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Long term outcomes to Fludarabine and Rituximab in Waldenström macroglobulinemia.

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Abstract

We report the long term outcome of a multicenter, prospective study examining fludarabine and rituximab in Waldenström macroglobulinemia (WM). WM patients with < 2 prior therapies were eligible. Intended therapy consisted of 6 cycles (25 mg/m²/day for 5 days) of fludarabine and 8 infusions (375 mg/m²/week) of rituximab. 43 patients were enrolled. Responses were: CR (n=2); VGPR (n=14); PR (n=21); MR (n=4); for an overall and major response rate of 95.3% and 86.0%, respectively. At best response, median bone marrow disease involvement declined from 55% to 5% (p<0.00001); serum IgM decreased from 3,840 to 443 mg/dL (p<0.00001); and hematocrit rose from 31.2% to 38.0% (p<0.0008). Median time to progression for all patients was 51.2 months, and was longer for untreated patients (p=0.017), and those achieving >VGPR (p=0.049). Grade > 3 toxicities included neutropenia (n=27); thrombocytopenia (n=7); pneumonia (n=6), including two patients who succumbed to non-PCP interstitial pneumonia. With a median follow-up of 40.3 months, we observed 3 cases of transformation to aggressive lymphoma and 3 cases of MDS/AML. The results of this study demonstrate that fludarabine and rituximab is highly active in WM, though short and long term toxicities need to be carefully weighed against other available treatment options. This study is registered with ClinicalTrials.gov under identifier NCT00020800.

Introduction

Waldenström macroglobulinemia (WM) is a distinct B-cell lymphoproliferative disorder characterized primarily by bone marrow infiltration with lymphoplasmacytic cells, along with demonstration of an IgM monoclonal gammopathy¹⁻³. Among treatment options for patients with WM, nucleoside analogues, as well as the CD20 directed monoclonal antibody rituximab have been commonly used. Response rates of 30-70% and durations of response of 20-24 months have been reported with the use of nucleoside analogues in WM patients⁴⁻¹³. Importantly, similar response rates were reported in these studies whether nucleoside analogues were employed as first line or salvage therapy. The use of rituximab has also been extensively evaluated in patients with WM. Using standard dose (i.e. 4 weekly infusions at 375 mg/m²), overall response rates of 25-30% have been observed. More recently, an extended dose regimen giving rituximab at 375 mg/m²/week for 4 weeks, then repeated again at week 12 has resulted in higher (40-50%) overall response rates^{4,14-19}.

In preclinical studies, the potential for rituximab and fludarabine to enhance each other's activity has been demonstrated, and may involve a spectrum of intracellular as well as extracellular mechanisms²⁰⁻²². Moreover, in other indolent B-cell malignancies, the combination of rituximab and fludarabine has led to higher response rates than those observed with either agent alone²³⁻²⁷. The potential for enhanced clinical benefit by giving rituximab with fludarabine concurrently versus sequentially has also been

reported in patients with CLL²⁶. In view of these considerations, we initiated a multicenter clinical trial of fludarabine and rituximab in patients with WM, which enrolled 43 subjects from March 7, 2001 to May 2, 2003. The outcome and long term follow-up of this study are presented in this report.

Patients and Methods

Patients with a clinicopathological diagnosis of WM requiring therapy who were naïve to fludarabine and rituximab, and who had 2 or less prior therapies, along with CD20 positive disease as determined by previous bone marrow immunohistochemistry or flow cytometry were eligible for this study. To meet eligibility patients had to demonstrate a monoclonal IgM protein, a minimum IgM level > 2 times the upper limit of normal, a baseline platelet count of $> 25,000/\mu\text{L}$, an absolute neutrophil count of $> 500/\mu\text{L}$, a serum creatinine of < 2.5 mg/dL (unless nephropathy was attributable to their WM), a serum total bilirubin and SGOT of < 2.5 times the upper limit of normal, and an ECOG performance status of 0-2. No chemotherapy, steroid therapy, or radiation therapy within 30 days of study entry was permitted. Patients who were pregnant or lactating, had serious co-morbid disease, had any uncontrolled bacterial, fungal or viral infection, or an active second malignancy were not eligible. All men and women of reproductive potential were required to agree to use an acceptable method of birth control before, during treatment, and for six months after completion of study treatment.

All patients provided informed written consent, in accordance with the Declaration of Helsinki, and the institutional review board approved the protocol at each participating site. A Data and Safety Monitoring Committee (DSMC) at the Dana Farber Cancer Institute oversaw adverse events from all participating centers connected to this study. A baseline evaluation was obtained for enrollment within 30 days prior to initiation of therapy and consisted of a medical history and physical exam, laboratory studies

consisting of a complete blood count and differential, chemistries, serum IgM levels, bone marrow biopsy and aspiration, and CT scans of the chest, abdomen and pelvis. Intended therapy consisted of 8 infusions of rituximab ($375 \text{ mg/m}^2/\text{week}$) administered at weeks 1-4, 17, 18, and 30, 31, along with 6 cycles of fludarabine ($25 \text{ mg/m}^2/\text{daily}$) given for 5 days at weeks 5,9,13, 19, 23, 27. Patients were assessed at week 12, and were eligible for continuation of therapy if they did not have progressive disease. Dose reduction of rituximab was not permitted; however, patients who demonstrated life threatening adverse events to rituximab infusion were allowed to have their rituximab discontinued and continued on fludarabine therapy. The use of diphenhydramine (50 mg intravenously), acetaminophen (1,000 mg orally), and at the treating physicians discretion corticosteroids (hydrocortisone 100 mg intravenously) was permitted for rituximab infusion prophylaxis. Dose modification for fludarabine was permitted on the basis of hematological toxicity. No dose modification for hematological grade 0-2 toxicity was required. For patients demonstrating grade 3 hematological toxicity, fludarabine was withheld until patient's platelet count, hemoglobin, and absolute neutrophil count (ANC) recovered to pre-treatment baseline values and then resumed omitting day 5 of fludarabine administration. For patients demonstrating grade 4 hematological toxicity, fludarabine was withheld until patient's platelet count, hemoglobin, and ANC recovered to pre-treatment baseline values and then resumed omitting days 4 and 5 of fludarabine administration. Granulocyte colony stimulating factor (G-CSF), erythropoietin, and transfusions of packed red blood cells or platelets were permitted to support patient's counts during therapy. Patients with an estimated creatinine clearance of $<50 \text{ ml/min}$ received fludarabine at a 50% dose reduction (i.e. $12.5 \text{ mg/m}^2/\text{day}$ for 5 days). The use of

herpes zoster prophylaxis was mandated for all patients after the enrollment of the first 21 patients, after the DSMC identified an increased risk of herpes zoster on this study. The DSMC strongly recommended the prophylactic use of plasmapheresis for patients demonstrating a serum viscosity level of >3.5 cp prior to the administration of rituximab after the enrollment of the first 15 patients after one patient suffered a hyperviscosity induced intracerebral bleed from a rituximab induced IgM spike^{28,29}.

Patients underwent re-assessment of their disease at weeks 12, 24, and 52, and thereafter every 12 weeks until progression of disease. Restaging visits consisted of a physical examination, complete blood count and differential, chemistries, serum IgM levels, a bone marrow biopsy and aspiration (to confirm complete remission), and CT scans of the chest, abdomen and pelvis (if extramedullary disease was present at baseline). Response determinations were made using modified consensus panel criteria from the Third International Workshop on WM³⁰, and response rates determined on intent to treat basis. A complete response was defined as having resolution of all symptoms, normalization of serum IgM levels with complete disappearance of IgM paraprotein by immunofixation, no evidence of disease by bone marrow examination, and resolution of any adenopathy or splenomegaly. Patients achieving a very good partial response (VGPR), partial response (PR), and a minor response (MR) were defined as achieving a $> 90\%$, $50-90\%$, and $25-50\%$ reduction in serum IgM levels, respectively. Patients with stable disease were defined as having $< 25\%$ change in serum IgM levels, in the absence of new or increasing adenopathy or splenomegaly and/or other progressive signs or symptoms of WM. Progressive disease was defined as occurring when a greater than 25% increase in serum

IgM level occurred from the lowest attained response value or progression of clinically significant disease related symptom(s). Time to disease progression (TTP) was calculated from the start of therapy using the Kaplan Meier method. The primary endpoints of this study were determination of overall response, median progression free survival, and toxicity. A 48 month landmark analysis was also performed comparing patients with untreated versus previously treated disease and for those patients achieving > VGPR versus < VGPR.

Statistical analysis

Comparison of pre- and post-treatment parameters was performed using a two-tailed students t-test on Microsoft Excel™ software. Non-parametric testing of pre- and post-treatment variables was performed by Fisher's Exact t-test (Vassar Stats). A p-value < 0.05 was deemed to be significant for the above studies.

Results

Patients and disease characteristics

Forty-three patients were enrolled in this study. The baseline characteristics for these patients are outlined in Table 1. The median age for all enrolled patients was 61 (range 52-75) years old. The median number of treatments was 0 (range 0-2 prior therapies). Twenty-seven (63%) patients had no prior therapy. Of the 16 previously treated patients, 14 and 2 patients were relapsed or refractory from their previous therapy, which included chlorambucil alone or with steroids (n=12), rituximab (n=2), vincristine, doxorubicin, dexamethasone (n=1), and autologous transplant (n=1). Median pre-therapy BM involvement with lymphoplasmacytic cells was 55% (range 5-100%), and median serum IgM level was 3,840 mg/dL (range 655 mg/dL to 10,500 mg/dL). Twenty-seven (63%) patients had an IgM level of >3,000 mg/dL. The median pre-therapy hematocrit and platelet count for all enrolled study patients was 31.2% (range 23.2-44.7%), and 252,000 (range 55,000-597,000/mm³), respectively. Fifteen (35%) and 4 (9.3%) of the patients had a hematocrit <30% and a platelet count of <100,000/mm³, respectively. Five patients (12%) had adenopathy and/or splenomegaly.

Of the 43 patients enrolled on study, 41 were eligible for and received therapy beyond week 12. One patient was removed from study at week 4 after experiencing an intracerebral bleed resulting from hyperviscosity induced by rituximab, while another

patient was removed for progressive disease after restaging at week 12. Thirty-five and 33 patients completed the intended 8 weekly infusions of rituximab and 6 cycles of fludarabine. The median number of cycles of fludarabine and infusions of rituximab administered was 6 (range 1-8) and 8 (range 4-8), respectively, and was the same for untreated and previously treated patients.

Response

The individual changes in serum IgM levels at best response for all patients are shown in Figure 1. Median IgM levels for all 43 patients declined from 3,840 mg/dL (range 655 to 10,400 mg/dL) pre-therapy to 443 mg/dL (range 43 to 7,040 mg/dL) at best response ($p < 0.00001$). Pre-therapy, 27/43 (62.8%) patients demonstrated an IgM level $> 3,000$ mg/dL; following treatment, only 3 of 43 (6.9%) had an IgM level $> 3,000$ mg/dL ($p < 0.00001$). Bone marrow involvement also decreased following therapy, with a decline in the median percentage of tumor cell involvement from 55% (range 5-100%) to 5% (range 0-95% ($p < 0.00001$)). Categorical responses were as follows: CR (n=2); VGPR (n=14); PR (n=21); MR (n=4); for an overall and major response rate of 95.3% and 86.0%. The overall (96.3% versus 93.8%; $p = 1.0$) and major (88.9% versus 81.3%; $p = 0.65$) response rates were similar for untreated and previously treated patients, respectively. Among responders, the median time to at least a 25% reduction in serum IgM was 3.9 (range 0.6-34.1) months, while the median time to best response for responding patients was 19.2 (range 4.2-61) months.

Time to progression

The median time to progression for all patients was 51.2 months (Figure 2), which was longer for untreated (77.6 months) versus previously treated (38.4 months) patients ($p=0.049$). With a median follow-up of 40.3 (range 1-88.3+) months, 31 patients are alive and 21 patients remain free of disease progression. Among responding patients (Figure 3), TTP was longer for patients achieving $>VGPR$ (>88.3 months) versus $<VGPR$ (36.9 months); $p=0.017$. For the 2 patients achieving a CR, the TTP as of 12/31/2007 was 60.9+ and 88.3+ months. A Forty-eight month landmark analysis was also performed for progression free survival for those patients who were untreated versus previously treated, and for those patients achieving $> VGPR$ versus $< VGPR$. Eighteen of 27 (67%) untreated and 6/16 (38%) previously treated patients were free of disease progression at 48 months ($p=0.028$). In as well, 12/15 patients (80%) achieving $> VGPR$ versus 12/28 (42.9%) achieving $<VGPR$ remained free of disease progression at 48 months ($p=0.021$).

Changes in hematological parameters in treated WM patients.

A significant increase in the median hematocrit was noted for all patients from 31.2% (range 23.2-44.7%) before therapy to 38.0% (range 24.9-45.9%) following treatment ($p<0.0008$), with 30 of the 43 (70%) patients demonstrating a hematocrit rise of $>2\%$. Conversely, the median platelet count decreased following treatment from 252,000/mm³ (range 55,000-597,000/mm³) to 204,000/mm³ (range 21,000-365,000/mm³); $p=0.002$,

though for most patients this decrease was not clinically significant. Pre-therapy, 15 (35%) and 4 (9.3%) of the 43 patients demonstrated a hematocrit <30% and a platelet count of <100,000/mm³, respectively. Following therapy, 6 (13.9%) and 6 (13.9%) of the 43 patients demonstrated a hematocrit of <30%, and platelet count of <100,000/mm³ (p=0.043 and 0.74, respectively).

Adverse Events

Toxicities encountered were mainly hematological and infectious. Encountered toxicities contributed to dose reduction (n=5) and/or truncation of intended therapy (n=9) in 13 patients. Intolerance to rituximab due to infusion related reactions (n=3) and rituximab mediated hyperviscosity (n=1) resulted in discontinuation of therapy for 4 of these patients, while prolonged myelosuppression on the basis of neutropenia (n=7) and/or thrombocytopenia (n=2) and/or peripheral neuropathy (n=2) from fludarabine resulted in discontinuation of therapy for 9 patients. Among the first 21 patients, 3 patients had grade 2 herpes zoster prompting institution of prophylaxis with either acyclovir or famcyclovir for the duration of therapy, and thereafter for one year at the recommendation of the DMSC. Following institution of herpes zoster prophylaxis, no further cases of herpes zoster were observed. Grade > 3 toxicities included neutropenia (n=27); thrombocytopenia (n=7); pneumonia (n=6), including in two patients who succumbed to non-PCP interstitial pneumonia; peripheral neuropathy (n=2); limbic encephalitis (n=1); and hemolytic anemia (n=1). The rate of grade >3 toxicities was similar among untreated and previously treated patients (p=0.48). With a median follow-up of 40.3 months, we

observed transformation to aggressive lymphoma (n=3); myelodysplasia (n=1); acute myelogenous leukemia (n=2); bladder carcinoma (n=1); and carcinoma of unknown primary (n=1) among 8 patients. The median time from initiation of protocol therapy to diagnosis of transformation was 21.1 (range 6-34.4) months, and for MDS/AML was 39.4 (range 11.9-40.8) months. Among patients who developed MDS or AML, 1 previously received chlorambucil, and another had previously undergone high dose chemotherapy with an autologous transplantation. Among the 3 patients who had transformation to diffuse large cell lymphoma, only one was previously treated and had received cyclophosphamide followed by rituximab. Among the two patients who developed adenocarcinoma, one was previously treated with chlorambucil.

Discussion

We examined the use of fludarabine in combination with rituximab in patients with WM given preclinical evidence suggestive of additive, and possibly synergistic mechanisms of action in various preclinical lymphoma models and clinical activity in other lymphoid malignancies. To our knowledge, this study represents one of the longest in terms of follow-up in the therapy of WM, and provides an important opportunity to assess both short and long term outcomes of this treatment. Intended therapy consisted of 6 cycles (25 mg/m²/day for 5 days) of fludarabine and 8 infusions (375 mg/m²/week) of rituximab over 31 weeks. On an intent to treat basis, we observed an overall and major response rate of 95.3% and 86.0%, respectively. Moreover, the median time to progression for all patients in this study was 51.2 months, which compares favorably to those previously reported with either fludarabine or rituximab alone, wherein response rates of 30-50% and median time to progression of 27-36 months have been reported. Median TTP was considerably longer (>88.3 versus 36.9 months) for previously untreated versus treated patients, respectively, suggesting that FR may represent a particularly good combination for initial long term disease control in suitable patients with WM.

An interesting observation in this study was that for responding patients achieving at least a very good partial response (i.e. at least a 90% reduction in disease burden), TTP was significantly greater versus patients attaining less than a VGPR (77.6 versus 38.4 months, respectively). While the importance of attaining a VGPR has been reported as a predictive variable for progression free survival in multiple myeloma^{31,32}, this study to

our knowledge, constitutes the first report of such potential benefit in WM. Studies addressing the impact of achieving at least a VGPR with other therapies would therefore seem appropriate in order to clarify whether VGPR attainment is categorical or therapy specific in terms of TTP benefit.

An intriguing finding in this study was the late response activity, as evidenced by the median time to best response which was 19.2 (range 4.2-61) months. Continued declines in serum IgM beyond one year have previously been reported by our group and Dimopoulos et al among patients receiving rituximab monotherapy^{18,19}. One possibility for this finding is that fludarabine and rituximab may more effectively target earlier members of the WM clone i.e. mature B-cells and lymphoplasmacytic cells, and spare (at least initially) more differentiated plasma cell members. Indeed, the relative resistance of malignant plasma cells to rituximab and/or fludarabine has previously been reported by us and others^{33,34}, and may account for the frequent detection of residual plasma cells in the absence of earlier B-cell precursors in the bone marrows of patients treated with either agent alone or in combination. The eventual clonal extinction of IgM producing plasma cells might then follow the initial elimination of precursor B-cells by fludarabine and rituximab thereby accounting for the slow, but continued declines in serum IgM observed in these studies.

An important consideration in this study was the differential impact of combined fludarabine and rituximab therapy on hematological parameters. A significant increase in the median hematocrit was noted for all patients from 31.2% to 38.0% following

treatment, with 30 of the 43 (70%) patients demonstrating a hematocrit rise of >2%. While the impact on hematocrit proved to be a positive outcome for patients, some patients experienced significant neutropenia and thrombocytopenia, which led to treatment discontinuation in 9 (21%) patients. Among these patients, 3 subjects experienced grade 4 neutropenia lasting more than 6 months. In two of these subjects, we observed a prompt recovery in neutrophil count following switch in cytokine support from granulocyte colony stimulating factor (G-CSF) to granulocyte-monocyte colony stimulating factor (GM-CSF), suggesting that the stimulation of earlier lineage leukocytes may overcome nucleoside analogue related prolonged neutropenia in certain patients.

In addition to hematological toxicities, infectious complications were encountered in this study and may have contributed to the death of two patients who succumbed to non-PCP related interstitial pneumonia, including one patient who was in a complete remission at time of death. Similar complications to nucleoside analogue therapy have previously been described³⁵. In addition, we observed herpes zoster in 3 of the first 21 patients prompting institution of herpes zoster prophylaxis with acyclovir or famcyclovir for the duration of treatment plus one year with good effect. The increased incidence of herpes zoster has also been reported by Czuczman et al²³ in patients with follicular NHL treated with fludarabine and rituximab, prompting prophylaxis in their study as well. Therefore, prophylaxis with an anti-viral agent appears warranted among WM patients receiving fludarabine and rituximab. Lastly, in one patient, we observed a case of limbic encephalitis, whose pathogenic basis remains unknown despite extensive workup but

suspected to be on the basis of a herpetic infection. Viral encephalitis suspected on the basis of a herpetic infection was previously reported in a WM patient receiving treatment with cladribine⁸.

In addition to the above short term toxicities, we also observed the development of diffuse large cell lymphoma (n=3), myelodysplasia and acute myelogenous leukemia (n=3), and carcinomas (n=2) among patients treated in this study with a median follow-up period of 40.3 months. While previous alkylator therapy may have been contributory in some of these cases to the development of secondary malignancies, only half of these patients received such therapy. The increased incidence of transformation to aggressive lymphoma and/or development of myelodysplasia and acute myelogenous leukemia following nucleoside analogue therapy have previously been reported by us³⁶ and others³⁷, and raises concerns about the long term consequences of these agents. Therefore, careful consideration of the candidacy for individual WM patients must be undertaken in order to discern if nucleoside analogue therapy is appropriate, as has also been recommended by the recent consensus panels on treatment of WM^{4,38}. The results of this study may also call into question whether a full 6 cycle course of fludarabine utilizing 5 days of therapy per cycle is warranted, particularly since 20% of patients experienced cytopenias resulting in treatment cessation. In most of these cases, treatment was truncated at or after 4 cycles of fludarabine. The impact of utilizing abbreviated courses of nucleoside analogues is therefore worthy of further study, as is also its potential impact on mitigating secondary malignancies.

Lastly, the sequencing of rituximab to fludarabine may be an important factor both from an efficacy standpoint, as well as avoidance of the IgM flare that commonly is observed following rituximab^{28,29} and which may have life threatening consequences as was the case in one patient in this study who experienced a hyperviscosity related intracerebral bleed following the first 4 weekly infusions of rituximab. Byrd et al²⁶ reported that the concurrent sequencing of fludarabine and rituximab produced a higher overall and complete remission response rate versus fludarabine followed by rituximab in CLL patients. The concurrent administration of these agents versus rituximab first followed by fludarabine as administered in this study might therefore lead to a better outcome in the treatment of WM patients. Moreover, as reported by Nichols et al⁴⁰ and Tedeshi et al³⁹, the concurrent administration of fludarabine and rituximab may also be associated with a decreased frequency of IgM flaring.

In conclusion, the results of this study demonstrate that fludarabine and rituximab is an active regimen in WM, though the ideal schedule and length of treatment for this combination needs to be better studied, and short and long term toxicities need to be carefully weighed against other available treatment options. The sequencing, combination, and duration of therapy to best optimize the combination of purine nucleoside analogues and rituximab remains to be defined by larger randomized studies.

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Authorship

SPT designed the trial, recruited and treated patients, and analyzed the data. ARB, LI, JDS, CJP served as clinical research coordinators for this trial, and collected, analyzed data. PW, CE, SRF, AL, PM, JM, SG, EK were site principal investigators, and recruited patients, collected data, and reviewed study outcome. BT was the biostatistician and provided data analysis for this study. SPT wrote the manuscript.

Conflicts of Interest

SPT, CE, SG have received honoraria from Genentech BioOncology and/or Biogen IDEC. SRF is presently an employee and shareholder of Hoffman LaRoche. Research funding in support of this study was provided by multiple sources, including Genentech BioOncology, Inc., Biogen IDEC Inc., and Berlex Inc.

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Median age	61 (52-75)
Median prior therapies	0 (0-2)
No prior therapy	27 (63%)
Median BM Involvement	55 (5-100%)
Adenopathy and/or splenomegaly	5 (12%)
Median IgM (mg/dL)	3,840 (655-10,500)
IgM >3,000 mg/dL	27 (63%)
Hct <30%	15 (35%)
PLT <100,000/mm ³	4 (9%)

Table 1. Baseline characteristics for all patients enrolled on study.

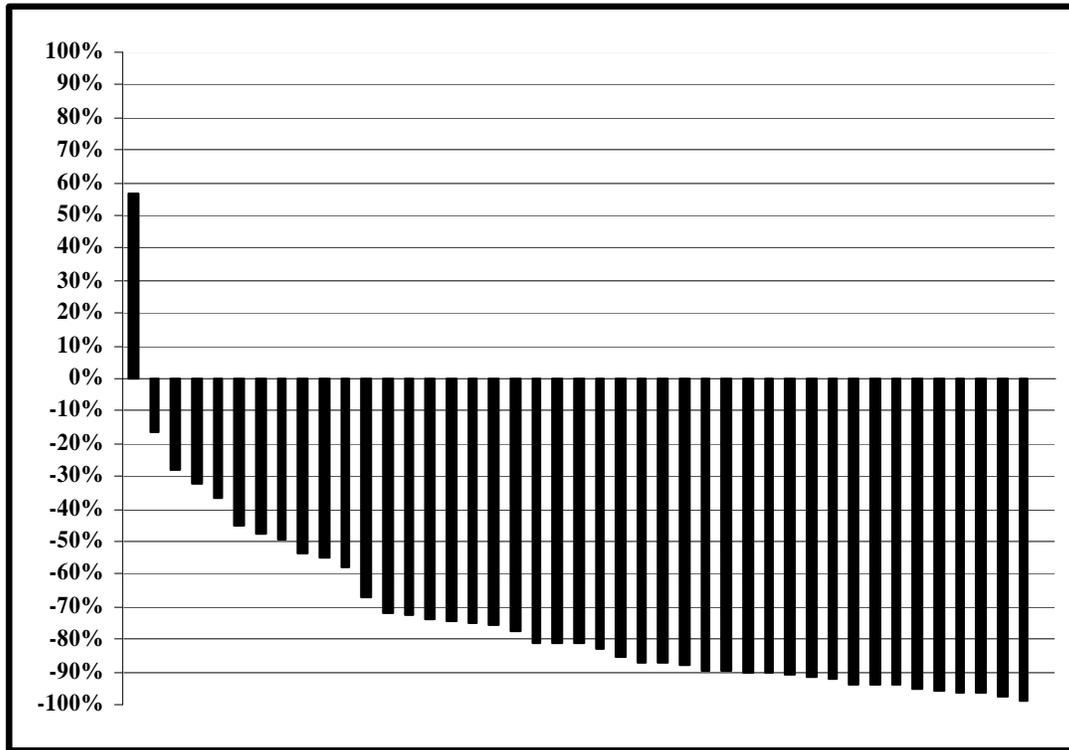
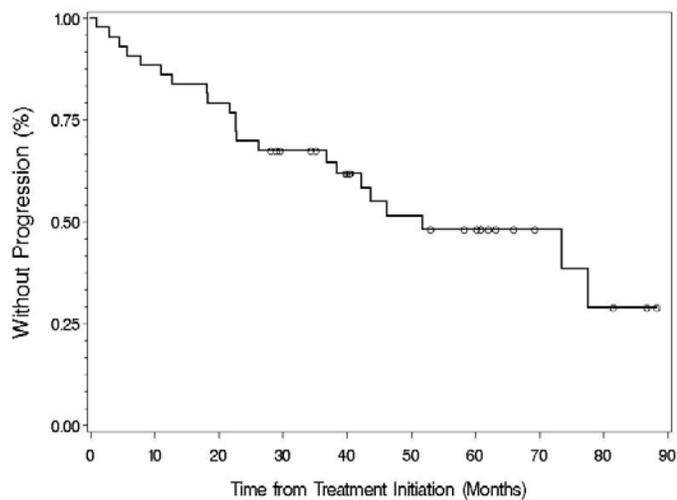


Figure 1. Individual changes (%) in serum IgM levels for following treatment with fludarabine and rituximab at best response.

A.



B.

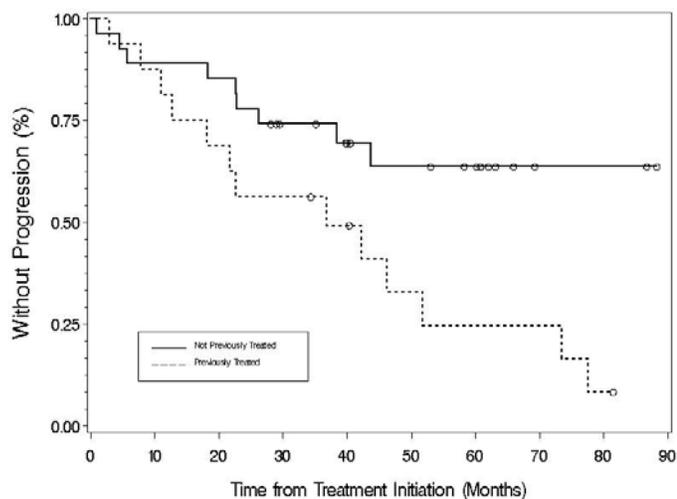


Figure 2. Time to progression for all patients (A) and for those patients (B) who were untreated or had received treatment prior to their therapy with fludarabine and rituximab. Open circles denote patients who had not progressed at last follow-up.

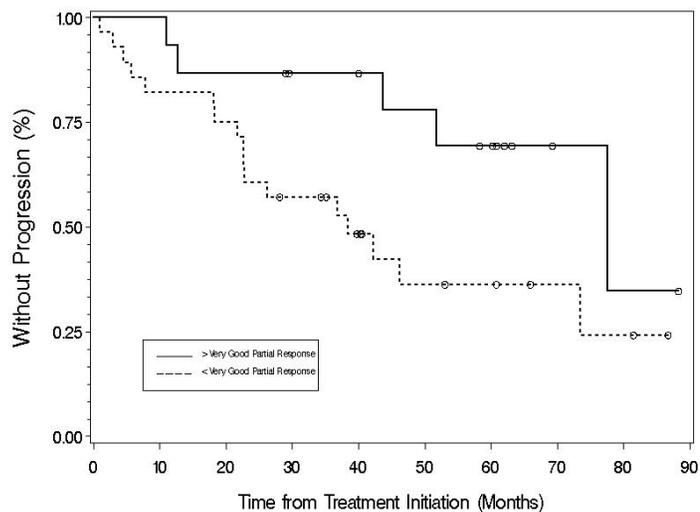


Figure 3. Time to progression for all responding patients based on achieving > VGPR or < VGPR following therapy with fludarabine and rituximab. Open circles denote patients who had not progressed at last follow-up.