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ORIGINAL ARTICLE

Farnesyltransferase inhibitor R115777 inhibits cell growth and induces apoptosis in mantle cell lymphoma

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Abstract
Introduction The cytotoxic activity of the farnesyltranseferase inhibitor R115777 was evaluated in cell lines representative of mantle cell lymphoma (MCL).
Methods Cell growth, proliferation,and apoptosis were analyzed in four human MCL cell lines (Granta, NCEB, REC, and UPN1)in presence of R115777, alone or in combination with vincristin, doxorubicin, bortezomib,cisplatin and cytarabine. Inhibition of farnesylation was determined by the appearance ofprelamin A. The antitumor activity of R115777, administered p.o. at 100, 250 and 500mg/kg, was determined in vivo in nude mice xenografted with UPN1 cells.
Results R115777inhibited the growth of MCL cell lines in vitro with inhibitory concentrations ranging between 2 and 15nM. A fifty percent decrease of cell viability was observed at concentrations comprised between 0.08 and 17μM. Apoptosis, evaluated by annexin V and activated caspase 3 staining, was induced in all cell lines, in 40 to 71% of the cells depending on the cell lines. In addition, R115777 significantly increased the cytotoxic effect of vincristine, doxorubicin, bortezomib, cisplatin and cytarabine ($p=0.001$, $p=0.016$, $p=0.006$, $p=0.014$ and $p=0.007$ respectively). Exposure of MCL cell lines to R115777 during 72 hours resulted in inhibition of protein farnesylation. R115777 administered p.o. twice daily for 8 consecutive days to mice bearing established s.c. UPN1 xenograft displayed cytostatic activity at the 500 mg/kg dosage.
Conclusion We have demonstrated that inhibition of farnesyltransferase by R115777 was associated with growth inhibition and apoptosis of MCL cell lines in vitro and tumor xenograft stability in vivo.

Keywords Farnesyltransferase · R115777 · Inhibitor · Mantle cell lymphoma

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Introduction

Mantle cell lymphoma (MCL) is a distinct entity in the World Health Organization (WHO) classification system for non-Hodgkin's lymphoma (NHL), which comprises ~5–8% of all lymphomas [30]. MCL express CD20 and CD5 antigens with a genetic hallmark, the translocation (11;14)(q13;q32) leading to the overexpression of cyclin D1 [44]. Clinically, MCL follows an aggressive clinical course with a transient and poor response to chemotherapy with progression typically occurring within 1 year after diagnosis and a median survival time of 3–4 years [7]. The best therapeutic options remain unclear [12]. Complete response (CR) to standard chemotherapy regimens is obtained in <50% of patients [26]; the role of anthracycline-containing regimen being controversial [12, 39, 47, 51],

with a higher response rate obtained with high-dose cytarabine-based regimens [39]. Purine analogs have been tested in small phase II studies with poor results, fludarabine and cladribine alone inducing a 33–41% [14, 22] and 81% overall response rate (ORR), respectively, with a median time to progression between 1.1 and 1.9 years. High-dose chemotherapy with autologous stem cell transplantation has given encouraging results with an improved overall survival [6, 25, 33, 46], particularly in first line therapy [25, 34]. Finally the monoclonal antibody rituximab has been reported to induce between 33 and 37% ORR in uncontrolled trials in MCL [11, 21], with higher response rate (up to 58%) when combined with CHOP [28] or with a combination of fludarabine, cyclophosphamide, and mitoxantrone [23].

Patients with MCL are clearly in need of innovative treatment. Gene-expression profiling (GEP) has remarkably improved our knowledge in cancer biology, particularly in B-cell lymphoma [2, 48]. Indeed, it has been shown that MCL exhibits specific molecular signatures that allow us to distinguish them from normal B-cells as well as from other B-cell lymphomas and predict patient survival [27, 45, 52]. Among the genes expressed in MCL, farnesyltransferase (FTase) is an enzyme whose α subunit transcript was found to be specifically overexpressed [52]. FTase catalyzes protein prenylation which consists in the covalent addition of a hydrophobic farnesyl (C15) group to the cysteine residue located at the COOH-terminus of several key cell cycle proteins, including most GTP-binding regulatory proteins such as members of the Ras superfamily [8, 10], several protein kinases and phosphatases, and a variety of proteins involved in nuclear integrity (lamins A and B) [17, 49] and centromere function (CENP-E/F) [3]. Prenylation is essential in the posttranslational modifications of proteins required for conversion to mature membrane-bound forms, allowing their participation in various signaling pathways regulating growth and survival [24]. Moreover, GEP has shown that the transcript expression of some FTase substrates is dysregulated, in particular for *N-Ras* whose expression is up-regulated more than tenfold in MCL tumor biopsies in comparison to non-malignant hyperplastic lymph nodes [27]. Recent studies have led to the development of a new anticancer drug class, known as FTase inhibitors (FTi) which have already demonstrated some therapeutic activity in hematological disorders in recent clinical trials [13, 31, 38, 54].

The aim of this preclinical study was to assess whether FTase could be validated as a therapeutic target in MCL. After having confirmed the overexpression of both α (FNTA) and β (FNTB) subunits of FTase transcripts by quantitative RT-PCR in tumor biopsies obtained from untreated patients with MCL, we analyzed the growth and viability of four human MCL cell lines in the presence of R115777, a competitive nonpeptidomimetic inhibitor of FTase. We also investigated the effects of R115777 in a mouse xenograft model of MCL. We showed that inhibition of FTase, as assessed by the appearance of unprocessed prelamin A, inhibited cell growth in vitro and induced apoptosis. Potentiation of antineoplastic drugs such as vincristine, doxorubicin, bortezomib, cisplatin, and cytarabine were observed in the presence of R115777. In vivo, administrations of R115777 were associated with cytostatic activity. These studies indicate that FTi possess potential antitumor activity against MCL.

Materials and methods

B-cell isolation, RNA preparation, and cDNA synthesis

Fresh-frozen tumor biopsies were obtained from 39 untreated patients after complete morphological analysis, including cytological, immunological, cytogenetic [conventional cytogenetic and fluorescent in situ hybridization (FISH)] and/or molecular analysis, to assess the diagnosis of typical MCL. All patients had signed informed consent for biopsy analysis. B-cells were isolated from these biopsies and from four hyperplastic non-neoplastic tonsils as controls. After tissue dilacerations, gradient centrifugation, and depletion of monocytes, NK cells and T cells, total RNA from B-cells was prepared using TriZol reagent (Invitrogen, Cergy Pontoise Cedex, France). For all samples, 1 μg of RNA was used to synthesize cDNA.

Quantitative real-time PCR

Levels of both FNTA and FNTB transcripts were evaluated in 39 selected biopsies and two MCL cell lines (NCEB and UPN1). Primers and TaqMan probes of FNTA, FNTB, and the reference gene PBGD were designed with the Primer Express software [4]. cDNA obtained from hyperplastic non-neoplastic tonsils were pooled and used as external calibrator. Quantitative RT-PCR was carried out in duplicate using ABI Prism 7000 Sequence Detector System (Applied Biosystems, Courtaboeuf Cedex, France). The comparative C_T method was adopted for the data analysis [20].

Chemical

R115777 (tipifarnib) and its less-active enantiomer R115776 were kindly supplied by DE (Johnson and Johnson Pharmaceutical Research and Development, Spring House, PA, USA). Solutions were prepared at 20 mM in dimethylsulfoxide (DMSO). Doxorubicin (DOX, Adriblastine®) and cytarabin (AraC, Aracytine®) were purchased from Pfizer,

New York, NY, USA. *Cis*-platinum (CDDP, Cisplatine®) and vincristin (VCR, Oncovin®) were purchased from Merck, Whitehouse Station, NJ, USA, and EG-Labo, Boulogne Billancourt Cedex, France, respectively. Bortezomib (PS-341, Velcade®) was a kind gift of Pr. Charles Dumontet (INSERM U590, Lyon, France).

Cell culture

Four human MCL cell lines were cultured as followed. Granta 519, NCEB, REC were cultured in RPMI-1640 supplemented with 2 mM L-glutamine, 10% FBS, streptomycin and penicillin whereas UPN1 was cultured in α-MEM supplemented with 2 mM L-glutamine, 10% FBS, streptomycin, and penicillin. SK-MEL-5, a melanoma cell line, served as positive control [16] and was cultured in the same conditions than UPN1.

Cell growth inhibition

Cells were treated under three conditions: (1) with R115777, (2) with its less active enantiomer R115776, (3) with DMSO during 72 h. Cell growth was assessed by cell count with trypan blue staining every 24 h during 72 h. This allowed us to define a cytostatic concentration for each cell line.

Western blot

After a 72-h incubation with cytostatic concentrations of R115777 or equivalent concentrations of DMSO, MCL cell lysates were prepared in lysis buffer (10 mM Tris–HCl, pH 7.6, 150 mM NaCl, 1% Triton-100, 1% β-mercaptoethanol, and 1 mM PMSF). Thirteen micrograms of protein was subjected to electrophoresis on SDS-polyacrylamide gels containing 10% acrylamide, transferred to nitrocellulose and probed with antibody directed against the NH_2-terminus of lamin A (sc-6215, Santa Cruz Biotechnology, Santa Cruz, CA, USA). Antigen-antibody complexes were detected using peroxidase-coupled secondary antibody with ECL reagents.

Cell viability

Cell viability was determined indirectly by the MTT assay [42]. In short, cells were seeded at a concentration of 0.2×10^6 cells/ml in a 96-well plate (20,000 cells/well) and exposed to different concentrations of R115777 and R115776. After 72 h, 20 µl of MTT (5 mg/ml) was added to each well. The absorbance of samples was measured at 550 nm. Cell viability of all MCL cell lines was analyzed three times under each condition. Cytotoxic concentrations were determined using semi-logarithmic curves.

Apoptosis assay

Mantle cell lymphoma cells were incubated with R115777 at concentrations inducing a 50% decrease in viability determined by the MTT assay. After 72 h, cells were harvested, washed three times in PBS. Phosphatidylserine externalization was evaluated by Annexin V binding as previously described [36]. Apoptotic cells were visualized by annexin V/propidium iodide double staining (Annexin V-FITC, Roche Applied Science, Meylan Cedex, France). Assays were performed in triplicate. For activated caspase-3 detection, cells were fixed on slides with 4% formaldehyde in PBS. Then cells were blocked and permeabilized for 1 h in blocking solution (0.8× PBS, 50 mM $NaCl_2$, 0.5% Triton X-100, 3% dry milk powder). Slides were incubated for 1 h with the active caspase-3 antibody diluted (1/10) in blocking solution (ab2302, Abcam, Cambridge, UK). After washes, slides were incubated for 1 h with secondary antibody (A-31572, Molecular Probes, Eugene, OR, USA), stained in DAPI diluted 1/5,000 in VECTASHIELD, and viewed under microscope.

R115777 in combination with cytotoxic drugs

Cytotoxic effects of vincristin, doxorubicin, bortezomib, cisplatin, and cytarabine on NCEB cells were examined in triplicate with or without the combination of R115777 by MTT assays. Cells were incubated for 72 h as follows (1) with various concentrations of vincristin, doxorubicin, bortezomib, cisplatin, or cytarabine alone, (2) with various concentrations of vincristin, doxorubicin, bortezomib, cisplatin, or cytarabine combined with R115777 at IC50 concentrations previously defined. The influence of combining R115777 with vincristin, doxorubicin, bortezomib, cisplatin, or cytarabine on cytotoxicity was assessed using the Student's *t*-test.

Human tumor xenografts

Five million UPN1 cells mixed with matrigel (v/v) [40] were injected as subcutaneous xenografts in female nude mice sublethally irradiated (5 Gy). At day 16 after injection, when 80% tumors had reached a volume of 100 mm^3, mice received either R115777 administered by oral gavages at doses of 100, 250, and 500 mg/kg, or H_2O. Dosing was twice daily for eight consecutive days. Tumor growth was assessed twice weekly in control and treated groups by measurements of the two largest diameters. Tumor volume was calculated using the following equation: $V = L \times S^2 \times \pi/6$, where L is the longer, and S is the shorter, of the two dimensions. The effect of the drug was determined by the growth delay as previously reported (difference in time for

the volumes of control (*C*) versus treated (*T*) tumors, $T/C \times 100$) [32].

Results

FNTA and FNTB overexpression in mantle cell lymphomas

The overexpression of FNTA and FNTB transcripts was validated in 39 MCL tumors and one MCL cell line using a quantitative RT-PCR method. Validation showed that both FNTA and FNTB mRNA were overexpressed in MCL samples and in NCEB. In the 39 MCL samples, the relative amounts of FNTA and FNTB mRNAs compared to that observed in normal B-cells ranged between 0.43 and 4.03 (mean 1.51), and 0.63 and 7.65 (mean 2.29), respectively (Fig. 1) ($P < 0.001$). Both tested MCL cell lines overexpressed FNTA and FNTB. The NCEB cells have relative amounts of 2.14 and 2.47 for FNTA and FNTB mRNAs, respectively, and UPN1 have relative amounts of 1.26 and 2.86 for FNTA and FNTB mRNAs, respectively.

R115777 reduced MCL cell growth and viability

The four MCL cell lines (Granta, NECB, REC, and UPN1) were incubated with different concentrations of R115777 and R115776 during 72 h. Concentrations needed to obtain a 50% decrease in cell count of all MCL cell lines ranged between 2 and 15 nM with R115777. Added to cell cultures at the same concentrations, R115776 did not lead to a 50% growth decrease (mean 15 ± 5% of growth decrease only). These results allowed us to conclude that R115777 has a cytostatic activity in all MCL cell lines at concentrations lower than 100 nM, in accordance with previous studies [16]. Using the MTT assay, R115777 cytotoxic concentrations were found to be ranged from 0.08 to 15 μM (Table 1). The viability of the SK-MEL-5 cell line, used as a reference line reported to be sensitive to R115777, was reduced with a concentration of 12 μM of this compound. We concluded that the cytotoxic activity of R115777 in MCL cell lines was similar to that observed in the sensitive control cell line. These cytotoxic concentrations were then used in subsequent experiments.

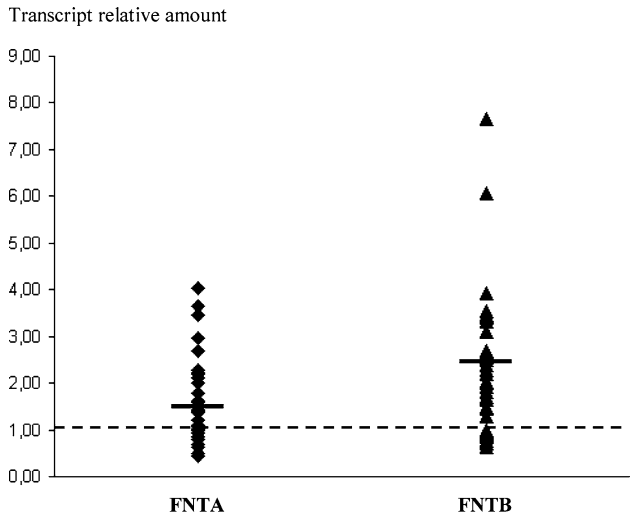

Fig. 1 Levels of both FNTA and FNTB mRNAs in MCL. Using quantitative real-time PCR, the relative amounts of FNTA and FNTB mRNAs were analyzed in 39 MCL samples and MCL cell lines compared to that observed in normal B-cells. FNTA primers were 5′-CTT CCC TTT GCC TGT GTT GTA-3′ and 5′-GTA GCA GCA GCA CCC AAG GA-3′. The FNTA probe was 5′-AAG TGC ATC ACA CAG GTA TTG CTT TTT AAC AAG AAC-3′. FNTB primers were 5′-TGG ATG TGA GAA GCG CAT ACT G -3′ and 5′-TCA AAG AGG TCT GGA GTG ATG ATG -3′. The FNTB probe was 5′-TGC CTC CGT AGC CTC GCT GAC C-3′

R115777 inhibited protein farnesylation

The effect of R115777 on prenylation was determined in all MCL cell lines by analyzing the electrophoretic profile of lamin A. In DMSO-treated cells, lamin A was essentially detected in the processed prenylated form. At cytostatic concentrations, R115777 inhibited the farnesylation of lamin A in all MCL cell lines as assessed by the increased amounts of the unfarnesylated precursor of lamin A and a decreased amount of the processed form (Fig. 2).

R115777 induced apoptosis in MCL cell lines

Apoptosis was assessed by flow cytometry using the annexin V assay in all MCL cell lines. Exposure to R115777 during 72 h resulted in an increase of the percentage of apoptotic cells in all MCL lines. The percentages of apoptotic cells were lower than 20% when cells were incubated with DMSO and rose to 40–71% after exposure to R115777. Apoptosis was also assessed using an active caspase-3 immunoassay. In the DMSO-exposed group the percentage of cells stained by the active caspase-3 antibody ranged between 6 and 9%. Exposure to R115777 resulted in a significant increase of the percentage of cells with activated caspase-3, ranging between 36 and 67% (Fig. 3) Therefore, R115777 was able to induce apoptosis in all MCL cell lines in vitro.

Synergistic effects of R115777 with vincristine, doxorubicin, bortezomib, cisplatin, and cytarabine

We used MTT assays to determine the effect of combinations of R115777 with other anticancer drugs on NCEB cells. We determined the concentrations required to reduce NCEB cell viability by 50% when vincristine, doxorubicin,

Table 1 R115777 cytostatic and cytotoxic concentrations on five MCL cell lines

Cell lines	R115777 cytostatic concentrations (C)					IC50 of R115777	
	C (nM)	R115776		R115777			
		Relative cell count (%)	Standard deviation	Relative cell count (%)	Standard deviation	Median (μM)	Standard deviation
Granta	8	108	10	57	6	2.7	1.5
NCEB	15	72	5	40	9	15	3.4
REC	2	83	8	48	4	0.08	0.02
UPN1	2	88	7	47	3	13.8	3.9
SKMEL 5	nd	nd	nd	nd	nd	12	3.9

Cytostatic concentrations: cell proliferation percentages were obtained by cell counts in blue trypan solution with DMSO-treated cells as references. Cell counts were performed in triplicate. Cytotoxic concentrations: median cytotoxic concentrations were determined by MTT assays in each MCL cell lines. MTT assays were performed three times

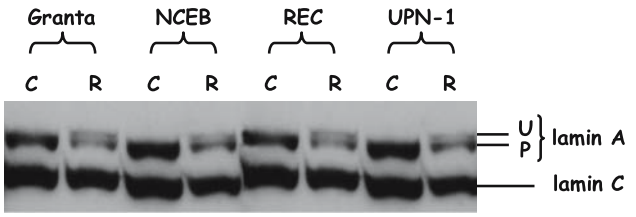

Fig. 2 Effects of R115777 on lamin A farnesylation. Increases of unfarnesylated precursor of lamin A in MCL cells treated with R115777 at cytostatic concentrations (R) compared with cells treated with DMSO (C). "U" and "P," unprocessed and processed proteins, respectively

bortezomib, cisplatin, and cytarabine were used alone or in combination with R115777 at IC20 values previously determined during MTT assays. Each experiment was repeated three times. The use of R115777 in combination with vincristine, doxorubicin, bortezomib, cisplatin, and cytarabine significantly enhanced their cytotoxic properties (0.11 nM vs. 0.55 nM, $P = 0.001$, 0.08 μM vs. 0.21 μM, $P = 0.016$, 5.4 nM vs. 12.6 nM, $P = 0.006$, 0.14 μM vs. 7.8 μM, $P = 0.014$, and 0.16 μM vs. 32.9 μM, $P = 0.007$, respectively), on MCL cells (Fig. 4).

R115777 in vivo

We studied the in vivo antitumor activity of R115777 in mice bearing s.c. UPN1 human MCL xenografts, using twice daily oral dosing for eight consecutive days. Dosing did not start until tumors were palpable (diameter of 5 mm). Groups of five mice were treated with R115777 at doses of 100, 250, and 500 mg/kg. Results showed that R115777 showed activity at 500 mg/kg ($P = 0.0013$), but had no activity at lower doses. There was no tumor shrinkage observed at any dose tested, but a cytostatic effect was observed in this model during, as well as 6 days after, therapy (Fig. 5).

A

Cell lines	Control	R115777
Granta	6%	67%
NCEB	7%	58%
REC	6%	63%
UPN1	9%	36%

B

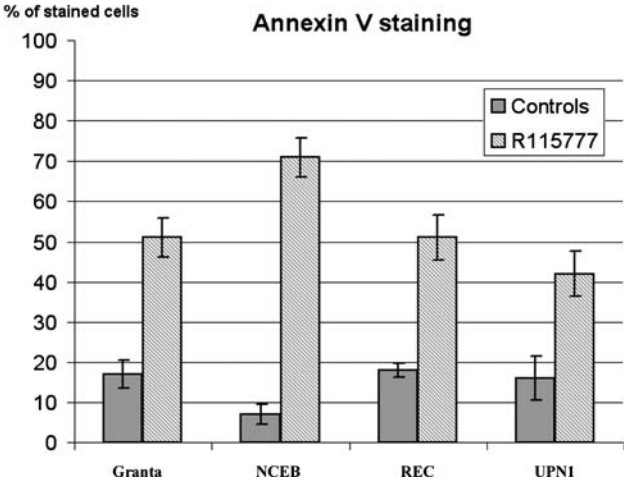

Fig. 3 Caspase-3 activation and annexin V staining in MCL cell lines treated with R115777. Activation of caspase-3 was assessed by indirect immunofluorescence. **a** Percentage of cells stained with caspase-3 activated antibody in presence of DMSO (vehicle) or R115777 at cytotoxic concentrations. **b** Percentage of cells stained with annexin V in presence of DMSO (vehicle) or R115777 in the four MCL cell lines analyzed

Discussion

Mantle cell lymphoma is a particularly chemoresistant subtype of B-cell lymphoma. Since the initial description of the translocation $t(11;14)$, the genetic hallmark of MCL

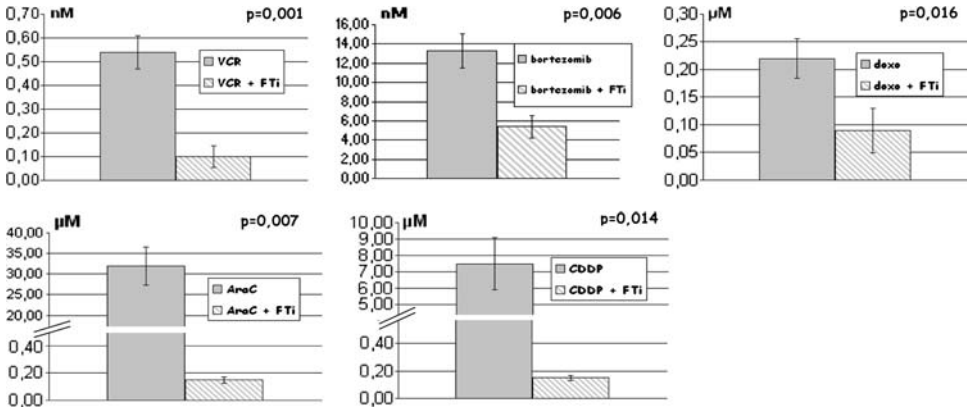

Fig. 4 Combination of vincristine, doxorubicin, bortezomib, cisplatine, and cytarabine with R115777. *Histograms* represent the concentration of each drug needed to reduce NCEB cell viability by 50% when vincristine (*VCR*), doxorubicin (*doxo*), bortezomib, cisplatin (*CDDP*), and cytarabine (*AraC*) were used alone or in combination with R115777 (FTi)

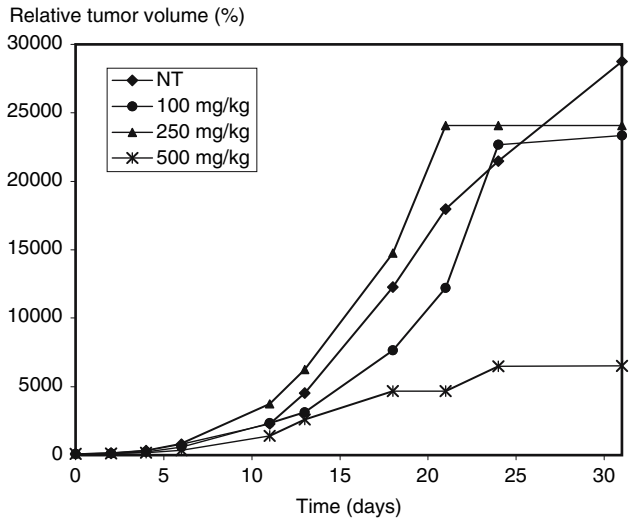

Fig. 5 Tumor growth curves of UPN1 human MCL xenografts Mice were treated with either H_2O (NT), or with varying doses of R115777 administrated twice daily at 100, 250, or 500 mg/kg for eight consecutive days

leading to deregulation and overexpression of *cyclin D1* [53], a number of additional oncogenic genetic events has been described by GEP approaches in MCL, with two pathogenic events emerging: the deregulation of cell cycle machinery and/or interference with the cellular response to DNA damage [19, 27, 41, 45, 52]. Moreover, these analyses described the overexpression of the FTase transcript in MCL tumors [52]. FTase catalyzes the first and essential posttranslational modification of several key cycle cell proteins [3, 8, 10, 18, 49] and specific inhibitors were developed as antitumor drugs. Among them, R115777 is a non-peptidomimetic competitive FTi that inhibits the proliferation of a variety of human tumor cell lines [16] and there are rapidly developing evidences for the use of R115777 in a wide range of hematological malignancies, including acute myeloid leukemia (AML) [31], chronic myeloid leukemia (CML) [13], myelodysplastic syndrome (MDS) [38], and aggressive NHL [54].

Using quantitative RT-PCR, we validated the overexpression of FTase transcripts of both subunits in MCL tumor biopsies and in our in vitro MCL model. These results prompted us to evaluate the effects of FTase activity inhibition by R115777 on MCL cell lines. In our study, we showed that R115777 induced significant growth inhibition of four MCL cell lines after 72 h of incubation. In agreement with End et al., who demonstrated the sensitivity of several human solid tumor cell lines to R115777 in vitro with 50% reduction in cell counts at concentrations lower than 100 nM [16], we concluded that MCL cell lines are sensitive to the antiproliferative effect of R115777. We specifically measured the difference of FT enzymatic activity with or without FTi by analyzing the prenylation of a targeted protein, lamin A, of which the unprenylated precursor is accumulated in MCL cells exposed to R115777 [1].

We further demonstrated that R115777 induced a cytotoxic effect in human MCL cell lines in vitro. IC50 values of R115777, ranging between 0.08 and 15 µM, were similar to that observed in the sensitive control cell line, SK-MEL-5 [16]. This cytotoxic effect was associated with apoptosis induction assessed by an increase of annexin V staining [17] and by the activation of caspase 3 in the FTi treated cells. This induction of caspase-3 activity could be implicated in the molecular mechanisms underlying the cytotoxic effects of FTi in MCL. Quantitative expression analysis of cell cycle and apoptosis-associated genes has already demonstrated that bcl-2 was up-regulated in 60% of MCL tumors [37]. Bcl-2 is an antiapoptotic protein, which is known to be a substrate of caspase-3 [9] and the Bcl-2 cleavage product promotes apoptosis with release of cytochrome c [35]. FTi have already been described to induce cytochrome c release from mitochondria in transformed cells [50]. However, some studies indicate that R115777 induces apoptosis via multiple intrinsic pathways. Even if the mitochondrial pathway is well documented, cell death induced by R115777 involves additional pathways that may cooperate with but are independent of mito-

chondrial apoptosis [5]. This cooperation of multiple pathways in R115777-induced apoptosis was probably the result of multiple mitogenic pathway inhibition related to the multiple FTase protein targets (Ras, Rho, Rheb, CENP-E/F...) [3, 10, 15, 29]. To identify biological pathways affected by FTi treatment, global analysis such as GEP could be useful. Indeed, effects of R115777 on AML cells revealed the combination of down-regulation of genes involved in proliferation and up-regulation of genes involved in apoptosis activation [43]. While FTi are originally designed to inhibit the Ras pathway, it becomes clear that other targets exist which could be interesting regarding their anticancer properties.

FTase inhibitors represent a promising class of small molecule inhibitors of cell signaling in MCL. Like other novel agents that inhibit key signaling proteins, these compounds may be more effective in combination with cytotoxic chemotherapy. Several preclinical studies have demonstrated cytotoxic synergy when A549 lung adenocarcinoma, T98G glioblastoma, BxPC-3 pancreatic, HCT-116 colon carcinoma cell lines were exposed to FTi in combination with some well-known anticancer drugs such as cisplatin, taxol, and gemcitabine. Moreover, MCL are particularly chemoresistant lymphomas and therapeutic regimens include a combination of several anticancer drugs. This prompted us to investigate whether R115777 combinations would be more effective. The present preclinical study demonstrated that R115777 synergized with vincristine, doxorubicin, bortezomib, cisplatin, and cytarabine to induce a decrease in viability in MCL cell lines. We showed that there was a synergistic effect with significantly enhanced cytotoxicity of vincristine, doxorubicin, bortezomib, cisplatin, and cytarabine when MCL cells were treated with those drugs in combination with R115777.

In this study, we were also able to investigate R115777 effects in vivo. Sublethally irradiated nude mice were xenografted with subcutaneous injections of one MCL cell line, UPN1. After 16 days of tumor growth, mice received either drug vehicle (H_2O) or varying doses of R115777 administrated by oral gavages. Dosing was twice daily for eight consecutive days. R115777 showed activity at 500 mg/kg, but no activity at inferior doses. In UPN1 human MCL xenografted mice, we observed only a cytostatic effect of R115777 during and also 6 days after therapy. In accordance with previous data in several human tumor xenografted models in nude mice, FTi effects consisted essentially in a marked decrease of tumor growth relative to controls rather than a reduction in tumor volume that would correspond to a cytotoxic effect. As a consequence of this cytostatic effect and of MCL's chemoresistant profile, the optimal clinical use of R115777 in MCL patients will probably be in combination with cytotoxic agents.

In summary, the FTi R115777 induced cell growth arrest and apoptosis in MCL cell lines and also displays cytostatic effects in a MCL mice xenograft model when administrated per os twice a day dosing at 500 mg/kg. On the basis of these preclinical results, R115777 could be investigated in clinical studies in patients with MCL.

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