Table 1. Flow diagram showing the updated results of randomized patients.

	HDT/ASCT arm (83)	CHT arm (80)	
Complete remission	76 (92%)	71 (89%)	
Deaths			
Overall	14	12	
During extended follow-up	6	3	
Relapses			
Overall	8	7	
During extended follow-up	–	3	
Failures*			
Overall	20	18	
During extended follow-up	1	4	
Median follow-up, months (alive pts.)	108 (42-172)	106 (9-158)	
10-year OS	85% (95% C.I. 78-90)	84% (95% C.I. 77-89)	p=0.7
10-year RFS	88% (95% C.I. 81-95)	89% (95% C.I. 83-93)	p=0.8
10-year FFS	79% (95% C.I. 72-85)	75% (95% C.I. 67-82)	p=0.8

*CR not achieved, relapse after CR, death in CR, second tumor.

patients at high risk of relapse candidates for intensification with HDT and ASCT.

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Clinical relevance of *MDM2* SNP 309 and *TP53* Arg72Pro in follicular lymphoma

Tumor protein 53 (TP53) is critical to cell cycle control and is the most common mutational target in germinal center lymphomas. However, these mutations occur infrequently at diagnosis (<10%) in follicular lymphoma (FL), and are more commonly associated with disease progression or transformation to more aggressive histology.[1-4] Inactivation of TP53 may also occur by upregulation of the human homolog of the *Murine Double Minute 2* protein (MDM2), which targets TP53 for degradation by the proteasome and is frequently amplified in FL and other malignancies; indeed *Mdm2* haplo-insufficiency in mice leads to enhanced TP53 function with delayed onset of lymphoma.[5] While many studies have examined the genomic events targeting these loci, less is known as to the functional role of polymorphic variants at the *MDM2* or *TP53* loci. The single nucleotide polymorphisms (SNPs), *MDM2* SNP 309 and *TP53* Arg72Pro, have at least in some studies an additive effect on cancer susceptibility with *MDM2* SNP 309 also predicting advanced disease at diagnosis.[6] Consequently, we investigated the impact of *MDM2* SNP 309 and *TP53* Arg72Pro on the clinical outcome of FL. *MDM2* SNP 309 characterizes a T>G substitution at nucleotide 309 of intron one and leads to higher *MDM2* mRNA and protein expression with lower apoptotic response. Significantly, homozygosity for the G allele correlates with earlier onset of *de novo* diffuse large B-cell lymphoma (DLBCL) in females[7] Its role in CLL is less clear as two recent studies provide contrasting results.[8,9] *TP53* Arg72Pro is a non-synonymous SNP involving G>C substitution at nucleotide 466 of exon 4 in

Table 1. Assessment of *MDM2* SNP 309 and *TP53* Arg72Pro against clinical parameters.

Outcome	*MDM2* and *TP53* allelic combinations				Total	*p* value
	Neither rare	Rare TP53	Rare *MDM2*	Both rare		
Gender						0.67
Male	27 (60%)	28 (52%)	30 (50%)	39 (58%)	124	
Female	18 (40%)	26 (48%)	30 (50%)	28 (42%)	102	
Stage						0.13
I-II	11 (27%)	6 (13%)	11 (23%)	20 (33%)	48	
III-IV	30 (73%)	39 (87%)	37 (77%)	40 (67%)	146	
Missing					32	
Response to first line therapy						0.18
CR	17 (39%)	13 (28%)	18 (32%)	16 (27%)	64	
PR	22 (50%)	26 (55%)	34 (60%)	28 (47%)	110	
NR	5 (11%)	8 (17%)	5 (9%)	16 (27%)	34	
Missing					18	
Best response to therapy						0.60
CR	19 (43%)	14 (30%)	19 (34%)	20 (33%)	72	
PR	23 (52%)	30 (64%)	36 (64%)	38 (64%)	127	
NR	2 (5%)	3 (6%)	1 (2%)	2 (3%)	8	
Missing					19	

Illustrated are results for four groups of allelic combinations of the two SNPs. The four groups are: "Neither rare" = cases homozygous for both common alleles of the two SNPs (MDM2 SNP 309 TT with TP53 Arg72Pro GG), "Rare TP53" = cases homozygous for the MDM2 SNP 309 common allele but having at least one copy of the TP53 Arg72Pro rare allele (MDM2 SNP 309 TT with TP53 Arg72Pro GC or CC), "Rare MDM2" = cases homozygous for the TP53 Arg72Pro common allele but having at least one copy of the MDM2 SNP 309 rare allele (TP53 Arg72Pro GG with MDM2 SNP 309 TG or GG), "Both rare" = cases homozygous for both rare alleles (TP53 Arg72Pro CC with MDM2 SNP 309 GG). No associations were seen between the three allelic combinations for MDM2 SNP 309 alone and these clinical parameters. When fitting an analysis of variance (ANOVA) model for age, neither MDM2 SNP 309 allelic combinations alone nor together with TP53 Arg72Pro allelic combinations show an association (p≥0.53). CR: complete response; PR: partial response; NR: no response.

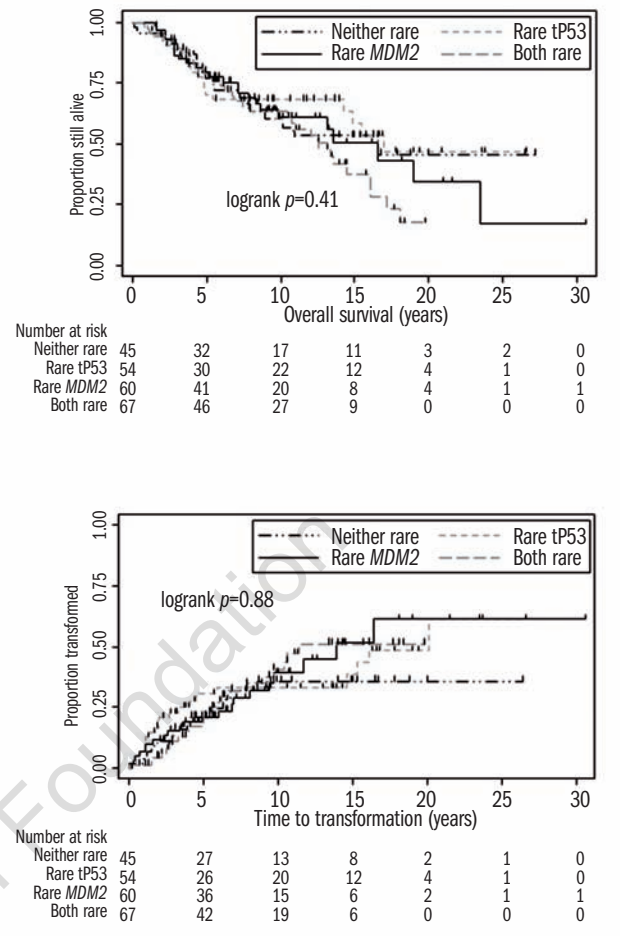

Figure 1. Kaplan-Meier plots and log rank test statistics for overall survival (A) and time to transformation (B) by *MDM2* SNP 309 and *TP53* Arg72Pro alleles. Illustrated are results for four groups of allelic combinations of the two SNPs. The four groups *Neither rare*, *Rare TP53*, *Rare MDM2* and *Both rare* are defined in Table 1. In addition, no associations were seen between the 3 allelic combinations for each SNP and the survival parameters, nor were associations seen on informal analyses of all possible allelic combinations between the two SNPs (*data not shown*).

TP53 which creates a TP53 protein with reduced potential to induce apoptosis or suppress cell transformation.[10] These SNPs are also associated with poor prognosis or increased cancer risk in certain solid malignancies.[11,12]

We investigated the relationship of the allelic combinations for both SNPs with the following clinical variables in cases of FL: age, gender, stage at diagnosis, response to first line therapy, best response to therapy, progression-free survival, relapse-free survival, overall survival and time to transformation (*Details of each variable are available in the Online Supplementary Table S1*). A real-time polymerase chain reaction Allelic Discrimination multiplexed endpoint assay protocol using the ABI PRISM™ 7700 Sequence Detector (Applied Biosystems, Foster City, CA, USA) was used to determine the SNP alleles present in DNA of 226 patient samples from bone marrow (n=207), peripheral blood (n=14) or lymph nodes (n=5). (Oligonucleotide sequences used are available on request.) Samples were obtained from the tissue archive at St Bartholomew's Hospital under Institutional Ethical Approval obtained from the Local Ethics Committee. Reactions were performed in duplicate including homozygous and heterozygous controls for each SNP genotype and a no DNA template control. Positive controls were confirmed by direct sequencing.

In this patient cohort, allelic frequencies were similar to previously published results at 44%, 43%, 13% for *MDM2* SNP 309 TT, TG, GG and at 46%, 46%, 8% for *TP53* Arg72Pro GG, GC, CC respectively (*Online Supplementary Table S2*) and both polymorphisms satisfied the Hardy-Weinberg equilibrium. Median age at diagnosis was 46 years, which is younger than that previously described for FL, and reflects our status as a tertiary referral center for patients with FL. There was no difference in the median age at diagnosis for each of the 3 allelic combinations of *MDM2* SNP 309 (both for the whole study population and for gender in contrast to findings in DLBCL[7]) and of *TP53* Arg53Pro (*Online Supplementary Table S2*).

Relationships between SNP alleles and clinical parameters were studied using χ^2 test for stage and gender, analysis of variance (ANOVA) for age and Kruskal-Wallis test for responses to therapy. The 3 allelic combinations for each SNP were informally assessed as well as formally assessing two groups of patient samples consisting of those homozygous for the common allele or those with at least one rare allele, enabling any dominant effects of the *MDM2* SNP 309 G or *TP53* Arg72Pro C alleles, as described in other malignancies, to be assessed in FL. As

MDM2 SNP 309 can lead to earlier onset or more advanced disease[6] and TP53 negatively regulates MDM2 expression, we assessed *MDM2* SNP 309 alleles in isolation or in combination with *TP53* Arg72Pro alleles against these clinical parameters; there was no evidence of any association (illustrated in Table 1 for gender, stage and responses to therapy; $p \geq 0.13$). Kaplan-Meier plots and log rank test statistics demonstrated no evidence for association of *MDM2* SNP 309 or *TP53* Arg72Pro allelic variants alone, or in combination, with overall survival (Figure 1A), progression free survival, relapse free survival or time to transformation (Figure 1B) ($p \geq 0.17$).

Consequently, whilst genomic lesions targeting the *MDM2-TP53* axis are an important feature of FL, *MDM2* SNP 309 and *TP53* Arg72Pro do not predict clinical outcome. In contrast to other malignancies these polymorphisms are not significant in FL.

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Pharmacokinetics of alemtuzumab in combination with fludarabine in patients with relapsed or refractory B-cell chronic lymphocytic leukemia

In recent years, chemoimmunotherapies that combine cytotoxic agents and monoclonal antibodies have been studied intensively for the treatment of B-cell chronic lymphocytic leukemia (CLL). Of particular interest are fludarabine-based combination regimens such as FluCam (fludarabine and alemtuzumab), FCR (fludarabine, cyclophosphamide, and rituximab), and FCCam (fludarabine, cyclophosphamide, and alemtuzumab).[1-4] Alemtuzumab (Campath), a humanized anti-CD52 monoclonal antibody, is currently approved in the United States as first-line, single-agent treatment of CLL and in the European Union as first-line treatment of CLL when fludarabine combination chemotherapy is not appropriate. When administered at the standard dosing schedule [30 mg intravenously (IV) 3 times a week (TIW) for up to 12 weeks], alemtuzumab demonstrated an overall response rate (ORR) of 33-50% in fludarabine-refractory patients [complete response (CR) rate, 0-4%][5-7] and 83-87% (CR rate, 19-24%) in previously untreated patients.[8,9] At this time, only limited data are available on the pharmacokinetics (PK) of alemtuzumab, and the approved dosing schedule of alemtuzumab monotherapy was developed empirically in the absence of detailed PK studies.

Recently, our group reported on the results of a phase II study that evaluated concomitant use of IV fludarabine and alemtuzumab administered with a novel schedule (FluCam regimen) in patients with relapsed/refractory CLL.[1] We conducted the present study to investigate the PK of alemtuzumab in patients who received the FluCam regimen. Fourteen patients with relapsed/refractory CLL were enrolled, all of whom gave written informed consent prior to study entry in accordance with the Declaration of Helsinki. Patient eligibility criteria have been previously described.[1] The study protocol was approved by the institutional review board. Following alemtuzumab dose escalation (3 to 10 to 30 mg over three days), fludarabine 30 mg/m^2 followed by alemtuzumab 30 mg was given IV on days 1-3 of a 28-day cycle for up to 6 cycles. Patients were managed as previously described.[1] In 5 patients, treatment cycles were extended to up to 42 days because of critical neutropenia. Response was assessed on the first day of cycle 4 and 1-3 months after the last cycle of therapy according to the 1996 National Cancer Institute Working Group criteria. Bone marrow aspiration and biopsy were performed two months after