Administration of Beta-Emitting Anti-CD37 Radioimmunoconjugate Lutetium ($^{177}$Lu) Lilotomab Satetraxetan as Weekly Multiple Injections Increases Maximum Tolerated Activity in Nude Mice with Non-Hodgkin Lymphoma Xenografts

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Abstract

Lutetium ($^{177}$Lu) lilotomab satetraxetan ($^{177}$Lu-lilotomab) is a novel anti-CD37 radioimmunoconjugate (RIC) currently in Phase 2b clinical trial for treatment of non-Hodgkin lymphoma (NHL). The aim of the current study was to investigate tolerability and anti-tumor efficacy of multiple dosing of $^{177}$Lu-lilotomab in vivo. Nude mice with subcutaneous (s.c.) NHL (Ramos) xenografts were given 2, 3 or 4 weekly injections of 300 MBq/kg $^{177}$Lu-lilotomab or NaCl. Treatment tolerability was assessed by body weight, hematology and histopathological evaluation. A separate experiment in mice without xenografts was performed to collect long term toxicity data. Mice in this study were dosed more conservatively with the intention that all mice should survive until end of experiment and were administered 2 or 4 weekly injections of 200 MBq/kg $^{177}$Lu-lilotomab or NaCl. Total accumulated activity of 900 MBq/kg $^{177}$Lu-lilotomab given as three weekly injections was tolerated by nude mice with s.c. Ramos xenografts, which is 50 % higher than the maximum tolerated activity (MTA) of a single injection of 530-600 MBq/kg. In the long-term toxicity animals, the highest accumulated activity tested, 800 MBq/kg, was also well tolerated. Radioactivity-dependent transient reduction in white blood cell and platelet counts occurred in all treated groups, with nadir 1-3 weeks after last injection. This toxicity was radioactivity dependent and consistent with histopathological changes in hemolymphopoietic tissues. Significant tumor growth delay measured as time to reach 4 times initial tumor volume was observed in all groups given multiple injections of 300 MBq/kg $^{177}$Lu-lilotomab compared to NaCl control group (p<0.001). In conclusion, weekly injections of $^{177}$Lu-lilotomab increase the accumulated MTA from 530 to 900 MBq/kg in nude mice, allowing the total injected activity and hence the radiation dose to tumor to be increased without increasing the toxicity to normal tissues.

Keywords: Lutetium ($^{177}$Lu) lilotomab satetraxetan, Non-Hodgkin lymphoma, Radioimmunoconjugate, Radioimmunotherapy, Molecular radiotherapy, Multiple injections

Abbreviations: FL: Follicular Lymphoma; i.v.: Intravenous; mAb: Monoclonal Antibody; MTA: Maximum Tolerated Activity; NHL: Non-Hodgkin Lymphoma; RIC: Radioimmunoconjugate; RIT: Radioimmunotherapy; s.c.: Subcutaneous
Introduction

Non-Hodgkin lymphoma (NHL) caused an estimated 200,000 cancer deaths worldwide in 2014 [1]. The most common sub-type of NHL is follicular lymphoma (FL), which represents approximately 25% of all newly diagnosed cases of NHL and 70% of indolent lymphomas. The 5-year overall survival rates for rituximab-refractory FL patients and those with early disease progression are 58% and 50%, respectively, compared to approximately 90% for all FL patients [2,3,4]. Indolent NHLs are usually diagnosed in the advanced stage and require systemic therapy. The standard of care for patients with advanced-stage, symptomatic or high tumor-burden FL is immuno-chemotherapy with alkylating agents in combination with an anti-CD20 monoclonal antibody (mAb) [5,6]. While immuno-chemotherapy regimens are initially effective in inducing responses, most patients inevitably relapse, and the same therapies show decreasing efficacy with repeated administration [2,3].

Radioimmunotherapies targeting the CD20 antigen have shown very good clinical responses for treatment of NHL [7,8,9]. Nevertheless, the treatments have been underused for various reasons, including overlapping target with the anti-CD20 antibody rituximab and cumbersome and laborious administration regimen including individual dosimetry and on-site production. Since the therapies were launched, many of these challenges have been overcome, but targeting of another antigen than CD20 would be beneficial.

CD37 is an internalizing transmembrane glycoprotein strongly expressed on mature B lymphocytes, including normal and neoplastic cells [10,11,12,13]. Expression of CD37 is independent of previous anti-CD20 therapy and could therefore be a relevant alternative antigen, as previous anti-CD20 therapies would not affect the target expression or block the binding to CD37. We thus developed the next generation beta-emitting radioimmunoconjugate (RIC) lutetium (\(^{177}\)Lu) lilotomab satetraxetan (Betalutin\(^{®}\), \(^{177}\)Lu-lilotomab) to target the CD37 antigen. Lutetium-177 has been successfully used in several clinical trials [14,15,16,17,18]. It is produced via beta decay of reactor-produced \(^{177}\)Yb and does not have radioactive daughter nuclides [19,20]. The ability to kill untargeted cancer cells within the 1.5 mm range of the beta-particles, known as the cross-fire effect, is one of the unique advantages of RICs compared to Antibody Drug Conjugates (ADCs), where only targeted cancer cells are killed. Thus, lack of internalization and payload release are not issues for RICs. The half-life of lutetium-177 is 6.7 days, which facilitates centralized production and shipment to hospitals. \(^{177}\)Lu-lilotomab is currently tested in a phase 1/2a clinical trial for treatment of relapsed B-cell NHL with promising safety and efficacy data [21].

NHL patients often have infiltration of tumor cells in the bone marrow, where multiple dosing with fractions of the total administered radioactivity might be more lenient than a single injection. Furthermore, multiple dosing might be easier to combine with other treatments, which are also often used as multiple injections, and it is expected to result in a more homogenous distribution of the RIC and the radiation dose within the tumor e.g. through radiation-induced increased leakiness of the tumor vessels [22,23].

Intervals between injections have varied widely in clinical trials testing fractionated radioimmunotherapy. One perspective has aimed at applying lower activity at weekly intervals, to increase the total activity administered while keeping the tumor activity levels (dose rate) high over a longer period of time. Another approach has been administration of a high activity (close to MTA) and wait until hematological toxicity recovers before administering a second dose [23]. Weekly fractions were chosen in the current study, as longer intervals would not have been feasible in this model with rapidly growing xenografts.

The present work is the first to explore the anti-tumor efficacy and tolerability of multiple dosing of the next generation RIC \(^{177}\)Lu-lilotomab in vivo in mice bearing NHL xenografts.

Materials and Methods

Radioimmunoconjugate

The lilotomab antibody was conjugated to p-SCN-Bn-DOTA (DOTA, satetraxetan, Macrocyclics, TX) and labeled with \(^{177}\)Lu (ITG, Garching, Germany) as previously described [24]. In brief, DOTA was dissolved in 0.005 M HCl, added to the antibody in a 6:1 ratio, pH adjusted to 8.5 using carbonate buffer and left to incubate for 45 minutes at 37°C. The reaction was stopped by adding glycine solution, and excess DOTA was removed by repeated washing with 0.9% NaCl using AMICON-30 centrifuge tubes (Milipore, Cork, Ireland). \(^{177}\)Lu labeling was performed within 24 hours prior to each injection. Before labeling with \(^{177}\)Lu, the pH was adjusted to 5.3 using 0.25 M ammonium acetate buffer. Between 100 and 250 MBq of \(^{177}\)Lu (ITG, Garching, Germany) was added to 1 mg of conjugated antibody and incubated for...
15 to 30 minutes at 37°C. Radiochemical purity (RCP) of the radioimmunoconjugate (RIC) was measured using instant thin layer chromatography. Purification using Econo-Pac 10 DG (Bio-Rad Laboratories, Hercules, CA, USA) or Sephadex G-25M (PD-10) desalting columns (GE Healthcare Life Sciences, Pittsburgh, PA, USA) was performed if RCP was below 95%. Immunoreactive fractions (IRF) of the RIC were evaluated, using single cell suspension of CD37-expressing Ramos cells applying a modified Lindmo method [25] using one cell concentration of 75 million cells/mL, ensuring a receptor-to-antibody ratio sufficiently high to bind all added antibodies (low end of Lindmo plot). IRF and specific activity of all RICs used in the reported experiments were in the ranges 75-81% and 127-202 MBq/mg antibodies, respectively.

Animals and xenografts

Institutionally bred female mice from the strain Hsd:Athymic Nude-Foxn1nu was maintained under pathogen-free conditions, and food and water were supplied ad libitum. Ramos Burkitt's lymphoma cells (LGC Standards, Boras, Sweden) were grown in RPMI 1640 medium supplemented with 10% heat-inactivated FCS, 1% L-glutamine and 1% penicillin-streptomycin (PAA, Linz, Austria) in a humid atmosphere with 95% air/5% CO₂. Ramos cells in serum-free medium (100 million cells/mL) were mixed with cell suspension from mechanically manipulated fresh xenografts from nude mice, and 100 µL of the suspension was injected s.c. into each flank. Tumor-bearing mice with tumor diameters between 5 and 12 mm were randomized to treatment groups. Tumor sizes at first therapy injection were in the range 37-662 mm³ (172 ± 112 mm³). To enable hematology monitoring at late time points 3-4 mice without tumor take were included in each dose group. A separate experiment in mice without xenografts was performed to collect long term toxicity data.

177Lu-lilotomab treatment

Nude mice injected s.c. with Ramos cells (age 5-7 weeks, 21-30 g) were given 2, 3 or 4 weekly injections of 300 MBq/kg 177Lu-lilotomab intravenously (i.v.) in the tail vein. Mice in the long-term toxicity study (age 4 weeks, 14-25 g) were dosed more conservatively with the intention that all mice should survive until end of experiment and were administered 200 MBq/kg 177Lu-lilotomab as 2 or 4 weekly injections. Control mice were given 4 weekly injections of 0.9% NaCl in both the efficacy and the long-term toxicity study. Treatment groups are listed in Table 1.

Measurements

Tumor size and body weight were measured 3 times a week. Blood samples from individual mice were collected from the lateral saphenous vein every 3 weeks. For mice without xenografts, blood samples were collected in groups of 5 mice, making hematology data available on a group level every 1.5 week. In mice injected with Ramos cells, samples were collected at baseline, 2, 5, 8 and 11 weeks.

Blood samples for hematology analysis were collected in 0.5 ml EDTA coated tubes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and analyzed using an automated hematology analyzer (Scil Vet abc, Horiba ABX, Montpellier, France).

Animals were euthanized by cervical dislocation on occurrence of humane end points; largest tumor diameter 20 mm (efficacy), loss of body weight 20% from maximum,

### Table 1: Treatment groups

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Activity (dose)</th>
<th>Number of mice</th>
<th>Number of Tumors</th>
<th>Tumor Volume (range, mm³)</th>
<th>Follow up Time (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.9% NaCl</td>
<td>4×100 µL</td>
<td>10 (3)</td>
<td>18b</td>
<td>37-662</td>
<td>19</td>
</tr>
<tr>
<td>¹⁷⁷Lu-lilotomab</td>
<td>2×300 MBq/kg</td>
<td>10 (4)</td>
<td>18b</td>
<td>61-519</td>
<td>20</td>
</tr>
<tr>
<td>¹⁷⁷Lu-lilotomab</td>
<td>3×300 MBq/kg</td>
<td>11 (3)</td>
<td>16b</td>
<td>36-272</td>
<td>20</td>
</tr>
<tr>
<td>¹⁷⁷Lu-lilotomab</td>
<td>4×300 MBq/kg</td>
<td>10 (3)</td>
<td>18b</td>
<td>56-434</td>
<td>20</td>
</tr>
<tr>
<td>0.9% NaCl</td>
<td>4×100 µL</td>
<td>10</td>
<td>-</td>
<td></td>
<td>16</td>
</tr>
<tr>
<td>¹⁷⁷Lu-lilotomab</td>
<td>2×200 MBq/kg</td>
<td>10</td>
<td>-</td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>¹⁷⁷Lu-lilotomab</td>
<td>4×200 MBq/kg</td>
<td>10</td>
<td>-</td>
<td></td>
<td>16</td>
</tr>
</tbody>
</table>

a: Mice with Ramos tumor xenografts; some additional mice without tumor take (number in brackets) were included to enable collection of samples for hematology evaluation at late timepoints. 
b: All mice were injected bilaterally with Ramos cells, meaning most mice had 2 tumors.
and/or illness or discomfort above a pre-defined score level using a score sheet taking into account posture, activity level and socialization (scored as toxicity when combined with low hematology counts). Necropsy was done of all animals, and abnormalities noted. In addition, femur, spleen, lymph nodes, and tumors were collected from all animals and lungs, heart, liver, kidneys, stomach, intestines, muscle, brain, skull, thyroid and ovaries were collected from 5 animals in control groups and highest activity groups in both studies (4·200 and 4·300 MBq/kg). Organs were stored in formalin free fixative (Accustain™, Sigma-Aldrich, St. Louis, MO, USA) and shipped to Accelera Srl, Nerviano, Italy for histopathology analysis.

Data analysis

Kaplan-Meyer log rank analysis and survival plots were made based on time to reach toxicity or efficacy related end points (time until 4-doubling of initial tumor volume) using Sigma Plot version 13.0 (Systat Software Inc, San Jose, CA). If a single mouse had more than one tumor, first tumor to reach the end point was counted. Blood cell counts were compared by one-way ANOVA on Ranks and Dunn’s and Holm-Sidak Pairwise Multiple Comparison tests in Sigma Plot. Significance level: p<0.05.

Results and Discussion

Survival of mice treated with multiple injections of $^{177}$Lu-lilotomab

No toxicity-related end point was met in the non-xenografted animals given weekly injections of 200 MBq/kg $^{177}$Lu-lilotomab (Figure 1a). A total accumulated activity of 900 MBq/kg $^{177}$Lu-lilotomab given as three weekly injections of 300 MBq/kg was tolerated without any toxicity related deaths for nude mice injected s.c. with Ramos cells (Figure 1b), while four weekly injections were found to be above the maximum tolerable activity. On day 37 after initial injection, i.e. 16 days after the last therapy injection, 5 of 12 mice had to be euthanized due to radiation toxicity, which manifested as rapid weight loss and severely reduced health condition in combination with low hematology values (see below). One mouse died due to the injection procedure in each of the NaCl and 4·300 MBq/kg groups. These mice were censored from the survival plot (Figure 1b).

In a study by Repetto-Llamazares et al., investigating toxicity of $^{177}$Lu-lilotomab in nude mice, 50% toxicity-related deaths were observed within 25 days after single injection of 800 MBq/kg $^{177}$Lu-lilotomab [24] while in the current study all mice survived when this activity was given in four weekly fractions.

![Figure 1: Tolerability of $^{177}$Lu-lilotomab therapy given as multiple injections.](image)

Body weight changes

In mice without xenografts, no effect on body weight was observed in mice given 2·200 MBq/kg $^{177}$Lu-lilotomab compared to the control mice. Stagnation in body weight increase was seen for some animals given 4·200 MBq/kg $^{177}$Lu-lilotomab. However continued growth was seen one week after last therapy injection (Figure 2a). In mice with xenografts an average decrease in body weight of 5% was seen for the 3·300 MBq/kg therapy group during the treatment period, with regained increase of body weight one week after last injection (Figure 2b). One mouse in the 3·300 MBq/kg group was euthanized due to rapid weight loss on study day 107, not considered radiation toxicity related. The rapid weight loss in the 5 mice suffering from radiation toxicity in the 4·300 MBq/kg therapy group brings the average body weight down prior to termination of mice on day 37.

Hematological toxicity

Hematology analysis showed transient reduction in white blood cells (WBC) and platelet counts in all groups receiving multiple injections of $^{177}$Lu-lilotomab, with mean nadir values (WBC: 9-42% of baseline values; platelets: 22-70% of baseline values) occurring 1-3 weeks after last therapy injection (Figure 3). Platelet counts returned to baseline levels within 4-6 weeks and WBC within 7-9 weeks after last injection, also for the survivors in the 4·300 MBq/kg group (40% of the mice were euthanized...
due to radiation toxicity). One mouse in the 3·300 MBq/kg group had low counts for all hematology parameters after 2 weeks (prior to 3rd injection). However, values were improved at 5 weeks and back to baseline 8 weeks after start of therapy. All 5 mice that were euthanized in the 4·300 MBq/kg group had low hematology counts at 5 weeks (WBC 0.3-0.5·10⁹/L, RBC 3.3-4.7·10¹²/L, PLT 111-225·10⁹/L). The mouse in the 3·300 MBq/kg group that was euthanized at day 107 never had low hematology counts, supporting its weight loss was not related to radiation toxicity.

Figure 2: Effect of ¹⁷⁷Lu-lilotomab multiple injections on body weight of nude mice. Average body weight as function of time after first ¹⁷⁷Lu-lilotomab injection in nude mice without injection of Ramos cells (a) and in mice with s.c. Ramos xenografts (b). Error bars are SD.

MTA of single injection ¹⁷⁷Lu-lilotomab in nude mice was in previous studies found to be between 530 and 600 MBq/kg with hematological toxicity as dose limiting toxicity [26,27]. In the current study the same strain of nude mice was found to tolerate 900 MBq/kg ¹⁷⁷Lu-lilotomab when split into 3 weekly injections. This MTA increase may be explained by the lower radiation burden to non-targeted hematopoietic stem cells due to the lower radiation per injection as well as partial washout and radioactive decay of the circulating RIC between injections, allowing repair of sublethal DNA damage prior to next injection of RIC [23]. The finding is in line with previously reported increase in total administered activity with no increase of dose-limiting hematological toxicity following fractionated RIT [28,29,30]. In studies by Morschhauser et al. and Ocean et al., giving ⁹⁰⁰Y-labeled antibodies as 2-3 weekly fractions enabled the total activity administered to be increased by a factor 2-2.5 [28,29].

Figure 3: Effect of ¹⁷⁷Lu-lilotomab multiple injections on blood cell counts in nude mice. Blood cell counts as function of time after first ¹⁷⁷Lu-lilotomab injection in nude mice without injection of Ramos cells (a) and in mice with Ramos xenografts (b). Blood samples collected from saphenous vein at baseline and every 1.5 weeks in groups of 5 mice in cohort without xenografts (a), at baseline, 2, 5, 8 and 11 weeks for xenograft-bearing mice (b).

Histopathological changes

Histopathological changes were observed in tissues from the hemolymphopoietic system (femoral bone marrow, spleen, and inguinal lymph nodes). Main findings are summarized in Table 2. For the femoral bone
Table 2: Incidence of main histopathological findings in nude mice with or without Ramos xenografts treated with multiple injections of $^{177}$Lu-lilotomab or 0.9% NaCl. Occurrence of findings in % and numbers relative to total number of organs investigated are indicated, independent of severity.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Histopathological finding</th>
<th>NaCl 0 (0/23)</th>
<th>2×200 MBq/kg (0/10)</th>
<th>4×200 MBq/kg (0/13)</th>
<th>2×300 MBq/kg (0/14)</th>
<th>3×300 MBq/kg (0/13)</th>
<th>4×300 MBq/kg (0/13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone marrow</td>
<td>Reduced cellularity</td>
<td>0 (0/23)</td>
<td>80 (8/10)</td>
<td>0 (0/13)</td>
<td>43 (6/14)</td>
<td>75 (9/12)</td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>Lymphoid depletion</td>
<td>13 (3/23)</td>
<td>90 (9/10)</td>
<td>100 (10/10)</td>
<td>92 (12/13)</td>
<td>100 (14/14)</td>
<td>100 (13/13)</td>
</tr>
<tr>
<td></td>
<td>Extramedullary hematopoiesis</td>
<td>96 (22/23)</td>
<td>100 (10/10)</td>
<td>90 (9/10)</td>
<td>92 (12/13)</td>
<td>100 (14/14)</td>
<td>85 (11/13)</td>
</tr>
<tr>
<td></td>
<td>Pigmented macrophages</td>
<td>70 (16/23)</td>
<td>100 (10/10)</td>
<td>90 (9/10)</td>
<td>77 (10/13)</td>
<td>86 (12/14)</td>
<td>92 (12/13)</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>Lymphoid depletion</td>
<td>32 (7/22)</td>
<td>100 (10/10)</td>
<td>100 (9/9)</td>
<td>46 (6/13)</td>
<td>100 (13/13)</td>
<td>100 (9/9)</td>
</tr>
<tr>
<td>Liver</td>
<td>Hepatocellular vacuolation</td>
<td>0 (0/10)</td>
<td>ND</td>
<td>0 (0/5)</td>
<td>ND</td>
<td>ND</td>
<td>60 (3/5)</td>
</tr>
<tr>
<td></td>
<td>Hepatocellular necrosis</td>
<td>0 (0/10)</td>
<td>ND</td>
<td>0 (0/5)</td>
<td>ND</td>
<td>ND</td>
<td>20 (1/5)</td>
</tr>
<tr>
<td>Kidney</td>
<td>Tubular dilatation</td>
<td>0 (0/10)</td>
<td>ND</td>
<td>20 (1/5)</td>
<td>ND</td>
<td>ND</td>
<td>40 (2/5)</td>
</tr>
<tr>
<td>Ovaries</td>
<td>Atretic follicles</td>
<td>0 (0/9)</td>
<td>ND</td>
<td>67 (2/3)</td>
<td>ND</td>
<td>ND</td>
<td>100 (4/4)</td>
</tr>
</tbody>
</table>

a: ND: Not done - tissues not harvested for analysis  
b: Occurred in mice with symptoms of acute radiation toxicity, euthanized 16 days after the last therapy injection.

marrow, reduced cellularity of minimal to slight severity was observed in the 4·200 MBq/kg and 3·300 MBq/kg groups, and up to severe changes in 8 of 12 animals in the 4·300 MBq/kg group. The severity of this finding was more prominent in mice that had to be euthanized early due to signs of radiation toxicity. An increase in incidence and severity of lymphoid depletion in spleen and lymph node with increasing activity was observed. Other less frequent and/or less severe changes were observed in the liver, ovaries and kidneys of animals from the highest activity groups. Hepatocellular vacuolation and necrosis were observed only in animals that were euthanized due to radiation toxicity (activity above MTA). Presence of atretic follicles was seen in all groups treated with $^{177}$Lu-lilotomab, in line with findings reported by Repetto-Llamazares et al. for single injection treatment and could potentially be explained by cross irradiation from the closely located kidneys during the elimination phase of the RIC [24].

The activity dependency in histopathological changes in tissues from the hemolymphopoietic system fits well with the hematology data in Figure 3. The high severity of abnormal changes in mice that were terminated due to radiation toxicity is reflected in the low cell counts for these animals on day 37 (Figure 3b). For mice in the 4·300 MBq/kg group that were euthanized later, pathological changes were less severe, indicating a recovery of changes that was further reflected in the hematology data.

**Tumor growth inhibition**

A tumor growth delay was observed in all groups given multiple injections of 300 MBq/kg $^{177}$Lu-lilotomab compared to control mice (Figure 4). Statistical differences in tumor growth delay were calculated on mouse level based on time to reach 4 times initial tumor volume by Kaplan-Meyer log rank analysis. In case mice had two tumors, the data for the fastest growing tumors were used. Data are shown in Table 3. All treated groups showed statistically significant delays compared to control group (p<0.001). Tumor growth delay was longer following 3 injections than 2 injections of 300 MBq/kg $^{177}$Lu-lilotomab; however, growth delay was not further prolonged by adding a 4th injection. Durable complete regression for more than 100 days was observed for 56% of the tumors in mice given 3 injections, compared to
28% in mice given 2 injections.

Using the same mouse model and method of analysis, significant tumor growth delay of single injection of 530 MBq/kg \textsuperscript{177}Lu-lilotomab compared to NaCl control was reported [26]. Interestingly, tumor growth in animals treated above MTA with single injection of 800 MBq/kg \textsuperscript{177}Lu-lilotomab was not statistically significant from tumor growth in control animals. Regrowth of tumors in several of the surviving animals treated with single injection of 800 MBq/kg \textsuperscript{177}Lu-lilotomab occurred (safety data published in [24], tumor data previously unpublished). Similar tumor regrowth in surviving mice treated above MTA with 4\times 300 MBq/kg \textsuperscript{177}Lu-lilotomab was observed in the current study (Figure 4).

Average tumor size at start of study was similar in the current study and in the single injection study (172 ± 112 mm\textsuperscript{3} vs. 139 ± 134 mm\textsuperscript{3}). In general, there was a wide range in tumor size. However, no significant difference in response was seen between tumors with small and large initial tumor size, neither in the control group or the therapy groups. The fast growth of the tumor model, with a tumor doubling time less than the half-life of \textsuperscript{177}Lu, may be some of the explanation why weekly multiple injections apparently did not improve efficacy compared to similar activity given as a single injection. Despite potentially improved targeting of the later injections, the lower dose-rate to tumor from the multiple injection regimen may have allowed many of the tumor cells to divide before accumulation of lethal radiation dose.

Tumor growth is heterogenous for Ramos xenografts. In some of the mice a spontaneous tumor regression occurred after initial growth, making efficacy evaluation a difficult exercise. Late spontaneous tumor regression also in the control groups was the main reason why 4 times initial tumor size was chosen as efficacy end point, both in the current and the single injection study. Natural tumor regression using lymphoma xenografts in nude mice has been previously reported [31,32], especially in Ramos xenografts [33,34,26]. In the previous single injection study by Repetto-Llamazares et al. tumor growth was more even and without spontaneous remissions in a control group receiving treatment with 530 MBq/kg of a non-specific RIC, \textsuperscript{177}Lu-IgG, [26]. It is well known that immunosuppression by whole body irradiation can improve growth of xenografts in mice [31,35,36,37]. We can therefore speculate that the reason why surviving mice in the highest activity groups above MTA showed inferior efficacy compared to the MTA groups could be an impaired immune system in these mice.

![Figure 4: In vivo efficacy of multiple injections of \textsuperscript{177}Lu-lilotomab in NHL xenograft model. Mean tumor volume as function of time after start of therapy in nude mice with s.c. Ramos xenografts. Time of multiple injections is indicated by grey arrows. Error bars are SE.](image)

Figure 4: In vivo efficacy of multiple injections of \textsuperscript{177}Lu-lilotomab in NHL xenograft model. Mean tumor volume as function of time after start of therapy in nude mice with s.c. Ramos xenografts. Time of multiple injections is indicated by grey arrows. Error bars are SE.

**Table 3:** Tumor growth inhibition. Growth delay shown as median time to reach 4 times initial tumor volume in nude mice with Ramos xenografts treated with multiple injections of \textsuperscript{177}Lu-lilotomab or 0.9% NaCl.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Activity (dose)</th>
<th>Number of mice</th>
<th>Median time to reach 4 times initial tumor volume (days)\textsuperscript{a}</th>
<th>p-value vs NaCl control</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.9% NaCl</td>
<td>4\times 100 µL</td>
<td>10</td>
<td>11 (8-14)</td>
<td>-</td>
</tr>
<tr>
<td>\textsuperscript{177}Lu-lilotomab</td>
<td>2\times 300 MBq/kg</td>
<td>10</td>
<td>55 (52-58)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>\textsuperscript{177}Lu-lilotomab</td>
<td>3\times 300 MBq/kg</td>
<td>11</td>
<td>&gt;134 (-)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>\textsuperscript{177}Lu-lilotomab</td>
<td>4\times 300 MBq/kg</td>
<td>10</td>
<td>70 (50-90)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

\textsuperscript{a}: Median (95% confidence interval)
We have showed that weekly injections of $^{177}$Lu-lilotomab increase tolerability compared to high dosage single injection, allowing the total injected activity and hence the radiation dose to tumor to be increased without increasing the toxicity to normal tissues. In a clinical setting, a chimeric or humanized version of the lilotomab antibody may be needed for a multiple injection treatment regimen, in order to minimize anti-drug antibody reactions. Nordic Nanovector is currently developing a chimeric version of the lilotomab antibody [38].

Conclusion

In conclusion, weekly injections increase tolerability of $^{177}$Lu-lilotomab compared to high dosage single injection, allowing the total injected activity and hence the radiation dose to tumor to be increased without increasing the toxicity to normal tissues. The study warrants further pre-clinical and clinical testing.

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Competing Interests

All authors are employees and shareholders of Nordic Nanovector ASA.

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Compliance With Ethical Standards

All procedures and experiments involving animals in this study were approved by the National Animal Research Authority and carried out according to the European Convention for the Protection of Vertebrates Used for Scientific Purposes.

References


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