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Molecular imaging in diagnostics and therapy of lymphoproliferative disorders

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Molecular imaging in diagnostics and therapy of lymphoproliferative disorders

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CHAPTER 1

Introduction

INTRODUCTION

Lymphoma

Together lymphomas account for approximately 3–4% of all malignancies worldwide [1]. The term lymphoma describes a heterogeneous group of malignancies derived from abnormal clones of B-cell-, T-cell- or natural killer (NK) origin at various degrees of differentiation [2]. Lymphomas are divided into two major categories. Hodgkin lymphoma (HL), characterized by a minority of tumour cells (Hodgkin/Reed-Sternberg cells) surrounded by an extensive infiltrate of immune- and stromal cells, is distinguished from other lymphoma entities called non-Hodgkin lymphomas (NHLs) [2]. The larger and more diverse group of NHLs is again subdivided into T-cell NHL (T-NHL) and B-NHL (B-NHL) that together account for over 30 distinct subtypes. Approximately 35 % of B-NHLs are classified as diffuse large B-cell lymphoma (DLBCL), the most common lymphoma in adults [2]. Haematologists further distinguish lymphomas based on clinical characteristics. Aggressive lymphoma subtypes such as DLBCL, primary mediastinal large B-cell lymphoma (PMBL), high-grade B-cell lymphoma (HGBCL), and NK/T-cell lymphoma are characterised by strong proliferative activity and fast clinical progression [3]. In contrast, indolent lymphoma subtypes such as low-grade follicular lymphoma (FL), marginal zone lymphoma (MZL), mucosa-associated lymphoid tissue (MALT) lymphoma, and lymphoplasmacytic lymphoma (LPL) exhibit low proliferative activity and slow disease progression, but tend to relapse often [3]. In a small proportion of patients, the indolent disease can undergo histological transformation into an aggressive lymphoma [4]. Another important group of lymphomas are those that develop because of suppression or deficiency of the immune system. Prominent subtypes of these immunodeficiency-associated lymphoproliferative disorders (IALPD) are those associated with primary immunodeficiencies, HIV infection, and immunosuppression after haematopoietic stem cell and solid organ transplantation (post-transplant lymphoproliferative disorder) [2]. Thus, within the heterogeneous group of malignancies we refer to as lymphomas, many different subtypes can be distinguished, each with specific clinicopathological features, prognosis and treatment.

Figure 1

Pie to pie chart depicting the most prominent subtypes of lymphoma categorized into B-cell, T-cell- and Hodgkin lymphoma, based on relative proportions of diagnoses from solid tissue biopsies [1]. AITL = angioimmunoblastic T-cell lymphoma, ALCL = anaplastic large cell lymphoma, ALK = anaplastic lymphoma kinase, ATLL = adult T-cell leukaemia/lymphoma, B-NHL = B-cell non-Hodgkin lymphoma, cHL = classical Hodgkin lymphoma, cHL-NS = nodular sclerosis cHL, cHL-MC = mixed cellularity cHL, cHL-LD = lymphocyte-depleted cHL, cHL-LR = lymphocyte-rich cHL, CLL/SLL = chronic lymphocytic leukaemia/small lymphocytic lymphoma, DLBCL = Diffuse large B-cell lymphoma, EATL = enteropathy-associated T-cell lymphoma, E NK/T = extranodal natural killer cell/T-cell lymphoma, FL = follicular lymphoma, HL = Hodgkin lymphoma, HGBL = High grade B-cell lymphoma, LPL = lymphoplasmacytic lymphoma, MCL = mantle cell lymphoma, MALT = mucosa associated lymphoid tissue lymphoma, NHL = non-Hodgkin lymphoma, NLPHL = nodular lymphocyte predominant Hodgkin lymphoma, NMZL = nodal marginal zone lymphoma, PMBL = primary mediastinal large B-cell lymphoma, PTCL = peripheral T-cell lymphoma, SMZL = splenic marginal zone lymphoma, T-NHL = T-cell non-Hodgkin lymphoma

Lymphoma subtypes investigated in this thesis

This thesis describes molecular imaging in the context of Hodgkin lymphoma (HL) for which the UMCG serves as a regional referral centre, diffuse large B-cell lymphoma (DLBCL) the most common lymphoma of adulthood for which an unmet medical need remains in patients with refractory and relapsed disease, and post-transplant lymphoproliferative disorder (PTLD), of which cases are clustered in large transplantation centres such as the UMCG. The following section will shortly introduce these three lymphoma subtypes. inflammatory infiltrate consisting of

lymphocytes, especially regulatory T-cells and T-helper 2 cells, histiocytes/macrophages, granulocytes such as eosinophils and neutrophils, mast cells, B-cells and fibroblasts. Although it has been established that Hodgkin/Reed-Sternberg (HRS) cells are of B-cell origin, these tumour cells have lost the vast majority of B-cell markers and can thus not be targeted with rituximab, a monoclonal antibody that targets the B-cell marker CD20. HL with HRS cells that do not exhibit a B-cell immunophenotype are characterised as classical Hodgkin lymphoma (cHL). According to certain histological features and the makeup of the tumour microenvironment cHL can be subdivided into the four histological categories, nodular sclerosis cHL, mixed cellularity cHL, lymphocyte rich cHL and lymphocyte-depleted cHL. Contrastingly, the tumour cells of nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL), exhibit a preserved B-cell phenotype making these cases eligible for immunotherapy with rituximab.

Diffuse large B-cell lymphoma (DLBCL) accounts for more than a third of all B-cell non-Hodgkin lymphomas, making it the most common form of lymphoma in adults. DLBCL is generally diagnosed as 'de novo' but can also arise through the histological transformation of less aggressive malignancies such as chronic lymphocytic leukaemia/small lymphocytic lymphoma (Richter transformation), follicular lymphoma, marginal zone lymphoma, or NLPHL. Despite attempts to subdivide DLBCLs based on morphological, biological and clinical characteristics, many DLBCL cases remain heterogeneous and are thus clustered into the group of DLBCL not otherwise specified (NOS). In 2000 researchers demonstrated that DLBCL NOS cases could be classified according to the gene expression profiles of their cell of origin, referring to germinal centre B-cells (GCB) or activated B-cells (ABC) [5]. In the context of humeral immune response, genetic rearrangement mechanisms within B-cells facilitate the expression of immunoglobulin molecules with the highest affinity for a given antigen. Recombinase activating genes (RAG) drive the rearrangement of the variable, diversity and joining (VDJ) fragments of the immunoglobulin gene. Two other processes crucial for physiological humeral immunity are somatic hypermutation and class-switch recombination, both of which are regulated by activation-induced cytidine deaminase (AID) [6, 7]. With these inbuilt machineries for genetic recombination B-cells have a higher chance of accumulating genetic aberrations that can result in malignancy. This risk is further increased when mutations render important germinal centre transcription factors and signalling mechanisms, such as B-cell lymphoma 6 (BCL-6), MYC and nuclear factor kappa-light-chain-enhancer of activated B-cells (NF- κ B) as perpetually active [8].

The *MYC* gene located on chromosome 8q24 encodes transcription factors that regulate metabolic activity in human cells by reinforcing the stimulus of growth-factor signalling on glucose metabolism and by influencing and controlling metabolic pathways necessary for DNA replication [9]. In DLBCL about 10–15% of cases harbour a *MYC* translocation, of which about a third involve genes encoding immunoglobulin heavy chains [10], leading to overexpression of *MYC*, aggressive clinical behaviour and worse prognosis. Recently the prognostic impact of this genetic aberrance has led to recognizing a novel lymphoma subtype by the WHO. High grade B-cell lymphoma (HGBL) with *MYC* and *BCL2* and/or *BCL6* rearrangements, often referred to as double or triple hit lymphomas, exhibit at least 2 of the most impactful genetic aberrations leading to a very high proliferative rate and aggressive clinical course, especially in cases with immunoglobulin encoding genes [11].

Post-transplant lymphoproliferative disorders (PTLDs) are a spectrum of haematological pre-malignant and malignant proliferations of lymphoid or plasmacytic origin that develop due to immunosuppression in the context of solid organ- or haematopoietic stem cell transplantation [1]. Based on morphological, genetic and (immuno-) phenotypic characteristics, PTLDs are categorised as either non-destructive-, polymorphic-, monomorphic- or classical Hodgkin lymphoma PTLD [1]. Non-destructive- and polymorphic PTLDs represent EBV-driven, pre-malignant entities of polyclonal origin that generally respond to reduction or cessation of immunosuppression [12, 13]. On the other hand, monomorphic PTLDs are defined by histopathological characteristics of aggressive NHLs (except EBV-positive MALT lymphoma), which develop from clonal expansions of B- or T-cell origin and require additional treatment with immunotherapy and chemotherapy regimens [14–16]. Classical Hodgkin lymphoma PTLD fulfils all characteristics of cHL in immunocompetent patients and is typically treated with the same combined chemo- and radiotherapy regimens [1].

Figure 2

Timeline indicating the most important developments in the treatment of lymphoid neoplasms since the 1960s. ADC = antibody drug conjugate, BH3 = Bcl-2 homology 3, BiTE = bispecific T-cell engager, BTK = bruton tyrosine kinase, CART = CAR-T-cell therapy, iCPI = immune checkpoint inhibitors, Imids = immunomodulatory drugs, PI3K = phosphoinositide 3-kinase, RIT = radioimmunotherapy,

Lymphoma treatment

Since their introduction in the 1960s, combined chemotherapy regimens with alkylating agents such as the CHOP (cyclophosphamide, doxorubicin, vincristine and prednisone) regimen have formed the foundation of treatment in malignant lymphoma. The successful introduction of the monoclonal antibody rituximab 20 years ago [17], heralded a new era of targeted immunotherapy in lymphoma. The most important developments in lymphoma therapy over time are depicted in Figure 2.

With survival benefits of up to 10–15 %, rituximab has become part of first-line treatment in almost all lymphomas expressing its molecular target CD20. This B-cell marker is involved in the B-cell receptor-mediated immune response. Even though CD20 expression is a prerequisite for rituximab therapy to work, not all patients with a CD20 immunophenotype benefit from rituximab treatment [18]. Further research is thus needed to identify which biological factors beyond CD20-expression influence the efficacy of rituximab treatment. In the Netherlands the recent and widespread introduction of rituximab biosimilars has significantly reduced the financial burden that came along with this highly effective targeted therapy. However, in the setting of DLBCL the efficacy of rituximab biosimilars has never been evaluated on a population level.

In recent years novel treatment approaches have led to improved outcomes and reduced toxicity in lymphoma subtypes like CLL/SLL, FL, MCL, and cHL [19, 20]. On

the other hand, patients with aggressive B-NHL (DLBCL, PMBCL, HGBL), PTCLs, and NK/T-cell lymphomas have only benefited marginally, or not at all from targeted therapy approaches.

To improve and personalize care for those patients, new therapeutic targets and drugs are required. However, with the constant advancement and development of therapeutics, diagnostics and risk stratification also need improvement. Moreover, even in subtypes with relatively good outcomes such as cHL and FL, the selection and early identification of patients with refractory disease and those at risk for relapse and histological transformation are crucial for improving outcomes. Especially in the era of immunotherapy and personalized medicine, an integration of both therapeutics and diagnostics into a so-called theragnostic approach might be implicated [21].

Diagnostics

The modern diagnostic workup of lymphoma rests on three pillars. The first is a clinical assessment based on a combination of the patients' medical history (e.g., treatment for previous malignancies, immunosuppression), symptoms (fever, night sweats, loss of weight, lymphadenopathy) and laboratory findings (e.g., increased serum LDH, EBV load, TARC, signs of bone marrow infiltration etc.) [22]. The second is the morphological classification and molecular characterization of lymphoma tissue acquired by histological biopsy. The third is molecular imaging in the form of ^{18}F -FDG PET/CT scans, which is currently used to assess anatomic location, stage, and response of ^{18}F -FDG-avid lymphoma subtypes. It should be noted that ^{18}F -FDG PET/CT is often employed before tissue diagnostics as it can be used to identify which tumour lesions is accessible for excision- or fine needle biopsy. In cHL ^{18}F -FDG PET/CT imaging can even be employed for adjusting treatment based on assessment during therapy (interim PET). While clinical assessment is mainly dependent on the treating physician's training and professional experience, the latter two diagnostic pillars might be improved upon to leverage a survival benefit for patients.

Apart from routine morphological assessment combined with immunohistochemistry, novel techniques for analysing the intricate molecular makeup, and pathophysiology of lymphoma include fluorescence in situ hybridization (FiSH), gene expression profiling (GEP), and next-generation sequencing (NGS). Slowly these techniques are being integrated into the routine assessment of lymphomas [23], e.g. FiSH for *MYC*, *BCL-2* and *BCL-6* translocations is now performed in patients

with DLBCL eligible for intensified treatment. Novel approaches such as ctDNA hold future potential for disease and response monitoring and insights into the genetic dynamics of lymphomagenesis and disease progression (see future perspective). Thus, in the modern workup of lymphoma a clinical suspicion is confirmed via histopathological and molecular analysis of a biopsy and staging, response evaluation and in some cases even interim adjustment of therapy is based on ^{18}F -FDG PET/CT imaging. This thesis focuses on the improvement and future development of molecular imaging for applications in lymphoma.

Prognostication

The heterogeneous character of lymphomas translates into the varying prognosis of patients with different lymphoma subtypes. Prognostication in NHL depends on many clinical, diagnostic, and histopathological characteristics and differs greatly between and even within disease entities. In DLBCL, the low-risk disease is associated with excellent prognosis with an overall survival (OS) of 80–90 %, while about half of high-risk cases have refractory disease or experience an early relapse [24]. To assess the risk of patients with DLBCL, the international prognostic index (IPI) is used [24, 25]. It combines the prognostic characteristics age, LDH serum levels, WHO-performance score, Ann Arbor stage, and involvement of more than one extra-nodal site (as assessed using ^{18}F -FDG PET/CT) into a prognostic model. The IPI has been further refined by adding a characteristic for tumour localisation to more readily predict CNS relapse (CNS-IPI) [26]. With modern treatment, the vast majority of cHL cases (> 90 %) can be cured. Nevertheless, patients with a high risk for relapse or treatment failure need to be identified. The basis for clinical risk assessment in cHL was laid by Hasenclever et al. in 1998 with the international prognostic score (IPS) assessing the seven clinical characteristics age above 45, albumin serum levels, white blood cell count, haemoglobin, lymphocyte count, male sex, and stage IV disease [27].

Along with changes in the diagnostic workup and management of lymphoma, recent advances in the molecular and biological characterization of lymphoma might be translated into improvements for risk stratification (see Discussion). Molecular risk factors such as the previously described cell of origin characteristic based on gene expression profiling [28] and *MYC*-rearrangements identified with FiSH [29] have all been linked to prognosis. Advances in analysing circulating tumour DNA allowed to identify minimal residual disease in lymphoma patients, identifying relapses before clinical signs or symptoms develop. In HL, risk assessment during treatment using changes in ^{18}F -FDG uptake between the pre-treatment and interim PET scans, can be used to adjust treatment regimens. Moreover,

novel ways of analysing imaging data from ^{18}F -FDG PET scans might see the inclusion of imaging parameters such as metabolic tumour volume (MTV) and total lesion glycolysis (TLG) into existing risk scores.

Molecular Imaging

Molecular imaging can be defined as the visualization of molecular processes, using medical imaging techniques such as magnetic resonance imaging (MRI), positron emission tomography (PET), single-photon emission computed tomography (SPECT) and ultrasound [30]. PET imaging with the tracer ^{18}F -FDG has been established as a gold standard for staging (Ann Arbor staging system) and response evaluation (Deauville criteria) of lymphomas [31, 32]. Novel ways of processing acquired PET images, such as semiquantitative analysis, provide new imaging metrics while increasing medical imaging standardization enables researchers to compare results between individuals and patient cohorts from different centres. However, while being highly sensitive for tissues engaged in glycolysis, the ^{18}F -FDG tracer is limited by low specificity in case of inflammation, in areas with physiologically high uptake of glucose (e.g., brain, kidneys, brown fat, heart), and indolent lymphoma subtypes [33]. ^{18}F -FDG remains the most important imaging technique for assessing stage, anatomical position, and response assessment, but evaluation of molecular targets beyond increased glucose metabolism have not found clinical application in lymphoma.

In the era of targeted therapies and personalized medicine, molecular imaging could provide insights into the expression of molecular targets for therapy, especially when employing whole-body imaging techniques such as PET and SPECT. Beyond established techniques such as ^{18}F -FDG PET, molecular imaging offers possibilities for the non-invasive and safe assessment of radioisotope labelled targeting molecules such as monoclonal antibodies [34]. Due to the intrinsic biological heterogeneity of lesions and metastases in cancer [35, 36], in-vivo, whole-body molecular imaging might be used to complement the current gold standard of biopsy-based biological assessment [37]. By directly labelling of therapeutic compounds, PET imaging could even be employed to identify patients that can benefit from targeted therapy. However, molecular imaging using radiolabelled monoclonal antibodies is not only restricted to visualizing and quantifying target expression. Clinical applications of molecular imaging beyond the assessment of target expression include improved staging before therapy [38, 39], monitoring of tumour uptake over time in individual patients [40], assessment of drugs interactions [41], dose-finding [42, 43] and prediction of response to therapy [44].

AIM OF THIS THESIS

This thesis aims to explore the impact of novel analysis techniques and the performance of the established molecular imaging technique ^{18}F -FDG PET/CT in different lymphoma subtypes (chapter 2–4), past, present and future efforts to introduce novel and clinically meaningful tracers for molecular imaging in lymphoma (chapter 5 and 7 - future perspectives), and the importance of validating revamped forms of standard treatment (chapter 6).

In **chapter 2** we retrospectively investigated whether translocation of the metabolic master regulator *MYC* in patients with DLBCL relates to changes metrics derived from the semiquantitative analysis of ^{18}F -FDG PET scans or visual parameters such as tumour necrosis and investigated their prognostic impact in an exploratory survival analysis.

In **chapter 3** we explored the relationship between semiquantitative ^{18}F -FDG PET measures and visually assessed tumour necrosis by retrospectively analysing 71 patients with classical Hodgkin lymphoma (cHL), and investigated the prognostic impact of these parameters in an exploratory survival analysis.

In **chapter 4** we retrospectively evaluated the diagnostic performance of ^{18}F -FDG PET/CT against a reference standard of histopathological reports and 2-year follow up in 91 suspected cases of PTLD and assessed which clinical risk factors affected the diagnostic yield of ^{18}F -FDG PET/CT scans in PTLD.

In **chapter 5** we searched the literature on non-FDG molecular imaging in lymphoma and reviewed the impact of 306 relevant studies on the biological understanding and clinical practise in lymphoma. Furthermore, we gave an overview of current developments in molecular imaging of lymphomas and discussed its potential applications and future role for diagnostics and targeted therapy in lymphoma.

In **chapter 6** we used clinical data from the Dutch National Cancer Registry along with pharmaceutical purchasing information from Dutch hospitals to evaluate the efficacy of rituximab-biosimilars in a population-based retrospective cohort study. After validating the pharmaceutical purchasing information, we compared the 3-year overall survival of 4429 Dutch patients with DLBCL treated with originator rituximab to the outcome of those treated with rituximab-biosimilars using cox-regression analysis.

In **chapter 7** we generally discuss the status quo of molecular imaging in lymphoma, the results presented in this thesis and aspects of lymphoma diagnostics and therapy not covered by previous chapters. We then go on to describe our on-going efforts by presenting two prospective molecular imaging studies in lymphoma patients. The first focuses on visualising the expression of the immune checkpoint programmed death-ligand 1 (PD-L1) in DLBCL patients using the radiolabelled therapeutic anti-PD-L1 antibody atezolizumab as a novel tracer for PET imaging. The second study aims at CD30-imaging in relapsed/refractory DLBCL patients using the Zirconium-89 (^{89}Zr)-labelled monoclonal antibody component of the anti-CD30 antibody drug conjugate brentuximab vedotin.

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CHAPTER 2

Tumour necrosis as assessed with ^{18}F -FDG PET is a potential prognostic marker in diffuse large B-cell lymphoma independent of *MYC* rearrangements

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ABSTRACT

Objectives: *MYC* gene rearrangements in diffuse large B cell lymphomas (DLBCLs) result in high proliferation rates and are associated with a poor prognosis. Strong proliferation is associated with high metabolic demand and tumour necrosis. The aim of this study was to investigate differences in the presence of necrosis and semiquantitative ^{18}F -FDG PET metrics between DLBCL cases with or without a *MYC* rearrangement. The prognostic impact of necrosis and semiquantitative ^{18}F -FDG PET parameters was investigated in an explorative survival analysis.

Methods: Fluorescence in situ hybridisation analysis for *MYC* rearrangements, visual assessment, semiquantitative analysis of ^{18}F -FDG PET scans and patient survival analysis were performed in 61 DLBCL patients, treated at a single referral hospital between 2008 and 2015.

Results: Of 61 tumours, 21 (34%) had a *MYC* rearrangement (*MYC*⁺). *MYC* status was neither associated with the presence of necrosis on ^{18}F -FDGPETscans (necrosis^{PET}; $P = 1.0$) nor associated with the investigated semiquantitative parameters maximum standard uptake value (SUV_{max} ; $P = 0.43$), single highest SUV_{max} ($P = 0.49$), metabolic active tumour volume (MATV; $P = 0.68$) or total lesion glycolysis (TLG; $P = 0.62$). A multivariate patient survival analysis of the entire cohort showed necrosis^{PET} as an independent prognostic marker for disease-specific survival (DSS) (HR= 13.9; 95% CI 3.0–65; $P = 0.001$).

Conclusions: *MYC* rearrangements in DLBCL have no influence on the visual parameter necrosis^{PET} or the semiquantitative parameters SUV_{max} , MATV and TLG. Irrespective of *MYC* rearrangements, necrosis^{PET} is an independent, adverse prognostic factor for DSS.

MATERIALS AND METHODS

Study design and case selection

For this retrospective single-centre study, consecutive patients with newly diagnosed, histologically confirmed DLBCL between 2008 and 2015 were identified in the electronic healthcare database of the University Medical Centre Groningen (UMCG), a reference centre for aggressive B cell lymphomas. Cases of primary cutaneous DLBCL, primary central nervous system lymphoma, primary mediastinal B cell lymphoma and immunodeficiency-associated lymphomas were excluded. The selection of cases for this study is summarised in Figure 1. Patients were stratified according to the National Comprehensive Cancer Network international prognostic index (NCCN-IPI) [22]. End of treatment response was assessed by ^{18}F -FDG PET/CT scan. Tumour responses were classified according to Lugano criteria [12]. Follow-up was registered until early October 2017. According to Dutch regulations, no medical ethical committee approval was required for this retrospective, non-interventional study. A waiver was obtained from the medical ethics committee of the UMCG on November 13, 2018. The study utilised rest material from patients, the use of which is regulated under the code for good clinical practice in the Netherlands and does not require informed consent in accordance with Dutch regulations.

Pathology review

Pathology review was done using the 2008 WHO classification of haematopoietic and lymphoid tissues (AD) [23]. Histological scoring for necrosis (necrosis^{Hist}) was done by microscopic assessment of haematoxylin & eosin-stained slides. Only microscopic areas with definite histopathological signs of necrosis (i.e. karyolysis) were scored as positive for necrosis^{Hist}.

MYC fluorescence in situ hybridization

For evaluation of a *MYC* rearrangement formalin-fixed paraffin-embedded tissue blocks of primary tumour samples were used. Interphase fluorescence in situ hybridization (FiSH) was performed on 4- μm thick whole tissue sections, using Vysis break apart probes (Abbot technologies) and standard FiSH protocols as previously described [24].

^{18}F -FDG PET imaging

All ^{18}F -FDG PET scans were performed prior to therapy. Patients were allowed to continue all medication and fasted for at least 6 hours before whole-body (from

Figure 1

Flow-chart of case selection.

skull vertex to mid-thigh level) three-dimensional PET images were acquired. This was done 60 minutes after intravenous administration of a standard dose of 3 MBq/kg (0.081 mCi/kg) bodyweight ^{18}F -FDG on a Biograph mCT (Siemens Medical Systems), according to the European Association of Nuclear Medicine (EANM) procedure guidelines for tumour imaging with FDG PET/CT (version 2.0) [25]. Acquisition was performed in 7 bed positions of 2 minutes emission scan for patients 60–90 kg. Patient with body weight less than 60 kg and more than 90 kg body weight, were scanned with 1 minute and 3 minutes per bed position, respectively. Low dose transmission CT was used for attenuation correction. Low dose CT and ^{18}F -FDG PET scans were automatically fused by use of three-dimensional fusion software (Siemens) with manual fine adjustments. Raw data were reconstructed through ultra-high definition (Siemens).

¹⁸F-FDG PET analysis

All ¹⁸F-FDG PET scans were visually assessed for the presence of tumour necrosis (necrosis^{PET}) by an experienced reader (TCK), who was blinded to clinical, laboratory, biopsy, and follow-up findings, as previously described [15]. Areas within any nodal or extranodal ¹⁸F-FDG PET avid lymphomatous lesions that showed no ¹⁸F-FDG uptake were registered as having necrosis^{PET} (Figure 2); no specific visual scale was used. Semiquantitative analysis was performed using an in-house tool for quantitative ¹⁸F-FDG PET/CT analysis, as previously described [26–28]. This program automatically preselects lesions using a SUV_{max} threshold of 4 and a metabolic volume threshold of 2.5 ml. Unwanted preselected FDG-avid regions, such as bladder and brain, are removed by user interaction. Finally, remaining FDG-avid segmentations are processed using a background corrected 50 % of SUV peak region growing method, as described by Frings et al [26], to obtain the final tumour segmentations. From the final segmentation the metabolic active tumour volume (MATV in ml), total lesion glycolysis (TLG = MATV × SUV_{mean}) and SUVs are derived for each lesion independently as well as summed over all lesions. Lesion selection and semiquantitative analysis was performed by MH under direct supervision of an experienced nuclear medicine physician (WN) and a nuclear physicist (RB). SUV_{max} was defined as the highest SUV per voxel within one lymphomatous lesion. In this paper SUV_{max} is reported as the mean of SUV_{max} across all lesions of an individual patient. SUV_{max} single highest was defined as the highest SUV_{max} of all lesions within an individual patient.

Statistical analysis

Comparison between continuous, non-normally distributed variables was estimated by Wilcoxon rank-sum test. Differences between two nominal variables were evaluated using Pearson's Chi square or Fisher's exact test (for expected groups sizes ≤ 5). For exploratory survival analysis the primary endpoints were OS, progression free survival (PFS) and diseases specific survival (DSS). OS was defined as time from diagnosis until death (from any cause). PFS was defined as the time from diagnosis until death or relapse or progression [12]. DSS was defined as the time from diagnosis until death from DLBCL. Surviving patients were censored at the last date of follow-up. Survival curves were estimated according to the Kaplan-Meier method. Cox regression was used for univariate and multivariate survival analysis and results were reported as hazard ratio (HR), 95 % confidence interval (CI) and *P*-value based on statistical Wald-test. A two-tailed *P*-value of less than 0.05 indicated statistical significance. All analyses were performed using R version 3.4.1 and R-studio version 1.0.153 software.

Figure 2

Visual assessment of necrosis and semi-quantitative ^{18}F -FDG PET review process. (A) 65-year-old man with diffuse large B-cell lymphoma (DLBCL) and tumour masses in the left dorsal chest wall and left pelvis, as shown on the coronal maximum intensity projection (MIP) ^{18}F -FDG PET image (arrows). Coronal fused ^{18}F -FDG PET/CT (B), axial CT (C) and axial fused ^{18}F -FDG PET/CT (D) show the tumour mass with photopenic areas (arrow heads), in keeping with tumour necrosis. Coronal and sagittal MIP ^{18}F -FDG PET images (E and F) show tumour segmentation (marked in red colour) for the calculation of metabolically active tumour volume (MATV), total lesion glycolysis (TLG), maximum standard uptake value (SUV_{max}), and single highest SUV_{max} .

INTRODUCTION

Diffuse large B cell lymphoma (DLBCL) accounts for 35% of all B cell non-Hodgkin lymphomas (B-NHL) [1]. Approximately 10–15% of DLBCL cases harbour a *MYC* gene rearrangement (*MYC*⁺), as assessed by fluorescence in situ hybridization (FISH) [2]. These lymphomas are characterized by a very high proliferation rate. Patients bearing a *MYC*⁺ lymphoma experience an aggressive clinical course and have a poor prognosis when treated with the standard regimen of rituximab, cyclophosphamide, doxorubicin, vincristine and prednisolone (R-CHOP) [3]. In 2017 the World Health Organization (WHO) established a new entity for *MYC* rearranged DLBCL, called 'high-grade B-cell lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangements' [1, 4].

MYC is an oncogenic transcription factor regulating a vast array of cellular processes and pathways [5, 6]. Tumour cells overexpressing *MYC*, meet their high energy demands by increased glucose uptake, glycolysis, lactate production and amino acid consumption [7, 8]. However, unlike physiological tissues, cancer cells frequently have acquired resistance to apoptosis and cannot regulate their energy expenditure during metabolic stress, resulting in cell death via necrosis when nutrient supply is compromised [9–11].

In B-NHL patients ^{18}F -fluorodeoxyglucose positron emission tomography (^{18}F -FDG PET) scans are used for staging and response assessment [12]. Tumour necrosis can be assessed by visual inspection of ^{18}F -FDG PET scans (necrosis^{PET}) [13]. Necrosis can be observed in 14–20 % of DLBCL cases and has been associated with an adverse prognosis [14, 15]. Semiquantitative assessment of ^{18}F -FDG PET allows for relative comparison of parameters based on the spatial distribution and degree of ^{18}F -FDG uptake, and is currently being investigated as a tool for therapy monitoring and assessing prognosis in B-NHL [16–18]. Still, data on the prognostic value of the semiquantitative parameters maximum standardized uptake value (SUV_{max}) and metabolically active tumour volume (MATV) in DLBCL are conflicting [19–21].

MYC rearrangement, tumour necrosis (necrosis^{PET}) and parameters derived from semiquantitative analysis of ^{18}F -FDG PET are fundamentally linked to metabolism, yet the relationship between these factors remains unknown.

The previously suggested prognostic impact of necrosis^{PET} [15] and semiquantitative parameters [16–18] in DLBCL might be accredited to their potential association with *MYC* rearrangements.

Therefore, the aim of this study was to investigate differences in the presence of necrosis^{PET} and semiquantitative ^{18}F -FDG PET metrics between DLBCL cases with or without a *MYC* rearrangement. The prognostic impact of these factors was explored by means of survival analysis.

RESULTS

Patient characteristics

Characteristics of the entire cohort (61 patients) are summarized in Table 1. A total of 21 patients (34%) had a DLBCL harbouring a *MYC* rearrangement. *MYC* rearrangement was observed in 11 patients (21.6%) primarily seen in the UMCG (n = 51) and 10 patients (100%) referred from affiliated hospitals (n = 10). *MYC* groups did not differ with regard to baseline characteristics (Table 1) except for serum LDH levels, which were higher in the *MYC*-positive group ($P = 0.036$) than cases without *MYC* rearrangement.

Table 1. Demographics and baseline disease characteristics of patients with diffuse large B-cell lymphoma according to MYC status.

	Total (n = 61)		MYC status				P-value
			MYC ⁻ (n = 40)		MYC ⁺ (n = 21)		
	No.	%	No.	%	No.	%	
Gender							1.0 ^a
Male	36	59.0	24	60.0	12	57.1	
Female	25	41.0	16	40.0	9	42.9	
Age							0.64 ^b
Median (range)	63 (26–91)		64 (26–91)		61 (30–79)		
Age ≤ 60y	24	39.3	14	35.0	10	47.6	
Age > 60y	37	60.7	26	65.0	11	52.4	0.5 ^a
Stage							0.97 ^a
I–II	22	36.0	15	37.5	7	33.3	
III–IV	39	63.9	25	62.5	14	66.7	
NCCN-IPi score							0.32 ^a
0–3	30	49.2	22	55.0	8	38.1	
4–8	31	50.8	18	45.0	13	61.9	
Serum LDH							0.04 ^b
Median (range)	282 (126–3037)		237 (126–1292)		381 (140–3037)		
Normal	29	47.5	22	55.0	7	33.3	
Elevated	32	52.5	18	45.0	14	66.7	0.18 ^a
Treatment							0.36 ^c
R-CHOP	56	91.8	37	92.5	19	90.5	
Intensive chemotherapy	3	4.9	1	2.5	2	9.5	
Palliative	2	3.3	2	5.0	0	0	

^a = Pearson's Chi-squared test with Yates' continuity correction^b = Wilcoxon rank sum test with continuity correction^c = Fischer's exact test for count data**MYC status, necrosis and semiquantitative ¹⁸F-FDG PET parameters**

Necrosis^{PET} was observed in 15 patients (25%). The relationships between MYC status and necrosis^{PET}, necrosis^{Hist} and semiquantitative ¹⁸F-FDG PET parameters are summarized in Table 2. MYC⁺ cases did not differ from cases without MYC rearrangement with regards to necrosis^{PET} ($P = 1.0$) or necrosis^{Hist} ($P = 0.52$). When the semiquantitative parameters SUV_{max}, SUV_{max} single highest, MATV and TLG, were studied, no difference between MYC groups was observed. There was no relation between the presence of necrosis^{PET} and necrosis^{Hist} ($P = 0.1$; data not shown).

Table 2. Necrosis and semiquantitative ¹⁸F-FDG PET parameters according to MYC status

	Total (n = 61)		MYC status				P-value
			MYC ⁻ (n = 40)		MYC ⁺ (n = 21)		
	No.	%	No.	%	No.	%	
Necrosis^{PET}							1.0 ^c
Absent	46	75.4	30	75.0	16	76.2	
Present	15	24.6	10	25.0	5	23.8	
Necrosis^{Hist}							0.52 ^c
Absent	42	68.9	28	70.0	14	66.7	
Present	16	26.2	11	27.5	5	23.8	
Not available	3	4.9	1	2.5	2	9.5	
SUV_{max}							0.43 ^b
Median (range)	13.0 (3.0–38.4)		13.1 (3.0–33.9)		10.4 (5.8–38.4)		
SUV_{max} single highest							0.49 ^b
Median (range)	18.8 (3.8–45.8)		19.7 (3.8–39.0)		14.2 (5.8–45.8)		
MATV							0.68 ^b
Median (range)	154.7 (1–3774)		156.0 (1 –2800)		154.7 (7–3774)		
TLG							0.62 ^b
Median (range)	1387.4 (3–29462)		1632.8 (3–29462)		1147.1 (47–20065)		

^b = Wilcoxon rank sum test^c = Fisher's exact test for count data

Survival analysis

The median follow up was 34 months. At 5-years OS was 67 % (95 % CI: 54–83 %), PFS was 65 % (95 % CI: 53–81 %) and DSS was 81 % (95 % CI: 70–93 %) for the entire cohort. Of the seven deaths unrelated to lymphoma, two were caused by metastatic adenocarcinoma, two were due to cardiac failure, one was due to acute on chronic renal failure and there were two cases of sudden deaths in patients in complete remission of DLBCL.

Results of the univariate Cox regression analysis (HR, 95% CI and *P*-value) are shown in Table 3. Univariate analysis for OS identified *MYC*, NCCN-IPI and SUV_{max} single highest as associated factors. In univariate analysis for PFS only NCCN-IPI was associated with outcome. In univariate analysis for DSS *MYC*, NCCN-IPI, SUV_{max} single highest and necrosis^{PET} were associated. Both SUV_{max} and SUV_{max} single highest showed negative beta-coefficients throughout the univariate survival analysis.

Table 3. Univariate analysis of patient characteristics and semiquantitative ^{18}F -FDG PET parameters on overall survival, progression free survival and disease specific survival.

	Hazard Ratio OS	95 % CI	P-value (Wald- test)	Hazard Ratio PFS	95 % CI	P-value (Wald- test)	Hazard Ratio DSS	95 % CI	P-value (Wald- test)
MYC									
MYC-negative	Reference			Reference			Reference		
MYC-positive	2.9	1.1–7.4	0.025*	2.3	0.97–5.7	0.058	6.3	1.7–24	0.007**
NCCN-IPI									
0–3	Reference			Reference			Reference		
4–8	3.0	1.0–8.3	0.04*	3.6	1.3–10	0.013*	10.7	1.4–84	0.024*
Necrosis^{PET}									
Absent	Reference			Reference			Reference		
Present	1.7	0.6–4.5	0.3	1.8	0.7–4.6	0.2	3.9	1.2–13	0.025*
SUV_{max}									
< Median	Reference			Reference			Reference		
≥ Median	0.4	0.1–1.1	0.08	0.4	0.2–1.1	0.08	0.2	0.05–1.1	0.06
SUV_{max} single highest									
< Median	Reference			Reference			Reference		
≥ Median	0.3	0.09–0.9	0.026*	0.4	0.2–1.1	0.07	0.1	0.01–0.8	0.028*
MATV									
< Median	Reference			Reference			Reference		
≥ Median	1.1	0.4–2.7	0.9	1.3	0.5–3.1	0.59	2.8	0.7–10.6	0.14
Single lesion MATV[†]									
< Median	Reference			Reference			Reference		
≥ Median	1.2	0.5–3.2	0.69	1.5	0.6–3.7	0.39	2.5	0.6–9.6	0.19
TLG									
< Median	Reference			Reference			Reference		
≥ Median	0.6	0.2–1.6	0.31	0.8	0.3–1.9	0.57	1.1	0.3–3.8	0.84

[†] = Volume of the single largest/necrotic lesion;

* = significance level of $P < 0.05$; ** = significance level of $P < 0.01$

Table 4. Multivariate analysis of patient characteristics on overall survival, progression free survival and disease specific survival

		Hazard Ratio OS	95 % CI	P-value (Wald-test)	P-value Model (Wald-test)
MYC	MYC-negative	Reference			0.004
	MYC-positive	3.1	1.1–8.7	0.029*	
NCCN-IPi	0–3	Reference			
	4–8	2.4	0.8–6.9	0.116	
Necrosis^{PET}	Absent	Reference			
	Present	2.6	0.9–7.7	0.079	
SUV_{max} single highest	< Median	Reference			
	≥ Median	0.3	0.1–0.9	0.027*	
		Hazard Ratio PFS	95 % CI	P-value (Wald-test)	P-value Model (Wald-test)
MYC	MYC-negative	Reference			0.005
	MYC-positive	2.4	0.9–6.3	0.07	
NCCN-IPi	0–3	Reference			
	4–8	3.2	1.1–9.0	0.028*	
Necrosis^{PET}	Absent	Reference			
	Present	2.6	1.0–7.0	0.06	
SUV_{max} single highest	< Median	Reference			
	≥ Median	0.4	0.2–1.1	0.08	
		Hazard Ratio DSS	95 % CI	P-value (Wald-test)	P-value Model (Wald-test)
MYC	MYC-negative	Reference			0.0007
	MYC-positive	14.6	2.6–82	0.002**	
NCCN-IPi	0–3	Reference			
	4–8	6.5	0.6–66	0.113	
Necrosis^{PET}	Absent	Reference			
	Present	13.3	2.8–63	0.001**	
SUV_{max} single highest	< Median	Reference			
	≥ Median	0.12	0.01–1.2	0.075	

* = significance level of $P < 0.05$; ** = significance level of $P < 0.01$

Figure 3 Disease specific survival according to *MYC* and necrosis^{PET} status

Kaplan-Meier curve showing disease specific survival according to combined analysis with *MYC* rearrangement status and necrosis^{PET} (log-rank test, $P = 0.00022$). No events were observed in patients without *MYC* rearrangement and who had no necrosis^{PET}.

For multivariate analysis the parameters *MYC*, NCCN-IPI, necrosis^{PET} and SUV_{max} single highest were used due to their prognostic impact on lymphoma-related deaths in univariate analysis (Table 4). Necrosis^{PET} did not contribute to the prognostic model for OS and PFS. However, for DSS, necrosis^{PET} had a large adverse prognostic impact and proved to be independent (HR = 13.9; 95% CI: 3.0–65; $P = 0.001$). Kaplan-Meier analysis for DSS showed no events during the 5-year follow-up period for patients who neither had *MYC* rearrangements, nor necrosis^{PET} (n = 30) (Figure 3).

DISCUSSION

This study shows that there is no association of *MYC* rearrangements with the presence of tumour necrosis assessed by ^{18}F -FDG PET or the semiquantitative ^{18}F -FDG PET parameters SUV_{max} , SUV_{max} single highest, MATV and TLG.

Our analyses demonstrate that necrosis^{PET} had a significant impact on DSS, thereby substantiating previous findings about the prognostic value of this visual marker [15]. The presented data show that presence of *MYC* rearrangement, in itself a powerful predictive factor, is not related to necrosis^{PET}. This allows for integration of *MYC* status and necrosis^{PET} into a prognostic model for DLBCL. When combined with *MYC*, NCCN-IPI and SUV_{max} single highest in multivariate analysis, necrosis^{PET} had the highest significance in predicting death due to lymphoma and a higher prognostic impact than NCCN-IPI, the currently most accurate prognostic index for DLBCL [22]. Thus, our results support the potential additive value of necrosis^{PET} as an important biomarker for risk stratification in the clinical setting [14, 15].

The lack of a relationship between *MYC* rearrangements and semiquantitative ^{18}F -FDG PET metrics might have several causes.

First, proliferation in DLBCL could be independent of *MYC* rearrangement. This would only partially explain the lack of relationship, since the median proliferation index (Ki-67 staining) of *MYC*⁺ DLBCL is universally high (> 90 %) in contrast to the much broader range observed in *MYC*⁻ DLBCL [29].

Second, overexpression of *MYC* via other mechanisms such as epigenetic pathways might explain increased glucose uptake in *MYC* FiSH negative DLBCL. This is supported by studies showing high *MYC* protein expression in 19–40 % of DLBCL cases [30–32]. Cottreau et al. previously reported a lack of relation between *MYC* protein expression and ^{18}F -FDG PET parameters in DLBCL [19]. However, FiSH analysis, which is considered the gold standard examination for *MYC* rearrangements [33–35], was not performed.

Third, high metabolic activity might be induced by alternative changes in metabolic drivers, such as mutations in *PTEN* (observed in approximately 15 % of DLBCL) that lead to activation of the P13K/AKT/mTOR pathway [29, 36–38].

Intriguingly, univariate survival analysis indicated a protective effect for cases with SUV_{max} and SUV_{max} single highest measurements above the median. Studies on the prognostic impact of these variables are conflicting [20, 39–41]. Gallicchio et al. published results similar to ours, alluding to lymphomas with high metabolic activity being more responsive to chemotherapy [20]. In light of conflicting data on the prognostic value of semiquantitative ^{18}F -FDG PET parameters [19–21, 42, 43], our results underline the need for larger, prospective studies with external validation cohorts [42].

This study has several limitations. First there is a referral bias with a high incidence of MYC^+ cases (34%) in our dataset. The enrichment in our study can largely be explained by the fact that, as a reference centre, aggressive and MYC^+ DLBCL cases (including suspected cases of Burkitt lymphoma which subsequently prove to be MYC^+ DLBCL) are referred to our site. Furthermore, patients were included irrespective of their comorbidities. Factors like differences in treatment regimen and non-cancer-related deaths might thus have a large impact on statistical analysis. This is supported by the difference between DSS and OS. Despite its limitations, the prognostic potential of MYC status and NCCN-IPI was reproduced in this dataset making it a representative set of DLBCL cases.

CONCLUSION

In this comprehensive analysis of MYC rearranged DLBCL we showed that a fundamental pathological change such as MYC rearrangement, which by itself has as significant impact on prognosis, has no influence on the presence of necrosis^{PET} or semiquantitative ^{18}F -FDG PET metrics. Explorative survival analysis suggests that the presence of necrosis determined by visual assessment of ^{18}F -FDG PET scans is an independent predictor of disease specific survival in patients with DLBCL, regardless of MYC status.

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SUPPLEMENTARY MATERIAL

Supplementary Figure 1

Venn diagram showing the overlap between necrosis^{PET} and necrosis^{Hist} scoring indicating poor concordance.

Supplementary Figure 2

Distribution of metabolically active tumour volume (MATV) and maximum standard uptake value (SUV_{max}) of single largest tumour lesions in relation to the absence or presence of necrosis^{PET}. MATV ($P = 0.0006$) and SUV_{max} ($P = 0.02$) were significantly higher in patients with necrosis^{PET}. MATV and SUV_{max} showed no significant difference between MYC groups.

Relationship between semiquantitative ^{18}F -fluorodeoxyglucose positron emission tomography metrics and necrosis in classical Hodgkin lymphoma

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ABSTRACT

Semiquantitative ^{18}F -fluoro-2-deoxy-D-glucose positron emission tomography (^{18}F -FDG PET) parameters have been proposed as prognostic markers in classical Hodgkin lymphoma (cHL). In non-Hodgkin lymphoma necrosis as assessed by ^{18}F -FDG PET or computed tomography (CT) (necrosis^{visual}) correlates with an adverse prognosis. We investigated whether semiquantitative ^{18}F -FDG PET metrics correlate with necrosis^{visual}, determined the incidence of necrosis^{visual} and explored the prognostic impact of these factors in cHL. From 87 cHL cases treated with ABVD, (escalated) BEACOPP or CHOP chemotherapy between 2010 and 2017, 71 had both a NEDPAS/EARL accredited ^{18}F -FDG PET and a contrast enhanced CT scan. Semiquantitative ^{18}F -FDG PET parameters were determined using Hermes Hybrid 3D software. Necrosis^{visual}, defined by photopenic tumour areas on ^{18}F -FDG PET and attenuation values between 10 and 30 Hounsfield units (HUs) on CT, was assessed blinded to outcome. Univariate Cox regression survival analyses of progression free survival (PFS) were performed. Necrosis^{visual} was observed in 18.3% of cHL patients. Bulky disease (tumour mass > 10 cm in any direction) ($P = 0.002$) and TLG ($P = 0.041$) but no other semiquantitative parameters were significantly associated with necrosis^{visual}. In explorative univariate survival analysis for PFS the covariates IPS, bulky disease, MTV and TLG were prognostic, while necrosis^{visual} was not.

INTRODUCTION

Classical Hodgkin lymphoma (cHL) is a B-cell neoplasm characterized by a minority of malignant Hodgkin-Reed Sternberg (HRS) cells in an inflammatory background [1]. Patients with cHL have a good prognosis with a long-term survival probability of over 90%. Nonetheless, about 10% of patients with early-stage (stage I–II) and 20–30% with advanced stage disease (III–IV) according to the Ann Arbor classification [2], are refractory or relapse after first line therapy [3]. Therefore, it is important to improve prognostic and predictive models, in order to optimize treatment results and reduce therapy-related toxicity. Several prognostic models are used for clinical risk stratification, including the international prognostic score (IPS) for advanced stage disease, the European Organization for Research and Treatment of Cancer (EORTC) score or the German Hodgkin Study Group (GHSG) classification for early stage disease. New prognostic biomarkers for cHL include serum levels of the Thymus and Activation Regulated Chemokine (TARC) [4–8] and tissue gene expression profiles (GEP) [9, 10]. Although promising, these markers are currently not routinely used to guide treatment.

Imaging markers evaluated in cHL have focused on Metabolic Active Tumour Volume (MTV) as quantified by ^{18}F -fluoro-2-deoxy-D-glucose positron emission tomography (^{18}F -FDG PET) [11–13]. However, results on the prognostic value of MTV in cHL are inconsistent [11, 14, 15]. Although efforts to standardize semiquantitative measurements have been made [16, 17], many study results are not comparable and so far, no uniform cut-off values for the interpretation of parameters such as MTV have been established [14]. The prognostic potential of total lesion glycolysis (TLG), defined as the product of mean standard uptake value (SUV_{mean}) and MTV, was recently shown in relapsed or refractory cHL [13] and early unfavourable cHL [18].

Tumour necrosis can be observed in the centre of fast-growing tumour lesions, illustrating the proposed underlying pathophysiological mechanism, in which tumours outgrow the existing nutrient and oxygen supplies [19, 20]. In non-Hodgkin lymphoma (NHL), necrosis as assessed by magnetic resonance imaging (MRI) and computed tomography (CT) correlates with high tumour volume and high clinical risk scores [21]. Tumour necrosis as assessed using ^{18}F -FDG PET scans and CT imaging (necrosis^{visual}) has been shown to be an adverse prognostic factor in NHL [22–25]. Previously, a study of 76 cHL patients with a thoracic mass treated

with MOPP-like regimens, reported necrosis as assessed with CT scans in 21 % of cases, but no significant impact on length of remission or overall survival (OS) [26]. However, with the introduction of ^{18}F -FDG PET into clinical routine, whole-body staging has significantly improved [27], warranting for a new investigation into the prognostic impact of necrosis^{visual}. In contrast to novel semiquantitative imaging markers, necrosis^{visual} can be easily detected using ^{18}F -FDG PET or CT scans and has a dichotomous outcome.

The aims of the current study were (I) to determine the incidence of necrosis^{visual} in cHL, (II) to evaluate its correlation with semiquantitative ^{18}F -FDG PET metrics, and (III) to explore the prognostic impact of these factors with regard to outcome.

METHODS

Study design and case selection

For this retrospective single centre study, a consecutive series of 87 patients with histologically confirmed cHL according to WHO classification 2008 [28] were initially identified in the electronic healthcare database of the University Medical Centre Groningen (UMCG). Patients were treated with doxorubicin, bleomycin, vinblastine, and dacarbazine (ABVD), bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisone (BEACOPP) or cyclophosphamide, doxorubicin, vincristine, prednisone (CHOP) between 2010 and 2017. Cases of nodular lymphocyte predominant HL, composite lymphoma and Hodgkin-like immuno-deficiency-associated lymphomas were excluded. Of the 87 patients 3 had only stand-alone diagnostic CT scans and were excluded from further analysis. In 13 cases acquisition methods of ^{18}F -FDG PET/CT scans did not comply with guidelines specified by the “Netherlands protocol for standardization of ^{18}F -FDG whole-body PET studies in multi-centre trials” (NEDPAS) [29] or “European Association of Nuclear Medicine Research Ltd.” (EARL) protocols [30]. Finally, a total of 71 cases were eligible for analysis (Figure 1). First and second order semiquantitative metrics were analysed. Patients were stratified according to international prognostic score (IPS) [31]. End of treatment response was assessed by ^{18}F -FDG PET/CT scan. Treatment response was classified as complete remission (CR), partial response (PR), stable disease (SD), or progressive disease (PD) according to Lugano criteria [32]. All cases with relapse were histologically confirmed. Follow-up was registered until February 2018. According to Dutch regulations, no medical ethical committee approval was required for

this retrospective, observational study. A waiver was obtained from the medical ethics committee of the UMCG on November 13th 2018. All data were coded before analysis.

Figure 1

Flow chart illustrating the case selection procedure for this study; cHL = classical Hodgkin lymphoma; PET = positron emission tomography; CT = computed tomography.

¹⁸F-FDG PET imaging

Integrated ¹⁸F-FDG PET/CT images were acquired on a Biograph mCT (Siemens Medical Systems, Knoxville, TN, USA) after a minimum fasting time of 6 hours. Whole body images (from the base of the skull to the mid-thigh) were acquired 60 minutes after intravenous administration of 3 MBq/kg ¹⁸F-FDG. PET image acquisition for patients between 60 and 90 kg bodyweight was performed in 7 bed

positions of 2-minute emission scans. Patients with bodyweight below 60 were imaged for 1.5 minute and above 90 kg for 3 minutes per bed position. Integrated ^{18}F -FDG PET/CT images were corrected for scatter and attenuation based on CT information and automatically fused through three-dimensional fusion software (Siemens). Raw data were reconstructed through ultra-high definition (Siemens).

Semiquantitative analyses

Semiquantitative analyses were performed using the “Tumor Finder” application and the Hermes Hybrid 3D (Hermes Medical Solutions AB, Stockholm, Sweden) software, as described previously [33]. Briefly, NEDPAS/EARL accredited ^{18}F -FDG PET and low-dose CT files were loaded from the UMCG electronic database. A spherical 3 cm³ volume of interest (VOI) over the right lobe of the liver was used as a reference according to PERCIST 1.0 criteria [34] and the “Tumor Finder” application automatically selected all VOIs with uptake $\geq 1.5 \times \text{mean SUV} + 2$ standard deviations (SDs) of this reference VOI. Volumes of high physiological uptake, not suspected for lymphoma were manually removed, while volumes suspected for disease involvement, but not automatically selected by the “Tumor Finder” application were selected manually using a threshold of ≥ 2.5 SUV corrected for body weight (SUVbw). Semiquantitative measurements included maximum standardized uptake value (SUV_{max}), SUV_{peak}, SUV_{mean}, MTV and TLG. SUV_{max} was defined as the SUV of the maximum intensity voxel within a region of interest (ROI), SUV_{peak} as the spherical 1 cm³ VOI within the borders of a lesion with the highest average SUV, SUV_{mean} as the average of SUV within a ROI, MTV as the total metabolically active volume of segmented tumour and TLG as the product of SUV_{mean} $\times$ MTV (summed over all lesions). To be representative of the entire lymphoma SUV_{max/peak/mean} are reported here as means calculated across all lesions.

CT imaging

Full-dose, intra-venous contrast enhancement, diagnostic CT scans of neck, chest and abdomen were acquired as part of an integrated ^{18}F -FDG PET/CT. CT scans were acquired using different multidetector row (≥ 16 -slice) CT scanners (Somatom Series, Siemens Healthineers, Erlangen, Germany). Presence of bulky disease was defined with lymphomatous lesion with a diameter of more than 10 cm in any direction, as assessed by full-dose, intra-venous contrast enhancement, diagnostic CT imaging.

Assessment of necrosis^{visual}

¹⁸F-FDG PET and CT review assessment of necrosis^{visual} was performed by an experienced reader (TCK) who was blinded to follow-up findings including patient outcome, as previously described [22, 23]. Briefly, all ¹⁸F-FDG PET and CT scans were visually assessed for the presence of tumour necrosis, within any nodal or extranodal ¹⁸F-FDG-avid lymphomatous lesion. Necrosis^{visual} was considered present if there were photopenic tumour areas on ¹⁸F-FDG PET and/or tumour areas with attenuation values between 10 and 30 Hounsfield units (HUs) on CT (Figure 2).

Figure 2

¹⁸F-FDG PET in a 21-year-old man with classical Hodgkin lymphoma. Coronal maximum intensity projection ¹⁸F-FDG PET shows a mediastinal tumour mass (A). Axial ¹⁸F-FDG PET with concomitant low-dose CT shows necrosis^{visual} with a photopenic area in the mediastinal tumour (B and C, arrows), with attenuation of around 18 HU on contrast-enhanced CT (D, arrow).

Statistical analysis

Baseline characteristics and semi quantitative parameters were described according to the presence of necrosis^{visual}. Categorical variables were expressed as counts and percentages. Differences between two nominal variables were evaluated using Pearson's Chi square or Fisher's exact test (for expected counts ≤ 5). Continuous variables were expressed as median with range. Univariate logistic regression analyses were used to determine the association of established prognostic parameters (stage, IPS, bulky disease) and semiquantitative parameters (SUV_{max} , SUV_{mean} , SUV_{peak} , MTV and TLG) with the presence of necrosis^{visual}. For survival analysis progression free survival (PFS) and overall survival (OS) were used as endpoints. PFS was defined as the time from diagnosis until death, histologically confirmed relapse or progression of disease (defined as increased diameter of lesions on CT in combination with higher FDG-signal on PET or increased PET signal with increased serum TARC levels) [32, 35], whichever came first. OS was defined as time from diagnosis until death (from any cause). Surviving patients were censored at the last date of follow-up. Survival curves were estimated according to the Kaplan-Meier method. Cox proportional hazards model was used for univariate and bivariate survival analysis and results were reported as hazard ratio (HR), 95 % confidence interval (CI) and *P*-value based on statistical Wald-test. A *P*-value of less than 0.05 indicated statistical significance. Continuous parameters such as MTV and TLG were dichotomized using the statistic median. Determination of an ideal cut-off via receiver operating characteristic (ROC) analysis was deliberately avoided due to the retrospective character of this study and the lack of a validation cohort [14]. All analyses were performed using R version 3.4.1 and R-studio version 1.0.153 software.

RESULTS

Baseline patient characteristics

The baseline characteristics of the 71 patients are summarized in Table 1. The median age across the entire patient population was 36.5 years with a range from 17–82 years. A majority of patients was treated with the ABVD regimen ($n = 55$, 77.5%).

Table 1. Baseline characteristics of patients with classical Hodgkin lymphoma according to necrosis^{visual} status as assessed by ¹⁸F-FDG PET and CT

	Total (n = 71)		Necrosis ^{visual} status				P-value
			Absent (n = 58)		Present (n = 13)		
	No.	%	No.	%	No.	%	
Gender							0.31 ^a
Male	43	60.6	33	56.9	10	76.9	
Female	28	39.4	25	43.1	3	23.1	
Age							0.74 ^b
Median (range)	36 (17–82)		39 (17–82)		30 (19–68)		
Age ≤ 45y	50	70.4	40	69.0	10	76.9	
Age > 45y	21	29.6	18	31.0	3	23.1	
Histology							0.54 ^b
NS	40	56.3	31	53.4	9	69.2	
MC	7	9.9	7	12.1	0	0.0	
LR	1	1.4	1	1.7	0	0.0	
NOS	23	32.4	19	32.8	4	30.8	
EBV*							0.71 ^b
Negative	45	63.4	35	60.3	10	76.9	
Positive	14	19.7	12	20.7	2	15.4	
B symptoms							0.12 ^a
No	33	46.5	30	51.7	3	23.1	
Yes	38	53.5	28	48.3	10	76.9	
Treatment							0.24 ^b
ABVD	55	77.5	47	81.0	8	61.5	
BEACOPP	12	16.9	8	13.8	4	30.8	
CHOP	4	5.6	3	5.2	1	7.7	
Radiotherapy							1.0 ^a
No	34	47.9	28	48.3	6	46.2	
Yes	37	52.1	30	51.7	7	53.8	

NS: nodular sclerosis; MC: mixed cellularity; LR: lymphocyte rich; NOS: not otherwise specified

^a = Pearson's Chi-squared test with Yates' continuity correction

^b = Fischer's exact test for count data

*: Missing in 24 cases

Semiquantitative parameters

The median SUV_{max} , SUV_{mean} and SUV_{peak} were 7.1 (range 3.4–20.9), 4.2 (range 2.7–8.7) and 5.3 (range 2.7–17.1), respectively (Table 2). SUV parameters showed a skewed distribution, with the majority of values lying below the mean (mean SUV_{max} = 8.7; mean SUV_{mean} = 4.6; mean SUV_{peak} = 6.8). MTV (median = 237.8, range = 3.8–1212) and TLG (median = 1169, range = 12.1–8048) were highly variable among examined cases. Both MTV and TLG exhibited distributions skewed towards smaller values (mean MTV = 309.9 ml, mean TLG = 1747.7). The range of TLG became larger with greater volumes (MTV) (Figure 3).

Table 2. Known prognostic parameters and semiquantitative measures according to necrosis^{visual} status

	Total (n = 71)		Necrosis ^{visual} status			
			Absent (n = 58)		Present (n = 13)	
	No.	%	No.	%	No.	%
Stage						
I–II	43	60.6	35	60.3	8	61.5
III–IV	28	39.4	23	39.7	5	38.5
IPS*						
0–2 (low risk)	50	70.4	42	72.4	8	61.5
3–7 (interm./high risk)	20	28.2	15	25.9	5	38.5
Bulky disease						
No	54	76.1	49	84.5	5	38.5
Yes	17	23.9	9	15.5	8	61.5
SUV_{max} Median (range)	7.1 (3.4–20.9)		7.0 (3.4–21.0)		7.9 (5.4–18.3)	
SUV_{mean} Median (range)	4.2 (2.7–8.7)		4.2 (2.7–8.7)		4.2 (3.3–8.2)	
SUV_{peak} Median (range)	5.3 (2.7–17.1)		5.3 (2.7–17.1)		5.3 (4.1–15.7)	
MTV Median (range)	237.8 (3.8–1212)		206.7 (3.8–1212)		398.7 (56.9–1151)	
TLG Median (range)	1169 (12.1–8048)		1093 (12.1–5775)		2079 (346–8048)	

*: Missing in 1 case

Figure 3

Dot plot showing the relation between metabolic tumour volume (MTV), total lesion glycolysis (TLG) and necrosis per investigated case (n = 71). Cases with necrosis^{visual} as determined by visual assessment of ¹⁸F-FDG PET scans and CT are depicted in red (n = 13).

Necrosis

Necrosis^{visual} was observed in 13 of the 71 cases (18.3%). CT (n = 13; 100%) and ¹⁸F-FDG PET (n = 12; 92.3%) agreed on the presence of necrosis in 12 of the 13 cases (92.3%). In one case lesions with attenuation values of 10–30 HU were observed on CT, however photopenic areas in ¹⁸F-FDG PET could not be distinguished due to low resolution of the ¹⁸F-FDG PET images. Of the examined prognostic parameters, bulky disease (OR = 8.5; 95% CI: 2.3–32.1; *P* = 0.002) and TLG (OR = 4.3; 95% CI: 1.1–17.2; *P* = 0.04) correlated with a higher risk for necrosis^{visual} (Figure 3), while stage and IPS did not. All other semiquantitative parameters were not associated with necrosis^{visual} (Table 3).

Table 3. Univariate logistic regression analyses for the association of investigated parameters with the presence of necrosis^{visual}

covariates		Odds ratio	95 % CI	P-value (Wald-test)
Stage	I–II	1		
	III–IV	0.9	0.27–3.2	0.9
IPS	0–2	1		
	3–7	1.75	0.5–6.2	0.39
Bulky disease	Absent	1		
	Present	8.53	2.3–32.1	0.002**
SUV_{max}	< Median	1		
	≥ Median	0.12	0.34–3.8	0.85
SUV_{mean}	< Median	1		
	≥ Median	0.89	0.27–3.0	0.85
SUV_{peak}	< Median	1		
	≥ Median	0.77	0.23–2.6	0.67
MTV	< Median	1		
	≥ Median	2.68	0.74–9.7	0.13
TLG	< Median	1		
	≥ Median	4.3	1.1–17.2	0.041*

* = significance level of $P < 0.05$; ** = significance level of $P < 0.01$

Survival analysis

The median follow up was 42 months (95 % CI: 34–60.7; IQR = 26.7–65.5). Twelve patients (16.9 %) experienced an event as defined for PFS. Seven patients had a histologically confirmed relapse and 4 patients had progressive disease (two with evident increase in diameter (CT) and ^{18}F -FDG signal (PET) and 2 with evident increase of ^{18}F -FDG signal and subsequent increase in TARC levels). There were 2 treatment related deaths: One due to complications of first line therapy (death counted as PFS event), the other due to complications of salvage therapy (one of the 4 patients with progressive disease). Two patients died due to lymphoma. The 5-year PFS and OS for the entire cohort was 80.1 % (95 % CI: 69.9–91.8 %) and 93.9 % (95 % CI: 88.3–99.9 %), respectively.

In univariate analysis for PFS IPS, bulky disease, MTV and TLG were prognostic factors, whereas necrosis was not (HR = 2.8; 95 % CI: 0.8–9.4, $P = 0.096$) (Table 4). Apart from MTV (HR = 5.7; 95 % CI: 1.3–26.2; $P = 0.024$) and TLG (HR = 5.5; 95 % CI: 1.2–25; $P = 0.028$), all other semiquantitative parameters (SUV_{max}, SUV_{mean}, SUV_{peak}) were not prognostic for PFS.

Table 4. Univariate Cox-regression analyses for progression free survival

Prognostic factor		Odds ratio	95 % CI	P-value (Wald-test)
Stage	I–II	1		
	III–IV	3.0	0.9–10.0	0.072
IPS	0–2	1		
	3–7	4.12	1.3–13.1	0.016*
Bulky disease	Absent	1		
	Present	5.3	1.7–16.8	0.005**
Necrosis^{visual}	Absent	1		
	Present	2.8	0.8–9.4	0.096
SUV_{max}	< Median	1		
	≥ Median	0.7	0.2–2.1	0.49
SUV_{mean}	< Median	1		
	≥ Median	0.5	0.2–1.7	0.28
SUV_{peak}	< Median	1		
	≥ Median	0.7	0.2–2.1	0.49
MTV	< Median	1		
	≥ Median	5.7	1.3–26.2	0.024*
TLG	< Median	1		
	≥ Median	5.5	1.2–25.0	0.028*

* = significance level of $P < 0.05$; ** = significance level of $P < 0,01$

DISCUSSION

In this retrospective study we performed a semiquantitative ^{18}F -FDG PET analysis and assessment of necrosis (necrosis^{visual}) by ^{18}F -FDG PET and CT in cHL.

Necrosis^{visual} was observed in 18 % of patients. Despite the additional use of ^{18}F -FDG PET imaging the observed rate of necrosis^{visual} in the current study was slightly lower than the incidence reported by Hopper et al. [26]. This might be explained by the focus on thoracic lesions in the latter study. Since there was a 92 % agreement between CT and ^{18}F -FDG PET to detect necrosis^{visual} we cannot make any statement about the superiority of one modality over the other. Nonetheless, by using two independent modalities we can confirm that necrosis^{visual} is present in a significant part of the cHL population, despite the distinct pathophysiologic characteristics of cHL.

The majority of cells in cHL lesions are infiltrating immune cells which have different proliferative characteristics than cancer cells [1]. However, the immune cell infiltration in cHL is a pathological process that is abnormal and extensive in nature [1, 36, 37]. In the physiological setting lymph nodes harbour a wide spread capillary bed which provides nutrients and oxygen for the reticular meshwork in which immune cell interaction takes place [38]. In cHL, the infiltration by a substantial number of immune cells frequently distorts and alters this highly vascularized lymph node architecture [36, 39]. Nevertheless, biological evidence from studies investigating the role of the cHL microenvironment, suggests that masT-cells, tumour associated macrophages, mesenchymal stromal cells as well as HRS cells themselves might be able to induce and contribute to angiogenesis [40]. In the light of this evidence, one might hypothesize that differences in proliferative activity between individuals, as well as an imbalance between proliferative activity of HRS cells and the infiltrate within individuals might explain why we observed necrosis^{visual} in almost 1 out of 5 patients in our cohort.

Parameters derived from semiquantitative analysis of ¹⁸F-FDG PET scans have been suggested as potential markers in lymphomas [18, 41]. We could confirm these findings, as baseline MTV and TLG were prognostic for PFS in univariate survival analysis. Several small studies have implied that MTV can be used as a prognostic marker for survival in cHL [11, 41–43]. In addition, previous investigations have suggested baseline TLG as a potentially useful prognostic marker for survival in cHL [13, 18] and other lymphomas [44–49]. However, as mentioned earlier, inconsistent results [14, 15], the challenge of standardising quantification [12, 16], as well as small study cohorts, raise doubts about the potential of MTV measurements in clinical practice. While in NHL attempts are being made to include standardized semiquantitative analysis in prospective trials [50], larger, prospective studies with standardized guidelines for semiquantitative analysis and appropriate validation cohorts for the determination of relevant cut-off values are needed to conclusively assess the role of semiquantitative measures in cHL [14, 41].

Our analyses demonstrate that necrosis^{visual} had no prognostic impact on PFSs and was strongly determined by the presence of bulky disease and TLG measures above the median. Since TLG had a significant impact on the presence of necrosis while MTV and SUV_{mean} did not, this points at a group of individual cases in which the interplay between volume and metabolic activity determines the development of necrosis.

The concept of tumour necrosis as a prognostic marker is based on the notion that it can develop when tumours outgrow their metabolic supply [19]. The correlation between proliferation rate and tumour necrosis, as exhibited by solid tumours such as clear cell renal cell carcinoma [51] and nodular cutaneous melanomas [52], is often stated to support this hypothesis. Necrosis as determined by visual assessment of ^{18}F -FDG PET and CT scans, has been identified as an independent adverse prognostic factor in NHL [22–25]. However, conflicting reports [53, 54], as well as findings suggesting a crucial role for necrosis-induced inflammation [55, 56] imply necrosis as one of the causes for, rather than the result of tumour growth [57, 58]. The relationship between necrosis and tumour volume might thus be very different in the setting of cHL with its distinct microenvironment, characterized by an extensive background of inflammatory infiltrate [40].

This study has several limitations. Only 71 patients had ^{18}F -FDG PET scans eligible for semiquantitative analysis, thereby reducing the power of the study. In the light of recent studies using ROC-analysis to find ideal cut offs for subsequent analysis, it is important to note that in the current study median values of semiquantitative ^{18}F -FDG PET parameters were used for the dichotomization of continuous covariates. While we are aware that dichotomization of continuous variables always leads to a loss of information and reduction in power [59], this option was chosen to avoid the introduction of arbitrary “optimal” cut-off values, which, in the absence of a validation cohort, can often be too specific for the retrospectively analysed patient population, leading to unwarranted conclusions [14].

CONCLUSION

In this retrospective cohort study necrosis^{visual}, as assessed by ^{18}F -FDG PET and CT, was observed in 18% of cHL patients. Tumor necrosis^{visual} was significantly associated with bulky disease and TLG. In exploratory survival analysis the semiquantitative ^{18}F -FDG PET parameters TLG and MTV were prognostic with regard to PFS, while necrosis^{visual} was not. Additional research is required to investigate the biological and clinical implications of tumour necrosis in cHL. While our results suggest no significant prognostic impact for necrosis^{visual} the role of semiquantitative ^{18}F -FDG PET parameters should be validated in prospective studies.

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SUPPLEMENTARY MATERIAL

Supplementary table 1. ESMO guidelines 2014 [1]: Risk factors

Large mediastinal mass (> 1/3 of maximum horizontal chest diameter)
Age ($\geq$ 50 years)
Elevated ESR (> 50 mm/h without B symptoms, > 30 mm/h with B symptoms)
$\geq$ 4 nodal areas

Supplementary table 2. ESMO guidelines 2014 [1]: Treatment

Limited	Limited stage with risk factors	Advanced stage
2/3 cycles of ABVD	4 cycles of ABVD or 2 cycles of escBEACOPP + 2 cycles of ABVD ($\leq$ 60 years)	6-8 cycles of ABVD or 6 cycles of escBEACOPP ($\leq$ 60 years)
20 Gy IFRT or ISRT	30 Gy IFRT or ISRT	localized RT to residual lymphoma > 1.5 cm (after ABVD) or localized RT to PET-positive residual lymphoma > 2.5 cm (after BEACOPP)

Supplementary Tables 1,2: References

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Diagnostic performance of FDG PET/CT of post-transplant lymphoproliferative disorder and factors affecting diagnostic yield

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ABSTRACT

Purpose: Post-transplant lymphoproliferative disorder (PTLD) is a serious complication after solid organ and hematopoietic stem cell transplantation, requiring a timely and accurate diagnosis. In this study, we evaluated the diagnostic performance of FDG PET/CT in patients with suspected PTLD and examined if lactate dehydrogenase (LDH) levels, Epstein Barr virus (EBV) load or timing of FDG PET/CT relate to detection performance of FDG PET/CT.

Methods: This retrospective study included 91 consecutive patients with clinical suspicion of PTLD and a total of 97 FDG PET/CT scans within an 8-year period. Pathology reports and a 2-year follow-up were used as the reference standard. Diagnostic performance of FDG PET/CT for detection of PTLD as well as logistic regression analysis for factors expected to affect diagnostic yield were assessed.

Results: The diagnosis of PTLD was established in 34 patients (35%). Fifty-seven FDG PET/CT scans (59%) were true negative, 29 (30%) were true positive, 6 (6%) false positive, and 5 (5%) false negative. Sensitivity of FDG PET/CT for the detection of PTLD was 85%, specificity 90%, positive predictive value 83%, and negative predictive value 92%, with good inter-observer variability ($k = 0.78$). Of the parameters hypothesized to be associated with a true positive FDG PET/CT result for the diagnosis of PTLD, only LDH was statistically significant (OR: 1.03, $P = 0.04$).

Conclusion: FDG PET/CT has a good diagnostic performance in patients suspected of PTLD, with a good inter-observer agreement. Only LDH levels seemed to influence the detection performance of FDG PET/CT. EBV-DNA load and timing of FDG PET/CT after transplantation did not affect FDG PET/CT diagnostic yield.

INTRODUCTION

Post-transplant lymphoproliferative disorder (PTLD) is a serious complication after solid organ (SOT) and hematopoietic stem cell transplantation (HSCT). PTLD encompasses a heterogeneous morphologic spectrum, ranging from EBV driven polyclonal proliferations to aggressive monomorphic large B-cell lymphomas. According to the World Health Organization, PTLD can be classified into 4 main groups: non-destructive lesions, polymorphic PTLD, monomorphic PTLD and classical Hodgkin lymphoma PTLD [1].

PTLD has a variable clinical presentation that may include B-symptoms, lymphadenopathy, organ/allograft dysfunction or a combination of non-specific symptoms. It is characterized by a bimodal presentation curve, with a peak incidence within 1 year after transplantation and a second peak after 4–5 years [2, 3]. However, the diagnosis of PTLD can be made at any time after transplantation and diagnostic work-up is usually initiated by the presence of B-symptoms and biochemical anomalies such as an increase in lactate dehydrogenase (LDH) and detectable Epstein-Barr virus (EBV) DNA. In PTLD, elevated LDH has been associated with a lack of response to initial therapy and worse prognosis [4–6]. EBV is recognized to play a crucial role in the immunopathogenesis of PTLD and EBV-DNA load monitoring is routinely performed for early detection of PTLD [7]. However, LDH and EBV-DNA load are non-specific for the detection of PTLD and particularly EBV-DNA load is not useful in EBV-negative PTLD which may compromise up to 50 % of PTLD cases [8–10]. During diagnostic work-up, ¹⁸F-fluoro-2-deoxy-D-glucose (FDG) positron emission tomography (PET)/computed tomography (CT) may also be performed, allowing for whole-body visualization of metabolic active lesions and direct biopsy localization.

Although FDG PET/CT is an established imaging modality in the detection of other FDG-avid lymphomas, with a reported median sensitivity of 90 % and specificity of 91 %, its diagnostic performance has not been extensively evaluated in PTLD [11,12]. FDG PET/CT may become an essential part of the diagnostic work-up in PTLD patients if proven to be of additional value for the detection of PTLD. However, biomarkers such as LDH, EBV-DNA load and timing of FDG PET/CT after transplantation may also influence its diagnostic yield. The purpose of this study was to evaluate the diagnostic performance of FDG PET/CT in patients with suspected PTLD and to examine if LDH levels, EBV-DNA load and timing after transplantation influence the detection performance of FDG PET/CT.

MATERIALS AND METHODS

Study design and patients

This retrospective study included all consecutive patients between January 2010 until January 2019 for whom an FDG PET/CT scan was requested. Indications for FDG PET/CT requests are described in Table 1. The first FDG PET/CT or in some patients the second scan after a 2-year negative follow-up period were included in the analysis (see “Reference standard”). Only patients 19-years and older were included. Patients with central nervous system involvement, complete tumour resection prior to FDG PET/CT evaluation and those without a biopsy or 2-year follow-up were excluded.

Table 1. Indications for FDG PET/CT request*

	n (%)
Blood panel disturbances (e.g.: complete blood count, biochemistry)	20 (20.6)
High EBV-DNA load	44 (45.3)
Physical symptoms (e.g.: B-symptoms, enlarged lymph nodes, other non-specific symptoms)	37 (38.1)
Anomalies previous examination (e.g.: colonoscopy, other non FDG PET/CT imaging)	8 (39.2)

* multiple indications possible for a single scan

Patient record review

Relevant clinical and biochemical data were collected from the electronic patient files at the University Medical Centre Groningen. These included: age, gender, organ transplanted, time between transplantation and FDG PET/CT, LDH levels, EBV-DNA load and PTLT morphology and histology.

FDG PET/CT acquisition and interpretation

All FDG PET/CT scans were performed on a Siemens Biograph 40- or 64 slice mCT (Siemens Healthineers, Erlangen, Germany) according to the European Association of Nuclear Medicine (EANM) procedure guidelines for tumour imaging [13]. Scans were performed after a minimum fasting time of 6 hours. Images from the mid-thigh to skull base were acquired 60 minutes after intravenous administration of 3 MBq/kg FDG. Integrated FDG PET/CT images were corrected for scatter and attenuation based on CT information. Scans were retrospectively reviewed by

3 readers (2 experienced nuclear medicine physicians (AG and WN) and 1 research fellow (FMJ) using syngo.via software (Siemens Healthineers, Erlangen, Germany). Readers reviewed the scans independently from each other and were blinded for other clinical/imaging findings and pathology results. Scans were considered positive for PTLD when FDG-avid lesions were present that could not be related to other pathology than PTLD. Scans were considered negative when no FDG-avid lesions suspicious for PTLD were found. Scans were considered equivocal when FDG-avid lesions were present, but this uptake could be due to either PTLD or due to other diseases/malignancy. In these cases, a differential diagnosis was noted. Discordant results between readers were re-evaluated in a consensus meeting and conclusively classified as positive or negative for PTLD.

Reference standard

Pathology reports were used as a reference standard for PTLD diagnosis. Two experienced hematopathologists (SR, AD) were consulted for morphology clarification when necessary. In case of a PTLD-negative biopsy or lack of tissue for pathological examination, a 2-year follow-up period without pre-emptive PTLD therapy was accepted as the reference standard. Absence of lymphoma during this period has been shown to be an accurate marker for lack of disease in other lymphomas [14, 15]. True positive cases were defined as a PTLD-positive FDG PET/CT and a PTLD-positive pathology result within 2 years after FDG PET/CT. True negative cases were defined as a PTLD-negative FDG PET/CT and no signs of PTLD within 2-year follow-up. False positive cases were defined as a PTLD-positive FDG PET/CT and no signs of PTLD within a 2-year follow-up. False negative results were defined as a PTLD-negative FDG PET/CT and pathology proven PTLD within a 2-year follow up period.

Statistical analysis

Baseline patient characteristics were summarized using medians with interquartile range (IQR) for non-normally distributed variables. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of FDG PET/CT for the detection of PTLD were calculated with a 95% confidence interval (CI). Logistic regression model analysis was carried out using mean serum LDH levels, mean EBV-DNA load (mean over a 31-day period before FDG PET/CT) and time between transplantation and FDG PET/CT with FDG PET/CT result as a dependent variable. FDG PET/CT results were dichotomized as true positive or not true positive (i.e. false positive, true negative, and false negative combined). The corresponding odds ratio (OR) and 95% CI were calculated. Statistical significance was set at

P -value ≤ 0.05 . Inter-observer variability between the 3 observers was calculated using Fleiss kappa. The kappa value was interpreted according to the method of Landis and Koch: poor (0 to 0.20), fair (0.21 to 0.40), moderate (0.41 to 0.60), good (0.61 to 0.80) and perfect agreement (0.81 to 1) [16]. All statistical analyses were performed using SPSS, version 23.0 (IBM Corporation, Armonk, NY, USA).

RESULTS

Patients

One-hundred-twelve potentially eligible patients were identified. Eleven patients were excluded due to central nervous system involvement, 6 due to complete tumour resection prior to FDG PET/CT evaluation and 4 due to lack of a reference standard. In total, 91 patients and 97 FDG PET/CT scans were included in this study (Table 2).

Table 2. Patient characteristics (n = 91)

Age at diagnosis (years)	Median	54
	Range	19–80
	IQR (Interquartile range)	25
Gender	Male	50 (55 %)
	Female	41 (45 %)
Transplanted organ	Lung	40 (44.0 %)
	Kidney	31 (34.1 %)
	Liver	11 (12.1 %)
	HSCT (Hematopoietic stem cell transplantation)	4 (4.4 %)
	Multi-organ	4 (4.4 %)
	Heart	1 (1.1 %)
Histology	Non-destructive	2 (5.9 %)
	Polymorphic	6 (17.6 %)
	Monomorphic	24 (70.6 %)
	Classic Hodgkin type	1 (2.9 %)
	Unclear	1 (2.9 %)
EBV status tumour	Positive	21 (62 %)
	Negative	13 (38 %)
Time between transplant and FDG PET/CT (years)	Median	5
	Range	0–28
	IQR (Interquartile range)	9

In 6 patients, 2 FDG PET/CT scans were included because of PTLD suspicion on two different occasions with a time interval of more than 2 years. There were 50 males (55%) and 41 females (45%) with a median age of 54 years. The most frequently transplanted organ was lung (n = 40, 44%) followed by kidney (n = 31, 34.1%), liver (n = 11, 12.1%), HSCT (n = 4, 4.4%), multi-organ (n = 4, 4.4%) and heart (n = 1, 1.1%). According to the reference standard, 34 patients (35%) were diagnosed with PTLD. There were 21 EBV-positive PTLDs (62%) and 13 EBV-negative PTLDs (38%). The median time between transplantation and FDG PET/CT was 5 years (IQR: 9).

Diagnostic performance of FDG PET/CT

After a consensus meeting, the three readers assessed 35 scans to be positive for PTLD and 62 scans negative. Pathology confirmation was the reference standard in 64 scans (66%) and 2-year follow up in 33 (34%). According to the reference standard, 57 scans (59%) were true negative, 29 (30%) were true positive, while 6 (6%) false positive and 5 (5%) false negative results were observed (Table 3, Figure 1). On a patient-based analysis, sensitivity of FDG PET/CT for the detection of PTLD was 85%, specificity 90%, PPV 83% and NPV 92% (Table 4).

Table 3. Classification of FDG PET/CT scans (n = 97)

	PTLD present No. (%)	PTLD absent No. (%)
PET positive	29 (29.9)	6 (6.2)
PET negative	5 (5.1)	57 (58.8)

Table 4. Detection performance of FDG PET/CT in PTLD

Analysis	Value %	95% CI
Sensitivity	85	68–94
Specificity	90	80–96
Positive predictive value	83	66–93
Negative predictive value	92	81–97
Accuracy	89	81–94

False positive and false negative scans

In total, 6 scans were found to be false positive (Figure 2). The final diagnoses of these false positive scans were: 1 case of condyloma acuminata in the recto-uterine pouch, 1 case of an adenomatoid tumour in the round ligament, 1 case of

Figure 1

A 49-year-old male presented with low LDH levels (251 U/l) and low EBV DNA (1010 copies/ml) load, 6 years after lung transplantation. FDG PET/CT was requested after palpable lymphadenopathy was clinically detected. Maximum intensity projection FDG PET image shows metabolically active supraclavicular and mediastinal lymph nodes, and a large confluent abdominal lesion. Axial fused PET/CT (top right) and CT (bottom right) show the metabolically active supraclavicular lymph node, which proved to be monomorphic PTLD after biopsy.

Figure 2

A 62-year-old male in which elevated LDH levels (347 U/l) and EBV-DNA load (1032500 copies/ml) were found after clinical monitoring within one year after kidney transplantation. FDG PET/CT was subsequently requested. Maximum intensity projection FDG PET shows disseminated metabolically active cervical, mediastinal and lung parenchymal lesions with focal pararenal, native kidney, mesenteric and liver lesions. Axial fused FDG PET/CT (top right) and CT (bottom right) show a metabolically active supraclavicular lymph node that proved to be a granulomatous inflammation due to a mycobacterium after biopsy.

small cell carcinoma in the transplanted lung, 1 case of systemic *Nocardia* infection, 1 case of aspergillus infection in the lungs and 1 case of spontaneous recovery of the suspected lesions without medical intervention, considered very unlikely to be PTLD. False positive results could be divided into two main categories: (1) other malignancies also showing high FDG uptake, making the differentiation between PTLD and other malignancy difficult and, (2) infections, also taking up FDG and leading to a differential diagnosis of PTLD or infection. Five scans were concluded to be false negative: 2 cases with focal uptake in the tonsils/adenoid, interpreted as physiological uptake but confirmed to be non-destructive PTLD, 1 case with focal uptake in the rectum, interpreted as physiological uptake but confirmed to be polymorphic PTLD, 1 case with uptake in the lungs, interpreted as a primary lung tumour or infection but confirmed to be classic Hodgkin type PTLD, and 1 case considered to be a lung infiltrate without FDG-uptake but confirmed to be monomorphic PTLD (Table 5).

Table 5. Description false positive/negative cases

	Readers' differential diagnosis*	Location FDG uptake	Final diagnosis/ outcome
False positive (n = 6)	PTLD Pelvic malignancy	Recto-uterine pouch	Condyloma acuminata
	PTLD	Round ligament, intra-abdominal, retro-peritoneal and inguinal lymph nodes	Adenomatoid tumour
	PTLD Liver abscess	Cervical, retroperitoneal lymph nodes, liver	Spontaneous recovery
	PTLD Disseminated infection	Supraclavicular, mediastinal, hilar and mediastinal lymph nodes, lung	Systemic (nocardia) infection
	PTLD Lung malignancy	Lung	Small cell carcinoma
	PTLD	Mediastinal lymph nodes, lung	Aspergillus infection
False negative (n = 5)	Inflammation PTLD	Tonsils	Non-destructive PTLD tonsils
	Pneumonia Lung malignancy PTLD	Lung	Classic non-Hodgkin PTLD lung
	Physiologic uptake	Tonsils, adenoids	Non-destructive PTLD tonsils
	Physiologic uptake	Pelvis	Polymorphic PTLD rectum
	Unspecific lung infiltrate	No uptake	Monomorphic PTLD lung

* in order of most likely diagnosis

Determinants of detection performance of FDG PET/CT

According to univariate logistic regression, the only statistically significant parameter associated with a true positive FDG PET/CT scan was serum LDH level with an OR of 1.03 ($P = 0.04$, 95 % CI: 1.001–1.06). Hence, for each 10 unit increase in LDH, the odds of having a true positive FDG PET/CT scan were 3% higher. The remaining parameters, EBV-DNA load (OR: 1.0, $P = 0.59$, 95 % CI: 1.00–1.00) and time between transplant and FDG PET/CT (OR: 1.05, $P = 0.23$, 95 % CI: 0.97–1.12) were not statistically significant in the univariate logistic regression analysis. For this reason, a multivariate logistic regression analysis was not performed (Table 6). Sub-analysis of only EBV-positive PTLD patients also revealed statistically insignificant results (OR: 1.0, $P = 0.64$, 95 % CI: 1.00–1.00).

Table 6. Association of parameters with a true positive FDG PET/CT result for the diagnosis of PTLD

Parameter	Univariate OR	95 % CI	<i>P</i>
Serum LDH levels	1.03	1.001–1.06	0.04
EBV-DNA load	1.00	1.00–1.00	0.59
EBV-DNA load (EBV-positive PTLD cases)	1.00	1.00–1.00	0.64
Time between transplant and FDG PET/CT	1.05	0.97–1.12	0.23

Inter-observer variability

From a total of 97 FDG PET/CT scans evaluated prior to consensus, discordant results were reported in 14 scans. The majority of discordant results were due to FDG uptake in the lung parenchyma ($n = 7$). The differential diagnosis for these lesions included: PTLD, primary lung tumour or infection. There were 5 cases of discordant results observed in the gastrointestinal tract and 1 discordant result for lesions in the pelvic area, in which observers reported difficulty in distinguishing between pathological and physiological uptake. Finally, 1 discordant finding, located in the adenoids of a young patient, was difficult to characterize as either pathological or physiological/infectious. From the 14 discordant FDG PET/CT scans, 2 were false positive and 3 were false negative. The inter-observer variability was found to be good at $k = 0.78$ (95 % CI: 0.68–0.88).

DISCUSSION

Although metabolic imaging with FDG PET/CT has an established role in the diagnosis of non-Hodgkin and Hodgkin lymphoma, few studies have been carried out to assert its detection performance in PTLD [11, 17]. In our study population, comprising of 97 FDG PET/CT scans in 91 patients with suspected PTLD, we found a sensitivity of 85 %, specificity of 90 %, PPV of 83 % and NPV of 92 % with good inter-observer variability ($k = 0.78$). Of the determinants hypothesized to influence detection performance of FDG PET/CT, only LDH levels were statistically significant with an OR of 1.03 ($P = 0.04$, 95 % CI: 1.001–1.06).

Current guidelines from the British Committee for Standards in Haematology, the British Transplantation Society and National Comprehensive Cancer Network include no concrete recommendations on the use of FDG PET/CT for diagnosing PTLD [18,19]. The good diagnostic performance demonstrated in this study indicates that FDG PET/CT is a valuable imaging modality for detecting PTLD. The good inter-observer variability also indicates that FDG PET/CT interpretation was minimally reader dependent between two nuclear physicians and a junior reader. We identified common limitations associated with FDG PET/CT false positive results such as inflammatory conditions and other malignancies in addition to false negative results in non-destructive and polymorphic PTLD, specific to this patient population. Because non-destructive lesions are commonly found in Waldeyer's ring, such lesion location may be prone to be interpreted as physiological uptake [20, 21].

Although the majority of studies published on the diagnostic performance of FDG PET/CT in PTLD are limited to case series, studies by Panagiotidis et al. and Dierickx et al. also reported good FDG PET/CT diagnostic performance [22, 23]. Panagiotidis et al. included 40 patients with suspected PTLD and compared FDG PET/CT diagnostic performance with CT. They concluded that FDG PET/CT plays a significant role in the diagnosis of PTLD with high detection accuracy (sensitivity 88 %, specificity 91 %, PPV 88 % and NPV 91 %) [22]. Some limitations of the study by Panagiotidis et al. included: a smaller study population, half of that included in our study, and FDG PET/CT scans were evaluated by a nuclear medicine physician and a radiologist without blinding for clinical data. Dierickx et al. investigated FDG PET/CT diagnostic performance in 125 cases and reported high sensitivity of FDG PET/CT in detecting PTLD (sensitivity 90 %, specificity 89 %, PPV 85 % and NPV 93 %) [23]. However, their study was performed between 2003 and 2010 with

43% of the study population scanned with a stand-alone PET system and no information on the duration of follow-up in negative cases. A recent systematic review on the clinical performance of different imaging modalities in the diagnosis and treatment response evaluation of PTLD patients reported FDG PET/(CT) to be the most frequently used imaging modality and a promising tool in this setting [24]. In the same review, false positive results were reported due to inflammatory conditions while false negative results occurred in areas of high physiological background activity and in non-destructive PTLD lesions. These results not only indicate the utility of FDG PET/CT in PTLD patients but also concur with some of the findings in this study regarding potential causes for false positive and false negative results.

From the parameters hypothesized to be associated with a true positive FDG PET/CT result for the diagnosis of PTLD, only LDH had a statistically significant odds increase. Uncontrolled proliferation of malignant T-cells with high cellular turnover is characterized by increased glycolysis and LDH release. High energy metabolism may translate into higher FDG uptake in tumour tissue and seemingly increase FDG PET/CT detection performance [25]. Nevertheless, the influence of LDH levels on the detection performance of FDG PET/CT should be confirmed in future research given the fact that the lower boundary of the 95% CI of the OR was close to 1 in the present study. The role of EBV in the pathogenesis of PTLD is well documented, however EBV-DNA load was not associated with true positive FDG PET/CT results in our study [26]. EBV monitoring is common clinical practice for early PTLD detection and various studies have advocated its importance as a predictor of PTLD development [3, 27–31]. Nonetheless, EBV-DNA load does not seem to affect the detection performance of FDG PET/CT. One potential explanation is the high percentage of EBV-negative PTLD in our study population, which may have decreased the clinical utility of the EBV-DNA load covariate. Yet, in a sub-analysis of only EBV-positive PTLD patients, we could not demonstrate a relationship between EBV DNA load and a true positive FDG PET/CT either. We hypothesize that a single time cut-off value may not be indicative of imminent PTLD and consequently the need for FDG PET/CT scanning. Instead, changes over time in EBV DNA load may yield more clinically relevant information as proposed by other studies [32, 33].

Due to the retrospective design of this study, information on immunosuppression adjustments were not available. As a consequence, it was not possible to evaluate EBV-DNA load changes over time as this is affected by immunosuppression intensity. PTLD patient population is inherently heterogeneous with regard to

medical history, immunosuppression regimens and treatment approaches. In our retrospective study, heterogeneity might have been introduced by the 8-year period inclusion time within various medical departments. Lack of standardization limits the analysis on how patient selection and timing of FDG PET/CT affect its diagnostic performance, potentially inducing selection bias. Likewise, due of the retrospective nature of this study we were not able to evaluate how different immunosuppressive regimens might have affected PTLD pathogenesis, which is of great clinical relevance and should be considered in future studies. Despite these limitations, the data presented reflects current clinical practice, as immunosuppression regimens are often department specific and there are currently no guidelines on the use FDG PET/CT for the diagnosis of PTLD. Additional future studies may also focus on the role of interim and end-of-treatment FDG PET/CT on survival, already explored by Keerberghen et al. and Zimmermann et. al., and on the role of FDG PET/CT in paediatric PTLD patients [34, 35]. Considering the marked differences in incidence, (imaging) presentation and prognosis of the disease between children and adults, paediatric specific studies are warranted.

CONCLUSION

FDG PET/CT has a good diagnostic performance in patients with suspected PTLD. False positive results were due to other malignancies or infections while false negative results occurred in cases interpreted as physiological uptake (adenoids and rectum), other malignancy and with 1 case of non-FDG-avid PTLD. Furthermore, a good inter-observer agreement was found, which further underlines the clinical utility of FDG PET/CT. Only LDH levels seemed to influence the detection performance of FDG PET/CT, while EBV-DNA load and time between transplant and FDG PET/CT did not. The results of this study may help to implement FDG PET/CT in future PTLD guidelines.

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Molecular imaging in lymphoma beyond ^{18}F -FDG PET: understanding the biology and its implications for diagnostics and therapy

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ABSTRACT

Mature lymphoproliferative diseases are a heterogeneous group of neoplasms arising from different stages of B-cell and T-cell development. With improved understanding of the molecular processes in lymphoma and novel treatment options, arises a growing need for the molecular characterisation of tumours. Molecular imaging with single-photon-emission CT and PET using specific radionuclide tracers can provide whole-body information to investigate cancer biology, to evaluate phenotypic heterogeneity, to identify resistance to targeted therapy, and to assess the biodistribution of drugs in patients. In this Review, we evaluate the existing literature on molecular imaging in lymphoma, other than ^{18}F -fluorodeoxyglucose molecular imaging. The aim is to examine the contribution of molecular imaging to the understanding of the biology of lymphoma and to discuss potential implications for the diagnostics and therapy of this disease. Finally, we discuss possible applications for molecular imaging of patients with lymphoma in the clinical context.

INTRODUCTION

Lymphomas account for 3–4% of all malignancies worldwide. They arise from abnormal B lymphocytes, T lymphocytes, and natural killer cells at various stages of maturation, and are primarily classified as Hodgkin lymphoma and non-Hodgkin lymphoma. Non-Hodgkin lymphoma is further subclassified into B-cell non-Hodgkin lymphoma and T-cell non-Hodgkin lymphoma, together accounting for more than 30 distinct subtypes with different diagnostic, prognostic, and therapeutic implications [1]. The definite diagnosis and molecular analysis of lymphoma relies predominantly on histopathological biopsies. However, because of the intrinsic heterogeneity of lymphoma, a single biopsy might not provide a complete characterisation of the lymphoma and the tumour microenvironment.

Clinical molecular imaging can provide phenotypic information of the entire tumour by non-invasively visualising and quantifying biological processes that are dysregulated in cancer [2]. The ability to characterise biological processes *in vivo* makes molecular imaging a valuable tool for visualising lymphoma biology and evaluating tumour heterogeneity, immune interactions, resistance patterns, and the biodistribution of drugs [2]. By providing crucial whole-body information, which is not obtainable through biopsies, molecular imaging could potentially improve patient management. Various modalities, such as single-photon-emission CT (SPECT), PET, MRI, magnetic resonance spectroscopy, optical imaging, and ultrasonography are applicable for molecular imaging. We focus on SPECT and PET imaging techniques because these modalities currently provide the most pragmatic solution for imaging of the whole body.

^{18}F -fluorodeoxyglucose (^{18}F -FDG) has become the most widely used molecular imaging tracer for routine diagnostic tests in patients with lymphoma, and its advantages and limitations are well documented. However, with the advent of targeted therapies, ^{18}F -FDG PET cannot be used to visualise tumour distribution of molecular targets at diagnosis and during therapy. Therefore, this Review will discuss molecular tracers other than ^{18}F -FDG in lymphomas.

The aim of this Review is to examine the contribution of molecular imaging to understanding the biology of lymphoma and its implications for treatment, thereby providing a framework for the future development of molecular tracers in the field of lymphoma imaging.

Figure 1

A BRIEF HISTORY OF MOLECULAR IMAGING IN LYMPHOMA

Several key developments have had an effect on the use of molecular imaging in lymphomas (figure 1). The Ann Arbor tumour staging system was introduced in 1971 for Hodgkin lymphoma and has since been adapted and used for most non-Hodgkin lymphomas. Around the same time, SPECT imaging with ^{67}Ga citrate was first studied as a tool for molecular imaging in lymphomas [3]. In human plasma, Ga^{3+} is mostly bound to carrier proteins and iron transport molecules, such as transferrin, because of the similarity between Ga^{3+} and ferric ion (Fe^{3+}). The ability of ^{67}Ga to target tumours relies on the upregulation of transferrin receptors on the surface of tumour cells because of the iron dependent synthesis of deoxyribonucleotides required for DNA replication [4]. The diagnostic performance of SPECT imaging, including ^{67}Ga citrate, was improved by combining CT with SPECT into a hybrid SPECT/CT modality in the early 1990s. However, important limitations of SPECT scanning with ^{67}Ga citrate include low specificity, low spatial resolution, and hepatic clearance, and have rendered this tracer obsolete in modern lymphoma testing [5].

The next milestone for molecular imaging in lymphomas was the introduction of ^{18}F -FDG, a radiolabelled glucose analogue, for PET imaging in lymphomas, with the first associated study published in 1987. An important characteristic of ^{18}F -FDG is the inability of cells to metabolise ^{18}F -FDG after phosphorylation, leading to intracellular trapping of the tracer. Compared with ^{67}Ga -SPECT, ^{18}F -FDG PET has higher sensitivity, higher spatial and temporal resolution, and easier tracer uptake quantification [6]. Despite limitations, such as reduced sensitivity in areas with high physiological glucose uptake, ^{18}F -FDG PET/CT has become an integral part of lymphoma management. ^{18}F -FDG PET/CT is currently recommended for the staging and response assessment of lymphomas avid for ^{18}F -FDG, with the Lugano classification and Deauville criteria [7]. In classical Hodgkin lymphoma, ^{18}F -FDG PET/CT has allowed for response-guided In the research field, molecular imaging followed similar developmental milestones to those in clinical lymphoma imaging. Early molecular imaging studies almost exclusively relied on SPECT imaging, whereas studies from the late 1990s began to use SPECT/CT in conjunction with radioimmunotherapy (figure 2). From 2005 onwards, a steady increase in studies investigating PET tracers other than ^{18}F -FDG can be observed, and a corresponding decline of SPECT imaging-based studies occurs, illustrating increased interest and applications of PET in lymphomas.

Figure 2

Number of preclinical and clinical imaging studies in lymphoma
 Counts are presented per year and categorised into different techniques. SPECT = single-photon-emission CT.

SEARCH RESULTS

We searched the online literature of PubMed, MEDLINE, and Embase for studies published between December, 1977, and April 16, 2019, with the search terms “lymphoma”, “lymphoid malignancy/malignancies”, “radioactive tracer/tracers”, “positron emission tomography”, “molecular imaging” and “single photon emission computed tomography” (Supplementary Material – Search Terms).

After elimination of duplicate articles, we initially identified 3014 publications. We excluded 2519 studies after manually screening the title and abstract, leaving 495 studies that were included for full text analysis. Another 189 studies were excluded after full text evaluation, resulting in 306 articles included for this Review (Supplementary Figure 1).

Data from these 306 articles concerning year of publication, country where the research was done, PubMed ID, radionuclide used, imaging technique, tracer target, type of research (in vitro, in vivo [animal models], and in humans), and type of lymphoma were manually gathered and imported into R (version 3.5.1) for analysis and data visualisation using RStudio (version 1.0.153).

By synthesising these data, we identified 139 individual tracers with 50 distinct molecular and functional targets, that were labelled with 22 different radionuclides. A structured overview providing detailed information on the study type, tracers, and targets is provided Supplementary Table 1. Seven (2%) of 306 studies featured in-vitro experiments, 57 (19%) studies described preclinical in-vivo imaging, 22 (7%) studies combined in-vitro and in-vivo experiments, 219 (72%) studies were done in humans, and one study combined in-vitro and in-vivo experiments in animal and human models (< 1%). B-cell lymphomas were most frequently studied, with 154 (50%) articles investigating B-cell non-Hodgkin lymphomas not otherwise specified, 59 (19%) articles studying aggressive B-cell non-Hodgkin lymphomas, 32 (11%) articles researching indolent B-cell non-Hodgkin lymphomas, and 18 (6%) articles investigating Hodgkin lymphomas. The T-cell non-Hodgkin lymphoma group comprised 19 (6%) studies, and 24 (8%) studies combined two or more of these lymphoma groups. Additionally, three ongoing trials were identified via ClinicalTrials.gov as of April 28, 2020 (Supplementary Table 2).

About half of all selected studies were done in the USA (145 [47%]). Other countries where molecular imaging in lymphoma research took place were Germany (28 [9%]), Italy (13 [4%]), the Netherlands (12 [4%]), Japan (11 [4%]), Taiwan (nine [3%]), and Australia (seven [2%]; Supplementary Figure 2).

TARGETING SURFACE MARKERS AND BIOLOGICAL PROCESSES

A total of 50 molecular targets for molecular imaging of lymphoma were identified following full text analysis (figure 3). The different targeting strategies for molecular imaging in lymphoma are visualised in figure 4. Seven targets were selected for detailed review on the basis of the number of publications or their potential clinical relevance: B-cell markers CD20 (MS4A1) and CD22, MHC class II molecule HLA-DR, apoptosis imaging, B-cell and T-cell lineage markers CD37 and CD30 (TNFRSF8), and T-cell marker CD5.

CD20

CD20 was the most frequently investigated target (99 [32%] of 306 studies). The expression of this cell-surface marker increases during the maturation of B-lineage lymphocytes and is lost upon differentiation towards plasma cells. Eight in vitro studies showed preserved binding affinity of anti-CD20 monoclonal antibodies after radiolabelling, for example with ⁶⁴Cu and ⁸⁹Zr [9, 10]. 19 studies

- B-cell lineage
- Non-specific targeting
- MHC class II
- Hormone receptor
- Apoptosis
- B-cell lineage or T-cell lineage
- T-cell lineage
- Other surface targeting
- Intracellular
- Chemokine receptor
- Chemo tracking
- Cell tracking
- Surface immunoglobulin
- Cell adhesion
- Microenvironment

Imaging targets are shown per target group (key). The target-circle size is proportional to the respective fraction of all included studies (total = 306).

investigated CD20 imaging in xenograft mouse models of non-Hodgkin lymphoma, supporting the stability of anti-CD20 tracers in vivo and showing the feasibility of PET imaging with monoclonal antibodies labelled with radionuclides, such as ^{64}Cu and ^{89}Zr [9, 11].

80 studies applied CD20-targeted imaging to a total of 2554 patients. 76 of these studies (2526 patients) used SPECT imaging for dosimetry of radioimmunotherapy. One study used the PET tracer ^{89}Zr -ibritumomab tiuxetan for dosimetry in seven patients with B-cell non-Hodgkin lymphoma [12]. Three studies were done without radioimmunotherapy to evaluate anti-CD20 tracer biodistribution.

PET-imaging studies investigating the tracer ^{89}Zr -rituximab provided important insights for the clinical application of CD20 imaging. ^{89}Zr -rituximab was initially investigated in five patients with indolent lymphomas who were scheduled to receive ^{90}Y -rituximab radioimmunotherapy [13]. This study showed that preloading with unlabelled rituximab had a marked effect on tracer biodistribution, with less background and higher lymphoma lesion tracer uptake (higher tumour-to-background ratio) compared with no preloading with unlabelled rituximab. ^{89}Zr -rituximab was later investigated in six patients with relapsed or refractory diffuse large B-cell lymphoma, showing that tracer uptake (as quantified using standard uptake values) corresponded with CD20 expression measured via immunohistochemistry in tumour biopsies [14].

Although no study specifically investigated the loss of CD20 expression after anti-CD20 immunotherapy, uptake of anti-CD20 tracers was observed in patients that had previously received rituximab and relapsed after anti-CD20 therapy [13, 14]. Three studies evaluated the CNS penetration of the anti-CD20 radioimmunotherapy compound ^{90}Y -ibritumomab tiuxetan, using SPECT imaging with ^{111}In -ibritumomab tiuxetan in 13 patients with relapsed or refractory primary CNS lymphoma [15–17]. All studies reported low rates of tracer accumulation in CNS lymphoma lesions, responses restricted to these original tumour sites, and rapid progression in other CNS locations [15–17]. The blood-brain barrier was hypothesised to be compromised at primary CNS lymphoma sites by extensive disease involvement, while preventing penetration of radioimmunotherapy compound in areas that later showed disease progression [15–17]. Despite being limited by small cohorts, these findings were corroborated by a phase 3 trial (HOVON 105/ ALLG NHL 24) in 2019 [18], which showed no additional benefit of rituximab in patients given high dose methotrexate and cytarabine for primary CNS lymphoma.

These data validate the feasibility of imaging the biodistribution of CD20 antibodies in humans [14]. However, important questions, such as the influence of different treatment regimens, B-cell lymphoma subtypes, B-cell depletion, and preload doses of unlabelled rituximab on tracer uptake and clearance [13], should be answered before routine clinical use of CD20 imaging.

CD22

CD22, an immunoglobulin-like lectin that binds sialic acid, is exclusively expressed on B-cells and involved in B-cell inhibition and interaction with T-cells [19]. From 16 studies (5% of 306) that investigated CD22, 12 were done by or in collaboration with one centre. More than half of the studies (nine of 16) focused on molecular imaging applications in antibody distribution, pharmacokinetics, and radiation dosimetry of radioimmunotherapy. In this context, the use of molecular imaging with different radionuclides allowed for evaluation of pharmacokinetic characteristics of monoclonal antibodies specific to CD22 in tumour tissue [20, 21]. Visualisation of physiological processes aided by molecular imaging and measurement of tumour-to-background ratios across different radioimmunoconjugates allowed for direct comparison of radioimmunotherapy compounds and more efficient treatment [21, 22]. Studies using PET and SPECT showed satisfactory tumour targeting with ^{64}Cu -labelled and ^{111}In -labelled tracers [23, 24]. Reported sensitivity of SPECT imaging on a lesion basis using ^{131}I and $^{99\text{m}}\text{Tc}$ tracers ranged from 60% to 89%, however no specificity was reported. Clinical scans did not provide adequate spatial resolution and showed low tumour-to-background ratios in the spleen [20]. Since late 2013, the status of a planned phase 3 trial with the monoclonal antibody epratuzumab is still unknown (NCT00022685). In 2018, a phase 3 trial with the antibody-drug conjugate inotuzumab ozogamicin showed no added benefit for survival over the investigator's choice of treatment [25], limiting the applicability for theragnostic imaging of CD22.

HLA-DR

18 studies (6%) imaged the MHC class II molecule HLA-DR. Loss of HLA-DR function by malignant T-cells allows them to escape immune surveillance and is associated with poor clinical outcomes [26]. Most of these studies (17 of 18) focused on applications of molecular imaging in the context of radioimmunotherapy. Molecular imaging was used to compare pharmacokinetics and biodistribution of various compounds, leading to the improvement of radioimmunotherapy [27, 28]. One study evaluated imaging of HLA-DR with $^{99\text{m}}\text{Tc}$ in a murine model [29], and showed that $^{99\text{m}}\text{Tc}$ -anti-HLA-DR targeted HLA-DR positive cells. However, no studies were done in humans, in whom visualisation and quantification of HLA-DR expression and loss could be of prognostic value [26].

Apoptosis

16 studies (5%) reported imaging of apoptosis in lymphoma. Most tracers in these cases targeted the phospholipid phosphatidylserine, which is transported to the cell surface of apoptotic cells, where it is involved in inducing phagocytosis via

macrophages [30]. Several preclinical studies reported the ability of CASP-3 and CASP-7 [31], La-antigen (SSB) [32], and phosphatidylserine-specific SPECT and PET tracers to visualise the apoptotic response of tumour cells to radiation and chemotherapy [33]. In humans, the use of molecular imaging allowed visualisation of apoptosis induced by therapy [34], predicted toxic effects in healthy tissue [35], and associated early tracer uptake with a favourable response to treatment [36]. However, poor radiotracer biodistribution with high retention in the liver and intestines, and non-specific binding and tumour heterogeneity, limit the applicability of these tracers in molecular imaging of lymphoma.

CD37

Targets shared by both B-cell and T-cell lineages were observed in 14 (5%) studies. CD37 (reported in ten [3%] of 306 studies) is a glycosylated protein that is mostly expressed by mature B-cells, and to a lesser extent by T-cells, macrophages, monocytes, granulocytes, and dendritic cells. Although the biological function of CD37 is not completely understood, it is thought to be involved in prosurvival and proapoptotic signalling, recruitment, adhesion, chemotaxis, and transendothelial migration [37]. Studies investigating CD37 were limited to imaging with ¹¹¹In-labelled, ¹³¹I-labelled, and ¹⁷⁷Lu-labelled tracers, which were used for evaluating dosimetry and biodistribution of radioimmunotherapy compounds [38–40]. Research focusing on molecular imaging of CD37 beyond radioimmunotherapy was scarce, with a small study reporting a lesion detection sensitivity of 40% using ¹³¹I-labelled anti-CD37 monoclonal antibodies [41]. This suboptimal targeting performance might be explained by variable CD37 expression in diffuse large B-cell lymphoma [42]. Further studies into CD37 as a target of radioimmunotherapy in non-Hodgkin lymphomas are underway (NCT01796171).

CD30

The tumour necrosis factor receptor CD30 (four studies [1%]) is expressed on activated T-cells and a subgroup of B-cells, where it plays an important role in cell survival and the crosstalk between T-cells and B-cells [43]. Brentuximab vedotin, an antibody-drug conjugate composed of the anti-CD30 monoclonal antibody brentuximab linked with monomethyl auristatin E (an antitubulin), has become a viable treatment option for patients with classical Hodgkin lymphoma and CD30-positive T-cell lymphomas [44, 45]. However, because not all patients respond to this treatment and responses to treatment can be variable, molecular imaging of CD30 might provide the necessary information to select appropriate treatment candidates. One murine study showed the high radiochemical yield, purity, and

Figure 4

Potential target groups with examples for molecular imaging applications in lymphoma
 $^{11}\text{C-MET}$ = $^{18}\text{C-methionine}$, $^{18}\text{F-FET}$ = $^{18}\text{F-fluoroethyltyrosine}$, $^{18}\text{F-FLT}$ = $^{18}\text{F-fluorothymidine}$.

tumour specific activity of ^{89}Zr -labelled anti-CD30 monoclonal antibody [46]. With non-specific uptake of the tracer being small and high tumour-to-background tissue contrast, ^{89}Zr -labelled anti-CD30 monoclonal antibodies are a potential tool for analysing and managing patients receiving brentuximab vedotin.

CD5

Ten imaging studies (3%) investigated T-cell lineage markers, in particular CD5 (eight [3%] of 306 studies). CD5 is a type 1 transmembrane glycoprotein that is expressed by lymphoid precursor cells, T-cells, and a small subset of mature B-cells [47]. CD5-mediated signalling suppresses regulatory T-cells [48], and inhibits T-cell and B-cell receptor-mediated signalling [47]. CD5 has been exclusively investigated as a target for the imaging of cutaneous T-cell lymphoma with ^{111}In or ^{131}I radionuclides. Studies using ^{111}In showed CD5-specific binding of the tracer to both cutaneous and systemic lesions in patients with cutaneous T-cell lymphoma [49]. Although CD5 is an attractive target for clinical imaging of T-cells, T-cell non-Hodgkin lymphomas, and CD5-targeted therapy, the development of novel tracers, specifically for PET imaging, is required before CD5 attains clinical usefulness.

ONGOING CLINICAL IMAGING STUDIES

There are three non- ^{18}F -FDG molecular imaging studies in lymphoma registered on ClinicalTrials.gov (Supplementary Table 2). One study is imaging CXCR4 with the ^{68}Ga -labelled agonist pentixafor with the aim of improving diagnosis and pretherapeutic evaluation (NCT03436342). Our own group is studying the use of ^{89}Zr -atezolizumab for evaluating PD-L1 expression in patients with diffuse large B-cell lymphoma (NCT03850028). The third trial aims at overcoming the diagnostic limitations in identifying primary CNS lymphoma with MRI by using the amino acid analogue ^{18}F -fluciclovine and ^{18}F -FDG with integrated PET/MRI scans (NCT03788354).

SUMMARY OF THE STATUS QUO

The production of stable tracers with high yield and high specific activity for selected targets is feasible. The application of molecular imaging was predominantly investigated in the context of radioimmunotherapy for pharmacokinetics, radiation dosimetry, and prediction of off-target effects with SPECT based tracers. Although

PET is clinically superior to SPECT, development of PET techniques over the past 50 years has been challenging because of the great investment and specialised knowledge required. Since the late 1980s, 106 studies have reported the use of SPECT/CT in 31 different radioimmunotherapy compounds (Supplementary Table 3). These studies allowed direct comparison between radioimmunoconjugates, and selection of compounds with more favourable tumour-specific radiation. Nevertheless, the clinical application of radioimmunotherapy is limited. Some explanations might include high myelotoxicity associated with radioimmunotherapy treatments, the establishment of rituximab therapy, and reluctance on the part of haematologists to use this novel and unconventional form of radiation therapy.

Outside the field of radioimmunotherapy, attempts to use molecular imaging to improve existing targeted treatments in lymphoma are limited to a few studies targeting CD20 (figure 1). Consequently, molecular imaging has not played a role in the development of novel targeted therapies for lymphoma to date. So far, studies evaluating the role of molecular imaging have been done in small and heterogeneous study populations. Additionally, most radionuclides were only investigated using SPECT, resulting in lower specificity and spatial resolution when compared with clinical PET cameras. Regarding innovative immunotherapeutic options, such as immune checkpoint inhibitors and chimeric antigen receptor T-cell therapy for lymphomas, we only identified one preclinical study in lymphoma that used multimodal imaging techniques to track genetically engineered T-cells [50]. Preclinical studies have mostly evaluated molecular imaging in the context of tracer development, rather than exploring new biological rationales or clinically relevant biological questions. This finding might be explained by the availability of practical and inexpensive techniques for visualisation and investigation of molecular processes such as immunofluorescence, optical imaging, flow cytometry, and immunohistochemistry in the preclinical setting. However, these techniques often cannot be used for in-vivo imaging and clinical applications.

The limited effect of molecular imaging with tracers other than ^{18}F -FDG in lymphomas is contrasted by developments in the field of solid oncology. Novel immunotherapeutic drugs have been used to produce tracers for molecular imaging in solid cancers to facilitate drug development [51]; to improve diagnosis [52]; to visualise biodistribution to predict toxic effects, off-target effects, and heterogeneity [53]; to evaluate drug combinations and dosimetry [54, 55]; to identify candidates for specific therapies [56]; and to predict target specific response (figure 1) [57].

FUTURE PERSPECTIVES

The development of novel molecular imaging tracers is a complex process requiring advanced multidisciplinary knowledge. Other challenges include the high costs of tracer development with good manufacturing practice requirements and PET imaging, little awareness in stakeholders or investors, a complicated reimbursement process for novel imaging techniques, and practical issues, such as difficult accrual of patients in imaging trials without treatment. The promotion of dedicated multidisciplinary research teams, sharing of resources and data between specialties, novel funding strategies, collaboration with the industry, and effective study design are necessary for future molecular imaging efforts [58]. For a detailed discussion of the obstacles to clinical molecular imaging research and suggestions for distinct measures on how to overcome them, we refer readers to an excellent review on this topic [58].

Many of the potential applications of molecular imaging could be of importance within the context of personalised medicine. In oncology and haematology, the trend towards highly sophisticated therapies, which are dependent on the genetic and phenotypic makeup of the tumour cells, presents opportunities for molecular imaging in medical decision making. In time, molecular imaging might become more cost-effective by enabling the selection of treatment candidates for expensive targeted therapies.

Certain existing tracers represent future opportunities for molecular imaging in lymphomas. CXCR4, a chemokine receptor that is frequently overexpressed in haematological malignancies, has been visualised using different SPECT and PET tracers in the lymphoma setting. CXCR4 expression has been linked to prognosis in several different lymphoma subtypes and preclinical research suggests a therapeutic synergy with rituximab [59]. Although clinical application of drugs that target CXCR4 is currently restricted to haematopoietic stem-cell transplantation, the therapeutic potential and the current development of tracers targeting CXCR4 for molecular imaging make it an intriguing candidate for future investigations. Novel tracers that are fibroblast activation protein (FAP) inhibitor based, which target tumour-associated fibroblasts, are candidates for general diagnostic imaging and might complement ^{18}F -FDG PET imaging, especially in indolent lymphoma subtypes [60].

One application for molecular imaging is during the early phase of drug development. In the field of immunotherapy, various novel compounds and treatment modalities

are being investigated, including intriguing candidates for clinical applications of molecular imaging (table [61–67]). Valuable information could be provided by future molecular imaging, including insights into the biodistribution of the molecular target (important in case of tumour heterogeneity or unexpected off-target effects), and molecular target expression over time (important for understanding the effects of standard therapy and resistance to targeted therapy). Additionally, future molecular imaging could allow selection of treatment candidates on an individual level, prediction of response to targeted therapy, dosimetry (determination of an effective and safe dose), and pathophysiology (e.g., correlating immunohistochemical target expression and clinical response with tracer uptake).

Immune checkpoint inhibitors are an important group of candidate drugs for molecular imaging. Monoclonal antibodies targeting PD-1, PD-L1, PD-L2, CTLA4, and CD47 have shown efficacy in patients with different lymphoma subtypes [68, 69]. For the treatment of advanced stage classical Hodgkin lymphoma, nivolumab was approved in 2016 and pembrolizumab was approved in 2017 (PD-1 checkpoint inhibitors). Although not effective as monotherapy in non-Hodgkin lymphoma, immune checkpoint inhibitors might still hold potential as part of combined treatment regimens [70]. PET imaging of the ^{89}Zr -labelled, PD-L1 checkpoint inhibitor, atezolizumab has been successfully done in a mixed cohort of 22 patients with bladder cancer, non-small cell lung carcinoma, and triple negative breast cancer [57]. These results suggest the feasibility and prognostic value of in-vivo imaging of tumour phenotypes in humans, and show the superiority of whole tumour characterisation with imaging over immunohistochemistry in histological biopsies from single sites. Nevertheless, further research and development is needed to validate these findings in bigger cohorts, acquire more safety data, and address issues such as cost-effectiveness.

Bispecific T-cell engagers and dual affinity retargeting compounds are another group of novel drugs relevant for molecular tracer development. Both formats of bispecific antibodies aim to directly link cytotoxic T-cells to tumour cells, thereby increasing their antitumour activity. By labelling these bispecific antibodies and separately targeting T-cell and tumour cell markers, the proposed mechanism of action and potential off-target sites could be visualised in vivo. A study in patients with gastrointestinal adenocarcinoma used a radiolabelled bispecific T-cell engager targeting CD3 and CEA (CEACAM5) for molecular imaging, and described both heterogeneous tracer uptake in tumour lesions and signal accumulation in the lymphoid compartment [53]. These results show how the binding affinity of

Table 1: Candidate drug groups and specific compounds for potential molecular imaging applications in lymphoma

	Target	Known efficacy in lymphoma	Specific applications of MI	General applications of MI
Immune checkpoint inhibitors				
Atezolizumab	PD-L1	15 % ORR (10 % CR) + obinutuzumab in R/R B-NHL (phase 1), 88 % ORR (78 % CR) + R-CHOP in untreated DLBCL (phase 1/2, interim)	<ul style="list-style-type: none">□ Correlate tracer uptake with response (effect through microenvironment?)	<ul style="list-style-type: none">□ Target bio-distribution<ul style="list-style-type: none">○ heterogeneity○ off-target effects (toxicity)□ Monitor target expression over time<ul style="list-style-type: none">○ effect of standard Tx○ effect of targeted Tx (e.g. resistance)□ Selection of treatment candidates□ Response prediction^[2] but predicting patient benefit using approved diagnostics is inexact, as some patients with PD-L1-negative tumors also show clinical benefit 1,2. Moreover, all biopsy-based tests are subject to the errors and limitations of invasive tissue collection 3–11. Preclinical studies of positron-emission tomography (PET)□ Determine effective and safe target dose (dosimetry)□ Insight into pathophysiology<ul style="list-style-type: none">○ correlate IHC expression and response with tracer uptake
Nivolumab/ Pembrolizumab	PD-1	Approved for R/R cHL; hope for combination therapy/ maintenance/consolidation in NHL	<ul style="list-style-type: none">□ Impact of standard therapy on PD-(L)1 expression (NHL)	
CAR T-cell therapy			<ul style="list-style-type: none">□ Monitor targeting ability and persistence of CAR-T-cells in vivo (cell tracking)	
Axicabtagene ciloleucel / Tisagenlecleucel	CD19	82 % ORR (54 % CR) and 52 % ORR (40 % CR), respectively in DLBCL (phase II)	Identify loss of the CD19 target ⁷⁵	
Bispecific antibodies				
AFM13	CD30 + CD16	In phase 2 for R/R cHL (NCT02321592)	<ul style="list-style-type: none">□ Monitor CD19 expression over time□ Identify loss of CD19 and early CD19-negative relapse□ Confirm mechanism of action (separate tumour and effector cell visualisation)	<ul style="list-style-type: none">□ Response prediction^[2] but predicting patient benefit using approved diagnostics is inexact, as some patients with PD-L1-negative tumors also show clinical benefit 1,2. Moreover, all biopsy-based tests are subject to the errors and limitations of invasive tissue collection 3–11. Preclinical studies of positron-emission tomography (PET)
Blinatumomab	CD19 + CD3	43 % ORR (19 % CR) in B-NHL (phase 2) ⁶⁵		
Mosunetuzumab	CD20 + CD3	41 % ORR (27 % CR) in B-NHL (phase 1, interim) ⁶⁶		
RG6026	CD20 + CD3	In phase 1 for R/R B-NHL (NCT03075696, CT03533283) and R/R B-NHL + untreated DLBCL (NCT03467373)		
REGN1979	CD20 + CD3	In phase 2 for R/R FL (NCT03888105)		
Antibody-drug conjugates				
Brentuximab vedotin	CD30	Approved for first line Tx in cHL (phase 2), ⁴⁴ not effective in PMLBCL (phase 2 trial terminated), 31 % ORR (12 % CR) in CD30+ DLBCL (phase 2)	<ul style="list-style-type: none">□ Identification of resistance (loss of CD30 in ALCL)□ Pinpoint biological differences in tracer uptake between lymphoma subtypes (no effect of BV in PMLBL)□ Selection of treatment candidates (e.g. CD30⁺ DLBCL)□ Impact of combination with other drugs (e.g. CPIs) on tracer uptake□ Potential impact of anti-CD20 therapy on CD79b expression	<ul style="list-style-type: none">□ Determine effective and safe target dose (dosimetry)□ Insight into pathophysiology<ul style="list-style-type: none">○ correlate IHC expression and response with tracer uptake
Canidanlumab tesirine	CD25	In phase 1 for R/R cHL and NHL (NCT024w32235)		
Loncastuximab tesirine	CD19	57 % ORR (34 % CR) in DLBCL (phase 1, interim) ⁶⁹		
Naratuximab emtansine	CD37	In phase 2 for R/R B-NHL (NCT02564744)		
Polatuzumab vedotin	CD79b	54 % ORR (21 % CR) + rituximab in DLBCL (phase 2) ⁶⁸		

ALCL = anaplastic large cell lymphoma, B-NHL = B-cell non-Hodgkin lymphoma, CAR = chimeric antigen receptor, CD = cluster of differentiation, CR = complete remission, CRS = cytokine release syndrome, cHL = classical Hodgkin lymphoma, CPI = checkpoint inhibitor, DLBCL = diffuse large B-cell lymphoma, FL = follicular lymphoma, IHC = immunohistochemistry, MI = molecular imaging, ORR = objective response rate, PD-1 = programmed death 1, PD-L1 = programmed death ligand 1, PMLBCL = primary mediastinal large B-cell lymphoma, R-CHOP = rituximab + cyclophosphamide, doxorubicin, vincristine and prednisolone, R/R = relapsed/refractory, Tx = therapy

the different arms of such bispecific antibodies can affect biodistribution [53]. The bispecific, tetravalent chimeric antibody construct AFM13, which targets both CD30 and CD16a (FCGR3A), is being evaluated in a phase 2 trial (NCT02321592) after showing a disease control rate of 61.5% in a phase 1b trial in patients with relapsed or refractory classical Hodgkin lymphoma [71]. For the bispecific T-cell engagers blinatumomab (targeting CD19 and CD3) and mosunetuzumab (targeting CD20 and CD3), which have both shown activity in non-Hodgkin lymphoma [61, 62], molecular imaging might provide information on target biodistribution, off-target effects, and potential heterogeneity.

The group of antibody-drug conjugates is being extensively studied for therapy in lymphomas [72]. The anti-CD30 compound brentuximab vedotin was evaluated for integration into first-line treatment of patients with high-risk classical Hodgkin lymphoma, showing a more favourable toxicity profile than standard therapy, with equal efficacy [44]. Meanwhile, novel compounds such as the anti-CD25 antibody-drug conjugate camidanlumab tesirine (ADCT-301) are entering clinical testing in classical Hodgkin lymphoma (NCT02432235). Encouraging results in the setting of diffuse large B-cell lymphoma have been reported for the anti-CD79b antibody-drug conjugate polatuzumab vedotin [66], the anti-CD19 antibody-drug conjugate loncastuximab tesirine [65], and the anti-CD37 antibody-drug conjugate naratuximab emtansine (NCT02564744). By radiolabelling the monoclonal antibody portion of an antibody-drug conjugate, the biodistribution of its target could be visualised. As discussed in earlier sections, studies have shown the feasibility of imaging targets like CD30 and CD37. However, tracers based on the naked antibody portion of the respective antibody-drug conjugate should be developed to be able to draw meaningful conclusions from molecular imaging studies.

Another area for future molecular imaging in lymphoma is the tracking of chimeric antigen receptor T-cells. Important preclinical work to image chimeric antigen receptor T-cell trafficking in models of solid malignancies was reported in 2020 [73]. Until now, molecular imaging of chimeric antigen receptor T-cell therapy in humans has been reported in only seven patients with glioma [74, 75]. By using a ^{18}F -labelled analogue of penciclovir, which is used in the treatment of herpes, researchers visualised the spatial expression of the herpes virus thymidine kinase reporter gene in CD8-positive cytotoxic T lymphocytes that were directly administered to the ventricular system for cellular therapy of patients with high-grade gliomas [74]. Early trials are underway to investigate imaging of cellular immunotherapy in lymphoma [50, 76]. In 2019, a preclinical study was able

to visualise the concentration of PSMA (FOLH1) transduced chimeric antigen receptor T-cell xenograft models of acute lymphoblastic leukaemia (Nalm6 cell line) via serial PET scanning with the PSMA-specific tracer 2-(3- {1-carboxy-5- [(6-[¹⁸F]fluoropyridine-3-carbonyl)amino]pentyl}ureido) pentanedioic acid (¹⁸F-DCFPyL) [77]. By visualising the biodistribution of chimeric antigen receptor T-cells, molecular imaging could provide valuable insights in targeting lymphoma lesions. Furthermore, molecular imaging of the CD19 target of chimeric antigen receptor T-cells might be used to monitor patients for loss of CD19, a major resistance mechanism in patients receiving this type of treatment [67].

CONCLUSION

Whether there will be a role for radionuclide tracers other than ¹⁸F-FDG PET in routine clinical diagnostics is unknown. Research suggests that molecular imaging is suited for patient and therapy tailored imaging in highly specific disease entities and patient cohorts, like in lymphoma. The target-specific information that can be obtained by molecular imaging becomes increasingly important in the context of immunotherapy and personalised medicine. Labelling of novel therapeutic compounds and imaging is feasible in patients with lymphoma. However, molecular imaging of lymphomas, outside the well-developed field of radioimmunotherapy, has not been used to its full potential. Most studies in humans used SPECT imaging for radioimmunotherapy based on small cohorts of patients and were done by a small number of research groups.

Improved study design and collaborations and coordination between different specialties and with the industry are necessary to effectively implement what the scientific community has learned from this research for future molecular imaging applications. In particular, the integration of molecular imaging efforts with drug development is essential. Building awareness for the potential opportunities and applications of molecular imaging in lymphoma is required before its full clinical utility can be realised.

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SUPPLEMENTARY MATERIAL

Search terms

PubMed:

("Lymphoma"[Mesh] OR lymphoma [tiab] OR lymphoid malignanc*[tiab]) AND
("Radioactive Tracers"[Mesh] OR "Positron Emission Tomography Computed
Tomography" [Mesh] OR "Molecular Imaging"[Mesh] OR "Single Photon Emission
Computed Tomography Computed Tomography" [Mesh] OR radiotracer* [tiab]
OR tracer* [tiab] OR immunopet*[tiab] OR 90Y* [tiab] OR 131I* [tiab] OR 177Lu*
[tiab] OR 89Zr* [tiab] OR 64Cu* [tiab] OR 99mTc* [tiab] OR 111In* [tiab] OR 125I*
[tiab] OR 124I* [tiab] OR Cy7* [tiab] OR 11C* [tiab] OR 68Ga* [tiab] OR 67Ga* [tiab]
OR 18F*[tiab] OR 225Ac* [tiab] OR 211At* [tiab] OR 212Bi* [tiab] OR 213Bi* [tiab]
OR 14C* [tiab] OR 51Cr* [tiab] OR 137Cs* [tiab] OR 67Cu* [tiab] OR 13N* [tiab] OR
186Re* [tiab] OR 188Re* [tiab] OR 103Ru* [tiab] OR 153Sm* [tiab] OR 149Tb* [tiab])
NOT

(Fluorodeoxyglucose F18 OR fdg [tiab] OR 18f-fdg [tiab] OR 18FDG [tiab] OR
18F-Fluorodeoxyglucose [tiab] OR Fluorodeoxyglucose [tiab] OR Case Reports
[ptyp] OR Review [ptyp])

Embase:

('lymphoma'/exp OR lymphoma:ab,ti)

AND

('tracer'/exp OR 'positron emission tomography'/exp OR 'molecular imaging'/
exp OR 'single photon emission computed tomography'/exp OR 'tracer*':ab,ti
OR 'immunopet':ab,ti OR 'yttrium 90':ab,ti OR 'iodine 131':ab,ti OR '177lu':ab,ti
OR 'zirconium 89':ab,ti OR 'copper 64':ab,ti OR 'technetium 99m':ab,ti OR 'indium
111':ab,ti OR 'iodine 125':ab,ti OR 'iodine 124':ab,ti OR 'cy7':ab,ti OR 'carbon
11':ab,ti OR 'gallium 68':ab,ti OR 'gallium 67':ab,ti OR 'fluorine 18':ab,ti OR 'actinium
225':ab,ti OR 'astatine 211':ab,ti OR 'bismuth 212':ab,ti OR 'bismuth 213':ab,ti OR
'carbon 14':ab,ti OR 'chromium 51':ab,ti OR 'cesium 137':ab,ti OR 'copper 67':ab,ti
OR 'nitrogen 13':ab,ti OR 'rhenium 186':ab,ti OR 'rhenium 188':ab,ti OR 'ruthenium
103':ab,ti OR 'samarium 153':ab,ti OR 'terbium 149':ab,ti)

AND

'article'/it

NOT

('fluorodeoxyglucose f18'/exp OR 'fluorodeoxyglucose'/exp OR
'fluorodeoxyglucose f 18':ab,ti OR 'fluorodeoxyglucose':ab,ti OR 'fdg*':ab,ti OR
'case report'/exp OR 'review'/it)

Supplementary Figure 1

Flow diagram depicting the study selection process; ^{18}F -FDG = ^{18}F -fluorodeoxyglucose; RIT = radioimmunotherapy; MI = molecular imaging.

Supplementary Figure 2

World map depicting the geographical distribution and density per country of included non-¹⁸F-fluorodeoxyglucose (non-¹⁸F-FDG) molecular imaging studies in lymphoma.

Supplementary Table 1. Overview of all tracers used in the 306 studies included in the present review

	Target	Tracer Type	Technique	Specific tracer
non-specific targeting	Functional uptake	labeled myethylene diphosphonate (MDP)	SPECT	^{99m} Tc-MDP
		tumor-targeting alkylphosphocholine	PET	⁸⁶ Y-NM600
		labeled chloride / pure isotope	SPECT	²⁰¹ Tl(-chloride)
		labeled diphosphine	SPECT	^{99m} Tc-tetrofosmin
		labeled Methoxy-Isobutyl-Isonitrile	SPECT	^{99m} Tc-MIBI
		labeled free isotope ion (GA3+)	SPECT	⁶⁷ Ga(-citrate)
		labeled σ 2-receptor ligand	PET	¹⁸ F-ISO-1
		labeled methylaminoisobutyric acid	PET	¹¹ C-MeAIB
		labeled essential nutrient	PET	¹¹ C-choline
		labeled artificial cyclic amino acid	PET	¹¹ C-1-aminocyclopentane-carboxylic acid (ACPC)
		labeled amphetamine	SPECT	¹²³ I-IMP (N-isopropyl-p- ¹²³ I-iodo-amphetamine / ¹²³ I-amphetamine)
		Labeled human IgG (HIG)	SPECT	^{99m} Tc-HIG
		labeled porphyrin derivative	SPECT, RIT	¹⁷⁷ Lu-porphyrin-BFCA
	DNA replication	labeled deoxyuridine analog	SPECT	¹³¹ I-5-iodo-(2-deoxy-2-fluoro- β -D-arabinofuranosyl)uracil (FIAU)
		labeled pyrimidine nucleoside	PET	¹⁸ F-fluorothymidine (FLT)
		labeled pyrimidine nucleoside	PET	¹¹ C-thymidine
	Protein synthesis	labeled amino acid	PET	L-1- ¹¹ C-tyrosine
				O-(2-(¹⁸ F)-fluoroethyl)-l-tyrosine (FET)
				¹¹ C-methionine (MET)
B lineage	CD19	Super Paramagnetic Iron Oxide Nanoparticles (SPION), labeled CAR-T-cells	PET	SPION and ⁶⁴ Cu labeled CAR-T-cells
	CD20	labeled mAb	PET	⁶⁴ Cu-rituximab
		labeled mAb	PET, NIRF	⁶⁴ Cu-DOTA-NuB2-AF750
		labeled mAb	NIRF	Cy7-obinutuzumab
		labeled mAb	PET	⁸⁹ Zr-obinutuzumab
		labeled mAb	PET	⁸⁹ Zr-ofatumumab and tositumomab
		labeled diabodies (scFv dimers)	NIRF	¹²⁴ I-scFv-8 and 124-Cys-Db
		labeled mAb	NIRF	Cy7-rituximab
		labeled mAb	PET	⁶⁴ Cu-Do-FN3
		labeled mAb	PET	¹²⁴ I-obinutuzumab
		Labeled recombinant mAb fragments	PET	¹²⁴ I-, ⁶⁴ Cu-scFv-CH3 dimer & -Fc fragment
		labeled single-domain Ab fragment (sdAb)	SPECT, PET	^{99m} Tc- and ⁶⁸ Ga-DTPA-sdAb 9079
		labeled mAb	PET	⁸⁹ Zr-rituximab
		labeled mAb	SPECT	¹¹¹ In-tositumomab
		labeled mAb	SPECT	^{99m} Tc-rituximab
		labeled mAb	SPECT	¹¹¹ In-ibrutumomab tiuxetan

	in vitro	in vivo	human	imaging	RIT	B-cell			T-cell
						HL	B-NHL		
							agressive	indolent	
								1	
								2	
						3-5	6-16 (CNS)	17	4
						18, 19			18 (histiocytic), 19-23
						24-26	16 (CNS)		24-26; 27 (mediastinal); 28-32
						3, 5, 33-38	39	40	33, 34, 37, 38; 41 (extranodal); 42-48
									49
									50
									51 (mediastinal)
						52			52
							53; 54 (PCNSL)		
						55			55
									56 (thymic)
									57
							58, 59	58-60	61 (mouse lymphoma); 62-64
									65
							66 (CNS)		
							67, 68 (CNS)		
						69	70-73 (CNS)		69
									74
							75		75-77
									78
							79		
							80		84
							80		
								81	
							82		
							83		83
									84
									85
									86
							75, 80, 87, 88		75, 89-91
							92		93
							82		94
						95 (CD20+ cells in TME)	96-98, 101, 102, 104; 99, 100, 103 (CNS)	102, 105-115	107, 116-137

Supplementary Table 1: continued

	Target	Tracer Type	Technique	Specific tracer
B lineage	CD20	labeled mAb	PET	⁸⁹ Zr-ibritumomab tiuxetan
		mAb (for pretargeting) + radio-rhodamine labeled dendrimer	pretargeted RIT, NIRF	A488-anti-CD20(DBCO) + Rhod-PAMAM(D- ⁸⁹ Y)
		labeled mAb	SPECT, RIT	¹³¹ I-rituximab
		labeled mAb	SPECT, RIT	¹³¹ I-tositumomab
		labeled mAb	SPECT, RIT	¹⁷⁷ Lu-rituximab
		labeled mAb	SPECT, RIT	¹³¹ I-1F5
		labeled mAb	SPECT, RIT	⁹⁰ Y- and ¹¹¹ In-veltuzumab
		labeled mAb	SPECT, RIT	¹⁸⁸ Re-anti-CD20
		labeled single-domain Ab fragment (sdAb)	SPECT, RIT	¹⁷⁷ Lu-DTPA-sdAb 9079
		labeled mAb	RIT	⁹⁰ Y-ibritumomab tiuxetan
		labeled mAb	RIT	⁹⁰ Y-tositumomab
	CD21 (CR2)	labeled mAb	SPECT, RIT	¹³¹ I-OKB7
	CD22	labeled Ab	SPECT	¹¹¹ In-biotin-anti-CD22
		labeled mAb (RIT)	PET	⁶⁴ Cu-DOTA-HB22.7
		labeled mAb (RIT)	SPECT	¹²⁵ I-LL2 (-IMP-R1/2-LL2; (Fab') ₂ , Fab' fragments)
		labeled mAb	SPECT	¹¹¹ In-LL2
		labeled mAb	SPECT	^{99m} Tc-LL2
		Labeled mAb (Fab'fragment)	SPECT	^{99m} Tc-LL2-Fab'
		labeled mAb	SPECT	¹¹¹ In-epratuzumab
		labeled mAb	SPECT, RIT	¹⁷⁷ Lu-huRFB4
		labeled mAb	SPECT, RIT	¹³¹ I-LL2 and ¹³¹ I-IMP-R1/2-LL2
		labeled mAb	RIT	⁹⁰ Y-epratuzumab
		labeled mAb	RIT	⁹⁰ Y-LL2
	CD38	labeled mAb	PET	⁸⁹ Zr-Df-daratumomab
B/T-lineage	CD30	labeled mAb	PET	⁸⁹ Zr-DFO-AC-10
		labeled mAb	SPECT	¹³¹ I-Ber-H2
		labeled mAb	SPECT, RIT	¹³¹ I-Ki-4
	CD30, CD16	labeled bispecific mAb	SPECT	¹¹¹ In-anti-CD30/anti-CD16
		labeled bispecific mAb	SPECT	¹²⁵ I-anti-CD30/anti-CD16
	TCR Vβ5	labeled mAb	SPECT	¹¹¹ In- and ⁹⁰ Y-LC4
	CD37	labeled mAb	SPECT	¹¹¹ In- and ⁹⁰ Y-MB-1
		labeled mAb	SPECT, RIT	¹⁷⁷ Lu-DOTA-HH1
		labeled mAb	SPECT, RIT	¹³¹ I-MB-1
T-lineage	CD2	labeled mAb	SPECT	¹²⁵ I-12-15A
	murine CD3	labeled mAb	PET, RL, PK/BD	⁸⁹ Zr-DFO-17A2
	CD5	labeled mAb	SPECT	¹¹¹ In-T101
		labeled mAb	SPECT, RIT	¹³¹ I-T101
		labeled mAb	RIT	⁹⁰ Y-T101

	in vitro	in vivo	human	imaging	RIT	B-cell			T-cell	
						HL	B-NHL			
							agressive	indolent		NOS
								135		
							138			
							139, 140	141, 142; 143 (LPL)	143-147	
						148	149	150-154	155-167	
								168, 169	170	
									171	
									172	
									173	
									86	
						95 (CD20+ cells in TME)	96-98, 101, 102, 104; 99, 100, 103 (CNS)	102, 105-115	107, 116-136	
									93	
									174	
							175			
									176	
							177			
							177		178	
									179	
									180, 181	
									182-184	
							185			
							177	143 (LPL)	143, 178, 186-188	
									172, 182-184, 189	
									178	
							190			
										191
						192				
						193				
									194	
									194	
										195
										195
							196			
									171, 197-199	
								200-202	203	
									204 (murine)	
										205
										206-211
										210, 212, 213
										208

Supplementary Table 1: continued

	Target	Tracer Type	Technique	Specific tracer
MHC class II	HLA-DR	labeled mAb	SPECT	^{99m} Tc-1D09C3
		labeled mAb	SPECT	¹⁸⁶ Re-Lym-1
		labeled mAb	SPECT	¹²⁵ I-Lym-1
		labeled mAb	SPECT	⁶⁷ Cu-Lym-1
		labeled mAb	SPECT	¹¹¹ In-2IT-BAD-Lym-1
		labeled mAb	SPECT	⁶⁷ Cu-2IT-BAT-Lym-1
		labeled mAb	SPECT	¹¹¹ In-Lym1
		labeled mAb	SPECT, RIT	¹³¹ I-Lym-1
		labeled mAb	RIT	⁹⁰ Y-2IT-BAD-Lym-1
		labeled mAb	RIT	⁹⁰ Y-2IT-BAT-Lym-1
chemokine receptor	CXCR4	labeled chemokinereceptor agonist	PET	⁶⁸ Ga-DOTA-4-FBn-TN14003
		Labeled monocyclam derivative	PET	¹⁸ F-MCFB
		labeled chemokinereceptor agonist	PET	⁶⁴ Cu-pentixather
		labeled chemokinereceptor agonist	PET	⁶⁸ Ga-pentixafor
		labeled chemokinereceptor agonist	PET	⁶⁸ Ga-pentixather
		labeled chemokinereceptor agonist	SPECT, RIT	¹⁷⁷ Lu-pentixather
apoptosis	phosphatidylserine	Labeled peptide	PET	⁶⁴ Cu-NOTA-Ava-PSBP-6
		Labeled C2A domain of synaptotagmin-I (NIRF and SPECT)	NIRF, SPECT	C2Am-AF750, ^{99m} Tc-C2Am and ¹¹¹ In-C2Am
		Labeled 2-(5-fluoropentyl)-2/methylmalonic acid, annexin V	PET	¹⁸ F-ML10, ¹²³ I-ML10 and ⁶⁸ Ga-Cys2-annexin V
		labeled annexin V	SPECT	¹¹¹ In-annexin V-CCPM
		labeled annexin V	PET	⁶⁸ Ga-Cys2-annexin V and ⁶⁸ Ga-Cys165-annexin V
		Labeled peptide and annexinV	PET	¹⁸ F-PS-binding peptides in competition with ⁶⁴ Cu-annexin V
		labeled annexin V	SPECT	^{99m} Tc-(HYNIC)-annexin V
	La-autoantigen	labeled mAb	SPECT	¹¹¹ In-3B9
		2 step imaging with biotin marked mAb + radiolabeled streptavidin	SPECT	DAB4 F(ab)2-LC-biotin + ¹¹¹ In-DOTA-streptavidin
		labeled mAb	SPECT	¹¹¹ In- DAB4
	caspase-3/7	labeled isatin sulfonamide	PET	¹⁸ F-ICMT-11
hormone receptor	somatostatin receptor	labeled somatostatin analog	SPECT	¹²⁵ I-ocreoitide
		labeled somatostatin analog	SPECT	¹¹¹ In-octreotide
		labeled somatostatin analog	SPECT	¹¹¹ In-pentetreotide
		labeled somatostatin analog	SPECT	^{99m} Tc-depreotide
		labeled somatostatin analog	SPECT	¹¹¹ In-lanreotide
		labeled somatostatin analog	PK/BD	¹⁷⁷ Lu-ocreoate
cell adhesion	α4β1	labeled high-specificity peptidomimetic ligand	PK/BD, AR, PET	¹¹¹ In-LLP2A-DOTA Polyethylene Glycol and ⁶⁴ Cu-LLP2A-CB-TE2A
	αvβ3	labeled peptide	PET	⁶⁸ Ga-NOTA-RGB (Arg-Gly-Asp)
	CD24	labeled mAb	SPECT	¹¹¹ In-BA-1
		labeled mAb	RIT	¹³¹ I-BA-1

	in vitro	in vivo	human	imaging	RIT	B-cell				T-cell
						HL	B-NHL			
							agressive	indolent	NOS	
						214				
						215				
						216				
						216, 217				
								218, 219		
						220		221-224		
								225		
								224-231		
								218		
								224		
									232	
						233				
						234				
						235, 236		236	236	
								237 (MALT)		
						238				
								239		
								240		
						241				
								242		
						243				
								244		
							245, 246	247-250		
								251		
								252		
								253		
								254		
									255	
						256-258	259 (MALT)	256-258, 260	255	
						261, 262		261-267	265 (CTCL)	
						268		268, 269		
								270		
								271		
						272				
								273		
								274		
								275		

Supplementary Table 1: continued

	Target	Tracer Type	Technique	Specific tracer
cell tracking	homing of specific cells	labeled DCs, CTLs and EL4 cells	PET, cell tracking	⁸⁹ Zr-oxine
	homing of labeled lymphocytes	cells labeled organic compound	SPECT	¹¹¹ oxine-labeled lymphocytes
	tracking of EBV-specific T-cells	Cell labeling via labeled thymidine analog	SPECT	¹³¹ I-2'-fluoro-2'-deoxy-β-d-5-iodouracil-arabinofuranoside (FiAU)
	dynamics of CD4+ lymphocytes	labeled CD4+ lymphocytes	SPECT	¹¹¹ In-CD4+ cells
intra-cellular	bortezomib induced EBV-TK expression	labeled thymidine analog	SPECT	¹²⁵ I- and ¹⁴ C-FiAU
	ferritin	labeled (polyclonal) Ab	SPECT	¹¹¹ In-antiferritin
		labeled (polyclonal) Ab	RIT	⁹⁰ Y-antiferritin
	BCL2	Labeled peptide nucleic acid (PNA)-peptide conjugates	SPECT	¹¹¹ In-DOTA-anti-bcl-2-Tyr3-ocreotate
			SPECT	⁶⁷ Cu-DOTA-anti-bcl-2-Tyr3-ocreotate
surface Ig	Ig-BCR	Core-shelled chitosan hyaluronic acid nanoparticles conjugated to Ig BCR-binding peptide	NIRF	Cy7-pA20-36-NPs
	variable-region antigenic determinants of the clonal surface Ig	labeled mAb	SPECT	¹¹¹ In-anti-idiotypic
		labeled mAb	RIT	⁹⁰ Y-anti-idiotypic
	membrane-bound mIgM markers	¹²⁹ Xe NMR nanoscale biosensors with a spherical MS2 viral capsid, Cryptophane A molecules, and DNA aptamers	NMR	MS2 viral capsid + Cryptophane A molecules + TD05.1 aptamer
micro-environment	eosinophil peroxidase	labeled mAb	SPECT	¹¹¹ In-EOS
	tenascin (ECM protein)	labeled mAb	SPECT, RIT	¹³¹ I-81C6
other surface markers	SLAM on MCL cells	engineered measles virus vaccine; Perthechnate and therapeutic isotope uptake and concentration via virus transfected Na/I symporter (NIS)	SPECT	Engineered measles virus vaccine: MV-NIS and MVvac2NIS Measuring uptake infection via: Sodium pertechnate: Na[^{99m} TcO ₄] Measuring isotope uptake via: ¹²⁵ I, ¹³¹ I
	ADP-ribosyltransferase ARTC2.2	labeled mAb compared to labeled single-domain nanobody	NIRF	AlexaFluor-680- Nika102 and s+16a
	Dalton's lymphoma associated antigen (DLAA)	labeled mAb	SPECT	^{99m} Tc-anti DLAA
		labeled mAb	SPECT	¹³¹ I-DLAB
	BLyS receptor	labeled gelonin toxin rGel/BLyS	SPECT, PK/BD	¹¹¹ In-DTPA-rGel/BLyS
	HER2-neu	labeled affibody (peptide antibody mimetic)	SPECT	¹¹¹ In-ZHER2:S1
	IFN-α receptor	labeled IFN-α		¹²⁵ I-IFN-α2a
chemo-tracking	bleomycin biodistribution	labeled cytostatic antibiotic	SPECT	¹¹¹ In-bleomycin
				⁵⁷ Co-bleomycin
	biodistribution of chemo-loaded nanoparticles	radiolabeled etoposide loaded nanoparticles	SPECT, PK/BD	^{99m} Tc-(polymeric nanoparticles)
		radiolabeled doxorubicin loaded nanoparticles	SPECT, PK/BD	^{99m} Tc-ETPL-nanoparticles
			SPECT	^{99m} Tc-DPBC

	in vitro	in vivo	human	imaging	RIT	B-cell			T-cell
						HL	B-NHL		
							agressive	indolent	
								275	
						276			
								277 (PTLD)	
						278			
							279		
						280, 281			
						280-284			
								285 (SLL)	
								286 (SLL)	
									287
									288, 289
									288
							290		
						291			291
									292
								293	
									294
									295 (murine)
									296 (murine)
							297		
							298		
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							301		
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									305
									306

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Supplementary Table 2: Ongoing molecular imaging trials in lymphoma registered on Clinical-Trials.gov

Number	Name	Intervention	Target	Institution	Status 11-2019
NCT03436342	Chemokine receptor CXCR4-targeting molecular imaging for metabolic characterisation of multiple myeloma and lymphoma	⁶⁸ Ga-Pentixafor in MM and lymphoma	CXCR4	Beijing Union Medical College Hospital, China	not yet recruiting
NCT03850028	Molecular imaging using radiolabelled atezolizumab to assess atezolizumab biodistribution in lymphoma patients	⁸⁹ Zr-atezolizumab PET scans in high risk DLBCL	PD-L1	University Medical Centre Groningen; VU University Medical Centre Amsterdam, The Netherlands	recruiting
NCT03188354	Diagnostic assessment of ¹⁸ F-fluciclovine and ¹⁸ F-FDG -PET/MRI of primary central nervous system lymphoma	¹⁸ F-fluciclovine and ¹⁸ F-FDG PET/MRI in PCNSL	¹⁸ F-fluciclovine uptake (mostly via sodium independent amino acid transport systems ASC and L) and ¹⁸ F-FDG uptake (mostly functional via transferrin receptor)	Norwegian University of Science and Technology, Trondheim, Norway	recruiting

BL = Burkitt lymphoma, CXCR-4 = chemokine receptor 4, DLBCL = diffuse large B-cell lymphoma, ¹⁸F = fluor-18, FDG = fluorodeoxyglucose, ⁶⁸Ga = gallium-68, MM = multiple myeloma, MRI = magnetic resonance imaging, PCNSL = primary central nervous system lymphoma, PD-L1 = programmed death ligand 1, PET = positron emission tomography, ⁸⁹Zr = zirconium-89

Supplementary Table 3: Overview of RIT studies identified during literature search for the present review

Reference	First author	PMID	Year	country	Isotope	Technique
1	Zimmer et al.	3258022	1988	USA	¹³¹ I	RIT+SPECT
2	Press et al.	2666588	1989	USA	¹³¹ I	RIT+SPECT
3	Vriesendorp et al.	2674084	1989	USA	⁹⁰ Y, ¹¹¹ In	RIT+SPECT
4	DeNardo et al.	2297713	1990	USA	¹³¹ I, ¹¹¹ In	RIT+SPECT
5	Bernstein et al.	2297714	1990	USA	¹³¹ I	RIT+SPECT
6	Goldenberg et al.	2066752	1991	USA	¹³¹ I	RIT+SPECT, PK/BD
7	Vriesendorp et al.	2033428	1991	USA	⁹⁰ Y, ¹¹¹ In	RIT+SPECT
8	Falini et al.	1329918	1992	Italy	¹³¹ I	RIT+SPECT
9	Kaminski et al.	1403053	1992	USA	¹³¹ I	RIT+SPECT
10	Bierman et al.	8478663	1993	USA	⁹⁰ Y, ¹¹¹ In	RIT+SPECT
11	Press et al.	7692295	1993	USA	¹³¹ I	RIT+SPECT
12	Kaminski et al.	7687326	1993	USA	¹³¹ I	RIT+SPECT
13	Czuczman et al.	8410126	1993	USA	¹³¹ I	RIT+SPECT
14	Kuzel et al.	7812346	1993	USA	¹³¹ I	RIT+SPECT
15	Herpst et al.	7666099	1995	USA	⁹⁰ Y, ¹¹¹ In	RIT+SPECT
16	Knox et al.	9816191	1996	USA	⁹⁰ Y, ¹¹¹ In	RIT+SPECT

	Target group	Target	HL	agressive B-NHL	indolent B-NHL	NOS B- NHL	T-NHL
	T-lineage	CD5					1
	B/T-lineage	CD37				1	
	intracellular	Ferritin	1				
	MHC class II	HLA-DR				1	
	B/T-lineage	CD37				1	
	B-lineage	CD22				1	
	intracellular	Ferritin	1				
	B/T-lineage	CD30	1				
	B/T-lineage	CD37				1	
	intracellular	Ferritin	1				
	B/T-lineage	CD20, CD37				1	
	B-lineage	CD20				1	
	other surface targets	CD21 (CR2)				1	
	MHC class II	HLA-DR				1	
	intracellular	Ferritin	1				
	B-lineage	CD20				1	

Supplementary Table 3: continued

Reference	First author	PMID	Year	country	Isotope	Technique
17	White et al.	8611688	1996	USA	^{90}Y , ^{111}In	RIT+SPECT
18	Foss et al.	9829731	1998	USA	^{90}Y , ^{111}In	RIT+SPECT
19	DeNardo et al.	9779698	1998	USA	^{131}I	RIT+SPECT
20	DeNardo et al.	9713461	1998	USA	^{67}Cu	RIT+SPECT, PK/BD
21	DeNardo et al.	10850360	1998	USA	^{131}I	RIT+SPECT
22	Liu et al.	9779701	1998	USA	^{131}I	RIT+SPECT
23	Linden et al.	10541377	1999	Sweden	^{131}I	RIT+SPECT
24	Witzig et al.	10577851	1999	USA	^{90}Y , ^{111}In	RIT+SPECT
25	Juweid et al.	10541378	1999	USA	^{131}I , ^{111}In , ^{90}Y	RIT+SPECT, PK/BD
26	Behr et al.	10541379	1999	Germany	^{131}I	RIT+SPECT
27	O'Donnell et al.	10616879	1999	USA	^{67}Cu	RIT+SPECT
28	DeNardo et al.	10450684	1999	USA	^{131}I	RIT+SPECT, PK/BD
29	Koral et al.	11041019	2000	USA	^{131}I	RIT+SPECT
30	Weiden et al.	10740649	2000	USA	^{90}Y , ^{111}In	RIT+SPECT
31	Vose et al.	10811451	2000	USA	^{131}I	RIT+SPECT
32	Wiseman et al.	10952488	2000	USA	^{90}Y , ^{111}In	RIT+SPECT
33	Vose et al.	10715303	2000	USA	^{131}I	RIT+SPECT
34	O'Donnell et al.	11268433	2000	USA	^{90}Y , ^{67}Cu	RIT, PK/BD, SPECT
35	DeNardo et al.	11707820	2000	USA	^{67}Cu , ^{131}I , ^{90}Y	RIT+SPECT
36	Weiden et al.	11578915	2001	USA	^{90}Y , ^{111}In	RIT+SPECT
37	DeNardo et al.	11535720	2001	USA	^{111}In	RIT+SPECT
38	Wiseman et al.	11418315	2001	USA	^{90}Y , ^{111}In	RIT+SPECT
39	Witzig et al.	12011122	2002	USA	^{90}Y , ^{111}In	RIT+SPECT
40	Scheidhauer et al.	12271407	2002	Germany	^{131}I	RIT+SPECT, PK/BD
41	Koral et al.	12621015	2003	USA	^{131}I	RIT+SPECT
42	Witzig et al.	12663713	2003	USA	^{90}Y , ^{111}In	RIT+SPECT
43	Sharkey et al.	14660727	2003	USA	^{111}In , ^{90}Y	RIT+SPECT
44	Sgouros et al.	12571219	2003	USA	^{131}I	RIT+SPECT
45	Tsimberidou et al.	15139064	2004	USA	^{90}Y , ^{111}In	RIT+SPECT
46	Gordon et al.	15453924	2004	USA	^{90}Y , ^{111}In	RIT+SPECT
47	Rizzieri et al.	15100153	2004	USA	^{131}I	RIT+SPECT, PK/BD
48	Forero et al.	14996706	2004	USA	^{90}Y , ^{111}In	RIT+SPECT, PK/BD
49	Kaminski et al.	15689582	2005	USA	^{131}I	RIT+SPECT
50	Bienert et al.	15937686	2005	Germany	^{131}I	RIT+SPECT
51	Watanabe et al.	16367911	2005	Japan	^{90}Y , ^{111}In	RIT+SPECT
52	Linden et al.	16033839	2005	Sweden	^{90}Y , ^{111}In	RIT+SPECT
53	Schnell et al.	16034043	2005	Germany	^{131}I	RIT+SPECT
54	Leahy et al.	16940276	2006	Australia	^{131}I	RIT+SPECT
55	Buchegger et al.	16685263	2006	Switzerland	^{131}I	RIT+SPECT
56	Iwamoto et al.	17932895	2007	USA	^{90}Y , ^{111}In	RIT+SPECT
57	Decaudin et al.	17762404	2007	France	^{90}Y , ^{111}In	RIT+SPECT
58	Doolittle et al.	17786706	2007	USA	^{90}Y , ^{111}In	RIT+SPECT
59	Vose et al.	17454625	2007	USA	^{90}Y , ^{111}In	RIT+SPECT
60	Cooney-Qualter et al.	17875803	2007	USA	^{90}Y , ^{111}In	RIT+SPECT
61	Schillaci et al.	17803447	2007	Italy	^{131}I	RIT+SPECT, PK/BD
62	Gopal et al.	17312330	2007	USA	^{131}I	RIT+SPECT

	Target group	Target	HL	agressive B-NHL	indolent B-NHL	NOS B-NHL	T-NHL
	surface Ig	variable-region antigenic determinants of the clonal surface Ig				1	
	T-lineage	CD5					1
	MHC class II	HLA-DR10				1	
	MHC class II	HLA-DR10				1	
	MHC class II	HLA-DR10				1	
	B-lineage	CD20				1	
	B-lineage	CD22				1	
	B-lineage	CD20				1	
	B-lineage	CD22				1	
	B-lineage	CD20, CD22			1	1	
	MHC class II	HLA-DR				1	
	MHC class II	HLA-DR				1	
	B-lineage	CD20			1		
	B-lineage	CD20				1	
	B-lineage	CD22				1	
	B-lineage	CD20				1	
	B-lineage	CD20				1	
	MHC class II	HLA-DR				1	
	MHC class II	HLA-DR				1	
	B-lineage	CD20				1	
	MHC class II	HLA-DR10				1	
	B-lineage	CD20				1	
	B-lineage	CD20			1	1	
	B-lineage	CD20				1	
	B-lineage	CD20				1	
	B-lineage	CD20			1		
	B-lineage	CD22				1	
	B-lineage	CD20				1	
	B-lineage	CD20		1			
	B-lineage	CD20				1	
	microenvironment	Tenascin (ECM protein)				1	
	B-lineage	CD20				1	
	B-lineage	CD20				1	
	B-lineage	CD20				1	
	B-lineage	CD20		1			
	B-lineage	CD22				1	
	B/T-lineage	CD30	1				
	B-lineage	CD20			1		
	B-lineage	CD20			1		
	B-lineage	CD20		1			
	intracellular	Ferritin	1				
	B-lineage	CD20		1			
	B-lineage	CD20				1	
	B-lineage	CD20				1	
	MHC class II	HLA-DR				1	
	B-lineage	CD20			1		

Supplementary Table 3: continued

Reference	First author	PMID	Year	country	Isotope	Technique
63	Ferrucci et al.	17979944	2007	Italy	⁹⁰ Y, ¹¹¹ In	RIT+SPECT
64	Mones et al.	17325895	2007	USA	¹³¹ I	RIT+SPECT
65	Iagaru et al.	18927323	2008	USA	⁹⁰ Y, ¹¹¹ In	RIT+SPECT
66	Morschhauser et al.	18854568	2008	France	⁹⁰ Y, ¹¹¹ In	RIT+SPECT
67	Krishnan et al.	18025438	2008	USA	⁹⁰ Y, ¹¹¹ In	RIT+SPECT
68	Torres-Garcia et al.	18068002	2008	Mexico	¹⁸⁸ Re	RIT+SPECT
69	Assié et al.	18298329	2008	France	⁹⁰ Y, ¹¹¹ In	RIT+SPECT
70	Wang et al.	19770379	2009	USA	⁹⁰ Y, ¹¹¹ In	RIT+SPECT
71	Fisher et al.	19289440	2009	USA	¹¹¹ In, ⁹⁰ Y	RIT+SPECT
72	Tobinai et al.	19018755	2009	Japan	⁹⁰ Y, ¹¹¹ In	RIT+SPECT
73	Chiesa et al.	19455328	2009	Italy	⁹⁰ Y, ¹¹¹ In	RIT+SPECT, PK/BD
74	Maza et al.	19060176	2009	Germany	⁹⁰ Y, ¹¹¹ In	RIT+SPECT
75	Younes et al.	19465429	2009	USA	⁹⁰ Y, ¹¹¹ In	RIT+SPECT
76	Delaloye et al.	19837764	2009	Switzerland	⁹⁰ Y, ¹¹¹ In	RIT+SPECT
77	Bethge et al.	20530284	2010	Germany	⁹⁰ Y, ¹¹¹ In	RIT+SPECT
78	Morschhauser et al.	20625137	2010	France	⁹⁰ Y, ¹¹¹ In	RIT+SPECT
79	Hoffmann et al.	21133720	2011	Austria	⁹⁰ Y, ¹¹¹ In	RIT+SPECT
80	Leahy et al.	20864582	2011	Australia	¹³¹ I	RIT+SPECT
81	Burdick et al.	20510542	2011	USA	⁹⁰ Y, ¹¹¹ In	RIT+SPECT
82	Hattori et al.	22955187	2012	USA	¹³¹ I	RIT+SPECT
83	Smith et al.	22851557	2012	USA	⁹⁰ Y, ¹¹¹ In	RIT+SPECT
84	Khoury et al.	22586182	2012	USA	⁹⁰ Y, ¹¹¹ In	RIT+SPECT
85	Forrer et al.	23358408	2012	Switzerland	¹⁷⁷ Lu	RIT+SPECT
86	Ferrer et al.	23358406	2012	France	⁹⁰ Y, ¹¹¹ In	RIT+SPECT
87	Bethge et al.	22504934	2012	Germany	⁹⁰ Y, ¹¹¹ In	RIT+SPECT
88	Forrer et al.	23572496	2013	Switzerland	¹⁷⁷ Lu	RIT+SPECT
89	Vose et al.	23478060	2013	USA	¹³¹ I	RIT+SPECT
90	Vaklavas et al.	23530878	2013	USA	⁹⁰ Y, ¹¹¹ In	RIT+SPECT
91	Wagner et al.	23765188	2013	Germany	¹³¹ I	RIT+SPECT
92	Dewaraja et al.	24842891	2014	USA	¹³¹ I	RIT+SPECT
93	Matesan et al.	25076159	2014	USA	⁹⁰ Y, ¹¹¹ In	RIT+SPECT
94	Boucek et al.	24134141	2014	Australia	¹³¹ I	RIT+SPECT
95	Witzig et al.	25150258	2014	USA	⁹⁰ Y	RIT+SPECT
96	Arnason et al.	25677780	2015	USA	⁹⁰ Y, ¹¹¹ In	RIT+SPECT
97	Elstrom et al.	24730538	2015	USA	¹³¹ I	RIT+SPECT, PK/BD
98	Berger et al.	25689832	2016	Switzerland	⁹⁰ Y, ¹¹¹ In	RIT+SPECT
99	Shin et al.	27577259	2016	South Korea	¹³¹ I	RIT+SPECT
100	Yadav et al.	26974315	2016	India	¹⁷⁷ Lu	RIT+SPECT
101	Blakkisrud et al.	27493270	2017	Norway	¹⁷⁷ Lu	RIT+SPECT
102	Blakkisrud et al.	27587710	2017	Norway	¹⁷⁷ Lu	RIT+SPECT
103	Krishnan et al.	28267593	2017	USA	⁹⁰ Y, ¹¹¹ In	RIT+SPECT
104	Chahoud et al.	29476021	2018	USA	⁹⁰ Y, ¹¹¹ In	RIT+SPECT
105	Stokke et al.	29470615	2018	Norway	¹⁷⁷ Lu	RIT+SPECT
106	Morschhauser et al.	30291297	2018	France	⁹⁰ Y, ¹¹¹ In	RIT+SPECT

	Target group	Target	HL	agressive B-NHL	indolent B-NHL	NOS B- NHL	T-NHL
	B-lineage	CD20				1	
	B-lineage	CD20				1	
	B-lineage	CD20				1	
	B-lineage	CD20			1		
	B-lineage	CD20				1	
	B-lineage	CD20				1	
	B-lineage	CD20				1	
	B-lineage	CD20			1		
	B-lineage	CD20				1	
	B-lineage	CD20			1		
	B-lineage	CD20				1	
	B-lineage	CD20		1			
	B-lineage	CD20 (B-cells in tumor microenvironment)	1				
	B-lineage	CD20			1		
	B-lineage	CD20				1	
	B-lineage	CD22				1	
	B-lineage	CD20				1	
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	B-lineage	CD20			1		
	B-lineage	CD20		1			
	B-lineage	CD20				1	
	B/T-lineage	CD37			1		
	B/T-lineage	CD37			1		
	B-lineage	CD20				1	
	B-lineage	CD20		1			
	B/T-lineage	CD37			1		
	B-lineage	CD20			1		

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Impact of rituximab biosimilars on overall survival in diffuse large B-cell lymphoma: a nationwide population-based study

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ABSTRACT

In 2017, the European Medicines Agency approved rituximab biosimilars (R-biosimilars) for treatment of diffuse large B-cell lymphoma (DLBCL). Thereafter, the Netherlands was one of the first countries to implement R-biosimilars, given lower costs as compared to rituximab originator (R-originator). This study's objective was to investigate whether overall survival (OS) of patients with DLBCL receiving R-biosimilars is similar to patients treated with R-originator. DLBCL patients ≥ 18 years, diagnosed between 2014 and 2018, who received at least one cycle of rituximab combined with cyclophosphamide, doxorubicin, vincristine, and prednisolone (R-CHOP) were identified in the Netherlands Cancer Registry. Patients were categorized into R-originator or R-biosimilars groups based on data from a central repository of the Dutch medicinal drug market. The primary endpoint was 3-year OS, defined as the time between diagnosis and all-cause death. By the end of 2018, 91% of purchased rituximab were biosimilars. In total, 4,429 patients were identified with 876 in the R-biosimilars group and 3,553 in the R-originator group. Patients in the R-biosimilars group less frequently received > 6 cycles of R-CHOP as compared to patients treated with R-originator (24 % vs. 30 %, $P = 0.003$). The 3-year OS did not differ between patients treated with R-originator or R-biosimilars (73 % vs. 73 %, $P = 0.855$). This was confirmed with a multivariable Cox regression analysis accounting for sex, age, IPI-score and number of R-CHOP cycles. In conclusion, the 3-year OS is similar for patients treated with CHOP in combination with R-originator or R-biosimilars and, therefore, favors the use of R-biosimilars in DLBCL treatment management.

INTRODUCTION

The standard treatment of patients with newly diagnosed diffuse large B-cell lymphoma (DLBCL) consists of the anti-CD20 monoclonal antibody rituximab combined with cyclophosphamide, doxorubicin, vincristine, and prednisolone (R-CHOP) [1]. In 2017, the rituximab biosimilars (R-biosimilars) CT-P10 and GP2013 were approved by the European Medicines Agency (EMA) for the treatment of DLBCL [2, 3]. This approval of R-biosimilars was based on preclinical data and prospective studies in patients with rheumatoid arthritis and naïve follicular lymphoma. Whereas efficacy data in naïve follicular lymphoma showed equal overall response rates (ORR) for R-biosimilars and R-originator, data on ORR and overall survival (OS) for patients with newly diagnosed DLBCL is limited to small post marketing studies [4, 5].

In 2017, the Dutch Association of Medical Specialists stated to adopt biosimilars into standard of care. Hence, due to considerably lower purchase costs of R-biosimilars as compared to R-originator (up to 50 %), the Netherlands was one of the first countries to implement R-biosimilars in its healthcare system. However, it is still uncertain if the effect of R-biosimilars on OS of patients with newly diagnosed DLBCL is truly equivalent to that of R-originator [6]. Complementary to prospective intervention studies, population-based studies can provide valuable information to assess treatment strategies and outcome in patients at the population level. Therefore, we evaluated the 3-year OS of newly diagnosed patients with DLBCL either treated with R-biosimilars or R-originator, using a contemporary, nationwide, population-based cancer registry in the Netherlands.

METHODS

Patients

Patients with newly diagnosed DLBCL ≥ 18 years diagnosed between January 1st, 2014, and December 31st, 2018, who received at least one cycle of R-CHOP, were identified in the Netherlands Cancer Registry (NCR). This population-based NCR is maintained and hosted by the Netherlands Comprehensive Cancer Organization (IKNL) and has, since 1989, nationwide coverage of at least 95 % of all malignancies. All newly diagnosed malignancies are reported to the NCR via the Nationwide Network of Histopathology and Cytopathology, and the National Registry of Hospital Discharges (e.g., inpatient and outpatient discharges). Information on patient characteristics and treatment (y/n) is routinely recorded in the NCR

by trained registrars of the NCR through retrospective medical records review. Unique to the NCR, since January 1st, 2014, detailed information on diagnostic and treatment characteristics for all hematological malignancies diagnosed in the Netherlands are also recorded in the NCR. For DLBCL, the available prognostic factors include Ann Arbor stage, serum lactate dehydrogenase (LDH) levels, WHO performance status, international prognostic index (IPI) score, type of therapeutic regimen, number of R-CHOP cycles, the initiation date of first-line therapy, and best response to first-line treatment.

Topography and morphology of hematological malignancies are coded according to the International Classification of Diseases for Oncology (ICD-O). Patients with newly diagnosed DLBCL were defined using morphology code 9680, thereby excluding patients with primary central nervous system lymphomas, primary mediastinal B-cell lymphoma, and primary testicular lymphoma. Information on the last known vital status for all patients (e.g., alive, death, or emigration) was obtained through annual linkage with the Nationwide Population Registries Network that holds vital statistics on all residents in the Netherlands.

Patients were categorized into R-originator or R-biosimilars groups using purchase information of the Dutch hospitals for every month, derived from FarmInform. This information was validated by the confirmation of transition date from R-originator to R-biosimilars, which was provided by the respective clinical pharmacists from the Dutch hospitals. In more detail, FarmInform maintains a central data pooling system of the medicinal drug market of pharmaceutical companies, importers, and wholesalers in the Netherlands with coverage of > 98 % of the total market. FarmInform performs internal and external audits to guarantee a high quality of the collected data and is monitored according to Dutch and European competition law. In the Netherlands, central pharmacies coordinate the distribution of medicines among and within hospitals. Therefore, we validated the categorization of patients into R-biosimilars or R-originator groups in a two-step approach. First, the registrars of the NCR performed an additional medical records review of a random sample of 30 % of the patients categorized in the R-biosimilars group, to validate the use of R-biosimilars according to purchase information from FarmInform. Detailed pharmacists' information about drug prescription was not accessible for all patients in the random sample via these medical records. Secondly, we therefore directly approached the responsible pharmacists of hospitals for which detailed information was not available in medical records with an inquiry about the exact transition date to R-biosimilars. Patients for whom the use of R-originator or R-biosimilars could not be conclusively deducted were excluded,

including patients who either switched from R-originator to R-biosimilars or vice versa during first-line treatment.

The primary endpoint was 3-year OS, defined as the time between DLBCL diagnosis and all-cause-death. The secondary endpoint was best response to first-line treatment. Best response – i.e. complete response (CR), partial response (PR) or stable (SD)/progressive disease (PD) – was determined by physicians' assessment.

According to the Central Committee on Research involving Human Subjects (CCMO), this type of observational study does not require approval from an ethics committee in the Netherlands. The Privacy Review Board of the NCR approved the use of anonymous data for this study.

Statistical Analysis

Age, sex, Ann Arbor stage, serum LDH level, WHO performance score, number of extranodal sites, IPI score, time until treatment, and the number of R-CHOP cycles were compared between R-originator and R-biosimilars treated patients. The Pearson chi-square test or the Fisher's exact test was applied to compare age (≤ 60 vs. > 60 years), sex, Ann Arbor stage, serum LDH level (elevated vs. normal), WHO performance score, number of extranodal sites, IPI-score, and number of cycles. The Kruskal-Wallis test was used for median age and time until treatment between R-originator and R-biosimilars treated patients. The Kaplan-Meier method served to estimate OS, and the log-rank test to examine differences in survival distributions between R-originator and R-biosimilars treated patients. Cox proportional hazard regression analyses were performed to investigate the effect of age, sex, Ann Arbor stage, WHO performance score, IPI score, and the number of R-CHOP cycles on OS. The results from the Cox regression analyses produce hazard ratios (HRs) with associated 95 % confidence intervals (CIs). The proportional hazard assumption was tested based on the Schoenfeld residuals [6]. Covariates were introduced in the regression models with a forward selection method, after adjusting for influence of those already selected. The final model was accomplished when the *P*-value for entering an additional covariate was above 0.05. Patients alive were censored on February 1st, 2021.

To account for differences in duration of follow-up between patients treated with R-originator and R-biosimilars, we additionally performed a sensitivity analysis. First, we selected patients with DLBCL diagnosed between April 1st, 2017 and December 31st, 2018 (the period following EMA approval of R-biosimilars) and

compared patients in the R-originator group with patients in the R-biosimilars group on OS. Second, we selected patients with DLBCL in the R-originator group and compared patients diagnosed in the pre-period EMA approval (pre-EMA) of R-biosimilars to patients diagnosed in the post-period EMA approval (post-EMA) of R-biosimilars.

P-values of less than 0.05 indicated statistical significance. All statistical analyses were performed with STATA version 16.1 (StataCorp, College Station, TX).

RESULTS

Patients

In total, we identified 4,968 patients with DLBCL treated with R-CHOP. For 539 patients with newly diagnosed DLBCL, information on the use of R-biosimilars was unknown and were excluded from analysis, leaving 4,429 patients in our study population.

Pre-EMA, e.g., between January 1st, 2014 and March 31st, 2017, 3,183 patients received R-originator. Post-EMA – from April 1st, 2017 onwards – the purchase percentage to R-biosimilars, as compared to R-originator, had risen to 91 % by the end of 2018 resulting in 370 patients receiving R-originator and 876 R-biosimilars (CT-P10 86 %, GP2013 14 %) (Figure 1). The baseline characteristics of all 4,429 patients, including age, sex, IPI score, and the number of R-CHOP cycles, are presented in Table 1, subdivided according to patients treated with R-originator (*n* = 3,553) and R-biosimilars (*n* = 876). In the R-biosimilars group, a higher proportion of patients had a WHO performance status of 0–2 (57 % vs. 53 %; *P* = 0.044), and a lower proportion of patients received > 6 cycles of R-CHOP (24 % vs. 30 %; *P* = 0.004) as compared to the R-originator group.

Response Rate

The ORR between R-originator and R-biosimilars was not significantly different (85 % vs. 84 %, *P* = 0.326) (Figure 2). In detail, 74 % of the patients in the R-originator group had a CR as compared to 72 % in the R-biosimilars group. In addition, the observed proportion of patients with a PR was similar in both groups (11 % and 11 %).

Table 1. Characteristics of DLBCL patients receiving rituximab originator or rituximab biosimilars as part of R-CHOP treatment regimen

Characteristic	Rituximab originator (n = 3,553)		Rituximab biosimilars (n = 876)		P-value
Female sex–no. (%)	1,466	(41.3)	344	(39.3)	0.283
Age years–median (range)	68	(18–95)	68	(21–95)	0.054
Age years–no. (%)					
≤ 60 years	1,092	(30.7)	232	(26.5)	0.014
Ann Arbor stage (%)					
I–II	1,282	(36.1)	305	(34.8)	0.333
III–IV	2,257	(63.5)	570	(65.1)	
Unknown	14	(0.4)	1	(0.1)	
Lactate dehydrogenase–no. (%)					
Normal	1,668	(46.9)	374	(42.7)	0.066
Elevated	1,819	(51.2)	487	(55.6)	
Unknown	66	(1.9)	15	(1.7)	
WHO performance status–no. (%)					
0–2	1,880	(52.9)	503	(57.4)	0.044
3–4	75	(2.1)	20	(2.3)	
Unknown	1,598	(45.0)	353	(40.3)	
Number of extranodal sites–no. (%)					
0–1	2,550	(71.8)	653	(74.5)	0.210
> 1	1,001	(28.2)	223	(25.5)	
Unknown	2	(0.06)	0	(0)	
IPI-score–no. (%)					
Low (0–1)	682	(19.2)	150	(17.1)	0.133
Low–intermediate (2)	464	(13.1)	129	(14.7)	
High–intermediate (3)	463	(13.0)	129	(14.7)	
High (4–5)	546	(15.4)	148	(16.9)	
Unknown	1,398	(39.4)	320	(36.5)	
Time until treatment–days, median	24	(15–36)	24	(14–38)	0.314
Number of cycles–no. (%)					
< 6 cycles	922	(25.9)	241	(27.5)	0.004
6 cycles	1,566	(44.1)	426	(48.6)	
> 6 cycles	1,062	(29.9)	209	(23.9)	
Unknown	3	(0.1)	0	(0)	

WHO denotes world health organization, IPI international prognostic index, IQR interquartile rate

Figure 1

Pace of conversion of rituximab originator (R-originator) to rituximab biosimilars (R-biosimilar) in the Netherlands. Line graph depicting the market share of rituximab biosimilars from their approval by the European Medicines Agency in 2017 until January 2019.

Overall Survival

The median follow-up for patients with DLBCL alive treated with R-originator and those who received R-biosimilars were 61.7 months (range 8.1–85.0 months) and 32.3 months (range 23.6–45.2 months), respectively. The 3-year OS of patients treated with R-originator or R-biosimilars was 73 % (95 % CI: 72 %–74 %) and 73 % (95 % CI: 70 %–76 %; $P = 0.855$), respectively, and is presented in Figure 3. When simultaneously adjusted for age at diagnosis, sex, IPI score, and the number of R-CHOP cycles, the risk of mortality was similar for patients treated with R-biosimilars (HR, 0.89; 95 % CI, 0.76–1.05; $P = 0.182$) and those treated with R-originator (Supplementary Table 1).

Best tumor response after first-line therapy with rituximab originator (R-originator) or rituximab biosimilars (R-biosimilars). Stacked bar graph depicting tumor response in patients with diffuse large B-cell lymphoma treated with rituximab originator (R-originator) or rituximab biosimilars (R-biosimilars) combined with standard CHOP chemotherapy regimens.

Sensitivity Analysis

To account for differences in duration of follow-up between patients receiving R-originator and R-biosimilars, we performed a sensitivity analysis using a two-way approach. First, all patients in the R-originator group diagnosed pre-EMA were excluded, leaving 370 patients who received R-originator and 876 patients who received R-biosimilars between April 1st, 2017 and December 31st, 2018. No differences in baseline characteristics were observed between both groups of patients, as well as in response rates (data not shown). The median follow-up was 42.7 months (range 15.8–46.0 months) for alive patients treated with R-originator and 32.3 months (range 23.6–45.2 months) for alive patients treated with R-biosimilars. The shorter median follow-up for the R-biosimilars treated patients was due to the timing R-biosimilars were introduced in routine clinical practice. The 3-year OS was 74 % (95 % CI: 70 %–79 %) for the R-originator group and 73 % (95 % CI: 70 %–76 %;) for the R-biosimilar group ($P = 0.798$).

Figure 3

Overall survival of patients treated with rituximab originator or rituximab biosimilars. Kaplan-Meier curves depicting 3-year overall survival in patients with DLBCL treated with rituximab originator (R-originator, in red) or rituximab biosimilars (R-biosimilars, in blue) combined with standard CHOP chemotherapy regimens. 95 % confidence intervals are depicted as blue/red shaded areas.

Secondly, all patients who received R-biosimilars were excluded, leaving 3,183 patients who received R-originator between January 1st, 2014 and March 31st, 2017, and 370 patients who received R-originator between April 1st, 2017 and December 31st, 2018. Here, patients who were diagnosed pre-EMA more often received > 6 cycles of R-CHOP as compared to patients diagnosed in the post-EMA (31 % vs. 22 %; $P < 0.001$). Otherwise, no differences in baseline characteristics and response rates were observed between patients diagnosed in the pre-EMA and patients post-EMA (data not shown). The median follow-up was 64.2 months (range 8.1–85.0 months) for alive patients treated with R-originator pre-EMA as compared to 42.7 months (range 15.8–46.0 months) for alive patients treated with R-originator post-EMA. The 3-year OS was 74 % for patients diagnosed post-EMA (95 % CI: 70 %–79 %) and 73 % for patients diagnosed in the R-originator group pre-EMA (95 % CI: 71 %–74 %; $P = 0.619$). Overall, the results of the sensitivity analyses were similar to those of the primary analyses.

DISCUSSION

We evaluated the 3-year OS of R-biosimilars as compared to R-originator in patients with newly diagnosed DLBCL using a contemporary, nationwide, population-based cancer registry in the Netherlands. The OS and the best response of patients with DLBCL did not differ between the R-biosimilars and R-originator groups.

To our knowledge, this is the first population-based study post-EMA of R-biosimilars to link treatment with R-biosimilars to survival among patients with newly diagnosed DLBCL. The 3-year OS in the current study was similar to the survival of the R-originator CHOP reported 15 years ago [7]. Collectively, our data show that patients with DLBCL benefit equally from R-originator or R-biosimilars combined with CHOP treatment. The R-originator was approved by the US Food and Drug Administration on November 26th, 1997 for treatment of relapsed/refractory CD20 positive follicular lymphoma and, subsequently, on June 2nd, 1998 by the EMA. It has since been the backbone of every treatment regimen in B-cell non-Hodgkin lymphomas. Although the glycoengineered anti-CD20 antibody obinatumab showed enhanced efficacy for follicular lymphoma and chronic lymphocytic leukemia, as compared to R-originator, this was not substantiated in DLBCL [8–10]. The approval of the subcutaneous R-originator for the treatment of DLBCL has not been translated into widespread use. Therefore, R-originator administered intravenously remained the standard of care for most patients with DLBCL [11].

Since the expiration of the patent of R-originator, several R-biosimilars have been approved by the EMA and subsequently by the FDA [2, 3, 12–14]. Despite the preclinical similarity between R-biosimilars and R-originator, the latter having a considerable share of the global market, the implementation of R-biosimilars into routine clinical practice fluctuates greatly between countries. In the current study, we objectivized that the transition to R-biosimilars in the Netherlands occurred within 12 months following EMA approval without an adverse impact on 3-year OS, thus supporting the use of R-biosimilars in DLBCL.

Global spending on cancer medicines has risen from \$96 billion in 2013 to \$150 billion in 2018. It might increase to \$200 billion by 2023 [15]. Biosimilars hold the promise of constraining healthcare spending with already 55 products approved by the EMA and 26 by the FDA in May 2020 [16]. Estimated cost reduction until 2026 is \$24 to \$150 billion for oncology products in the United States alone. [17]. With

global sales of \$7.6 billion of R-originator in 2017, R-biosimilars may have major health-economic consequences. With a worldwide R-originator sales increase of 1% in 2017, purchase percentages of R-originator decreased by 8% and 4% in 2018 and 2019, respectively [18]. The highest conversion rate occurred in Europe with a 33% purchase reduction of R-originator in 2019 [18]. Despite approval in 2018, the first R-biosimilar was only marketed in November 2019 in the United States. In that same year, the relative expenditure on R-originator in the Netherlands was 43% lower than in 2015. [19].

The main strength of this study is the use of a nationwide population-based cancer registry over clinical trial data as clinical trial data is based on selections of patients. Secondly, with the availability of comprehensive data on first-line treatment for a substantial number of patients, treatment practices could be assessed and linked to outcome. Moreover, by combining clinical data from the NCR and validated purchase information on rituximab from FarmInform, we were able to distinguish patients treated with R-originator and R-biosimilar. Limitations of this study mainly pertain to the lack of detailed information on dosage per cycle at the patient level and treatment-related toxicities, including infusion-related reactions. Second, we observed a change in standard-of-care treatment strategy for patients with DLBCL over time with a reduction of the number of cycles R-CHOP used. However, 6 cycles of R-CHOP did not negatively affect survival, as compared to 8 cycles of R-CHOP, regardless of whether patients with DLBCL received R-originator or R-biosimilars in combination with CHOP [10, 21]. Third, while 2-year event free-survival EFS is considered a robust endpoint in DLBCL [20, 23] because the relapse rate after this time point is low – we acknowledge that 2-year EFS is lacking as an outcome parameter in our study. Since OS is affected by the efficacy of salvage treatment and a 2-year OS does not guarantee a similar 2-year EFS between R-originator and R-biosimilars, we evaluated a longer term OS, i.e. 3-years. Despite the abovementioned limitations, this cancer registry represents an important tool to gain insight into the clinical data and outcome of large numbers of patients pre- and post-EMA approval, since all newly diagnosed patients are captured.

In conclusion, in this nationwide, population-based study, we observed no difference in OS between patients with DLBCL treated with R-biosimilars and patients with DLBCL treated with R-originator. These results favor the use of R-biosimilars in the first-line treatment of DLBCL, as this helps to constrain worldwide healthcare spending without negatively affecting OS.

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SUPPLEMENTARY MATERIAL

Supplementary Table 1. Cox regression analyses for overall survival in DLBCL patients treated with rituximab originator or rituximab biosimilars

	Univariate			Multivariate		
	HR	95 % CI	P-value	HR	95 % CI	P-value
Rituximab biosimilar						
No	1	reference	–	1	reference	–
Yes	0.98	0.83–1.15	0.775	0.89	0.76–1.05	0.182
Age at diagnosis	1.04	1.03–1.04	< 0.001	1.02	1.01–1.03	< 0.001
Gender						
Male	1	reference	–	1	reference	–
Female	0.90	0.79–1.02	0.107	0.86	0.75–0.97	0.017
Ann Arbor stage						
I–II	1	reference	–	–	–	–
III–IV	2.88	2.45–3.40	< 0.001	–	–	–
Unknown	7.71	3.80–15.67	< 0.001	–	–	–
WHO performance status						
0–2	1	reference	–	–	–	–
3–4	4.03	2.98–5.4	< 0.001	–	–	–
unknown	1.72	1.51–1.96	< 0.001	–	–	–
IPI-score						
Low (0–1)	1	reference	–	1	reference	–
Low – intermediate (2)	2.20	1.57–3.08	< 0.001	3.06	2.18–4.30	< 0.001
Intermediate – high (3)	3.92	2.88–5.34	< 0.001	5.76	4.20–7.89	< 0.001
High (4–5)	7.86	5.91–10.47	< 0.001	10.16	7.58–13.61	< 0.001
Unknown	3.99	3.02–5.27	< 0.001	4.76	3.60–6.30	< 0.001
Number of cycles						
6	1	reference	–	1	reference	–
< 6	3.30	2.86–3.79	< 0.001	4.00	3.47–4.63	< 0.001
> 6	0.80	0.67–0.97	0.020	0.89	0.74–1.08	0.237
Unknown	5.90	1.47–23.67	0.012	6.80	1.68–27.48	0.007

WHO denotes world health organization, IPI international prognostic index, HR hazard ratio, CI confidence interval

CHAPTER 7

General discussion and future perspective

GENERAL DISCUSSION

¹⁸F-fluor-deoxyglucose PET/CT

Approximately three decades after its introduction into the clinics, ¹⁸F-FDG PET imaging has become an integral part of lymphoma care. It fulfils an important role in diagnosis and response evaluation.

However, the field of ¹⁸F-FDG PET imaging in lymphomas is still in development. In the last decade, many efforts have been made to use ¹⁸F-FDG PET/CT for response-adapted therapy in lymphoma. The notion of adjusting a patient's regimen according to the response during therapy is intriguing as it might aid in providing the most effective treatment while limiting toxicity. In early stage CHL, these so-called interim PET evaluations can now be used for an escalation approach in which patients with a positive ¹⁸F-FDG PET/CT scan after two courses of adriamycine, bleomycin, vinblastine and dacarbazine (ABVD) will receive escalated bleomycin, etoposide, adriamycine, cyclophosphamide, vincristine, procarbazine and prednisone (escBEACOPP) and involved node radiotherapy (INRT) [1]. A recent randomized clinical trial with patients receiving four cycles of escBEACOPP showed that radiotherapy could be safely omitted in patients with a negative ¹⁸F-FDG PET scan after four weeks [2]. In patients with advanced stage CHL who achieve complete metabolic remission on two courses of escBEACOPP, de-escalation to four instead of the conventional six or eight cycles of escBEACOPP is possible without reduction in PFS or OS [3]. A large American trial demonstrated that PET-guided escalation from ABVD to escBEACOPP in patients with advanced CHL is feasible [4]. In the case of DLBCL, the implications of ¹⁸F-FDG PET guided therapy are less clear-cut. While many small retrospective trials claim that interim ¹⁸F-FDG PET scans in DLBCL can be used for guiding treatment decisions, other studies, most importantly the multicentre phase III PETAL trial, show no benefit for this approach [5]. This is due to the limited positive predictive value of ¹⁸F-FDG PET imaging in patients with DLBCL that have received R-CHOP therapy [6], which might be explained by the modality's inability to differentiate between persisting tumour tissue and therapy induced inflammatory response.

It has been suggested that semiquantitative measurements, such as the reduction in standard uptake value (SUV) between baseline and interim ¹⁸F-FDG PET scans (delta SUV), might provide a benefit over visual assessment using the Deauville scoring system that was designed for lymphoma PET response evaluation at the end of treatment [7]. However, while the measurement of semiquantitative

parameters such as delta SUV might seem an objective approach at first glance, these metrics require rigorous standardization of scans and are highly dependent on lesion selection. As reported in Chapter 1, we found an unexpected level of tumour heterogeneity in individual patients of our own DLBCL cohort. A recent study was able to demonstrate that highly heterogeneous ^{18}F -FDG uptake on PET/CT scans of patients with DLBCL is prognostic for worse overall- and event-free survival [8]. However, further research and standardisation of semiquantitative measurements is needed to determine if analysis of certain lesions (e.g., highest, or average uptake) or the entire tumour (total lesion glycolysis, total metabolic tumour volume, mean of SUVmax etc.) is best suited for prediction. Another point of improvement for scientific studies in the field of semiquantitative imaging biomarkers is the inadequate scrutiny of cut-off values. Smaller studies will often use receiver operator curves (ROCs) to determine so-called “optimal cut-offs” for variables. Dichotomization of datasets based on these cut-offs will increase the chances of producing significant results for survival analyses of the specific dataset at hand. This is problematic, as future studies will struggle to reproduce the results using non-validated cut-off values that have been determined with limited data. Validation in independent datasets is necessary and should be the standard to identify whether these cut-off values can be applied to unrelated cohorts.

In Chapter 2 we investigated whether molecular tumour characteristics translate into changes in imaging biomarkers in the setting of DLBCL. We were, however, unable to correlate fundamental biological changes such as aberrations in the metabolic master switch *MYC* with semiquantitative measures based on ^{18}F -FDG uptake on PET scans. Our current limitation in understanding lymphoma biology might explain this lack of correlation. In a molecular heterogeneous disease with constitutively high proliferative activity such as DLBCL, we might overestimate the influence that single aberrations like *MYC* translocation have on the metabolic phenotype as visualized and quantified using ^{18}F -FDG PET/CT. This notion is supported by findings that correlate the proliferative marker Ki-67 in tissue biopsies with ^{18}F -FDG uptake in lymphoma [9–11]. It could also mean that higher-order variables including the makeup of the local tumour vasculature or the tumour microenvironment, might influence, obscure, and distort metabolic changes at the molecular level. These influences might be so subtle that they cannot be discerned using current technology and modes of analysis. Interestingly, we were able to illustrate that the easily extractable visual characteristic of tumour necrosis is a negative prognostic factor for survival in patients with DLBCL, while in cHL this

was not confirmed. This might be attributed to the different pathophysiologic makeup of these malignancies with the majority of DLBCL lesions consisting of lymphoma cells, while in cHL the bulk of the tumour lesions is generally made up of a heterogeneous immune infiltrate. In DLBCL tumour necrosis might thus be a more direct results of the tumour outgrowing its oxygen and nutritional support, while in cHL tumour necrosis is more indirect, as necrotic tissue largely consists of inflammatory infiltrate that forms the tumour microenvironment rather than HRS cells themselves.

Constant progress in PET scanners' technical development has led to a significant increase in scan resolution with every new generation of PET camera [12]. Although this increased sensitivity for tracer signal generates problems of its own (e.g., interpretation of ^{18}F -FDG PET signals that were formerly undetectable), it also decreases the detection threshold for novel, lymphoma specific tracers improving the visualization of targets that are less abundantly expressed (e.g., CD30 expression in cHL with a minority of tumour cells) and further helps to discern inter- and intra-lesion heterogeneity.

Dynamic and total-body PET imaging, i.e., using novel scanners that encompass the entire body and thus allow for speedy acquisition of imaging data enabling the assessment of tracer distribution over time, are intriguing developments to improve ^{18}F -FDG PET. While current protocols ensure high scan quality, breaking the limitation of imaging at one particular time point might unlock additional tumour specific information, similar to contrast-enhanced CT scans [13]. ^{18}F -FDG uptake during specific timeframes might be able to discern between different lymphoma subtypes. Given the broad spectrum of lymphoma subtypes and pathophysiology, differences in ^{18}F -FDG uptake dynamics between HL and NHL or aggressive and indolent are theoretically likely. Recent prototype PET scanners for total-body imaging promise a 24-fold increase in sensitivity for the commonly applied head to thigh imaging setting [14]. Furthermore, integrated dynamic-total body PET scanning offers additional technical advantages resulting in reduced scanning time, less background signal, and improved quantification, amongst others [13].

Finally, recent efforts in improving the post-processing of raw imaging data from molecular imaging via machine learning algorithms, holds great potential for artefact correction, signal enhancement, novel radiomic tumour characterization, and for reducing radiation exposure [15, 16].

Many of the above-mentioned restrictions to ^{18}F -FDG PET/CT imaging are based on the limited specificity of ^{18}F -FDG uptake. Novel tracers with highly specific molecular targeting ability might offer new ways for future tumour imaging and allow for the extraction of highly detailed and clinically meaningful information from imaging data. As illustrated in Chapter 5 and the future perspectives section, novel tracers beyond ^{18}F -FDG can be of value in the context of drug development and patient selection for targeted therapy. In addition to diagnostic imaging with ^{18}F -FDG PET/CT as an established clinical tool, novel tracers could provide relevant biological information of the lymphoma at hand and help to select patients who could benefit most from treatment options exploiting these phenotypic tumour characteristics.

In conclusion, despite certain limitations, recent and future developments in PET technology and image analysis could improve future lymphoma workup and management, even 30 years after the introduction of ^{18}F -FDG PET.

Molecular imaging in solid oncology

In contrast to molecular imaging in lymphomas several new molecular tracers beyond ^{18}F -FDG have been developed and clinically investigated in solid malignancies. Molecular imaging has the potential to facilitate oncologic drug development [17], and several targeted therapeutics have been used to produce tracers for molecular imaging. ^{89}Zr -trastuzumab has been used for HER2/*neu* imaging in breast cancer and epidermal growth factor receptor (EGFR) imaging was achieved with ^{11}C -erlotinib in patients with non-small cell lung carcinoma (NSCLC), showing that tracers other than ^{18}F -FDG can be used for diagnostic imaging [18, 19]. By visualising the biodistribution of tracers like ^{111}In -trastuzumab, ^{18}F -fluorestradiol and ^{89}Zr -atezolizumab researchers have discovered that the expression of therapeutic targets like HER2/*neu*, oestrogen receptor (ER) and programmed death-ligand 1 (PD-L1) is much more heterogeneous than previously thought [20–22]. In advanced NSCLC PET imaging of ^{89}Zr -bevacizumab enabled the visualisation of VEGF target expression and thus bevacizumab biodistribution [23]. By labelling the bispecific T-cell engager (BiTE) AMG 211, researchers showed tracer uptake in gastrointestinal adenocarcinomas and CD3-rich lymphoid tissue, alluding to a role for molecular imaging in predicting potential toxicity from targeting physiologic tissues as well as off-target effects of novel immunotherapeutic drugs [24]. Imaging with ^{89}Zr -trastuzumab and ^{89}Zr -bevacizumab has been used to investigate the effect of heat shock protein 90 (HSP90) inhibition and everolimus treatment on the expression of molecular targets in patients with

metastatic breast cancer and metastatic renal cell carcinoma, respectively [25, 26]. Moreover, molecular imaging can be used for dosimetry of targeted drug therapy and radioimmunotherapy (RIT) [27, 28]. By quantitative imaging of targets like EGFR, ER, and androgen receptor (AR) molecular imaging has been used to identify treatment candidates for targeted therapies in NSCLC, metastatic breast cancer, and advanced head and neck cancer [29–32]. Finally, recent studies imaging ^{89}Zr -atezolizumab in a basket trial and ^{89}Zr -trastuzumab show that molecular imaging of molecular targets before immunotherapy can predict clinical response [33, 34].

The successes in molecular imaging of solid malignancies illustrate the potential that molecular imaging of treatment targets can have in the clinical context and provide a scaffold that could be adopted for future tracer development in hematology.

Caveats in molecular imaging and implications of tumour heterogeneity

Molecular imaging studies in solid malignancies have shown that tumour heterogeneity concerning specific molecular targets is prevalent (see above). Results from the current gold standard of histologic biopsy to determine molecular target expression are thus susceptible to sampling errors that might result in the erroneous selection or exclusion of candidates for receiving targeted therapy. This has obvious implications for future clinical practice [35]. In the future and after necessary validation studies, whole-body molecular imaging might add to existing diagnostic techniques as an inherently quantitative, fast, and non-invasive diagnostic tool that provides phenotypic information of all tumour lesions.

As shown in Chapters 2 and 3, heterogeneous uptake of ^{18}F -FDG is a widespread phenomenon in DLBCL and cHL. That should be kept in mind when discussing semiquantitative measures for prognostication, disease monitoring, and evaluating therapy response. This is especially important in lymphoma, in which the vast majority of patients present with several lesions rather than a single large tumour mass. With evident tumour heterogeneity, selecting a lesion that is representative of the entire disease process, and thus suited for disease monitoring, will likely remain a challenge for future molecular imaging studies. An interesting finding in Chapter 2 was the lack of correlation between the metabolic master-switch *MYC* and semiquantitative imaging parameters derived from ^{18}F -FDG uptake on PET scans. When critically appraising these findings, the complexity of biological processes and mechanisms controlling and influencing glycolysis in tumour cells and tissue might not be adequately described by relatively simplified measures

such as SUV, MTV, and TLG. This limits the interpretability and conclusions, which can be drawn from these imaging parameters. The better the biological and pathophysiological background of a molecular target is understood, and the more specific it is for tumour tissue, the better it is suited for molecular imaging. In this context, well-studied molecular targets that are only marginally present in physiological tissues, and especially those that are tumour-specific, offer a major advantage compared to ^{18}F -FDG PET.

Challenges to tracer development

The development of molecular tracers is complex and involves many diverse fields, including chemistry, pharmacology, hematology, nuclear medicine, radiologist, biological and physiological research, specialists in health technology assessment, and shareholders from pharmaceutical and technical industries [36]. Due to this complexity, a multidisciplinary approach, which integrates specialists of the respective field for each stage, greatly facilitates tracer development. Naturally, not all centres house such a broad range of resources and the need to share knowledge, experience, protocols, data, and experimental and fully developed tracers between research groups, departments, and institutes has been stressed previously [36].

As a proof of principle, promising new molecular tracers and targeting rationales can be investigated and assessed using animal models before clinical testing. The number of preclinical studies exploring molecular imaging within recent decades (see chapter 5) clearly shows the continued interest in molecular imaging of lymphoma. However, more effort should be invested in implementing these imaging strategies into clinical care and for patients' benefit. Raising awareness about the potential of molecular imaging could help recruit financial support from shareholders such as the pharmaceutical and technical industry, patient representatives, and through grants. Integrating molecular imaging into the drug development process (see future perspectives section) could help in understanding the pharmacological behaviour of these compounds, while at the same time alleviating the financial burden that molecular imaging research entails for investigator-initiated studies. Especially in the field of hematology, where studies frequently seek for novel application of old and new drugs, parallel or integrated imaging studies could provide novel insights and stimulate new collaborations between clinical investigators and the industry.

Radioimmunotherapy

Molecular imaging plays an essential role in the dosimetry for RIT. This therapeutic approach uses antibodies' specific targeting ability to deliver lethal payloads of radiation from isotopes such as ^{90}Y -ttrium, ^{131}I -iodine and ^{177}Lu -lutetium directly to tumour cells. By coupling the gamma ray emitting isotope ^{90}Y to a monoclonal anti-CD20 antibody, the resulting antibody-radionuclide compound (ARC) ^{90}Y -ibritumomab tiuxetan can be used in the treatment of B-NHL [37]. For the treatment of B-NHLs two radioimmunotherapy compounds, both targeting the B-cell marker CD20, ^{90}Y -ibritumomab tiuxetan and ^{111}In -tositumomab have received approval from EMA and FDA. However, RIT is dose-limited by prolonged myelosuppression, and most trials excluded patients with more than 25 % marrow involvement [38]. Despite convincing evidence of efficacy, anti-CD20 RIT has not found its way into the routine management of patients with B-NHL. Along with reimbursement problems and other economic issues, ^{111}In -tositumomab was discontinued in 2014 [39], leaving ^{90}Y -ibritumomab tiuxetan as the only FDA approved compound for RIT of lymphoma. Moreover, the necessary multidisciplinary approach, technical know-how, and financial requirements along with the simultaneous advent of immunotherapy, might have acted as culprits for the implementation of RIT. As Chapter 5 demonstrates, investigation and drug development of RIT compounds are on-going, including research projects investigating targeting CD37 with a ^{177}Lu -labelled monoclonal antibody for RIT in B-NHL [40, 41]. However, given its lack of clinical use, toxicity, and complicated implementation, it is unknown whether RIT can assert itself in clinical practice in an era of highly effective immunotherapeutic treatment modalities.

Liquid Biopsies

This thesis has illustrated the ways in which molecular imaging can and could be used to improve diagnostics, staging, and lymphoma treatment. By combining different tools for the molecular and biological characterization of lymphomas limitations of molecular imaging such as detection thresholds, visualization of only a single molecular target at a time and its technical and financial drawbacks might be compensated. In the last decade molecular analysis of the genetic makeup of malignancies derived from liquid biopsies have been tested for clinical application and might offer novel opportunities for monitoring lymphomas over time [42]. During tumour proliferation and progression, tumour cells that do not sufficiently adapt to the tumour microenvironment's ever-changing conditions (changes in pH, oxygen supply, host immune response, etc.) die regularly. In these abnormal cells, apoptosis mechanisms are frequently distorted, leading to the release of cellular

contents into the circulation in the course of their demise. Unrelated to necrotic tumour cell demise, DNA fragments have also been found in apoptotic and secretory vesicles. However, not only tumour cells release DNA fragments into the bloodstream. The sum of DNA that can be analysed from a so-called “liquid biopsy” is referred to as cell free DNA (cfDNA). Often only a fraction of cfDNA is derived from tumour cells, and thus is referred to as circulating tumour DNA (ctDNA). State of the art next-generation sequencing can be used to analyse the minute quantities of ctDNA to detect genetic signatures and alterations characteristic for malignancies [43]. In a retrospective analysis of patients with DLBCL, who received complete remission after treatment, the analysis of ctDNA identified patients with a high risk of recurrence on average 3.5 months before the relapse became clinically evident [44]. Analysing ctDNA can detect minimal residual disease in DLBCL and thus holds great potential for disease monitoring, and response guided therapy, especially since a consensus for the use of interim PET scans to guide treatment is still debated for these patients [45]. In cHL, ctDNA derived from HRS cells can be used for prognosticating patient outcome [46]. Furthermore, ctDNA profiling from blood samples taken before, during, and after therapy was able to identify patients relapsing after chemotherapy and those with partial remission maintained by immunotherapy, based on characteristic clonal evolution patterns [46]. As suggested by the authors, ctDNA analysis in cHL could be of benefit when integrated with molecular imaging to improve on inaccuracies of interim PET/CT scanning in this patient population [46].

An important limitation of liquid biopsy analyses is the amount of circulating tumour DNA present (also referred to as ctDNA burden), with factors such as tumour location, earlier treatment and disease burden influencing the amount of ctDNA available for analysis and thus affecting clinical application [47]. This makes the application of ctDNA analysis in the context of cancer screening with the goal to detect malignancies at the earliest stage possible, particularly ambitious. However, rapid development of sequencing technology in the past decade has significantly decreased the detection threshold and several large-scale studies such as SUMMIT (NCT03934866) and STRIVE (NCT03085888) are under way to investigate the utility of ctDNA based cancer screening [47]. Liquid biopsy besides ctDNA approaches that hold potential in the setting of lymphomas include the analysis of DNA derived circulating tumour cells and miRNAs [48].

Thus, liquid biopsies might in the future complement biological and genetic assessment, molecular imaging, and histopathological examination to better characterize, diagnose, monitor and potentially screen for lymphomas.

The financial cost of modern lymphoma treatment

The rate at which novel lymphoma treatments become available has increased tremendously after the introduction of immunotherapy [49, 50]. While some novel therapeutic approaches translated into a real survival benefit for some patients, those with biologically heterogeneous lymphoma entities, such as DLBCL, have not yet benefitted. Given this context, the efficacy of CAR-T cell therapy with about 45 % long-term remission in relapsed/refractory lymphoma has sparked patients' and clinicians' hopes. Despite the unmet therapeutic need in this patient category and the promising results in larger trials the significant cost of CAR-T cell therapy has been subject to criticism from public reimbursement institutions and patient representatives. In the Netherlands, CAR-T-cell therapy is available (registered and reimbursed) for relapsed/refractory DLBCL patients since June 2020. Similarly, recent results from clinical studies of the antibody drug conjugate polatuzumab vedotin in combination with bendamustine and rituximab in the setting of relapsed/refractory DLBCL have demonstrated a 58 % survival benefit (i.e. a hazard ratio of 0.42) compared to patients treated with bendamustine and rituximab alone [51]. Currently negotiations for the reimbursement of polatuzumab vedotin treatment are underway. However, the fact that pricing might restrict accessibility to highly effective treatment options is worrying. Here the essential role of regulatory authorities and governmental bodies becomes apparent. With the power to limit patents, set competitors against each other and even restrict market access for certain drugs these bodies try to limit health costs. Nevertheless, the expense of oncologic treatments has been ever rising, resulting in the promotion of generic alternatives for expensive, branded drugs. In chapter 6 we demonstrate how large the percentage of biosimilar use in the context of rituximab has grown in the Netherlands. A biosimilars' efficacy should be confirmed in large population-based studies, regardless of the reduction in annual expenditure that can be achieved. In our retrospective population-based study of Dutch patients with DLBCL the considerably cheaper rituximab biosimilar showed equivalent efficacy with rituximab originator.

To conclude, the recent development of increasingly active targeted compounds in the treatment of lymphoma has led to an increase in cost. Instruments to restrain health costs in lymphoma care include price regulation and reimbursement schemes, competition amongst pharmaceutical manufacturers, and the use of validated biosimilars. Of course, these biosimilar compounds' non-inferiority needs to be demonstrated and their efficacy validated on a population level before they can be recommended as viable alternatives. Another approach to limiting health

costs is by improving the identification of patients who might benefit from these targeted treatments. In the near future novel techniques such as liquid biopsies and novel applications of molecular imaging could facilitate and refine patient selection. The future perspectives section introduces two clinical studies that investigate molecular imaging as a tool to measure molecular target expression in patients, thus taking the first step towards identifying treatment candidates in the future.

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FUTURE PERSPECTIVES

The integration of molecular imaging with clinical trials

In Chapter 5, the potential of a theragnostic approach of integrating molecular imaging applications with clinical testing of novel immunotherapeutic compounds, is explored.

In the course of this PhD project, we designed two molecular imaging side-studies parallel to clinical trials testing immunotherapeutic compounds for the treatment of DLBCL. In this section, the concept of application of molecular imaging in the clinical setting will be illustrated by providing an overview of the scientific rationales and designs of these studies.

⁸⁹Zr-atezolizumab PET imaging for assessing PD-L1 expression in patients with DLBCL

Background

Patients with DLBCL with an IPI score > 2, have a high risk of tumour relapse even after achieving a metabolic complete remission with R-CHOP chemo-immunotherapy. Outcome after relapse is dismal [1]. To reduce relapses in this patient population, the HOVON 151 trial (NCT03463057), evaluates consolidation treatment with the monoclonal antibody atezolizumab after a complete metabolic remission has been achieved with R-CHOP. By binding to program death ligand 1 (PD-L1) atezolizumab blocks inhibitory signalling of programmed death 1 (PD-1), an immune checkpoint. The rationale of atezolizumab consolidation is to enhance an effective T-cell mediated immune response to tumour cells (by inhibiting PD-L1 mediated inhibitory signal).

The observed percentage of PD-L1 positive tumour cells in DLBCL cases ranges from 13 to 31% [2, 3]. While immunohistochemistry (IHC) for PD-L1 expression in tumour biopsies is used as a predictive marker in certain tumour types [4], this methodology has important limitations. The proposed cut-off value of minimum PD-L1 expression necessary for anti-PD-L1 immunotherapy to be considered as beneficial varies between malignancies [5]. Moreover, there is considerable variation in PD-L1 expression between biopsies from different tumour sites within a patient (intra-individual variation) [6], and within tumours lesions themselves (intra-tumour heterogeneity) [7]. Furthermore, patients with tumours without objective IHC PD-L1 expression have responded to anti-PD-L1 checkpoint inhibition therapy

[8, 9]. In addition, IHC assays for PD-L1 expression, vary concerning sensitivity and specificity, are not inherently quantifiable, and cannot reliably predict future responders [10, 11]. Therefore, PD-L1 expression assessed with IHC based on biopsy is susceptible to sampling errors and might not represent the entire tumour.

Molecular imaging offers the possibility to assess the biodistribution of monoclonal antibodies [12] noninvasively. For the visualization of full antibodies, the radio metal zirconium-89 (^{89}Zr) is an excellent candidate for radiolabelling, as its half-life ($t_{1/2} = 3.27$ days) is long enough to still have a PET signal after the time needed for the antibody to reach and accumulate in target tissues. In a small basket trial of patients with solid malignancies, the uptake of the ^{89}Zr -atezolizumab tracer by tumour tissue has been suggested as an independent prognosticator of tumour response to immune checkpoint inhibition with atezolizumab [12]. Using this experience in solid malignancies, the feasibility of imaging PD-L1 target expression can now be tested using an optimized imaging protocol. To achieve an optimal PET signal, a tracer dose consisting of unlabelled atezolizumab and ^{89}Zr -atezolizumab (in total 10 mg protein and 37 MBq) is administered 4 days before the ^{89}Zr -atezolizumab PET-scan.

Design

In this PD-L1 imaging study, parallel to the HOVON 151 study, a maximum of 20 DLBCL patients shall undergo a ^{89}Zr -atezolizumab PET scan at diagnosis (part A) and after standard therapy with R-CHOP (part B). Patients with a high-risk DLBCL (IPI score > 2) who achieve a complete remission with R-CHOP will then be treated with atezolizumab (HOVON 151). For patients in whom a relapse is suspected during or after atezolizumab therapy, a third ^{89}Zr -atezolizumab PET scan will be performed (Part C) (Figure 1).

Figure 1

This study is designed as a prospective observational multicentre trial that is conducted in the University Medical Centre Groningen (UMCG) and the Amsterdam University Medical Centre (AUMC).

Implications

PET scans with the novel tracer ^{89}Zr -atezolizumab enable the collection of data concerning the biodistribution of the monoclonal antibody atezolizumab and thus the expression of PD-L1 in patients with DLBCL. Together with molecular tumour characterization and the collection of circulating tumour DNA, the acquired data will contribute to a better understanding of PD-L1 expression in DLBCL patients and its dynamics during treatment. The results of this study will guide patient selection, response mechanisms, toxicity and the relation with minimal residual disease in future investigations of PD-L1 immune checkpoint inhibition.

^{89}Zr -brentuximab imaging to evaluate CD30-expression in relapsed/refractory DLBCL

Background

Approximately 10%–15% of patients with DLBCL exhibit primary refractory disease (no response or relapse within 3 months of therapy), and an additional 20% to 25% of patients relapse following initial tumour response to therapy [13]. With the vast majority of patients receiving rituximab during induction chemotherapy, only 15% of the relapse/refractory (R/R) patients achieve long-term survival [14]. High-dose chemotherapy and autologous stem cell transplant (ASCT) have provided the best chance of cure for patients with chemosensitive relapse [15]. No particular agent or regimen has demonstrated superiority over another in this setting [14]. However, the DHAP (dexamethasone, high-dose cytarabine, cisplatin) regimen followed by ASCT is the most commonly used salvage treatment for R/R DLBCL in the Netherlands.

CD30, encoded by the *TNFRSF8* gene, is a member of the tumour necrosis factor receptor superfamily of proteins. In contrast to the universal expression of CD30 in classical Hodgkin lymphoma (cHL) [16, 17], only 10–20% of primary DLBCL samples express CD30 as examined by IHC [18, 19]. There are no published data about the incidence of CD30 expression in relapsed DLBCL. An analysis of a Dutch pathology registry database found CD30 expression in 28% of patients with a first

relapse and 30 % of patients with 2 or more relapses (Prof. Dr. D. de Jong, 2015, personal communication). Targeting CD30 with the antibody-drug conjugate (ADC) brentuximab vedotin is efficacious as therapy for relapsed cHL and systemic anaplastic large cell lymphoma, both expressing CD30 [20–22]. In a small cohort of 48 heavily pre-treated patients with CD30-positive DLBCL, treatment with brentuximab vedotin lead to an overall response rate of 44 %, with 17 % of patients achieving CR [23]. This evidence led to the initiation of the HOVON 136 study. During this multicentre trial, patients with CD30-positive DLBCL will receive brentuximab-vedotin in addition to DHAP and ASCT to improve outcome.

Interestingly, CD30 expression, as assessed by IHC did not correlate with treatment outcome in the study mentioned above [23]. Other studies in T-cell NHL [24, 25] and B-NHL [26] support these findings. This implies phenomena such as tumour heterogeneity, drug uptake in the tumour microenvironment, immune modifying effects of targeting CD30 positive immune cells, or CD30 expression levels below the IHC detection threshold to play a role in CD30 targeting.

PET imaging of zirconium-89 (^{89}Zr) labelled brentuximab (^{89}Zr -brentuximab) could serve to evaluate whole-body CD30 expression in patients with DLBCL, and visualize antibody biodistribution and tumour heterogeneity. ^{89}Zr is the radioisotope that lends itself best for molecular imaging of antibodies. Its half-life of 78 h allows imaging even after longer waiting times, needed for adequate antibody accumulation and cellular internalisation in target tissues. Moreover, because of its longer half-life, ^{89}Zr lends itself for centralized production and distribution [27]. Based upon previous studies, the optimal moment of readout in PET imaging of 37 MBq (1 mCi) of ^{89}Zr labelled antibody lies between 3 to 7 days after tracer injection. For a PET study with an ADC, like brentuximab vedotin, the naked antibody is labelled, since radiolabelling of the complete compound could destabilize the resulting tracer. Given variable CD30 expression in DLBCL [18] we also expect varying levels of ^{89}Zr -brentuximab tumour uptake in patients with R/R DLBCL. Moreover, a study imaging the labelled naked antibody in patients later receiving the ADC, not only observed differences in tracer uptake between subjects but also heterogeneous uptake within individuals [28]. Therefore, we expect to observe tumour uptake of the tracer to varying between and within patients.

Aim

In this imaging study patients with R/R DLBCL will undergo PET scans with the novel ^{89}Zr -brentuximab tracer to assess biodistribution, tumour uptake and pharmacokinetic properties of brentuximab, before treatment with brentuximab vedotin in the HOVON 136 trial. Furthermore, we hypothesize that the results of this side-study, together with insights from the HOVON 136 trial, might be used to facilitate the identification of DLBCL patients that would benefit from CD30-targeted treatment with brentuximab vedotin.

Design

This prospective observational single-centre trial is conducted in the University Medical Centre Groningen (UMCG) in 2 parts:

Part 1: To establish the feasibility of ^{89}Zr -brentuximab PET imaging, a cohort of at least 5 patients with R/R DLBCL will undergo PET scans at 3 different time points (1, 4, and 7 days after tracer injection) (Figure 2). Two of those patients will only receive ^{89}Zr labelled brentuximab (1 Mbq, ~ 1 mg). In case the concentration of tracer in the circulation is insufficient, 3 patients will receive an additional cold brentuximab dose (~ 9 mg). Depending on the scan quality achieved during dose finding, the cohort will either be expanded for further optimization of the imaging protocol, or the study will proceed to part 2.

Part 2: In the second part of this imaging study, 15 patients with R/R DLBCL will undergo ^{89}Zr -brentuximab PET scans according to the optimized imaging protocol established in part 1 (Figure 3). Provided eligibility criteria are met, patients will be able to proceed to the HOVON 136 treatment trial, after the imaging study.

Figure 2

Part 1: Initial dose finding (minimum of 5 patients)

Figure 3

Part 2: Expansion phase with optimized ^{89}Zr -brentuximab imaging (15 pat.)

Implications

If PET scans with ^{89}Zr -brentuximab prove feasible, molecular imaging might allow the prediction of brentuximab vedotin biodistribution and the expression of CD30 in patients with DLBCL. Together with molecular characterization of tumour tissue obtained through biopsy and data on patient outcome, this study may contribute to a better understanding of CD30 targeting in patients with DLBCL. Furthermore, exploratory survival analysis might indicate whether CD30 expression, assessed with ^{89}Zr -brentuximab PET imaging, can be used for patient selection.

CONCLUDING REMARKS

Novel ways of interpreting ^{18}F -FDG PET derived data such as semiquantitative imaging parameters hold potential for prognostication in lymphoma. However, our results suggest that conclusions with regards to biology and clinical behaviour of lymphomas need to be drawn with caution, interpreted with regard to different subtypes, and validated in large-scale clinical studies. Current and future development in ^{18}F -FDG PET imaging might further promote the role of molecular imaging in diagnosis, prognostication, disease monitoring, and imaging guided therapy. In the context of novel modalities for targeted therapy new molecular tracers beyond ^{18}F -FDG could be used for visualizing molecular target expression in patients with the ultimate goal of selecting patient most likely to benefit from targeted treatment. Future developments in machine learning algorithms might improve medical imaging as a whole and reveal novel imaging-based prognosticators. Despite the great potential of molecular imaging, the future workup, prognostication, risk assessment and disease monitoring of

lymphoma will most likely see a combination of imaging with advanced molecular analysis techniques such as liquid biopsy analysis. Despite its efficacy in certain NHL entities, radioimmunotherapy has failed to establish its own niche in modern lymphoma treatment and the only available compound is currently outperformed by novel immunotherapeutic modalities such as CAR-T-cell therapy and bispecific antibodies. With the armamentarium of targeted immunotherapy for lymphoma steadily growing, biosimilars offer a realistic opportunity to limit the ever-increasing financial burden on modern health care systems. For these generic drugs to become embedded in the routine treatment of lymphoma, validation of their efficacy in large population-based studies is imperative.

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CHAPTER 8

Summary

SUMMARY

This thesis evaluated the impact of novel analysis techniques and the performance of the established molecular imaging modality ^{18}F -fluoro-2-deoxy-D-glucose positron emission tomography/computed tomography (^{18}F -FDG PET/CT) in the lymphoma subtypes diffuse large B cell lymphoma (DLBCL), classical Hodgkin lymphoma (cHL), and post-transplant lymphoproliferative disorder (PTLD), reviewed and identified past, present, and future efforts in the development of molecular imaging tracers in lymphoma, and examined the effectiveness of rituximab (R)-biosimilars after their widespread introduction into clinical practice in a Dutch population-based study.

Chapter 1 introduces the lymphoma subtypes discussed in this thesis and provides a short overview of lymphoma treatment and currently used risk assessment tools. It also briefly defines molecular imaging, summarizes its role in lymphoma care and point out its potential future applications.

MYC gene rearrangements in DLBCLs result in high proliferation rates and are associated with a poor prognosis. Strong proliferation is associated with high metabolic demand and tumour necrosis. In **chapter 2** we investigated the differences in the presence of necrosis and semiquantitative ^{18}F -FDG PET metrics between DLBCL cases with or without a *MYC* rearrangement. The prognostic impact of tumour necrosis on PET/CT scans and semiquantitative ^{18}F -FDG PET parameters was investigated in an explorative survival analysis. Fluorescence in situ hybridisation analysis for *MYC* rearrangements in patient material, visual assessment, semiquantitative analysis of ^{18}F -FDG PET scans and patient survival analysis were performed in 61 patients with DLBCL, treated at the UMCG between 2008 and 2015. Twenty-one (34%) of the 61 lymphomas had a *MYC* rearrangement. *MYC* status was neither associated with the presence of necrosis on ^{18}F -FDG PET scans (necrosis^{PET}; $P = 1.0$) nor associated with the investigated semiquantitative parameters maximum standard uptake value (SUV_{max} ; $P = 0.43$), single highest SUV_{max} ($P = 0.49$), metabolic active tumour volume (MATV; $P = 0.68$) or total lesion glycolysis (TLG; $P = 0.62$). A multivariate patient survival analysis of the entire cohort showed necrosis^{PET} as an independent prognostic marker for disease-specific survival (DSS) (HR: 13.9; 95% CI: 3.0–65; $P = 0.001$). In conclusion, *MYC* rearrangements in DLBCL do not influence the visual parameter necrosis^{PET} or the semiquantitative parameters SUV_{max} , MATV and TLG. Irrespective of *MYC* rearrangements, necrosis^{PET} is an independent, adverse prognostic factor for DSS.

Semiquantitative ^{18}F -FDG PET parameters have been proposed as prognostic markers in cHL. Since necrosis as assessed by ^{18}F -FDG PET or CT (necrosis^{visual}) correlates with an adverse prognosis in DLBCL, **chapter 3** investigated the incidence of tumour necrosis, its relation to semiquantitative ^{18}F -FDG PET metrics, and the prognostic impact of these imaging parameters in cHL. Out of the 87 patients with cHL treated with ABVD, (escalated) BEACOPP or CHOP chemotherapy between 2010 and 2017, 71 had both a NEDPAS/EARL accredited ^{18}F -FDG PET and a contrast-enhanced CT scan. Semiquantitative ^{18}F -FDG PET parameters were determined using Hermes Hybrid 3D software. Necrosis^{visual}, defined by photopenic tumour areas on ^{18}F -FDG PET and attenuation values between 10 and 30 Hounsfield units (HUs) on CT, was assessed blinded to outcome. Univariate Cox regression survival analyses of progression free survival (PFS) were performed. Necrosis^{visual} was observed in 18.3% of patients with cHL. Bulky disease (tumour mass > 10 cm in any direction) ($P = 0.002$) and total lesion glycolysis (TLG) ($P = 0.041$) but no other semiquantitative parameters were associated with necrosis^{visual}. In explorative univariate survival analysis for PFS the covariates internal prognostic score (IPS), bulky disease, metabolic tumour volume (MTV) and TLG were prognostic, while necrosis^{visual} was not.

PTLD is a serious complication after solid organ and hematopoietic stem cell transplantation, requiring a timely and accurate diagnosis. In **chapter 4**, we evaluated the diagnostic performance of ^{18}F -FDG PET/CT in patients with suspected PTLD and examined its correlation with lactate dehydrogenase (LDH) levels, Epstein Barr virus (EBV) load or timing of FDG PET/CT. This retrospective study included 91 consecutive patients with clinical suspicion of PTLD and a total of 97 ^{18}F -FDG PET/CT scans within an 8-year period. Pathology reports and a 2-year follow-up served as the reference standard. This study assessed the diagnostic performance of ^{18}F -FDG PET/CT to detect PTLD and used logistic regression analysis to identify factors that affect diagnostic yield. The diagnosis of PTLD was established in 34 patients (35%). Fifty-seven ^{18}F -FDG PET/CT scans (59%) were true negative, 29 (30%) were true positive, 6 (6%) false positive, and 5 (5%) false negative. The sensitivity PET/CT for the detection of PTLD was 85%, specificity 90%, positive predictive value 83%, and negative predictive value 92%, with good interobserver variability ($k = 0.78$). Of the parameters hypothesized to be associated with a true positive ^{18}F -FDG PET/CT result for the diagnosis of PTLD, only LDH was significant (OR: 1.03, $P = 0.04$). ^{18}F -FDG PET/CT has a good diagnostic performance in patients suspected of PTLD, with a good inter-observer agreement. Only LDH levels seemed to influence the detection performance of ^{18}F -FDG PET/CT. EBV-DNA load

and timing of ^{18}F -FDG PET/CT after transplantation did not affect ^{18}F -FDG PET/CT diagnostic yield of ^{18}F -FDG.

With improved understanding of the molecular processes in lymphoma and novel treatment options, arises a growing need for the molecular characterisation of tumours. Molecular imaging with single-photon-emission CT and PET using specific radionuclide tracers can provide whole-body information to investigate cancer biology, to evaluate phenotypic heterogeneity, to identify resistance to targeted therapy, and to assess the biodistribution of drugs in patients. In **chapter 5**, we evaluated the existing literature on molecular imaging in lymphoma, other than ^{18}F -FDG molecular imaging. The aim was to examine the contribution of molecular imaging to the understanding of lymphoma biology and to discuss potential implications for the diagnostics and therapy of these diseases. Finally, we discuss possible applications for molecular imaging of patients with lymphoma in the clinical context.

In 2017, the European Medicines Agency approved rituximab biosimilars (R-biosimilars) for treatment of diffuse large B-cell lymphoma (DLBCL). Thereafter, the Netherlands was one of the first countries to implement R-biosimilars, given lower costs as compared to rituximab originator (R-originator). In **chapter 6** we investigated whether overall survival (OS) of patients with DLBCL receiving R-biosimilars is similar to patients treated with R-originator. DLBCL patients ≥ 18 years, diagnosed between 2014 and 2018, who received at least one cycle of rituximab combined with cyclophosphamide, doxorubicin, vincristine, and prednisolone (R-CHOP) were identified in the Netherlands Cancer Registry. Patients were categorized into R-originator or R-biosimilars groups based on data from a central repository of the Dutch medicinal drug market. The primary endpoint was 3-year OS, defined as the time between diagnosis and all-cause death. By the end of 2018, 91% of purchased rituximab were biosimilars. In total, 4,429 patients were identified with 876 in the R-biosimilars group and 3,553 in the R-originator group. Patients in the R-biosimilars group less frequently received > 6 cycles of R-CHOP as compared to patients treated with R-originator (24 % vs. 30 %, $P = 0.003$). The 3-year OS did not differ between patients treated with R-originator or R-biosimilars (73 % vs. 73 %, $P = 0.855$). This was confirmed with a multivariable Cox regression analysis accounting for sex, age, IPI-score and number of R-CHOP cycles. In conclusion, the 3-year OS is similar for patients treated with CHOP in combination with R-originator or R-biosimilars and, therefore, favors the use of R-biosimilars in DLBCL treatment management.

APPENDIX

NEDERLANDSE SAMENVATTING

Jaarlijks wordt bij zo'n 4500 mensen in Nederland de diagnose lymfeklierkanker (lymfoom) gesteld. Hiermee behoren deze tumoren tot de 10 meest voorkomende soorten van kanker. Lymfeklierkanker is een overkoepelend begrip voor een heterogene groep van ziektebeelden die ontstaan uit een maligne onttaarding van rijpe B-cellen of T-cellen op verschillende momenten van de normale ontwikkeling. Er worden ongeveer 70 verschillende subtypes onderscheiden.

In dit proefschrift worden 3 soorten lymfoom onderzocht. De oudste bekende vorm van lymfoom betreft het relatief weinig voorkomende Hodgkin lymfoom (HL). Deze vorm is vernoemd naar zijn ontdekker Thomas Hodgkin, die het ziektebeeld in 1832 heeft beschreven. Kenmerkend voor het HL is dat het voor een kleine deel (1–10 %) bestaat uit tumorcellen en verder uit ontstekingsinfiltraat. De overige vormen van lymfklierkanker worden non-HL (NHL) genoemd. Ze ontstaan voor het grootste deel uit B-cellen (rond 90 %), met als meest frequente type het diffuus grootcellig B-cel lymfoom (DLBCL). Kenmerkend voor het DLBCL zijn de grote B-cellen die diffuus voorkomen in de tumor. Het DLBCL wordt gerekend tot de agressieve vormen van lymfklierkanker. Recidieven na eerstelijnsbehandeling hebben een slechte prognose. Een specifieke vorm van lymfklierkanker is het post-transplantatie lymfoom (PTLD). Dit type lymfeklierkanker ontstaat doordat bij patiënten met een transplantaat het afweersysteem onderdrukt wordt met medicatie. Door deze medicatie kan het afweersysteem beschadigde B-cellen niet meer herkennen en kunnen deze B-cellen zich ontwikkelen tot een lymfoom. Volwassenen die een orgaantransplantatie hebben ondergaan hebben een risico van 1 tot 2 % om een PTLD te ontwikkelen.

Het laatste decennium is er veel kennis vergaard over de biologie en ontstaanswijze van de verschillende soorten lymfklierkanker. De uitdaging waar we voor staan is om deze nieuwe inzichten te vertalen naar een betere behandeling. Er zijn verschillende mogelijkheden om een verbeterslag te maken.

Allereerst lijkt het in de toekomst potentieel mogelijk om al bij diagnose hoog-risico patiënten te herkennen die baat kunnen hebben bij nieuwe behandelingen. Tegelijkertijd zou het helpen om bij diagnose patiënten met een goede prognose te kunnen identificeren. Voor deze patiënten is wellicht de-escalatie mogelijk van toxische therapie.

Ten tweede zou het nuttig zijn om in een vroeg stadium van behandeling patiënten te kunnen identificeren die falen op therapie.

Ten derde kan er mogelijk een verbetering plaatsvinden door nieuwe medicijnen in te zetten op basis van (specifieke) tumoreigenschappen. Hierbij moet uitgezocht worden welke patiënten het meest profiteren van een specifieke therapie. Zorgvuldige selectie van gerichte therapiemogelijkheden zal behandelingen ook het meest kosteneffectief maken.

In dit proefschrift wordt onderzoek beschreven waarin gekeken is naar een relatie tussen biologische tumorkarakteristieken en parameters afgeleid van ^{18}F -fluor-2-deoxy-D-glucose positronenemissie-tomografie/computertomografie (^{18}F -FDG PET/CT) scans in patiënten met een DLBCL en HL. Er is ook onderzocht hoeveel ^{18}F -FDG PET/CT scans bijdragen aan de diagnose en behandeling van patiënten met een PTLD is. Naast de ^{18}F -FDG PET/CT studies, wordt een overzicht gegeven over eerdere, huidige en toekomstige mogelijkheden van moleculaire beeldvorming. Tenslotte is de effectiviteit van rituximab biosimilars in de behandeling van DLBCL in Nederland onderzocht.

^{18}F -FDG PET bij lymfomen

^{18}F -FDG PET is een manier van moleculaire beeldvorming waarbij gekeken wordt naar de distributie van radioactief gelabeld glucose (^{18}F -FDG). ^{18}F -FDG wordt in de cel opgenomen, maar kan vervolgens niet gemetaboliseerd worden. Hierdoor stapelt het zich op in weefsels die veel glucose gebruiken. Aangezien lymfomen veelal metabool actief zijn, dus veel glucose gebruiken, kan een ^{18}F -FDG-PET scan de distributie van lymfoomlocalisaties in het lichaam laten zien. Vaak combineert men ^{18}F -FDG-PET met een computertomografie (CT) scan (^{18}F -FDG PET/CT) teneinde de anatomische ligging van de weefsels die ^{18}F -FDG opnemen te correleren met de betreffende anatomische structuur.

^{18}F -FDG-PET/CT scans worden tegenwoordig routinematig gebruikt bij de stadiëring, respons evaluatie en follow-up van patiënten met lymfklierkanker. Stadiëring betekend dat voor de start van behandeling in kaart gebracht wordt hoe uitgebreid de lymfeklierkanker bij een patiënt aanwezig is. Bij responsevaluatie wordt tijdens en na de behandeling gekeken in hoeverre het lymfoom op de therapie gereageerd heeft. Tijdens follow up na behandeling wordt de patiënt regelmatig poliklinisch gecontroleerd en kan er bij verdenking op ziekteactiviteit een nieuwe ^{18}F -FDG-PET/CT scan worden verricht. Er zijn ook lymfoomsubtypes die

vanwege hun langzame groei weinig ^{18}F -FDG opnemen waardoor zij niet op een PET scan afgebeeld worden. Verder zijn er zeldzame ziektebeelden zoals PTLD waarvoor de diagnostische waarde van ^{18}F -FDG PET nog helemaal niet in kaart is gebracht.

In de afgelopen jaren is er veel onderzoek gedaan om de rol van ^{18}F -FDG-PET/CT voor therapie aanpassing in kaart te brengen. Indien aangetoond kan worden dat eventuele therapie aanpassing op basis van een ^{18}F -FDG-PET/CT scan gewenst is, dan zou dat kunnen helpen bij het geven van de meest effectieve behandeling met beperkte toxiciteit. In HL kunnen ^{18}F -FDG-PET/CT scans inmiddels gebruikt worden voor de-escalatie van behandeling naar minder toxische chemotherapiekuren. Bij onvoldoende respons kan escalatie naar effectievere en toxischere behandeling plaatsvinden.

Het is nog niet bekend of bepaalde karakteristieken van een ^{18}F -FDG PET scan overeenkomen met bekende biologische biomarkers. De laatste tijd probeert men met nieuwe gegevens die afgeleid worden van de mate van ^{18}F -FDG stapeling op PET scans (semikwantitatieve parameters) de lymfomen en respons beter in kaart te brengen. In dit proefschrift hebben we onder ander de relatie van bekende tumorkarakteristieken en nieuwe parameters afgeleid uit ^{18}F -FDG PET scans, in verschillende soorten lymfeklierkanker onderzocht.

MYC en ^{18}F -FDG in DLBCL

In DLBCL wordt in 10 % van de gevallen een breuk geconstateerd in het *MYC*-gen. De *MYC*-herschikkingen geven de tumorcellen een sterk groei voordeel en leiden tot een hoge celdeling (proliferatie). DLBCL met een *MYC*-breuk hebben daarom een slechte prognose. Een hoge proliferatie wordt in verband gebracht met een grote energiebehoefte en dus een hoog suiker verbruik. Tegelijkertijd kan een sterke proliferatie aanleiding geven tot tumornecrose omdat aanvoer van voeding voor de tumor de groei niet kan bijhouden. In **hoofdstuk 2** onderzochten we de verschillen in semikwantitatieve ^{18}F -FDG PET parameters en tumornecrose in patiënten met een DLBCL met en zonder een *MYC*-herschikking. De aanwezigheid van *MYC*-herschikking, dat invloed op de metabole fenotype van DLBCL heeft, had evenwel geen relatie met de semikwantitatieve ^{18}F -FDG PET parameters. Wel vonden wij dat, ongeacht de *MYC*-herschikking, de aanwezigheid van tumornecrose op een ^{18}F -FDG-PET scan een onafhankelijke, ongunstige prognostische factor voor ziekte specifieke overleving was.

Tumor necrose en ^{18}F -FDG in HL

Ook in HL worden semikwantitatieve ^{18}F -FDG PET-parameters door onderzoekers als prognostische markers voorgesteld. Aangezien necrose op ^{18}F -FDG PET of CT (necrose^{visueel}) met een ongunstige prognose voor patiënten met in DLBCL correleert, onderzochten wij in **hoofdstuk 3** de incidentie van tumornecrose en de relatie met semikwantitatieve ^{18}F -FDG PET parameters. Bij 18 % van de 71 patiënten met HL was necrose aantoonbaar. Van de semikwantitatieve parameters was de totale laesie glycolyse, het volume van alle tumorlaesies en hun glucose gecombineerd, geassocieerd met necrose. Tumornecrose was echter niet prognostisch in HL. Bekende waardes zoals de IPS, laesiegrote van >10 cm en ook nieuwe parameters zoals het totale tumor volume en totale laesie glycolyse waren wel prognostisch.

^{18}F -FDG PET in PTLD

In patiënten met een PTLD is een tijdige en nauwkeurige diagnose soms lastig te stellen. In **hoofdstuk 4** evalueerden we de diagnostische waarde van ^{18}F -FDG PET/CT bij patiënten met verdenking op PTLD en onderzochten we de correlatie met serum lactaat dehydrogenase (LDH) concentraties, Epstein Barr virus (EBV)-load in het bloed en het tijdstip van ^{18}F -FDG PET/CT scans. Alleen een verhoogde LDH-concentratie was positief gecorreleerd met de diagnostische opbrengst van ^{18}F -FDG PET/CT scan. De hoogte van de EBV-load en het tijdstip waarop de ^{18}F -FDG PET/CT na transplantatie werd uitgevoerd hadden geen invloed op de diagnostische opbrengst. In deze studie had een normale ^{18}F -FDG PET/CT een hoge negatief voorspellende waarde voor de aanwezigheid van PTLD.

Moleculaire beeldvorming in lymfomen met specifieke radionuclide-tracers

Door een beter begrip van de moleculaire processen in lymfomen en nieuwe therapie opties, ontstaat er een groeiende behoefte aan niet invasieve moleculaire karakterisering. Met behulp van specifieke radionuclide-tracers kan moleculaire beeldvorming op basis van uitstraling van enkelvoudige fotonen (single photon emission computed tomography = SPECT) en positronen (positron emission tomography = PET), informatie over het hele lichaam opleveren. Deze data kunnen gebruikt worden om niet invasief de kankerbiologie te onderzoeken, fenotypische heterogeniteit te evalueren, resistentie tegen gerichte therapieën te identificeren en de biologische distributie van geneesmiddelen bij patiënten te beoordelen. In **hoofdstuk 5** hebben we de bestaande literatuur over moleculaire beeldvorming, anders dan ^{18}F -FDG PET/CT, bij patiënten met lymfomen geëvalueerd. Het

doel was om de bijdrage van moleculaire beeldvorming aan het begrip van lymfoombiologie te onderzoeken en mogelijke implicaties voor de diagnostiek en therapie van deze ziekten te bespreken. Verder werden mogelijke toepassingen voor moleculaire beeldvorming van patiënten met lymfoom in de klinische context besproken.

Zoals in **hoofdstuk 5** en **7** geïllustreerd, kunnen nieuwe tracers, naast ^{18}F -FDG, mogelijk van waarde zijn in de context van ontwikkeling van geneesmiddelen en bij patiëntenselectie voor gerichte therapie. Nieuwe meer gerichte tracers kunnen relevante biologische informatie van het betreffende lymfoom opleveren waardoor patiënten mogelijk geselecteerd kunnen worden, die het meeste baat kunnen hebben bij behandelingsopties die gebruikmaken van deze fenotypische tumoreigenschappen. Wel moet de transitie van preklinische ontwikkeling van nieuwe tracers na klinische implementatie nog verbeterd worden opdat zorgvuldige studies kunnen gebeuren voordat de precieze rol van deze nieuwe analyse-technieken voor patiënten helder is.

Effectiviteit van rituximab biosimilars bij DLBCL

Een biosimilar is een biologisch geneesmiddel dat heel erg lijkt op een eerder reeds goedgekeurde biologische medicijn en daardoor een gelijk werkingsmechanisme heeft. Geregistreerde biosimilars zijn dus versies van zogenoemde originator geneesmiddelen. In 2017 keurde het Europees Geneesmiddelenbureau rituximab (R)-biosimilars goed voor de behandeling van DLBCL. Nederland was een van de eerste landen die R-biosimilars in de kliniek implementeerde. In **hoofdstuk 6** onderzochten we of de 3-jaars overleving van Nederlandse patiënten met DLBCL die R-biosimilars kregen, vergelijkbaar is met die van patiënten die met R-originator behandeld werden. De 3-jaars overleving in patiënten die worden behandeld met CHOP in combinatie met R-originator of R-biosimilars was vergelijkbaar. Dit bevestigt de gelijkwaardigheid van R-biosimilars voor de behandeling van DLBCL.

Toekomstige ontwikkelingen en studies met moleculaire beeldvorming in lymfomen

Een van de mogelijke manieren om de transitie van preklinisch onderzoek naar toepasbare beeldvorming in patiënten te verbeteren is de zogenoemde theragnostische methode die moleculaire beeldvorming met klinische trials voor gerichte therapieën verbindt.

In de loop van dit promotietraject hebben we twee imaging studies op het gebied van moleculaire beeldvorming ontworpen. Deze studies lopen parallel aan klinische studies die immuun-therapeutische behandelingen voor DLBCL onderzoeken. **Hoofdstuk 7** illustreert het concept van de toepassing van moleculaire beeldvorming in de setting van klinische studies.

Binnen de HOVON 151 en HOVON 152 studies worden patiënten met een DLBCL en hooggradig B-cel lymfoom met een *MYC*-breuk en een *BCL2*- of *BCL6*-breuk (HGBCL-DH) behandeld met een immuuncheckpointremmer. Ter evaluatie van de PD-L1 expressie bij diagnose en follow-up wordt in de PD-L1-imaging studie (NCT03850028) bij patiënten met een DLBCL de biodistributie van de anti-PD-L1 immuuncheckpointremmer atezolizumab in kaart gebracht met sequentiële PET scans met zirkonium-89 gelabeld atezolizumab (^{89}Zr -atezolizumab).

In een tweede, geplande imaging studie zullen patiënten met een recidief of refractair DLBCL PET-scans ondergaan met de nieuwe ^{89}Zr -brentuximab-tracer om de biodistributie van deze tracer, de tracer opname door lymfoomlaesies en de farmacokinetische eigenschappen van brentuximab te beoordelen. Na de ^{89}Zr -brentuximab PET scan zullen patiënten binnen de HOVON 136-trial met brentuximab vedotin worden behandeld. De resultaten van dit onderzoek, samen met inzichten verkregen in het HOVON 136-onderzoek, kunnen bijdragen aan de identificatie van die DLBCL-patiënten die baat hebben bij CD30-gerichte behandeling met brentuximab vedotin.

Afsluitende gedachten

De snelle ontwikkeling van nieuwe behandelingsmethoden en de steeds grotere kennis over de biologische processen in lymfomen bieden veel mogelijkheden voor verder onderzoek. Nieuwe manieren om data van routine ^{18}F -FDG-PET scans te interpreteren en nieuwe methodes zoals liquid tumorbiopsieën zouden in de toekomst een impact op de diagnostiek en het vervolgen van ziekteactiviteit kunnen hebben. Naast ^{18}F -FDG zijn er een veelvoud aan moleculaire tracers die gebruikt zouden kunnen worden om in vivo informatie over het hele lymfoom in kaart te brengen. Al deze nieuwe ontwikkelingen vereisen dat resultaten juist geïnterpreteerd en gevalideerd worden om te zorgen dat middelen zorgvuldig ingezet worden om de zorg betaalbaar te houden. Rituximab-biosimilars zijn belangrijk om de kosten binnen de lymfoomzorg te beperken.

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