FDG PET/CT and Dosimetric Studies of $^{177}$Lu-Lilotomab Satetraxetan in a First-in-Human Trial for Relapsed Indolent non-Hodgkin Lymphoma—Are We Hitting the Target?

Ayca Løndalen$^{1,2}$, Johan Blakkisrud$^{1,3}$, Mona-Elisabeth Revheim$^{1,2}$, Jostein Dahle$^{4}$, Arne Kolstad$^{5}$, and Caroline Stokke$^{1,3}$

$^1$Division of Radiology and Nuclear Medicine, Oslo University Hospital, Oslo, Norway
$^2$Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, Oslo, Norway
$^3$Department of Physics, University of Oslo, Oslo, Norway
$^4$Nordic Nanovector ASA, Oslo, Norway
$^5$Department of Oncology, Oslo University Hospital, Radiumhospital, Oslo, Norway

Abstract

**Purpose:** $^{177}$Lu-lilotomab satetraxetan, a novel CD37 directed radioimmunotherapy (RIT), has been investigated in a first-in-human phase 1/2a study for relapsed indolent non-Hodgkin lymphoma. In this study, new methods were assessed to calculate the mean absorbed dose to the total tumor volume, with the aim of establishing potential dose–response relationships based on 2-deoxy-2-[18F]fluoro-D-glucose (FDG) positron emission tomography (PET) parameters and clinical response. Our second aim was to study if higher total tumor burden induces reduction in the $^{177}$Lu-lilotomab satetraxetan accumulation in tumor.

**Procedures:** Fifteen patients with different pre-dosing (non-radioactive lilotomab) regimens were included and the cohort was divided into low and high non-radioactive lilotomab pre-dosing groups for some of the analyses. $^{177}$Lu-lilotomab satetraxetan was administered at dosage levels of 10, 15, or 20 MBq/kg. Mean absorbed doses to the total tumor volume ($tTAD$) were calculated from posttreatment single-photon emission tomography (SPECT)/computed tomography (CT) acquisitions. Total values of metabolic tumor volume ($tMTV$), total lesion glycolysis ($tTLG$) and the percent change in these parameters were calculated from FDG PET/CT performed at baseline, and at 3 and 6 months after RIT. Clinical responses were evaluated at 6 months as complete remission (CR), partial remission (PR), stable disease (SD), or progressive disease (PD).

**Results:** Significant decreases in $tMTV$ and $tTLG$ were observed at 3 months for patients receiving $tTAD \geq 200$ cGy compared to patients receiving $tTAD < 200$ cGy ($p = .03$ for both). All non-responders had $tTAD < 200$ cGy. Large variations in $tTAD$ were observed in responders. Reduction in $^{177}$Lu-lilotomab satetraxetan uptake in tumor volume was not observed in patients with higher baseline tumor burden ($tTMV$).

**Conclusion:** $tTAD$ of $\geq 200$ cGy may prove valuable to ensure clinical response, but further studies are needed to confirm this in a larger patient population. Furthermore, this work indicates that higher baseline tumor burden (up to 585 cm$^3$) did not induce reduction in radioimmunoconjugate accumulation in tumor.

**Keywords** Indolent non-Hodgkin lymphoma · FDG PET/CT · SPECT/CT · Radioimmunotherapy · Tumor absorbed dose
Introduction

Individualized treatments in modern oncology demand accurate measurement of the pharmaceutical amount reaching the target. Pharmacokinetic (PK) studies are often applied as indirect methods to theoretically determine the distribution both in normal tissue and tumor. Radiolabeled targeted therapies have the advantage of enabling the direct measure of radiopharmaceutical amount accumulating in normal tissue and tumor. Such measurements became more feasible with advances in hybrid imaging technologies.

Targeted therapies like monoclonal antibodies (mAbs) administered as single agents or in combination with other agents have changed the course of non-Hodgkin lymphoma (NHL). Clusters of differentiation (CD) 20 targeting mAb, rituximab, was the first of its kind. Variations in response were reported when rituximab was given as single agent since its introduction [1]. Several studies in early 2000s investigated if this variation may be explained by factors like tumor burden, antigen concentration in tumor, circulating antigens or genetic factors [2, 3]. In recent years, tumor volume measurements have gained increased interest as a parameter to guide individual dose adjustments. Precise measurement of tumor burden before treatment was proposed as part of individualized therapies [4]. Before the introduction of positron emission tomography/computer tomography (PET/CT), tumor burden was solely determined by computer tomography (CT) as the sum of perpendiculars of all lesions, sum of perpendiculars of target lesions or longest diameter of the largest involved node. With the introduction of metabolic tumor volume (MTV) as a 2-deoxy-2-[18F]fluoro- [177Lu]Lu-lilotomab satetraxetan (Betalutin® (Nordic Nanovector ASA, Oslo, Norway) has been investigated in the first-in-human phase 1/2a study LYMRIT-37–01 for treatment of relapsed indolent NHL [10]. We have previously investigated absorbed doses to normal tissues, and for selected individual lesions [11, 12]. No absorbed dose–response relationships were then found for single lesions [11]. In the current sub-study of LYMRIT-37–01, we aimed to investigate 177Lu-lilotomab satetraxetan radioimmunoconjugate uptake parameters on the whole-body level, and developed method to calculate tTAD. The potential therapeutic effect of tTAD was then analyzed, based on changes in FDG PET parameters from baseline to 3 and 6 months after treatment (ΔtMTV_3months, ΔTLG_3months, ΔtMTV_6months and ΔTLG_6months) and clinical response after 6 months. Furthermore, we investigated if higher baseline tumor burden (tMTV_baseline) induces reduction in the amount of radiopharmaceutical uptake and tumor absorbed dose.

Material and Methods

Patient Characteristics and Treatment

Fifteen patients with relapsed/refractory indolent non-Hodgkin B-cell lymphoma from the multicenter phase 1/2a LYMRIT-37–01 (ClinicalTrials.gov Identifier—NCT01796171) non-randomized trial led by Oslo University Hospital were included in this work. Table 1 shows patient characteristics. Only patients from our center, eligible for dosimetry, were included to assure image standardization. CD37 status of patients were confirmed by immunohistochemistry. Histological subtypes were follicular lymphoma (FL) grade I-IIIA and mantle cell lymphoma (MCL). The LYMRIT 37–01 trial was approved by the regional ethics committee, and all patients had signed an informed consent form.

Arm 1, 4 and 5 patients at three different dosage levels were included. Arms 2 and 3 without pre-dosing with lilotomab were not included due to the discontinuation of these arms and the limited number of patients in these groups. Patients received a single injection of 177Lu-lilotomab satetraxetan; either 10, 15, or 20 MBq/kg body weight. Administered mass: mean 1465 MBq (SD +/− 388) and administered mass: mean 6.4 mg (SD +/− 2.1). All patients were pre-treated with rituximab, and non-radioactive lilotomab was injected as pre-dosing 1–3 h before injection of 177Lu-lilotomab satetraxetan (Table 2) (Fig. 1). Patients were also grouped further based on pre-dosing, defining arm 1 with 40 mg lilotomab (standard flat dose to all patients in this arm regardless of body weight and body surface area) as the “low lilotomab” group and arms 4 and 5 receiving 100 mg/m² and 60 mg/m², respectively, as the “high lilotomab” group (Fig. 1).

FDG PET/CT Imaging and Quantification

FDG PET was performed at baseline (PET_baseline) and repeated 3 months (PET_3months) and 6 months (PET_6months) after 177Lu-lilotomab satetraxetan treatment. PET/CT images...
were acquired using a Biograph 16 (Siemens Healthineers) and Discovery MI (GE Healthcare). Acquisitions were performed from vertex to mid-thigh 58–85 min after intravenous administration of 267 to 405 MBq FDG. All PET scans were reconstructed to comply with the EARL standard. tMTV and $tTLG$ were measured at all three time-points according to EANM procedure guidelines for tumor imaging: version 2 [6]. Syngo.via software solution VB30 (Siemens Healthineers) was used, and a threshold of 41% of $SUV_{max}$ applied. Figure 2a illustrates the entire metabolic tumor uptake volume at $PET_{baseline}$ in one of the patients. Changes in these parameters from baseline to $PET_{3months}$ and $PET_{6months}$ were calculated as percent reduction from baseline value, defined as $\Delta tMTV_{3months}$, $\Delta tTLG_{3months}$, $\Delta tMTV_{6months}$, and $\Delta tTLG_{6months}$. Negative values represent increase in $tMTV$ or $tTLG$. All measurements were performed by an experienced nuclear medicine physician. Two patients did not undergo $PET_{3months}$ and $PET_{6months}$ (one of these patients did not undergo contrast enhanced CT (ceCT) either). Data from these patients were used in the analyses regarding the effect of baseline $tMTV/tTLG$ and effect of dosage levels on $tTAD$ (Fig. 3 and Fig. 4, respectively). One patient did not undergo $PET_{6months}$; thus, only $PET_{3months}$ were used in the analyses regarding $\Delta tMTV/\Delta tTLG$.

Table 1. Patient characteristics in the entire population. Median values (range) are indicated for continuous variables. Distributions of gender and type of lymphoma are given as number and as percentage.

| Characteristic                   | Value               |
|---------------------------------|---------------------|
| Age (y), median (range)         | 70 (38–78)          |
| Gender, $n$ (%)                 | Male                |
|                                 | Female              |
|                                 | 13 (87%)            |
|                                 | 2 (13%)             |
| Body weight (kg), median (range)| 85 (56–111)         |
| Body surface area (m²), median (range) | 1.99 (1.54–2.35) |
|Histology, $n$ (%)               | Follicular lymphoma, grad I 5 (33%) |
|                                 | Follicular lymphoma, grad II 8 (53%) |
|                                 | Follicular lymphoma, grad III 1 (7%) |
|                                 | Mantle cell lymphoma 1 (7%) |

Table 2. Patient treatment. Median value (range) is given for the total injected activity in the entire population. Numbers of patients in each dosage level, stratified by arm, are also given.

| Amount | Patients ($n$) |
|--------|----------------|
| Total injected activity (MBq), median (range) | 1434 (746–2189) 15 |
| Injected activity/body weight (MBq/kg) | Arm 1 10 2 |
| | Arm 1 15 2 |
| | Arm 1 20 2 |
| | Arm 4 15 1 |
| | Arm 4 20 7 |
| | Arm 5 20 1 |

Fig. 1. Study design: three different dosage levels, 10, 15, or 20 MBq/kg, were investigated in the LYMRIT-37–01 study. The zero-hour time point on the grey time line indicates administration of $^{177}$Lu-lilotomab satetraxetan. The current study included arms with three different pre-dosing regimens given 1–3 h before $^{177}$Lu-lilotomab satetraxetan injection. Based on pre-dosing, patients were here divided into two groups as indicated; low and high lilotomab. Pre-treatment regimens were given 28 and 21 days before or 14 days before the radioimmunoconjugate. FDG PET was performed as baseline investigation and at 3 and 6 months.
Patients underwent SPECT/CT at day 4 and day 7 post-injection of $^{177}$Lu-lilotomab satetaxetan in arm 1 and at day 1, 4, and 7 post-injection in arm 4 and arm 5 (Fig. 1). SPECT/CT scans were acquired with a dual-head Symbia T16 (Siemens Healthineers) scanner. Scanner protocol and reconstruction parameters have been described previously [13]. SPECT/CT data were segmented using the software program PMOD (version 3.6; PMOD Industries) and later post-processed with in-house written python software (version 2.7). Total radioimmunoconjugate tumor volume ($tRTV$) with $^{177}$Lu-lilotomab satetaxetan uptake was determined on the day 4 and 7 SPECT/CT scans by a semi-automatic approach. An initial manual segmentation was performed by a nuclear medicine specialist to exclude physiological uptake in normal tissue in close proximity to lesions. Then, a thresholding with a 26% cut-off based on the voxel with the highest uptake in the initial segmentation was carried out. This threshold was chosen after a visual optimization that fitted the tumor volumes. The total radioimmunoconjugate lesion uptake ($tRLU$) was defined as the total activity inside the $tRTV$. $tRLU$ normalized by dosage level was defined as $tRLU_{\text{dosage}}$ ($^{RLU}_{\text{dosage level}}$) (MBq/MBq/kg). Cumulative activity concentration was calculated by assuming a mono-exponential wash-out of the activity, as previously used for individual tumors [13]. Total tumor-absorbed dose, defined as $tTAD$, was calculated from the time-integrated activity curve and the tumor volume, by assuming a local dose deposition of all electron radiation particles, equating to 0.0853 GY/(MBqhrs/g) and a tissue density of 1 g/ml [14]. $tTAD_{\text{dosage}}$ ($^{TAD}_{\text{dosage level}}$) (cGy/MBq/kg).

Response Assessment

Responses were assessed by FDG PET and ceCT at 3 and 6 months after RIT according to the Cheson criteria [15].
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Results

Overall mean (range) imaging-based values were: \(tMTV_{baseline}\) 212 cm\(^3\) (44–585 cm\(^3\)), \(tTLG_{baseline}\) 1427 g (275–4170 g), \(tRTV\) (day 4) 236 cm\(^3\) (39–531 cm\(^3\)), \(tRLU\) (day 4) 18.2 MBq (1.1–56.6 MBq), \(tTAD\) 170 cGy (40–420 cGy). Mean changes in FDG PET parameters were \(\Delta tMTV_{3months}\) 69% (19–100%), \(\Delta tTLG_{3months}\) 66% (8–100%), \(\Delta tMTV_{6months}\) 50% (−78 to 100%), and \(\Delta tTLG_{6months}\) 46% (−134 to 100%) (negative values represent increase). These measures were also stratified by low and high lilotomab groups, as presented in Table 3 and Table 4. Individual values are provided in Supplementary Table 1.

Tumor volumes on PET\(_{baseline}\) (\(tMTV_{baseline}\)) and SPECT day 4 and day 7 (\(tRTV - day4\) and day7) correlated significantly (both \(p < 0.01\)) as expected. Supplementary Fig. 1a shows data for \(tRTV - day4\). Interestingly, there were also strong correlations between glucose consumption, \(tTLG_{baseline}\), and radioimmunoconjugate uptake normalized by dosage, \(tRLU_{dosage}\) day4 and day 7 (both, \(p < 0.01\)), an indication that \(^{177}\)Lu-lilotomab satetraxetan successfully targets FDG avid tumor tissue. Supplementary Fig. 1b shows data for \(tRLU_{dosage}\) day 4. However, radioimmunoconjugate activity concentration (expressed as \(tRLU_{dosage}/volume\)) and baseline \(SUV_{mean}\) correlation were not significant (\(p = 0.07\)), indicating that consumption of glucose and CD37 expression on tumor cells does not correspond (Supplementary Fig. 1d).

We tested if increasing baseline tumor volumes have reducing effect on radioimmunoconjugate uptake, a probable sign of antibody shortage for higher target antigen burden. A significant positive correlation between \(tRLU_{dosage}\) and \(tRTV\) indicates that the total tumor uptake of radioimmunoconjugate does not decrease, but contrarily increases with larger tumor volumes (\(p < 0.01\)) (Supplementary Fig. 1c). Another way of testing this was by analyzing the correlation between \(tMTV\) and \(tTAD_{dosage}\). This analysis demonstrated that \(tTAD_{dosage}\) increased slightly with larger \(tMTV_{baseline}\) (Fig. 3a). Even if the correlation was not significant, it is still indicating that larger tumor volumes probably do not cause shortage of radioimmunoconjugate. A similar trend was observed between glucose consumption (\(tTLG_{baseline}\)) and \(tTAD_{dosage}\) (Fig. 3b).

Higher total tumor absorbed doses (\(tTAD\)) were observed with increasing \(^{177}\)Lu-lilotomab satetraxetan dosage levels, but the differences were not significant (\(p = 0.10\)). It should be noted that there are 2 patients in the 10 MBq/kg group which makes this analysis prone to uncertainty (Fig. 4).

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Table 3. FDG PET parameters stratified by low and high lilotomab pre-dosing. Mean (range) values are given for each parameter. The \(\Delta\) values are calculated from the change relative to baseline, and increases are given as negative values.

|            | \(tMTV_{baseline}\) (cm\(^3\)) | \(tTLG_{baseline}\) (g) | \(\Delta tMTV\) (3 months) (%) | \(\Delta tTLG\) (3 months) (%) | \(\Delta tMTV\) (6 months) (%) | \(\Delta tTLG\) (6 months) (%) |
|------------|---------------------------------|-------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Low lilotomab | 138 (63–289)                         | 735 (434–1540)                          | 87 (44–100)                | 90 (53–100)                | 79 (7–100)             | 81 (15–100)             |
| High lilotomab | 261 (44–585)                         | 1888 (275–4170)                         | 58 (19–100)                | 52 (8–100)                | 30 (–78 to 100)         | 21 (–134 to 100)         |
Table 4. SPECT/CT parameters stratified by low and high lilotomab pre-dosing. Mean (range) values are given for each parameter: \( tRTV \), \( tRLU \), and \( tRLUDosage \) in the first three columns are day 4 values

|            | \( tRTV \) (cm³) | \( tRLU \) (MBq) | \( tRLUDosage \) (MBq/MBq) | Effective half-life for \( tRLU \) (days) | \( tTAD \) (cGy) | \( tTAD_{Dosage} \) (cGy/MBq) |
|------------|------------------|-----------------|-----------------------------|------------------------------------------|-----------------|-----------------------------|
| Low lilotomab | 141 (39–199) | 6.2 (1.1–9.8) | 0.4 (0.1–0.7) | 3.2 (1.7–4.8) | 142 (40–420) | 8.6 (4.0–21.0) |
| High lilotomab | 298 (114–531) | 26.1 (6.4–56.6) | 1.4 (0.3–3.0) | 3.2 (2.7–3.8) | 189 (60–380) | 9.8 (3.0–19.0) |

\( tTAD_{Dosage} \) was slightly higher in the high lilotomab group (Table 4), but the differences were not significant across low and high lilotomab groups \((p = 0.61)\).

Reduction in metabolic tumor volumes \((\Delta tMTV_{3months})\) and glucose consumption \((\Delta tTLG_{3months})\) after RIT were significant for the \( tTAD \geq 200\) cGy group compared to the group receiving < 200 cGy \((p = 0.03)\) (Fig. 5a and c). A similar correlation was shown at PET \(_{6months}\) \((\Delta tMTV_{6months} \text{ and } \Delta tTLG_{6months})\) but did not reach significance \((p = 0.07\) for both) (data not shown).

Tumor volume shrinkage and decrease in glucose consumption expressed as \(\Delta tMTV_{3months}\), \(\Delta tTLG_{3months}\), \(\Delta tMTV_{6months}\), and \(\Delta tTLG_{6months}\) were statistically significantly correlated with increasing \( tTAD \) in the high lilotomab group. Such correlation could not be demonstrated in the low lilotomab group (Fig. 5b and d for \(\Delta tMTV_{3months}\), \(\Delta tTLG_{3months}\), respectively) (data not shown for \(\Delta tMTV_{6months}\), \(\Delta tTLG_{6months}\)). However, higher mean \(\Delta tMTV_{3months}\), \(\Delta tTLG_{3months}\), \(\Delta tMTV_{6months}\), and \(\Delta tTLG_{6months}\) were observed in this group, and the lack of a correlation can be explained by the small variations in response (Table 3).

Five patients had CR, two had PR, five had SD, and two had PD (Fig. 6a and Supplementary Table 1). \( tTAD \) was statistically significantly higher in responders (CR + PR) compared to non-responders (SD + PD) in the high lilotomab group \((p = 0.04)\) but not in the low lilotomab group \((p = 1.0)\) (Fig. 6b), similar to the results from \(\Delta tMTV / \Delta tTLG\) analyses. Large variations in \( tTAD \) were observed in responders in low lilotomab group (range 40–420 cGy) (Fig. 6b) (Supplementary Table 1). Across the entire cohort, independent of amount of pre-dosing, all non-responders had \( tTAD < 200\) cGy; however, large variations in \( tTAD \) were observed in responders; especially in the low lilotomab group (Fig. 6).

### Discussion

In this era of precision medicine and personalized therapy, it is imperative to explore the best way of delivering a treatment with precise dosing tailored for each individual patient. Although time-consuming, tumor and normal tissue dosimetry is a crucial part of targeted radiotherapies, and should be standard both in the clinical setting and in trials. Radioimmunoconjugate uptake determined by post-therapy SPECT-derived metrics is an accurate method of analyzing the amount of radioactivity accumulating in tumor; an option unavailable for non-radioactive mAb treatments. In this sub-study of LYMRIT-37–01, the total amount of \(177\)Lu-lilotomab satetraxetan accumulated in tumor \((tRLU)\), total tumor uptake volume \((tRTV)\), and total tumor absorbed doses \((tTAD)\) were calculated from post-therapy SPECT/CT. Our results indicate that \(177\)Lu-lilotomab satetraxetan targets FDG avid tumor tissue without a reduction in uptake in larger tumor volumes; hence, no indication of radioimmunoconjugate shortage was found. Furthermore, especially for the high lilotomab group, \( tTAD \) showed an impact on both \(\Delta tMTV\) and \(\Delta tTLG\), and on clinical response.

Standard PK methods to theoretically calculate the amount of a radiopharmaceutical reaching the tumor volumes outside blood compartment without molecular imaging-based support is not straightforward. This is mainly because of changes in biodistribution between tumor and normal tissue as shown by Stokke et al. for \(177\)Lu-lilotomab satetraxetan [12]. Image-based measurement of the amounts accumulating in the tumor mass is feasible for targeted radiotherapies where it also enables the calculation of tumor absorbed doses. Despite this advantage, tumor dosimetry is still an underutilized method. From such measurements, several interesting findings were derived for \(177\)Lu-lilotomab satetraxetan in this work. A strong correlation between \( tRLUDosage \) and \( tRTV \) implicates that increasing tumor volumes do not reduce \(177\)Lu-lilotomab satetraxetan accumulation in tumor (Supplementary Fig. 1c). This was also demonstrated by larger \( tMTV_{Baseline} \) not resulting in reduced \( tTAD_{Dosage} \) (Fig. 3a). It is therefore fair to assume that the injected amount of radioimmunoconjugate was sufficient for all tumor volumes studied and larger tumor volumes of up to 585 cm³ do not result in shortage of \(177\)Lu-lilotomab satetraxetan. Recent PK studies have reported that tumor burden influences availability of two different CD20 mAbs, rituximab and obinutuzumab, in NHL patients. It was proposed that the standard dose given may not reach sufficient therapeutic levels of mAbs in cases with high tumor burden [4, 17, 18]. While reduction of \( tRLU \) or \( tTAD \) with increasing tumor burden was not demonstrated in our study, a lower mean tumor volume (212 cm³) in our population compared to Tout et al. (313 cm³) [4] and Ternant et al. (600 cm³) [18] might explain why we did not observe such effects. However, Ternant et al. used different methodology to measure \(tMTV\); thus, a direct comparison with our study is not possible. Different levels of CD20 and CD37 expressed by cells, and different administration protocols and pharmacological properties of rituximab versus \(177\)Lu-lilotomab...
satetraxetan hinder direct comparisons. By another approach, whole body (WB) absorbed doses for $^{131}$I-tositumomab were used to demonstrate availability of radioimmunoconjugate. By this method, dosing and pre-dosing regimens and the possibility of fractionation to reach high WB absorbed doses and longer half-life of radioimmunoconjugate were evaluated [19]. Changes in biodistribution after different pre-dosing regimens have previously been demonstrated for $^{177}$Lu-lilotomab satetraxetan [12]. Thus, the approach using WB absorbed doses is probably not precise enough to reflect the amount reaching the tumor for $^{177}$Lu-lilotomab satetraxetan.

Application of $tTLG$ in treatment planning or changes in $tTLG$ to evaluate response during, and after treatment in lymphoma has been proven useful [20, 21]. In our study, lack of correlation between baseline $tTLG$ and $tTAD_{\text{dosage}}$ indicates that absorbed dose cannot be predicted by FDG uptake intensity at baseline FDG PET (Fig. 3b). There was strong correlation between $tTLG$ and $tRLU_{\text{dosage}}$ (Supplementary Fig. 1b), but activity concentration defined by $tRLU_{\text{dosage}} / \text{volume}$ and $SUV_{\text{mean}}$ (calculated across the total tumor tissue) was not significant (Supplementary Fig. 1d). Thus, the $tTLG_{\text{baseline}}$ vs $tRLU_{\text{dosage}}$ correlation can possibly be attributed to the fact that these parameters were derived from their respective volumes rather than a similarity between consumption of glucose and CD37 expression on these cells. While this still supports that $^{177}$Lu-lilotomab satetraxetan successfully targets the viable tumor cells in the volume of interest determined from baseline FDG PET, it also indicates that FDG uptake intensity does not necessarily correlates with CD37 expression in tumor.
We have previously investigated lesion-based tumor-absorbed doses and dose–response relationships, by analyzing 1–5 selected lesions per patient [11]. The criteria for lesion inclusion were then strictly defined for individual dosimetry of each tumor. Significant intra-patient variations were observed and absorbed dose–response relationship at lesion level could not be demonstrated based on changes in FDG PET parameters and Deauville 5-point-scale [11]. In the current study, by measuring $tTAD$, we averaged out intra-patient variations and most importantly avoided possible selection bias. In addition, arms 2 and 3 without pre-dosing with lilotomab were not included to assure a more homogeneous group which can be analyzed as one, for some of the analyses. Traditionally, radioimmunotherapy of lymphoma includes pre-dosing with non-radioactive mAbs; therefore, comparisons with earlier studies are assumed to be more accurate by including only patients receiving non-radioactive mAb as pre-dosing before treatment. While it can be argued that mean absorbed dose is not an adequate metric, and that local low-dose areas are relevant for the overall response, this parameter has been demonstrated as a significant predictor for $^{131}$I-tositumomab treatment [7, 8]. Mean $tTAD$ in our study was 170 cGy (median 130 cGy). This is lower than the median value of between 341 and 275 cGy reported with $^{131}$I-tositumomab (Bexxar®) by Dewaraja et al. [7, 8]. Methodologies applied in these two studies are partly comparable to ours, although the CT-driven approach for tumor delineation, performed for $^{131}$I-tositumomab, can potentially result in a lower mean tumor absorbed dose (i.e. $tTAD$) compared to our current method which may exclude tumor tissue with very low uptake. Also, post-therapy dosimetry was based on imaging at day 2, 5, and 7–9 for $^{131}$I-tositumomab and day 4 and 7 in the present study. While imaging data for day 1 were available for arm 4 and 5, this time-point was not included in the dosimetry calculation due to harmonization between arms. While a previous publication showed the mean difference between 2 and 3 time-points to be 5.5% (maximum error 16%) [13], this is a possible limitation in the current work.
In addition, Dewaraja et al. took into account the non-radiative antibody effect which we did not because of limited cell killing effect of lilotomab demonstrated by in-vitro cell studies [8, 22].

Based on the proposal by Dewaraja et al. [8], we decided to pursue a 200 cGy tTAD threshold by investigating the changes in FDG PET parameters and response status stratified by this threshold in our population. ∆tMTV3months, ∆tTLG3months, ∆tMTV6months, and ∆tTLG6months were higher in tTAD ≥ 200 cGy group and this difference was significant for ∆tMTV3months and ∆tTLG3months (Fig. 5a and c), indicating that there is indeed an absorbed dose–response correlation also for 177Lu-lilotomab satetraxetan and that the same threshold can be applied. All four patients with tTAD ≥ 200 cGy had ∆tMTV3months ≥ 90%. Variations in response in the lower tTAD (<200 cGy) group were larger. While the patient with the lowest tTAD (37 cGy) had ∆tMTV3months = 96% and ∆tMTV6months = 89%, a patient with progression (∆tMTV6months = −77%; negative value represents increase) had tTAD = 100 cGy. One of the patients with progressive disease was the only mantle cell lymphoma in our study with tTAD = 77 cGy. Even though mantle cell lymphomas have been characterized as radiosensitive [23], like follicular lymphomas, this patient unfortunately did not respond to 177Lu-lilotomab satetraxetan treatment. There are few patients in our study and these dissident findings may be random, but it is likely that absorbed doses ≥200 cGy gives a more predictable effect, whereas the response to lower absorbed doses (<200 cGy) may be more dependent on individual radiosensitivity. While the threshold of 200 cGy may seem low, it is also in relative accordance with low dose involved field external beam radiotherapy (2×2 Gy) inducing high response rates for indolent lymphomas [24]. Even if direct comparisons with external beam radiotherapy cannot be made due to different beam qualities, dose rates, etc., this is in the same order of magnitude.

When analyzing the effect of pre-dosing on absorbed doses, we observed a slight but not significantly higher tTAD dosage and tTAD in high lilotomab group. Interestingly, mean ∆tMTV3months, ∆tTLG3months, ∆tMTV6months, and ∆tTLG6months were lower in this group despite slightly higher tTAD (Table 3 and 4). A clear dose–response relationship was illustrated for this group, with higher tTAD inducing statistically significant metabolic tumor volume shrinkage and reduction in lesion glycolysis (Fig. 5b and d for ∆tMTV3months and ∆tTLG3months; Data not shown for 6 months data). On the contrary, the low lilotomab group with slightly lower tTAD dosage and tTAD had higher mean ∆tMTV3months, ∆tTLG3months, ∆tMTV6months, and ∆tTLG6months (Table 3 and 4). Dose–response relationships could not be demonstrated in this group (Fig. 5b and d). This is expected since the overall high response rate could mask a possible dose–response relationship. Why such a difference in response as higher mean ∆tMTV3months, ∆tTLG3months, ∆tMTV6months, and ∆tTLG6months was observed in low lilotomab group and whether other factors that may influence the response are still open questions. A possible explanation may be the differences between baseline mean tMTV between low and high lilotomab groups (Table 3). However, the differences were not significant in the current population (p = 0.27).

The LYMRIT 37–01 PK study demonstrated an increase in blood activity adjusted exposure (area under the curve) with higher lilotomab pre-dosing levels. According to this PK analysis, arm 4 (high lilotomab) demonstrated the highest exposure, the lowest clearance, and the longest biological half-life of 177Lu-lilotomab satetraxetan, slightly higher than arm 1 (low lilotomab) [10]. Furthermore, lower bone marrow and spleen absorbed doses in arm 4 [12] in addition to higher blood exposure shown by PK study [10] indicates that more 177Lu-lilotomab satetraxetan is available for tumor uptake in this arm. This proposed effect was supported in our study by slightly higher tTAD dosage in the high lilotomab group (arm 4 and 5), even though this was not significant. Larger tTAD dosage variations were also observed in the high lilotomab group, in line with our previous lesion-based tumor-absorbed dose analysis [11].

Evaluation of clinical response versus tTAD also supports the assumption of absorbed dose–response relationships and a 200 cGy threshold. Patients with CR had large variations in tTAD (range 69.5–418.3 cGy) (Supplementary Table 1), while all patients with SD or PD had tTAD < 200 cGy (Fig. 6a and c). Only two patients had PR; one just above a tTAD of 200 cGy and one below. Notably, all patients with tTAD ≥ 200 cGy were responders, whereas all non-responders had tTAD < 200 cGy (Fig. 6c). Based on this analysis, we propose a threshold of 200 cGy to ensure CR, while for <200 cGy large variations in response may be expected. Our methodology for tTAD can exclude tumor volumes with low uptake. However, the inclusion of low uptake tumor volumes ensures not to overestimate the patients’ mean tumor absorbed doses. This means that our conclusions with respect to the 200 cGy limit are conservative and can be safely employed regardless of methodology. Applying a different approach, resulting in lower tTADs, would not misplace any <200 cGy patients in the ≥200 cGy group (only CR). Thus, the observation that all non-responders had tTAD < 200 cGy would also hold true using a different approach. When comparing responders and non-responders in low and high lilotomab groups, a similar pattern as for the PET response evaluation was revealed. tTAD was statistically significantly higher in responders (CR + PR) compared to non-responders (SD + PD) in the high lilotomab group (p = 0.04). In the low lilotomab group, the response rates were generally higher, and there were only two patients with SD + PD (Fig. 6b). The reason for the difference between the high and low lilotomab groups is not clear, as discussed above, but regardless of pre-dosing, all non-responders had tTAD < 200 cGy.

We observed increasing tTAD with increasing 177Lu-lilotomab satetraxetan dosage levels in this study (Fig. 4), but the differences were not significant (p = 0.1). This illustrates that increasing the amount of activity administered will not necessarily increase the absorbed dose significantly as this value will also depend on patient-specific uptake.
and kinetics. $\Delta MTV_{3\text{months}}$, $\Delta TLG_{3\text{months}}$, $\Delta MTV_{6\text{months}}$, and $\Delta TLG_{6\text{months}}$ did not either vary between the 3 dosage levels ($p = 1$, $p = 1$, $p = 0.8$, and $p = 0.8$ respectively), but notably, there was a difference for these parameters according to $tTAD$ with threshold 200 cGy, as discussed above. This finding indicates that response does not necessarily directly rely on dosage levels, and that absorbed dose can be further investigated as a solitary predictor.

**Conclusion**

In this study, $^{177}$Lu-lilotomab satetraxetan total tumor absorbed doses were calculated and an absorbed dose–response relationship in indolent NHL patients was revealed in the high lilotomab pre-dosing group. Our results suggest that prediction of response with tumor absorbed doses $\geq 200$ cGy is reasonable, while large variations of response should be expected for tumor-absorbed doses $<200$ cGy.

Higher baseline tumor burden did not induce reduction of $^{177}$Lu-lilotomab satetraxetan uptake in tumor, indicating that the amount of radioimmunoconjugate given was sufficient for all tumor volumes studied. However, further studies are needed to establish this in a patient population with a larger range of volumes.

Well-designed dosimetric studies are the most direct method to measure the uptake of radioimmunoconjugates in targeted radiotherapies. This provides valuable information to determine the optimal dosage levels and pre-dosing regimens to attain the highest possible absorbed dose to tumor while maintaining acceptable absorbed doses to normal tissues.

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**Declarations**

**Ethics Approval** “All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.”

**Conflict of Interest** Arne Kolstad was in part supported by grants from the Norwegian Cancer Society. Arne Kolstad is member of the Scientific Advisory Board of Nordic Nanovector ASA. Jostein Dahle is an employee and shareholder of Nordic Nanovector ASA. Ayca Løndalen has no conflict of interest. Johan Blakkisrud has received grants from South-Eastern Norway Regional Health Authority during the conduct of the study. Mona-Elisabeth Revheim has no conflict of interest. Caroline Stokke has no conflict of interest.

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