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CLINICAL TRIAL REPORT

Phase II trial and prediction of response of single agent tipifarnib in patients with relapsed/refractory mantle cell lymphoma: a Groupe d'Etude des Lymphomes de l'Adulte trial

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Abstract
Purpose Farnesyltransferase (Ftase) was identified by gene-expression profiling and by preclinical evaluation in in vitro and in vivo mantle cell lymphoma (MCL) models as a rational therapeutic target in MCL, one of the most refractory B-cell lymphomas. We conducted a multicenter phase II study of a potent Ftase inhibitor, tipifarnib, in patients with relapsed or refractory MCL.

Methods Tipifarnib was administered at 300 mg orally twice daily for the first 21 days of each 28-day cycle for 4 cycles, and in case of response for 6 cycles. Study endpoints were objective response at 4 and 6 cycles, progression free survival (PFS), overall survival, and toxicity. Prediction of response was retrospectively evaluated in the initial tumor biopsy by the *RASGRP1/APTX* gene expression ratio, and the *AKAP13* expression level.

On behalf of the GELA.

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Results Eleven patients (median age, 71 years) were enrolled. Patients received a median number of three prior therapies (range 1–11). Nine patients completed at least 3 cycles of tipifarnib. No grade III–IV hematological toxicities were recorded. One patient presented a complete response (CR) after 4 and a persistent CR at 6 cycles (ORR = 9%). Median PFS was 3 months (range 0.7–14.2). The *RASGRP1/APTX* gene expression ratio was higher in the responder ($n = 1$) while the *AKAP13* expression was higher in the non-responders ($n = 2$). This corresponds to the expected result for predicting response to tipifarnib.
Conclusion Treatment with tipifarnib relapsed or refractory MCL is associated with low response rates. Limited gene expression studies suggest that response may be associated with molecular targets.

Keywords Tipifarnib · Mantle cell lymphoma · Clinical trial · Molecular predictor

Introduction

Mantle cell lymphoma (MCL) is a rare B cell non-Hodgkin lymphoma (NHL) representing only 5–8% of all NHL in America and in Europe [1]. Beside an indolent clinical presentation at initial diagnosis, this lymphoma is characterized by a poor survival with a median around 5 years, translating to only 10–15% of long-term survivors, although improvement has been recently demonstrated with combination of new drugs and high-dose therapy.

The best therapeutic options remain unclear while conventional therapy has hardly altered the continuously declining survival curve [2]. Complete remission (CR) to standard chemotherapy regimens such as R-CHOP is poor. Less than 50% of patients will respond to R-CHOP as front line therapy [3]. The role of anthracyclin-containing regimens is controversial [4]. Purine analogs have been tested in small phase II studies with poor results. Fludarabine and cladribine alone induce 33 and 44% overall response rates (RR), respectively, with a median time to progression between 1.1 and 1.9 years [5]. In combination with other drugs, fludarabine, or cladribine induced a higher RR but also a higher hematologic toxicity. Other combinations with novel agents, such as bendamustine, bortezomib, and lenalidomide in combination are reported with promising results [6–8]. High-dose cytarabine (Ara-C) seems to achieve high-response rates (over 80%) when associated to multidrug regimens such as DHAP or HyperCVAD [9]. High-dose chemotherapy with autologous stem cell transplantation has been considered for these patients. While some pilot studies have given encouraging results, particularly when realized in first line therapy [10, 11], follow-up has generally showed no evidence of cure or long-term remission [12]. The monoclonal antibody Rituximab has been reported to induce 33–37% overall response rates in uncontrolled trials [13], with higher response rates achieved when combined with CHOP or FCM [11, 14, 15]. Despite this complex treatment, patients will relapse and there is clearly a need of alternative innovative treatment.

Gene expression profiling (GEP) has remarkably improved our knowledge of B-cell lymphoma biology. Indeed, MCL, characterized by a t (11;14) translocation inducing the overexpression of CCND1 not sufficient to induce lymphoma development [16], exhibits specific molecular signatures which distinguish them from normal B-cells as well as from other B-cell lymphomas [17–19]. Among the up-regulated genes, farnesyltransferase (FTase) gene was consistently overexpressed among the MCL samples [19]. FTase is an enzyme important for the maturation of a certain number of proteins, including protooncogenes, such as Ras, Rho-B [20–23], Rac, Rheb, and also nuclear lamins [24, 25] and centromeric proteins CENP-E/F [26]. These proteins are synthesized in the cytoplasm as precursor proteins that require additional posttranslational modifications in order to have a good subcellular localization, a prerequisite for their biological activities, i.e. the signal transduction. These modifications are accomplished by a prenylation reaction involving the attachment of a 15-carbon farnesyl group to the C-terminal cysteine residue, mediated by an enzyme, the FTase.

Based on the assumption that interruption of prenylation may prevent cellular events that depend on the function of those substrates, several classes of FTase inhibitors (FTI) have been designed as antineoplastic therapies in solid tumors [27] as well as in hematologic malignancies, including acute myeloid leukemia, chronic myelogenous leukemia, myelodysplastic syndromes, and myeloproliferative disorders [28–34]. R115777 (tipifarnib, ZARNESTRA™, Ortho Biotech Research and Development, Titusville, NJ, USA) is a potent non-peptidomimetic FTI with significant antitumor effects in preclinical studies [35]. In MCL, we have previously demonstrated the activity of tipifarnib in in vitro and in vivo models [36]. Tipifarnib specifically inhibited the growth of 4 MCL cell lines (Granta, NCEB, REC, UPN1), and induced apoptosis in 40–70% of MCL cells depending on the MCL cell lines. In addition, when used in combinations at IC20, tipifarnib significantly enhanced the cytotoxic effect of doxorubicin, cytarabin, cisplatin, vincristin, or bortezomib with a marked reduction of their IC50 (ranged from 57 to 99%), as also demonstrated by others [37]. We further demonstrated that tipifarnib administered p.o. twice daily for 8 consecutive days at the 500 mg/kg dosage displayed cytostatic activity in a xenograft mouse model bearing established s.c. UPN1 [36].

These results indicate that patients with MCL could benefit from treatment targeting protein farnesylation. In this

study, we investigated the activity of tipifarnib in patients with relapsed/refractory MCL in a phase II, multicentric, open-label, non-randomized, non-competitive prospective clinical trial. Because of the clinical importance of patient stratification, we also evaluated the ability to predict the response to tipifarnib using previously described molecular biomarkers [38, 39] in initial biopsies of responder and non-responder patients.

Patients and methods

Patients

Adult patients aged 18 years or older, with relapsed or refractory MCL were eligible for the study. None of them should be eligible for receiving high-dose autologous stem cell transplantation. They were recruited over six centers of the GELA between July 2007 and January 2008. All the patients signed informed consent. This clinical trial has been registered in the ICMJE-approved public trial registry with the identity number NCT00847223 (http://www.ClinicalTrials.gov).

Eligibility criteria were as follows: (1) a histologically proven MCL with the presence of t (11;14) by classical karyotype or FISH analysis, and/or high expression of cyclin D1 by immunohistochemistry; (2) Eastern Cooperative Oncology Group (ECOG) performance status of 0,1 or 2; (3) adequate organ functions, defined as serum total bilirubin level lower than 1.5 times ULN, serum creatinin level lower than 15 µmol/L, serum aspartate transaminase (ASAT) level lower than 2.5 times ULN and serum alanine transaminase (ALAT) level lower than 2.5 times ULN. Moreover, the study required an absolute neutrophil count (ANC) of 10^9/L or higher, and a platelet count of $\geq 75 \times 10^9$/L or higher; and (4) at least one measurable lymph node mass in a total body CT scan, that is >1.5 cm in two perpendicular dimensions without previously irradiated or which has grown since previous irradiation.

Patients were ineligible if they were infected with the human immunodeficiency virus, pregnant, had a malignancy other than NHL within 5 years before inclusion, symptomatic peripheral neuropathy of any grade, psychiatric illness likely to interfere with participation in this clinical study. Moreover, patients were ineligible if they had anti-neoplastic (including therapeutic antibody, radioimmunoconjugates or toxin immunoconjugates) or radiation therapy within 2 week before inclusion or if they were known or suspected to be allergic to imidazole drugs.

Prior to the start of therapy, all patients had a complete history taken, and received a complete physical examination, a complete blood count, a complete biochemical test (total protein, albumin, creatinine, glucose, uric acid, total bilirubin, alkaline phasphatase, lactate dehydrogenase (LDH), alanine aminotransferase, calcium, phosphorus, and a computed tomography-scan of thorax, abdomen and pelvis.

Treatment schedule

The proposed schedule of tipifarnib administration was based on preclinical and clinical studies already reported [40–42]. Patients received tipifarnib at 300 mg administrated orally twice daily for the first 21 days of each 28-day cycle for 4 cycles (Fig. 1). Two additional cycles were administrated for patients showing at least a partial response. Toxicity was evaluated at each cycle and it was programmed that patients who developed grade 3 or higher toxicity (graded according to the NCI Common Toxicity Criteria v2.0) had their treatment interrupted, and the treatment reinstituted upon improvement of the toxicity to grade 1 or lower with a two-step dosage reduction: first step, 300 mg administered orally twice daily for the first 14 days of each 28-day cycle; second step, 200 mg administered orally twice daily for the first 14 days of each 28-day cycle.

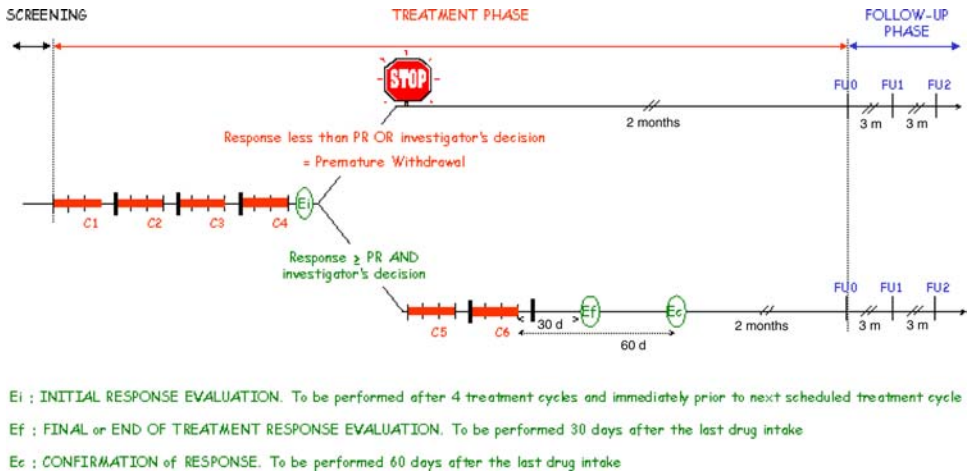

Fig. 1 Design of the therapeutic trial. Tipifarnib was administered to patients with relapsed/refractory MCL at 300 mg orally twice daily for the first 21 days of each 28-day for 4 cycles, and in case of response for 6 cycles. Follow-up was organized for the responders with a clinical evaluation every 3 months

Study design and endpoints

This multicentric, open-label, non-randomized, non-competitive prospective clinical trial was to determine disease response rate therapy of tipifarnib in subjects presenting relapsed, refractory, or progressive MCL, not able to receive high dose autologous stem cell transplantation. The primary objective of this study was to determine the response rate (complete response [CR], complete response unconfirmed [CRu], and partial response [PR]) to tipifarnib as a single agent, according to the criteria of the NCI Sponsored International Working Group guidelines [43]. The secondary objectives were to determine the overall CR rate (CR + CRu), progression-free survival (PFS), the overall survival, and to evaluate the safety and tolerability of tipifarnib.

For response and toxicity evaluation, physical examination was assessed on day 1 of each cycle before tipifarnib intake as well as biochemical tests (calcium, sodium, potassium, glucose, albumin, creatinine, LDH, ASAT, ALAT, and bilirubine). The complete blood cell counts were performed on days 1, 8, 15, and 22 of each cycle. After completion of 4 and 6 cycles of treatment, patients underwent the same biological tests with a thorax, abdomen, and pelvis CT-scan locally assessed by the radiologist of each center. Blood immunophenotyping and bone marrow aspirate or biopsy were performed if indicated.

Sample consideration and statistical analyses

Sample size was calculated according to an optimal two-stage design [44] with a NCSS-PASS 2002 software. The following hypotheses were used: a disease response rate less or equal to 10% if the drug is not effective (H0), and a disease response rate at 35% or more if the drug is effective (H1), with alpha and beta risk of 5 and 10%, respectively. Eleven patients were necessary in the first stage and 16 in the second stage that is a total of 27 patients in absence of early termination. According to ICH E9 recommendations (http://www.ich.org), all patients included in the trial and who receive any amount of tipifarnib were considered for the efficacy and safety analyses.

Correlative biological analyses for prediction of response to tipifarnib

We secondly analyzed response prediction to tipifarnib in responder and non-responder patients. Lymphoma biopsies realized at diagnosis or during the evolution of the disease were retrospectively collected. The expression of *AKAP13*, *RASGRP1*, and *APTX* genes were quantified by RT-qPCR using fresh frozen tissues or cells. A hyperplasic non-malignant lymph node was used as an external calibrator for the real time-qPCR.

Briefly, total RNA was isolated either from 20 fresh frozen tissue sections of 10 µm in thickness or from frozen cells obtained after fine needle aspirate, using mirVana kit (Ambion) according to the manufacturer's instructions. The RNA quantity was assessed by OD_{260}. For each sample, 1 µg total RNA was reversed-transcripted using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems) according to the manufacturer's instructions. Primers and MGB-probes for *AKAP13* and control gene *HMBS* were those described before by Raponi et al. [38, 39], while those for *RASGRP1* and *APTX* genes were available as Assay-on-Demand from Applied biosystems [39]. Real-time qPCR was performed using the ABI Prism 7900HT sequence detection system (Applied Biosystems) with all samples run in duplicate. Each reaction contained 10 µl TaqMan Universal PCR Master Mix containing uracil-N-glycosylase (Applied biosystems), 2 µl of previously synthesized cDNA and 1 µl of 20× Assay-on-Demand Gene Expression Assay Mix (Applied Biosystems) or 9 pmol both forward and reverse primer and 2.5 pmol probe in a total volume of 20 µl. For each gene, the comparative Ct method was adopted for relative expression evaluation [45]. The *RASGRP1/APTX* gene expression ratio was reckoned with the 2^-ΔΔCt formula using the non-malignant hyperplasic lymph node as control and using [ratio = 2^-((*RASGRP1* sample − *RASGRP1* control) − (*APTX* sample − *APTX* control))].

Results

Clinical characteristics of the patients

Eleven patients with relapsed/refractory or progressive MCL were enrolled between June 2007 and January 2008. The median age was 71 (range 66–79.5) (Table 1). All patients presented with stage III–IV disease and with good performance status. The median number of prior cytotoxic chemotherapy regimens was 3 (range 1–11). All patients received prior to the inclusion a combined chemotherapy based on anthracyclin and rituximab, and/or a combined chemotherapy associating cytarabine and rituximab, except one. One patient had received intensive chemotherapy with autologous stem cell transplantation before tipifarnib.

Treatment received and response to tipifarnib

Patients received a median of 4 cycles (range 1–6 cycles) of tipifarnib. One patient achieved a CR at 4 cycles. At the time of inclusion, this patient presented with a bulky disease including peripheral and visceral adenopathies at 4 cm, splenomegaly with nodular infiltration, and left pleural

Table 1 Patients characteristics ($n = 11$)

Characteristic	No.	%
Sex		
Male	8	72
Female	3	27
Age (years)		
Median	71	
Range	66–79.5	
ECOG PS		
0–1	11	100
≥2	0	0
LDH		
Normal	4	36
Elevated	7	63
Stage		
III	3	27
VI	8	72
Prior systemic therapy regimens		
1	1	9
2	4	36
3	2	18
4	1	9
≥5	2	18
HDT/ASCT	1	9
Additional radiotherapy	2	18

ECOG PS Eastern Cooperative Oncology Group performance status, *LDH* lactate dehydrogenase, *HDT* high-dose therapy, *SCT* autologous stem-cell transplantation

effusion (Fig. 2). He presented, however, with a good performance status (PS = 1). B symptoms were present with night sweats. LDH level was normal. Blood counts were normal. An objective clinical response was seen after the first cycle. After 4 cycles, clinical examination showed a disappearance of cervical adenopathy, disappearance of the splenomegaly and a normal pulmonary exam. Body CT-scan confirmed the complete response (Fig. 2). This patient received two additional cycles. The clinic evaluation at 6 cycles showed a persistence of CR.

The 10 other patients presented a progressive disease. Two patients progressed very early during or after the first cycle. Nine of 11 patients (81%) experienced a freedom-from progression (FFP) for ≥2 cycles, and one patient experienced a FFP for ≥4 cycles. Three patients (27%) progressed after 3 cycles and 5 (46%) patients after 4 cycles. Median PFS for the 10 patients that progressed under treatment was 88.5 days (range 21–177 days). Median PFS for all patients was 3 months (range 0.7–14.2). The OOR was 9%.

At the time of writing, seven patients died. Median time to death was 10.5 months (range 0.6–14 months). Four patients are alive, with a median follow-up of 13 months (range 11.3–14.7 months).

Result of the first interim analysis

The first interim analysis was realized after the completion of 4 cycles of treatment and concerned the 11 first patients. Response rate was evaluated at 9%, with 1 patient in CR and 10 patients in progressive disease. This reached the H0 hypothesis corresponding for tipifarnib to a non-efficient drug for patients with relapsed/refractory or progressive MCL. The decision was made to interrupt the clinical trial at this point of the trial.

Toxicity profile

No grade III–IV hematological toxicities were recorded. No reduction of doses was realized during the treatment. Peripheral neuropathy was not observed. One patient presented an unrelated neurological symptom after the first dose administered but no side effect was recorded at the tipifarnib reintroduction a few days later. Nausea and vomiting were not reported by the patients. In total, tipifarnib presented an excellent safety profile.

Molecular biomarkers predicts response to tipifarnib

We investigated whether the tipifarnib predictors of response used in acute myeloid leukemia could be applied to predict the response to tipifarnib in the context of MCL. We collected fresh frozen tumoral tissues from 3 of the 11 MCL patients enrolled in the clinical trial (1 responder [named MCL-1] and 2 non-responders [named MCL-2 and MCL-3]). These were all initial biopsies taken prior to any treatment. The relative expressions were determined by the comparative Ct method using *HMBS* Ct as control gene Ct. Relative expression of *APTX* gene was lower in responder than in non-responders tumor biopsies, whereas relative expressions of *RASGRP1* were higher in responder than in non-responders. The *RASGRP1/APTX* ratio was increased in the responder (2.89) and was decreased in the non-responders (0.28 and 0.32, for MCL-2 and MCL-3 patient, respectively) (Fig. 3). Relative expression of *AKAP13* gene was lower in responder (1.33) than in non-responders (2.69 and 3.49, for MCL2 and MCL3) tumor biopsies.

Discussion

We report for the first time the result of tipifarnib administered as single-agent for 21 consecutive days of 28-day cycles in patients with relapsed/refractory or progressive

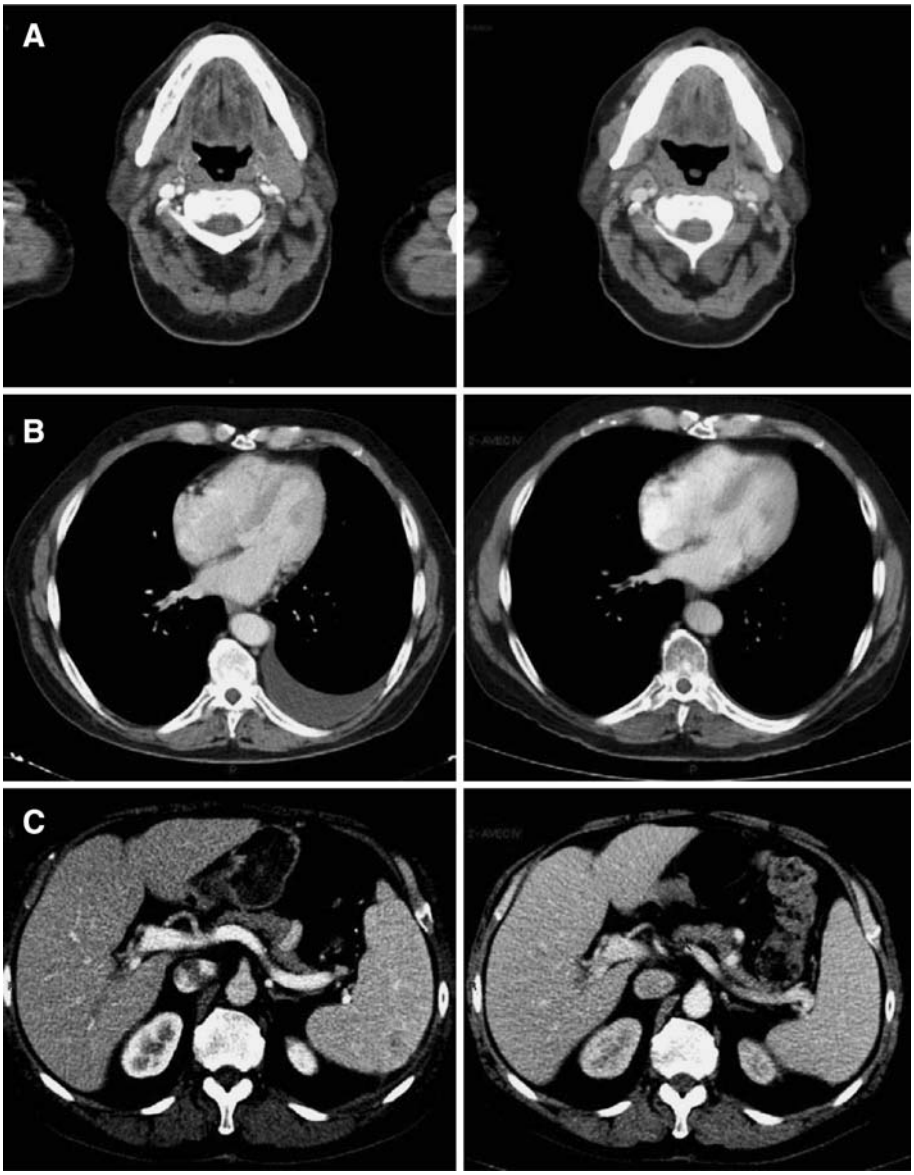

Fig. 2 CT scan features of the responder patient at inclusion and after 4 cycles. Before tipifarnib administration, the patient presented a bulky disease with superficial and visceral adenopathies, particularly at the cervix (**a**), left pleural effusion (**b**), and a splenomegaly with nodular infiltration (**c**). After 4 cycles, CR was observed and persists after 6 cycles

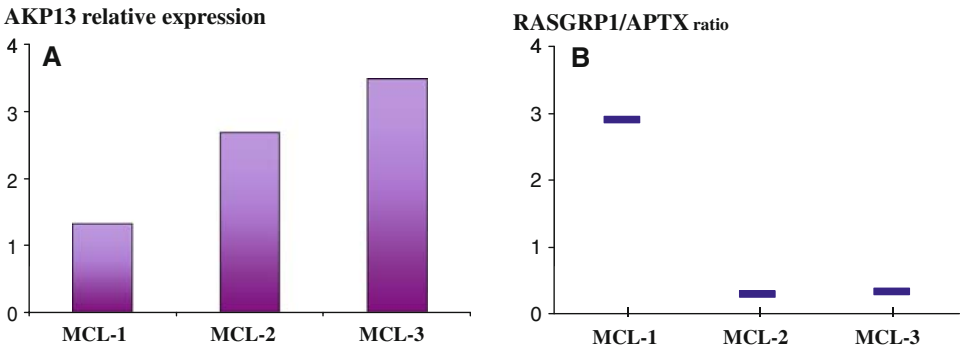

Fig. 3 Response prediction to tipifarnib. Prediction of response was retrospectively evaluated in the initial tumor biopsy by the analysis of the *AKAP13* expression level (**a**) and the *RASGRP1/APTX* gene expression ratio (**b**). The *RASGRP1/APTX* gene expression ratio was higher in the responder while the *AKAP13* expression was higher in the non-responders. This corresponds to the expected result to the response prediction to tipifarnib

MCL. At the first interim analysis when 11 patients were included, no adverse side effects were recorded but only one patient achieved a CR (9%) while the other patients presented a progressive disease. This result led to an early interruption of the trial.

This trial is based on the result of a GEP analysis comparing three subtypes of non-follicular B cells lymphomas including MCL, marginal zone lymphoma (MZL) and small lymphocytic lymphoma (SLL) [19]. The FTase gene was found to be significantly overexpressed in MCL samples compared to MZL and SLL samples. Efficacy of FTase inhibition was analyzed in MCL models, in vitro as well as in vivo [36], prior to designing the clinical trial. However, results of the clinical trial are disappointing with only a response in one patient, even if the response was a CR. To our knowledge, only two clinical trials have been set up in the same context of post-GEP analyses in lymphomas, and concerned diffuse large B-cells lymphomas (DLBCL) [46–48]. The first trial evaluated the efficacy of a PKCβ inhibitor administered as single agent in 55 patients with refractory DLBCL [47]. Overall response rate was reported at 7% (4/55 patients), including 3 CR and 1 stable disease. The second trial evaluated the efficacy of a SYK (spleen tyrosine kinase) inhibitor administered as single agent in 59 patients with refractory B-cell NHL. In refractory DLBCL, the subtype of lymphoma initially analyzed by GEP, the overall response rate was evaluated at 23% (1 CR, 4 PR out of 23 patients) [48].

These results demonstrated the absolute necessity of molecularly categorizing patients to select those that might respond to targeted therapies. Raponi et al. [38, 39] have already performed pharmacogenomic analysis to identify GEP signatures associated with response to tipifarnib as single agent therapy in two previous studies: one including patients with refractory AML and one including elderly adults with newly diagnosed AML. Among these signatures, three genes, *RASGRP1*, *APTX*, and *AKAP13* were the best in predicting the response. *RASGRP1* and *AKAP13* are guanine nucleotide exchange factors (GEF) that activates RAS and RHO, respectively, meanwhile *APTX* is involved in DNA excision repair. The *RASGRP1/APTX* ratio predicted a good response to tipifarnib [39], whereas the *AKAP13* expression level predicted a poor response [38]. In our series of relapsed/refractory MCL, we showed that the two molecular predictors of response (*AKAP13* and *RASGRP1/APTX* ratio) could be used to predict the response to tipifarnib. Although only three patient biopsies could be molecularly analyzed, the results are in accordance with previous reports. More interestingly, biopsies analyzed were the biopsy prior any treatment, meaning that classical chemotherapy may have not changed profiles of tumors considering this particular pathway.

In fact, reliable biomarkers to predict response to targeted therapies have become increasing valuable in the clinical management of patients with neoplastic disorders. For example, Seidman et al. [49] demonstrated that trastuzumab administration was more beneficial in terms of overall response rate for women with HER2-overexpressing metastatic breast cancer than for women with HER2-normal expressing metastatic breast cancer. Similar results were obtained with anti-EGFR treatment in colorectal cancer where the response was correlated to an increased EGFR copy number [50]. Those observations suggest that patients might be selected for target therapy treatment based on molecular predictors. In the case of MCL, the initial evaluation *RASGRP1/APTX* ratio and *AKP13* expression level would be useful to select patients who are more likely to respond to tipifarnib. However, we can deduce from the re-analysis of GEP data in MCL (data not shown) that *RASGRP1* transcript is probably not very often overexpressed in MCL, *AKP13* being not evaluable because imprecise measurements [18].

Tipifarnib was administrated at 300 mg twice daily. While it is possible that the antineoplastic effects of tipifarnib will be enhanced by adjusting the dose or schedule, an alternative approach would be to combine tipifarnib with drugs already used in MCL treatment. On one hand, this assumption was supported by the fact that in the preclinical evaluation of tipifarnib in MCL we demonstrated that cytotoxic effects of vincristine, doxorubicine, cisplatin, cytarabine, and bortezomib were significantly increased when used in combination with tipifarnib [36]. On the other hand, the combination of tipifarnib with other drugs was already evaluated in other hematological malignancies. In a phase I clinical study of tipifarnib in combination with etoposide in patients with newly diagnosed AML, Karp et al. [51] concluded that this combination was a promising regimen in elderly adults who are not candidates for conventional induction chemotherapy. A synergistic effect when tipifarnib was administrated in combination with imatinib was also described in CML patients with Abl kinase domain mutations [52]. Regarding these interesting results, it is conceivable that the combination of tipifarnib with agents acting through a different mechanism might result in an increased response rate in MCL patients.

In conclusion, single-agent tipifarnib in patients with relapsed/refractory MCL is well tolerated but induces a moderate clinical activity. Importantly, in the few patients with available biopsies, the response to tipifarnib in MCL could be predicted by two specific molecular predictors evaluated in the initial tumor biopsies prior to therapy. Future directions in the molecular characterization of MCL may aid in the identification of patients prior to treatment.

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