Phase II Study of Perifosine and Sorafenib Dual-Targeted Therapy in Patients with Relapsed or Refractory Lymphoproliferative Diseases

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STATEMENT OF TRANSLATIONAL RELEVANCE

Treatment of patients with relapsed/refractory lymphoproliferative diseases represents an unmet medical need that urgently requires development of molecularly targeted agents. We conducted a phase II study testing the AKT inhibitor perifosine in combination with the multikinase inhibitor sorafenib in patients with relapsed/refractory lymphoproliferative diseases. This is the first study investigating the clinical activity and pharmacodynamic effects of perifosine and sorafenib combination therapy in this patient population. Results of the present study indicate that the combination therapy is feasible and results in clinical responses in heavily pretreated patients, with an objective response rate of 22% among the entire cohort and 28% among relapsed/refractory Hodgkin’s lymphoma patients. Interestingly, levels of extracellular signal-regulated kinase (ERK) and AKT phosphorylation in peripheral blood lymphocytes were predictive of clinical responses to the combination therapy. Promising activity observed in Hodgkin’s lymphoma patients warrants additional clinical trials to evaluate kinase inhibitors in association with new and conventional drugs.
ABSTRACT

Purpose: To evaluate safety and activity of perifosine and sorafenib combination therapy in patients with lymphoproliferative diseases.

Experimental Design: Patients with relapsed and refractory lymphoproliferative diseases received perifosine (50 mg BID) for one month. Patients achieving less than partial response (PR) after perifosine alone were administered the combination therapy [perifosine plus sorafenib (400 mg BID)] until progressive disease (PD) or unacceptable toxicity occurred. The phosphorylation of extracellular signal-regulated kinase (pERK) and AKT (pAKT) in peripheral blood lymphocytes as well as serum cytokine levels were investigated as predictive biomarkers of response.

Results: Forty patients enrolled in this study. After one month of perifosine alone, 36 who achieved less than PR went on to combination therapy, whereas 4 CLL patients who achieved PR continued with perifosine alone for a median of 10 months (range, 4 - 21). The most common drug-related toxicities were grade 1-2 anemia (17%), thrombocytopenia (9%), diarrhea (25%), joint pain (22%) and hand-foot skin reaction (25%). Three patients experienced grade 3 pneumonitis. Eight patients (22%) achieved PR, 15 (42%) achieved stable disease, and 13 (36%) experienced PD. A 28% PR rate was recorded for 25 HL patients. Among all patients, median overall survival and progression-free survival were 16 and 5 months, respectively. Early reductions in pERK and pAKT significantly correlated with the probability of clinical response.

Conclusions: Perifosine and sorafenib combination therapy is feasible with manageable toxicity and demonstrates promising activity in HL patients. The predictive value of pERK and pAKT should be confirmed in a larger patient cohort.
INTRODUCTION

Chemo-immunotherapy and peripheral blood stem cell transplantation (SCT) have established roles in the management of patients with high-risk non-Hodgkin lymphoma (NHL) (1, 2), relapsed follicular lymphoma (FL) (3, 4) and relapsed Hodgkin’s lymphoma (HL) (5, 6). However, a consistent proportion of high-risk NHL (20 – 30%) and relapsed or refractory HL (50 - 60%) patients are refractory to standard treatment and ultimately succumb to their disease (7). Moreover, patients with chronic lymphocytic leukemia (CLL) refractory to fludarabine or with high-risk disease exhibit a poor prognosis with standard regimens (8). Currently available compounds do not cure these patients; thus, new treatment strategies are needed.

In recent years, significant advances have been made to elucidate the mechanism(s) involved in the pathogenesis of lymphomas. Studies from several laboratories have elucidated a variety of constitutively activated signaling pathways, such as the phosphatidylinositol 3-kinase/mammalian target of rapamycin (PI3K/mTOR), nuclear factor-kB (NF-kB), Bcl-2, Bcl-6 and extracellular signal-regulated kinase (ERK) pathways. These pathways play critical roles in regulating tumor cell growth, proliferation, and survival, which collectively contribute to lymphomagenesis (9-12). Based on these findings, a wide spectrum of new agents is currently being tested in patients with lymphoproliferative diseases.

Perifosine (Æterna Zentaris GmbH, Germany) is an orally active synthetic alkylphospholipid that inhibits AKT, a protein involved in the PI3K/mTOR pathway (13). Activated AKT phosphorylates a variety of substrates that have a crucial role in cell cycle regulation. In lymphoma cells, the PI3K pathway is activated by the B-cell receptor (BCR) and exposure to survival factors in the microenvironment (14, 15). Perifosine has been tested as a single agent in phase 2/3 studies in patients with a variety of solid tumors, and limited antitumor activity was demonstrated (16-19). In combination studies, perifosine was shown to enhance the antitumor effects of dexamethasone in multiple myeloma and capecitabine in colorectal cancer (20-22).
Antiapoptotic and prosurvival signals in lymphoma cells are derived from the activation of the Ras/Raf/mitogen-activated protein (MAP)/ERK kinase (MEK) [or mitogen-activated protein kinase (MAPK)] pathway, which is implicated in cell proliferation, differentiation and survival in a variety of solid tumors and leukemic cell lines (23-25). Sorafenib (Nexavar®, Bayer) is an oral multikinase inhibitor that inhibits the MAPK pathway and several tyrosine kinase receptors involved in angiogenesis and lymphangiogenesis. In lymphoma cells, sorafenib downregulates myeloid cell leukemia-1 (Mcl-1), an anti-apoptotic Bcl-2 family member protein implicated in cell survival (25-28). Recently, sorafenib has demonstrated limited anti-lymphoma activity in patients with lymphoproliferative diseases (29, 30). Complex cross talk between the PI3K and MAPK pathways suggests that concomitant therapeutic targeting of these signal transduction pathways may have relevant clinical implications (31).

Recent preclinical data have demonstrated that perifosine combined with sorafenib induces gene expression profiling and signaling alterations associated with synergistic cytotoxic activity against lymphoma cell lines in vitro and in vivo, thus providing a strong rationale for evaluating the efficacy of dual-targeted therapy in lymphoma patients (32).

We therefore conducted a phase II study aimed at evaluating the safety and activity of perifosine and sorafenib combination therapy in patients with relapsed or refractory lymphoproliferative diseases. Additionally, this study also investigated a variety of predictive biomarkers, including (i) ERK and AKT phosphorylation in peripheral blood lymphocytes (PBLs) (33-35) and (ii) serum levels of cytokines involved in angiogenesis and lymphangiogenesis (36).
PATIENTS AND METHODS

Patient Selection. Patients with advanced lymphoproliferative diseases who had failed second-line or subsequent salvage chemo-radiotherapy were enrolled in this study. The eligibility criteria included age ≥18 years, 0-1 Eastern Cooperative Group (ECOG) performance status and measurable disease as assessed by computed tomography (CT). Adequate renal [creatinine ≤1.5 x upper limit of normal (ULN)], bone marrow (absolute neutrophil count ≥1,000/µl, hemoglobin value >9 gr/dl and platelet count ≥75,000/µl) and liver (serum bilirubin ≤1.5 x ULN, AST and ALT ≤2.5 x ULN, alkaline phosphatase ≤4 x ULN) function was required. Previous chemotherapy, radiotherapy and surgery must have been completed at least 4 weeks before registration and autologous or allogeneic SCT must have been completed at least 2 months before registration. Previous sorafenib treatment was permitted. The major exclusion criteria were a history of cardiac disease (i.e., congestive heart failure >NYHA class 2, active coronary artery disease, cardiac arrhythmia or uncontrolled hypertension), active infection or symptomatic metastatic brain or meningeal tumor. All patients provided written informed consent in accordance with the Declaration of Helsinki prior to enrollment in this study. The Institutional Review Board and Ethical Committee approved this study.

Study Design and Treatment. The primary objective of this open-label, single-center, phase 2 study was to assess the overall response rate (ORR), including the rates of complete remission (CR) and partial remission (PR). The secondary objectives included assessments of safety and tolerability, overall survival (OS), progression-free survival (PFS), time to progression (TTP) and duration of response (DOR). Enrolled patients received therapy with 50 mg BID perifosine [kindly provided by Aeterna Zentaris (Frankfurt, Germany, EU)] alone for 1 month followed by a response assessment. Patients achieving at least PR with perifosine alone were removed from the study and continued with perifosine treatment until progression of disease (PD) or unacceptable toxicity occurred. Patients achieving less than PR were eligible for perifosine and sorafenib [Nexavar®, Bayer AG (Leverkusen, Germany, EU)] combination therapy. Based on a previous phase I study evaluating the safety and maximum tolerated dose (MTD) of perifosine and sorafenib combination therapy in renal cell carcinoma (37), patients in our phase 2 study were administered 400 mg BID sorafenib one hour before or two hours after a meal and 50 mg
BID perifosine with a meal. The treatment was administered continuously until PD or unacceptable toxicity occurred. In the case of grade 2 perifosine-related clinically significant toxicity, treatment was reduced to 50 mg daily. With regard to grade 3 or 4 toxicity, treatment was withheld until improvement to grade ≤1 was achieved. Grade 2 sorafenib-related toxicity required a 200 mg twice daily or 200 mg once daily dose reduction, whereas grade 3 or 4 toxicity required temporary treatment interruption. After resolution of the related toxicities, dose re-escalation was permitted. If symptoms did not resolve after 3 weeks of treatment interruption, the patients were removed from the study.

**Study Assessments.** Tumor assessment by physical examination and CT was performed at baseline, after treatment with perifosine alone, after 4 weeks of combination therapy and every 8 weeks thereafter. Disease assessment by fluorodeoxyglucose (FDG) positron emission tomography (PET) was performed at baseline in all HL and NHL patients and during therapy in those patients who achieved a clinical response or disease stabilization as detected by CT scan. Tumor responses were assessed according to the revised response criteria for malignant lymphoma of the International Working Group (38) and the updated guidelines of the International Workshop on CLL (39). Responding patients were followed until disease progression, initiation of subsequent therapy or death. Serum samples and peripheral blood mononuclear cells were collected for biomarker analysis at baseline and monthly during the first four months of therapy. In HL patients serum levels of thymus- and activation-regulated chemokine (TARC) were assessed monthly by an enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, USA). Safety assessments, including blood pressure monitoring, complete blood cell count analysis, serum chemistry analysis and physical examination, were performed weekly during the first 8 weeks of therapy and monthly thereafter. The National Cancer Institute Common Toxicity Criteria of Adverse Events (CTCAE) version 3.0 was used for adverse event classification. Attributable toxicity was defined as an adverse event that was classified as possibly, probably or definitely related to the treatment.

**ERK and AKT Phosphorylation.** Unstimulated or phorbol myristate acetate (PMA)-stimulated (2 µM; Sigma, Milano, Italy, EU) PBLs were stained with a phospho-Akt- or phospho-ERK1/2-specific monoclonal antibody (33, 34). Briefly, the cells were fixed with 2%
paraformaldehyde for 10 min to arrest signaling activity, washed in 2 ml of staining buffer, and permeabilized with 90% methanol at 4°C. The cells were then incubated with an Alexa Fluor 647-conjugated monoclonal antibody specific for S473-phosphorylated Akt and an Alexa Fluor 488-conjugated monoclonal antibody specific for T202/Y204-phosphorylated ERK1/2 (Becton-Dickinson Biosciences, Milano, Italy, EU). The permeabilized cells were also stained with Alexa Fluor 647- or 488-conjugated mouse immunoglobulin G1 isotype control antibodies (BD Biosciences) to determine background staining. The samples were analyzed with a dual-laser fluorescence-activated cell sorter (FACSCalibur, Becton-Dickinson Biosciences) using CellQuest software (BD Biosciences). The results are expressed as the percentage of cells stained with pAkt or pERK1/2 antibody in PMA-stimulated cultures after subtraction of the staining detected in unstimulated PBLs. PBLs from normal donors were also included as an internal control.

**Serum Biomarker Analysis.** After centrifugation of blood samples at room temperature, sera were frozen and stored at -80°C until use. Custom-made antibody-based chemiluminescent SearchLight® multiplex arrays (Aushon BioSystems, Billerica, MA, USA) were designed to detect and quantify the following soluble molecules: CD40-L, M-CSF, VEGF-A, CD30, TRAIL and PDGF-AA. In addition to patient sera, a pool of sera from 5 healthy donors was also evaluated for each of these molecules. All serum samples were evaluated in duplicate. Briefly, sera from the patients and controls were incubated with biotinylated antibodies as described previously (36). Next, streptavidin-horseradish peroxidase (SA-HRP) was added. SA-HRP then reacted with a chemiluminescent substrate (SuperSignal® ELISA Femto Chemiluminescent Substrate) to produce a chemiluminescent signal that was detected by a CCD camera (SearchLight Plus CCD Imaging System). The SearchLight images were analyzed using Array Analyst software (Aushon BioSystems). Nonlinear regression analysis via Prism software (GraphPad) was then performed to fit the following variable-slope, four-parameter standard curve equation: 

\[ Y = \text{Bottom} + \frac{\text{Top} - \text{Bottom}}{(1 + 10^{(\log EC50 - X)})^\text{HillSlope}} \]

**Statistical Analysis.** This is a Simon’s two-stage phase II (40). The study was designed to detect a difference of 25% between the null hypothesis proportion of a 15% response rate and the alternative hypothesis of a 40% response rate using a two-sided hypothesis test...
with a target significance level of 5% and a power of 90%. After testing the drug in 13 patients in the first stage, the trial would be permanently closed if less than three responded. If three or more responses were observed, a total of 36 patients would be enrolled to receive the combination therapy. More than 9 responses in 36 patients would be considered evidence that this agent has some activity. Efficacy and toxicity were evaluated in 36 patients receiving combination therapy. The ORR was defined as the proportion of patients achieving CR and PR. Time-to-event end point distributions were estimated using the Kaplan-Meier method (41). Correlation between changes of ERK and AKT phosphorylation levels during the first two months of therapy and the probability of obtaining a clinical response were calculated using the logistic regression model. Analysis of variance (ANOVA) followed by the SNK multiple comparison test was used to compare cytokine levels at different times or in different subgroups of patients. All p values are two-sided and were considered significant if $p \leq 0.05$. The statistical analysis was performed using the statistical package Prism v6.0e (GraphPad Software, San Diego, CA, USA) on a Macintosh Pro personal computer (Apple Computer, Inc.).
RESULTS

Patient Characteristics. From July 2008 to November 2011, 40 patients were enrolled in this study and treated with perifosine alone for 1 month. Four patients with relapsed or refractory CLL achieved PR after treatment with perifosine alone and were not eligible for combination therapy based on the study design. The remaining 36 patients who achieved less than a PR after 1 month of perifosine therapy alone were subsequently administered the combination therapy. The clinical and demographic characteristics of the patients who received combination therapy are summarized in Table 1. Patients had a histologically confirmed diagnosis of relapsed or refractory lymphoproliferative disease, including diffuse large B cell lymphoma (DLBCL) (n = 3), follicular lymphoma (FL) (n = 3), CLL (n = 4), Waldenstrom macroglobulinemia (WM) (n = 1) and HL (n = 25). The median time from diagnosis to study entry was 5 years (range, 1 – 15), and the median time since the last therapy was 3 months (range, 1 – 24). At study entry, patients had undergone a median of 5 (range, 2 – 11) prior treatment regimens and had relapsed (n = 11) or refractory (n = 25) disease. Refractory disease was defined as a failure to achieve a CR or PR after the last treatment. Twenty-five patients (69%) had undergone autologous SCT, and 15 patients had also received an allogeneic SCT (42%). The majority of patients had advanced-stage disease, with extranodal involvement at study entry in 20 patients (64%). Since 25 of 36 patients receiving the combination therapy had a diagnosis of HL, their clinical characteristics are described in detail in Supplementary Table 1. None of these patients had received Brentuxinab Vedotin.

Treatment Delivered. Thirty-six patients received at least one month of perifosine and sorafenib combination therapy and were evaluated for response and toxicity. The median duration of combination therapy was 4 months (range, 2 – 18). Due to side effects, 17 patients (47%) required transient sorafenib discontinuation for a median of 7 days. The main reason for discontinuation was grade 3 or prolonged grade 2 hand-foot skin reaction (HFSR; n = 9). In addition, 14 patients (39%) required transient discontinuation of both agents for a median of 7 days. Reasons included gastrointestinal symptoms, fever, skin abscess, blurred vision, ulcerative keratitis and thrombocytopenia.
The majority of patients (90%) required a transient dose reduction of sorafenib mainly due to gastro-intestinal symptoms or HFSR, with 15 patients (42%) requiring a definitive sorafenib dose reduction to 200 mg twice daily. A transient reduction of perifosine, which lasted a median of 10 days, was required in 9 patients (25%) due to grade 2 toxicity (joint pain and gastrointestinal symptoms). Definitive treatment cessation because of disease progression occurred in 31 patients. Recurrent anorexia with weight loss and grade 3 pneumonitis prompted cessation in 1 and 2 patients, respectively. Two patients with availability of an identical donor discontinued study treatment after achievement of PR and underwent allogeneic SCT.

**Treatment Efficacy.** According to the study design, 4 patients with CLL who achieved a PR after 1 month of perifosine therapy alone were removed from the study and continued perifosine treatment alone for a median of 10 months (range, 4 – 21). The remaining 36 patients who achieved SD (n = 21) or PD (n = 15) with perifosine alone were administered the combination therapy. Eight of the 36 patients treated with perifosine/sorafenib obtained a PR, with an ORR of 22%. No patients achieved a CR. However, 15 patients (42%) achieved SD, and 13 patients experienced PD. Tumor lesion shrinkage was detected in 7 patients who achieved SD; therefore, a total of 15 patients (42%) exhibited a reduced tumor burden (**Fig. 1**). The median time to PR was 4 months (range, 1 – 8). After a median follow-up time of 33 months (range, 22–62), the median OS, PFS, TTP and DOR for all study patients was 16 (range, 2 – 62), 5 (range, 2 – 17), 5 (range, 2 – 17) and 4 (range, 1 – 12) months, respectively (**Fig. 2**). Median OS, PFS, TTP and DOR for 25 HL patients was 18 (range, 2 – 62), 5 (range, 2 – 17), 5 (range, 2 – 17) and 3 (range, 1 – 12) months, respectively. One patient was alive without disease after treatment with perifosine, sorafenib and allo-SCT, and 12 patients (33%) were alive with disease. In contrast, 22 (61%) patients died from disease progression, and 1 patient (3%) died from pneumonitis. HL patients achieving PR or SD by CT scans were also assessed by FDG-PET scans. Of note, in three of the 8 SD patients and in 2 of the 7 PR patients, FDG-PET scans displayed necrosis of lymphadenopathy.

PR was observed in HL (n = 7) and CLL (n = 1) patients. The clinical responses according to histological subtype are detailed in **Table 2**. Given the promising response to treatment observed among the HL patients enrolled during the initial study period, we encouraged the
inclusion of patients with this histological subtype. Therefore, the majority of study patients (25/36) were affected by HL. Among the HL patients, 7 obtained a PR (28%), 8 achieved SD (32%), and 10 (40%) experienced PD, with a 60% disease control rate (PR + SD) in this subgroup of patients. The study treatment was ≥ the 5th line of therapy in responding HL patients. In total, 3 patients were primary refractory, and 4 and 3 patients were classified as refractory and relapsed, respectively, following the last treatment (Supplementary Table 2).

Two pretreated HL patients underwent allo-SCT after achieving a significant PR with perifosine and sorafenib; one of these patients is still in remission 47 months after allo-SCT, and the other patient experienced complete remission that lasted 15 months. In HL patients achieving PR the median PFS was 12 months (range, 5 - 17) and the time to response ranged from 2 to 9 months. During treatment, median reductions of 75% (range, 53 – 81) and 35% (range, 0 – 66) in serum TARC levels were observed in HL patients who achieved PR and SD, respectively. A reduction in serum TARC levels was not observed in PD patients.

**Safety and Tolerability.** The adverse events (AEs) that occurred in patients during the first month of treatment with perifosine alone are listed in Supplementary Table 3. The most frequent toxicities were grade 3 increase in AST/ALT values in three patients (8%), grade 2 skin abscess in 2 patients (6%), grade 2 fever in 2 patients (6%) and grade 1 skin rash in 2 patients (6%). The hematological and non-hematological toxicities recorded during sorafenib and perifosine combination therapy are detailed in Table 3. Hematological AEs included grade 1 and 2 thrombocytopenia and anemia, which were observed in 9% and 17% of the patients, respectively. Grade 4 neutropenia occurred in 1 patient (3%). The non-hematological AEs observed in more than 10% of the patients included grade 2 diarrhea (25%), grade 1 weight loss (19%), grade 1 abdominal pain and nausea (11%), grade 1 fever (11%) and grade 2 and grade 3 HFSR (25% and 14%, respectively). Grade 3 pneumonitis was observed in 3 patients (8%). Unexpected toxicities observed in this study were grade 2 arthritis/joint pain in 8 patients (22%), grade 2 scalp pain in 3 patients (8%), grade 1-2 sterile skin abscess in 4 patients (12%) and grade 2 ulcerative keratitis in one patient. The majority of toxicities were completely reversible after temporary cessation of the study drugs. A HL patient who had received four prior regimens including autologous and allogeneic transplants three years before study entry, developed a pneumonitis five months after starting the combination therapy to whom she was responding. This patient had a history of
recurrent infections (pneumonitis and cutaneous abscesses) before enrollment and was heavily immunocompromised due to previous transplants and long history of disease. She died for sequelae of the pneumonitis (bone marrow and multiorgan failure) 30 days after the last assumption of study drug.

ERK and AKT Phosphorylation. pERK and pAKT levels were measured in PBLs at baseline and monthly for four months in 32 patients who received perifosine/sorafenib therapy. The percentages of pERK- and pAKT-positive PBLs detected in individual patients prior to therapy are presented in Figure 3A-B. Values of baseline pERK- and pAKT-positive cells preferentially were above the median values in responsive (PR + SD) and preferentially below in non responsive (PD) patients, respectively. Analysis of the continuous distributions of pERK and pAKT values allowed to discriminate responsive and non-responsive patients (p = 0.005, p = 0.009, respectively). Then, the percentage variation between day 60 and day 0 in pERK- and pAKT-positive PBLs was calculated for each patient. As shown in Figure 3C-D, 8 of the 12 patients who experienced PD displayed an increased percentage variation of pERK and pAKT, whereas 18 of the 20 patients who achieved PR and SD displayed reduced percentage variations of pERK and pAKT. Finally, normalized percentage variation values of phosphorylated cells were investigated as a marker of clinical response using logistic regression model. A reduction in the percentage variations correlated well with the probability of response (p = 0.003 for pERK and p = 0.005 for pAKT).

Serum Biomarker Analysis. Serum samples obtained from 22 consecutive patients, including 6 PR patients, 8 SD patients and 8 PD patients, at baseline and at various times during therapy were evaluated using the SearchLight Multiplex array approach. Six of the molecules investigated (CD40-L, M-CSF, VEGF-A, sCD30, TRAIL and PDGF-AA) were detected in the majority of serum samples from all the patients. None of these factors showed significant changes during therapy that could be associated with response (data not shown). However, after analyzing all available serum samples, we observed that M-CSF, CD30, TRAIL and PDGF-AA displayed significantly different levels in the three response groups (PR vs. SD vs. PD; Supplementary Figure 1 for CD30 data and Supplementary Table 4 for results for all 4 molecules).
DISCUSSION

In the present study, we assessed the efficacy and tolerability of sorafenib and perifosine dual-targeted therapy in relapsed and refractory lymphoma patients. Ras/Raf/MAPK and PI3K/AKT/mTOR are critical pathways in the development and proliferation of lymphoproliferative diseases and solid tumors. Recently, published observations have demonstrated that the blockage/inhibition of only one of the pathways can result in the activation of the other pathway (31, 42). Therefore, based on a specifically designed preclinical study (32), we sought to test the effects of concomitant MAPK and AKT inhibition in a clinical setting to investigate the possibility of achieving synergistic activity against lymphoma cells. Results reported herein show a clinical activity of the combination regimen in 8 of 36 patients with a PR being detected in 7 out of 25 HL patients. Despite PFS data suggest a higher activity of perifosine/sorafenib compared to sorafenib alone (29), no definitive conclusion can be drawn on the comparative efficacy of the single agent and combination regimen.

We designed the study to assess the therapy in a minimum of three patients representing each of the major lymphoma subtypes to determine a target population for subsequent studies. Our initial results demonstrated a significant proportion of clinical responses in the HL group; therefore, we subsequently enrolled primarily HL patients. The results of the study indicate a 28% ORR and a 60% disease control rate (PR and SD patients) in this histological subgroup. Overall, the observed ORR of the study was 22% and the number of patients achieving a clinical response was slightly inferior to the 10 responses requested by the statistical design to demonstrate evidence of some activity of the study drugs. However, although the study design was not planned to investigate activity only in the HL subgroup, it is important to point out the imbalance between the 7 responses observed among 25 HL patients and absence of responses observed among the remaining NHL patients. In contrast, we observed significant efficacy with perifosine alone in the CLL patients; 4 of the 8 CLL patients responded to perifosine alone, and one of the 4 patients who received combination therapy achieved PR. This observation is consistent with recent studies demonstrating either PI3K/AKT pathway activation or anti-PI3K/AKT drug efficacy in CLL. In particular, the activity of the PI3K inhibitor CAL-101 observed in relapsed and refractory CLL patients suggests that new molecules directed against this pathway should be developed (43).
Future clinical trials studying perifosine alone or in combination with other agents in CLL are warranted.

The response rate observed in the HL group is noteworthy given the refractoriness and number of previously administered therapies reported in this patient group. Moreover, the efficacy of the study drugs was confirmed by the clinical history of two pretreated HL patients. In these patients, perifosine and sorafenib treatment acted as a bridge to allogeneic SCT, and the patients subsequently experienced a considerable amount of time in complete remission. In responsive HL patients, the observed median PFS of 12 months and the time to response ranging from 2 to 8 months suggest that the anti-lymphoma activity of the study drugs is not exclusively demonstrated by the achievement of a clinical response. In fact, numerous patients remained on therapy without the development of PD and achieved a PR after many months of unchanged disease. Our findings could be explained by the fact that molecular drugs act on lymphoma cells via slow, continuous activity that inhibits the constitutive proliferative and antiapoptotic signals. In contrast to traditional chemotherapies, molecular drugs do not induce rapid cellular death. These observations along with a recent publication by Younes et al. suggest that treatment should not be discontinued when SD is achieved during treatment with targeted agents and that new response criteria are needed (44).

The ORR in HL patients reported herein is consistent with data from several recent studies investigating the activity of new agents, such as histone deacetylase inhibitors, everolimus and lenalidomide in HL patients (45-47). In contrast, the new drug-conjugated antibody brentuximab vedotin showed a potent anti-lymphoma activity, as reported in the pivotal phase II study, the ORR was 75% with 34% of patients achieving CR, patients in complete remission presented a median PFS of 21.7 months whereas the PFS for all study patients was limited to 5.6 months (48). The anti-lymphoma activity of these new agents should be confirmed by additional studies investigating methods to optimize the drug combination, timing and dosing.

The response rate and duration of response reported in this study suggest that dual inhibition is not sufficient to induce durable regression in the majority of lymphoma
patients. This observation can be explained by differences in pathway activation between histological subtypes as well as patients. The inherent complexity of regulatory growth, proliferation and survival mechanisms may also be involved. In addition, lymphoma cells can utilize alternate pathways to escape drug-induced damage. Interesting findings emerged from the HL patient group suggesting that this subgroup displays increased responsiveness to combination therapy compared with other lymphoma subgroups. We can infer that increased activation of the PI3K and ERK pathways in HL cells could play a role in sensitivity to the study drugs.

The therapy was administered in an outpatient setting, and hospital admissions were limited to one clinical visit per month. The toxicities were mild, and most of the events could be managed by a transient dose reduction. Doses of study drugs were planned based on a recent phase I study conducted in renal cell carcinoma patients (37), however in the present study we observed a higher percentage of patients with hematological toxicities and non hematological recurrent grade 2 toxicities. An increased rate of hematological toxicity reported in this study is in agreement with our previous observation in patients treated with sorafenib alone and with data reported in DLBCL patients receiving the kinase inhibitor sunitinib (49). In fact, inhibition of VEGFR, c-kit and Flt-3 expressed by myeloid and lymphoid marrow progenitors seems play a role in the pathophysiology of sorafenib-induced marrow toxicity (50). We hypothesize that lymphoma patients display a reduced tolerance to perifosine/sorafenib combination due to previous extensive exposure to cytotoxic drugs and long history of disease. In particular, the known toxicity of targeted agents on hematopoietic cells when combined with a previous damage induced by chemo-radiotherapy might result in an increased hematological toxicity. Grade 3 or extended grade 2 HFSR, a common sorafenib-related toxicity, represented the main non-hematological toxicity causing transient sorafenib discontinuation; patients requiring transient discontinuation of both agents experienced joint pain, skin abscesses and ocular toxicity. We observed grade 3 pulmonary infections requiring cessation of the therapy in one patient who had received an allogeneic transplant and in a CLL patients with disease-related immunodeficiency. Although these patients were already immunocompromised due to previous transplant or advanced disease, it is possible that the combination treatment could have contributed to immunodepression. In future studies, the administration of
immunoglobulin and antibiotic prophylaxis in CLL and transplanted patients is recommended.

We performed translational analyses of the present study to establish whether serum biomarkers could predict treatment response. Predictive biomarkers are useful for overcoming the risk of poor outcome related to inadequate patient selection in future studies and for optimizing the use of new drugs. The results indicate that pERK and pAKT levels measured in PBLs are predictive of response, decrease of these values after two months of therapy indicates a clinical response. Blood biomarkers that indicate the status of ERK and AKT phosphorylation could serve as a simple tool with which to select patients who will benefit from dual-targeted therapy. In contrast, cytokine measurements failed to correlate with clinical response; thus, increased CD30 and M-CSF levels observed in non-responsive patients most likely reflect the presence of aggressive disease and do not correlate with drug activity.

In conclusion, combination therapy with perifosine and sorafenib exerts anti-lymphoma activity and is tolerated by heavily pretreated lymphoma patients. Frequent toxicities such as HFSR and infections didn’t compromise prolonged treatment but required careful management. The promising clinical responses observed in the relapsed and refractory HL patients suggest that this subgroup could serve as target population for new studies. Patients who display an early reduction of pERK and pAKT in PBLs could potentially benefit from dual-targeted therapy; thus, this test should be investigated in future studies with a larger patient cohort. The results from this study encourage the planning of future investigations to assess targeted therapies as well as molecular and conventional drug combinations in lymphoma patients. Biomarker studies should be included in these new studies to identify responsive patients. The identification of predictive biomarkers could aid in the optimization of new drugs, garner more significant results from phase II studies and improve the cure rate.
REFERENCES


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Final approval of manuscript: A. Guidetti, C. Carlo-Stella, W. Malorni, A. Anichini, M. Di Nicola, P. Corradini, A.M. Gianni
Table 1. Clinical characteristics of the patients administered perifosine/sorafenib combination therapy at study entry

<table>
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<tr>
<th>Characteristic</th>
<th>No. of Patients</th>
<th>%</th>
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<tr>
<td><strong>Age, years</strong></td>
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</tr>
<tr>
<td>Median</td>
<td>41</td>
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<tr>
<td>Range</td>
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<tr>
<td><strong>Males/Females</strong></td>
<td>22/14</td>
<td>61/39</td>
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<td>8</td>
</tr>
<tr>
<td>FL</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>WM</td>
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<td>4</td>
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<tr>
<td>HL</td>
<td>25</td>
<td>69</td>
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<td><strong>Stage (Ann Arbor) of the 31 lymphoma patients</strong></td>
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<tr>
<td>I/II</td>
<td>6</td>
<td>19</td>
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<tr>
<td>III/IV</td>
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<td>81</td>
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<tr>
<td><strong>Extranodal involvement</strong></td>
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<tr>
<td><strong>Time from diagnosis, years</strong></td>
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<tr>
<td>Median</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>1 - 15</td>
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<tr>
<td><strong>Time from last therapy, months</strong></td>
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<td>Median</td>
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<td>Range</td>
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<td><strong>No. of prior treatment regimens</strong></td>
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<td>Median</td>
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<tr>
<td>Range</td>
<td>2 - 11</td>
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</tr>
<tr>
<td>2 regimens</td>
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<td>4 regimens</td>
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<td>22</td>
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<tr>
<td>≥5 regimens</td>
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<tr>
<td><strong>Best response to most recent therapy</strong></td>
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<tr>
<td>CR</td>
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<td>PR</td>
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<td>SD</td>
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<td>PD</td>
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<td><strong>Prior autologous SCT</strong></td>
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<tr>
<td><strong>Prior allogeneic SCT</strong></td>
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</table>

**Abbreviations:** DLBCL, diffuse large B cell lymphoma; FL, follicular lymphoma; WM, Waldenstrom macroglobulinemia; CLL, chronic lymphocytic leukemia; HL, Hodgkin’s lymphoma; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; SCT, stem cell transplantation.
Table 2. Best response to perifosine and sorafenib combination therapy according to disease type

<table>
<thead>
<tr>
<th>Disease type</th>
<th>N</th>
<th>CR (%)</th>
<th>PR (%)</th>
<th>SD (%)</th>
<th>PD (%)</th>
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<td>15 (42)</td>
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<td>7 (28)</td>
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<td>0</td>
<td>2 (67)</td>
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<td>0</td>
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<td>2 (67)</td>
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<td>0</td>
<td>1 (100)</td>
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<td>1 (25)</td>
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</table>

**Abbreviations**: DLBCL, diffuse large B cell lymphoma; FL, follicular lymphoma; WM, Waldenstrom macroglobulinemia; CLL, chronic lymphocytic leukemia; HL, Hodgkin’s lymphoma; CR, complete remission; PR, partial remission; SD, stable disease; PD, progressive disease.
## Table 3. Adverse events in patients administered perifosine and sorafenib

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<td>%</td>
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</tbody>
</table>
**FIGURE LEGENDS**

**Figure 1** - Waterfall plot of the maximum decrease in tumor size from baseline (pre-therapy) for 36 patients treated with perifosine and sorafenib who experienced PD, SD or PR. Asterisks indicate HL patients.

**Figure 2** - (A) Overall survival, (B) progression-free survival, (C) time to progression and (D) duration of response in study patients receiving perifosine and sorafenib.

**Figure 3** – Baseline values of pERK (A) and pAKT (B) in individual patients. Red, green and black bullets represent PR, SD and PD patients, respectively. Horizontal lines represent the median baseline values. Waterfall plot of percentage variations in pERK (C) and pAKT (D).
Figure 1

Tumor Size (% Change from Baseline)

Individual Patients (n = 36)

PD
SD
PR
Figure 2

A

Overall Survival (%)

n = 36, 12 censored
Median OS: 16 ms

0 12 24 36 48 60 72

Months

B

Progression-Free Survival (%)

n = 36, 5 censored
Median PFS: 5 ms

0 6 12 18

Months

C

Time to Progression (%)

n = 36, 6 censored
Median TTP: 5 ms

0 6 12 18

Months

D

Duration of Response (%)

n = 23, 15 censored
Median DOR: 4 ms

0 6 12 18

Months
Figure 3

A

Baseline pERK+ Cells (%)

Individual Patients (n = 32)

B

Baseline pAKT+ Cells (%)

Individual Patients (n = 32)

C

% Variation of pERK+ Cells

Individual Patients (n = 32)

D

% Variation of pAKT+ Cells

Individual Patients (n = 32)
Clinical Cancer Research

Phase II Study of Perifosine and Sorafenib Dual-Targeted Therapy in Patients with Relapsed or Refractory Lymphoproliferative Diseases

Anna Guidetti, Carmelo Carlo-Stella, Silvia L Locatelli, et al.

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