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

Table 1. Patients' demographics.

Characteristics	N=14
Median age, years (range)	59 (49-73)
Time since diagnosis of CLL, years (range)	6.2 (2.2-14.6)
Binet stage at study enrollment, n (%)	
A	0 (0)
B	5 (36)
C	9 (64)
Median n. of prior regimens (range)	2.5 (1-5)
Type of prior therapies administered, n (%)	
Fludarabine monotherapy or combination	8 (57)
Chlorambucil monotherapy	5 (36)
Bendamustine monotherapy	4 (29)
Rituximab monotherapy or combination	4 (29)
Fludarabine + alemtuzumab	3 (21)
Alemtuzumab monotherapy	2 (14)

CLL: chronic lymphocytic leukemia.

demonstration of CR by clinical and laboratory assessments. Patient serum samples were collected on day 0 (the day before start of cycle) and on days 1 (before drug administration), 4, 7, 14, 21, and 28 (and days 35 and 42 in patients with extended cycles). Samples were stored at -70°C until analysis. Plasma concentrations of alemtuzumab were determined using the previously described method.[10] Mean plasma concentration on a defined day (e.g., day 1) of a treatment cycle was calculated as the mean value across all treatment cycles. Correlation coefficient was calculated using Spearman's rank method. Patient serum samples were not assessed for antiglobulin response.

Patients' demographics are shown in Table 1. Half of the patients received 3 or more prior lines of therapy. All patients had extensive bone marrow infiltration at baseline, and the majority had lymphadenopathy and/or splenomegaly. Patients received a median of 4 cycles of FluCam (range, 1-6). There were 5 patients with CR, 7 patients with partial response (PR), and 2 patients with progressive disease (PD) (ORR 86% and CR rate 36%). Three patients had received 4 cycles of FluCam as their last treatment prior to study enrollment, and in this study they received fludarabine 25 mg/m^2, cyclophosphamide 200 mg/m^2, and alemtuzumab 30 mg on the same schedule, with 1 CR and 2 PR achieved. For PK analysis, a total of 158 patient serum samples were collected, of which 120 were tested. Within a treatment cycle, the mean plasma concentration of alemtuzumab increased from days 1 to 4, reaching 1.97 µg/mL on day 4, then decreased to 0.28 µg/mL on day 7. By day 21, plasma concentration of alemtuzumab decreased to undetectable levels (Figure 1A). There was a trend toward a higher plasma alemtuzumab level in patients with a better response [correlation coefficient r=0.527, p=0.078 (two-sided)], although it did not reach a statistically significant level, probably because of the small patient cohort. The highest plasma level achieved in a given patient in all treatment cycles averaged 3.35 µg/mL in patients with CR, 0.98 µg/mL in patients with PR, and 0.32 µg/mL in patients with PD (Figure 1B). We did not observe a clear correlation between initial white blood cell count and plasma alemtuzumab level (*data not shown*). No significant correlation was observed between plasma alemtuzumab level and CD4$^+$ T-cell level or incidence of infection (*data not shown*).

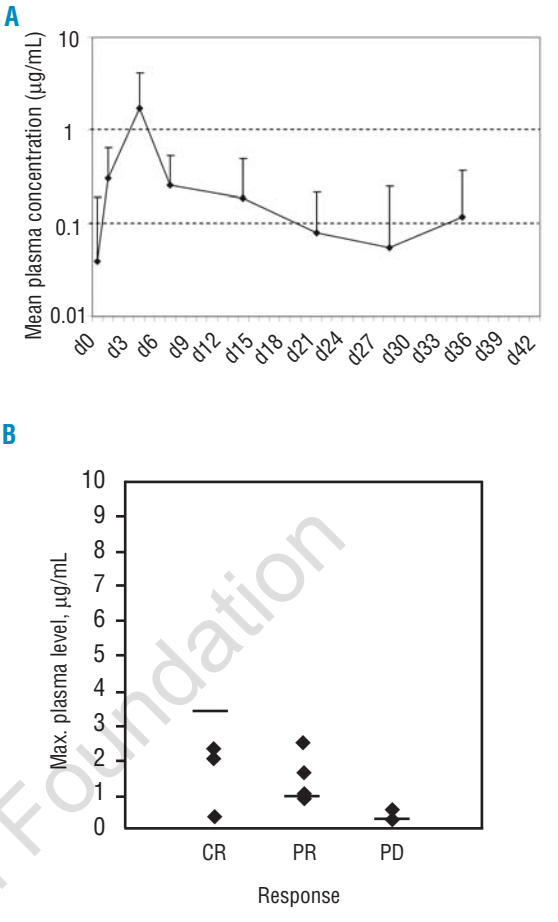

Figure 1. (A) Mean plasma concentration of alemtuzumab in patients receiving the FluCam regimen. Data are plotted in log-scale. Error bars indicate standard deviation. (B) Relationship between maximal plasma level of alemtuzumab and clinical response. Plasma concentration data are available for 4 patients with complete response (CR), 6 patients with partial response (PR), and 2 patients with progressive disease (PD). Horizontal bars denote the mean values of individual patient groups.

At present, PK data on alemtuzumab are limited. PK of alemtuzumab was characterized by a two-compartment model with nonlinear elimination. Interpatient variability was large, probably reflecting differences in tumor burden and disease activity among patients.[11] Positive correlations between plasma alemtuzumab level and clinical response have been demonstrated by Hale *et al.* and Montillo *et al.*[10,12] Our data further confirm such positive correlations. Based on *in vitro* results, a concentration of 1.0 µg/mL has been previously suggested as the minimum alemtuzumab concentration required for lympholytic activity.[10] However, no *in vivo* data are available to define the plasma alemtuzumab concentration required for clinical activity. Considering the high response rate achieved with the FluCam regimen in this study, we propose that a plasma alemtuzumab level lower than 1.0 mg/mL can still be clinically effective in combination therapy, possibly because of potential synergy between fludarabine and alemtuzumab.

Based on the positive results of the phase II FluCam trial, a large, international phase III trial (CAM314) comparing the FluCam regimen with fludarabine alone has

been initiated. Because plasma drug level positively correlates with response to alemtuzumab-containing therapy, future clinical studies of alemtuzumab-containing therapy should ideally include measurement of plasma drug concentration to evaluate the feasibility of PK-directed dosing, with the aim of improving patient response and minimizing toxicity.

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Respiratory depression and somnolence in children receiving dimethylsulfoxide and morphine during hematopoietic stem cells transplantation

Dimethylsulfoxide (DMSO) has been commonly used for the past 20 years as a well-known cryo-protectant agent. It acts by penetrating the cell and binding water molecules; by doing so, it blocks the efflux of water and prevents cellular dehydration, maintains stable pH, intracellular salt concentration, and the formation of the ice crystals which endanger cell integrity. The most common clinical application of such a procedure is the cryo-protection of stem cells frozen in liquid nitrogen for their subsequent reinfusion in autologous stem cell transplantation.[1]

The infusion of DMSO may have several side effects, such as vasoconstriction, nausea, vomiting, abdominal cramps, cardiovascular and respiratory problems, and a variety of neurological events.[2-4] Despite this, it is used by transplant specialists worldwide.

Morphine is widely used to control pain in patients with different clinical conditions. Its wider use has been repeatedly advocated by scientists, patients' associations and the media in order to combat old prejudices restricting its use to terminal cancer patients. Patients undergoing hematopoietic stem cell transplantation (HSCT) may face pain for different causes, including severe mucositis.[5] Thus the use of morphine and DMSO may concur in such patients. To our knowledge, reports of adverse events resulting from their interaction are not available.

Recently, we observed 3 patients who unexpectedly developed somnolence and clinically significant oxygen desaturation soon after reinfusion of autologous stem cells. Their main features are summarized in Table 1. Neurological alterations rarely occur as a primary complication during or soon after stem cell infusion. In a recent survey, only 3 out of 179 consecutive patients experienced neurological complications during stem cell infusion.[6] In order to understand the pathogenesis of such unexpected events, we performed a thorough critical re-evaluation of the clinical course of the patients. We noted that all of them were already receiving i.v. morphine infusion at the time of stem cell infusion; this is quite unusual in our experience, since severe pain is more frequently observed later during the course of transplantation.[5] Thus, we wondered if morphine interaction with any other transplant-related agent might