

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

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Abstract

The cytotoxic activity of the farnesyltransferase inhibitor R115777 was evaluated in cell lines representative of mantle cell lymphoma (MCL). 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 of prelamin 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. R115777 inhibited 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. 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.

MESH Keywords Animals ; Antineoplastic Agents ; administration & dosage ; pharmacology ; Antineoplastic Combined Chemotherapy Protocols ; pharmacology ; Apoptosis ; drug effects ; Cell Line, Tumor ; Cell Proliferation ; drug effects ; Cell Survival ; drug effects ; Dose-Response Relationship, Drug ; Drug Synergism ; Farnesyltransferase ; antagonists & inhibitors ; Female ; Humans ; Lymphoma, Mantle-Cell ; drug therapy ; Mice ; Mice, Nude ; Nuclear Proteins ; drug effects ; metabolism ; Protein Precursors ; drug effects ; metabolism ; Quinolones ; administration & dosage ; pharmacology ; Xenograft Model Antitumor Assays

Author Keywords farnesyltransferase ; R115777 ; inhibitor ; mantle cell lymphoma

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 approximately 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 follow an aggressive clinical course with a transient and poor response to chemotherapy with progression typically occurring within one year after diagnosis and a median survival time of 3 to 4 years (7). The best therapeutic options remain unclear (12). Complete response (CR) to standard chemotherapy regimens is obtained in less than 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% to 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 signalling 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 10 fold 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 farnesyltransferase 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 farnesyltransferase (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 analysed the growth and viability of 4 human MCL cell lines in the presence of R115777, a competitive non-peptidomimetic inhibitor of farnesyltransferase. 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.

MATERIAL 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 4 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, France). For all samples, 1 μ g of RNA was used to synthesise cDNA.

Quantitative real-time PCR

Levels of both FNTA and FNTB transcripts were evaluated in 39 selected biopsies and two MCL cell line (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 were carried out in duplicate using ABI Prism 7000 Sequence Detector System (Applied Biosystems, 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, USA). Solutions were prepared at 20 mM in dimethyl-sulfoxide (DMSO). Doxorubicin (DOX, Adriblastine®) and cytarabine (AraC, Aracytine®) were purchased from Pfizer. Cis-platinum (CDDP, Cisplatine®) and vincristin (VCR, Oncovin®) were purchased from Merck and EG-Labo, 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 2mM L-glutamine, 10% FBS, streptomycin and penicillin whereas UPN1 was cultured in α -MEM supplemented with 2mM 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 3 conditions: 1/with R115777, 2/with its less active enantiomer R115776, 3/with DMSO during 72 hours. Cell growth was assessed by cell count with trypan blue staining every 24 hours during 72 hours. This allowed us to define a cytostatic concentration for each cell line.

Western blot

After a 72 hour-incubation with cytostatic concentrations of R115777 or equivalent concentrations of DMSO, MCL cell lysates were prepared in lysis buffer (10mM Tris-HCl, pH7.6/150mM NaCl/1% Triton-100/1% β -mercaptoethanol/1mM PMSF). Thirteen micrograms

of protein were subjected to electrophoresis on SDS-polyacrylamide gels containing 10% acrylamide, transferred to nitrocellulose and probed with antibody directed against the NH₂-terminus of lamin A (sc-6215, Santa Cruz Biotechnology). 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 hours, 20 μ L of MTT (5mg/ml) were added to each well. The absorbance of samples was measured at 550 nm. Cell viability of all MCL cell lines was analysed three times under each condition. Cytotoxic concentrations were determined using semi-logarithmic curves.

Apoptosis assay

MCL cells were incubated with R115777 at concentrations inducing a 50% decrease in viability determined by the MTT assay. After 72 hours, 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). 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 permeabilised for 1 hour in blocking solution (0.8 \times PBS/50mM NaCl₂/0.5% triton \times 100/3% dry milk powder). Slides were incubated 1 hour with the active caspase-3 antibody diluted (1/10) in blocking solution (ab2302, Abcam). After washes, slides were incubated for 1h with secondary antibody (A-31572, Molecular Probes), stained in DAPI diluted 1/5000 in VECTASHIELD and viewed under the 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 during 72 hours as followed 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 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 (5Gy). At day 16 after injection, when 80% tumors had reached a volume of 100mm³, mice received either R115777 administered by oral gavages at doses of 100, 250 and 500mg/kg, or H₂O. Dosing was twice daily for 8 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 (Figure 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 4 MCL cell lines (Granta, NECB, REC, and UPN1) were incubated with different concentrations of R115777 and R115776 during 72 hours. Concentrations needed to obtain a fifty percent decrease in cell count of all MCL cell lines ranged between 2 to 15nM with R115777. Added to cell cultures at the same concentrations, R115776 did not lead to a fifty percent growth decrease (mean=15% \pm 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 100nM, in accordance with previous studies (16). Using the MTT assay, R115777 cytotoxic concentrations were found to be ranged from 0.08 to 15 μ M (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 μ M 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.

R115777 inhibited protein farnesylation

The effect of R115777 on prenylation was determined in all MCL cell lines by analysing 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 (Figure 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 hours 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 to 67% (Figure 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 anti-cancer drugs on NCEB cells. We determined concentrations required to reduce NCEB cell viability by 50% when vincristine, doxorubicin, 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 (0.11nM vs 0.55nM, $p=0.001$, 0.08 μ M vs 0.21 μ M, $p=0.016$, 5.4nM vs 12.6nM, $p=0.006$, 0.14 μ M vs 7.8 μ M, $p=0.014$ and 0.16 μ M vs 32.9 μ M, $p=0.007$ respectively) on MCL cells (Figure 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 8 consecutive days. Dosing did not start until tumors were palpable (diameter of 5mm). Groups of 5 mice were treated with R115777 at doses of 100, 250 and 500mg/kg. Results showed that R115777 showed activity at 500mg/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 (Figure 5)

DISCUSSION

Mantle cell lymphoma (MCL) is a particularly chemoresistant subtype of B-cell lymphoma. Since the initial description of the translocation t(11;14), the genetic hallmark of MCL 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 (27, 41, 45, 52, 19). Moreover, these analyses described the overexpression of the farnesyltransferase (FTase) transcript in MCL tumors (52). FTase catalyses 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 it is rapidly developing evidences in 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 non-Hodgkin lymphomas (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 hours incubation. In agreement with D. End et al, who demonstrated sensitivity of several human solid tumor cell lines to R115777 in vitro with 50% reduction in cell counts at concentrations lower than 100nM (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 μ M, 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 have 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 mitochondrial 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 anti-cancer properties.

FTis represent a promising class of small molecule inhibitors of cell signalling in MCL. Like other novel agents that inhibit key signalling 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 anti-cancer drugs, such as cisplatin, taxol and gemcitabine. Moreover, MCL are particularly chemoresistant lymphomas and therapeutic regimens include a combination of several anti-cancer 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 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 (H₂O) or varying doses of R115777 administrated by oral gavages. Dosing was twice daily for 8 consecutive days. R115777 showed activity at 500mg/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 500mg/kg. On the basis of these preclinical results, R115777 could be investigated in clinical studies in patients with MCL.

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References:

1. Adjei AA , Davis JN , Erlichman C , Svingen PA , Kaufmann SH Comparison of potential markers of farnesyltransferase inhibition. *Clin Cancer Res.* 6: 2318- 2325 2000;
2. Alizadeh AA , Eisen MB , Davis RE , Ma C , Lossos IS , Rosenwald A , Boldrick JC , Sabet H , Tran T , Yu X , Powell JI , Yang L , Marti GE , Moore T , Hudson J , Lu L , Lewis DB , Tibshirani R , Sherlock G , Chan WC , Greiner TC , Weisenburger DD , Armitage JO , Warnke R , Levy R , Wilson W , Grever MR , Byrd JC , Botstein D , Brown PO , Staudt LM Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature.* 403: 503- 511 2000;
3. Ashar HR , James L , Gray K , Carr D , Black S , Armstrong L , Bishop WR , Kirschmeier P Farnesyl transferase inhibitors block the farnesylation of CENP-E and CENP-F and alter the association of CENP-E with the microtubules. *J Biol Chem.* 275: 30451- 30457 2000;
4. Baseggio L , Bienvenu J , Charlot C , Picollet J , Felman P , Coiffier B , Salles G Higher LPS-stimulated TNF-alpha mRNA levels in peripheral blood mononuclear cells from non-Hodgkin's lymphoma patients. *Exp Hematol.* 29: 330- 338 2001;
5. Beaupre DM , Cepero E , Obeng EA , Boise LH , Lichtenheld MG R115777 induces Ras-independent apoptosis of myeloma cells via multiple intrinsic pathways. *Mol Cancer Ther.* 3: 179- 186 2004;
6. Blay JY , Sebban C , Surbiquet C , Ouachée M , Philip I , Philip T , Biron P High-dose chemotherapy with hematopoietic stem cell transplantation in patients with mantle cell or diffuse centrocytic non-Hodgkin's lymphomas: a single center experience on 18 patients. *Bone Marrow Transplant.* 21: 51- 54 1998;
7. Campo E , Raffeld M , Jaffe ES Mantle-cell lymphoma. *Semin Hematol.* 36: 115- 127 1999;
8. Casey PJ , Solski PA , Der CJ , Buss JE p21ras is modified by a farnesyl isoprenoid. *Proc Natl Acad Sci U S A.* 86: 8323- 8327 1989;
9. Cheng EH , Kirsch DG , Clem RJ , Ravi R , Kastan MB , Bedi A , Ueno K , Hardwick JM Conversion of Bcl-2 to a Bax-like death effector by caspases. *Blood.* 278: 1966- 1968 1997;

- 10. Clark GJ, Kinch MS, Rogers-Graham K, Sebti SM, Hamilton AD, Der CJ The Ras-related protein Rheb is farnesylated and antagonizes Ras signaling and transformation. *J Biol Chem.* 272: 10608- 10615 1997;
- 11. Coiffier B, Haioun C, Ketterer N, Engert A, Tilly H, Ma D, Johnson P, Lister A, Feuring-Buske M, Radford JA, Capdeville R, Diehl V, Reyes F Rituximab (anti-CD20 monoclonal antibody) for the treatment of patients with relapsing or refractory aggressive lymphoma: a multicenter phase II study. *Blood.* 92: 1927- 1932 1998;
- 12. Coiffier B, Hiddemann W, Stein H Mantle cell lymphoma: a therapeutic dilemma. *Ann Oncol.* 6: 208- 210 1995;
- 13. Cortes J, Albitar M, Thomas D, Giles F, Kurzrock R, Thibault A, Rackoff W, Koller C, O'Brien S, Garcia-Manero G, Talpaz M, Kantarjian H Efficacy of the farnesyl transferase inhibitor R115777 in chronic myeloid leukemia and other hematologic malignancies. *Blood.* 101: 1692- 1697 2003;
- 14. Decaudin D, Bosq J, Tertian G, Nedellec G, Bennaceur A, Venuat AM, Bayle C, Carde P, Bendahmane B, Hayat M, Munck JN Phase II trial of fludarabine monophosphate in patients with mantle-cell lymphomas. *J Clin Oncol.* 16: 579- 583 1998;
- 15. Du W, Prendergast GC Geranylgeranylated RhoB mediates suppression of human tumor cell growth by farnesyltransferase inhibitors. *Cancer Res.* 59: 5492- 5496 1999;
- 16. End DW, Smets G, Todd AV, Applegate S, Fuery CJ, Angibaud P, Venet M, Sanz G, Poignet H, Skrzat S, Devine A, Wouters W, Bowden C Characterization of the antitumor effects of the selective farnesyl protein transferase inhibitor R115777 in vivo and in vitro. *Cancer Res.* 61: 131- 137 2001;
- 17. Fadok VA, Voelker DR, Campbell PA, Cohen JJ, Bratton DL, Henzon PM Exposure of phosphatidylserine on the surface of apoptotic lymphocytes triggers specific recognition and removal by macrophages. *J Immunol.* 148: 2207- 2216 1992;
- 18. Farnsworth CC, Wolda SL, Gelb MH, Glomset JA Human lamin B contains a farnesylated cysteine residue. *J Biol Chem.* 264: 20422- 20429 1989;
- 19. Fernandez V, Hartmann E, Ott G, Campo E, Rosenwald A Pathogenesis of mantle-cell lymphoma: all oncogenic roads lead to dysregulation of cell cycle and DNA damage response pathways. *J Clin Oncol.* 23: 6364- 6369 2005;
- 20. Fink L, Seeger W, Ermert L, Hänge J, Stahl U, Grimminger F, Kummer W, Bohle RM Real-time quantitative RT-PCR after laser-assisted cell picking. *Nat Med.* 4: 1329- 1333 1998;
- 21. Foran JM, Cunningham D, Coiffier B, Solal-Celigny P, Reyes F, Ghielmini M, Johnson PW, Gisselbrecht C, Bradburn M, Matthews J, Lister TA Treatment of mantle-cell lymphoma with Rituximab (chimeric monoclonal anti-CD20 antibody): analysis of factors associated with response. *Ann Oncol.* 11 : (Suppl 1) 117- 121 2000;
- 22. Foran JM, Rohatiner AZ, Coiffier B, Barbui T, Johnson SA, Hiddemann W, Radford JA, Norton AJ, Tollerfield SM, Wilson MP, Lister TA Multicenter phase II study of fludarabine phosphate for patients with newly diagnosed lymphoplasmacytoid lymphoma, Waldenström's macroglobulinemia, and mantle-cell lymphoma. *J Clin Oncol.* 17: 546- 553 1999;
- 23. Forstpointner R, Dreyling M, Repp R, Hermann S, Hänel A, Metzner B, Pott C, Hartmann F, Rothmann F, Rohrberg R, Böck HP, Wandt H, Unterhalt M, W; H German Low-Grade Lymphoma Study Group The addition of rituximab to a combination of fludarabine, cyclophosphamide, mitoxantrone (FCM) significantly increases the response rate and prolongs survival as compared with FCM alone in patients with relapsed and refractory follicular and mantle cell lymphomas: results of a prospective randomized study of the German Low-Grade Lymphoma Study Group. *Blood.* 104: 3064- 3071 2004;
- 24. Gelb MH Protein prenylation, et cetera: signal transduction in two dimensions. *Science.* 275: 1750- 1751 1997;
- 25. Haas R, Brittinger G, Meusers P, Murea S, Goldschmidt H, Wannemacher M, Hunstein W Myeloablative therapy with blood stem cell transplantation is effective in mantle cell lymphoma. *Leukemia.* 10: 1975- 1979 1996;
- 26. Hiddemann W, Unterhalt M, Herrmann R, Wöltjen HH, Kreuser ED, Trümper L, Reuss-Borst M, Terhardt-Kasten E, Busch M, Neubauer A, Kaiser U, Hanrath RD, Middeke H, Helm G, Freund M, Stein H, Tiemann M, Parwaresch R Mantle-cell lymphomas have more widespread disease and a slower response to chemotherapy compared with follicle-center lymphomas: results of a prospective comparative analysis of the German Low-Grade Lymphoma Study Group. *J Clin Oncol.* 16: 1922- 1930 1998;
- 27. Hofmann WK, de Vos S, Tsukasaki K, Wachsmann W, Pinkus GS, Said JW, Koeffler HP Altered apoptosis pathways in mantle cell lymphoma detected by oligonucleotide microarray. *Blood.* 98: 787- 794 2001;
- 28. Howard OM, Gribben JG, Neuberger DS, Grossbard M, Poor C, Janicek MJ, Shipp MA Rituximab and CHOP induction therapy for newly diagnosed mantle-cell lymphoma: molecular complete responses are not predictive of progression-free survival. *J Clin Oncol.* 20: 1288- 1294 2002;
- 29. Hussein D, Taylor SS Farnesylation of Cenp-F is required for G2/M progression and degradation after mitosis. *J Cell Sci.* 115: 3403- 3414 2002;
- 30. Jaffe ES, Harris NL, Stein H, Vardiman JW Editor: Lyon Press I World Health Organisation Classification of tumours. Pathology and genetics of tumours of hematopoietic and lymphoid tissues. 2001; 168- 170
- 31. Karp JE, Lancet JE, Kaufmann SH, End DW, Wright JJ, Bol K, Horak I, Tidwell ML, Liesveld J, Kottke TJ, Ange D, Buddhharaju L, Gojo I, Highsmith WE, Belly RT, Hohl RJ, Rybak ME, Thibault A, Rosenblatt J Clinical and biologic activity of the farnesyltransferase inhibitor R115777 in adults with refractory and relapsed acute leukemias: a phase I clinical-laboratory correlative trial. *Blood.* 97: 3361- 3369 2001;
- 32. Kelland LR, Smith V, Valenti M, Patterson L, Clarke PA, Detre S, End DW, Howes AJ, Dowsett M, Workman P, Johnston SRD Preclinical antitumor activity and pharmacodynamic studies with the farnesyl protein transferase inhibitor R115777 in human breast cancer. *Clin Cancer Res.* 7: 3544- 3550 2001;
- 33. Ketterer N, Salles G, Espinouse D, Dumontet C, Neidhardt-Berard EM, Moullet I, Bouafia F, Berger F, Felman P, Coiffier B Intensive therapy with peripheral stem cell transplantation in 16 patients with mantle cell lymphoma. *Ann Oncol.* 8: 701- 704 1997;
- 34. Khouri IF, Romaguera J, Kantarjian H, Palmer JL, Pugh WC, Korbling M, Hagemeyer F, Samuels B, Rodriguez A, Giralt S, Younes A, Przepiora D, Claxton D, Cabanillas F, Champlin R Hyper-CVAD and high-dose methotrexate/cytarabine followed by stem-cell transplantation: an active regimen for aggressive mantle-cell lymphoma. *J Clin Oncol.* 16: 3803- 3809 1998;
- 35. Kirsch DG, Doseff A, Chau BN, Lim DS, de Souza-Pinto NC, Hansford RKM, Lazebnik YA, Hardwick JM Caspase-3-dependent cleavage of Bcl-2 promotes release of cytochrome c. *J Biol Chem.* 274: 21155- 21161 1999;
- 36. Koopman G, Reutelingsperger CP, Kuijten GA, Keehnen RM, Pals ST, van Oers MH Annexin V for flow cytometric detection of phosphatidylserine expression on B cells undergoing apoptosis. *Blood.* 84: 1415- 1420 1994;
- 37. Korz C, Pscherer A, Benner A, Mertens D, Schaffner C, Leupolt E, Döhner H, Stilgenbauer S, Lichter P Evidence for distinct pathomechanisms in B-cell chronic lymphocytic leukemia and mantle cell lymphoma by quantitative expression analysis of cell cycle and apoptosis-associated genes. *Blood.* 99: 4554- 4561 2002;
- 38. Kurzrock R, Albitar M, Cortes JE, Estey EH, Faderl SH, Garcia-Manero G, Thomas DA, Giles FJ, Ryback ME, Thibault A, De Porre P, Kantarjian HM Phase II study of R115777, a farnesyl transferase inhibitor, in myelodysplastic syndrome. *J Clin Oncol.* 22: 1287- 1292 2004;
- 39. Lefrère F, Delmer A, Suzan F, Levy V, Belanger C, Djabarri M, Arnulf B, Damaj G, Maillard N, Ribrag V, Janvier M, Sebban C, Casasnovas RO, Bouabdallah R, Dreyfus F, Verkarre V, Delabesse E, Valensi F, McIntyre E, Brousse N, Varet B, Hermine O Sequential chemotherapy by CHOP and DHAP regimens followed by high-dose therapy with stem cell transplantation induces a high rate of complete response and improves event-free survival in mantle cell lymphoma: a prospective study. *Leukemia.* 16: 587- 593 2002;
- 40. Lepelletier Y, Camara-Clayette V, Jin H, Hermant A, Coulon S, Dussiot M, Arcos-Fajardo M, Baude C, Canionni D, Delarue R, Brousse N, Benaroch P, Benhamou M, Ribrag V, Monteiro RC, Moura IC, Hermine O Prevention of mantle lymphoma tumor establishment by routing transferrin receptor toward lysosomal compartments. *Cancer Res.* 67: 1145- 1154 2007;
- 41. Martinez N, Camacho FI, Algara P, Rodriguez A, Dopazo A, Ruiz-Ballesteros E, Martin P, Martinez-Climent JA, Garcia-Conde J, Menarguez J, Solano F, Mollejo M, Piris MA The molecular signature of mantle cell lymphoma reveals multiple signals favoring cell survival. *Cancer Res.* 63: 8226- 8232 2003;
- 42. Mosmann T Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods.* 65: 55- 63 1983;
- 43. Raponi M, Belly RT, Karp JE, Lancet JE, Atkins D, Wang Y Microarray analysis reveals genetic pathways modulated by tipifarnib in acute myeloid leukemia. *BMC Cancer.* 4: 56- 67 2004;
- 44. Rosenberg CL, Wong E, Petty EM, Bale AE, Tsujimoto Y, Harris NL, Arnold A PRAD1, a candidate BCL1 oncogene: mapping and expression in centrocytic lymphoma. *Proc Natl Acad Sci U S A.* 88: 9638- 9642 1991;
- 45. Rosenwald A, Wright G, Wiestner A, Chan WC, Connors JM, Campo E, Gascoyne RD, Grogan TM, Muller-Hermelink HK, Smeland EB, Chiorazzi M, Giltnane JM, Hurt EM, Zhao H, Averett L, Henrickson S, Yang L, Powell J, Wilson WH, Jaffe ES, Simon R, Klausner RD, Montserrat E, Bosch F, Greiner TC,

- Weisenburger DD , Sanger WG , Dave BJ , Lynch JC , Vose J , Armitage JO , Fisher RI , Miller TP , LeBlanc M , Ott G , Kvaloy S , Holte H , Delabie J , Staudt LM The proliferation gene expression signature is a quantitative integrator of oncogenic events that predicts survival in mantle cell lymphoma. *Cancer Cell*. 3: 185- 197 2003;
- 46. Rummel MJ , Chow KU , Karakas T , Jäger E , Mezger J , von Grünhagen U , Schalk KP , Burkhard O , Hansmann ML , Ritzel H , Bergmann L , Hoelzer D , Mitrou PS Reduced-dose cladribine (2-CdA) plus mitoxantrone is effective in the treatment of mantle-cell and low-grade non-Hodgkin's lymphoma. *Eur J Cancer*. 38: 1739- 1746 2002;
 - 47. Samaha H , Dumontet C , Ketterer N , Moullet I , Thieblemont C , Bouafia F , Callet-Bauchu E , Felman P , Berger F , Salles G , Coiffier B Mantle cell lymphoma: a retrospective study of 121 cases. *Leukemia*. 12: 1281- 1287 1998;
 - 48. Savage KJ , Monti S , Kutok JL , Cattoretti G , Neuberger D , De Leval L , Kurtin P , Dal Cin P , Ladd C , Feuerhake F , Aguiar RC , Li S , Salles G , Berger F , Jing W , Pinkus GS , Habermann T , Dalla-Favera R , Harris NL , Aster JC , Golub TR , Shipp MA The molecular signature of mediastinal large B-cell lymphoma differs from that of other diffuse large B-cell lymphomas and shares features with classical Hodgkin lymphoma. *Blood*. 102: 3871- 3879 2003;
 - 49. Sinensky M , Fantle K , Trujillo M , McLain T , Kupfer A , Dalton M The processing pathway of prelamin A. *J Cell Sci*. 107: 61- 67 1994;
 - 50. Suzuki N , Urano J , Tamanoi F Farnesyltransferase inhibitors induce cytochrome c release and caspase 3 activation preferentially in transformed cells. *Proc Natl Acad Sci U S A*. 95: 15356- 15361 1998;
 - 51. Teodorovic I , Pittaluga S , Kluin-Nelemans JC , Meerwaldt JH , Hagenbeek A , van Glabbeke M , Somers R , Bijmens L , Noordijk EM , Peeters CD Efficacy of four different regimens in 64 mantle-cell lymphoma cases: clinicopathologic comparison with 498 other non-Hodgkin's lymphoma subtypes. European Organization for the Research and Treatment of Cancer Lymphoma Cooperative Group. *J Clin Oncol*. 13: 2819- 2826 1995;
 - 52. Thieblemont C , Nasser V , Felman P , Leroy K , Gazzo S , Callet-Bauchu E , Loriod B , Granjeaud S , Gaulard P , Haioun C , Traverse-Glehen A , Baseggio L , Bertucci F , Birnbaum D , Magrangeas F , Minvielle S , Avet-Loiseau H , Salles G , Coiffier B , Berger F , Houlgatte R Small lymphocytic lymphoma, marginal zone B-cell lymphoma, and mantle cell lymphoma exhibit distinct gene-expression profiles allowing molecular diagnosis. *Blood*. 103: 2727- 2737 2004;
 - 53. Vandenberghe E , De Wolf-Peters C , van den Oord J , Wlodarska I , Delabie J , Stul M , Thomas J , Michaux JL , Mecucci C , JJ C Translocation (11;14): a cytogenetic anomaly associated with B-cell lymphomas of non-follicle centre cell lineage. *J Pathol*. 163: 13- 18 1991;
 - 54. Witzig TE , Maurer MJ , Johnston PB , Colgan JP , Kaufmann SH , Inwards DJ , Micallef IN , Ansell SM , Zent CS , Allmer C , Weiner GJ , Wooldridge JE , Link BK , Habermann TM Oral Tipifarnib (R115777) Has Single Agent Anti-Tumor Activity in Patients with Relapsed Aggressive Non-Hodgkin Lymphoma (NHL): Results of a Phase II Trial in the University of Iowa/Mayo Clinic Lymphoma SPORE (CA97274). [ASH annual meeting abstract]. *Blood*. 108: 530- 2006;

Figure 1

Levels of both FNTA and FNTB mRNAs in MCL

Using quantitative real-time PCR, the relative amounts of FNTA and FNTB mRNAs were analysed 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'.

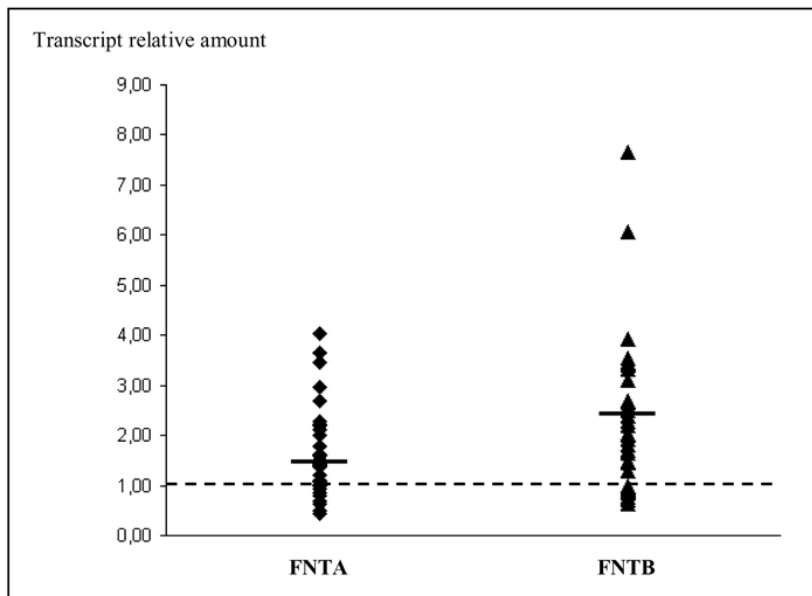


Figure 2

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.

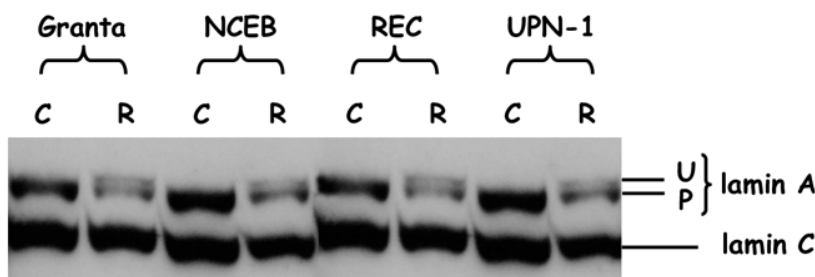


Figure 3

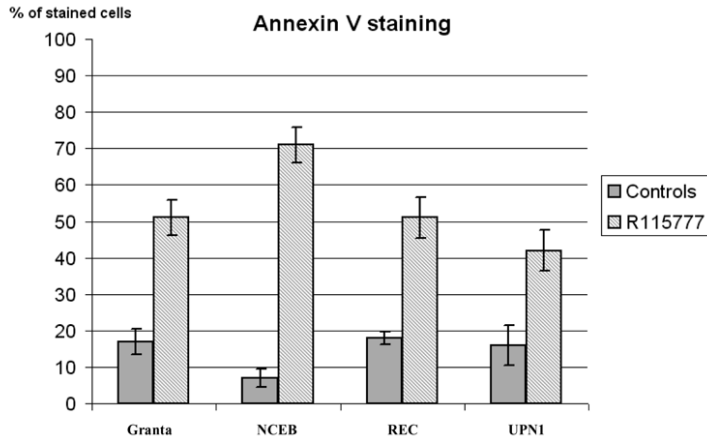
Caspase-3 activation and annexin V staining in MCL cell lines treated with R115777

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 5 MCL cell lines analyzed.

A

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

B

**Figure 4**

Combination of vincristine, doxorubicin, bortezomib, cisplatin 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).

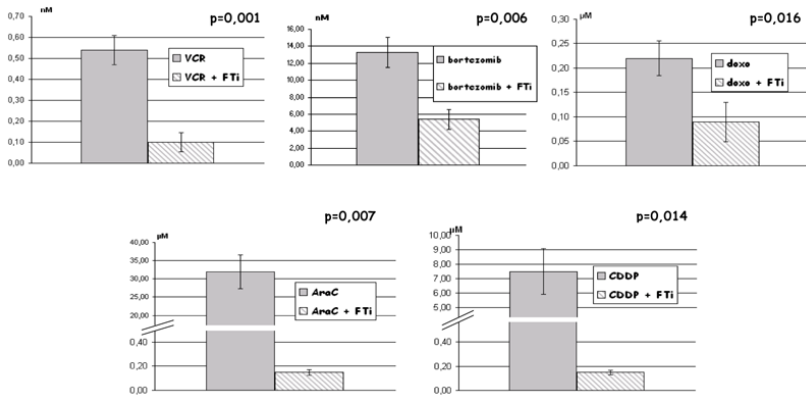


Figure 5

Tumor growth curves of UPN1 human MCL xenografts

Mice were treated with either H₂O (NT), or with varying doses of R115777 administrated twice daily at 100, 250 or 500mg/kg for 8 consecutive days.

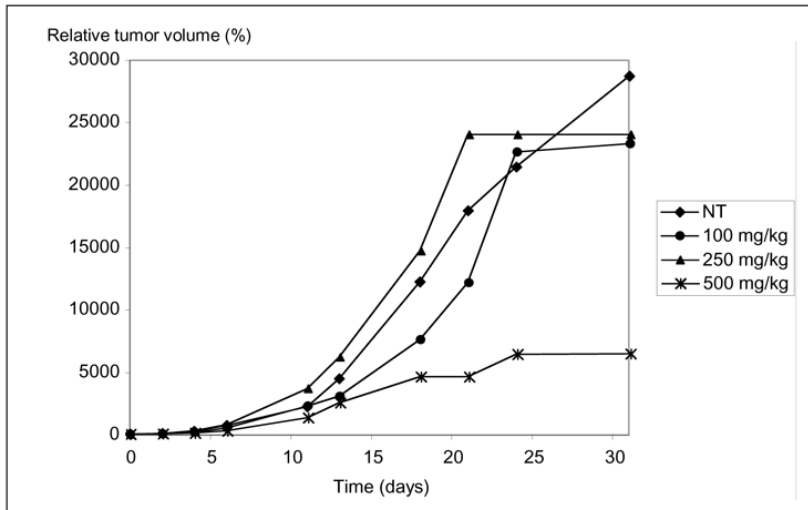


Table 1

R115777 cytostatic and cytotoxic concentrations on five MCL cell lines

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.

Cell lines	R115777 cytostatic concentrations (C)						IC50 of R115777	
	C (nM)	R115776		R115777		Median (μ M)	Standard deviation	
		Relative cell count (%)	Standard deviation	Relative cell count (%)	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	
SKMEL5	nd	nd	nd	nd	nd	12	3,9	