Sepantronium Bromide (YM155) Enhances Response of Human B-cell non-Hodgkin Lymphoma to Rituximab

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Running title: Combination effect of YM155 with Rituximab in B-NHL

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Abbreviations: ASCT, autologous stem cell transplant; B-NHL, B-cell non-Hodgkin lymphoma; DLBCL, diffuse large B-cell lymphoma; $^{18}$F-FDG, 2’-deoxy-2’-$^{18}$F-fluoro-D-glucose; FL, follicular lymphoma; $^{18}$F-FLT, 3’-$^{18}$F-fluoro-3’-deoxythymidine; MCL, mantle cell lymphoma; PET, positron emission tomography; SCID, severe combined immunodeficient

Section: Drug Discovery and Translational Medicine
Abstract

In the treatment of B-cell non-Hodgkin lymphoma (B-NHL), rituximab improves long-term survival in combination with conventional chemotherapy. However, as the majority of B-NHL patients eventually relapse, the development of more effective therapies is needed. Here, we evaluated the antitumor effects of a combination treatment involving sepantronium bromide (YM155), a first-in-class survivin suppressant, and rituximab in B-NHL xenograft mice models. To determine the efficacy of the combination treatment, YM155 and rituximab-treated B-NHL cell xenografted mice were monitored for tumor size and survival, and were subjected to $^{18}$F-FDG and $^{18}$F-FLT PET imaging. In addition, the cell proliferation status of excised tumors was examined by Ki-67 immunohistochemistry. In DB, WSU-DLCL-2, and Mino xenograft-bearing mice, the combination treatment of YM155 and rituximab induced significant tumor growth inhibition and tumor regression compared to either single agent. On day 3 after the initiation of treatment, a significant decrease in both $^{18}$F-FDG and $^{18}$F-FLT tumor uptake from pre-treatment levels was observed in combination treatment groups. The Ki-67 proliferation index was significantly decreased on day 3 in the xenograft models treated with combination treatment, suggesting that the combination of YM155 plus rituximab reduced cell proliferation as well as glucose metabolism. Furthermore, compared to monotherapy, combination treatment prolonged survival times of severe combined immunodeficient (SCID) mice with disseminated WSU-FSCCL and Jeko B-NHL tumors. Our findings demonstrate that YM155 and rituximab combination treatment enhances antitumor activity in B-NHL xenografts, and that $^{18}$F-FLT- and
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18F-FDG-PET imaging may allow the early functional evaluation of treatment responses in B-NHL patients.
Introduction

B-cell non-Hodgkin lymphoma (B-NHL) is a heterogeneous group of B-cell malignancies that display considerable genetic diversity, which reflects the differentiation status of lymphocytes at the time of transformation (Kuppers, 2005). The anti-CD20 antibody rituximab (Rituxan®) is standard therapy for many types of CD20-positive B-cell lymphomas and improves long-term survival in combination with conventional chemotherapy (Cheson and Leonard, 2008). As the majority of B-NHL patients eventually relapse (Coiffier, 2007), high-dose chemotherapy followed by autologous stem cell transplant (ASCT) is the accepted second-line treatment for diffuse large B-cell lymphoma (DLBCL), grade 3 follicular lymphoma (FL), and mantle cell lymphoma (MCL). However, as many patients are not candidates for ASCT because of advanced age or co-morbidities, a need exists for the development of novel therapeutic strategies to supplement current B-NHL treatment regimens.

In B-NHL patients, increased expression levels of the anti-apoptotic protein survivin are typically observed in malignant lymphocytes (Ambrosini et al., 1997). Survivin positivity in B-NHL patients is also associated with increased chemo-resistance and worse clinical outcomes (Adida et al., 2000; Markovic et al., 2011). In addition, functional knock down of survivin by shRNA induces cell apoptosis and growth inhibition of B-NHL cells (Ansell et al., 2004). Taken together, these findings suggest that survivin-targeted therapy would be suitable for the treatment of B-NHL.
Sepantronium bromide (YM155) is a first-in-class compound that functions selectively to suppress survivin expression (Nakahara et al., 2007). We previously demonstrated that YM155 displays potent anticancer activity against a broad spectrum of human cancer cell lines and various human-derived tumor xenograft mouse models (Nakahara et al., 2011a; Yamanaka et al., 2011b). In aggressive B-NHL models, YM155 treatment resulted in potent and sustained antitumor activity (Kita et al., 2011). Immunohistochemical analyses of non-small cell lung cancer (NSCLC), melanoma, and B-NHL tumors have indicated that YM155-induced tumor regression is associated with the down-regulation of intratumoral survivin expression, growth inhibition, and apoptotic induction of tumor cells (Kita et al., 2011; Nakahara et al., 2011b; Yamanaka et al., 2011a). Therefore, we hypothesized that YM155 treatment in combination with rituximab would be effective against B-NHL, and evaluated the efficacy of combination treatment using human B-NHL-xenografted mice models on tumor size and survival.

One powerful technique for evaluating the therapeutic efficacy of drugs in the clinical setting is positron emission tomography (PET) imaging, which allows non-invasive assessment of functional and metabolic alterations in tumor cells. The glucose analog 2’-deoxy-2’-18F-fluoro-D-glucose (18F-FDG) is the most commonly used PET tracer for diagnosis, staging, and response monitoring in patients with malignant lymphoma including DLBCL (Klabbers et al., 2003; Shankar et al., 2006). A second attractive PET tracer is 3’-18F-fluoro-3’-deoxythymidine (18F-FLT), which is a substrate of the DNA synthetic pathway and is considered to be highly specific for proliferating tumor cells (Shields et al., 1998; Vesselle...
et al., 2002). $^{18}$F-FLT uptake correlates with the rate of tumor proliferation, as measured by Ki-67 tumor staining, in NSCLC patients (Vesselle et al., 2002). However, the potential utility of $^{18}$F-FDG- and $^{18}$F-FLT-PET for the assessment of tumor response to YM155 treatment has not been demonstrated. Here, we evaluated the antitumor activity of YM155 combined with rituximab against B-NHL by interim $^{18}$F-FDG- and $^{18}$F-FLT-PET imaging using human B-NHL xenografted mice models.
Materials and Methods

*Sepantronium bromide (YM155) and rituximab*

YM155 was synthesized in house by Astellas Pharma, Inc. (Nakahara et al., 2007). For *in-vivo* experiments, YM155 was dissolved and diluted in saline immediately before administration. Dose levels are expressed in terms of YM155, the cationic moiety of the drug substance. Rituximab was obtained from Chugai Pharmaceutical Co., Ltd. (Tokyo, Japan) and was diluted in saline immediately before administration.

*Cell lines*

The human DLBCL cell line DB and the human mantle cell line Mino were purchased from the American Type Culture Collection (ATCC; Manassas, VA). The human DLBCL cell line WSU-DLCL-2, human FL cell line WSU-FSCCL, and human MCL cell line Jeko were purchased from the German Resource Center for Biological Material (DSMZ, Braunschweig, Germany). The cells were maintained in RPMI 1640 medium (Life Technologies, Gaithersburg, MD) supplemented with 10% heat-inactivated fetal bovine serum (Invitrogen, Grand Island, NY) in a humidified incubator with an atmosphere containing 5% CO₂ at 37 °C.

*Animals for in-vivo studies*
All animal experimental procedures were approved by the institutional animal care and use committee of Astellas Pharma, Inc. Furthermore, Astellas Pharma, Inc., Tsukuba Research Center was awarded Accreditation Status by the AAALAC International. Animals were euthanized if tumor burden exceeded 10% of the host animal’s body weight.

**In vivo antitumor activity in subcutaneous tumor xenograft models**

Five-week-old male BALB/c nude mice (CAnN.Cg-Foxn1nu/CrlCrlj) were obtained from Charles River Japan (Kanagawa, Japan). Cultured DB, WSU-DLCL-2, and Mino cells were harvested, suspended in a phosphate buffer solution, and then subcutaneously injected into the flanks of mice (3×10⁶ cells/0.1 mL/mouse). Body weight and tumor volume were assessed twice weekly. Tumor volume was estimated by calculating tumor dimensions using calipers (length × width² × 0.5). When tumor volumes reached >300 mm³, animals were randomly assigned into groups based on tumor volume. In the combination treatment study, the first day of drug treatment was designated as day 0, and observations continued until day 21, representing one full treatment cycle in the clinical study of YM155. YM155 was administered as a seven-day continuous infusion using a micro-osmotic pump (Alzet Model 1003D and 1007D; Durect Corp., Cupertino, CA). Rituximab was administered intravenously at 50 mg/kg on days 0 and 2. Percent tumor regression was calculated using the following formula: 100 × [1 − (mean tumor volume of each group on day 21) / (mean tumor volume of each group on day 0)].
18F-FDG- and 18F-FLT-PET imaging

18F-FDG was purchased from Nihon Medi-Physics (Hakui, Japan). 18F-FLT was synthesized in house from its precursors. Briefly, 18F was produced via an 18O(p,n)18F nuclear reaction by proton bombardment (12 MeV, 50 μA) of an 18O-water target using a cyclotron-target system (OSCAR-12; JFE Plant & Service Corp., Yokohama, Japan). 18F-FLT was then synthesized by nucleophilic fluorination of 3−N−Boc−5′−O−dimethoxytrityl−3′−O−nosyl−thymidine. For PET imaging studies, 5×10^6 cells/0.1 mL/mouse were inoculated subcutaneously in the right hind leg. 18F-FDG and 18F-FLT uptake were evaluated using a small-animal Inveon PET scanner (Siemens, Knoxville, TN) on days 0 and 3 of treatment. Selected mice for 18F-FDG-PET scans were fasted for over 12 h prior to imaging. For imaging, 6-12 MBq aliquots of 18F-FDG or 18F-FLT were administered by tail vein injection to conscious animals, which were then maintained in cages for 1 h to allow for 18F-FDG and 18F-FLT uptake into tumors. Under 2.0–2.5% isoflurane gas anesthesia, mice were placed on the scanning stage and a 5-min emission scan was performed using the PET scanner. Images were reconstructed by OSEM3D and analyzed with ASIPro software (Seimens Medical Solutions). Regions of interest were drawn around the boundaries of tumors on axial slices and the observed maximum pixel value was normalized to the injected dose and body weight to give the maximum standardized uptake value (SUVmax).

Immunohistochemical analysis of lymphoma xenografts
Tumors were excised from mice, weighed, fixed in 4% paraformaldehyde, embedded in paraffin, and then sectioned at 5-µm thickness. The sections were dewaxed in xylene and were then subjected to antigen retrieval by placing samples in 10 mM citrate buffer, followed by autoclaving for 15 min at 121 °C. The sections were then incubated with mouse monoclonal anti-Ki-67 antibody (Dako, CA) for 1 h. Antibody binding was visualized using the Envision System (Madison, NJ). The sections were then incubated with diaminobenzidine and counterstained with hematoxylin. The Ki-67 proliferation index of each group was calculated as follows: Ki-67 positive cells (per 1000 cells) × tumor weight (g).

Studies with disseminated xenografts

Four-week-old female SCID mice (CB17/Icr-Prkdc<sup>scid</sup>/CrlCrlj) were purchased from Charles River Japan. WSU-FSCCL and Jeko cells were injected intravenously (4×10<sup>6</sup> cells/0.2 mL/mouse), and dissemination and tumor growth was allowed to proceed for 7 days. The mice were then divided into 4 comparable groups of 10 animals each based on body weight using SAS software (SAS Institute, Cary, NC), and a clinical dose of either YM155 (2 mg/kg 7-day continuous infusion), rituximab (50 mg/kg intravenously), or YM155 (2 mg/kg 7-day continuous infusion) plus rituximab (50 mg/kg intravenously) was administered once every 3 weeks for 3 courses in WSU-FSCCL and 1 course in Jeko models, respectively. Animals were monitored daily for the occurrence of mortality, and were euthanized while under diethyl ether anesthesia.
Mice were scored as dead if any of the following signs of suffering were observed: cachexia, weakness, and difficulty in moving or eating.

**Statistical analysis**

Differences in tumor volume on day 21 between the combination treatment group and each single-compound group were evaluated using the Student’s *t*-test. Immunohistochemistry and PET imaging data were analyzed using analysis of variance (ANOVA) followed by *post hoc* Tukey’s test. For survival analysis, Kaplan-Meier curves were generated, and the differences between the control and drug-treated groups were assessed using the log-rank test. All statistical analyses were performed using GraphPad Prism 5 (GraphPad Software, CA), and *P* values less than 0.05 were considered to be significant. All values are expressed as means and standard errors (SEM) of the means.
Results

*Sepantronium bromide (YM155) enhances antitumor activity of rituximab against subcutaneous xenograft tumors*

We first evaluated the antitumor effects of YM155 in combination with rituximab in B-NHL xenograft-bearing mice models (Fig. 1). As our preliminary studies demonstrated that a 2 mg/kg dose of YM155 induced tumor regression, including a complete response, a dose lower than 2 mg/kg was used in the present study. In each xenograft model, rituximab monotherapy showed slight antitumor activity. For YM155 monotherapy, although a low dose of YM155 (0.5 or 1 mg/kg/day) caused a delay in tumor growth, tumors began to regrow during the cessation of treatment, and none of the tumors showed complete regression by day 21. In contrast, combination treatment with YM155 and rituximab resulted in significant tumor regression (>100% inhibition). The percentage of tumor regression in the combination treatment group on day 21 was 76%, 29%, and 9% for the DB-, WSU-DLCL-2-, and Mino-xenografted mice models, respectively (Figs. 1A-C). A complete response was achieved in two of five DB-xenografted mice. In addition, no animals in any group exhibited significant body-weight loss at any time point during treatment (data not shown).

*18F-FDG- and 18F-FLT-PET imaging of WSU-DLCL-2 xenograft-bearing mice*

To assess functional changes of tumor xenografts by the combination treatment, we performed 18F-FDG-PET and 18F-FLT-PET imaging as indicators of glucose metabolism and cell
proliferation, respectively. Representative $^{18}$F-FDG (Fig. 2A) and $^{18}$F-FLT (Fig. 2B) images of WSU-DLCL-2 xenograft-bearing mice taken at baseline (before treatment, day 0) and 3 days post-treatment are shown. A marked decrease of tumoral FDG and FLT uptake 3 days after the initiation of combination treatment was observed (arrows). In contrast, FDG and FLT uptake remained high in the control and rituximab monotherapy mice. Tumoral uptake of both FDG or FLT into the xenografts of all mice were analyzed and the results are summarized in Table I. Within three days of treatment, a decrease (-42%) in $^{18}$F-FDG uptake was achieved in the combination treatment group ($P<0.05$ vs. control). YM155 monotherapy and combination treatment significantly reduced the tumor uptake of $^{18}$F-FLT by -32% and by -39%, respectively ($P<0.05$; $P<0.01$ vs. control). Combination treatment group also decreased $^{18}$F-FLT uptake compared to rituximab monotherapy group (-36%) ($P<0.05$).

**Histology and Ki-67 immunohistochemistry**

To examine the feasibility of PET analysis as a complementary technique to immunohistochemistry in routine clinical applications, we also analyzed tissue samples using a standard Ki-67 immunostaining assay as an indicator of cell proliferation status. Representative sections from treated and untreated tumors are shown in Fig. 3. We found that sections obtained from untreated WSU-DLCL-2 xenotransplants showed high proliferative activity. In YM155-monotherapy (-46%) and rituximab-monotherapy (-23%), and combination treated animals (-56%), a significant decrease in the Ki-67 proliferation index was detected on day 3.
(Table II; $P<0.01$, vs. control). A significant decrease was observed in combination treatment tumors compared to rituximab monotherapy ($P<0.01$).

**YM155 in combination with rituximab against disseminated aggressive B-NHL xenografts**

Advanced B-NHL commonly develops clinically as disseminated disease. To examine the survival benefit of YM155 and rituximab combination treatment, we evaluated the effects of the clinical dosage of YM155 (2 mg/kg/day) alone or in combination with rituximab in the disseminated SCID/WSU-FSCCL and SCID/Jeko lymphoma models (Fig. 4). The administration of either YM155 or rituximab alone extended the median survival from 35 days to 60 and 42 days, respectively, in WSU-FSCCL-xenografted mice, and 1 from 54 days to 67 and 90 days, respectively, in Jeko-xenografted mice. Notably, the addition of YM155 to rituximab treatment significantly prolonged survival compared with the respective mono-therapies in both examined models.
Discussion

The present study clearly shows that survivin suppressant, sepantronium bromide (YM155), in combination with rituximab results in significant improvements in tumor regression and survival in B-NHL xenograft models. We also demonstrated that the antitumor effect of combined YM155 and rituximab treatment is accompanied by decreases in tumor glucose metabolism and cell proliferation, as assessed by small-animal $^{18}$F-FDG- and $^{18}$F-FLT-PET imaging.

In the DB-, Mino-, and WSU-DLCL-2-xenograft models, although rituximab alone exhibited slight antitumor activity, the combination of low dose YM155 (0.5 or 1 mg/kg/day) with rituximab significantly improved tumor response compared to the monotherapy groups (Fig. 1). Advanced B-NHL commonly develops clinically as disseminated disease. Here, the combination of YM155 and rituximab also significantly prolonged the survival of disseminated B-NHL models compared with either drug alone (Fig. 4). As increased survivin dependency for tumor growth is reported to be associated with poor clinical outcomes, the combination of a survivin suppressant, YM155, and an anti-CD20 antibody may represent a potent strategy to treat B-NHL (Adida et al., 2000; Markovic et al., 2011).

A number of studies have suggested that the combination of rituximab with various agents potentially improves their therapeutic potential (Di Gaetano et al., 2001; Ghetie et al., 2001; Alas et al., 2002; Chow et al., 2002; Emmanouilides et al., 2002; Boye et al., 2003; Jazirehi et al., 2003; Ackler et al., 2010). The mechanisms of action of rituximab are not fully understood and remain a matter of debate. Two immune-mediated mechanisms, antibody-dependent
cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) have attention as possible processes linked to rituximab action. To investigate whether NK-dependent ADCC activity is the important for enhancing the effectiveness of YM155 treatment \textit{in vivo}, we evaluated the combination regimen in lymphoma xenografts established in NOD/SCID mice, which have impaired T/B cell lymphocyte development and natural killer-cell function (Shultz et al., 1995). Combined rituximab and YM155 treatment resulted in a significant enhancement of antitumor activity over rituximab alone, a finding that is consistent with the results in BALB/c nude mice (data not shown). This result suggests that the therapeutic interaction between these two agents does not appear to arise from the antibody-mediated effector functions of rituximab and that the rituximab-mediated inhibition of intracellular survival pathways is important for enhancing the effectiveness of YM155 treatment \textit{in vivo}. Mechanistically, rituximab has been reported to abrogate the intracellular signal transduction of survival pathways involving p38, MAPK, and NF-κB, resulting in decreased Bcl-2 expression and sensitization to drug-induced apoptosis. Rituximab also upregulates Raf kinase inhibitor protein, thus decreasing the activity of the ERK1/2 pathway, leading to reduced Bcl-xL expression (Bonavida, 2007; Vega et al., 2011). Previously, we have demonstrated that concomitant treatment with YM155 enhanced chemosensitivity to Bcl-2 inhibitor in DLBCL (data not shown). We postulate that the anticancer effects of YM155 and rituximab observed here result from the simultaneous inhibition of different cell-survival pathways, the survivin pathway by YM155 and the Bcl-2/Bcl-xL pathway by rituximab. However, western blot analysis revealed that rituximab did not suppress the expression of Bcl-2/Bcl-xL in the DLBCL cell line (data not shown). Thus, further investigations
are required to identify the signaling pathways that are affected by the combined exposure to YM155 and rituximab.

We explored whether $^{18}$F-FDG and $^{18}$F-FLT accumulation are sufficiently large to be quantitatively imaged with PET in early in the treatment. The uptake of $^{18}$F-FDG and $^{18}$F-FLT was significantly decreased in the combination-treated group by day 3, on the other hand no apparent decrease was observed in the rituximab monotherapy group (Fig. 2). Both PET imaging techniques could detect early responses to treatment based on tumor activity, with results that were consistent with tumor regression determined by caliper measurements (Figs. 1 and 2). Recent animal and preliminary clinical studies have suggested that $^{18}$F-FLT may be more sensitive than $^{18}$F-FDG for assessing tumor response (Troost et al., 2010; Kahraman et al., 2011). Our present findings indicate that $^{18}$F-FDG and $^{18}$F-FLT have comparable sensitivities for detecting early changes in tumor cell activity after combined YM155 and rituximab treatment in a B-NHL xenograft model (Fig. 2), indicating that the combination treatment induces suppression of glucose metabolism and cell proliferation. Importantly, the significant decrease in $^{18}$F-FLT uptake observed here in combination treated mice was also consistent with a decrease in Ki-67 immunostaining (Fig. 3). Thus, the similar trends observed between PET output metrics and the standard histological marker, Ki-67, suggest that PET imaging can be used to monitor cellular proliferation in tumors. These results suggest that PET imaging with $^{18}$F-FDG or $^{18}$F-FLT could evaluate the response of tumors to YM155 and rituximab treatment within several days of initiating combined treatment, as this would allow non-responding tumors to be identified.
In conclusion, combined YM155 and rituximab treatment has shown promising antitumor effects in B-NHL xenograft mice models. Additionally, PET imaging data suggest that tumor responses to combination treatment of YM155 and rituximab can be monitored using the tracer $^{18}$F-FLT with a similar sensitivity to that of $^{18}$F-FDG-PET. Based on the positive preclinical results of YM155 in B-NHL, a phase II open-label study of YM155 in combination with rituximab in relapsed patients with CD20-positive B-NHL was initiated and is currently underway.
Acknowledgements

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Authorship Contributions

Participated in research design: Kita, Mitsuoka, Miyoshi, Sasamata

Conducted experiments: Kita, Mitsuoka, Kaneko, Nakata, Jitsuoka, Miyoshi

 Contributed new reagents or analytic tools: Jitsuoka

 Performed data analysis: Kita, Mitsuoka, Noda, Kaneko, Nakata, Jitsuoka

 Wrote or contributed to the writing of the manuscript: Kita, Mitsuoka, Noda, Kaneko, Jitsuoka, Yamanaka, Mori, Nakahara, Sasamata
References


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Figure Legends.

**Figure 1. Antitumor activity of sepantronium bromide (YM155) against aggressive B-NHL xenografts.** BALB/c nude mice bearing DB (A), WSU-DLCL-2 (B), and Mino (C) xenografts were treated with YM155 and rituximab. YM155 was given as a 7-day continuous subcutaneous (sc) infusion starting on day 0, and rituximab was administered intravenously (iv) by bolus injection on days 0 and 2. Tumor volume on day 21 was compared between each single-treatment group and the combination group using Student’s *t*-test *P*<0.05, **P*<0.01 vs. YM155 monotherapy group; ##P*<0.01, ###P*<0.001 vs. rituximab monotherapy group. Mean, SEM (n=5 mice/group).

**Figure 2. PET imaging identifies early metabolic changes after chemotherapy treatment.** Representative images of ^18^F-FDG (A) and ^18^F-FLT (B) in WSU-DLCL-2 xenograft-bearing mice on days 0 and 3 of treatment. An arrow indicates the WSU-DLCL-2 xenograft.

**Figure 3. Representative tumor sections subjected to Ki-67 staining.** Tumor xenografts were stained with H&E (upper) or cell proliferation marker Ki-67 (lower). Bar, 100 μm.

**Figure 4. Efficacy of YM155 and rituximab combination treatment against disseminated, aggressive B-NHL xenografts.** Animals (n=10/group) were monitored daily for survival up to
day 63 for WSU-FSCCL xenografts (A) and up day 90 day for Jeko xenografts (B). Statistical differences were determined using the log-rank test. *$P<0.05$, ***$P<0.001$ vs. YM155 monotherapy group; ##$P<0.01$, ###$P<0.001$ vs. rituximab monotherapy group
Table I. Quantitative analysis of PET images.

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<tr>
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<th>Control</th>
<th>YM155</th>
<th>Rituximab</th>
<th>Combination</th>
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<tbody>
<tr>
<td><strong>FDG-PET</strong></td>
<td>0.71 ± 0.03</td>
<td>0.60 ± 0.08</td>
<td>0.64 ± 0.09</td>
<td>*0.42 ± 0.06</td>
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<tr>
<td><strong>FLT-PET</strong></td>
<td>1.01 ± 0.04</td>
<td>*0.69 ± 0.10</td>
<td>0.97 ± 0.08</td>
<td><strong>#0.62 ± 0.04</strong></td>
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Tumor activity (SUV$_{\text{max}}$) change from baseline (day 0) by $^{18}$F-FDG or $^{18}$F-FLT on day 3. Mean, SEM (n=4/group). *P<0.05, **P<0.01 vs. control group; #P<0.05 vs. rituximab monotherapy group (ANOVA with Tukey’s post hoc test).
Table II. Ki-67 proliferation index.

<table>
<thead>
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<th>Control</th>
<th>YM155</th>
<th>Rituximab</th>
<th>Combination</th>
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<tr>
<td><strong>Proliferation Index</strong></td>
<td>396 ± 17</td>
<td><strong>215 ± 21</strong></td>
<td>*306 ± 24</td>
<td><strong>174 ± 17</strong></td>
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Mean, SEM (n=4/group) *P<0.05, ***P<0.01 vs. control group; #P<0.05, ##P<0.01, vs. rituximab monotherapy group (ANOVA with Tukey’s test).
Figure 1

(A) DB

- Control
- YM155
- Rituximab
- Combination

YM155 1 mg/kg, s.c. infusion
Rituximab 50 mg/kg, i.v. injection

(B) WSU-DLCL-2

YM155 0.5 mg/kg, s.c. infusion
Rituximab 50 mg/kg, i.v. injection

(C) Mino

YM155 1 mg/kg, s.c. infusion
Rituximab 50 mg/kg, i.v. injection
Figure 2

(A) FDG-PET

Control

YM155

Rituximab

Combination

Day 0

Day 3

SUV

2.3

0

(B) FLT-PET

Control

YM155

Rituximab

Combination

Brain

Bladder

Kidneys

Tumor
Figure 3

Control | YM155 | Rituximab | Combination
---|---|---|---
H&E | | | |
KI-67 | | | |
Figure 4

(A) WSU-FSCCL

(B) Jeko

Survival (%) vs. Time (day)

Control
YM155 2 mg/kg, s.c. infusion
Rituximab 50 mg/kg, i.v. injection
Combination

Control
YM155 2 mg/kg, s.c. infusion
Rituximab 50 mg/kg, i.v. injection
Combination

* ,###
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