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RITUXIMAB IN COMBINATION WITH HIGH DOSE METHYLPREDNISOLONE FOR THE TREATMENT OF CHRONIC LYMPHOCYTIC LEUKEMIA

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Abstract

We observed that high-dose methylprednisolone (HDMP) and rituximab (R) was well tolerated and had promising activity when used in combination to treat patients with fludarabine-refractory chronic lymphocytic leukemia (CLL). This prompted us to evaluate the use of these agents in frontline therapy. Twenty-eight patients with a median age of 65 enrolled in this study. Patients received HDMP at 1 g/m^2 each day for three days during each of the three four-week cycles together with rituximab and prophylactic anti-microbial therapy. The treatment was well tolerated with few adverse events of grade III or higher. The overall response rate was 96% (N=27). Nine patients (32%) achieved a complete remission (CR), two of which were without detectable minimal residual disease (MRD). Six patients with MRD received consolidation with alemtuzumab; five of these patients achieved an MRD-negative CR. With over three years of follow-up median progression free survival was 30.3 months with only 39% of patients requiring additional therapy, and an overall survival was 96%. This study demonstrates that HDMP and rituximab is an effective non-myelosuppressive treatment combination for patients with CLL that warrants consideration particularly for patients with limited myeloid reserve that might not tolerate standard treatment regimens.

INTRODUCTION

Myelosuppression is associated with significant morbidity and mortality in chronic lymphocytic leukemia (CLL) and is often exacerbated by currently available treatments for

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AUTHORSHIP

T.J.K. and J.E.C. designed, performed research, and wrote the paper. D.F.J. performed research, analyzed data, and wrote the paper. T.J.K., J.E.C., and D.F.J. treated the patients. J.D.S. and S.J. analyzed data and wrote the paper. J.B. performed research and treated the patients. L.Z.R. performed research.

this disease. Progress in the treatment CLL has been made with the introduction and the Food and Drug Administration (FDA) approval of novel agents including fludarabine, alemtuzumab, and bendamustine. However, treatment with any one of these agents is often complicated by hematologic toxicity. (1, 2) Chemoimmunotherapy regimens such as those that combine fludarabine monophosphate with rituximab (FR), cyclophosphamide (FC), cyclophosphamide and rituximab (FCR), cyclophosphamide, and mitoxantrone (FCM), have improved response rates and progression free survival (PFS) over single-agent therapy.(3–10) However, the risk of therapy-related myelosuppression is high with combination chemoimmunotherapy, particularly in elderly patients, and those with compromised marrow function. Consequently, many patients cannot tolerate the full-dose therapy or experience treatment delays due to hematologic toxicity.(3–5) Accordingly, the development of novel treatment strategies that lack significant myelotoxicity is desirable.

Rituximab (RituxanR) is FDA approved for treatment of B-cell non-Hodgkin's lymphoma and rheumatoid arthritis. Rituximab has modest activity in CLL, even when used at higher doses than those used in lymphoma.(11, 12) High-dose methylprednisolone (HDMP) also has activity in CLL, including those cases with loss of p53 and high-risk cytogenetic abnormalities.(13) However, complete remissions are rarely observed with either agent administered alone.

There is growing recognition of the integral role of the tumor microenvironment in CLL. (14–17) When CLL cells are cultured with marrow stromal or nurselike cells, they are rescued from spontaneous apoptosis and protected from drug-induced cytotoxicity *in vitro* and conceivably *in vivo*.(18–20) In pre-clinical studies, rituximab acted synergistically with methylprednisolone to induce apoptosis of CLL cells, particularly in the presence of nurse-like cells.(21) Therefore, we speculated that rituximab might have enhanced activity in CLL if administered with glucocorticoids. Accordingly, we implemented a single-institution study to evaluate the combination of HDMP-rituximab in fludarabine-refractory patients. (22, 23) Excellent response rates and tolerability of this non-chemotherapy combination prompted us to evaluate this regimen in treatment-naive CLL patients.

PATIENTS AND METHODS

Patients

Patients were required to have diagnosis of CLL and an indication for treatment as per the National Cancer Institute Working Group Guidelines (NCI-WG)(24). Each patient had not received prior monoclonal antibody and/or chemotherapy for CLL and had a performance status of 2 or less and normal renal and hepatic function. We excluded patients who had a history of diabetes, steroid-induced psychosis, active peptic ulcer disease, history of recent gastrointestinal bleeding, diverticulitis, or pancreatitis, and patients who had active infection or evidence of past infection with hepatitis B or C or Human Immunodeficiency Virus. Patients provided written informed consent for this protocol, which was approved by the UCSD Human Research Protections program and conducted in accordance with the Declaration of Helsinki.

Study Design

To evaluate dosing and toxicity we conducted a study of HDMP-rituximab in chemotherapy naive CLL patients. To assess if higher doses of rituximab could augment the clinical response rate to the combination we evaluated two different doses of rituximab. Patients in group one received 1 gm/m^2 of HDMP for three days in combination with rituximab at 375 mg/m^2 weekly for three 4-week cycles. Group two patients received same schedule of HDMP, but with rituximab 750 mg/m^2 administered following two schedules to determine pharmacokinetic (PK) variation with consecutive daily or weekly dosing. Primary objectives of the studies were to evaluate the clinical activity, safety, and tolerability of this combination. Clinical activity was evaluated by NCI-WG criteria with four-color flow cytometry to detect minimal residual disease (MRD) in the marrow.(25)(26) Secondary objectives evaluated PFS, treatment free survival (TFS), overall survival (OS), rituximab PK, incidence of infection, and the impact of therapy on hematologic and immune parameters.

Treatment And Monitoring

Cycles were administered as outpatient infusions every four weeks for a total of 3 cycles. All patients received HDMP 1 gm/m^2 intravenously over 90 minutes on days 1–3 of each cycle with intravenous cimetidine for premedication. To decrease initial infusion reactions the first dose of rituximab was divided over the first two days of cycle one. Rituximab infusions were premedicated with both acetaminophen and diphenhydramine. Group 1 patients (N =16) received twelve doses of rituximab at 375 mg/m^2 weekly over three 4-week cycles. Group 2 patients (N =12) received nine doses of rituximab at 750 mg/m^2 administered following two different schedules. Group 2A received rituximab 750 mg/m^2 daily times three administered at the start of each cycle. Group 2B received rituximab 750 mg/m^2 weekly times three during cycle one and daily times three for cycle two and three. Genentech Inc., San Francisco, CA, provided the rituximab for this study.

Patients received antibiotic prophylaxis with trimethoprim-sulfamethoxazole, acyclovir, and fluconazole or equivalents throughout treatment and for two months following completion of therapy. Allopurinol at a dose of 300 mg/day was administered three days prior to the start of therapy and during treatment for tumor lysis prophylaxis. Patients with blood glucose of 200mg/dl or more on the days of treatment with HDMP received subcutaneous regular insulin following a routine sliding scale.

Five patients who had evidence of minimal residual disease (MRD) following HDMP-rituximab chose to participate in an ongoing consolidation protocol evaluating alemtuzumab 30mg three times weekly administered subcutaneously for one (one patient) or two (four patients) 4-week cycles. All patients who met inclusion criteria of the alemtuzumab consolidation protocol patients in a PR or CR were eligible for this therapy. One patient with residual disease elected to undergo consolidation with an outside provider and received 12 weeks of alemtuzumab administered intravenously at a dose of 30mg three times weekly. Alemtuzumab was administered at least four months (average 26 weeks) following completion of treatment with HDMP-rituximab.

Evaluations And Follow-Up

Pretreatment evaluation consisted of a history, physical, complete blood count, serum chemistry, quantitative immunoglobulins, CD4 T-lymphocyte count, serum beta 2-microglobulin (β_2M), and marrow biopsy and aspiration with immunophenotyping, metaphase karyotype analysis, and fluorescence *in situ* hybridization (FISH) analysis for the most common chromosomal abnormalities in CLL(27). Patients with deletions of 11q or 17p, trisomy 12, or complex genetic abnormalities were considered to have unfavorable cytogenetics.(27) CLL-cell expression of the 70-kD zeta-associated protein (ZAP-70) and CD38 were reported as elevated using a threshold of >20% and >34%, respectively, as described.(28),(29) We determined the sequence of the expressed Ig heavy chain variable region (IGHV) gene. Those cases that used IGHV with 98% or greater homology to a known germ-line gene were classified as using unmutated IGHV.

Patients underwent a physical and laboratory studies prior to each cycle, two months after completion of treatment, and every 3–6 months until additional therapy was administered or death. A marrow biopsy was performed on all patients at least two-months following completion of HDMP-rituximab with MRD assessment by 4-color flow cytometry evaluating for CD5, CD19, CD20, and CD79b, as previously described.(26) Patients who received alemtuzumab consolidation were followed until they received additional treatment

Toxicity Assessment

Patients were assessed for adverse events throughout the study. Non-hematologic toxicity was graded accordingly with the NCI Common Toxicity Criteria (http://ctep.cancer.gov/reporting/ctc.html). Hematological toxicity was graded according to NCI-WG guidelines. (24, 25)

Response Assessment

Patients were evaluated for response at least two months following completion of therapy using the 1996 NCI-WG guidelines.(24, 25) Those without evidence of MRD in the marrow who also satisfied criteria for a CR were designated as having had an MRD-negative CR.

Pharmacokinetic Studies

Group 2 patients had serum levels of rituximab determined by an enzyme-linked immunoabsorbent assay (ELISA) that uses affinity purified polyclonal goat anti-rituximab as the capture reagent and goat antibody to mouse IgG F(ab')2 as the detection reagent. Rituximab PK was assessed on days 1, 4, 15, 29, 31, 57, and 59 prior to rituximab and on days 4, 15, 31, and 59 following rituximab infusion, and at one or three months after treatment.

Statistical Methods

The initial evaluation of the combination of HDMP rituximab was a Simon 2-stage design powered for the primary endpoint of response and enrolled 16 patients (Group 1). Analysis of the first stage of this study met the defined endpoint to enroll in the second stage with an ORR >60%. However, the CR rate was lower than expected when compared to our results of

a similar regimen (with higher total doses of steroids) reported in the fludarabine refractory population.(22) Therefore, a second study was designed to evaluate the safety and efficacy of HDMP in combination with higher doses of rituximab in 12 patients (Group 2). Group 2 patients were enrolled on an alternating basis to receive rituximab along two different dose schedules during cycle 1 to assess for pharmacokinetic variation. Descriptive statistics were used to summarize changes in hemoglobin, platelet or lymphocyte count, and lymph node or spleen size.

PFS, TFS, and OS were analyzed by the Kaplan-Meier method for various categories of response to therapy.(30) Differences among response to therapy were evaluated via the log-rank test.(31) Time intervals were measured from the first day of treatment until progressive disease, additional CLL treatment per NCI-WG guidelines, or death. Patients were followed until they received alternative treatment for relapsed disease or death. Analysis was performed on an intention-to-treat basis. Fisher's exact test and multivariable logistic regression identified pre-treatment patient characteristics associated with treatment response. P-values less than 0.05 were considered significant. Analyses were performed using the R-software (version 2.6.2, http://www.r-project.org/).

RESULTS

Patient Characteristics

Patient characteristics are listed in Table 1. The patients who participated in this study had a median age of 65 years. Eight (29%) were over age 70. Thirteen patients (48%) had high-risk disease by the modified Rai classification, 14 (50%) had CLL cells with highZAP-70 expression, and 16 (57%) had CLL cells that used unmutated IGHV genes. Eleven patients (39%) had leukemia-cell cytogenetics abnormalities classified as unfavorable based on the detection of leukemia cell deletion of 11q or 17p, or trisomy 12. Three patients (11%) had leukemia cell deletions in 11q with one patient (4%) having CLL cells with deletions in 17p. Seven patients had trisomy 12 with four of these patients have additional abnormalities on metaphase cytogenetics including 14q abnormalities (N=2), deletion of 6q (N=1), and coinciding loss of 13q14 (N=1). Two male patients had leukemia cells with loss of the Y chromosome, which was not considered an adverse cytogenetics feature. None of the patients had leukemia cells with detectable translocations. All patients had an ECOG performance status of either 0 or 1 at study entry. There were no significant differences in pretreatment characteristics between groups one and two.

Therapy

Patients received 3 consecutive cycles of HDMP-rituximab every 4 weeks without dose reduction or delay. Only one patient received less than the planned amount of therapy due to transient psychosis likely related to HDMP. Five patients with evidence of MRD following HDMP-rituximab elected to participate in a consolidation protocol evaluating intravenous versus subcutaneous dosing of alemtuzumab for one or two 4-week cycles. One patient elected to receive such therapy at an outside institution. Five patients received alemtuzumab administered subcutaneously over two cycles (N = 4) or one cycle (N = 1) and one patient received twelve weeks of intravenous alemtuzumab administrations.

Toxicity

Toxicity consisted primarily of fatigue, hyperglycemia, sinusitis, and dyspepsia. Adverse events during treatment are listed in Table 3 and for one-year from treatment initiation in Table 4. Hyperglycemia was transient and observed only during the administration of methylprednisolone. No patients had sustained hyperglycemia, developed diabetes, or required more than sliding scale regular insulin on the days of HDMP infusion.

Fever and neutropenia were observed, but there were no episodes of sepsis. Sinusitis and upper respiratory tract infections were the most frequently observed infectious events during one-year of follow-up. Two grade III pneumonias were treated on an empiric basic with broad coverage of common bacterial pathogens, results of cultures were negative, and both patients recovered completely. We assessed for opportunistic infections during extended follow-up (until new therapy or death) and found that there were no opportunistic infections except for two episodes of herpes zoster that occurred at month 23 (individual also received alemtuzumab consolidation) and 44 following treatment-initiation with HDMP-rituximab. An episode of GI bleeding secondary to a duodenal ulcer was assessed as grade III in severity. This did not result in hemodynamic compromise and resolved completely without requiring blood transfusion. Hematologic toxicity was minimal and consisted primarily of neutropenia. For a total of 81 administered cycles there was only one red blood cell transfusion and no platelet transfusions. None of the patients developed Ritcher transformation, myelodysplasia, or acute myelogenous leukemia. There were no deaths during treatment or immediate follow-up period. One patient died 21 months following HDMP-rituximab during treatment for relapsed disease.

Hematologic and Immune Parameters

Amelioration of impaired marrow function was observed throughout the treatment period with improvement in thrombocytopenia or anemia (Figure 1). Hemoglobin and platelet counts improved significantly from pre-treatment values and lymphocyte counts decreased to normal levels in almost all cases (Figure 2).

Of the 28 treated patients, 14 had IgM levels below detectable levels (< 25 mg/dL) before and after therapy. Two patients with IgM levels of 112 mg/dL and 431 mg/dL prior to therapy had IgM levels below detectable levels (< 25 mg/dL) after therapy. The 12 patients who had detectable levels of IgM after therapy, had a median pre-treatment serum IgM level 54 mg/dL (± 101, standard deviation (S.D.)) and a post-treatment median serum IgM level of 40 mg/dL (±139, S.D.), which were not significantly different.

The patients' median post treatment serum IgG level of 351 mg/dL (±250, S.D.) was significantly less than the median pre-treatment serum IgG level of 628 mg/dL (± 417, S.D.) ($P < 0.05$, Student t test). Moreover, the patients' median post-treatment serum IgA level of 47 mg/dL (± 44, S.D.) was significantly less than the median pre-treatment serum IgG level of 32 mg/dL (± 26, S.D.) ($P<0.05$, Student t test). The IgG or IgA levels at one year post-treatment, however, were not significantly different from that observed prior to therapy. We conclude that HDMP-rituximab caused worsening IgG and IgA hypogammaglobulinemia, particularly in the immediate post-treatment period.

One of the enrolled patients was receiving IVIG at 24 gm every 4 weeks because of pre-treatment hypogammaglobulinemia associated with recurrent infections. This patient continued to receive IVIG during and after treatment with HDMP-rituximab. Two other patients with low IgG levels of 568 mg/dL and 590 mg/dL prior to therapy were initiated on treatment with IVIG after receiving two cycles of HDMP-rituximab when they developed grade III sinusitis or grade III bacterial pneumonia. These two patients continued to receive monthly IVIG after completion of HDMP-rixtuximab therapy.

On the other hand, CD4 T-cell lymphopenia was not observed following HDMP-rituximab. The median CD4 count for 22 patients evaluated two months following treatment was 848 cells/μl (+/−184, 95% confidence interval) with no patients experiencing a CD4 count less than 200 cells/μl.

Response to Therapy

ORR was 96% (N =27). Nine patients (32%) achieved a CR, 2 (8%) of which were MRD-negative (Table 2). In patients older than 70 years (N =8), the ORR was 100% with three patients (38%) achieving a CR, one of which was MRD negative. Of the patients with CLL cells harboring deletions at 11q (N = 3) or 17p (N = 1), all achieved at least a partial response to therapy. One patient who had CLL cells with del(11q) achieved a CR. There was no significant difference in CR rates for patients over age 70, those with high-risk modified Rai stage, or those with high-level leukemia-cell expression of ZAP-70 or CD38, use of unmutated IGHV genes, or adverse cytogenetics. Univariate logistic regression identified an elevated β2M (Odds ratio (OR) 0.15, p= 0.039) or splenomegaly palpable at least 5 cm below the costal margin (OR 0.08, p= 0.009) as being associated with failure to achieve a CR in response to therapy. There was a trend for patients who received higher dose rituximab compared to 375 mg/m^2 to be more likely to achieve a CR (OR 4.33, p= 0.089). Multivariable logistic regression identified only splenomegaly >5 cm as an independent adverse predictor for achieving a CR (OR 0.10, p= 0.031).

Five of the six patients (83%) who received consolidation with alemtuzumab following HDMP-rituximab achieved MRD-negative CRs. Three patients converted from a PR to MRD-negative CR and 2 CRs became MRD-negative. The final CR rate was 43% (N =12), with 25% (N =7) achieving an MRD-negative CR when we factor in the responses of the six patients who received alemtuzumab consolidation.

Pharmacokinetics

Despite different dosing schedules of rituximab during cycle 1, both Group 2A and Group 2B achieved similar peak and trough levels by cycle 2. (Figure 5) Four of six patients who achieved a serum rituximab level greater than 1 mg/ml experienced a CR whereas only two of five of those with lower levels achieved a CR. However, there was no significant association between achieving a CR and PK parameters including average pre-infusion level, maximum rituximab level, or attaining a level of 1 mg/ml or greater.

Follow-up

With a median follow-up three years (36.3 months) the OS is 96%. Forty-six percent (N =13) of patients have progressed and 39% (N =11) of patients have required additional therapy, median PFS and TFS of 30.5 and 33.3 months, respectively. Patients who achieved a CR (MRD-positive or MRD-negative) in response to HDMP-Rituximab enjoyed a median PFS of 40.3 months with a median TFS that has not been reached. Those who did not achieve a CR experienced a median PFS and TFS of 23.9 and 31.5 months, respectively.

Achieving an MRD-negative CR (N = 7), with or without alemtuzumab consolidation, was associated with prolonged PFS and TFS with a median that has not been reached (log-rank p-values are 0.025 and 0.015). No patient who achieved an MRD-negative CR has required additional therapy (median follow-up: 40.4 months). (Figure 2 and Figure 3 those who did not receive consolidation with alemtuzumab)

DISCUSSION

Patients treated with HDMP-rituximab achieved a high ORR (96%), CR (32%), PFS (54%), TFS (61%), and OS (96%) during a median follow-up period of 3 years. Treatment with HDMP-rituximab was well tolerated, with a safety profile that compares favorably with that of combination chemoimmunotherapy and approved single agents for the initial treatment of patients with CLL. Importantly, HDMP-rituximab was associated with a lower risk of myelosuppression, a factor that is associated with significant morbidity and mortality in patients with CLL, particularly the elderly.

All patients who received 3 cycles of HDMP-rituximab achieved a PR or CR by NCI-WG criteria. Nine patients (32%) achieved a CR, two (22%) of which had no evidence of MRD after treatment. Single agents approved for the initial treatment of CLL, namely alemtuzumab, chlorambucil, or bendamustine, have yielded ORR and CR rates of 83% and 24%, 26–55% and less than 1–4%, or 59% and 8%, respectively.(1, 32, 33) Combination chemoimmunotherapy administered over six cycles, such as FR or PCR, are associated with ORR of 90% and CR rates of approximately 40%.(6, 7) Higher CR rates have been reported with FCR and FCM, however such responses were noted in younger patients who had a median age of approximately 57.(8, 9)

The median age of CLL patients at diagnosis is approximately 70.(34, 35) However, patients treated in most clinical studies have a median age that is less than 60. Age-related co-morbidities and decreased myeloid reserve complicate the tolerance of therapy and often preclude patients over age 65 from completing standard chemoimmunotherapy protocols. (36) The median age of the patients treated in this study was 65, and 8 patients (29%) were older than 70. Because treatment with HDMP-rituximab was associated with less myelosuppression than treatment with standard chemoimmunotherapy regimens, it could be administered without dose reductions or delays. This regimen was both well tolerated and effective even in patients over age 70.

Patients with leukemia cells that have high-level expression of ZAP-70 or CD38, use unmutated IGHV, or have adverse cytogenetic features, and patients who have bulky

lymphadenopathy, achieved CRs in response to HDMP-rituximab at rates that appeared similar to those who did not have such disease characteristics. On the other hand, univariate analysis revealed that patients who had an elevated β2M (N =20) or splenomegaly palpable at least 5 cm below the costal margin (N =17) had a lower rate of achieving CR, with CR occurring in only 20% and 12% of such patients, respectively. In contrast, CRs were achieved in 63% of those with a normal β2M (N =8) and in 64% of patients with smaller spleens (N =11). Interestingly, similar ORR rates were seen with HDMP-rituximab in fludarabine refractory patients as in the frontline setting.(23) Other regimens such as FCR are associated with much lower response rates in refractory patients when compared with a treatment naive population.(3, 9) The similar response rates in these different treatment settings might be attributable to HDMP being an effective agent in patients with p53 abnormalities.(13)

Only 19% (N =3) of patients in group one who received the lowest amounts of rituximab achieved a CR. This CR rate was lower than that noted in our previous study evaluating this regimen in a salvage setting (36%).(22) Possibly this was a consequence of the reduction in the duration of treatment with methylprednisolone for each cycle from 5 days to 3. However, group 2 patients who received higher amounts of rituximab had a collective CR rate of 50% (N =6), including two patients who achieved an MRD-negative CR. Univariate analysis suggested a trend for an increased CR rate in patients who received rituximab at 750 mg/m^2 compared with those who received 375 mg/m^2. However, comparison of rituximab dose and pharmacokinetics with this small sample size is limited. At this point given the cost of higher doses of rituximab we would not yet advocate using a dose of rituximab greater than 375mg/m^2. Further evaluation of HDMP with rituximab at 750mg/m^2 in an ongoing study will provide sufficient power to detect a clinically relevant difference in CR rate between standard and higher dose rituximab.

Patients who achieved an MRD-negative CR had a prolonged PFS when compared with patients who had persistent MRD after treatment, an association that has been previously reported.(36–39) In fact, none of the patients who achieved MRD-negative CR have required subsequent therapy over a median follow-up period of 40.4 months. Six patients received alemtuzumab consolidation and 83% (N =5) experienced eradication of MRD. Initial therapy of CLL with alemtuzumab resulted in 7% achieving a MRD-negative CR.(32) This suggests that alemtuzumab might be more effective following initial “debulking” with a regimen such as HDMP-rituximab. Of note, fludarabine containing regimens used for the treatment of CLL have been associated with CD4 T-lymphopenia, a characteristic associated with increased infectious complications in patients who receive alemtuzumab consolidation. (40) However, the median CD4 T-lymphocyte count two months following HDMP-rituximab was 848 cells/μl and all treated patients maintained CD4 count greater than 200 cells/μl. In any case, it should be noted that use of alemtuzumab for consolidation can be associated with significant toxicity, is not part of standard of care treatment for CLL, and should not be administered outside the context of clinical protocols or without the guidance of clinicians who are experienced in this modality of therapy.(37–39, 41, 42)

Other studies evaluating use of HDMP in the relapsed or refractory setting have reported more infectious toxicity, including early deaths, which were not observed in the current

study evaluation.(13, 23, 43, 44) These studies used five days of methylprednisolone at 1 gm/m^2. The reduction of steroid dose by 40% from 5 to 3 consecutive days of each cycle was instituted to reduce steroid attributable toxicity and improve the tolerability of the regimen. Additional differences that might be associated with reduced infectious toxicity in the present study include treatment in the frontline setting, good performance status of the patients, use of prophylactic antimicrobials throughout the treatment period and for 2 months following therapy, and the administration of IVIG to those patients with hypogammaglobulinemia and infections.

In summary, twelve weeks of therapy with HDMP-rituximab has encouraging clinical activity that compares favorably with that of currently available treatment regimens for CLL. Hematologic toxicity was minimal with the majority (80%) of all adverse events limited to grade I or II in severity. Treatment with HDMP-rituximab was well tolerated, notably in elderly patients who are frequently less tolerant to combination chemoimmunotherapy. This regimen is of particular interest for the treatment of patients who are older, have poor myeloid reserve, pretreatment cytopenias, bulky disease, or adverse cytogenetics.

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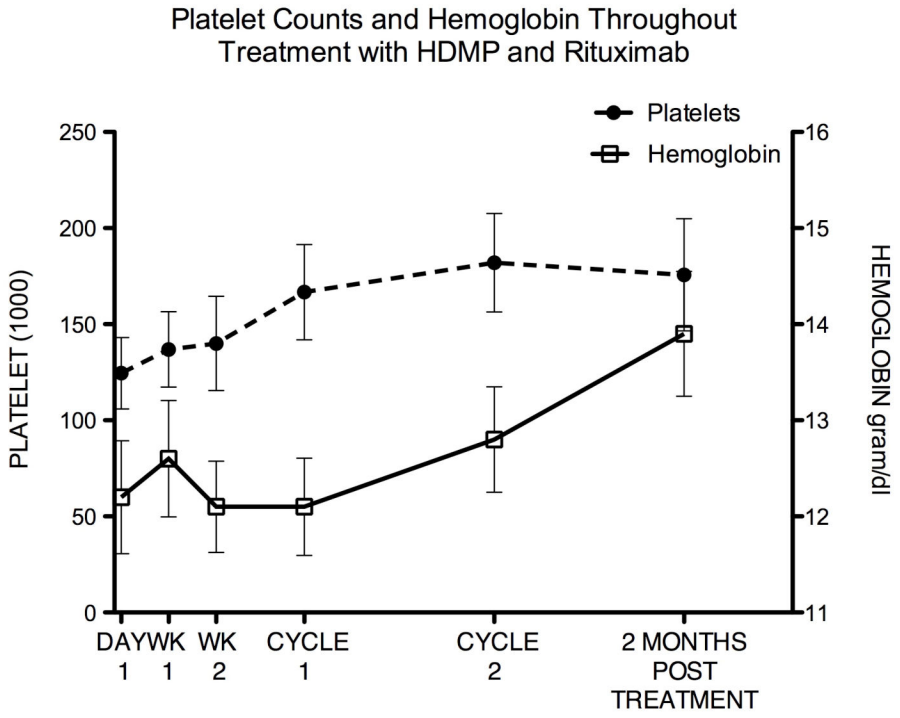

Figure 1. Platelet counts and hemoglobin throughout treatment with high-dose methylprednisolone (HDMP) and rituximab

Mean platelet counts (solid line) and hemoglobin levels (dashed line) of 28 patients from pre-treatment values on day one assessed throughout therapy until two month following treatment completion. Error bars represent standard error. Abbreviations: Wk, week; gm, gram; dl, deciliter; ml, microliter.

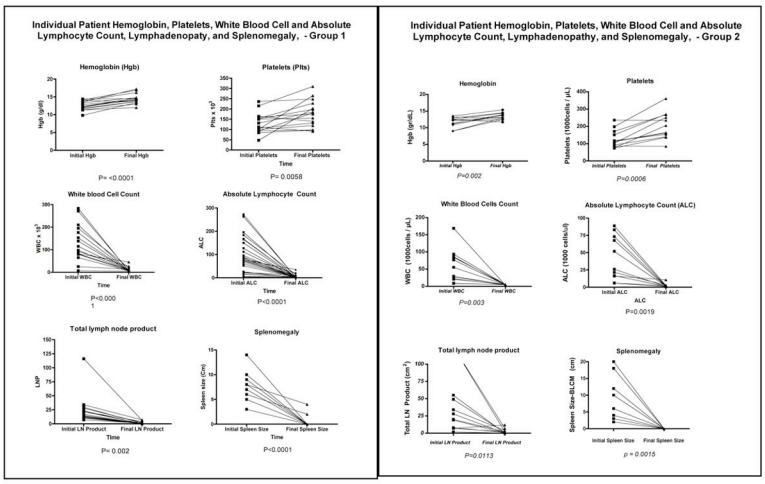

Figure 2. Individual patient hemoglobin, platelet count, white blood cell and absolute lymphocyte count, total lymph node product, and spleen size prior to and following treatment with High-dose Methylprednisolone (HDMP) and Rituximab

Clinical disease characteristic prior to and following treatment with HDMP and Rituximab for individual patients in Group A (Left Panel) and Group B (right panel). Baseline and Final Hemoglobin (Hgb), platelets (plts), total white blood cell (WBC) count, absolute lymphocyte count (ALC), Bidimensional lymph node (LN) product, and splenomegaly measured centimeter (cm) below left costal margin (LCM).

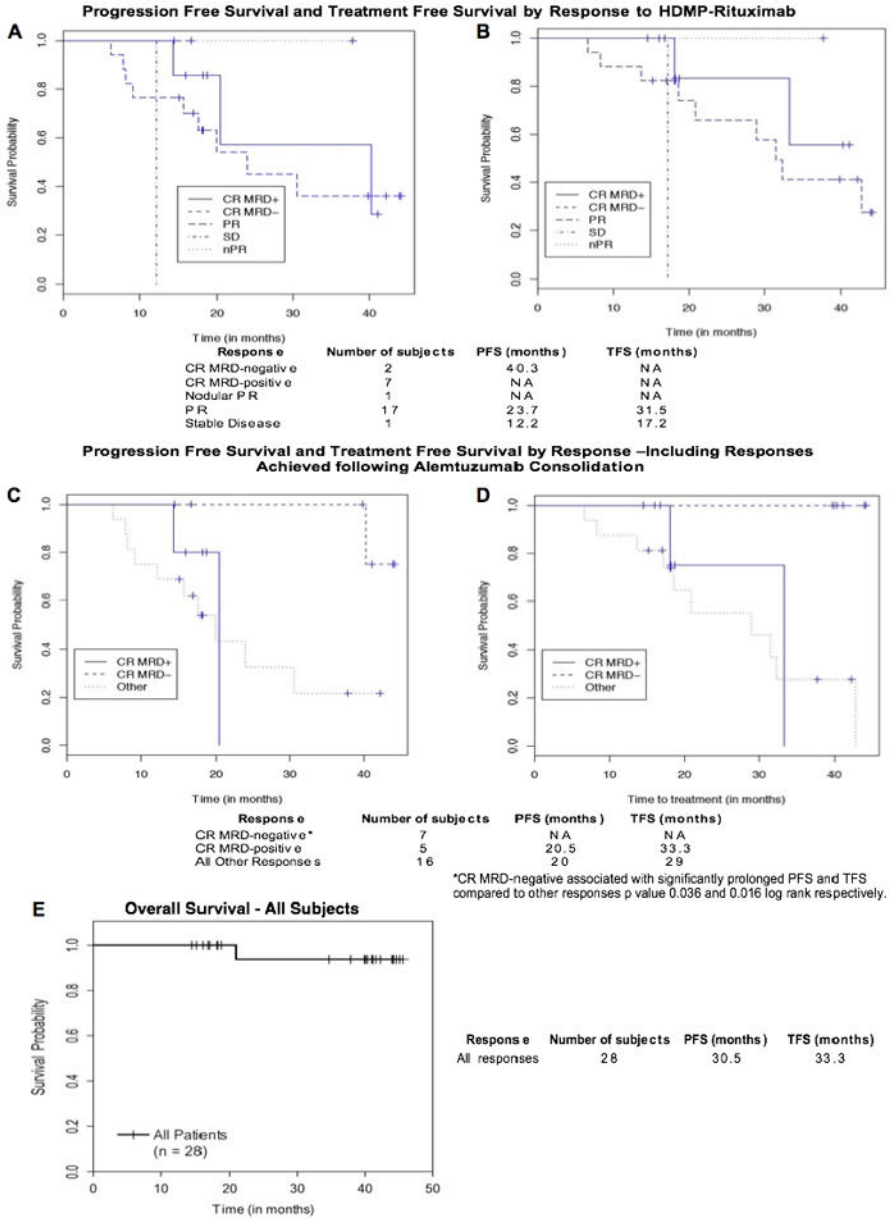

Figure 3. Progression free survival (PFS), treatment free survival (TFS) and overall survival for patients treated with High-Dose Methylprednisolone (HDMP) and Rituximab

Kaplan-Meier curves for (A) PFS and (B) TFS by response to HDMP and Rituximab. (C) PFS and (D) TFS including responses following consolidation with alemtuzumab. (E) Overall survival over median 36.3 months follow-up. Abbreviations: Complete Response (CR) with or without (+ / −) Minimal Residual Disease (MRD), Partial Response (PR), Stable Disease (SD), nodular PR (nPR), NA signifies median time to PFS or TFS has not been reached.

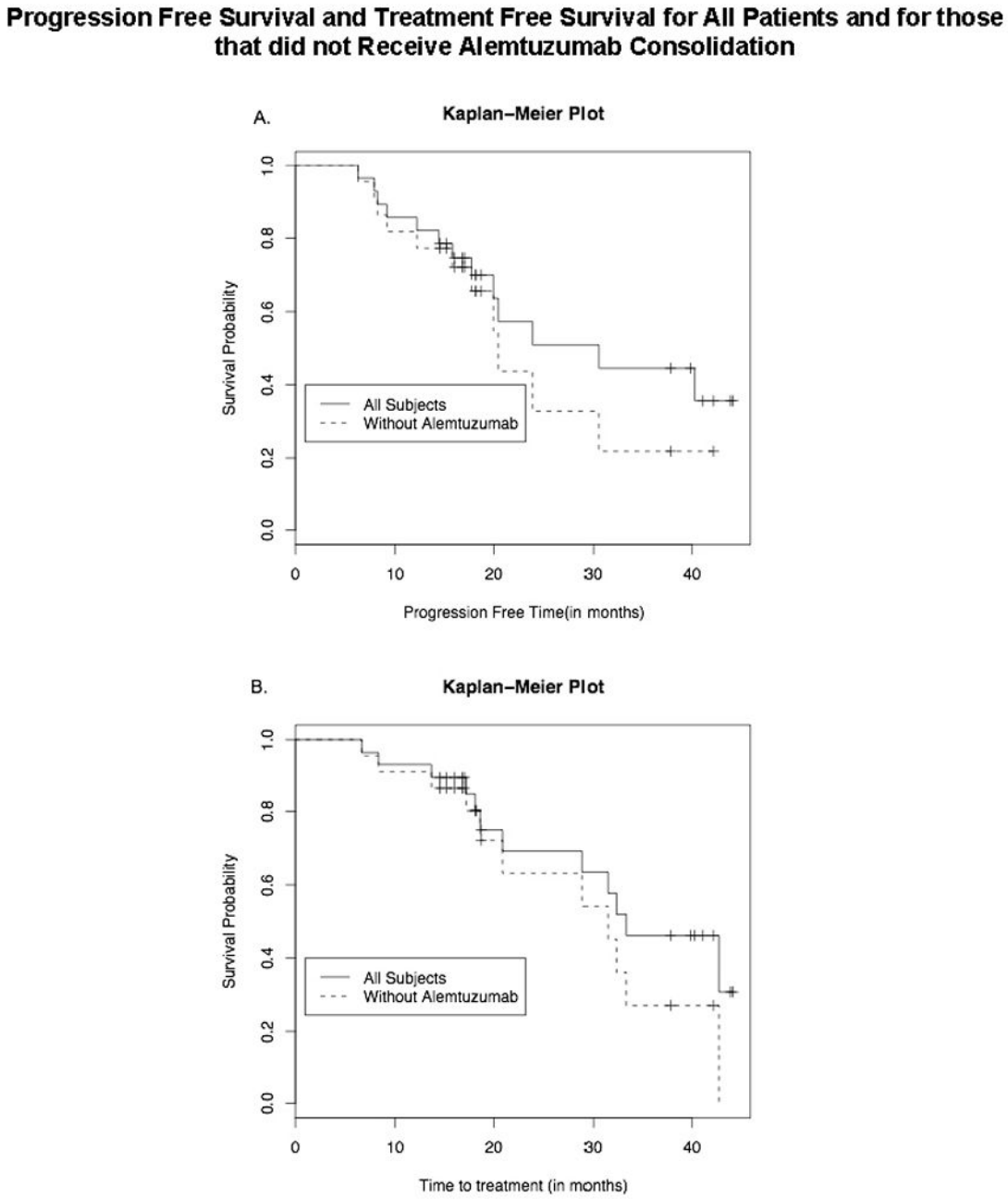

Figure 4 . Progression free survival (PFS) and treatment free survival (TFS) for all patients and excluding those patients who received aleumtuzumab as consolidation

Kaplan-Meier Curves demonstrate Median PFS (Top) and TFS (Bottom) projected at 30.5 and 33.3 months for 28 patients, excluding the six patients who received alemtuzumab consolidation PFS and TTNT is 20.5 and 31.5 months respectively.

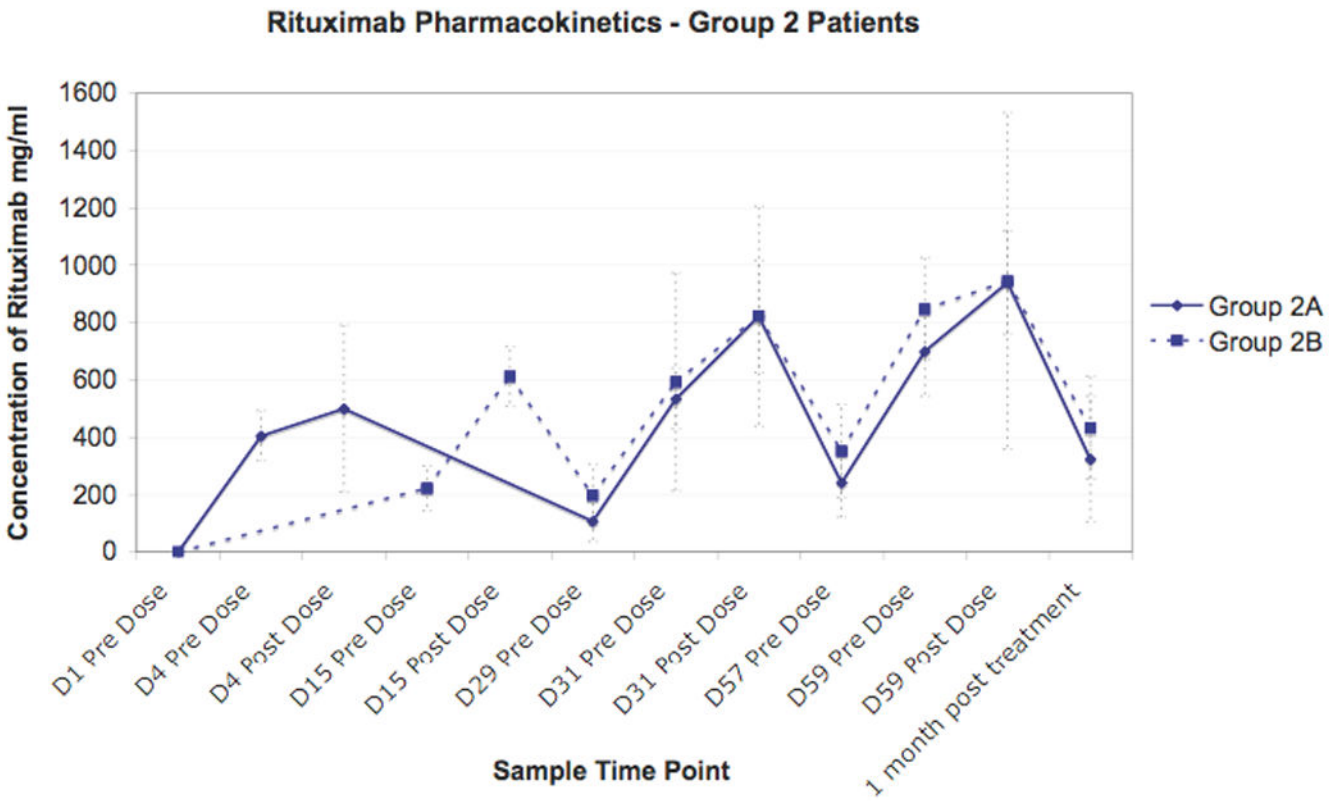

Figure 5. Pharamcokinetics of rituximab for group 2 patients

Mean rituximab concentration for six group 2A patients (solid line) treated with rituximab 750 mg/m^2 administered as consecutive daily dosing during cycle 1 and five group 2B patients (dashed line) treated with weekly rituximab- error bars represent standard deviation. Abbreviations: mu, microgram; ml, milliliter.

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Table 1

Baseline patient characteristics

Characteristics	All patients (Groups 1 & 2 Combined)		Groups 1		Groups 2	
	Number of patients	% of total patients	Number of patients	% of patients	Number of patients	% of patients
	28	100%	16	57%	12	43%
Rai stage						
0 to II	15	54%	11	69%	4	33%
III to IV	13	46%	5	31%	8	67%
Age						
< 55 years	2	7%	1	6%	1	8%
55–69 years	18	64%	11	69%	7	58%
70 years or older	8	29%	4	25%	4	33%
Gender						
Male	18	64%	11	69%	7	58%
Female	10	36%	5	31%	5	42%
Spleen						
0–4 cm below LCM	11	39%	5	31%	6	50%
5 cm below LCM	17	61%	11	69%	6	50%
Beta2-microglobulin						
< = 2.4 mg/L	8	29%	4	25%	4	33%
> 2.4 mg/L	20	71%	12	75%	8	67%
Marrow cellularity, biopsy						
< 90%	16	57%	10	63%	6	50%
>=90%	12	43%	6	38%	6	50%
ZAP-70 elevated[1]	14	50%	6	38%	8	67%
ZAP-70 normal	14	50%	10	63%	4	33%
Unmutated IGHV[2]	15	54%	10	63%	5	42%
Mutated IGHV	13	46%	6	38%	7	58%
CD38% High[3]	9	32%	5	31%	4	33%

Characteristics	All patients (Groups 1 & 2 Combined)		Groups 1		Groups 2	
	Number of patients	% of total patients	Number of patients	% of patients	Number of patients	% of patients
CD38% normal	19	68%	11	69%	8	67%
Cytogenetics Unfavorable[4]	11	39%	5	28%	6	50%
Cytogenetics Favorable[5]	17	61%	11	65%	6	50%
FISH deletion of 11q or 17p	4	14%	2	13%	2	17%

[1] Elevated ZAP-70 expression is defined as >20%(1),

[2] Unmutated is >98% homology to germline,

[3] CD38 elevated is >34%(1),

[4] Cytogenetics Unfavorablerefers to metaphase cytogenetics or FISH revealing loss of 17p, 11q, trisomy 12, or multiple cytogenetic abnormalities,

[5] Cytogenetics favorable refers to metaphase cytogenetics andFISH that were normal diploid or sole 13q14-.

* There were no significant differences in these baseline categorical variables between group 1 and group 2 using Fisher's exact test and a p >0.05.

Abbreviations: LCM, Left Costal Margin; FISH, fluorescent in situ hybridization.

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Table 2

Response to treatment according to baseline characteristics

	All patients (Groups 1 & 2 Combined)						
Characteristic	**Number of patients**	**ORR**	**CR**	**%CR MRD -**	**PR**	**nPR**	**SD**
All patients	28	96%	32%	22%	61%	4%	4%
Group 1 (Rituximab 375 mg/m^2)	16	94%	19%	0%	69%	6%	6%
Group 2 (Rituximab 750 mg/m^2)	12	100%	50%	33%	50%	0%	0%
Rai stage							
0 to II	15	93%	27%	11%	60%	7%	7%
III to IV	13	100%	38%	11%	62%	0%	0%
Age							
< 55 years	2	100%	0%	0%	100%	0%	0%
55–69 years	18	94%	33%	11%	56%	6%	6%
70 years or older	8	100%	38%	11%	63%	0%	0%
Gender							
Male	18	94%	39%	22%	56%	0%	6%
Female	10	100%	20%	0%	70%	10%	0%
Spleen							
0–4 cm below LCM	11	100%	64%	22%	36%	0%	0%
5 cm below LCM*	17	94%	12%	0%	76%	6%	6%
Beta2-microglobulin							
< = 2.4 mg/L	8	100%	63%	11%	38%	0%	0%
> 2.4 mg/L*	20	95%	20%	11%	80%	5%	5%
Marrow cellularity, biopsy							
< 90%	16	94%	38%	11%	56%	0%	6%
>=90%	12	100%	25%	11%	67%	8%	0%
Bulky Lymphadenopathy[1]	5	100%	20%	0%	80%	0%	0%
ZAP-70 elevated[2]	14	93%	43%	11%	50%	0%	7%
ZAP-70 normal	14	100%	21%	11%	71%	7%	

	All patients (Groups 1 & 2 Combined)						
Characteristic	**Number of patients**	**ORR**	**CR**	**%CR MRD -**	**PR**	**nPR**	**SD**
Unmutated IGHV[3]	15	93%	33%	0%	60%	0%	7%
Mutated IGHV	13	100%	31%	22%	62%	8%	0%
CD38% High[4]	9	89%	33%	11%	56%	0%	11%
CD38% normal	19	100%	32%	11%	63%	5%	0%
Cytogenetics Unfavorable[5]	11	91%	36%	25%	55%	0%	9%
Cytogenetcs Favorable[6]	17	100%	29%	20%	65%	6%	0%
FISH -deletion of 11q or 17p	4	100%	25%	0%	75%	0%	0%

[1] Bulky lymphadenopathy is defined as a palpable lymph node >5cm,

[2] Elevated ZAP-70 expression is >20%[1]

[3] Unmutated is >98% homology to germline.

[4] CD38 elevated is >34%

[5] Cytogenetics Unfavorable refers to metaphase cytogenetics or FISH revealing loss of 17p, 11q, trisomy 12, or multiple cytogenetic abnormalities,

[6] Cytogenetics favorable refers to metaphase cytogenetics and FISH that were normal diploid or sole 13q14-

* Prognostic factors that had significance for predicting a complete response in univariate analysis $p<0.05$ denoted with *.

Abbreviations: ORR, Overall Response Rate; CR, Complete Response; MRD -, No evidence of Minimal Residual Disease; PR, Partial Response; nPR, nodular Partial Response; SD, Stable Disease; LCM, Left Costal Margin.

Table 3

Adverse events during treatment with HDMP and rituximab

ADVERSE EVENT	Grade I-II	Grade III-IV	Total patients (%)
Fatigue	6		6/28 (21.4%)
Hyperglycemia	4	2	6/28 (21.4%)
Sinusitis	3	1	4/28 (14.2%)
Dyspepsia	4		4/28 (14.2%)
Fever	3		3/28 (10.7%)
Anxiety	3		3/28 (10.7%)
Insomnia	3		3/28 (10.7%)
Neutropenia	2	1	3/28 (10.7%)
Dyspnea	2		2/28 (7.1%)
Paresthesisas	2		2/28 (7.1%)
Pneumonia		2	2/28 (7.1%)
Edema	2		2/28 (7.1%)
Anemia	1	1	2/28 (7.1%)
Upper Respiratory Infection	1		1/28 (3.5%)
Neutropenic Fever		1	1/28 (3.5%)
Thrombocytopenia	1		1/28 (3.5%)
Anorexia	1		1/28 (3.5%)
Migraine	1		1/28 (3.5%)
Epistaxis	1		1/28 (3.5%)
Memory Impairment	1		1/28 (3.5%)
Psycosis		1	1/28 (3.5%)
Hyperlipidemia	1		1/28 (3.5%)
Transaminase Elevation	1		1/28 (3.5%)
Drug Infusion reaction	1		1/28 (3.5%)
Cellulitis	1		1/28 (3.5%)
Upper GI bleeding		1	1/28 (3.5%)
Diarrhea		1	1/28 (3.5%)
Arthralgias/Bone pain	1		1/28 (3.5%)
Deep Venous Thrombosis	1		1/28 (3.5%)
Superficial Thrombophlebitis	1		1/28 (3.5%)
Dysphonia	1		1/28 (3.5%)
Agitation	1		1/28 (3.5%)

Table 4

Additional adverse events – 9 months following completion of HDMP and rituximab

ADVERSE EVENT	Grade I-II	Grade III-IV	Total patients (%)
Upper Respiratory Infection	8		8/28(28.5%)
Sinusitis	4		4/28 (14.2%)
Cough	4		4/28 (14.2%)
Fatigue	3		3/28 (10.7%)
Constipation	2		2/28 (7.1%)
Myalgias	2		2/28 (7.1%)
CMV Reactivation	2		2/28 (7.1%)
Autoimmune thrombocytopenia		1	1/28 (3.5%)
Hyperglycemia	1		1/28 (3.5%)
Depression	1		1/28 (3.5%)
Anxiety	1		1/28 (3.5%)
Diarrhea	1		1/28 (3.5%)
Abdominal pain	1		1/28 (3.5%)
Lymphadenitis	1		1/28 (3.5%)
Coronary Heart Disease	1		1/28 (3.5%)
Rhinitis	1		1/28 (3.5%)
Arthralgias	1		1/28 (3.5%)
Osteoporosis	1		1/28 (3.5%)
Atrial Fibrilation	1		1/28 (3.5%)
Peripheric arterial insufficiency	1		1/28 (3.5%)
Paresthesias	1		1/28 (3.5%)
Herpes Zoster	1		1/28 (3.5%)
Genital Herpes	1		1/28 (3.5%)
Weight loss	1		1/28 (3.5%)
Urinary Track Infection	1		1/28 (3.5%)
Hypertrygliceridemia	1		1/28 (3.5%)
Dyspnea/wheezing	1		1/28 (3.5%)

Cytomegalovirus (CMV) was viremia in 33% of alemtuzumab consolidated patients there were no cases of CMV disease.