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ORIGINAL ARTICLE

Temsirolimus acts as additive with bendamustine in aggressive lymphoma

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Abstract The mammalian target of rapamycin (mTOR) is a protein kinase involved in the phosphatidylinositol 3-kinase (PI3K)/AKT signalling pathway. It plays a pivotal role in the control of cell proliferation, survival, and angiogenesis with multiple and frequent dysregulations of this pathway in human tumors. Temsirolimus is an intravenous drug, specifically inhibiting the mTOR pathway. Bendamustine is well known for its clinical activity in indolent non-Hodgkin-lymphoma (NHL) and has lately shown clinical activity in mantle cell lymphoma (MCL). Here, we present a case report of temsirolimus in combination with bendamustine and rituximab leading to a CR in a pretreated male. In addition, our in vitro data underlines the additive and synergistic efficacy in cell growth reduction of temsirolimus combined with bendamustine in MCL cell lines and in DLBCL cell lines. Furthermore, as an underlying mechanism of this additive, effects on cell cycle inhibition and apoptosis induction could be identified.

Keywords MCL · DLBCL · Temsirolimus · Bendamustine

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Introduction

Mantle cell lymphoma (MCL) is an aggressive subtype of B cell non-Hodgkin lymphoma (NHL) associated with a poor prognosis, comprising about 7 % of all NHL. MCL is characterized by the translocation t(11;14)(q13;q32), observed by fluorescence in situ hybridization (FISH), resulting in the overexpression of the cyclin D1 [1]. Chemotherapy is the frontline treatment for almost all patients with aggressive lymphoma such as MCL or diffuse large B cell lymphoma (DLBCL), using combination chemotherapy regimens like rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP); regimes containing high-dose cytarabine and rituximab (R-DHAP); or hyperfractionated cyclophosphamide, vincristine, doxorubicin, dexamethasone, and rituximab (hyperCVAD). Despite high initial response rates in these regimens, almost all of the patients with MCL and many with DLBCL relapse within a few years of treatment and become more difficult to treat in relapse because of evolving drug resistance in the tumor and cumulative toxicity of the agents [2, 3].

Therefore, finding efficacious treatments for relapsed or refractory disease is a goal of current research. However, with the advent of newer agents and targeted therapies, the options are expanding; the mammalian target of rapamycin (mTOR) is a pivotal multi-protein complex, responsible for processing cell signals from growth factors, hormones, and nutrients and communicating energy status commonly deregulated in human cancers. Various oncogenes and regulatory proteins for cell cycle progression and apoptosis are processed by this pathway [4, 5]. Temsirolimus is the first mTOR inhibitor that has shown clinical efficacy in relapsed and refractory MCL [6–9] and aggressive B cell NHL [10, 11]. It is reported to induce a cell cycle G1 phase arrest in various cancer cells [10, 12, 13].

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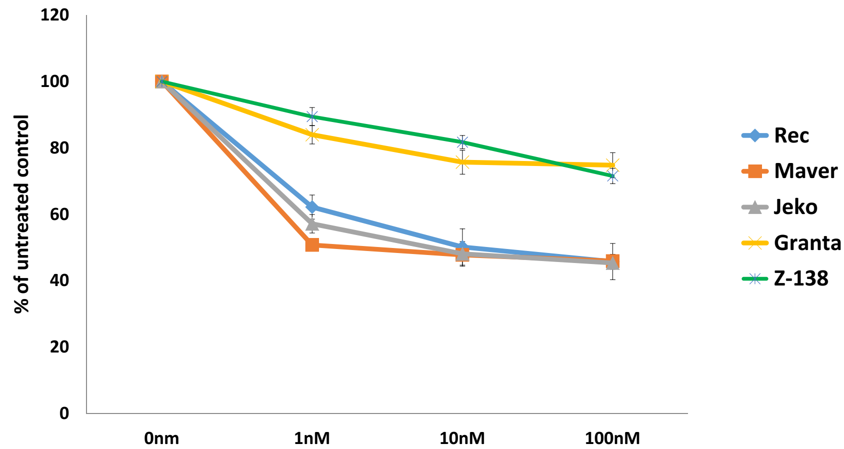

Fig. 1 Temsirolimus 72-h treatment MCL cell lines

It has shown superiority over other investigator's choice agents in heavily pretreated MCL patients [14]. However, complete remission rates are low and median duration of response is still short. These limitations may be overcome by combining temsirolimus with conventional chemotherapy agents in aggressive lymphoma.

Bendamustine is widely used in indolent NHL and is reported to have cytotoxic effects [6, 15, 16]. Further, it currently gains importance in the treatment of MCL [17–19]. To use the potential additive effects on cell growth inhibition of both drugs, combination treatment was analyzed.

We present one case report of a combination of bendamustine, rituximab, and temsirolimus leading to a CR in a patient transformed from indolent lymphoma and further in vitro data detecting additive effects not only in MCL cell lines but also in DLBCL cell lines.

Case report

A 65-year-old male with a known yet untreated B cell lymphocytic leukemia, Stadium Binet A, presented himself with a rapidly growing lymphadenopathy and unspecific abdominal pain in November 2008. The otherwise healthy patient, except for a paroxysmal atrial fibrillation, was diagnosed with a mantle cell lymphoma in an excised cervical lymph node. The further staging showed a Stadium IV E with bone marrow involvement, a diffuse infiltration of the GI tract in gastro and colonoscopy, and a generalized lymphadenopathy. The patient was treated from November 2008 to May 2009 with 6 cycles of R-CHOP resulting in a partial remission. An autologous stem cell transplantation was planned, but the patient developed a severe necrotizing fasciitis of his right upper torso after Dexa-BEAM mobilization chemotherapy and had to be

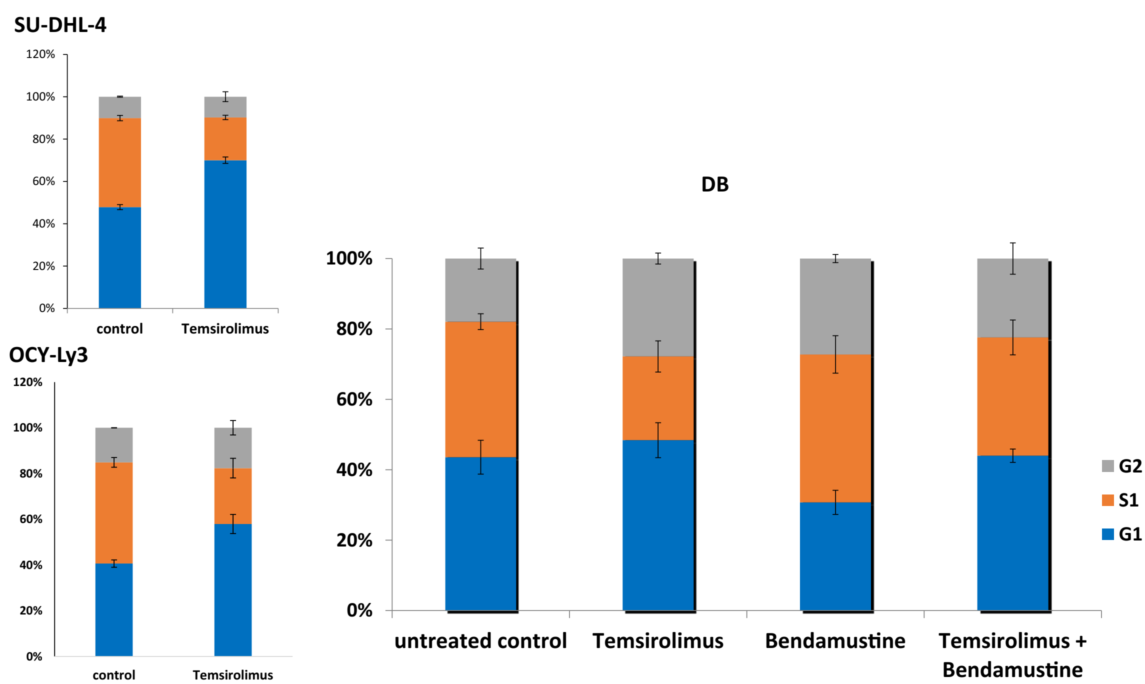

Fig. 2 Cell cycle DLBCL cell line

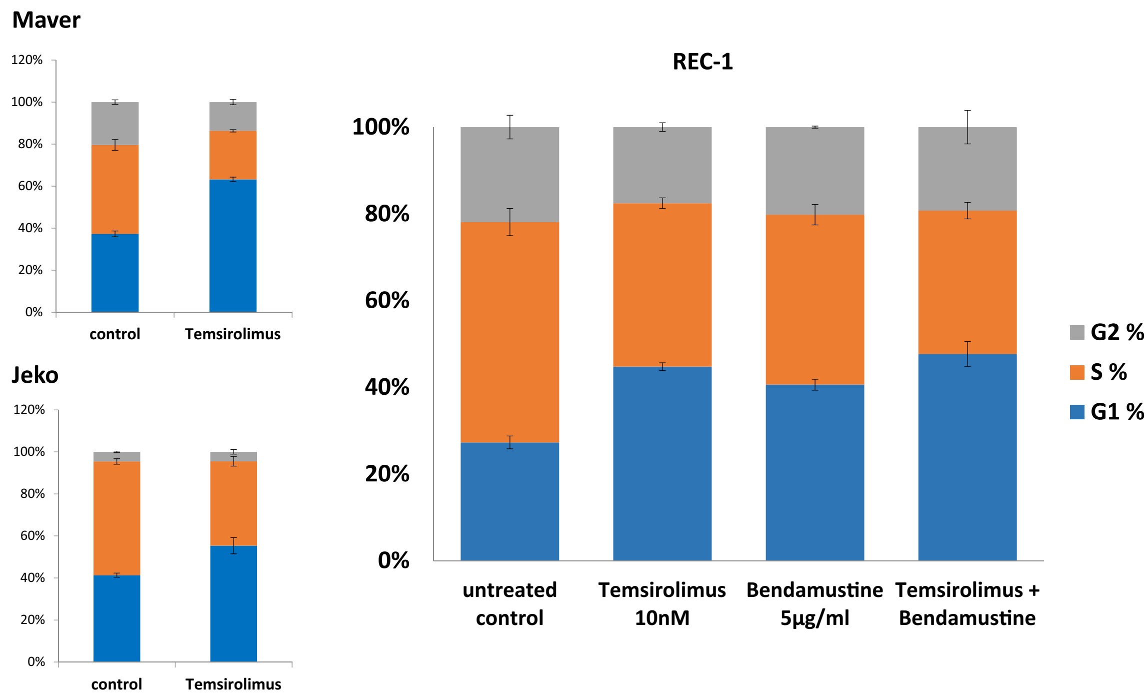

Fig. 3 Cell cycle MCL cell lines

treated in ICU for 4 weeks due to a severe septic complication and was not eligible for autologous stem cell transplant (SCT) after this ordeal. In July 2011, the patient presented with a progression of his MCL; the CT scan showed a growing generalized lymphadenopathy. We decided to treat the patient with a combination of rituximab (375 mg/m^2) bendamustine, and temsirolimus (BERT protocol). The patient was planned to receive 4 cycles of the BERT regimen. The overall toxicity was moderate; the patient developed grade III thrombocytopenia and leukopenia leading to postponement of chemotherapy after cycle one; the dose of bendamustine was tapered to 60 mg/m^2, 2 d1+2 in the following courses; and G-CSF prophylaxis was administered thereafter. After the third cycle, the patient developed an uncomplicated pneumonia which was treated by antibiotics but postponing the fourth cycle for 2 weeks. After 4 cycles, the patient had a complete remission and was referred to the allogenic transplantation unit where he received an uncomplicated SCT. The patient is relapse free an in good health by April 2015.

Materials and methods

Cell lines and cell culture

A panel of established MCL (Granta 519, Jeko-1, Maver-1, Mino, Rec-1, and Z-138) and DLBCL cell lines (SU-DHL-4, Will-2, HBL-1, OCI-LY-3, U2932, and DB) were used. All MCL cell lines were purchased from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany), except Z-138 (LGC Standards GmbH, Wesel, Germany), and cultured in RPMI 1640 culture medium (PAM, Aidenbach, Germany) at 37 °C in a humidified atmosphere containing 5 % carbon dioxide. OCI-Ly3 was cultivated in Iscove's modified essential medium with 20 % heparinized human plasma, penicillin, streptomycin, and β-mercaptoethanol. The DLBCL cell lines HBl-1, OCI-LY-3, and U2932 were kindly supplied by G. Lenz, Münster, Germany.

Cell proliferation quantification by trypan blue staining

Cells were seeded at a density of 5×10^5/mL and counted at 24, 48, and 72 h using the Vi-CELL Cell Viability Analyzer (Beckman Coulter, Brea, USA). To detect synergism,

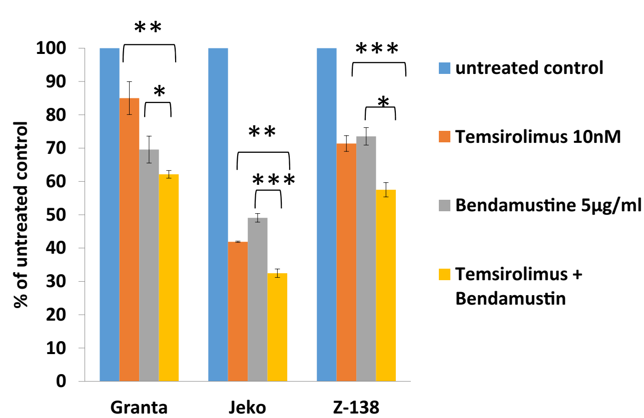

Fig. 4 Temsirolimus + bendamustine MCL cell lines

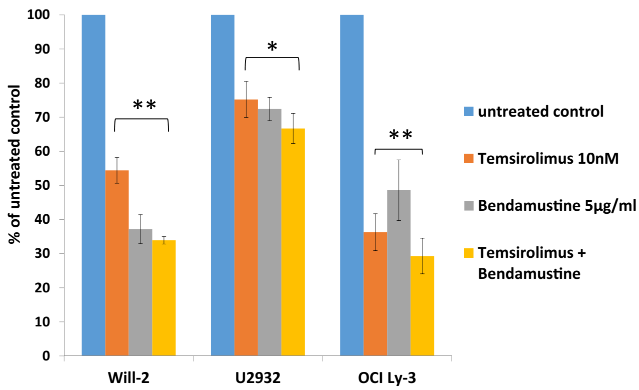

Fig. 5 Temsirolimus + bendamustine DLBCL cell lines

additivism, or antagonism, the relative decrease of viability was compared to the viability of the untreated cells (fraction affected) using Webb's formula [(1−FA substance A)×(1−FA substance B)]−(1−FA combination substances A+B) [20]. Results higher than +0.1 were regarded as synergistic; results smaller than −0.1 were regarded as antagonistic. Values between both thresholds were regarded as additive. Further, a paired student *t* test was applied to the combination treatment data. *P* values <0.05 were regarded as significant. *P* values are indicated as $^*P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$.

Cell cycle and apoptosis analysis

For detection of cell cycle distribution and cell death, experiments were performed as described above, incubated for 24 and 48 h, and stained with propipium iodide-based lysis buffer (Natriumcitrat 100 mg; Triton X-100 100 µL; Propidiumiodid 2 mg; Aqua purificata 100 mL; pH 8.0 with HCl) or with Annexin V and 7AAD using Annexin V-PE Apoptotis Detection Kit 1 (BD Pharmingen, San Jose, USA) according to the manufacturer's instructions. Cells were sorted using a FACSCalibur II and analyzed with FlowJo (version 7.6.5, Treestar, Ashland, USA), Excel 2013 (Microsoft, Redmond, USA), and GraphPad Prism (San Diego, USA) software.

Results and discussion

In MCL, cell lines Maver, Jeko, and Rec were equally sensitive toward temsirolimus treatment, while cell lines Granta and Z-138 were less sensitive (Fig. 1). Interestingly, doses up to 100 nM did not significantly increase cell growth reduction (Fig. 1).

As discussed in [10] in detail, 10 nM temsirolimus decreased cell proliferation in relation to untreated control to 66–30 % in DLBCL cell lines with cell line DB being resistant to treatment. Interestingly, no difference in efficacy was detected in germinal center B (GCB) and activated B cell (ABC) subtypes.

In cell cycle analysis, temsirolimus induced a G1 phase arrest in DLBCL (Fig. 2), increasing—depending on cell line—the G1 phase up to 71 %. Similar effects were detected in MCL cell lines, with a G1 phase increment up to 63 % (Fig. 3).

Of bendamustine, 5 µg/mL reduced cell growth in relation to untreated control from 73 to 37 % in the cell lines (Figs. 4 and 5). In contrast to temsirolimus, bendamustine induced apoptosis in DLBCL and MCL cell lines (Fig. 6).

Combination with temsirolimus and bendamustine induced additive effects in cell growth reduction in MCL and DLBCL cell lines after 72 h of treatment (Figs. 4 and 5).

After combination treatment, both cell cycle G1 phase arrest and apoptosis induction could be detected (Figs. 2, 3, and 6).

Temsirolimus has shown significant clinical activity in lymphoid neoplasm [6, 14]. In this study, temsirolimus effectively inhibited cell growth in all DLBCL cell lines besides DB with no detected difference between GCB and ABC subtypes. Interestingly, the same dose induced equal effects in

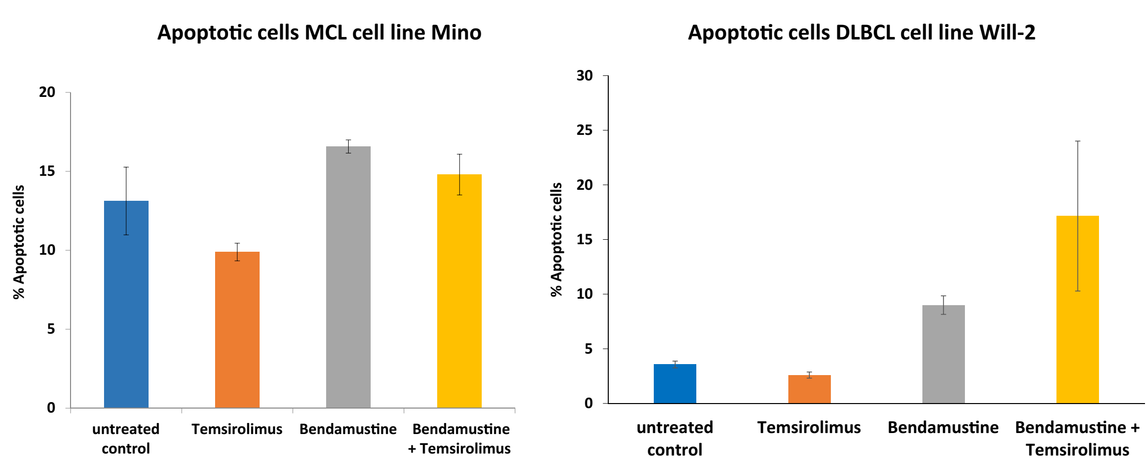

Fig. 6 Temsirolimus + bendamustine apoptosis

MCL cell lines Maver, Jeko, and Rec with the line Granta and Z-138 being less sensitive. Bendamustine has lately shown significant clinical activity in mantle cell and diffuse large B cell lymphoma [16, 21, 22]. Temsirolimus is known to induce cell growth reduction by initiating a G1 phase arrest [23]; therefore, a combination treatment with the apoptotic agent bendamustine targets cytostatic and cytotoxic cell growth reduction and as shown additively reduces cell growth in DLBCL and MCL cell lines.

As we show here, a combination of both temsirolimus and bendamustine reduces cell growth additively by inducing a G1 phase increase as well as apoptosis.

Furthermore, the published data from the BERT study [8] underlines the relevance of our patient's reported clinical course.

In summary, the presented in vitro studies and the described case report emphasize the efficacy of the combination treatment with temsirolimus and bendamustine in NHL.

Compliance with ethical standards

Conflict of interest Martin Dreyling received research funding and speaker's honoraria from Pfizer, Mundipharma, and Roche. Georg Hess received research funding and honoraria from and is on the advisory committee of Pfizer and research funding from Mundipharma and honoraria from and is on the advisory committee of Roche. All other authors have no conflict of interest to declare.

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