td>
<td>28%</td>
<td>8/84 (10%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>B-symptoms</td>
<td>53%</td>
<td>11/70 (16%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Stage IV</td>
<td>91%</td>
<td>65/91 (71%)</td>
<td>0.006</td>
</tr>
<tr>
<td>β_{2}-microglobulin &gt; normal</td>
<td>17/22 (77%)</td>
<td>20/34 (59%)</td>
<td>–</td>
</tr>
<tr>
<td>Median β_{2}-microglobulin/ULN (range)</td>
<td>2.0 (0.5–7.3)</td>
<td>1.08 (0.6–5.2)</td>
<td>0.079</td>
</tr>
<tr>
<td>Serum LDH &gt; normal</td>
<td>38/51 (75%)</td>
<td>16/50 (32%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Median LDH/ULN (range)</td>
<td>1.26 (0.1–11.9)</td>
<td>0.84 (0.52–1.65)</td>
<td>–</td>
</tr>
<tr>
<td>Median WBC, ×10^9/l (range)</td>
<td>10.9 (2.8–351)</td>
<td>8.0 (4.1–470)</td>
<td>0.058</td>
</tr>
<tr>
<td>ECOG ≥2</td>
<td>16/43 (30%)</td>
<td>12/68 (18%)</td>
<td>0.026</td>
</tr>
<tr>
<td>Ki-67 ≥30%</td>
<td>70%</td>
<td>4/8 (50%)</td>
<td>0.69</td>
</tr>
<tr>
<td>Median Ki-67 (range)</td>
<td>53.5% (5–90%)</td>
<td>22.5% (8–80%)</td>
<td>0.44</td>
</tr>
<tr>
<td>High MIPI score (≥6)</td>
<td>25/41 (61%)</td>
<td>14/50 (28%)</td>
<td>0.0018</td>
</tr>
</tbody>
</table>

analysed the actuarial risk of CNS involvement amongst 113 patients at PMCC according to baseline characteristics to derive a predictive risk model. Amongst the 44 ‘high risk’ patients (defined as ≥1 of the above risk factors) the actuarial risk of CNS involvement was 15% at 5 years, compared to 0% in the 67 ‘low risk’ patients (defined by the absence of high risk features), P=0.0005 (Figure 16).
Figure 16 CNS relapse-free survival by baseline characteristics at Peter MacCallum Cancer Centre. ‘High-risk’ defined by one or more of: serum LDH raised, ECOG 2 or more, B symptoms and blastoid histology. ‘Low-risk’ defined by the absence of high-risk features. Reproduced from Cheah et al\textsuperscript{322} with permission.

**Clinical features at time of CNS involvement**

Symptoms at presentation varied, but included weakness (28%), confusion (24%), ocular disturbance (20%) and headache (19%). CSF cytology was positive in 86% cases and flow-cytometry was positive in 91%. One case negative by cytology was positive by flow-cytometry. The median CSF cell count \((n=37)\) was 79 (range 0-41093) cells/\(\mu\)L. The CSF protein \((n=29)\) was raised in 69% cases. CSF LDH was infrequently measured \((n=10)\) but was elevated in 90%.

Neuroimaging was performed in 47 cases (82%). Patients with normal imaging had CNS disease proven by positive CSF cytology and/or flow-cytometry, therefore leptomeningeal disease [positive cytology with no/normal neuroimaging \((n=26)\), or leptomeningeal abnormalities \((n=15)\), total \(n=41\) (72%)] was more frequent than parenchymal \((n=17, 29\%)\), though seven patients with leptomeningeal disease also had parenchymal lesions. Of 17 patients with parenchymal lesions, 10 also harboured MCL cells in the CSF. Therefore isolated parenchymal CNS disease constituted just 12% of cases.
The median number of prior chemotherapy regimens was two (range 0 – 6) with 60% previously exposed to a CHOP-like regimen, 38% rituximab (with chemotherapy), 18% Hyper-CVAD and 20% receiving prior intrathecal chemotherapy. Therefore previous treatment with CNS penetrating doses of anti-metabolites and intrathecal prophylaxis did not completely protect from subsequent CNS relapse. Of the CNS relapses 48% occurred at first relapse, 25% at second, 20% were primary refractory and 7% at third or subsequent relapse. The median time to CNS relapse was 15 (range 2 – 167) months (Figure 17); 78% occurred within 36 months of initial diagnosis; the remaining 22% were spaced over many years (up to 14 years from diagnosis).

Figure 17 Time to CNS event (patients with CNS involvement at diagnosis excluded). Median time to CNS relapse 15.2 months, with 65% occurring within 2 years of initial diagnosis.

I compared 34 patients with early relapse (<36 months from initial diagnosis) to 10 with late (≥36 months) and found no differences in baseline characteristics or survival (data not shown).
**CNS directed treatment strategies and outcomes**

Once CNS involvement was confirmed, treatment strategies included chemotherapy alone in 72% combined chemo-radiotherapy in 13%, radiotherapy alone in 4%, and palliative care in 10%. The most frequently utilised chemotherapy strategy contained high-dose methotrexate (defined as $\geq 3 \text{ g/m}^2$, or $\geq 2 \text{ g/m}^2$ if age $> 60$) and/or cytarabine (defined as $\geq 3 \text{ g/m}^2$) in 40%, in various forms including alone, as part of (R) Hyper-CVAD or maxiCHOP/HDAC. Rituximab was incorporated in 13% and intrathecal therapy in 79%. Intrathecal therapy consisted of methotrexate, cytarabine and steroid in 15 cases, methotrexate and cytarabine in 12 cases and methotrexate alone in nine. Liposomal cytarabine was employed in three cases.

Of 47 patients with data, eight (17%) received autologous stem cell transplant in consolidation and one (2%) allogeneic. The conditioning regimens for the autologous transplants were BEAM (busulfan, etoposide, cytarabine, melphalan) in six and busulphan-melphalan in two. The allogeneic transplant was reduced intensity conditioning with fludarabine and total body irradiation. Seven of these patients had received high-dose methotrexate or cytarabine containing regimens as CNS treatment. The median age of these nine patients was 56 (range 38-67) years. Three had prior autologous stem cell transplantation. Patients consolidated with high dose chemotherapy and stem cell rescue had attained both systemic (CR in 6) and CNS (CR $n= 4$, PR $n=2$) responses. Two patients received whole brain radiotherapy following transplant. Amongst patients not receiving transplant Treatment ranged in intensity from high-dose cytarabine and/or methotrexate to palliative care, with no significant differences in response rates.

The median overall survival from time of CNS involvement was 3.7 (range 0.2 – 69.3) months (Figure 18).
Figure 18  Overall survival from time of central nervous system involvement.

Nine patients were alive at time of reporting with a median follow-up from time of CNS event of 15.2 (range 6.6 – 69.3) months (Online figure 1), with the remaining 47 having died (42 with progressive disease, four of sepsis and one cause unknown). The only long-term survivors from time of CNS diagnosis received high-dose anti-metabolite therapy and transplant (Figure 19).
Eight patients survived ≥12 months from recognition of CNS involvement. Comparing the baseline data of these patients to those surviving <12 months showed no significant differences in age, white cell count, LDH, or pattern of relapse, although median Ki-67 staining (24% v 53%; $P=0.04$) and MIPI score ($P=0.02$) were lower in those surviving ≥12 months.
**Prognostic factors**

Four factors in univariate analysis were associated with improved overall survival from time of CNS involvement (Table 25). Although age alone (≥60y vs <60y) was not associated with outcome, the cohort treated with chemotherapy regimens containing high-dose anti-metabolites had median age 56 versus 64 years in those not treated with these agents ($P=0.006$). B-symptoms, number of extranodal sites, LDH, ECOG, bulky disease, positive neuroimaging findings, and CSF cell count/protein were not associated with survival. There was no difference in survival between patients with proven histological/cytological involvement and those without (10 vs 4 months; $P=0.62$). Benefit of prior treatment with rituximab approached, but did not reach, statistical significance (HR 0.47, 95% CI 0.21 to 1.05; $P=0.065$).

*Table 25 Prognostic factors associated with improved survival from time of diagnosis of central nervous system involvement. WBC indicates white cell count; CNS, central nervous system*

<table>
<thead>
<tr>
<th>Prognostic factor</th>
<th>HR (95% CI)</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC &lt;10.9 x 10^9/L (at baseline)</td>
<td>0.44 (0.21 - 0.91)</td>
<td>0.02</td>
</tr>
<tr>
<td>Treatment of CNS disease with high-dose anti-metabolites</td>
<td>0.43 (0.20 - 0.94)</td>
<td>0.03</td>
</tr>
<tr>
<td>Consolidation with stem cell transplant</td>
<td>0.42 (0.19 - 0.91)</td>
<td>0.03</td>
</tr>
<tr>
<td>Achievement of CNS complete response to salvage</td>
<td>0.23 (0.10 - 0.53)</td>
<td>0.0006</td>
</tr>
</tbody>
</table>
Investigation of NMP in combination with anti-CD20 monoclonal antibodies on NHL cell lines

Hypothesis: NMP enhances rituximab induced ADCC on NHL cell lines

In this chapter I outline the investigation of NMP as an immune potentiating agent and explore its potential for use in lymphoma. To begin with, I performed pilot ADCC experiments to establish the correct dose of anti-CD20 mAb to use in combination with immunomodulatory drugs (IMiDs).

The ADCC protocol was performed as described in “Methods” on page 64. In brief, untreated bulk PBMCs (obtained from a single healthy donor) were thawed and used as effectors; Raji cells labelled with $^{51}$Cr served as targets. The PBMCs were not treated with IMiD. The targets were treated with either rituximab or obinutuzumab at 3 dose levels: 1, 5 and 25μg/ml. Figure 20 shows varying effector-target ratios (E:T) and rituximab dose levels. At a PMBC:target ratio of 20:1, 25μg/ml of rituximab was needed to enhance ADCC, however at an E:T of 50:1 even 1μg/ml was sufficient to enhance ADCC over baseline. I also explored obinutuzumab (GA101) with similar results, although even at 20:1 5μg/ml was enough to increased ADCC.
Figure 20 Rituximab dose titration ADCC experiment. X-axis demonstrates varying effector:target ratios (using bulk PBMCs). Results depict a single experiment using PBMCs from a single donor performed in triplicate.

Figure 21 Obinutuzumab dose titration ADCC experiment. X-axis demonstrates varying effector-target ratios (using bulk PBMCs). Results depict a single experiment using PBMCs from a single donor performed in triplicate.
Having established an optimum concentration of rituximab and obinutuzumab (25μg/ml for 20:1 PBMCs and 5 or 25μg/ml for 50:1) the next step was to pretreat the PBMCs with IMiDs. In the first attempt, the PBMCs were treated with a dose titration of NMP or lenalidomide for 7 days in low dose IL-2 and the anti-CD20 mAb used was rituximab (or no mAb). The results are shown in Figure 22.

Figure 22 ADCC experiment with IMiD dose titration and anti-CD20 mAb (no mAb, rituximab 20μg/ml or obinutuzumab 20μg/ml). For the initial experiment, PMBCs were treated with varying concentrations of NMP (A,B) or lenalidomide (C,D) and incubated in low dose IL-2 (20IU/ml) for 7 days. The PBMCs were then washed of excess IMiD and resuspended in fresh RPMI culture media, viability assessed using trypan blue in a haemocytometer and resuspended in a fixed concentration. The PBMCs were then added at 20:1 to Raji (A,C) or Daudi (B,D) targets labelled with 51Cr, and co-incubated for 4 hours. Results shown are a single experiment performed in triplicate.
Figure 23

B. NMP treated PBMC 20:1

C. len treated PBMCs 20:1
Rituximab significantly enhanced ADCC consistent with the prior experiment but the addition of IMiDs had no impact on either cell line regardless of the dose used. The extended treatment with IMiD was pinpointed as a potential problem, as the previous literature\textsuperscript{128} suggested an overnight incubation was sufficient. Additionally, preclinical data from the same paper suggested a dose of lenalidomide of 10\textmu M might be optimal for NK-cell activation.

Therefore, the next experiment used an overnight (approximately 12 hours) pretreatment with IMiD and higher doses of both lenalidomide and NMP. Raji targets were used, and the results shown in Figure 24. Puzzlingly at 20:1 (PBMCs) the addition of IMiDs did not increase ADCC, however at 40:1 there appeared to be enhancement of rituximab-induced ADCC but not obinutuzumab. This may be because obinutuzumab already demonstrated high natural ADCC, with the vehicle treated PBMCs causing approximately 40\% lysis. Furthermore, it appeared that the reason for the apparent enhancement in ADCC with rituximab and NMP/lenalidomide was a reduction in a failure of rituximab to enhance ADCC in the vehicle treated PBMCs.
Figure 24 Bulk PBMCs treated with IMiD (10μM lenalidomide, NMP or vehicle (DMSO) with anti-CD20 mAbs (none, rituximab or obinutuzumab) at an effector:target ratio of 20:1 (A) or 40:1 (B). Results shown reflect a single experiment in triplicate.

To further investigate this, I used MACS purified NK cells as there are data that NK cells are important effectors of rituximab-induced ADCC. Because NK cells represent only 5-10% of lymphocytes in healthy donors, a large numbers of PBMCs were required to obtain NK-cells in sufficient quantities. The next experiment used MACS purified NK cells at 25:1, with the results shown in Figure 25. Both NMP and
Lenalidomide showed highly significant enhancement of ADCC in the absence of anti-CD20 mAb, however the mAbs failed to enhance ADCC.

Figure 25 ADCC experiment with MACS purified NK-cells against Raji targets. The dose of IMiD was increased to 10μmol, closer to the optimum levels in the previous literature.  

To troubleshoot this failure I considered three possibilities:

1. the Raji cells (now passaged 7-8 times) had developed antigenic drift and lost CD20 expression
2. the anti-CD20 mAbs had deteriorated
3. IL-2 at 20IU/ml was rate limiting, and a higher dose of IL-2 was needed

To exclude these possibilities, I harvested Raji cells from a confluent monoculture at the same passage number and performed FACS analysis for CD20 expression. In brief, 100% of Raji cells expressed CD20. To exclude deterioration of anti-CD20 mAbs, fresh stock was obtained and tested in an ADCC assay against Raji cell lines and confirmed to be functional. Finally, to explore whether IL-2 was rate limiting, I repeated the experiment at two dose levels: 20IU/ml and 2500IU/ml. The latter dose was chosen because in the original paper, 10pg/ml was equivalent. The resultant experiment is shown in Figure 26. It became apparent that NMP did enhance rituximab-induced ADCC with low dose IL-2 when conditions were optimised; increasing the dose of IL-2
to 2500IU/ml resulted in “super-activation” of NK-cells to such an extent that tumour killing approached 80-90% in almost all conditions, obscuring the ability to distinguish the incremental additive effect of immune activation with IMiD.

Figure 26 Top panel: NMP enhances rituximab-induced ADCC and displays a non-significant trend toward enhancing obinutuzumab-induced ADCC. Single experiment, performed in triplicate. Upper panel (A): low dose IL-2 (20IU/ml) Lower panel (B): identical experiment using high dose (2500IU/ml) IL-2.

ADCC purified NK cells v Raji (10:1) IL2 20U/ml

ADCC purified NK cells v Raji (10:1) IL2 2500U/ml
Results IV

Rationale clinical trial design incorporating scientific correlative endpoints in the treatment of patients with lymphoproliferative disorders

The protocols presented were written in accordance with the Australian Code for the Responsible conduct of Research, the International Conference on Harmonisation Guideline for Good Clinical Practice Guidelines and the National Health and Medical Research Council (NHMRC) National Statement on Ethical Conduct in Human Research”. The ethical principles have their origin in the Declaration of Helsinki and applicable privacy laws.

During the process of protocol design, key stakeholders including clinicians, diagnostic and laboratory scientists, clinical trial nurses, pharmacists and biostatisticians.

Feasibility assessment

I gave detailed consideration to the following factors at the two centres (PMCC and RMH): 1) total number of patients with the eligible disease groups 2) current and future studies in competition 3) study appeal to clinicians and patients 4) past recruitment rates in studies targeting similar patient populations

Protocol 1 NMP in relapsed and refractory myeloma

Based on the number of patients with multiple myeloma at PMCC and RMH (n=250 for both centres) it is expected around 2-4 patients per month who relapse or are refractory to standard therapies will be potentially eligible for this study. Allowing for a 50% screening failure or declination rate, it is estimated that recruitment will be 1-2 patients per month. This equates to approximately 24 months to complete enrolment, a time period that is feasible for the conduct of a “proof of principle” phase I study. At
the time of writing, there are 4 phase I/II studies open at PMCC available for patients with relapsed/refractory MM. At RMH there are currently 2 competing studies (1 in phase I and 1 phase III respectively). It is likely the appeal of oral administration and likely favourable side effect profile will make this an appealing option for patients. A study targeting a similar patient population enrolled 25 patients in 26 months.\textsuperscript{324} Based on these data this study appears feasible.

**Protocol 2 ISCOMAB and rituximab in indolent B-cell NHL**

Based on the number of patients with indolent B-cell lymphoma at PMCC and RMH (n=250 for both centres) it is expected that 2-4 patients per month will be potentially eligible for this study. Allowing for a 50% screening failure or declination rate, the estimated recruitment for this study is 1-2 patients per month. This equates to approximately 12-24 months to complete enrolment. At present, there are 3 competing studies in relapsed/refractory indolent B-cell NHL at PMCC and 1 competing study at RMH. At the two centres, a phase I study targeting a similar population has recruited 20 patients in just over 12 months.\textsuperscript{325} Furthermore, it is predicted that the appeal of a non-chemotherapy approach and likely favourable side effect profile will make this protocol attractive to patients and clinicians. Based on these data this study appears feasible.

**Protocol 3 αgalcer and CpG in indolent B-cell NHL and CLL**

Based on the number of patients with indolent B-cell lymphoma at PMCC and RMH (n=250 for both centres) it is expected 2-4 patients per month to be potentially eligible for these studies. This protocol will also be available to patients with CLL, a larger population (n=300). Allowing for a 50% screening failure or declination rate, the estimated recruitment for this study is 2-3 patients per month. This equates to approximately 12-18 months to complete enrolment. At present, there are 3 competing studies in relapsed/refractory indolent B-cell NHL at PMCC and 1 competing study at RMH. For patients with relapsed or refractory CLL there are currently 3 studies at PMCC and 2 studies at RMH in competition. Phase I studies in
similar population of patients with CLL\textsuperscript{326} and NHL\textsuperscript{325} have recruited at the rates estimated above. Furthermore, it is predicted that the appeal of a non-chemotherapy approach and likely favourable side effect profile will make this protocol attractive to patients and clinicians. Based on these data this study appears feasible.

**Protocol review by collaborators**

**Clinical trials unit**

For all studies, detailed input was sought from the head of the Clinical Trials Unit at Peter MacCallum Cancer Centre. They were consulted early during the protocol and had input into the design of the patient informed consent form, case report form and schedule of assessments to ensure that each aspect was easily understood and logistically achievable. and that optimal use was made of trial nursing time, a major cost in the conduct of clinical trials.

**Correlative scientific analyses**

For all studies, detailed consultation was undertaken with translational scientists (Dr Paul Neeson, Dr Jake Shortt, Cancer Immunology Program, Peter MacCallum Cancer Centre) to ensure that timing, handling and nature of correlative sample collection was appropriate to address correlative endpoints. Furthermore, for protocol 1 (NMP in relapsed refractory myeloma) a pharmacokineticist (Prof Jennifer Martin, University of Queensland) was consulted regarding the timing of collection for pharmacokinetic analysis and dose escalation guidance.

The head of clinical trials pharmacy at Peter MacCallum Cancer Centre (Carol Rice) was engaged to assist with the practicalities of drug dispensing, storage and handling.

**Drug supply and formulation**

**Protocol 1 NMP in relapsed and refractory myeloma**

NMP is available as 2M pharmaceutical grade stock solution from Sigma-Aldrich Pharmaceuticals. In order to convert this to a form suitable for oral dosing, several options were investigated. I collaborated with Dr Jonathon Fairweather (Research and
Development Manager, Sypharma). Initial experiments to formulate NMP as a capsule were unsuccessful, as NMP is a solvent. This led to the capsule being dissolved over a 48 hour period, rendering this method of administration unachievable. An alternate delivery system of differing strengths of solution in a glucose mixture formulated in a plastic, child-proof bottle was developed (Figure 27).

*Figure 27 Sample formulations of NMP for oral administration developed for use in protocol 1 (Phase I open label study of orally administered NMP in patients with relapsed or refractory myeloma)*

For protocol 2 (ISCOMAB and rituximab) the ISCOMAB will supplied by CSL Limited and rituximab will used in accordance with its Pharmaceutical Benefits Scheme (PBS) listing. For protocol 3 (αgalcer and CpG in indolent B-cell NHL or CLL) αgalcer will be obtained from Industrial Research Laboratories (Lower Hutt, New Zealand).

**Statistical considerations**

For all protocols, power calculations for adverse event rates were made using STATA version 12.1 (College Station, TX) and verified with a biostatistician from the Centre for Biostatistics and Clinical Trials (BaCT), located at Peter MacCallum Cancer Centre.
Protocol 1: NMP in myeloma

**Table 26 Synopsis of protocol for NMP in relapsed refractory**

<table>
<thead>
<tr>
<th><strong>Title</strong></th>
<th>A phase I, open label dose escalation trial of orally administered N-methyl-pyrrolidone (NMP) in patients with relapsed or refractory myeloma</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Short title</strong></td>
<td>NMP in relapsed/refractory myeloma</td>
</tr>
<tr>
<td><strong>Sponsor</strong></td>
<td>Peter MacCallum Cancer Centre</td>
</tr>
<tr>
<td><strong>Study design</strong></td>
<td>Open label, dose-escalation, safety, pharmacokinetic, and pharmacodynamic study.</td>
</tr>
<tr>
<td><strong>Expected sample size</strong></td>
<td>20 evaluable patients will be enrolled in this trial. The dose escalation phase of this study will enrol approximately 15 patients and up to 5 additional patients will participate in the expansion portion of the study to further evaluate NMP at the recommended Phase 2 dose level. An evaluable patient begins cycle 1 of treatment and is available for DLT assessment.</td>
</tr>
<tr>
<td><strong>recruiting period</strong></td>
<td>2 years</td>
</tr>
<tr>
<td><strong>Investigational product</strong></td>
<td>NMP</td>
</tr>
</tbody>
</table>
| **Inclusion criteria** | 1. Histologically confirmed diagnosis of plasma cell myeloma  
2. Relapsed, refractory or intolerant of both bortezomib and lenalidomide  
   Definitions:  
   1) **refractory** at least 4 weeks of therapy administered, with less than a partial response by IMG criteria  
   2) **relapsed** previous response (PR or greater) to therapy, with subsequent disease progression (as defined by fall in Hb of 20g/L, platelet count <100 x 109/L, development of hypercalcemia or new lytic bone lesions, or an asymptomatic increase in serum M protein of 5g/L OR absolute increase of involved serum free light chain of >250mg/L  
   3) **intolerant**: grade 2 or higher toxicity unresponsive to dose adjustment  
3. Prior autologous stem cell transplant (if eligible)  
4. age ≥18 years  
5. ECOG performance status >2 |
6. Measurable disease as defined by at least one of:
   - serum M protein $\geq 5$g/L
   - urine M protein $\geq 200$mg/24hrs
   - involved serum free light chain $\geq 100$mg/L
   - measurable soft tissue (not bone) plasmacytoma (STPC)

7. The following values within 14 days of commencing NMP (blood transfusions prior to study entry are permitted)
   - haemoglobin $>80$g/L
   - absolute neutrophil count $>1.0 \times 10^9$/L
   - platelet count $\geq 25 \times 10^9$/L
   - Creatinine clearance $>30$ml/min (by Cockcroft/Gault)
   - Bilirubin $\leq 3 \times$ upper limit of normal (ULN)
   - ALT $\leq 3 \times$ ULN

8. Left ventricular ejection fraction (LVEF) $\geq 45\%$ (by gated cardiac blood pool scan or echocardiography)

9. Life expectancy $> 3$ months

10. Able to give written informed consent

11. In the opinion of the investigator, willing and able to comply with required study procedures

12. Able to take oral medications (no malabsorptive condition)
### Exclusion criteria

1. Pregnant or breast feeding female patients
2. Female of child bearing potential unwilling or unable to use two methods of contraception
3. Received chemotherapy, immunotherapy or biological therapy within two weeks of enrolment
4. Any uncontrolled serious medical condition or laboratory abnormality, which would in the opinion of the investigator make participation unsafe
5. any condition which would impair interpretation of toxicity from the agent/establishing MTD
6. uncontrolled diarrhoea, nausea or vomiting
7. concomitant exposure to another investigational agent

### Primary endpoint

To determine the maximum tolerated dose (MTD) of NMP by oral administration when administered daily in patients with relapsed or refractory multiple myeloma

### Secondary endpoints

1. To determine the safety of repeated dosing of NMP by oral administration
2. To determine the pharmacokinetic properties of NMP after oral administration in patients with relapsed/refractory myeloma
3. To assess the immunological and anti-tumour activity of NMP in patients with MM using correlative assays to determine the effect of NMP on IL2, CD4+ T cells and NK cell function; BET-bromodomain inhibitory activity and CRBN/DDB1/CUL4 signalling.
4. To determine the response rate (partial response or greater as defined by the International Myeloma Working Group uniform response criteria)

### Dose escalation scheme

Accelerated dose escalation schema until DLT occurs in cycle 1, see Figure 28

Standard dose escalation schema thereafter, see Figure 29

### Treatment duration

Treatment for each patient will continue in the absence of disease progression or unacceptable toxicity.

### Follow up schedule

Patients will be followed up on D1 of each cycle during treatment for 12 months and then 2 monthly thereafter as minimum. After treatment is stopped patients will be followed up every 2 months until the next treatment

### DLT definition

A DLT is defined by the occurrence during the first cycle of any one of the following:

1) Haematological toxicity
|   | a) grade 4 neutropenia on Day 1 of the next cycle despite ≥5 days of G-CSF therapy  
|   | b) grade 4 thrombocytopenia on Day 1 of next cycle  
|   | c) requirement for platelet transfusion which in the opinion of the investigator is not due to underlying disease  
|   | d) Febrile neutropenia  
| 2) | Any grade 3 or higher non-haematological toxicity which is considered probably related to study drug, and not attributable to disease progression except for alopecia and anorexia  
| 3) | Treatment delay of ≥2 weeks due to prolonged recovery from drug related toxicity  

For the purpose of assessment of DLT, the following guidelines will be used in conjunction to above criteria.

1) Time of occurrence. Any new ≥ Grade 3 toxicity as described in DLT definitions occurring within the first treatment cycle of the patient, in which the relationship to investigational therapy cannot be ruled out, with the exception of fever, nausea/vomiting, and/or diarrhoea which will only be considered a DLT if they reach ≥ Grade 3 severity despite adequate supportive care measures (>24 hours of treatment) and are considered probably related to study drug.

2) Duration of laboratory toxicity. Any Grade 3 non-haematological toxicity for > 7 days and that requires treatment for correction (i.e., exclude those for < 7 days and/or do not require corrective treatment.)

3) Treatment delays and dose modification
   a) Treatment delays > 28 days due to toxicity.  
   b) Dose modifications are required due to toxicity.

4) Toxicities that will not be determined as DLT
   a) Grade 3 nausea, vomiting, diarrhoea, and fever that respond to therapy.  
   b) Grade 3 nausea, vomiting, diarrhoea, and fever < 24 hours without any treatment  
   c) Any Grade 3 non-haematologic laboratory abnormalities with a baseline assessment of grade 1 or 2 (e.g. LFTs.)  
   d) Lymphopenia, hyperuricemia, electrolyte abnormalities that respond to therapy within 48 hours  
   e) Alopecia
<table>
<thead>
<tr>
<th><strong>Efficacy assessments</strong></th>
<th>Patients will be evaluated for response after every 28 day cycle using the appropriate disease assessment criteria for multiple myeloma.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PK assessments</strong></td>
<td>Plasma PK analysis for NMP will be performed on Day 1 of the first treatment cycle for all patients enrolled into this study. Serial heparinized blood samples for PK analysis will be collected from all patients before dosing; at 30 minutes after oral administration; then at 2 hours, 4 hours, hours and 8 hours after oral administration. Subsequently, all patients will return to the clinic on Days 2, 3, and 4, for additional blood sample collections at 24, 48 and 72 hours after oral administration on Day 1 and immediately prior to the next scheduled dose. Additional blood samples for pharmacokinetic assay may also be collected at unscheduled time points for patient safety-associated reasons.</td>
</tr>
<tr>
<td><strong>Safety assessments</strong></td>
<td>Adverse events and serious adverse events will be reported as described in the protocol. Stopping rules: • Death in first cycle possibly drug related • SUSAR which is possibly drug related • Anaphylaxis • Recommendation from safety committee • Evidence becoming available during the accrual phase of the trial, which clearly demonstrates that it is unethical to register patients to the trial • Inadequate recruitment, such as less than 1 patient per 6 months</td>
</tr>
<tr>
<td><strong>Interim analysis</strong></td>
<td>This will be carried out after every patient is enrolled by the Dose Escalation and Safety monitoring committee and at specified timepoints.</td>
</tr>
<tr>
<td><strong>Ethical considerations</strong></td>
<td>This study will be conducted in accordance with applicable laws and regulations including but not limited to, the ACRCR Nat Statement International Conference on Harmonisation Guideline for Good Clinical Practice (GCP) and the ethical principles that have their origins in the Declaration of Helsinki. The institutional review board (IRB)/independent ethics committee (IEC) must review and approve the protocol and informed consent form before any subjects are enrolled. Before any procedures specified in the protocol are performed the subject (and caregiver where applicable) must sign and date the IRB/IEC</td>
</tr>
<tr>
<td>approved informed consent form.</td>
<td></td>
</tr>
</tbody>
</table>
Figure 28 Accelerated phase cycle 1 dose escalation for NMP in myeloma. Only toxicities experienced during cycle 1 apply. (Dose Level 7 is the highest level). Moderate toxicities are assessed only during the accelerated dose escalation and the incidence is cumulative across dose levels. If early termination criteria are met for the first patient on dose level 1, 3 patients may be enrolled at dose level-1 if considered appropriate by the DMSC.

Figure 29 Standard dose escalation schema for NMP in myeloma. Only toxicities experienced during cycle 1 apply. (Level X is the dose level of the last patient entered in the accelerated phase in cycle 1). Accrual of patients in the Standard phase will be sequential.
Protocol 2 synopsis: ISCOMAB and rituximab in indolent B-cell NHL

<table>
<thead>
<tr>
<th><strong>Title</strong></th>
<th>A phase Ib, open label dose escalation trial of ISCOMAB and rituximab in patients with relapsed or refractory indolent B-cell NHL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Short title</strong></td>
<td>ISCOMAB and rituximab in indolent B-cell NHL</td>
</tr>
<tr>
<td><strong>Sponsor</strong></td>
<td>Peter MacCallum Cancer Centre</td>
</tr>
<tr>
<td><strong>Study design</strong></td>
<td>Open label, dose-escalation, safety, pharmacokinetic, and pharmacodynamic study.</td>
</tr>
<tr>
<td><strong>Expected sample size</strong></td>
<td>20 evaluable patients will be enrolled in this trial. The dose escalation phase of this study will enrol approximately 15 patients and up to 5 additional patients will participate in the expansion portion of the study to further evaluate ISCOMAB and rituximab at the recommended Phase 2 dose level. An evaluable patient begins cycle 1 of treatment and is available for DLT assessment.</td>
</tr>
<tr>
<td><strong>recruiting period</strong></td>
<td>2 years</td>
</tr>
<tr>
<td><strong>Investigational product</strong></td>
<td>ISCOMAB and rituximab</td>
</tr>
</tbody>
</table>
| **Inclusion criteria** | 1. Histologic diagnosis of indolent B-NHL (Follicular lymphoma, Marginal Zone Lymphoma, Small Lymphocytic Lymphoma) with relapse after ≥1 prior regimen of systemic therapy  
2. Ability to provide informed consent  
3. Measurable disease on CT scanning  
4. ECOG performance status ≤2  
5. Ability to adhere to contraception and provide informed consent  
6. Evidence of past sensitivity to rituximab (defined by partial response or greater lasting ≥ 6 months)  
7. age ≥18 years  
8. The following values within 14 days of commencing ISCOMAB (blood transfusions prior to study entry are permitted)  
   - haemoglobin >80g/L  
   - absolute neutrophil count >1.0 x 10^9/L  
   - platelet count ≥ 25 x 10^9/L  
   - Creatinine clearance >30ml/min (by Cockcroft/Gault)  
   - Bilirubin ≤ 3x upper limit of normal (ULN) |
• ALT ≤ 3 x ULN

9. Left ventricular ejection fraction (LVEF) ≥45% (by gated cardiac blood pool scan or echocardiography)

10. Life expectancy > 3 months

11. In the opinion of the investigator, willing and able to comply with required study procedures
### Exclusion criteria
1. Pregnant or breast feeding female patients
2. Female of child bearing potential unwilling or unable to use two methods of contraception
3. Received chemotherapy, immunotherapy or biological therapy within two weeks of enrolment
4. Any uncontrolled serious medical condition or laboratory abnormality, which would in the opinion of the investigator make participation unsafe
5. Any condition which would impair interpretation of toxicity from the agent/establishing MTD
6. Concomitant exposure to another investigational agent

### Primary endpoint
To determine the recommended phase II dose (R2PD) of ISCOMAB + rituximab by subcutaneous administration in patients with relapsed or refractory indolent B-cell NHL

### Secondary endpoints
1. To determine the safety of repeated dosing of ISCOMAB + rituximab
2. To assess the immunological and anti-tumour activity of ISCOMAB + rituximab in patients with indolent B-cell NHL
3. To determine the response rate (partial response or greater as defined by the International Working Group uniform response criteria)\(^\text{328}\)

### Dose escalation scheme
ISCOMAB will be administered by intramuscular injection on days 0, 7, 14, 21 and 28 for a total of 5 doses. Single agent ISCOMAB will be administered on day zero to ensure that unexpected side effects of are not seen in patients with iB-NHL either as a consequence of the disease type or prior rituximab exposure. Rituximab will then be administered intravenously on Days 7, 14, 21 and 28. On days when ISCOMAB formulation and rituximab are both administered, ISCOMAB will be administered 1 hour prior to the start of the rituximab infusion. Up to 3 dose levels will be explored; the initial 6 patients will receive 45 ISCO\(^\text{®}\) units and the subsequent 6 patients will receive 90 ISCO\(^\text{®}\) units. If no patients in cohort 2 have experienced dose limiting toxicity (DLT) and two or fewer patients in the second cohort achieve a partial response, a third cohort of 180 ISCO\(^\text{®}\) units will enrol up to 6 patients. Patients will undergo routine baseline iB-NHL staging and screening procedures summarised in Figure 7. Due to the superior sensitivity for staging and response assessment of PET-CT scans, as an exploratory outcome measure we will also perform \(^{18}\text{F-FDG-PET-CT}\) at baseline (day -28 to 0) and day 72±7.

Patients removed from the study for toxicity or progression will be followed until stabilisation of an adverse event or initiation of the next anti-cancer therapy.
<table>
<thead>
<tr>
<th><strong>Treatment duration</strong></th>
<th>Total duration of treatment will be 28 days. See Figure 30.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Follow up schedule</strong></td>
<td>Patients will be followed weekly during treatment, and then until Day 72 (end of study visit). See Figure 30.</td>
</tr>
<tr>
<td><strong>DLT definition</strong></td>
<td>A DLT is defined by the occurrence of any one of the following:</td>
</tr>
<tr>
<td></td>
<td>1) Haematological toxicity</td>
</tr>
<tr>
<td></td>
<td>a) grade 4 neutropenia on Day 1 of the next cycle despite $\geq5$ days of G-CSF therapy</td>
</tr>
<tr>
<td></td>
<td>b) grade 4 thrombocytopenia on Day 1 of next cycle</td>
</tr>
<tr>
<td></td>
<td>c) requirement for platelet transfusion which in the opinion of the investigator is not due to underlying disease</td>
</tr>
<tr>
<td></td>
<td>d) Febrile neutropenia</td>
</tr>
<tr>
<td></td>
<td>2) Any grade 3 or higher non-haematological toxicity which is considered probably related to study drug, and not attributable to disease progression except for alopecia and anorexia</td>
</tr>
<tr>
<td></td>
<td>3) Treatment delay of $\geq2$ weeks due to prolonged recovery from drug related toxicity</td>
</tr>
<tr>
<td></td>
<td>For the purpose of assessment of DLT, the following guidelines will be used conjunction to above criteria.</td>
</tr>
<tr>
<td></td>
<td>5) Time of occurrence. Any new $\geq$ Grade 3 toxicity as described in DLT definitions occurring within the first treatment cycle of the patient, in which the relationship to investigational therapy cannot be ruled out, with the exception of fever, nausea/vomiting, and/or diarrhoea which will only be considered a DLT if they reach $\geq$ Grade 3 severity despite adequate supportive care measures (&gt;24 hours of treatment) and are considered probably related to study drug.</td>
</tr>
<tr>
<td></td>
<td>6) Duration of laboratory toxicity. Any Grade 3 non-haematological toxicity for $&gt;7$ days and that requires treatment for correction (i.e., exclude those for $&lt;7$ days and/or do not require corrective treatment.)</td>
</tr>
<tr>
<td></td>
<td>7) Treatment delays and dose modification</td>
</tr>
<tr>
<td></td>
<td>a) Treatment delays $&gt;28$ days due to toxicity.</td>
</tr>
<tr>
<td></td>
<td>b) Dose modifications are required due to toxicity.</td>
</tr>
<tr>
<td></td>
<td>8) Toxicities that will <strong>not</strong> be determined as DLT</td>
</tr>
<tr>
<td></td>
<td>a) Grade 3 nausea, vomiting, diarrhoea, and fever that respond to therapy.</td>
</tr>
<tr>
<td></td>
<td>b) Grade 3 nausea, vomiting, diarrhoea, and fever $&lt;24$ hours without any treatment</td>
</tr>
<tr>
<td></td>
<td>c) Any Grade 3 non-haematologic laboratory abnormalities with a baseline assessment of grade 1 or 2 (e.g. LFTs.)</td>
</tr>
</tbody>
</table>
d) Lymphopenia, hyperuricemia, electrolyte abnormalities that respond to therapy within 48 hours

e) Alopecia

**Efficacy assessments**
Patients will be evaluated for response with 7 days of Day 72 by International Working Group criteria. \(^{328}\)

**Safety assessments**
Adverse events and serious adverse events will be reported

Stopping rules:
- Death in first cycle possibly drug related
- SUSAR which is possibly drug related
- Anaphylaxis
- Recommendation from safety committee
- Evidence becoming available during the accrual phase of the trial, which clearly demonstrates that it is unethical to register patients to the trial
- Inadequate recruitment, such as less than 1 patient per 6 months

**Interim analysis**
This will be carried out after every patient is enrolled by the Dose Escalation and Safety monitoring committee and at specified timepoints.

**Ethical considerations**
This study will be conducted in accordance with applicable laws and regulations including but not limited to, the ACRCR Nat Statement International Conference on Harmonisation Guideline for Good Clinical Practice (GCP) and the ethical principles that have their origins in the Declaration of Helsinki. The institutional review board (IRB)/independent ethics committee (IEC) must review and approve the protocol and informed consent form before any subjects are enrolled. Before any procedures specified in the protocol are performed the subject (and caregiver where applicable) must sign and date the IRB/IEC approved informed consent form.

---

**Figure 30 Schedule of assessments for ISCOMAB and rituximab.**

<table>
<thead>
<tr>
<th>schedule of treatments and assessments</th>
<th>screening</th>
<th>end of study visit</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISCOMAB (intramuscular injection)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rituximab (375mg/m2 by intravenous infusion)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical examination</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Full blood count</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Biochemistry (renal and liver function)</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>peripheral blood sample for correlative immunology</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>bone marrow biopsy</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>PET-CT</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>CT neck to pelvis</td>
<td>x</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Day</th>
<th>-28 to 0</th>
<th>0</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
<th>56</th>
<th>72</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISCOMAB</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>rituximab</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
</tbody>
</table>

131
Protocol 3 synopsis: intratumoural αgalcer and CpG in relapsed indolent B-cell NHL

<table>
<thead>
<tr>
<th><strong>Title</strong></th>
<th>A phase Ib, open label dose escalation trial of intra-tumoural αgalcer and CpG in patients with relapsed or refractory indolent B-cell NHL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Short title</strong></td>
<td>intra-tumoural αgalcer and CpG in indolent B-NHL</td>
</tr>
<tr>
<td><strong>Sponsor</strong></td>
<td>Peter MacCallum Cancer Centre</td>
</tr>
<tr>
<td><strong>Study design</strong></td>
<td>Open label, dose-escalation, safety, pharmacokinetic, and pharmacodynamic study.</td>
</tr>
<tr>
<td><strong>Expected sample size</strong></td>
<td>20 evaluable patients will be enrolled in this trial.</td>
</tr>
<tr>
<td><strong>recruiting period</strong></td>
<td>2 years</td>
</tr>
<tr>
<td><strong>Investigational product</strong></td>
<td>alphagalactoceramide and CpG</td>
</tr>
</tbody>
</table>
| **Inclusion criteria** | 1. Histologic diagnosis of indolent B-NHL (Follicular lymphoma, Marginal Zone Lymphoma, Small Lymphocytic Lymphoma) with relapse after ≥1 prior regimen of systemic therapy  
2. Ability to provide informed consent,  
3. In the opinion of the investigator, willing and able to comply with required study procedures  
4. At least 3 sites of disease (one for biopsy, one palpable area for treatment and one that can be measured radiologically)  
5. ECOG performance status ≤2  
6. Ability to adhere to contraception and provide informed consent  
7. Evidence of past sensitivity to rituximab (defined by partial response or greater lasting ≥ 6 months)  
8. age ≥18 years  
9. The following values within 14 days of commencing αgalcer + CpG (blood transfusions prior to study entry are permitted)  
9.1. haemoglobin >80g/L  
9.2. absolute neutrophil count >1.0 x 10⁹/L  
9.3. platelet count ≥ 25 x 10⁹/L  
9.4. Creatinine clearance >30ml/min (by Cockcroft/Gault)  
9.5. Bilirubin ≤ 3x upper limit of normal (ULN)  
9.6. ALT ≤ 3 x ULN  
10. Left ventricular ejection fraction (LVEF) ≥45% (by gated cardiac blood pool scan or echocardiography) |
|   | 11. Life expectancy > 3 months |
### Exclusion criteria

1. **aggressive lymphoma** as determined by the principle investigator and defined by:
2. histological subtype (including but not limited to: diffuse large B-cell lymphoma, Burkitt lymphoma, lymphoblastic lymphoma, histologically transformed low grade lymphoma, Hodgkin lymphoma)
3. clinical behaviour (including but not limited to: constitutional symptoms, lymphadenopathy causing organ compromise, rapidly enlarging lymph node mass)
4. Pregnant or breastfeeding female patients
5. Female of child bearing potential unwilling or unable to use two methods of contraception
6. chemotherapy, immunotherapy or biological therapy within two weeks of enrolment
7. concomitant immunosuppression with two weeks (defined as doses of prednisolone 10mg/day or greater, or any dose of: tacrolimus, cyclosporine, azathioprine, mycophenelate mofetil)
8. active autoimmune disease (such as SLE, Sjogrens Syndrome, Rheumatoid Arthritis)
9. any medical disease, laboratory abnormality or condition which in the opinion of the principal investigator makes the subject unsuitable.
10. Current HIV, HBV (defined as HBV DNA or HbsAg positive) or HCV (HCV RNA positive) infection
11. concomitant exposure to another investigational agent
12. second malignancy which would result in a life expectancy of < 2 years

### Primary endpoint

To determine the recommended phase II dose (R2PD) of αGalCer + CpG by intratumoral administration in patients with relapsed or refractory indolent B-cell NHL

### Secondary endpoints

1. To measure the specific immunological response induced by αGalCer and CpG in patients with low grade lymphoma
   a. flow cytometric analysis with staining for CD45Ro, CD3, CD4, CD8, CD137, CD16, CD56 and PE-conjugated αGalCer loaded-CD1d tetramer
   b. intracellular cytokine assessment using Golgi-stop, followed by staining for IFNg, TNFa, IL-2
   c. assessment of peripheral blood NKT cell subsets (identified on the basis of CD3 expression and reactivity with αGalCer loaded-CD1d tetramer)
   d. to correlate ex-vivo T<sub>reg</sub> enhancement with clinical
2. To determine the response rate (partial response or greater as defined by the International Working Group uniform response criteria)\textsuperscript{328}

### Dose escalation scheme

aGalCer and CpG will be administered at a fixed doses by direct injection into a palpable lymph node on days 1-4, then weekly for a further 8 weeks (12 doses in total).

Patients will be assessed for toxicity every week or at any unplanned visit, and for response every 4 weeks. Intra-patient dose escalation will be allowed providing:

1. At the first efficacy assessment (after 4 weeks on any given dose) there has been less than a minor response and;

2. There have been no grade 3 toxicities (which will mandate a dose reduction) in that patient in the preceding two cycles.

<table>
<thead>
<tr>
<th>Dose level</th>
<th>aGalCer</th>
<th>CpG</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1mg</td>
<td>6mg</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>2mg</td>
<td>6mg</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>2mg</td>
<td>12mg</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>4mg</td>
<td>12mg</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>4mg</td>
<td>18mg</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>6mg</td>
<td>18mg</td>
<td>3</td>
</tr>
<tr>
<td>7</td>
<td>6mg</td>
<td>24mg</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>8mg</td>
<td>24mg</td>
<td>3</td>
</tr>
</tbody>
</table>

### Treatment duration and follow up

See Figure 31

### DLT definition

A DLT is defined by the occurrence of any one of the following:

1) Grade 3-4 injection site reaction
2) Grade 3-4 fevers, arthralgia or myalgia
3) Grade 3-4 nausea, vomiting or diarrhoea
4) Any other clinically significant non haematological toxicity
### Schedule of assessments, phase I study of aGalCer and CpG

<table>
<thead>
<tr>
<th></th>
<th>screening</th>
<th>induction (day)</th>
<th>consolidation (week)</th>
<th>observation phase (week)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 2 3 4</td>
<td>1 2 3 4</td>
<td>5 6 7 8</td>
<td>10 12 14 16 18 20 24 28 32 36 40 44 48 52</td>
</tr>
<tr>
<td>aGalCer/CpG (intratumoral injection)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>informed consent</td>
<td>x</td>
<td>x x x x</td>
<td>x x x x x x</td>
<td></td>
</tr>
<tr>
<td>demographics</td>
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<tr>
<td>medical history</td>
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<tr>
<td>vital signs</td>
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<td>weight</td>
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<td>ECOG PS</td>
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<tr>
<td>concomitant medications</td>
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<tr>
<td>echocardiogram</td>
<td>x</td>
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<td>ECG*</td>
<td>x</td>
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</tr>
<tr>
<td>Physical examination</td>
<td>x</td>
<td>x x x x x x</td>
<td>x x x x x x</td>
<td>x x x x x x x x x x x x</td>
</tr>
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<td>FBC + diff~</td>
<td>x</td>
<td>x x x x x x</td>
<td>x x x x x x</td>
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</tr>
<tr>
<td>UEC, LFT, Ca, Mg ~</td>
<td>x</td>
<td>x x x x</td>
<td>x x x x x x</td>
<td>x x x x x x x x x x x x</td>
</tr>
<tr>
<td>Correlative samples</td>
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<td>x x x x x x</td>
<td>x x x x x x x x x x x x</td>
</tr>
<tr>
<td>LDH, h2m</td>
<td>x</td>
<td>x x x x x</td>
<td>x x x x x x</td>
<td>x x x x x x x x x x x x</td>
</tr>
<tr>
<td>SPEP, immunoglobulins</td>
<td>x</td>
<td>x x x x x x</td>
<td>x x x x x x</td>
<td>x x x x x x x x x x x x</td>
</tr>
<tr>
<td>pregnancy test*</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CT body</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PET-CT</td>
<td>#</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Continuous assessment: if clinically indicated

Screening assessments up to 4 weeks prior to commencing treatment

* females patients of child bearing potential only

~ during treatment to be performed within 48 hours of dosing

# optional
Current status of clinical trial protocols

All three protocols are now ready for ethics submission.

Protocol 1 (NMP in myeloma)

At the time of writing, protocol 1 (NMP in relapsed/refractory myeloma) was about to be submitted to the Human Research and Ethics Committee of Peter MacCallum Cancer Centre. I was instrumental in successfully obtaining full funding ($530,000 AUD) from the National Health and Medical Research Council (NHMRC) project grant for 2014 which fully covers the cost of the study and correlative scientific analyses. At the time of writing, ethics submission, patient informed consent form, investigator brochure, case report form, regulatory notification, database design and other setup procedures are underway. The trial is expected to open at both PMCC and the Royal Melbourne Hospital in quarter 3, 2014. The forecast study timelines are as follows:
Table 27 Timeline of expected events for protocol 1 (NMP in myeloma)

<table>
<thead>
<tr>
<th>time</th>
<th>Milestone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q2 2014</td>
<td>HREC submission</td>
</tr>
<tr>
<td></td>
<td>study setup development</td>
</tr>
<tr>
<td></td>
<td>Regulatory notification</td>
</tr>
<tr>
<td>Q3 2014</td>
<td>HREC approval (PMCC and RMH)</td>
</tr>
<tr>
<td></td>
<td>Study opens, site initiation visits</td>
</tr>
<tr>
<td></td>
<td>First patient enrolled</td>
</tr>
<tr>
<td>Q4 2014 – Q2 2016</td>
<td>Continue patient enrollment (expect 1-2 patients per month)</td>
</tr>
<tr>
<td></td>
<td>Dose escalation and safety monitoring board meetings</td>
</tr>
<tr>
<td>Q2 2016</td>
<td>Planned Safety analysis for American Society of Haematology (ASH)</td>
</tr>
<tr>
<td></td>
<td>annual meeting abstract submission (August 2016)</td>
</tr>
<tr>
<td></td>
<td>Begin correlative immunology studies</td>
</tr>
<tr>
<td>Q4 2016</td>
<td>Report on interim findings at ASH 2016</td>
</tr>
<tr>
<td>Q2 2017</td>
<td>Final analysis of primary endpoint and correlative immunology</td>
</tr>
<tr>
<td>Q3 2017</td>
<td>Manuscript preparation</td>
</tr>
<tr>
<td>Q4 2017</td>
<td>Manuscript submission</td>
</tr>
</tbody>
</table>

Protocol 2 (ISCOMAB and rituximab in indolent B-cell lymphoma)

Protocol 2 is subject to a 2015 NHMRC project grant (CIA Prof David Ritchie). The outcome of this will be available in July 2014, and if sufficiently ranked, outcome of rebuttals will be available in October 2014.

Protocol 3 (αgalcer and CpG in indolent B-cell NHL and CLL)

Applications to fund this study will be made in the next round of NHMRC project grants, the Leukemia and Lymphoma Society and Lymphoma Research Foundation.
Discussion

In the course of my fellowship I have undertaken projects that explore the biology, monitoring, outcome and novel therapies in B-cell lymphoproliferative disorders. These projects have made a significant contribution to the field of lymphoma clinical research. In the following discussion I will outline how my data should be interpreted in the current standing of lymphoma research.

PET-CT in DLBCL

Surveillance PET-CT in DLBCL

My data suggests that PET-CT scanning has both a low yield, and for most patients with DLBCL achieving CMR at the completion of primary therapy is not justified unless there is clinical suspicion of relapse. The only potential subgroup in whom a surveillance strategy warrants further investigation is patients with baseline IPI score \( \geq 3 \) in the first 18 months from completion of therapy, when the risk of relapse is greatest. In this study I did not demonstrate a difference in either second-line IPI or OS for patients with subclinical compared to symptomatic relapse, though the number of relapses was small. Underpinning the desire for earlier detection is the theoretical benefit of better outcomes from salvage therapy \(^{70}\) although I acknowledge that poor outcomes seen in this group of patients may reflect aggressive biology rather than late detection of relapse. A prospective study of patients with DLBCL and baseline IPI \( \geq 3 \) in first remission randomised to PET-CT surveillance versus no surveillance with a primary endpoint of overall survival would be required to address this issue.

The low rate of relapse among patients achieving CMR at the completion of treatment combined with the lower sensitivity and specificity of CT than PET\(^{329}\) suggests that diagnostic CT is even less likely to be worthwhile in a surveillance setting. PET-CT detected six (46%) relapses before clinical manifestations, a numerically greater proportion than using CT alone but still a suboptimal surveillance test.\(^{62,63}\) This could be improved by a shorter time-interval surveillance strategy but this may also increase false positives, cost and radiation exposure. Two thirds of the subclinical relapses

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would not have been detected using CT alone, further strengthening the case for use of PET-CT over CT alone in surveillance.

I confirm the finding of other investigators that PET-CT is both sensitive and specific for the detection of relapsed DLBCL (Table 28)\(^{65,66,68}\). The NPV of 99-100% means that patients with negative scans can be reassured that a CMR truly reflects ongoing remission from DLBCL.

**Table 28** Existing literature on the use of PET-CT in post remission surveillance of diffuse large B-cell lymphoma. Abbreviations: DLBCL = diffuse large B-cell lymphoma, PPV = positive predictive value, NNS = number needed to scan, NR = not reported/calculable. * Only proportion of patients with “aggressive Non Hodgkin Lymphoma”, not specifically DLBCL, reported.

<table>
<thead>
<tr>
<th>Reference</th>
<th>n (%DLBCL)</th>
<th>Surveillance protocol</th>
<th>Median F/U (mo)</th>
<th>Subclinical relapses</th>
<th>False positives (%)</th>
<th>PPV</th>
<th>No. needed to scan</th>
</tr>
</thead>
<tbody>
<tr>
<td>65</td>
<td>421 (43%)*</td>
<td>6 monthly for 2 years, then annual for 2 yrs</td>
<td>39</td>
<td>31%</td>
<td>16/1789 (0.9%)</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>66</td>
<td>75(100%)</td>
<td>non standard</td>
<td>16.5</td>
<td>13%</td>
<td>NR</td>
<td>85%</td>
<td>NR</td>
</tr>
<tr>
<td>67</td>
<td>125(65%)</td>
<td>non standard</td>
<td>NR</td>
<td>38%</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>68</td>
<td>52(83%)</td>
<td>6 monthly for 2 years, then annual for 3 yrs</td>
<td>18</td>
<td>100%</td>
<td>15/138 (10.3%)</td>
<td>21%</td>
<td>34.5</td>
</tr>
<tr>
<td>69</td>
<td>625(100%)</td>
<td>non standard</td>
<td>60</td>
<td>26%</td>
<td>NR</td>
<td>NR</td>
<td>120</td>
</tr>
<tr>
<td>current</td>
<td>116 (100%)</td>
<td>non standard</td>
<td>53</td>
<td>46%</td>
<td>6/456 (1.3%)</td>
<td>IPI&lt;3 56%</td>
<td>IPI&lt;3 92</td>
</tr>
<tr>
<td>study</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IPI≥3 80%</td>
<td>IPI≥3 22</td>
</tr>
</tbody>
</table>

False positive scans were also infrequent, with six (1.3%) identified. My findings (86% of inconclusive scans being negative on follow-up) are consistent with the results of
Zinzani et al. It is important to recognise common patterns of uptake unlikely to represent lymphomatous recurrence. The majority of false-positive and inconclusive scans occurred in the head and neck or mediastinum, often at sites of lymphomatous involvement at baseline. Increased tonsillar activity is common following chemotherapy, and usually represents reactive lymphoid hyperplasia. Similar findings occur in lymphoid tissue in the mediastinum and para-appendiceal region, with symmetric uptake in the mediastinum and linear uptake in the para-appendiceal region suggesting benign pathology. Mild-to-moderate uptake in cervical nodes, especially following upper respiratory tract infection, should not be mistaken for recurrent lymphoma. It should be highlighted that CT alone would have missed two-thirds of subclinical relapses and therefore cannot be recommended as an alternative surveillance strategy.

I have not made formal economic evaluation of surveillance PET-CT imaging, however health resources are scarce and in the real world must be considered when recommending any surveillance procedures. The true cost of surveillance includes not only that of the PET-CT scans themselves (an amount which varies considerably between health systems) but the additional costs of investigating indeterminate or false positive scans (either with repeat interval scanning or unnecessary biopsy). Another potential harm of surveillance PET-CT is additional radiation exposure. The radiation dose varies depending on the CT protocol and sex of the patient, but typically from a combined modality scan of the body is approximately 12-15mSv per scan. The subsequent risk of second malignancy is highest in younger patients, and particularly in adolescents and young adults minimisation of radiation exposure should be an important consideration when determining the risks and benefits of a surveillance strategy. Surveillance PET-CT scanning detected second primary malignancies in eight patients (7%), leading to curative procedures in four. Whether this impacted on mortality is uncertain, as these may have been detected without PET. I acknowledge that detection of second malignancies does not constitute a reason to perform surveillance PET-CT, however it is a useful by-product.
My data has limitations as a retrospective study. I took great lengths to ensure data quality, however some information is nevertheless missing or incomplete. Although departmental recommendations for post remission scanning existance, adherence was non-uniform; accordingly my results reflect time periods rather than a precise schedule. I observed refinement in reporting styles of nuclear medicine physicians over time, with greater recognition of phenomena such as the characteristic appearance of rebound lymphoid hyperplasia in recent compared to earlier reports, but my analyses are based on the actual report generated at the time of the scan. Finally, my findings with regard to the performance characteristics of surveillance PET-CT scans are specific to this setting, an academic tertiary referral centre with high imaging volume and physician expertise.

**Surveillance PET-CT in transformed indolent NHL**

In this cohort of patients with TrIL, who were managed mostly with upfront transplantation and rituximab containing chemotherapy and achieved CMR, I was unable demonstrate clinical benefit from a PET-CT surveillance strategy weighted towards more frequent scanning at the time of greatest likelihood of lymphoma relapse. Patterns of relapse were somewhat surprising: all relapses with low grade histology (follicular lymphoma in this study) were subclinical, and occurred within two years from completion of therapy. In contrast, large cell relapses occurred up to 3.5 years after completion of therapy and all nine were accompanied by signs or symptoms of relapse - prompting unscheduled early review and PET-CT scanning. The finding of subclinical relapse of low grade histology is of limited clinical benefit, as such patients rarely merit further therapy based on imaging findings alone. In contrast, patients with relapsed large cell lymphoma, who may have theoretically benefited from early detection of relapse were not detected by the surveillance strategy. Separate analysis of Groups 1 and 2 showed similar patterns of relapse and test performance.

One finding from this study is the value of end of treatment PET-CMR to identify a subset of patients with excellent outcomes. This prognostic information can be of
great reassurance to patients. Although further negative surveillance PET-CT scans had high negative predictive value for relapsed lymphoma and can give reassurance to patients of ongoing remission, this must be weighed against the anxiety arising from indeterminate or false positive scans, cost and inconvenience of a small number of unneeded biopsies and radiation exposure. Notwithstanding, the false positive rate of PET-CT in this series was very low.

I analysed a number of prognostic factors in an attempt to define a high-risk population of patients who may benefit from a surveillance strategy. Age at transformation >60 was the only significant predictor of inferior PFS on univariate analysis; the presence of B symptoms displayed a non-significant trend ($P=0.06$). The most consistently identified prognostic factors for patients with TrIL in previous studies is raised serum LDH at time of transformation,$^{3,4,72,332}$ although numerous other factors have been proposed (Table 29).
Table 29 Outcomes of selected recent studies in transformed indolent lymphoma. Abbreviations: n – number of patients, ASCT – autologous stem cell transplantation, PFS – progression free survival, OS – overall survival, NR – not reported, FL – follicular lymphoma *this study included 22 patients (13%) who underwent allogeneic stem cell transplantation

Although I identified age >60 as a prognostic factor, the pattern of relapses were such that confining a surveillance strategy to such patients would not have resulted in clinical benefit. It is important to point out that although there is limited benefit from surveillance PET-CT, there is even less of a role for CT, which has higher false positive rates, poor sensitivity for extranodal disease and is only able to detect 11-17% of relapses prior to clinical evidence of relapses.61-63
The actuarial 3-year PFS of 77% observed in this cohort is promising, but must be interpreted in the context of 1) the exclusion of patients with refractory disease 2) most patients (69%) undergoing ASCT and 3) most patients (80%) receiving rituximab. The outcomes are superior to the reported median PFS of 13-26 months in published series of patients with TrIL undergoing autologous stem cell transplantation (ASCT) prior to the incorporation of rituximab.\textsuperscript{332,333-341} Published series of patients with TrIL receiving rituximab containing chemotherapy as part of initial therapy are limited, but outcomes appear to be improving, with 5-year OS from recent series 48-75%.\textsuperscript{4,333-336,342,343} Selected recent studies of patients with TrIL receiving ASCT are displayed in Table 29.

There are several limitations to this study. The patient population studied was selected (young, good performance status and primary refractory patients excluded) and was managed aggressively with ASCT performed in over two-thirds of cases. However, patients falling outside these criteria (either unfit for stem cell transplantation or chemo-refractory) have limited options for treatment and therefore a surveillance strategy to detect early relapse is rarely of clinical benefit. The number of relapses was relatively small, in part due to the favourable outcomes seen from this treatment strategy. Although a departmental scanning protocol was in place, adherence was dependent on the individual treating physician and thus adherence was variable, as evidenced by the number of patients otherwise eligible who did not receive surveillance PET-CT scans. This, a consequence of the retrospective design introduces the possibility of bias. The study spans a period of almost a decade during which time interpretation of PET findings has improved. In particular, symmetric tonsillar and associated cervical nodal activity which constituted several of the false positive results is now recognised a feature of lymphoid repopulation or hyperplasia post chemotherapy. Finally, my findings with regard to the performance characteristics of surveillance PET-CT scans are specific to our setting, an academic tertiary referral centre with high imaging volume and physician expertise.
Interim PET-CT in primary mediastinal B-cell lymphoma

This analysis of consecutive patients with PMBL managed at my institution, emphasizes the high NPV of PET-CT, whether performed at an interim time point or at the completion of therapy. Thus patients who achieve negative ePET can be assured of excellent outcomes (4-year PFS 94% in this cohort). The high NPV of a negative ePET is important as the focus of current efforts is to spare these patients (who are often young and female) the potential long-term morbidity of involved field radiotherapy. The exploratory analysis of iPET scans found that they were positive in around one-third of patients and had poor predictive value for relapse. It should be noted that interpretation of the PPV of iPET in this study is particularly difficult because treatment was escalated in response to positive scans.

The interpretation of positive ePET (25-32% of patients) is less clear. I feel that it is instructive highlighting the three cases in whom despite escalation of therapy (including high dose consolidation) residual FDG avid masses were shown on biopsy to be due to granulomatous inflammation and necrosis rather than viable lymphoma. There are several potential explanations why patients with PMBL may have persistently FDG avid masses during and after therapy. Patients with PMBL tend to have bulky disease at baseline and in patients treated with R-CHOP (or similar) radiotherapy is frequently used. Both of these factors may lead to persistence of FDG avidity immediately following treatment. The three patients who underwent biopsy of persistently FDG avid mediastinal masses were found to have granulomatous inflammatory response and necrosis, which may reflect the particular biology of the disease, and response to treatment. I may speculate that overexpression of the glucose transporter GLUT1 may play a role in the persistent FDG avidity in such patients. However, I did not demonstrate a relationship between presence of bulk and positive iPET or ePET. Thus, one of the key messages arising from this study is the need for clinicians to exercise caution in the interpretation of FDG avid ePET residual masses in patients with PMBL. Although in general it is preferable to re-biopsy FDG avid residual masses, for DLBCL NOS, in practice this is occasionally omitted for reasons such as sites which are anatomically difficult to access. However,
Discussion

when treating patients with PMBL positive ePET should not be assumed to reflect treatment failure – escalation of therapy should be reserved for either unequivocal progression or histologically confirmed viable tumour.

Positive ePET using semi-quantitative visual analysis or 5-point scale had moderate PPV of 43% and predicted for inferior PFS. The true PPV may be underestimated in this study due to the use of PET to select patients for intensified treatment approaches. Furthermore, given the overall favourable prognosis of PMBL, the a priori risk of relapse is relatively low and even a moderate number of false positive results result in poor PPV, as demonstrated by my results (particularly for iPET).

The published experience using PET in PMBL patients is limited. To the best of my knowledge, five studies (all reported in abstract form) have specifically examined iPET (Table 30). Avigdor et al reported on 16 patients treated with R-VACOPB or R-CHOP and found 8/16 50% of iPET were positive, with no impact on outcome. Moskowitz et al reported a larger cohort of 51 patients treated with R-CHOP consolidated with ICE (a radiotherapy free regimen) and found similar results: 24/51 (47%) iPET positive, with a positive scan not adversely impacting on outcome. Prahladan et al using DA-EPOCH-R, found that although 90% of patients were positive for PET2, a positive result did not predict outcome. Algrin et al explored the role of PET2 and PET4 in 24 patients with PMBL with details of therapy unavailable. Whilst the earlier timepoint did not predict outcome, patients with a positive PET4 had inferior PFS. Chong et al reported on 26 patients with PMBL uniformly treated with R-CHOP, of whom 22 (85%) had iPET after 2 or 4 cycles. Of these, 17 (77%) were positive. They found the 1-yr PFS of iPET positive patients 68.9% vs 100% for patients with negative iPET, however the PPV was 43%. Based on the available data I conclude that iPET is frequently positive but has poor PPV for relapse and therefore should not be performed as part of standard care.
Table 30. Studies reporting interim ± end of treatment PET-CT findings in patients with primary mediastinal B-cell lymphoma. Abbreviations: PMBCL = primary mediastinal B-cell lymphoma; PET = positron emission tomography; iPET = interim PET; ePET = end of treatment PET; PPV = positive predictive value; ΔSUVmax = change in maximum standardised uptake value; 5PS = Deauville 5-point score; IHP = International Harmonisation Project; DA-EPOCH-R = dose adjusted etoposide, prednisolone, doxorubicin, cyclophosphamide, vincristine, rituximab; R-CHOP = rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone; Hyper-CVAD-R = hyper-fractionated cyclophosphamide, doxorubicin, dexamethasone, vincristine, rituximab; PFS = progression free survival; OS = overall survival.

*range given to account for different methods of interpretation of scans.

<table>
<thead>
<tr>
<th>Reference</th>
<th>year</th>
<th>n with iPET</th>
<th>chemotherapy</th>
<th>iPET timing</th>
<th>method of interpretation</th>
<th>iPET +ve</th>
<th>ePET +ve</th>
<th>iPET PPV</th>
<th>difference in outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>352</td>
<td>2007</td>
<td>16</td>
<td>R-VACOPB R-CHOP</td>
<td>not stated</td>
<td>not stated</td>
<td>8/16 (50%)</td>
<td>not stated</td>
<td>25%</td>
<td>no</td>
</tr>
<tr>
<td>353</td>
<td>2010</td>
<td>51</td>
<td>RCHOP/ICE</td>
<td>not stated</td>
<td>ΔSUVmax</td>
<td>24/51 (47%)</td>
<td>not stated</td>
<td>not stated</td>
<td>no</td>
</tr>
<tr>
<td>350</td>
<td>2010</td>
<td>24</td>
<td>N/A</td>
<td>post cycle 2,4</td>
<td>visual 5PS ΔSUVmax</td>
<td>PMBCL not reported separately</td>
<td>-</td>
<td>N/A</td>
<td>+ve iPET 4 cycles → inferior EFS (visual, 5PS)</td>
</tr>
<tr>
<td>349</td>
<td>2012</td>
<td>10</td>
<td>DA-EPOCH-R</td>
<td>post cycle 2</td>
<td>5PS</td>
<td>9/10 (90%)</td>
<td>0/7 (0%)</td>
<td>16%</td>
<td>no</td>
</tr>
<tr>
<td>351</td>
<td>2013</td>
<td>26</td>
<td>R-CHOP</td>
<td>post cycle 2,4</td>
<td>IHP</td>
<td>17/22 (77%)</td>
<td>6/15 (40%)</td>
<td>50%</td>
<td>+ve ePET → inferior PFS</td>
</tr>
<tr>
<td>current study</td>
<td>2014</td>
<td>28</td>
<td>R-CHOP DA-EPOCH-R Hyper-CVAD-R</td>
<td>post cycle 2-4</td>
<td>visual 5PS ΔSUVmax</td>
<td>29-37%*</td>
<td>24-32%*</td>
<td>12-50%*</td>
<td>+ve ePET → inferior PFS (visual, 5PS 4-5)</td>
</tr>
</tbody>
</table>
Discussion

Table 31 Studies reporting end of treatment PET-CT findings in patients with primary mediastinal B-cell lymphoma. Abbreviations: PMBCL = primary mediastinal B-cell lymphoma; PET = positron emission tomography; iPET = interim PET; ePET = end of treatment PET; PPV = positive predictive value; ΔSUVmax = change in maximum standardised uptake value; 5PS = Deauville 5-point score; IHP = International Harmonisation Project304; DA-EPOCH-R = dose adjusted etoposide, prednisolone, doxorubicin, cyclophosphamide, vincristine, rituximab; R-CHOP = rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone; Hyper-CVAD-R = hyper-fractionated cyclophosphamide, doxorubicin, dexamethasone, vincristine, rituximab; PFS = progression free survival; OS = overall survival. *range given to account for different methods of interpretation of scans. *125 patients treated but 10 excluded as primary refractory or insufficient PET data

<table>
<thead>
<tr>
<th>Reference</th>
<th>year</th>
<th>n with ePET</th>
<th>chemotherapy</th>
<th>radiotherapy</th>
<th>method of interpretation</th>
<th>ePET +ve</th>
<th>ePET PPV</th>
<th>difference in outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>347</td>
<td>2012</td>
<td>59</td>
<td>R-CHOP 59</td>
<td>23/24 (96%)</td>
<td>ePET +ve 2/35 (6%) ePET -ve</td>
<td>not stated</td>
<td>35/59 (59%)</td>
<td>17%</td>
</tr>
<tr>
<td>354</td>
<td>2012</td>
<td>115*</td>
<td>R-CHOP 20</td>
<td>106/119 (89%)</td>
<td>SPS IHP</td>
<td>SPS ≥3 61/115 (53%) SPS ≥4 34/115 (30%)</td>
<td>5PS ≥3 15% 5PS ≥4 26%</td>
<td>5PS≥4 → inferior PFS + OS</td>
</tr>
<tr>
<td>160</td>
<td>2013</td>
<td>36</td>
<td>DA-EPOCH-R 36</td>
<td>2/36 (6%)</td>
<td>IHP</td>
<td>18/36 (50%)</td>
<td>17%</td>
<td>not reported</td>
</tr>
<tr>
<td>355</td>
<td>2013</td>
<td>71</td>
<td>R-CHOP 71</td>
<td>PET-pos 93%</td>
<td>PET-neg 43%</td>
<td>IHP SUVmax</td>
<td>29/71 (41%)</td>
<td>21%</td>
</tr>
<tr>
<td>356</td>
<td>2013</td>
<td>37</td>
<td>R-CHOP 8</td>
<td>100%</td>
<td>SPS</td>
<td>SPS ≥3 25/37 (68%) SPS ≥4 18/37 (49%)</td>
<td>5PS ≥3 3/25 (12%) 5PS ≥4 3/18 (17%)</td>
<td>5PS≥4 → inferior OS</td>
</tr>
<tr>
<td>357</td>
<td>2013</td>
<td>74</td>
<td>R-MACOP-B</td>
<td>51/51 (100%)</td>
<td>ePET +ve 0/23 ePET -ve</td>
<td>not stated</td>
<td>51/75 (69%)</td>
<td>4/51 (8%)</td>
</tr>
</tbody>
</table>
Several studies have reported findings from ePET (but not iPET) in PMBL (Table 31). Although choice of chemotherapy regimen and criteria for PET interpretation varied between studies, 41-59% of patients remained positive; the PPV for relapse was 11-22%. The impact of positive ePET on outcome is difficult to determine due to the heterogeneity of chemotherapy used. The prospective IELSG-26 study (using a combination of R-CHOP, R-MACOPB or R-VACOP-B, with radiotherapy permitted but not defined according to PET result) found patients with 5-point scale 4 or 5 experienced inferior PFS/OS compared to patients with negative scans (score 0-3). The authors concluded that patients with residual uptake in the mass of greater intensity than liver may be candidates for early intensification of therapy.

ePET has been explored as a means of sparing patients consolidative radiotherapy. Savage et al performed a retrospective analysis of (R)-CHOP treated patients, dividing them into two eras. In the “RT era” was considered standard of care, with 80% of patients treated, whilst in the “PET era” patients were referred for radiotherapy if ePET was positive, and 38% were treated. Amongst 56 patients treated with R-CHOP with a median 5.4 years of follow up, omission of radiation in ePET-negative patients did not compromise outcome. Vassilakopoulos et al also analysed R-CHOP treated patients in whom ePET was performed. They irradiated 93% of PET-positive patients and 43% of PET-negative patients. Using this approach, the 2-year PFS was non-significantly higher in the PET-negative group (93 v 73%, P=0.06). Zinzani et al treated 74 patients with R-MACOP-B and only irradiated the 69% of patients with positive ePET, with no significant differences in disease free or overall survival at the time of reporting.

How best to manage patients with residual FDG avid masses remains a clinical challenge. In both this study (66 v 100%, P=0.001) and that of Vassilakopoulos et al ePET SUVmax >5 predicted inferior 2-yr PFS (53 v 92%, P=0.05). In the NCI report of DA- EPOCH-R Dunleavy et al noted that the three patients with positive end of treatment PET were found to have residual lymphoma all had SUVmax >5 (5.9, 10.2
Discussion

and 14.5). From these data, it is reasonable to observe patients with minimal residual FDG uptake with serial imaging, whilst indeterminate cases should undergo biopsy for histologic confirmation prior to salvage therapy.

The role of radiotherapy in PMBL remains unclear. The excellent outcomes reported in the NCI study suggest that in patients receiving DA-EPOCH-R, radiotherapy may be safely omitted.\textsuperscript{160} Two retrospective analyses using anthracycline-based chemotherapy without rituximab found radiotherapy improved PFS.\textsuperscript{359,360} In contrast, Savage \textit{et al} (discussed above) found a PET-directed approach to risk stratification of radiotherapy spares a significant proportion radiotherapy toxicity without apparently compromising outcome.\textsuperscript{347} Filippi \textit{et al} recently reported a series of 37 patients treated with R-CHOP, R-MACOPB or R-VACOPB and radiotherapy and despite 49% being ePET positive, excellent 3-year PFS and OS of approximately 90% were achieved.\textsuperscript{356} The prospective IELSG-37 study may resolve this unanswered question.\textsuperscript{361}

These data have several limitations – in particular the use of iPET (and to a lesser extent ePET) to guide escalation of therapy may have salvaged suboptimal responses and therefore reduced the PPV. Thus caution must be exercised in the interpretation of these results. Because of the retrospective nature, despite great lengths to ensure completeness some data is missing or incomplete. As a single centre experience of a rare disease with a favourable outcome, the number of patients experiencing relapse is low, limiting my power to make firm conclusions about the predictive value of PET.

CNS relapse in aggressive lymphoma

CNS relapse in DLBCL

Whilst acknowledging the limitations of this retrospective analysis, in a group of patients specifically selected for high risk of CNS relapse the addition of high-dose IV MTX with or without cytarabine was associated with a reduction in the rate of subsequent CNS relapse. The actuarial 3-year risk of CNS relapse was 18.4% in patients who received CHOP\textpm{}R with IT MTX; 6.9% in patients who received CHOP\textpm{}R, IT MTX and high-dose IV MTX and 2.3% in patients receiving chemotherapy regimens containing high-dose IV MTX and cytarabine ($P=0.009$). The non-significant trend
toward lower incidence of CNS relapse seen in group 3 vs 2 may reflect the high dose cytarabine administered to these patients, or potentially superior systemic disease control, as fewer systemic relapses occurred in the group 3.

The high relapse rate in patients treated with IT alone support findings from other studies that this approach is inadequate.\textsuperscript{110,112,117,208} Over 60% of CNS relapses involved brain parenchyma and were not prevented by IT chemotherapy alone. In fact, there was a non-significant trend toward fewer leptomeningeal relapses in patients who received prophylaxis with IV MTX. This result is difficult to explain, however it should be noted that the total number of CNS relapses was low and >80% of patients in groups 2 and 3 also received IT MTX.

Soon after two of our institutions changed policy to incorporate IV MTX for CNS prophylaxis in 2003, rituximab was reimbursed for the treatment of patients with DLBCL in Australia in 2005. This coincidence accounts for the imbalance in rituximab use between groups. The published data dealing with the impact of rituximab on CNS relapse are mixed - some studies have shown no impact \textsuperscript{110,116,362,363} whilst others have shown a significant reduction.\textsuperscript{109,112,115} A meta-analysis of pooled data from eight studies comparing rituximab with chemotherapy to chemotherapy alone found the addition of rituximab slightly reduced the risk of CNS relapse from 5.7% to 4.7%.\textsuperscript{364} In this analysis, the use of rituximab as part of induction therapy did not appear to influence the risk of CNS relapse either in the cohort as a whole, or when subgroup analyses were performed within groups 1 and 3. Therefore I conclude that the impact from the lack of rituximab in group 1 is likely to be modest and does not completely account for the marked difference in CNS relapse risk seen. There were some imbalances in CNS risk factors between the groups: group 3 were younger, had higher serum LDH and more patients with B symptoms. In spite of this, the risk of CNS relapse was lowest in this group.

High-dose IV MTX is not without disadvantages. It is administered in an inpatient setting at our institutions, which has both financial and physical resource implications. Although grade 3+ adverse events were rare, despite careful fluid management and
urinary alkalinization, grade 2+ renal impairment occurred in 15% of patients. Whilst no patients developed irreversible renal impairment or need for haemodialysis, in 8% of cycles the renal impairment resulted in delayed MTX clearance and prolonged hospitalization.

A few other studies have been performed which suggest high-dose anti-metabolite therapy provides effective CNS prophylaxis in patients with DLBCL. Tilly et al conducted a prospective comparison between ACVBP and CHOP for intermediate grade lymphoma. The ACVBP regimen included a consolidation phase (IV MTX 3g/m²) and four doses of IT MTX. Although stratification by CNS risk was not pre-specified, the randomization resulted in balanced distribution of CNS risk features (such as raised serum LDH and multiple extranodal sites) between arms. Patients treated with ACVBP (which included both IT and high-dose IV MTX) had CNS relapse risk of 2.8% compared with 8.3% in patients treated with CHOP alone (P=0.004). The follow-up LNH03-2B study was a randomized phase III comparison between R-ACVBP and eight cycles of R-CHOP21 (with four doses of IT MTX) in patients with DLBCL, aIPI ≥1 aged 18-65. Patients in this study was neither specifically selected nor stratified for CNS relapse risk, and the low incidence of CNS relapse overall (0/192 (0%) for R-ACVBP v 2/183 (1%) for R-CHOP) makes it difficult to draw conclusions regarding the effectiveness of the MTX in this study. Abramson et al treated 65 patients with high risk for CNS involvement as defined by published risk models or high-risk extranodal sites. Patients treated with a median of three cycles of MTX at a dose of 3.5g/m² but only four (6%) received a dose of IT MTX at time of diagnostic LP. Despite this, after a median follow up of 33 months only two patients had developed CNS relapse, with a resultant estimated CNS recurrence rate of 3%. Holte et al conducted a phase II study of young, high-risk patients (aged <65 years, age adjusted IPI 2-3) in which the treatment protocol was specifically designed to minimize CNS relapse. Patients were treated with rituximab, cyclophosphamide, doxorubicin, etoposide, and prednisolone (R-CHOEP14) followed by four doses of IV cytarabine (2-3g/m²) and one cycle of MTX (1.5-3g/m²). They treated 156 patients and found the toxicity manageable (grade 3/4 haematological 79%, grade 3/4 infections 7%) and
deliverable. With a median follow up of 52 months, seven CNS relapses have occurred, a crude incidence of 4.5% - lower than might be expected in a high-risk population.

It should be noted that flow cytometric analysis of CSF was not uniformly performed at baseline at all centres; this policy was adopted from 2007 at PMCC. Seven patients developed CNS relapse within six months of diagnosis. I acknowledge that occult CNS involvement at baseline in these patients may not have been detected, however the distribution of cases with missing baseline CSF cytology did not differ between treatment groups. Recently many groups have incorporated both more rigorous baseline CNS staging (with mandatory CSF flow cytometric analysis) and earlier CNS directed therapies (both systemic and IT) into future treatment protocols.

The optimal timing of systemic high dose chemotherapy as CNS prophylaxis is a critical unresolved question. Studies have consistently shown that CNS relapse is most frequent in the first 12 months from completion of primary therapy. This pattern of early CNS failures suggests occult CNS disease present at diagnosis and has led some groups, such as the German High Grade Lymphoma Study Group to incorporate the first dose of systemic high-dose MTX prior to the commencement of chemo-immunotherapy, during steroid pre-phase (Michael Pfreundschuh, personal communication). Although providing early CNS prophylaxis, such scheduling risks delaying chemo-immunotherapy if toxicity occurs. Given the paucity of comparative data evaluating the efficacy of differing doses and timing of administration of systemic anti-metabolite therapy for CNS prophylaxis it is clear that these remain contentious issues.

The major limitation of this study lies in the retrospective nature, and heterogeneity in baseline risk and treatment factors (particularly rituximab) amongst the three groups, leading to potential bias. Nonetheless, this finding adds to the growing body of non-randomized data suggesting the incorporation of high-dose IV MTX ± cytarabine into treatment protocols may lower the risk of CNS relapse in patients with DLBCL at high risk of the complication. Ideally this hypothesis would be tested in an adequately
Discussion

powered, prospective study randomizing patients to R-CHOP + IT MTX ± high dose IV MTX and/or cytarabine, with the primary endpoint 2-year rate of CNS relapse. There are however, several practical difficulties with performing such a study. Many clinicians believe sufficient evidence exists to support the efficacy of high dose IV MTX ± cytarabine for CNS-directed prophylaxis and may be uncomfortable enrolling patients to a protocol with a chance of not receiving it. Secondly, because CNS relapse remains a rare complication, adequately powering a study is costly and difficult. Limiting the study to only high risk patients (with estimated CNS relapse risk of around 15%) would reduce the sample size needed, but such patients comprise <10% of DLBCL overall. This probably explains why a prospective study addressing this question has yet to be completed to my knowledge.

CNS relapse in mantle cell lymphoma

I have presented the largest collection of cases of patients with MCL and CNS involvement reported to date. The crude incidence of 4.1% suggests that CNS involvement is uncommon in the clinical course of MCL, being toward the lower end of previously published estimates (Table 32). However, due to non-uniform staging procedures the true incidence of asymptomatic CNS disease may be under-reported.
Table 32 Summary of studies on central nervous system involvement in mantle cell lymphoma. CNS indicates central nervous system. From Cheah et al, with permission.\textsuperscript{196}

<table>
<thead>
<tr>
<th>Reference</th>
<th>at any time at diagnosis</th>
<th>median time to CNS disease (months)</th>
<th>1\textsuperscript{st} line HD MTX/ara-c</th>
<th>1\textsuperscript{st} line IT chemo</th>
<th>median survival from CNS event (months)</th>
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<tbody>
<tr>
<td>199</td>
<td>4/94 (5%) 0 (0%)</td>
<td>51</td>
<td>some</td>
<td>0</td>
<td>0.8</td>
</tr>
<tr>
<td>200</td>
<td>10/108 (9%) 2/108 (1.8%)</td>
<td>15.5</td>
<td>unknown</td>
<td>0</td>
<td>Unknown</td>
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<tr>
<td>308</td>
<td>11/82 (13%) 1/62 (1.6%)</td>
<td>25</td>
<td>20%</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>194</td>
<td>4/62 (6.5%) 1/62 (1.6%)</td>
<td>12</td>
<td>16%</td>
<td>10%</td>
<td>3</td>
</tr>
<tr>
<td>198</td>
<td>11/142 (7.7%) 0</td>
<td>13.8</td>
<td>13%</td>
<td>NR</td>
<td>6.3</td>
</tr>
<tr>
<td>Current study</td>
<td>57/1396 (4.1%) 13/1396 (0.9%)</td>
<td>15.2</td>
<td>20%</td>
<td>20%</td>
<td>3.7</td>
</tr>
</tbody>
</table>

Comparison with a CNS non-involved cohort of patients identified blastoid histology, B-symptoms, increased serum LDH, poor ECOG performance status and high MIPI score as possible indicators of CNS risk. Though not derived from the entire cohort, I nonetheless developed a predictive risk model for CNS involvement based on the presence of ≥1 high risk feature(s) at baseline. In the absence of any risk features I did not observe cases of CNS involvement whilst 15% of patients with risk features developed CNS involvement by 5 years. Thus, these results support the findings of previous investigators that highly proliferative MCL is a risk factor for CNS involvement.\textsuperscript{312,367-369}

Given that blood involvement at diagnosis is common\textsuperscript{368}, I recommend that patients with high risk of CNS involvement undergo CSF assessment once the peripheral blood lymphoma cells are cleared, acknowledging that delaying initial CSF sampling until after clearance risks underestimating the presence of CNS involvement if CNS-
penetrating doses of antimetabolites have been used prior to CSF sampling. I also recommend performing flow-cytometry on CSF, due to the improved sensitivity over cytology alone.\(^{370-372}\) If traumatic tap occurs, performing blood count, film and blood flow-cytometry can assist the pathologist in differentiating sample contamination from true CSF involvement.

Given these findings, I recommend that CNS prophylaxis be given to patients at high risk for CNS involvement, particularly when the primary chemotherapy strategy does not contain CNS-penetrating doses of methotrexate or cytarabine. As has been discussed extensively in the DLBCL literature\(^ {208}\) the effectiveness of CNS prophylaxis cannot be readily determined from retrospective studies. It is clear that despite prophylaxis some patients developed CNS relapse. Only prospective, randomised allocation of CNS prophylaxis is able to determine the benefit of such an approach.

One observation to emerge from this study is the predilection of MCL to result in leptomeningeal disease in apparent contrast to DLBCL, where parenchymal relapse is more frequent. In a meta-analysis of 28 studies by Siegal and Goldschmidt, 59% of 693 cases of CNS relapse in patients with DLBCL were parenchymal with 41% leptomeningeal\(^ {209}\).

The treatment approaches once CNS involvement was established which were associated with superior overall survival were therapy with CNS-penetrating doses of anti-metabolites, and consolidation with stem cell transplantation. Stem cell transplantation was the only strategy that provided remissions beyond 12 months, making this approach the therapeutic goal for patients with adequate performance status and organ function. However, this group of patients were younger, had better performance status, achieved at least PR and sustained their responses long enough to undergo transplantation.

These data have weaknesses. As a pooled retrospective multicentre case series, CNS staging, investigation and management policies varied widely between centres and this non-uniform data collection lessens the quality and reliability of data. In particular, primary treatment strategies varied considerably and the majority of patients did not
receive rituximab. I am unable to provide an analysis of frontline therapy and risk of CNS relapse, which would be of interest. This predictive index is based on single institution cohort of patients, some of whom were treated before the era of highly active regimens containing CNS penetrating doses of anti-metabolites. As such, prospective validation in a larger cohort should be a goal of future studies in the field.
NMP and anti-CD20 monoclonal antibodies in NHL

Using a series of refinements to experimental methodology I was able to show that NMP enhances rituximab-induced ADCC in vitro. Given some of the similarities between lenalidomide and NMP it may be hypothesised that the mechanism of this potentiation may be similar, though this remains to be proven. Although not reaching significance in my experiment, there was a trend toward improvement of ADCC in obinutuzumab also (Figure 26). The results obtained from this experiment provide preliminary evidence that NMP should be explored as an immunotherapy in patients with lymphoma as well as myeloma. A good starting point for this investigation would be a phase I clinical trial using daily oral NMP in conjunction with rituximab in patients with relapsed or refractory indolent B-cell NHL. Such a design would ideally enrol patients relatively early in their disease course, when immune function remains preserved. In this way, the chance of a missing a significant response rate would be minimised.
Conclusion

In this thesis I have outlined methods in which patients with lymphoproliferative disorders are failing standard therapies. By applying the dual functional-structural imaging modality of PET-CT I have shown that Surveillance PET-CT has no role in patients with DLBCL in achieving CMR with the possible exception of patients with baseline IPI ≥3 in the 18 months following completion of primary therapy. A prospective study would be required to address this. Furthermore, in patients with TrIL achieving CMR, PET-CT detected subclinical relapses of low-grade histology with high sensitivity but with a low false-positive rate. This is of limited clinical benefit as the initiation of further therapy in these circumstances is rarely based on imaging findings alone. In contrast, all DLBCL relapses in this cohort were accompanied by clinical symptoms. Thus, surveillance imaging of patients with TrIL achieving CMR is not indicated. PET-CT should be reserved for evaluation of suspected relapse. The use of PET-CT in PMBL showed positive ePET predicts inferior PFS but an inflammatory response resulting in metabolic abnormality is frequently observed and is difficult to differentiate from residual disease. Metabolically active residual masses after the completion of treatment should be biopsied to confirm viable lymphoma if salvage therapy is planned. Another method of preventing treatment failure in NHL is identification of patients at highest risk for CNS relapse, and applying CNS-directed prophylaxis. I was able to show that the addition of high-dose IV MTX, either at the completion of R-CHOP or as part of dose intensive chemotherapy strategies, is associated with a reduction in CNS relapse risk in DLBCL and should be considered in patients with high risk for this complication. Additionally, I identified several risk factors which are markers of highly kinetic disease to predict risk in patients with MCL.

Once patients have failed existing treatments, it is clear that alternative treatment options are needed. I shoed that NMP enhances rituximab-induced ADCC in vitro against B-cell lymphoma, providing preclinical rationale for the evaluation of this
combination in phase I clinical trial designs enrolling patients with relapsed or refractory B-cell NHL. Finally, I developed three phase I clinical trial protocols for patients with lymphoid malignancies with opportunities for correlative scientific analyses to better predict markers of disease response and failure. Lymphoid malignancies are a heterogeneous group of disorders with diverse biologic and clinical characteristics. Accordingly, a “one-size fits all” approach is unlikely to be universally successful. Using this multifaceted approach targeting different aspects of the disease I have shown that with small incremental benefits, the outcomes of patients with lymphoid malignancy can be improved.

In the course of completing my thesis I have developed skills that lay the foundations for my career as an academic Haematologist. The projects involving clinical datasets enabled the acquisition of skills in database design and coding to gather enough information to answer clinically meaningful questions without wasting time on extraneous data. I acquired a sound understanding of the basic principles and application of biostatistics, which will be invaluable in the design of future clinical investigations. My contributions to the field regarding CNS relapse in aggressive lymphoma and clinical utility of PET in DLBCL have influenced clinical practice locally and internationally.

Importantly, I have learnt how to ask the right questions, assess the feasibility, plan, design, execute and publish clinical investigations. The experience gained writing grants will prove invaluable me in obtaining funding to conduct future investigations – critical in an increasingly competitive environment. The next phase in my career will be further enhancing these skills in a Lymphoma Fellowship with the Department of Lymphoma/Myeloma at the University of Texas MDACC. I plan to return to Australia as a comprehensively trained clinical and translational investigator equipped to make further improvements in care for patients with lymphoid malignancy.
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## List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>5PS</td>
<td>5-point scale</td>
</tr>
<tr>
<td>age-adjusted International Prognostic Index</td>
<td></td>
</tr>
<tr>
<td>ABC</td>
<td>activated B-cell</td>
</tr>
<tr>
<td>ADCC</td>
<td>antibody dependent cell mediated cytotoxicity</td>
</tr>
<tr>
<td>ALT</td>
<td>alanine transaminase</td>
</tr>
<tr>
<td>ASCT</td>
<td>autologous stem cell transplantation</td>
</tr>
<tr>
<td>BCCA</td>
<td>British Columbia Cancer Agency</td>
</tr>
<tr>
<td>BCG</td>
<td>bacillus Calmette-Guérin</td>
</tr>
<tr>
<td>BLNI</td>
<td>British National Lymphoma Investigation</td>
</tr>
<tr>
<td>CDC</td>
<td>complement dependent cytotoxicity</td>
</tr>
<tr>
<td>CLL</td>
<td>chronic lymphocytic leukaemia</td>
</tr>
<tr>
<td>CMR</td>
<td>complete metabolic response</td>
</tr>
<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>CR</td>
<td>complete response</td>
</tr>
<tr>
<td>CRu</td>
<td>unconfirmed complete response</td>
</tr>
<tr>
<td>CSF</td>
<td>cerebrospinal fluid</td>
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<tr>
<td>CT</td>
<td>computed tomography</td>
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<td>DLBCL</td>
<td>diffuse large B-cell lymphoma</td>
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<tr>
<td>DLT</td>
<td>Dose limiting toxicity</td>
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<tr>
<td>DMSC</td>
<td>Data Monitoring and Safety Committee</td>
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<td>DSHNHL</td>
<td>Deutsche Studiengruppe für Hochmaligne Non-Hodgkin-Lymphome</td>
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<tr>
<td>EBV</td>
<td>Epstein-Barr virus</td>
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<td>ECOG</td>
<td>Eastern Cooperative Oncology Group</td>
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<td>EFS</td>
<td>event-free survival</td>
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<tr>
<td>EMCLN</td>
<td>European Mantle Cell Lymphoma Network</td>
</tr>
<tr>
<td>ePET</td>
<td>end of treatment positron emission tomography scan</td>
</tr>
<tr>
<td>FACS</td>
<td>fluorescence activated cell sorting</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
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<td>---------</td>
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<tr>
<td>FDG</td>
<td>fluorodeoxyglucose</td>
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<tr>
<td>FISH</td>
<td>fluorescence in situ hybridisation</td>
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<td>follicular lymphoma</td>
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<td>FLIPI</td>
<td>follicular lymphoma international prognostic index</td>
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<tr>
<td>GCB</td>
<td>germinal centre B-cell</td>
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<tr>
<td>GCP</td>
<td>Good Clinical Practice</td>
</tr>
<tr>
<td>GELA</td>
<td>Groupe d’Etude des Lymphomes de l’Adulte</td>
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<tr>
<td>HDT</td>
<td>high-dose therapy</td>
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<tr>
<td>HIV</td>
<td>human immunodeficiency virus</td>
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<td>HL</td>
<td>Hodgkin lymphoma</td>
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<tr>
<td>hPEBP4</td>
<td>human phosphatidylethanolamine binding protein</td>
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<td>iB-NHL</td>
<td>indolent B-cell non-Hodgkin lymphoma</td>
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<td>IEC</td>
<td>independent ethics committee</td>
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<td>International Extranodal Lymphoma Study Group</td>
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<td>interferon-γ</td>
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<tr>
<td>IHC</td>
<td>immunohistochemistry</td>
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<tr>
<td>IMiD</td>
<td>immunomodulatory drug</td>
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<td>interim positron emission tomography scan</td>
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<td>International Prognostic Index</td>
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<td>Institutional Review Board</td>
</tr>
<tr>
<td>IT</td>
<td>intrathecal</td>
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<tr>
<td>IV</td>
<td>intravenous</td>
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<td>IVL</td>
<td>intravascular large B-cell lymphoma</td>
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<tr>
<td>KIR</td>
<td>killer immunoglobulin receptor</td>
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<tr>
<td>LDH</td>
<td>lactate dehydrogenase</td>
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<td>LPD</td>
<td>lymphoproliferative disorder</td>
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<tr>
<td>LVEF</td>
<td>Left ventricular ejection fraction</td>
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<td>mAb</td>
<td>monoclonal antibodies</td>
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<td>MACS</td>
<td>magnetic activated cell sorting</td>
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<td>MALT</td>
<td>mucosa associated lymphoid tissue lymphoma</td>
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<td>MCL</td>
<td>mantle cell lymphoma</td>
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<td>MCP-1</td>
<td>monocyte chemotactic protein-1</td>
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<td>Definition</td>
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<tr>
<td>MDACC</td>
<td>MD Anderson Cancer Center</td>
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<td>mantle cell lymphoma international prognostic index</td>
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<td>MM</td>
<td>multiple myeloma</td>
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<tr>
<td>MMC</td>
<td>Monash Medical Centre</td>
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<tr>
<td>MTD</td>
<td>maximum tolerated dose</td>
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<tr>
<td>MTX</td>
<td>methotrexate</td>
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<tr>
<td>MZL</td>
<td>marginal zone lymphoma</td>
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<tr>
<td>NCCN</td>
<td>National Comprehensive Cancer Network</td>
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<td>NHL</td>
<td>non-Hodgkin lymphoma</td>
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<td>NHMRC</td>
<td>National Health and Medical Research Council</td>
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<tr>
<td>NK</td>
<td>natural killer</td>
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<tr>
<td>NMP</td>
<td>N-Methyl-2-pyrrolidone</td>
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<tr>
<td>NOS</td>
<td>not otherwise specified</td>
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<tr>
<td>NPV</td>
<td>negative predictive value</td>
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<tr>
<td>OBD</td>
<td>optimum biologic dose</td>
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<tr>
<td>OR(R)</td>
<td>objective response (rate)</td>
</tr>
<tr>
<td>OS</td>
<td>overall survival</td>
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<tr>
<td>PD</td>
<td>progressive disease</td>
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<tr>
<td>PET</td>
<td>positron emission tomography</td>
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<td>PFS</td>
<td>progression-free survival</td>
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<td>PMBL</td>
<td>primary mediastinal B-cell lymphoma</td>
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<td>partial metabolic response</td>
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<td>positive predictive value</td>
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<td>RMH</td>
<td>Royal Melbourne Hospital</td>
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<td>RP2D</td>
<td>recommended phase II dose</td>
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<tr>
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<td>severe combined immunodeficient</td>
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<td>stable disease</td>
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<td>small lymphocytic lymphoma</td>
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<tr>
<td>SUSAR</td>
<td>suspected unexpected serious adverse reaction</td>
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<tr>
<td>TrIL</td>
<td>transformed indolent lymphoma</td>
</tr>
<tr>
<td>ULN</td>
<td>upper limit of normal</td>
</tr>
<tr>
<td>ΔSUVmax</td>
<td>change in maximum standardised uptake value</td>
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</table>
List of chemotherapy protocols

**R-CHOP**

rituximab 375mg/m² D1  
cyclophosphamide 750mg/m² D1  
doxorubicin 50mg/m² D1  
vincristine 1.4mg/m² IV capped at 2mg D1  
prednisolone 100mg D1-5 po

**R-dose adjusted EPOCH**

rituximab 375mg/m² D1  
Infused agents  
etoposide 50 mg/m²/day IVI D 1-4 (96 hours)  
doxorubicin 10 mg/m²/day IVI D 1-4 (96 hours)  
vincristine 0.4 mg/m²/day IVI D 1-4 (96 hours)  
Bolus agents  
cyclophosphamide 750 mg/m²/day IV D 5  
prednisone 60 mg/m²/BD Oral D 1-5

**R-maxi-CHOP (Nordic regimen)**

rituximab  
cyclophosphamide 750mg/m² D1  
doxorubicin 50mg/m² D1  
vincristine 1.4mg/m² IV capped at 2mg D1  
prednisolone 100mg D1-5 po

**R-DHAP**

rituximab 375mg/m² D1  
dexamethasone 40mg D1-4  
cytarabine 2g/m² BD D2
cisplatin 100mg/m\(^2\) D3

\(R^2\)

rituximab 375mg/m\(^2\)
lenalidomide 20mg daily, D1-21

\((R)-\text{Hyper-CVAD/R-MA}\)

\textbf{A cycle}

(rituximab 375mg/m\(^2\) D1)

cyclophosphamide 300mg/m\(^2\) IV twice daily D1-3

methotrexate 12mg IT D1

doxorubicin 50mg/m\(^2\) IV D3

vincristine 1mg/m\(^2\) (max 2mg) IV D3,11

dexamethasone 40mg PO D1-4 and 11-14

\textbf{B cycle}

(rituximab 375mg/m\(^2\) D1)

methotrexate 1g/m\(^2\) IVI (over 24 hours) D1

cytarabine 3g/m\(^2\) IVI twice daily D2,3

methotrexate 12mg IT D1

\textit{CODOXM-IVAC}

(rituximab 375mg/m\(^2\) D1)

cyclophosphamide 800mg/m\(^2\) IV D1

cyclophosphamide 200mg/m\(^2\) IV D2-5

vincristine 1.5mg/m\(^2\) (max 2mg) IV D1,8

doxorubicin 40mg/m\(^2\) IV D1

cytarabine 70mg IT D1,3

methotrexate 1g/m\(^2\) IVI (over 24 hours)

ifosfamide 1.5g/m\(^2\) IV D1-5

etoposide 60mg/m\(^2\) IV D1-5
cytarabine 2g/m² IV twice daily D1-2
methotrexate 12mg IT D5

(R)-MACOPB
(rituximab 375mg/m² D1)
methotrexate 400mg/m² IV weeks 2,6,10
doxorubicin 50mg/m² IV weeks 1,3,5,7,9,11
cyclophosphamide 350mg/m² IV weeks 1,3,5,7,9,11
vincristine 1.4mg/m² (capped at 2mg) IV weeks 2,4,6,8,10,12
prednisolone 75mg daily
bleomycin 10mg/m² IV weeks 4,8,12

m-BACOD
methotrexate 200mg/m² D8, 15
bleomycin 4mg/m²
doxorubicin 45mg/m²
cyclophosphamide 600mg/m²
vincristine 1mg/m²
dexamethasone 6mg/m² D 1 - 5

ProMACE-CytaBOM
prednisone 60mg/m² D 1 – 14 PO
doxorubicin 25mg/m² IV D1
cyclophosphamide 650mg/m² IV D1
etoposide 120mg/m² IV D1
cytarabine 300mg/m² IV D8
bleomycin 5mg/m² IV D8
vincristine 1.4mg/m² IV D8
methotrexate 120mg/m2 IV D8

(R)-ACVBP
4 induction courses (Q21 days)
(rituximab 375mg/m² D1)
doxorubicin 75 mg/m² IV D1,
cyclophosphamide 1200 mg/m² intravenously D1
vindeistine 2 mg/m² on D1,5
bleomycin 10 mg D1,5
prednisolone 60 mg/m² orally D1-5
methotrexate 15 mg IT D2
Consolidation therapy (Q14 days)
methotrexate 3 g/m² IV plus leucovorin rescue x 2
etoposide 300 mg/m² IV x 4
ifosfamide 1500 mg/m² IV x4
cytosine-arabinoside 100 mg/m² subcutaneously D1-4

R-CHOEP-14 + cytarabine + methotrexate (Nordic high risk DLBCL protocol)
rituximab 375 mg/m²
cyclophosphamide 750 mg/m² IV
doxorubicin 50 mg/m² IV
vincristine 1.4 mg/m² (maximum 2.0 mg) IV day 1
etoposide 100 mg/m² IV on days 1–3
prednisone 100 mg daily PO for days 1–5.

systemic CNS prophylaxis
cytarabine 3 g/m² IV twice daily for 4 doses

high-dose methotrexate (H- MTX; course number 8) 3 g/m² IV over 24h infusion.
Appendices

Full text of publications

1. A multicentre retrospective comparison of central nervous system prophylaxis strategies among patients with high-risk diffuse large B-cell lymphoma.
*Br J Cancer* 2014 Sep;111(6):1072

*Final published available at:* [http://annonc.oxfordjournals.org/content/24/8/2119.full](http://annonc.oxfordjournals.org/content/24/8/2119.full)

3. Limited role for surveillance PET-CT scanning in patients with diffuse large B-cell lymphoma in complete metabolic remission following primary therapy.
*Br J Cancer* 2013 Jul 23;109(2):312-7

4. Limited clinical benefit for surveillance PET-CT scanning in patients with histologically transformed lymphoma in complete metabolic remission following primary therapy.
*Ann Hematol* Epub March 5, 2014

5. The utility and limitations of PET-CT in patients with primary mediastinal B-cell lymphoma: a single centre experience and literature review.
*Leuk Lymphoma* epub 14 April 2014
A multicentre retrospective comparison of central nervous system prophylaxis strategies among patients with high-risk diffuse large B-cell lymphoma

C Y Cheah1,2, K E Herbert1,2,3, K O’Rourke4, G A Kennedy4,5, A George1, P L Fedele6, M Gilbertson6,7, S Y Tan6, D S Ritchie1,2, S S Opat6,7, H M Prince1,2,3, M Dickinson1,2, K Burbury1,2, M Wolf1,2,3, E H Januszewicz1, C S Tam1,2, D Westerman1,2, D A Carney1,2, S J Harrison1,2 and J F Seymour*,1,2

1Department of Haematology, Peter MacCallum Cancer Centre, Locked Bag 1, A’Beckett Street, Melbourne, Victoria 8006, Australia; 2Sir Peter MacCallum Department of Oncology, University of Melbourne, Parkville, Victoria, Australia; 3Cabrini Medical Centre, Malvern, Victoria, Australia; 4Department of Haematology, Royal Brisbane and Women’s Hospital, Brisbane, Queensland, Australia; 5University of Queensland, St Lucia, Queensland, Australia; 6Department of Haematology, Monash Health, Clayton, Victoria, Australia and 7Department of Haematology, Monash University, Clayton, Victoria, Australia

Background: Central nervous system (CNS) relapse in diffuse large B-cell lymphoma (DLBCL) is a devastating complication; the optimal prophylactic strategy remains unclear.

Methods: We performed a multicentre, retrospective analysis of patients with DLBCL with high risk for CNS relapse as defined by two or more of: multiple extranodal sites, elevated serum LDH and B symptoms or involvement of specific high-risk anatomical sites. We compared three different strategies of CNS-directed therapy: intrathecal (IT) methotrexate (MTX) with (R)-CHOP ‘group 1’; R-CHOP with IT MTX and two cycles of high-dose intravenous (IV) MTX ‘group 2’; dose-intensive systemic antimetabolite-containing chemotherapy (Hyper-CVAD or CODOXM/IVAC) with IT/IV MTX ‘group 3’.

Results: Overall, 217 patients were identified (49, 125 and 43 in groups 1–3, respectively). With median follow-up of 3.4 (range 0.2–18.6) years, 23 CNS relapses occurred (12, 10 and 1 in groups 1–3 respectively). The 3-year actuarial rates (95% CI) of CNS relapse were 18.4% (9.5–33.1%), 6.9% (3.5–13.4%) and 2.3% (0.4–15.4%) in groups 1–3, respectively (P = 0.009).

Conclusions: The addition of high-dose IV MTX and/or cytarabine was associated with lower incidence of CNS relapse compared with IT chemotherapy alone. However, these data are limited by their retrospective nature and warrant confirmation in prospective randomised studies.

Diffuse large B-cell lymphoma (DLBCL) is the most frequent form of non-Hodgkin’s lymphoma (Martelli et al, 2013). With current chemoimmunotherapy, ~60% of patients achieve long-term disease-free survival (Feugier et al, 2005; Swerdlow et al, 2008). With improving systemic disease control, an important mode of treatment failure is secondary involvement of the central nervous system (CNS), a complication that is typically rapidly fatal.

Both the accurate quantification of risk and optimisation of prevention of CNS involvement in patients with DLBCL have been the focus of many studies and several reviews (Ferreri et al, 2009;
Herrlinger et al, 2009; Siegal and Goldschmidt, 2012). In an unselected population with DLBCL treated with R-CHOP or similar regimens, the risk of CNS involvement is 4–7% (van Besien et al, 1998; Zinzani et al, 1999; Hollender et al, 2002; Bjorkholm et al, 2007). This low rate combined with the apparent lack of impact of intrathecal (IT) chemotherapy as prophylaxis has led some to question the value of CNS-directed prophylaxis in the era of R-CHOP primary therapy (Chua et al, 2002; Bernstein et al, 2009; Guirguis et al, 2012; Kumar et al, 2012; Zhang et al, 2014). Despite this, even when treated with R-CHOP and IT prophylaxis, a substantial proportion of the truly high-risk population of patients still experience CNS relapse. In an analysis of the large, prospective RICOVER-60 study in which elderly patients with DLBCL were treated with CHOP-14 with or without rituximab, Boehme et al (2009) identified the following factors as independent predictors of CNS relapse risk: elevated serum level of lactate dehydrogenase (LDH), >1 extranodal site of disease and the presence of B symptoms. The 2-year actuarial risk of CNS relapse for patients with all three risk factors, which comprised 4.8% of the cohort, was 33.5%. Although neither systematically delivered nor randomly allocated, the use of IT prophylaxis did not significantly reduce the risk of CNS relapse in this cohort, a finding also shown in other studies (Chua et al, 2002; Guirguis et al, 2012; Kumar et al, 2012). It is clear that for these patients a more effective strategy for reducing secondary CNS lymphoma beyond IT chemotherapy alone is needed.

There are several potential explanations for the suboptimal efficacy of IT methotrexate (MTX) alone. IT chemotherapy has poor penetration into brain parenchyma, the site of the majority of CNS relapses (Siegal and Goldschmidt, 2012). Intravenous (IV) MTX achieves more even drug distribution within the neuroaxis than IT administration. (Kimelberg et al, 1977; Balis et al, 2000). Pharmacokinetic studies have shown continuous infusion of IV MTX results in a ‘therapeutic’ serum level longer than bolus administration (Hryniuk and Bertino, 1969). Hence, there is conceptual appeal to treating patients with doses of MTX and cytarabine capable of penetrating brain parenchyma.

Based on the report by Tilly et al (2003b) showing that the application of CNS prophylaxis with four doses of IT MTX and two courses of IV MTX at 3 g m$^{-2}$ reduced CNS relapse in patients with intermediate grade lymphoma, the CNS prophylaxis strategy for DLBCL at Peter MacCallum Cancer Centre (PMCC) was altered to include high-dose MTX either at the completion of R-CHOP therapy or in combination with cytarabine as part of the Hyper-CVAD regimen (Koller et al, 1997). Other Australian institutions subsequently adopted this policy and herein we evaluate the outcome of patients so treated.

### Table 1. Comparison of treatment strategies for contributing centres

<table>
<thead>
<tr>
<th></th>
<th>Royal Brisbane and Women’s Hospital</th>
<th>Monash Medical Centre</th>
<th>Peter MacCallum Cancer Centre</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of patients</strong></td>
<td>35</td>
<td>39</td>
<td>143</td>
</tr>
<tr>
<td><strong>Primary chemotherapy (age &lt; 60 years, aIPI 2, 3)</strong></td>
<td>R-Hyper-CVAD/R-MA</td>
<td>CODOXM/IVAC</td>
<td>R-Hyper-CVAD/R-MA</td>
</tr>
<tr>
<td><strong>Primary chemotherapy (all others)</strong></td>
<td>R-CHOP</td>
<td>R-CHOP, R-MACOPB</td>
<td>R-CHOP</td>
</tr>
<tr>
<td><strong>Year HD-MTX commenced</strong></td>
<td>2003</td>
<td>2007</td>
<td>2003</td>
</tr>
</tbody>
</table>

Abbreviations: aIPI = age-adjusted international prognostic index; CODOXM/IVAC = cyclophosphamide, vincristine, doxorubicin, methotrexate, ifosfamide, etoposide, cytarabine; HD = high dose; R-CHOP = rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone; R-Hyper-CVAD = rituximab, hyperfractionated cyclophosphamide, doxorubicin, vincristine, dexamethasone; R-MA = rituximab, high-dose methotrexate, high-dose cytarabine; R-MACOPB = rituximab, methotrexate, doxorubicin, cyclophosphamide, vincristine, prednisolone, bleomycin. See Appendix for dosage and administration details.

We conducted a retrospective, multicentre analysis comparing three different forms of therapy on the incidence of CNS relapse in patients with DLBCL judged at high risk of this complication. Patients were identified by searching institutional databases from 1996 to 2011 (to allow a minimum of 2 years of follow-up) for patients with a confirmed histologic diagnosis of DLBCL by WHO criteria (Swerdlow et al, 2008). Patients with DLBCL following histologic transformation of low-grade lymphoma and HIV-associated DLBCL were included; however, patients with Burkitt or Burkitt-like lymphoma and patients with CNS involvement at diagnosis were excluded. Data collection was compliant with local Institutional Review Board requirements at each site. Patients were selected for CNS prophylaxis strategies by their primary managing haematologist if they fulfilled two or more of the following criteria: (1) multiple extranodal sites; (2) elevated serum LDH; (3) B symptoms. In addition, involvement of specific high-risk anatomical sites, that is, bone marrow (with large cell lymphoma) (Bos et al, 1998), breast (Ryan et al, 2006), testis (Zauca et al, 2003), kidney (Villa et al, 2010), adrenal glands (Tomita et al, 2012), paranasal sinus, nasopharynx, liver and paravertebral (Ferreri et al, 2009) was also considered an indication for CNS prophylaxis.

The features of patients by treatment groups are summarised in Table 1. In brief, from 1991 to 2003, patients received CHOP and IT MTX (group 1). This group also included patients who received MACOP-B ± rituximab, as the dose of IV MTX is <1 g m$^{-2}$ and thus unlikely to reach therapeutic levels in brain parenchyma. As previously described, our units adopted a policy of adding high-dose IV MTX at different times: PMCC and the Royal Brisbane and Women’s (RBWH) in 2003 and Monash Medical Centre (MMC) in 2007. This consisted of 1–3 g m$^{-2}$ (tailored according to renal function) by 24-h continuous IV infusion, followed by leucovorin rescue delivered on days 1 and 15, commencing 2 to 4 weeks after the completion of the CHOP-like regimen (group 2). Patients <65 years of age with age-adjusted IPI of ≥2 were treated with dose intensive therapy containing anti-metabolites (Hyper-CVAD or CODOXM-IVAC, with rituximab after it became available (group 3)). Patients also received IT MTX (12 mg via lumbar puncture) with each cycle of chemotherapy, aiming for a total of six doses unless contraindicated, patients refused or unacceptable toxicity developed. A summary of chemotherapy protocols used can be found in the Appendix.

Central nervous system staging with lumbar puncture and cerebrospinal fluid (CSF) analysis for cytology, flow cytometry (at PMCC, from 2007) and biochemical analysis was typically performed at baseline; however, baseline neuroimaging was typically only performed in the presence of clinical evidence for CNS involvement.
suggesting CNS lymphoma or positive CSF cytology or flow cytometry. The CNS involvement was defined by one or more of (1) histologically confirmed CNS involvment; (2) neuroimaging findings compatible with CNS involvement with lymphoma, in conjunction with consistent clinical presentation and the absence of other clinically feasible diagnosis; or (3) positive CSF (lymphoma cells detected by cytology and/or flow-cytometry).

Statistical analysis. Continuous variables are expressed as median and range and compared using the Mann–Whitney U-test. Categorical variables are reported as proportions, and compared using \( \chi^2 \) or Kruskal–Wallis tests, as appropriate. Progression-free survival (PFS), overall survival (OS) and time to CNS relapse were determined from date of diagnosis using the method of Kaplan and Meier (1958) and compared using log-rank analysis. An ‘event’ for PFS was defined by CNS or systemic relapse, or death from any cause. Cumulative incidence of CNS relapse was calculated using the Kaplan–Meier method and competing risk regression analysis using Fine and Gray’s proportional hazard model (Fine and Gray, 1999). In this analysis, death without CNS relapse was defined as the competing risk. Statistical analysis was performed using STATA version 12.1 (Statacorp, College Station, TX, USA) and any \( P \)-value of < 0.05 was considered significant.

RESULTS

We identified 217 patients with DLBCL judged as high risk for CNS involvement by the stated criteria. A total of 35 patients (15%) were treated at RBWH, 39 (18%) at MMC and 143 (69%) at PMCC. Group 1 (reference) was drawn from PMCC and MMC, whereas all centres contributed cases to groups 2 and 3. The baseline characteristics of patients in the three groups are summarised in Table 2. Fewer patients in group 1 received rituximab given the timeframe of its availability within Australia and, as expected, patients selected for intensive approaches (group 3) were younger and had higher risk disease features (higher normalised serum LDH, B symptoms). The distribution of extranodal sites for each of the three groups was similar, with a nonsignificant trend towards greater frequency of epidural/paraspinal disease in group 1. The majority of patients (84%) underwent baseline CSF analysis; the remaining 16% had no clinical evidence of CNS involvement at baseline and the first available CSF cytology was negative. The proportion of patients without baseline CSF analysis was similar between the three groups.

CNS prophylaxis. In group 2, 109 (80%) received both intended cycles of systemic high-dose MTX, with 25 (20%) receiving only one (for reasons described under ‘toxicity’ below). For patients in group 2, the median length of inpatient admission to receive high-dose IV MTX was 4 (range 2–16) days per cycle. Compliance with planned IT MTX was high, with a median of 5, 6 and 5 doses of IT MTX in groups 1–3 respectively. All patients in group 1 received at least one dose of IT MTX, compared with 81% and 85% of patients in groups 2 and 3 respectively (\( P = 0.005 \)).

Outcomes. The median follow-up in the entire cohort was 3.4 (range 0.2–18.6) years. Among groups 1–3, the median follow-up was 5.8, 3.0 and 3.8 years, respectively. During this time, 23 CNS relapses have occurred (12, 10 and 1 in groups 1–3, respectively). The median time to CNS relapse was 10.8 (range 4–109.6) months from initial diagnosis. The number and distribution of CNS relapses, 3-year cumulative incidence rates of CNS relapse and OS by treatment group are displayed in Table 3 and Figures 1 and 2. Briefly, the CNS relapse risk was highest in group 1 (\( P = 0.006 \)). Although the CNS relapse risk appeared numerically lower in group 3 compared with group 2, direct comparison between the two showed no statistically significant difference (\( P = 0.16 \)). The actuarial 3-year PFS rates were 65.5% (49.8–77.3%), 82.9% (74.7–88.6%) and 70.6% (53.9–82.2%) in groups 1–3, respectively (\( P = 0.051 \), Figure 3). Isolated CNS relapse (in the absence of systemic relapse) occurred in 20 out of 23 (87%) patients with CNS relapse, with the remaining 3 (12%) occurring in conjunction with systemic relapse. Of the 17 patients with sufficient data, the distribution of CNS relapses was leptomeningeal alone in 6 (35%), parenchymal alone in 9 (53%) and both in 2 (12%) patients. The pattern of localisation did not differ between groups (\( P = 0.16 \), Table 3). While recognising that all patients were considered to be at high risk for CNS relapse, we explored several potential risk factors identified in other studies (van Besien et al, 1998; Haioun et al, 2000; Shimazu et al, 2009; Schmitz et al, 2012). By univariate analysis, the only factor affecting CNS relapse risk was treatment group (Table 4). We also performed a multivariate analysis that included treatment group, use of rituximab, age > 60 years, ECOG ≥ 2, IPI ≥ 3, raised serum LDH, B symptoms, multiple extranodal sites, paraspinal disease and treatment era (dichotomised at 2000) – only treatment group affected CNS relapse (Table 5). The hazard ratio (HR) for group 2 was 0.26 (95% CI 0.08–0.81, \( P = 0.02 \)) and for group 3 was 0.07 (0.01–0.55, \( P = 0.01 \)). Number of doses of IT MTX did not affect the risk of CNS relapse (HR for 4 or more doses compared with 3 or less 0.84 (95% CI 0.29–2.40, \( P = 0.75 \)).

Impact of rituximab. In total, 159 out of 217 (73%) patients received rituximab as part of induction therapy. Nearly all patients in group 2, but only 37% and 42% of patients in groups 1 and 3, received rituximab. However, use of rituximab had no impact on CNS relapse when all groups were considered collectively (HR 0.62, 95% CI 0.47–0.95, \( P = 0.009 \)).

Toxicity (described for patients in group 2 only). Despite routine urinary alkalisation, the most frequent toxicity of systemic MTX was renal impairment of any grade, occurring in 70% of cycles overall, the majority (55%) grade 1 severity. Most of these events were minor and transient elevations of serum creatinine without clinical consequences. In two cases, grade 1 renal impairment was associated with delayed MTX clearance (defined as > 5 days). Grade 2 renal impairment occurred in 14% of cycles and grades 3 and 4 were rare (1%). All patients recovered renal function without need for haemodialysis. The second cycle was omitted in 20 cases because of renal impairment and delayed MTX clearance (\( n = 8 \)), grade 3 + alanine transaminase (ALT) elevation (\( n = 3 \)), CNS toxicity (\( n = 1 \)), sepsis (\( n = 2 \)) and reason not specified (\( n = 4 \)). Dose reductions for the second cycle occurred in 11 out of 104 patients (10.6%) because of renal impairment (\( n = 4 \)), painful neuropathy (\( n = 1 \)), delayed clearance with normal renal function (\( n = 1 \)) and reason not documented (\( n = 4 \)). Asymptomatic elevation of ALT resolved in all cases spontaneously without complication.

DISCUSSION

While acknowledging the limitations of this retrospective analysis, in a group of patients specifically selected for high risk of CNS relapse, the addition of high-dose IV MTX with or without cytarabine was associated with a reduction in the rate of subsequent CNS relapse. The actuarial 3-year risk of CNS relapse was 18.4% in patients who received CHOP + R with IT MTX; 6.9% in patients who received CHOP + R, IT MTX and high-dose IV MTX; and 2.3% in patients receiving chemotherapy regimens containing high-dose IV MTX and cytarabine (\( P = 0.009 \)). The nonsignificant trend towards lower incidence of CNS relapse seen in group 3 vs 2 may reflect the high dose of cytarabine
administered to these patients, or potentially superior systemic disease control, as fewer systemic relapses occurred in the group 3. Although three patients in group 3 received CODOXM IVAC (which contains both high-dose cytarabine and ifosfamide), the low numbers make it difficult to comment meaningfully on their effect on CNS relapse. The high relapse rate in patients treated with IT alone support findings from other studies that this approach is inadequate (Chua et al, 2002; Boehme et al, 2009; Villa et al, 2010; Tai et al, 2011). Over 60% of CNS relapses involved brain parenchyma and were not prevented by IT chemotherapy alone. In fact, there was a nonsignificant trend towards fewer leptomeningeal relapses in patients who received prophylaxis with IV MTX. This result is difficult to explain, however, it should be noted that the total number of CNS relapses was low and >80% of patients in groups 2 and 3 also received IT MTX.

Soon after two of our institutions changed policy to incorporate IV MTX for CNS prophylaxis in 2003, rituximab was reimbursed for the treatment of patients with DLBCL in Australia in 2005. This coincidence accounts for the imbalance in rituximab use between groups. The published data dealing with the impact of rituximab on CNS relapse are mixed – some studies have shown no impact (Feugier et al, 2005; Yamamoto et al, 2010; Mitrovic et al, 2012). Nevertheless, others have shown a significant reduction (Shimazu et al, 2009; Villa et al, 2010; Miyazaki et al, 2011; Tai et al, 2011) whereas others have shown a significant reduction (Shimazu et al, 2009; Mitrovic et al, 2012). A meta-analysis of pooled data from eight studies comparing rituximab with chemotherapy with chemotherapy alone found that addition of rituximab slightly reduced the risk of CNS relapse from 5.7% to 4.7% (Zhang et al, 2014). In our analysis, the use of rituximab as part of induction therapy did not appear to influence the risk of CNS relapse either in the cohort as a whole or when...
subgroup analyses were performed within groups 1 and 3. Therefore, we conclude that the impact from the lack of rituximab in group 1 is likely to be modest and does not completely account for the marked difference in CNS relapse risk seen. There were some imbalances in CNS risk factors between the groups: group 3 were younger, had higher serum LDH and more patients with B symptoms. In spite of this, the risk of CNS relapse was lowest in this group.

High-dose IV MTX is not without disadvantages. It is administered in an in-patient setting at our institutions, and has both financial and physical resource implications. Although grade 3+ adverse events were rare, despite careful fluid management and urinary alkalinisation, grade 2+ renal impairment occurred in 15% of patients. Although no patients developed irreversible renal impairment or need for haemodialysis, in 8% of cycles the renal impairment resulted in delayed MTX clearance and prolonged hospitalisation. The optimal schedule for delivery of high-dose IV MTX remains unclear and both shorter and 24-h infusion times are used, with both appearing to have utility (Joerger et al, 2012).

A few other studies have been performed that suggest that high-dose anti-metabolite therapy provides effective CNS prophylaxis in patients with DLBCL (Tilly et al, 2003a; Abramson et al, 2010; Holte et al, 2013). Tilly et al (2003b) conducted a prospective comparison between ACVBP and CHOP for intermediate grade lymphoma. The ACVBP regimen included a consolidation phase (IV MTX 3 g m\(^{-2}\)) and four doses of IT MTX. Although stratification by CNS risk was not prespecified, the randomisation resulted in balanced distribution of CNS risk features (such as

| Table 3. Overall and CNS relapse-free survival by group |
|----------------|----------------|----------------|
|                | Group 1         | Group 2         | Group 3         |
| Number         | 12              | 10              | 1               |
| CHOP ± R + IT MTX | CHOP ± R + IT + IV HD MTX | HyperCVAD or CODOXMIVAC ± R | P-value         |
| Leptomeningeal  | 5               | 1               | 0               | 0.16            |
| Parenchymal     | 4               | 5               | 0               |                |
| Both            | 2               | 0               | 0               |                |
| Unknown         | 1               | 4               | 1               |                |
| 3-Year cumulative incidence of CNS relapse (95% CI) | 18.4% (9.5–33.1%) | 6.9% (3.5–13.4%) | 2.3% (0.3–15.4%) | 0.009 |
| 3-Year overall survival | 68.0% (52.4–79.3%) | 85.9% (77.6–91.3%) | 89.2% (73.7–95.8%) | 0.029 |

Abbreviations: CI = confidence interval; CNS = central nervous system; CODOXMIVAC = cyclophosphamide, vincristine, doxorubicin, methotrexate, ifosfamide, etoposide, cytarabine; HD = high dose; IT = intrathecal; IV = intravenous; MTX = methotrexate; R = rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone; R-HyperCVAD = rituximab, hyper fractionated cyclophosphamide, doxorubicin, vincristine, dexamethasone; R-MTX-ara-c = rituximab, high-dose methotrexate, high-dose cytarabine. Bold denotes P<0.05.
et al draw conclusions regarding the effectiveness of the MTX in this (although CNS penetration of its metabolite, 4-OH-ifosfamide is regimen also contains four cycles of ifosfamide, an agent that is by published risk models (van Besien treated 65 patients with high risk for CNS involvement as defined (2010) comparison between R-ACVBP and eight cycles of R-CHOP21 2003b). The follow-up LNH03-2B study was a randomised phase 6 www.bjcancer.com | et al aged 18–65 years (Recher dose IV MTX) had CNS relapse risk of 2.8% compared with 8.3% raised serum LDH and multiple extranodal sites) between arms. neither specifically selected nor stratified for CNS relapse risk, and was already selected for high risk of CNS relapse. Bold denotes 

<table>
<thead>
<tr>
<th>Table 4. Cox regression univariate analysis of risk factors for CNS relapse among patients selected for high risk of this complication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk factor (univariate)</td>
</tr>
<tr>
<td>Age &gt; 60 years</td>
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<tr>
<td>Stage III/IV</td>
</tr>
<tr>
<td>Histologic transformation</td>
</tr>
<tr>
<td>ECOG performance status &gt; 2</td>
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<tr>
<td>Serum LDH &gt; ULN</td>
</tr>
<tr>
<td>Multiple extranodal sites</td>
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<td>IPI 3–5</td>
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<tr>
<td>B symptoms</td>
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<tr>
<td>Paraspinal disease</td>
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<tr>
<td>Group 1 (high-dose IV MTX)</td>
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<tr>
<td>Group 3 (high-dose IV MTX-ara-c)</td>
</tr>
<tr>
<td>Rituximab</td>
</tr>
<tr>
<td>Number of doses of IT MTX (&gt;4 vs 0–3)</td>
</tr>
<tr>
<td>Decade of treatment (1990–2000 vs 2000 onwards)</td>
</tr>
<tr>
<td>0.85 (0.30–2.30)</td>
</tr>
</tbody>
</table>

Abbreviations: CI = confidence interval; CNS = central nervous system; ECOG PS = Eastern Cooperative Oncology Group Performance Status; HR = hazard ratio; IPI = international prognostic index; IT = intrathecal; IV = intravenous; LDH = lactate dehydrogenase; MTX = methotrexate; MTX-ara-c = high-dose methotrexate, high-dose cytarabine; NA = not available; ULN = upper limit of normal. Note that this population of patients was already selected for high risk of CNS relapse. Bold denotes P < 0.05.

<table>
<thead>
<tr>
<th>Table 5. Cox regression multivariate analysis of risk factors for CNS relapse among patients selected for high risk of this complication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk factor (multivariate)</td>
</tr>
<tr>
<td>Group 2 (high-dose IV MTX)</td>
</tr>
<tr>
<td>Group 3 (high-dose IV MTX-ara-c)</td>
</tr>
</tbody>
</table>

Abbreviations: CI = confidence interval; CNS = central nervous system; HR = hazard ratio; IV = intravenous; MTX = methotrexate; MTX-ara-c = high-dose methotrexate, high-dose cytarabine.

The major limitation of this study lies in the retrospective nature, and heterogeneity in baseline risk and treatment factors (particularly rituximab) among the three groups, leading to potential bias. Nonetheless, this finding adds to the growing body of nonrandomised data suggesting that incorporation of high-dose IV MTX ± cytarabine into treatment protocols may lower the risk of CNS relapse in patients with DLBCL at high risk of the complication. Ideally, this hypothesis would be tested in an adequately powered, prospective study randomising patients to R-CHOP + IT MTX ± high-dose IV MTX and/or cytarabine, with the primary end point 2-year rate of CNS relapse. There are, however, several practical difficulties with performing such a study. Many clinicians believe sufficient evidence exists to support the efficacy of high-dose IV MTX ± cytarabine for CNS-directed prophylaxis and may be uncomfortable enrolling patients to a protocol with a chance of not receiving it. Second, because CNS relapse remains a rare complication, adequately powering a study is costly and difficult. Limiting the study to only high-risk patients (with estimated CNS relapse risk of ~15%) would reduce the sample size needed, but such patients comprise <10% of DLBCL overall (Schmitz et al, 2013). This probably explains why a prospective study addressing this question has yet to be completed to our knowledge.
CONCLUSION

The addition of high-dose IV MTX, either at the completion of R-CHOP or as part of dose-intensive chemotherapy strategies, is associated with a reduction in CNS relapse risk in DLBCL. This finding should ideally be tested in prospective, randomised studies.

ACKNOWLEDGEMENTS

This study was funded in part by the Victorian Cancer Agency Grant Number CTCB11_18 and the Haematology Society of Australia and New Zealand (New Investigator Scholarship).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

CJC collected and analysed data and wrote the manuscript; KEH, KO’R, MG, AG, GAK, PF, SYT, SSO, DSR, HMP, MD, KB, DW, MW, EHJ, DAC and SHJ contributed patients and collected data; JFS designed the study, analysed data and co-wrote the manuscript. All authors participated in manuscript revision and approved the final version.

REFERENCES


vincristine 1.5 mg m

750 mg m

D1; doxorubicin 50 mg m

D1; vincristine 1.4 mg m

IV capped at 2 mg D1; prednisolone 100 mg D1–5 p.o.

(R) Hyper CVAD A cycle (rituximab 375 mg m

Cyclophosphamide 300 mg m

2 IV twice daily D1–3; methotrexate 12 mg IT D1; doxorubicin 50 mg m

2 IV D3; vincristine 1 mg m

2 (max 2 mg) IV D3, 11; dexamethasone 40 mg p.o. D1–4 and 11–14. B cycle (rituximab 375 mg m

2 D1) Methotrexate 1 g m

IVI (over 24 h) D1; cytarabine 3 g m

2 IVI twice daily D2, 3; methotrexate 12 mg IT D1.

CODOXM (rituximab 375 mg m

2 D1) Cyclophosphamide 800 mg m

2 IV D1; cyclophosphamide 200 mg m

2 IV D2–5; vincristine 1.5 mg m

2 (max 2 mg) IV D1, 8; doxorubicin 40 mg m

2 IV D1; cytarabine 70 mg IT D1, 3; methotrexate 1 g m

2 IVI (over 24 h). IVAC Ifosfamide 1.5 g m


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Chemotherapy protocols

(R) CHOP (rituximab 375 mg m

2 D1) Cyclophosphamide 750 mg m

2 D1; doxorubicin 50 mg m

2 D1; vincristine 1.4 mg m

2 IV capped at 2 mg D1; prednisolone 100 mg D1–5 p.o.

(R) Hyper CVAD A cycle (rituximab 375 mg m

Cyclophosphamide 300 mg m

2 IV twice daily D1–3; methotrexate 12 mg IT D1; doxorubicin 50 mg m

2 IV D3; vincristine 1 mg m

2 (max 2 mg) IV D3, 11; dexamethasone 40 mg p.o. D1–4 and 11–14.

B cycle (rituximab 375 mg m

2 D1) Methotrexate 1 g m

IVI (over 24 h) D1; cytarabine 3 g m

2 IVI twice daily D2, 3; methotrexate 12 mg IT D1.

CODOXM (rituximab 375 mg m

2 D1) Cyclophosphamide 800 mg m

2 IV D1; cyclophosphamide 200 mg m

2 IV D2–5; vincristine 1.5 mg m

2 (max 2 mg) IV D1, 8; doxorubicin 40 mg m

2 IV D1; cytarabine 70 mg IT D1, 3; methotrexate 1 g m

2 IVI (over 24 h). IVAC Ifosfamide 1.5 g m

2 IV D1–5; etoposide 60 mg m

2 IV D1–5; cytarabine 2 g m

2 IV twice daily D1–2; methotrexate 12 mg IT D5.

(R)-MACOPB (rituximab 375 mg m

2 D1) Methotrexate 400 mg m

2 IV weeks 2, 6, 10; doxorubicin 50 mg m

2 IV weeks 1, 3, 5, 7, 9, 11; cyclophosphamide 350 mg m

2 IV weeks 1, 3, 5, 7, 9, 11; vincristine 1.4 mg m

2 (capped at 2 mg) IV weeks 2, 4, 6, 8, 10, 12; prednisolone 75 mg daily bleomycin 10 mg m

2 IV weeks 4, 8, 12.

ACVBPM

Four induction courses (Q21 days)

Methotrexate 75 mg m

2 IV D1; cyclophosphamide 1200 mg m

2 intravenously D1; vindesine 2 mg m

2 on D1, 5; bleomycin 10 mg D1, 5; prednisone 60 mg m

2 orally D1–5; methotrexate 15 mg IT D2.

Consolidation therapy (Q14 days)

Methotrexate 3 g m

2 IV plus leucovorin rescue × 2; etoposide 300 mg m

2 IV × 4; ifosfamide 1500 mg m

2 IV × 4; cytosine-arabinoside 100 mg m

2 subcutaneously D1–4.

APPENDIX
Limited role for surveillance PET–CT scanning in patients with diffuse large B-cell lymphoma in complete metabolic remission following primary therapy

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Background: The usefulness of positron emission tomography with computed tomography (PET–CT) in the surveillance of patients with diffuse large B-cell lymphoma (DLBCL) in complete metabolic remission after primary therapy is not well studied.

Methods: We performed a retrospective review of our database between 2002 and 2009 for patients with de novo DLBCL who underwent surveillance PET–CT after achieving complete metabolic response (CMR) following primary therapy.

Results: Four-hundred and fifty scans were performed in 116 patients, with a median follow-up of 53 (range 8–133) months from completion of therapy. Thirteen patients (11%) relapsed: seven were suspected clinically and six were subclinical (all within first 18 months). The positive predictive value in patients with international prognostic index (IPI) \(\leq 3\) was 56% compared with 80% in patients with IPI \(\geq 3\). Including indeterminate scans, PET–CT retained high sensitivity 95% and specificity 97% for relapse.

Conclusion: Positron emission tomography with computed tomography is not useful in patients for the majority of patients with diffuse large B-cell lymphoma in CMR after primary therapy, with the possible exception of patients with baseline IPI \(\geq 3\) in the 18 months following completion of primary therapy. This issue could be addressed by a prospective clinical trial.
symptoms despite surveillance scans (Weeks et al, 1991; Eliz et al, 2002; Guppy et al, 2003). Positron emission tomography combined with computer tomography (PET–CT) has become the modality of choice for initial staging and end of treatment assessment in DLBCL (Hicks et al, 2005; Cheson, 2011). The improved sensitivity of PET–CT suggests advantages over CT in the detection of subclinical relapse. Few studies have examined the role of PET–CT surveillance in patients with DLBCL achieving remission after primary therapy (Zinzani et al, 2009; Petraschul et al, 2010; El-Galaly et al, 2011; Goldschmidt et al, 2011; Abel et al, 2012). Liedtke et al (2006) found patients with subclinical relapse were more likely to have lower second-line IPI (RR 4, 95% CI 0.58–27.6) with a non-significant trend towards survival benefit (actuarial 5 year survival of 54% vs 43%; P = 0.13). The aim of our study was to evaluate the role of \(^{18}\)F-fluorodeoxyglucose (FDG) PET–CT scans in the surveillance of patients achieving complete metabolic response (CMR) after primary therapy for DLBCL, and define a risk-adapted strategy for surveillance imaging.

**MATERIALS AND METHODS**

We conducted a retrospective review of patients with DLBCL who underwent PET–CT scanning at the Peter MacCallum Cancer Centre. Data collection was compliant with the institutional ethics requirements. In the period analysed, departmental protocol recommended 6-monthly PET–CT scans for patients in CMR, for the first 2 years, and then annually until 5 years after completion of therapy for patients in whom there existed intention to intervene if subclinical relapse was identified. In most cases, this intervention consisted of intensive salvage chemotherapy following by autologous stem cell transplantation. Implementation was at the discretion of the treating physician. We included patients who had a confirmed diagnosis of de novo DLBCL treated at our centre between 1st January 2002 and 31st December 2009 who had achieved CMR at the completion of primary therapy and underwent at least one surveillance PET–CT scan.

We identified 200 patients with DLBCL within the specified time period. Eighty-four were ineligible for the following reasons: histological transformation from a variety of indolent lymphoma subtypes (n = 29), no surveillance PET–CT scans performed (primary treatment, patients aged over 70 or otherwise unfit for treatment, n = 26), did not achieve CMR (n = 14), end of treatment PET positive for another reason for example, sarcoidosis or infection (n = 7), palliative management only (n = 5), had prior chemotherapy at another institution (n = 3). Only two patients without surveillance PET scans relapsed within 6 months of completing therapy (3.2 and 5.4 months) only one of whom was a suitable candidate for autologous stem cell transplant.

Of the cohort (n = 116) analysed, the median was age 59 years (range 16–85), 54% were male and 51% had an elevated serum LDH. Eastern Cooperative Oncology Group performance status score \(\leq 1\) in 96% of patients, with \(< \text{2 sites of extranodal involvement in 75% and baseline international prognostic index (IPI; 1993)—determined using PET–CT and bone marrow biopsy was }< 3\text{ in 77 (66%) and }\geq 3\text{ in 37 (32%) of patients. In two patients, baseline IPI could not be calculated due to missing data. Initial immunochemotherapy was R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine and prednisolone) in 110 (95%), while six (5%) received R-Hyper-CVAD (rituximab, hyper-fractonated cyclophosphamide, vincristine, doxorubicin and dexamethasone) alternating with high-dose methotrexate and cytarabine at the discretion of the treating clinician due to the presence of high-risk features. Sixty-six patients (57%) received radiotherapy as consolidation for bulky or localised disease.

**Data collection.** For each patient, we collected baseline characteristics including sex, performance status, age, serum LDH, number of extranodal sites, IPI (A predictive model for aggressive non-Hodgkin's lymphoma, 1993), primary therapy, date and details of follow-up PET–CT scans, and follow-up data including the date and site of relapse, type (subclinical or suspected), relapse IPI, biopsy results, second malignancies, cause and date of death.

The primary end point was determination of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of PET–CT for the detection of relapse. \(^{18}\)F-FDG PET–CTs were obtained on a dedicated PET/CT scanner (Discovery LS, GE Medical Systems, Milwaukee, USA; Discovery STE, GE Medical Systems, Milwaukee, USA or Biograph 64, Siemens Medical Solutions, Knoxville, USA) from the skull-base to upper-thigh level, unless there was suspicion or known disease outside this field-of-view. Patients were fasted for 6 h before administration of 5 MBq kg \(^{-1}\) \(^{18}\)F-FDG, to a maximum of 400 MBq adapted for weight and imaged after a \(\geq 60\)-min uptake phase.

**Definitions.** PET reports were reviewed and classified as positive, negative or indeterminate for relapsed lymphoma by one (clinician) investigator blinded to patient outcome. In generating the original PET report, the imaging specialist had access to prior investigation results, including the baseline and post treatment FDG PET–CT studies. It should be noted that the time period covered by the study was mostly before the publication of both the International Harmonisation Project (Juweid et al, 2007) and the Deauville criteria (Meignan et al, 2009). A positive scan suggested relapsed lymphoma, with true-positive results requiring either biopsy confirmation or unequivocal scan progression. A false-positive scan was refuted by biopsy and/or follow-up showing resolution of areas of increased FDG uptake. A negative scan was interpreted as negative for relapsed lymphoma: true negatives had no clinical relapse and false negatives manifest relapse within 3 months from the date of the scan. Cases in which uncertainty in the interpretation of the scan existed (n = 26) were referred to a three member review panel (which included one imaging specialist) and re-scored with majority opinion accepted. For seven scans, no determination could be made and they were recorded as ‘indeterminate’. A ‘suspected relapse’ was defined as relapse preceded by signs, symptoms or other clinical features (such as rising serum LDH). A ‘subclinical relapse’ was defined as relapse detected without the above features, on the basis of imaging findings.

**Statistical analysis.** Continuous variables are expressed as median and range and compared using the unpaired t-test. Non-normally distributed variables are expressed as median and range, and compared using Mann–Whitney U-test. Categorical variables are reported as percentages, and compared using Fisher’s exact test. Event-free survival, overall survival (OS) and time to relapse were determined using the method of Kaplan and Meier, with curve comparisons using log-rank analysis. A P-value <0.05 was considered significant.

**Results.** In 116 patients, 450 surveillance PET–CT scans were performed with a median of four scans per patient (range 1–10). At 1st January 2012, with a median of 53 (range 8–133) months follow-up from completion of therapy, 13 patients (11%) had relapsed and 97 (84%) remain relapse-free in ongoing complete remission. Features associated with relapse in these patients are displayed in Table 1. Of those who relapsed, eight died from progressive disease and five are in remission after salvage therapy. Six patients died from other causes: gastric cancer (n = 2), pneumonia complicating oesophageal cancer (n = 1), ruptured abdominal aortic aneurysm (n = 1), metastatic squamous cell carcinoma (n = 1) and cause unknown, while in clinical remission (n = 1).
Test performance of PET–CT surveillance scanning. There were 13 true-positive scans, six false positives, no false negatives and 424 true negatives. The PPV was 68% and the NPV 100%. Of the seven indeterminate scans, six were shown to follow-up to be negative for lymphoma and one was biopsy confirmed to be positive. If we include indeterminate scans by scoring the former as false positives and the latter as false negatives, respectively, test performance remained robust with revised sensitivity 95%, specificity 97%, PPV 60% and NPV 99%.

However, when considering patients with baseline IPI $\geq 3$ ($n = 37$) there were eight true positives, two false positives, no false negatives, 112 true negatives and two indeterminate scans. While sensitivity, specificity and NPV (100%) were essentially unchanged, the PPV increased to 80%. In patients with baseline IPI $< 3$ ($n = 77$), there were five true positives, four false positives, no false negatives, 312 true negatives and five indeterminate scans. This resulted in a lower PPV (56%). Most relapses (and therefore true-positive scans) occurred within the first 18 months. The number of scans needed to detect one subclinical relapse was analysed as a function of both baseline IPI ($\geq 3$ vs $< 3$), as well as time following completion of primary therapy. Averaged over the first 18 months following completion of therapy, 92 scans were performed to detect one subclinical relapse in patients with baseline IPI $< 3$, but only 22 scans in patients with baseline IPI $\geq 3$ (86 scans to detect four subclinical relapses). Surveillance PET–CT had low yield after 18 months regardless of baseline IPI, with only one (clinically suspected) true-positive result in a patient (baseline IPI 3) from a total of 170 scans (Table 2).

Patterns of relapse. Two-thirds of relapses occurred within 18 months of completing chemotherapy and 85% within 2 years, with a median time to relapse of 12.8 months. The time distribution of surveillance PET–CT scans in the 13 relapsing patients is displayed in Figure 1.

Relapses were detected clinically in seven patients (54%) with examination findings ($n = 4$), fever ($n = 2$) or collapse ($n = 1$). Five (71%) suspected relapses occurred at sites, which were previously uninvolved by DLBCL. PET–CT was concordant in all seven cases, with confirmatory biopsies including one case (intra-abdominal relapse) where PET directed the biopsy. In the remaining six cases, biopsy site was selected clinically. The remaining six relapses were subclinical, with three (50%) occurring at previously uninvolved sites. Four (67%) subclinical relapses detected by PET–CT would very likely have been missed by CT alone as either nodal disease was $< 15$ mm ($n = 2$) or relapse was extranodal (bony without structural abnormality $n = 2$). There was no difference in OS between the two groups ($P = 0.76$, Figure 2). Of six subclinical relapses, four had second-line IPI $< 3$ and two cases $\geq 3$. Among seven suspected relapses, four had second-line IPI $< 3$, one case second-line IPI was 3 and in two cases not evaluable due to serum LDH at relapse not being performed. There was no difference in second-line IPI between the two groups ($P = 1.00$).

Management of relapse. The median age of the 13 patients who relapsed (at the time of relapse) was 64 (range 21 to 82) years. All patients received salvage therapy, 11 with R-ICE (rituximab, ifosfamide, carboplatin and etoposide), one (who was 82) with R-CVP and one (who relapsed with follicular histology) with $^{131}$I-rituximab (Leahy et al, 2006). Of the 11 patients receiving R-ICE, seven proceeded to cyclophosphamide, carmustine, etoposide conditioned autologous stem cell transplant. The remaining four patients did not proceed to transplant because their disease was refractory ($n = 2$) or they did not tolerate ($n = 2$) salvage chemotherapy.

Six false-positive scans for recurrent lymphoma occurred at a median of 9.0 (range 3.9–25.1) months following completion of

### Table 1. Factors associated with relapse after achieving a complete remission at the end of therapy (univariate analysis)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Relapse n = 13</th>
<th>No relapse n = 103</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (years)</td>
<td>59</td>
<td>59</td>
<td>0.96</td>
</tr>
<tr>
<td>PET stage 3/4 at diagnosis</td>
<td>11 (84%)</td>
<td>35 (34%)</td>
<td>0.005</td>
</tr>
<tr>
<td>PET IPI 3–5</td>
<td>8 (62%)</td>
<td>29 (28%)</td>
<td>0.02</td>
</tr>
<tr>
<td>2+ EN sites</td>
<td>7 (54%)</td>
<td>22 (21%)</td>
<td>0.02</td>
</tr>
<tr>
<td>ECOG &gt; 1</td>
<td>3 (23%)</td>
<td>1 (1%)</td>
<td>0.004</td>
</tr>
<tr>
<td>Median LDH (IU l$^{-1}$)</td>
<td>634</td>
<td>514</td>
<td>0.21</td>
</tr>
</tbody>
</table>

Abbreviations: EN = extranodal; ECOG = Eastern Cooperative Oncology Group; IPI = international prognostic index; LDH = lactate dehydrogenase. PET IPI is calculated using the stage based on PET rather than contrast CT.

### Table 2. Distribution of PET–CT results as a function of time elapsed from completion of primary chemotherapy for all patients

<table>
<thead>
<tr>
<th>Months post treatment</th>
<th>True negative</th>
<th>True positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–6</td>
<td>91</td>
<td>66</td>
</tr>
<tr>
<td>6–12</td>
<td>96</td>
<td>68</td>
</tr>
<tr>
<td>12–18</td>
<td>50</td>
<td>51</td>
</tr>
<tr>
<td>18–24</td>
<td>66</td>
<td>68</td>
</tr>
<tr>
<td>24–36</td>
<td>31</td>
<td>31</td>
</tr>
<tr>
<td>36–48</td>
<td>22</td>
<td>22</td>
</tr>
</tbody>
</table>

Abbreviation: PET–CT = positron emission tomography with computed tomography.

![Graphical representation of timing of PET–CT scans performed in the 13 patients who experienced relapse.](image-url)
SURVEILLANCE PET–CT IN DIFFUSE LARGE B–CELL LYMPHOMA

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Two false positives were in patients with baseline IPI \( \geq 3 \) and four occurred in those with baseline IPI \( < 3 \). The sites involved were the tonsils \((n = 2)\), a cervical lymph node \((n = 1)\), mediastinal nodes \((n = 2)\) and a peri-duodenal node \((n = 1)\). In all cases either biopsy \((n = 3)\) or clinical follow-up and resolution \((n = 3)\) demonstrated no recurrent lymphoma. There were seven indeterminate scans; two in patients with baseline IPI \( \geq 3 \) and five in patients with baseline IPI \( < 3 \). The sites involved were lung in the setting of a chest infection \((n = 1)\), tonsils \((n = 2)\), cervical \((n = 1)\), suboccipital \((n = 1)\), mediastinal \((n = 1)\) and inguinal nodes \((n = 1)\). In all but the final case (biopsy proven recurrent DLBCL), repeat scanning showed resolution of changes. In the terminology used by Zinzani, there were six ‘inconclusive negative’ and one ‘inconclusive positive’ scans (Zinzani et al, 2009). In 67% of false-positive and indeterminate scans combined, the region of interpretative uncertainty was a nodal site involved on baseline PET–CT.

Second malignancies were detected by surveillance PET–CT in eight (7%) patients (Table 3). In addition, PET prompted colonoscopy and polypectomy in one patient. There were two false-positive scans suggesting second malignancy, with PET–CT suggesting possible breast cancer in one patient (mammogram normal, colonoscopy normal).

DISCUSSION

Our data suggests that PET–CT scanning has both a low yield, and for most patients with DLBCL achieving CMR at the completion of primary therapy is not justified unless there is clinical suspicion of relapse. The only potential subgroup in whom a surveillance strategy warrants further investigation is patients with baseline IPI score \( \geq 3 \) in the first 18 months from completion of therapy, when the risk of relapse is greatest. In this study, we did not demonstrate a difference in either second-line IPI or OS for patients with subclinical compared with symptomatic relapse, though the number of relapses was small. Underpinning the desire for earlier detection is the theoretical benefit of better outcomes from salvage therapy (Liedtke et al, 2006), although we acknowledge that poor outcomes seen in this group of patients may reflect aggressive biology rather than late detection of relapse. A prospective study of patients with DLBCL and baseline IPI \( \geq 3 \) in first remission randomised to PET–CT surveillance vs no surveillance with a primary end point of OS would be required to address this issue.

The low rate of relapse among patients achieving CMR at the completion of treatment combined with the lower sensitivity and specificity of CT than PET (Wagner-Johnston and Bartlett, 2011) suggests that diagnostic CT is even less likely to be worthwhile in a surveillance setting. PET–CT detected six (46%) relapses before clinical manifestations, a numerically greater proportion than using CT alone but still a suboptimal surveillance test (Weeks et al, 1991; Guppy et al, 2003). This could be improved by a shorter time-interval surveillance strategy but this may also increase false positives, cost and radiation exposure. Two-thirds of the subclinical relapses would not have been detected using CT alone, further strengthening the case for use of PET–CT over CT alone in surveillance.

We confirm the finding of other investigators that PET–CT is both sensitive and specific for the detection of relapsed DLBCL (Table 4) (Zinzani et al, 2009; Petrausch et al, 2010; El-Galaly et al, 2011). The NPV of 99–100% means that patients with negative scans can be reassured that a CMR truly reflects ongoing remission from DLBCL.

False-positive scans were also infrequent, with six (1.3%) identified. Our findings (86% of inconclusive scans being negative on follow-up) are consistent with the results of Zinzani et al (2009). It is important to recognise common patterns of uptake unlikely to represent lymphomatous recurrence. The majority of false-positive and inconclusive scans occurred in the head and neck or mediastinum, often at sites of lymphomatous involvement at baseline. Increased tonsillar activity is common following chemotherapy, and usually represents reactive lymphoid hyperplasia. Similar findings occur in lymphoid tissue in the mediastinum and para-appendiceal region, with symmetric uptake in the mediastinum and linear uptake in the para-appendiceal region suggesting benign pathology. Mild-to-moderate uptake in cervical nodes, especially following upper respiratory tract infection, should not be mistaken for recurrent lymphoma. It should be highlighted that CT

Table 3. Second malignancies detected by PET–CT during surveillance scanning

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Sex</th>
<th>Second tumour</th>
<th>Months post treatment</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>M</td>
<td>Gastric (recurrent)</td>
<td>7</td>
<td>Death (pyloric obstruction)</td>
</tr>
<tr>
<td>65</td>
<td>F</td>
<td>Hepatocellular</td>
<td>25</td>
<td>Resection, alive in remission</td>
</tr>
<tr>
<td>70</td>
<td>M</td>
<td>SCC</td>
<td>30</td>
<td>Palliative radiotherapy, death</td>
</tr>
<tr>
<td>62</td>
<td>M</td>
<td>Oesophageal</td>
<td>30</td>
<td>Resection, survived 28 m</td>
</tr>
<tr>
<td>72</td>
<td>M</td>
<td>Prostate</td>
<td>6</td>
<td>Alive, on anti-androgen Rx</td>
</tr>
<tr>
<td>63</td>
<td>M</td>
<td>SCC</td>
<td>13</td>
<td>T1N1 left perifom fossa, curative RT</td>
</tr>
<tr>
<td>57</td>
<td>F</td>
<td>Breast</td>
<td>5</td>
<td>Mastectomy, alive in remission</td>
</tr>
<tr>
<td>81</td>
<td>F</td>
<td>Breast</td>
<td>6</td>
<td>Lumpectomy/radiotherapy → remission, death cause unknown 50 months</td>
</tr>
</tbody>
</table>

Abbreviations: F = female; M = male; PET–CT = positron emission tomography with computed tomography; SCC = squamous cell carcinoma.

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alone would have missed two-thirds of subclinical relapses and, therefore, cannot be recommended as an alternative surveillance strategy.

We have not made formal economic evaluation of surveillance PET–CT imaging, however health resources are scarce and in the real world must be considered when recommending any surveillance procedures. The true cost of surveillance includes not only that of the PET–CT scans themselves (an amount which varies considerably between health systems) but the additional costs of investigating indeterminate or false-positive scans (either with repeat interval scanning or unnecessary biopsy). Another potential harm of surveillance PET–CT is additional radiation exposure. The radiation dose varies depending on the CT protocol and sex of the patient, but typically from a combined modality scan of the body is approximately 12–15 mSv per scan (Murano et al., 2011). The subsequent risk of second malignancy is highest in younger patients, and particularly in adolescents and young adults minimisation of radiation exposure should be an important consideration when determining the risks and benefits of a surveillance strategy (Rathore et al., 2012). Surveillance PET–CT scanning detected second primary malignancies in eight patients (7%), leading to curative procedures in four. Whether this impacted on mortality is uncertain, as these may have been detected without PET. We acknowledge that detection of second malignancies does not constitute a reason to perform surveillance PET–CT, however it is a useful by-product.

Our data has limitations as a retrospective study. We took great lengths to ensure data quality, however, some information is nevertheless missing or incomplete. Although we had departmental recommendations for post-remission scanning, adherence was non-uniform; accordingly our results reflect time periods rather than a precise schedule. We observed refinement in reporting styles of nuclear medicine physicians over time, with greater recognition of phenomena such as the characteristic appearance of rebound lymphoid hyperplasia in recent compared with earlier reports, but our analyses are based on the actual report generated at the time of the scan. Finally, our findings with regard to the performance characteristics of surveillance PET–CT scans are specific to our setting, an academic tertiary referral centre with high imaging volume and physician expertise.

Conclusion. Surveillance PET–CT has no role in patients with DLBCL in achieving CMR with the possible exception of patients with baseline IPI ≥3 in the 18 months following completion of primary therapy. A prospective study would be required to address this.

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AUTHOR CONTRIBUTIONS

CC collected and interpreted the data and wrote the manuscript. JPS designed the study, interpreted the data and wrote the manuscript. MH and RJH interpreted the data and wrote the manuscript. MD, DW, DSR, DAC, KH, HMP, KB and SH provided patients and wrote the manuscript.

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