

## Allogeneic Transplant

# Reduced-intensity rituximab-BEAM-CAMPATH allogeneic haematopoietic stem cell transplantation for follicular lymphoma is feasible and induces durable molecular remissions

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### Summary:

The indolent non-Hodgkin's lymphomas are theoretically curable through allogeneic haematopoietic stem cell transplantation (allo-HSCT). The applicability of standard conditioning allo-HSCT is, however, restricted by its toxicity. Recently, reduced-intensity conditioning regimens have demonstrated efficacy with significantly reduced early morbidity and mortality. We examined the safety and efficacy of rituximab-BEAM-CAMPATH as reduced-intensity conditioning for allo-HSCT in follicular lymphomas. Minimal residual disease was assessed by polymerase chain reaction (PCR) for bcl-2/IgH translocations, and chimerism by X,Y-FISH or PCR amplification of short tandem repeat sequences. At a median follow-up of 521 days (371–719), four of five patients were alive and three were in complete molecular remission. Three patients required pre-emptive treatment for CMV reactivation. One succumbed to a perforated viscus and one had slowly progressive disease. A graft-versus-lymphoma effect was demonstrable and quantitative PCR for bcl-2/IgH may allow better accuracy in scheduling post-allograft rituximab and/or donor lymphocyte infusions. Although follow-up is short, reduced-intensity allo-HSCT with BEAM-CAMPATH conditioning appears to be safe, effective in inducing a molecular remission and is potentially curative. Further recruitment and much longer follow-up will be necessary to determine if this impacts favourably upon disease-free and overall survival.

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Follicle centre cell lymphoma usually presents at an advanced median age of approximately 60 years and in an advanced stage. However, a significant proportion of

patients present at a younger age. Although it has an indolent natural history with a median survival of 7–9 years and complete clinical remission may be achieved with standard chemotherapy, a chronic relapsing course with progressively shorter periods of remission is typical, with most patients eventually succumbing to their disease.<sup>1</sup>

The t(14;18)(q32;q21) is the karyotypic hallmark of follicular lymphomas. This results in an overexpression of the bcl-2 gene as it comes under the influence of the IgH promoter and immortalisation of the cell. In the majority (approximately 70%) of patients, the translocation breakpoint on chromosome 18 occurs within the major breakpoint region (MBR) within exon III of the bcl-2 gene.<sup>2</sup> Polymerase chain reaction (PCR) methods, both qualitative and quantitative, targeted at these breakpoints have been developed, which enable the detection of as few as one cell harbouring the bcl-2/IgH translocation in 10<sup>5</sup>–10<sup>6</sup> normal cells. However, the threshold above which t(14;18) positivity becomes significant is unknown<sup>3</sup> as the translocation can also be detected in normal healthy individuals.<sup>4–6</sup>

High-dose therapy and autologous transplantation have been undertaken to improve disease-free survival. However, autologous transplantation of unmanipulated haematopoietic stem cells (HSCT) is associated with a high relapse rate.<sup>7,8</sup> Relapse is strongly associated with the re-emergence or persistence of molecular marker positive cells detected by PCR.<sup>9–12</sup> *In vivo* or *in vitro* purging of the graft with monoclonal antibodies has demonstrated an improvement in disease-free survival, most marked in patients whose infused cells were negative for molecular markers.<sup>11,13–15</sup> Persistent marrow negativity is strongly predictive of continued complete remission,<sup>9</sup> with peripheral blood being less predictive.<sup>16</sup> Unfortunately, the risk of developing treatment-related myelodysplastic syndrome is substantial, at up to 19.8% at 10 years with no evidence of a plateau.<sup>17</sup>

At present, the only treatment modality that may be delivered with curative intent is allogeneic HSCT (allo-HSCT), with its attendant graft-versus-lymphoma (GvL) effects.<sup>18,19</sup> However, the transplant-related mortality (TRM) associated with conventional transplant conditioning is high at 25–40%,<sup>20,21</sup> resulting in no apparent improvement in overall survival although disease-free survival is significantly improved.<sup>8,21–23</sup> The high toxicity and TRM of conventional transplant conditioning restricts

its applicability in an 'indolent' disease in a group of advanced age and often with significant comorbidity.

Within the last few years, 'reduced-intensity' conditioning regimens have been shown to be feasible and applicable to a wide range of malignant and nonmalignant disorders. The improvements in toxicity and accompanying TRM are impressive, with rapid engraftment and demonstrable graft-versus-malignancy effects.<sup>24-28</sup>

Rituximab is a chimeric monoclonal anti-CD20 antibody with proven activity against follicular lymphoma.<sup>29,30</sup> In combination with CHOP, molecular remissions have been demonstrable,<sup>31</sup> which translates to significantly improved relapse-free survival.<sup>32</sup> Its use as an *in vivo* purging agent in the autologous transplant scenario can lead to PCR negative harvests, and there is suggestion of a synergistic effect with cytotoxic drugs.<sup>14</sup>

We therefore established an allogeneic transplant protocol in follicular lymphoma utilising conditioning with 'BEAM', a proven conditioning regimen for autologous<sup>33</sup> and allogeneic<sup>34</sup> transplantation, together with CAMPATH-1H (anti-CD52) to minimise the risk of rejection, graft-versus-host disease<sup>35</sup> and to maximise tumour bulk reduction. Previous series have demonstrated its feasibility.<sup>34,36</sup> Rituximab delivered within 120 days preconditioning was used as *in vivo* purging. Molecular monitoring of MRD was achieved through qualitative and quantitative PCR for the bcl-2/IgH translocation, and chimerism by XY-FISH or PCR of short tandem repeat sequences (STR).

## Materials and methods

### Qualitative *t(14;18)* PCR

Genomic DNA was extracted from the mononuclear cell fraction of clinical blood and bone marrow samples following density-gradient centrifugation on Ficoll-Histopaque®-1077 (Sigma-Aldrich, Dorset, UK) using the Wizard® Genomic DNA Purification Kit (Promega UK Ltd, Southampton). Precipitated DNA was rehydrated in 10 mM Tris-HCl (pH 7.4) and 1 mM EDTA (pH 8.0) and quantitated by measuring the optical density at 260 nm. Positive control DNA was extracted from the DOHH-2 cell line (DSMZ, Berlin, Germany) and negative control DNA from the K562 cell line (DSMZ, Berlin, Germany) by an identical methodology.

DNA integrity was confirmed by amplification with  $\beta$ -actin primers (forward AAA TCT GGC ACC ACA CCT TC) (reverse AAC GGC AGA AGA GAG AAC CA) (MWG Biotech, Milton Keynes), 0.6 U Taq DNA polymerase (Promega UK Ltd), 1 mM dNTPs (Roche Molecular Diagnostics, UK) and 1.5 mM magnesium chloride in PCR buffer (Sigma-Aldrich, Dorset, UK) in a total volume of 25  $\mu$ l. Reactions were denatured at 95°C for 3 min, subject to 40 cycles of 95°C/60 s; 60°C/60 s; 72°C/60 s, and a final elongation step of 72°C for 7 min in a GeneAmp PCR System 2700 thermal cycler (Applied Biosystems, Cheshire, UK).

Four replicates of 0.1  $\mu$ g of DNA were amplified with 400 nM forward primer MBR1 (TAT GGT GGT TTG ACC TTT AG), 400 nM consensus JH primer (ACC TGA

GGA GAC GGT GAC) (MWG Biotech, Milton Keynes),<sup>37</sup> 0.6 U Taq DNA polymerase (Promega UK Ltd), 1 mM dNTPs (Roche Molecular Diagnostics, UK) and 1.5 mM magnesium chloride in PCR buffer (Sigma-Aldrich, Dorset, UK) in a total volume of 25  $\mu$ l. Reactions were denatured at 95°C for 3 min, subject to 40 cycles of 95°C/60 s, 60°C/60 s, 72°C/60 s, and a final elongation phase of 72°C for 7 min. The products were visualised after electrophoresis through an ethidium bromide stained 2% agarose gel. The reproducible sensitivity of this assay was approximately 1:1000 cells.

A 1  $\mu$ l aliquot of this first round product was subjected to a second round reaction using 250 nM internal primer MBR2 (GAG TTG CTT TAC GTG GCC TG), 250 nM consensus JH (ACC TGA GGA GAC GGT GAC) and 125 nM dual labelled internal hydrolysis probe (6FAM-TCA ACA CAG ACC CAC CCA GAG CCC(TAM-RA)Tp) (TIB Molbiol, Berlin, Germany), with 1 mM dNTPs, 0.6 U Taq DNA polymerase, 1.5 mM magnesium chloride in PCR buffer (Sigma-Aldrich, Dorset, UK) in a total volume of 20  $\mu$ l. The reaction mixture was subjected to 1 min denaturation at 95°C, then 20 cycles of 95°C/15 s and 58°C/30 s in glass capillary tubes within a LightCycler® (Roche Diagnostics, Lewes) instrument. The combined reproducible sensitivity was approximately 1:10 000 cells.

### Quantitative *t(14;18)* PCR

Two replicates of 0.1  $\mu$ g of DNA were amplified with primer MBR2 (GAG TTG CTT TAC GTG GCC TG), 250 nM consensus JH (ACC TGA GGA GAC GGT GAC) and 125 nM dual labelled internal hydrolysis probe (6FAM-TCA ACA CAG ACC CAC CCA GAG CCC (TAMRA)Tp), with 1 mM dNTPs, 0.6 U Taq DNA polymerase, 1.5 mM magnesium chloride in PCR buffer (Sigma-Aldrich, Dorset, UK) in a total volume of 20  $\mu$ l. The reaction mixture was subjected to 1 min denaturation at 95°C, then 60 cycles of 95°C/15 s and 58°C/30 s in glass capillary tubes within a LightCycler® (Roche Diagnostics, Lewes). Replicate 0.1  $\mu$ g aliquots were amplified in parallel with 250 nM of each  $\beta$ -actin primer (forward ACC CAC ACT GTG CCC ATC TA) (reverse CGG AAC CGC TCA TTG CC) and 125 nM probe (6FAM-ATG CCC XT CCC CCA TGC CAT CCT GCG Tp) utilising identical reaction mixes and conditions. A semiquantitative result was derived by utilising the ratio of the calculated *t(14;18)* to the calculated  $\beta$ -actin. The sensitivity of the quantitative assay was approximately 1:2000 cells.

### Chimerism analysis

DNA was extracted from whole blood using the QIAamp DNA blood mini-kit (Qiagen UK Ltd). The resulting DNA was processed using GenePrint® Fluorescent STR Multiplex-CSF1PO, TPOX, TH01, vWA and GenePrint® Fluorescent STR Multiplex-GammaSTR™ systems (Promega UK Ltd) following the manufacturer's instructions, and analysed on an ABI Prism 377 DNA sequencer (Applied Biosystems, Cheshire, UK). Interphase FISH analysis was performed on mononuclear cells separated from whole blood using a direct labelled Chromosome X/Y probe

**Table 1** Patient characteristics at transplant

Patient no.	Age/sex	Diagnosis to transplant (years)	IPI	ECOG	Diagnosis (stage)	Previous therapy
1	55.8/m	3.1	2	1	Follicular NHL(IVB); local HG-transformation (Ia); 2nd PR	CHOP(5); RT; CHOP(6); mini-BEAM
2	54.8/m	3.0	2	1	Follicular NHL (IVB); 1st PR	Chl/pred (8); FMD(4); CHOP(6); rituximab (1*)
3	50.8/f	1.5	2	1	Follicular NHL (IVB); 1st PR	COP(3); FAD(6); rituximab(1*)
4	50.9/f	3.8	2	1	Follicular NHL (IVB); 3rd PR	FAD(6); CMOP(3); rituximab(1*); BEAM auto; rituximab(1*); DHAP(3); rituximab(1*)
5	42.6/f	3.2	2	1	Composite follicular/mantle cell NHL (IIIA); 2nd CR	Intensified CHOP (HOVON26) (6); CVEP-rituximab(1*)

CHOP: cyclophosphamide, doxorubicin, vincristine, prednisolone; mini-BEAM: BCNU 60 mg/m<sup>2</sup> Day 1, etoposide 75 mg/m<sup>2</sup> days 2–5, cytarabine 100 mg/m<sup>2</sup> bd Days 2–5, melphalan 30 mg/m<sup>2</sup> Day 6; chl/pred: chlorambucil/prednisolone; FMD: fludarabine, mitoxantrone, dexamethasone; COP: cyclophosphamide, vincristine, prednisolone; FAD: fludarabine, adriamycin, dexamethasone; CMOP: cyclophosphamide, mitoxantrone, vincristine, prednisolone; CVEP: cyclophosphamide, vincristine, etoposide, prednisolone; DHAP: dexamethasone, cisplatin, cytarabine; RT: radiotherapy; HOVON 26 intensified CHOP: cyclophosphamide 1000 mg/m<sup>2</sup>, doxorubicin 70 mg/m<sup>2</sup>, vincristine 2 mg, prednisolone 100 mg Days 1–5, G-CSF Days 2–11, 14-day cycle: 1\* indicates one course of four weekly infusions of 375 mg/m<sup>2</sup>. PR: partial remission; CR: complete remission; IPI: International Prognostic Index; ECOG: Eastern Cooperative Oncology Group performance status.

cocktail (Qbiogene, UK) following the manufacturer's instructions.

### Patients

Patients with CD20 positive follicular lymphoma, refractory, in 1st partial or 2nd complete remission were eligible. The study protocol was approved by the local ethics committee and informed consent was obtained from each patient. Rituximab (Mabthera<sup>®</sup>) was delivered at a dose of 375 mg/m<sup>2</sup> weekly for 4 weeks within 12 weeks prior to conditioning. Reduced-intensity conditioning comprised 'BEAM-CAMPATH', scheduled as follows: CAMPATH-1H 20 mg once daily i.v. on Days –5 to –1; BCNU 300 mg/m<sup>2</sup> i.v. on D-6; cytarabine 200 mg/m<sup>2</sup> 12 hourly i.v. on Days –5 to –2; etoposide 200 mg/m<sup>2</sup> once daily i.v. on Days –5 to –2; melphalan 140 mg/m<sup>2</sup> i.v. Day –1; HSC infusion D0. G-CSF (filgrastim, Neupogen<sup>®</sup>) 300 µg daily was administered from D+7 to neutrophil recovery. Immunosuppression was with cyclosporin, titrated to achieve plasma levels of 150–200 ng/l and tapered from Day +58 in the absence of clinical GvHD. Toxicity was assessed utilising the abbreviated Common Toxicity Criteria of the NCIC<sup>38</sup> and response assessment was made using the guidelines of the International workshop.<sup>39</sup> GvHD was graded according to the Consensus Conference on GvHD grading.<sup>40</sup>

All patients received standard nursing and supportive care protocols for neutropenic patients. Infection prophylaxis comprised itraconazole suspension 200 mg p.o. twice daily, high-dose acyclovir 500 mg/m<sup>2</sup> 8 hourly i.v. from Day –5 in high-risk patients (patient and/or donor CMV positive), otherwise 200 mg 8 hourly p.o. and aerosolised pentamidine 300 mg on Day –10. Blood products were universally leucodepleted and irradiated to 25 cGy. CMV seronegative recipients received CMV seronegative blood products. Prior to August 2000, CMV monitoring was performed using a fluorescent antigen detection methodology (CENFA). Subsequently, a PCR-based DNA assay several-fold more sensitive than the CENFA was utilised. All patients were monitored weekly until Day +100 or until immunosuppression was completely withdrawn and

no graft-versus-host disease (GvHD) was present. Two positive PCR assays or a single positive CENFA were indications for pre-emptive therapy with intravenous ganciclovir.

All data were censored on 30 June 2002.

### Results

At the time of reporting, a total of five patients (two male; three female), with a median age at transplant of 50.9 years (42.6–55.8), had been recruited, over a period of approximately 6 months. The median age at diagnosis was 49.3 (39.4–52.7) years and at transplant 50.9 (42.6–55.8) years. At a median follow-up of 521 days (371–719), four of five patients were alive and three were in complete radiological and molecular remission. Patient and disease characteristics are shown in Table 1.

At transplant, all were in complete or partial remission after a median of three previous courses of chemotherapy.<sup>2–7</sup> All initial stage IV patients had continued marrow infiltration morphologically. Residual clinical/radiological evidence of pathological lymphadenopathy was present in two patients and three had detectable bcl-2/IgH translocations. Four received peripheral blood stem cells from a sibling donor with a median CD34 cell count of  $5.21 \times 10^6$ /kg ( $2.9 \times 10^6$ – $6.95 \times 10^6$ ) and one bone marrow from a volunteer-unrelated donor with  $3.45 \times 10^6$ /kg CD34+ cells.

The immediate post-transplant toxicities were limited to NCIC grade 1–2 stomatitis, nausea, vomiting and grade 3 febrile neutropenia. The median time to neutrophil ( $>0.5 \times 10^9$ /l) and platelet ( $>20 \times 10^9$ /l) recovery was 12 days (Table 2). There was no delayed or secondary neutropenia following regeneration.

One patient (Patient no. 1) pancytopenic with secondary myelodysplasia, did not receive pre-transplant rituximab, as it was felt that this would result in an unnecessary delay to allografting. Following demonstration of a persistent marrow infiltrate, predominantly recipient chimerism and rapidly progressive nodal disease at D+80, cyclosporin was completely withdrawn and rituximab delivered on

**Table 2** Transplant and clinical follow-up

Patient no.	Donor /source/sex	CD34 dose (kg)	CMV recipient/donor	Neutrophil recovery (days)	Platelet recovery (days)	CT findings pre-transplant	CT response post-transplant
1	Sibling/PBSC/f	$5.66 \times 10^6$	Pos/pos	11	10	PR	D + 77SD D + 138SD D + 323CR
2	Sibling/PBSC/m	$2.9 \times 10^6$	Pos/pos	20	23	PR	D + 99SD D + 185SD D + 246SD D + 574PD
3	Sibling/PBSC/f	$6.95 \times 10^6$	Pos/pos	11	12	CR	D + 56CR D + 168CR D + 382CR
4	VUD/BM/f	$3.45 \times 10^6$	Neg/pos	29	50	CR	D + 180CR D + 356CR
5	Sibling/PBSC/m	$4.76 \times 10^6$	Pos/neg	12	12	CR	D + 120CR D + 294CR

CR, complete remission; PR, partial remission; PD, progressive disease; SD, stable disease; VUD, volunteer unrelated donor; PBSC, peripheral blood stem cells; BM, bone marrow; neutrophil recovery defined as neutrophils  $> 0.5 \times 10^9/l$  for  $\geq 2$  consecutive days; platelet recovery defined as unsupported platelet count  $> 20 \times 10^9/l$  for  $\geq 2$  consecutive days.

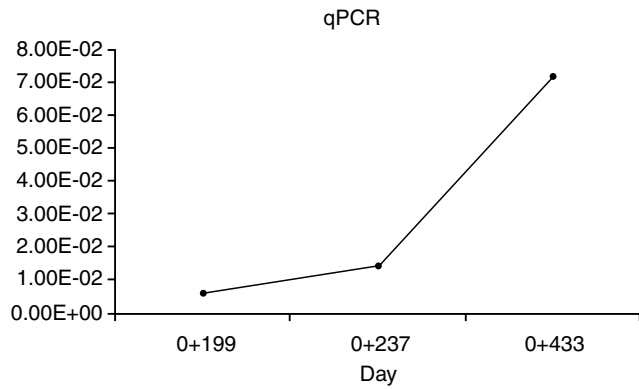
**Table 3** Molecular follow-up and GvHD

Patient no.	Chimerism (% donor)	BM morphology		<i>t(14;18)</i>		GvHD	Follow-up
		Pre-tpt	Post-tpt	Pre-tpt	Post-tpt		
1	FISH D + 68 45% D + 163 55% D + 331 98%	LG paratrabeular infiltrate	D + 68 infiltrated  D + 156 neg	Not avail	D + 68 pos  D + 177 neg D + 344 neg	Gr III GIT and liver (post-DLI D + 204, $5 \times 10^6$ )	D + 371 (deceased)
2	STR D + 181 pD D + 409 pR D + 433 R D + 497 R	LG paratrabeular infiltrate	D + 90 neg  D + 181 neg D + 248 neg D + 497 infiltrated	Not avail	D + 199 pos (PB)  D + 237 pos (PB) D + 433 pos (PB) D + 497 pos (PB) *qPCR	0	D + 719  PD
3	STR D + 110 D D + 362 D	LG paratrabeular infiltrate	D + 56 neg  D + 110 neg D + 200 neg D + 362 neg	MBR pos	D + 56 pos  D + 110 pos D + 200 neg D + 362 neg	Limited chronic skin (D + 129)	D + 521  CR
4	STR D + 110 D D + 293 D D + 356 D	LG paratrabeular infiltrate	D + 68 neg  D + 110 neg D + 203 neg D + 375 neg	MBR pos	D + 68 neg  D + 110 neg D + 202 neg D + 357 neg	0	D + 528  CR
5	FISH D + 28 87% D + 63 100% D + 98 96% D + 230 88% D + 266 74% D + 391 85%	no infiltrate	D + 28 neg D + 63 neg D + 98 neg D + 248 neg	MBR pos	D + 29 neg D + 63 neg D + 98 neg D + 248 neg	0	D + 439 CR

FISH = fluorescent *in situ* hybridisation; STR = short tandem repeats; qPCR = quantitative PCR; PD = progressive disease; CR = complete remission; pD = predominantly donor; pR = predominantly recipient; D = donor; R = recipient.

D + 82. Although he then achieved morphological clearance of his bone marrow and bcl-2/IgH negativity, he failed to attain MRD because of the persistence of pathological lymphadenopathy. DLI was delivered on D + 204 with

$5 \times 10^6$  CD3+ /kg resulting in complete remission and conversion to predominantly donor chimerism but with grade III gastrointestinal and liver GvHD. He succumbed to a perforated viscus on D + 371 (Table 3).



**Figure 1** Patient no. 2 quantitative PCR post-allograft, y-axis shows the ratio of bcl-2/IgH positive cells to  $\beta$ -actin positive cells.

Patient no. 2 failed to achieve either a clinical or molecular response to the transplant or to post-transplant rituximab delivered from D+199, despite morphological clearance of marrow, with stable disease on imaging. Late graft failure with autologous regeneration was demonstrated by chimerism studies on D+409. Quantitative PCR demonstrated a progressive relative increase in bcl-2/IgH positivity, preceding the initial clinical evidence of progressive disease on D+497 (Figure 1). At the time of reporting, he had slowly progressive disease and is awaiting a second reduced-intensity allograft.

Two patients (Patient nos. 4 and 5) achieved clinical and molecular remissions at first assessment following allograft. Patient no. 3 achieved a clinical and morphological remission and full donor chimerism, but demonstrated persistent bcl-2/IgH positivity on D+110. Chronic limited cutaneous GvHD developed at D+129 following complete withdrawal of cyclosporin, with subsequent bcl-2/IgH negativity. These three patients remain in complete clinical and molecular remission.

Donor lymphocyte infusions were delivered to three patients: Patient no. 1 for disease progression and declining donor chimerism, Patient no. 4 for the emergence of a small t(8;14) clone on D+482 and Patient no. 5 for declining donor chimerism. DLI was effective against the indications for administration.

CMV reactivation was demonstrated in three patients: Patient no. 4 at D+249, Patient no. 5 at D+38, Patient no. 3 at D+54, D+242 and D+345. All responded well to intravenous gancyclovir. In addition, Patient no. 3 required aerosolised ribavirin therapy for community-acquired RSV upper respiratory tract infection at D+53 and D+74.

## Discussion

At present, allo-HSCT represents the only therapeutic modality for the indolent lymphomas, which may be delivered with curative intent. Its applicability, however, is limited by the availability of an allogeneic HSC donor and the toxicity of the conditioning regimen.

Despite the dramatic improvements achieved to date in reducing transplant-related complications and mortality, it

may be difficult to justify allo-HSCT as ‘up-front’ therapy for an ‘indolent’ disease with a long median survival. The optimal timing of allogeneic HSCT is unresolved. For this study, we therefore selected patients with relatively resistant disease beyond 1st partial remission, or patients beyond 2nd complete remission, with an available molecular marker for minimal residual disease and with a suitable sibling or volunteer-unrelated donor. These criteria have undoubtedly limited accrual into the study.

Nevertheless, reduced-intensity conditioning with rituximab-BEAM-CAMPATH appears to be safe and efficacious in permitting durable engraftment. Although effective against marrow disease, conditioning appears to have a limited effect against nodal disease present at transplant as evidenced by an absence of response in the two patients with residual lymphadenopathy. This is in keeping with the findings of other studies that chemosensitive disease and minimal disease state at the time of allografting are significantly associated with survival.<sup>8,13</sup>

A graft-versus-lymphoma effect has been demonstrated, most marked following DLI and withdrawal of immunosuppression, with regression of nodal disease in one patient and the achievement of PCR negativity in a second. Rituximab delivered post-transplant, although safe and complication-free, did not impact significantly upon residual lymphoma, again in the two patients with residual nodal disease. A more formal purging schedule with rituximab immediately prior to HSC infusion and closely thereafter may improve disease control<sup>41</sup> when used in conjunction with specific conditioning protocols. While CAMPATH-1H was administered for the sole purpose of immunosuppression to minimise GvHD and graft rejection,<sup>35,42</sup> the antilymphoma properties of CAMPATH-1H in this setting cannot be discounted.<sup>6</sup>

There appears to be a high incidence of CMV reactivation, with three of five patients requiring pre-emptive therapy. While the numbers in this study are far too small to draw any conclusions, delays in immune reconstitution following CAMPATH-1H may play a role.<sup>43,44</sup> This may be due to delayed T-cell reconstitution or effects on antigen-presenting cells.<sup>45</sup>

Although follow-up is short, reduced-intensity allo-HSCT with rituximab-BEAM-CAMPATH conditioning appears to be safe, effective in inducing durable donor engraftment and molecular remissions and is potentially curative. Quantitative PCR for bcl-2/IgH may allow more accurate targeted scheduling of rituximab and/or DLI in the post-HSCT setting, although the ‘threshold’ for instituting therapy has yet to be determined. The majority of reduced-intensity conditioning protocols to date have included fludarabine because of its excellent immunosuppressive and antilymphoma properties. However, with the inclusion of fludarabine as first line or salvage chemotherapy in lymphoma, the adoption of a nonfludarabine-containing transplant conditioning regimen may be advantageous and worthy of further investigation. It is far too early to determine if a reduced-intensity allograft approach will affect the overall or disease-free survival in this indolent lymphoma, but these early results appear encouraging. Further follow-up with a larger cohort of patients is essential, as is investigation into the role of rituximab in the

pre- and post-transplant setting as well as into any potential antilymphoma properties of CAMPATH.

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