Connecting the dots in classical Hodgkin Lymphoma

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Connecting the dots in classical Hodgkin Lymphoma

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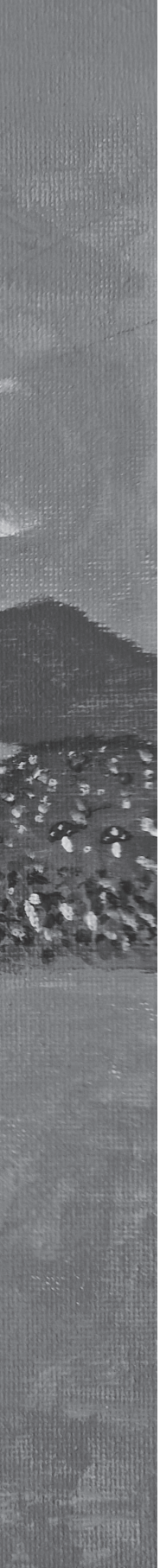

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General introduction

GENERAL INTRODUCTION

Classical Hodgkin Lymphoma

Classical Hodgkin Lymphoma (cHL) is the most common lymphoma subtype in adolescents in the Western world, and accounts for about 15% of all lymphomas.[1] cHL is a heterogeneous malignancy derived from germinal center B-cells, characterized by a low number of Hodgkin and Reed-Sternberg (HRS) cells within a reactive tumor microenvironment (TME) abundant in immune cells.[2] The disease is named after Thomas Hodgkin, who described several cases of an illness characterized by lymphadenopathy and hepatosplenomegaly in 1832.[3] In the late 1890s to early 1900s, Dorothy Reed and Carl Sternberg unveiled the presence of large, predominantly double-nucleated HRS cells within biopsies of enlarged lymph nodes.[4] Their discovery played a pivotal role in dispelling the belief within the medical community of that era that Hodgkin's disease was a form of tuberculosis, due to shared symptoms like night sweats, weight loss, fever, and lymphadenopathy.[5] This eventually led to the recognition of cHL as a malignancy.[2,3,5] Over the past years, cHL has undergone a transformative journey in terms of understanding its biology, classification and therapeutic strategies.[6-12] These advancements have translated into outstanding outcomes for the majority of patients undergoing contemporary treatment.[11-15] Current challenges in cHL management are primarily centered around limiting toxicity by optimizing treatment strategies to reduce late adverse effects in the first-line setting, and around enhancing outcomes in the relapsed/refractory (R/R) setting.

Epidemiology

cHL has an incidence rate of approximately 3 per 100,000 persons in Western countries.[1,16] The disease is one of the more common cancers occurring in adolescent and young adults aged 15-40 years.[17] Of note, cHL has a bimodal age distribution with two peaks, namely among young (15-35 years) and older adults (>60 years).[18] Overall, the 5-year relative survival rate is around 80%.[13,19] However, for patients who do not respond to first-line treatment or who experience relapse, the chance of cure with conventional salvage treatment and autologous stem cell transplantation (ASCT) is only 40-60%, and it is particularly disappointing in patients who are primary refractory to, or relapsing early after first-line treatment.[20-24]

Pathogenesis and Tumor microenvironment

There has been a long debate about the origin of the HRS cell due to its immunophenotype, which deviates significantly from that of any known immune cell type. HRS cells are almost always of germinal center B-cell origin, but show loss of typical B-lineage markers such as CD20, CD79a and the B-cell receptor, with aberrant expression of CD30, MUM1 (IRF4), CD15, weak expression of PAX5, and lack of CD45.[2,6,25] Therefore, these HRS cells are sometimes called 'crippled' B cells. The non-classical Nodular Lymphocyte Predominant Hodgkin Lymphoma (NLPHL) has distinct biological features such as the expression of CD20 and the absence of CD30.[2]

Despite its classification within the Hodgkin lymphoma family, its characteristics are more akin to indolent B-cell non-Hodgkin lymphoma.[2] Therefore, this thesis focusses exclusively on cHL.

cHL is morphologically classified into several subtypes based on the composition of the TME and characteristics of the HRS cells. Nodular sclerosis (NS) is the most common subtype (about 80% of cases), characterized by large HRS cells surrounded by small lymphocytes, eosinophils and histiocytes, a nodular growth pattern and sclerotic bands. Mixed cellularity (MC, about 15% of cases) is characterized by a diffuse growth pattern, relatively smaller binucleated HRS cells with a mixed inflammatory background, and more often EBV positivity.[26] Lymphocyte-depleted cHL is relatively rare (<5% of cases) and is characterized by a paucity of lymphocytes and a higher percentage of HRS cells, in contrast to lymphocyte-rich (<5%) cHL which has a diffuse growth pattern with a background rich in lymphocytes.[2,27]

Genetic analysis has revealed that HRS cells carry immunoglobulin (Ig) heavy and light chain gene rearrangements and somatic mutations in the Ig genes, which is an indicator of somatic hypermutation, thus confirming the germinal center B-cell origin of HRS cells.[28] Mechanisms inducing malignant transformation are largely unknown, but probably involve escape from programmed cell death since non-functional or destructive mutations in the Ig gene rearrangements, that are expected to induce apoptosis of germinal center B cells, are frequently present in HRS cells.[6,29] HRS cells universally express CD30, a transmembrane glycoprotein also known as tumor necrosis factor receptor superfamily member 8 (*TNFRSF8*), which is considered a hallmark of cHL.[2] The CD30 receptor can be targeted with brentuximab vedotin (BV), a monoclonal antibody-drug conjugate.[30,31] CD30 is involved in proliferation, survival and apoptosis, mainly through activation of NF-κB and JAK-STAT pathways.[32] HRS cells show constitutive activation of these pathways through CD30 signaling, but also by genetic mutations. Genetic analysis is hampered by the low percentage of HRS cells in biopsies, but analysis on microdissected HRS cells revealed frequent mutations in the NF-κB and JAK-STAT pathways, with amplification of *JAK2* in some cases, contributing to the activation of these pathways.[33,34] Constitutive activation of the NF-κB pathway is known to cause upregulation of anti-apoptotic genes, which likely contributes to escape from apoptosis during the germinal center reaction.[33] The JAK-STAT pathway is a main player in the cytokine signaling by HRS cells, which is an important factor in attracting immune cells to the TME and in shaping the function of these cells.[34] Furthermore, approximately 75% of patients show copy number gains and amplifications of *programmed death ligand-1* (*PD-L1*) and *PD-L2* genes in HRS cells.[35] PD-L1 and PD-L2 bind to PD1, which impedes the activation of immune cells expressing PD1, such as cytotoxic T cells. This can be targeted through the use of anti-PD-L1 immune checkpoint inhibitors.[35,36] In contrast to normal B cells, HRS cells express CD137 (*TNFRSF9*), which is transferred to nearby HRS and antigen-presenting cells expressing CD137 ligand (*TNFSF9*) through trogocytosis.[37] This results in the internalization of the CD137-CD137L complex, causing disappearance of CD137L on the surface of these cells. Consequently, this diminishes co-stimulation of T cells and reduces IFN-γ

release. Another mechanism of immune evasion involves mutations in β-2-microglobulin, which inhibit the recognition of HRS cells by CD8+ T cells by abrogating MHC class I expression.[38]

The Epstein-Barr virus (EBV) genome is identified in 30-50% of cases worldwide, with a lower percentage in western countries of about 20-30%.[26] EBV positive cHL is reflected by expression of the EBV derived proteins EBNA1, LMP1 and LMP2. Mutations in genes of the NF-κB pathway are less prevalent in EBV positive cHL, while LMP1, which is a strong NF-κB activator, is highly activated in most of these cases.[39] It is thought that EBV causes virally driven malignant transformation of B cells in the face of a failing immune response, as the viral genomes in these cases are monoclonal, and EBV-specific T cells are frequently found in EBV positive patients.[26]

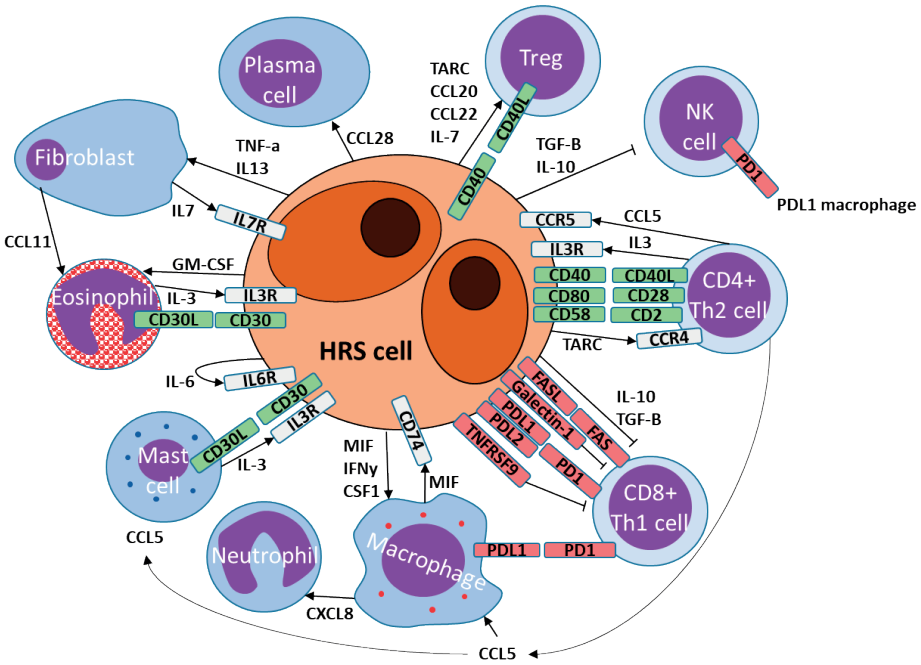

Figure 1. Tumor microenvironment and immune evasion mechanisms in cHL. This figure shows the main interactions between HRS cells and immune cells in the TME. HRS cells secrete several cytokines and chemokines that recruit CD4+ Th2 cells, macrophages, mast cells, eosinophils, fibroblasts, and plasma cells. In addition, immune evasion mechanisms are shown that mainly inhibit the function of CD8+ Th1 cells and NK cells and promote Tregs.

HRS cells secrete various cytokines and chemokines that recruit reactive immune cells to the TME.[40] The TME is primarily composed of CD4+ T helper (Th) cells, alongside regulatory T cells (Tregs), CD8+ cytotoxic T cells, eosinophils, macrophages, plasma cells, neutrophils, NK cells, B cells and mast cells [**Figure 1**].[27,41] The extensive immune infiltrate appears to lack the ability to mount an effective immune response against HRS cells. Instead, the CD4+ T cells seem to paradoxically support the survival of HRS cells and induce evasion of attacks by cytotoxic T

cells.[27] Approximately 90-95% of cHL patients have elevated serum levels of Thymus and Activation Regulated Chemokine (TARC, also known as CCL17), which is secreted by HRS cells.[42] Immunohistochemistry staining of TARC and CD30 is shown in **Figure 2**. Its expression by HRS cells is significantly elevated compared to its normal physiological expression in dendritic and thymus epithelial cells.[42,43] TARC can therefore be used as a tumor cell specific marker, and elevated TARC levels can be detected years before actual diagnosis of cHL in the peripheral blood.[44] In newly diagnosed cHL patients, serum TARC levels have been shown to correlate with disease stage and response to treatment.[45,46] TARC attracts CD4+ T cells by binding to their CCR4 receptor, which causes the formation of T cell rosettes around HRS cells.[41] This may protect HRS cells from attacks by cytotoxic T cells and natural killer (NK) cells.[40,41] Several other cytokines contribute to this immune evasion, such as CCL20, which mainly recruits Tregs that suppress the activation of CD4+ T cells, CD40 that binds to CD40 ligand on CD4+ T cells and interleukin (IL)-7 which enables differentiation of naïve CD4+ T cells into Tregs.[27,47,48] Moreover, HRS cells suppress a Th1 response by the production of anti-inflammatory cytokines IL-10 and TGF-β, and the expression of FAS ligand and galectin-1, that induce apoptosis of cytotoxic T cells.[27] HRS cells produce several autocrine growth factors, such as IL-6, of which the secretion is stimulated by the presence of CD30L expressed on eosinophils and mast cells.[49] Furthermore, paracrine growth factors contribute to HRS cell proliferation, for example CCL5, which is a chemokine produced by T cells that binds to the CCR5 receptor on HRS cells.[50]

Macrophages are also abundant in the TME, with M2 macrophages predominating, characterized by expression of CD163 and associated with promoting tumor growth. Studies have shown that macrophages in cHL often upregulate PD-L1, which contributes to the suppression of PD1+ T cells.[51] This could possibly explain the adverse prognosis associated with the presence of CD68+ tumor-associated macrophages.[52,53]

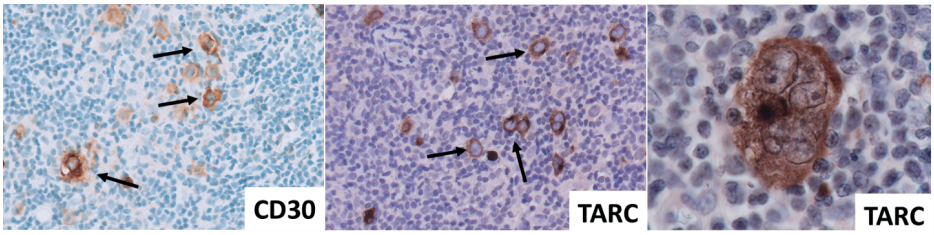

Figure 2. Immunohistochemistry staining of TARC and CD30 in lymph node biopsies of cHL patients. Arrows indicate HRS cells.

In conclusion, the interplay between HRS cells and the TME plays a pivotal role in the pathogenesis of cHL. Characterized by the unique immunophenotype of HRS cells and the diverse immune cell composition within the TME, cHL demonstrates complex mechanisms of immune evasion.

Clinical presentation and diagnosis

Patients with cHL mainly present with lymphadenopathy that is often located in the cervical and mediastinal regions.[54] Patients often present with fatigue and pruritus, and some patients experience alcohol-induced pain in enlarged lymph nodes.[55] Indicators of more aggressive disease are the presence of B symptoms, which include unexplained weight loss, night sweats and fever. Diagnosis relies on biopsy of a suspected lymph node or organ (e.g. spleen, bone marrow). Due to the low ratio of HRS cells to non-tumor cells and the need for architectural information an excisional or large incisional biopsy is required at diagnosis.[56] Laboratory tests may reveal anemia, elevated erythrocyte sedimentation rate (ESR) and elevated lactate dehydrogenase (LDH). Staging is conducted through assessment of an ^{18}F-fluorodeoxyglucose (FDG)-positron emission tomography (PET)-computed tomography (CT) scan, which is also used for interim and end-of-treatment response assessment and detection of recurrent disease during follow-up. Staging is standardized according to the Ann Arbor staging system, which is based on the number of involved lymph node stations, disease involvement on one or both sides of the diaphragm, and presence of extranodal disease [**Table 1**]. Additionally, the presence of B symptoms is denoted by adding "B" to the stage (e.g., IIB for stage II with B symptoms).[54,57] Response evaluation is based on PET-CT scans after a number of treatment cycles and is based on the visual Deauville criteria, but may also be measured quantitatively by the reduction in standard uptake value (SUV) of affected lymph nodes [**Table 2**].[54]

Table 1. Ann-Arbor staging criteria for classical Hodgkin lymphoma

Stage	Lymph node involvement	Extranodal status
Limited		
I	One single lymph node region	One single extranodal lesion without nodal involvement (IE)
II	Two or more lymph node regions on the same side of the diaphragm	Localized involvement of an extralymphatic organ or site plus its regional lymph nodes, with or without involvement of other lymph node regions on the same side of the diaphragm (IIE)
Advanced		
III	Involvement of lymph node regions on both sides of the diaphragm	Localized involvement of an extralymphatic organ or site (IIIE), spleen (IIIS), or both (IIIE+S).
IV	Disseminated involvement of one or more extralymphatic organs, with or without associated lymph node involvement or isolated extralymphatic organ involvement with distant (non-contiguous) regional lymph node involvement.	-

Table 2. Deauville score for response assessment on the PET-CT

Deauville score	
Complete metabolic response (CMR)*	
1	No uptake above background
2	Uptake ≤ mediastinal blood pool
3	Uptake > mediastinal blood pool but ≤ liver
Partial metabolic response (PMR)	
4	Uptake moderately increased compared to the liver at any site.
5	Uptake markedly increased compared to the liver at any site.
X	New areas of uptake unlikely to be related to lymphoma.

*CMR is commonly defined as DS 1-3, however in specific clinical trials, CMR has been defined as DS 1-2.

Contemporary treatment landscape in the first-line setting

Treatment for newly diagnosed patients is risk stratified and is based on Ann Arbor stage, presence of B symptoms, mediastinal chest ratio for tumor bulk, age and ESR. Treatment for patients with limited-stage cHL (i.e. stage I-II) generally consists of 2-4 cycles of adriamycin, bleomycin, vinblastine and dacarbazine (ABVD) followed by involved-node radiotherapy (INRT), or the so called 2+2 regimen consisting of 2 cycles of escalated bleomycin, etoposide, adriamycin, cyclophosphamide, vincristine, procarbazine, prednisone esc(BEACOPP) followed by 2 cycles of ABVD.[10,15,58] For patients who do not achieve a complete metabolic response after ABVD (CMR, most commonly defined as Deauville score 1-3), treatment can be intensified to escBEACOPP.[15,58] In patients with advanced-staged disease (stage III-IV), treatment generally consists of 4-6 cycles of ABVD or escBEACOPP, with radiotherapy on PET-positive residual lesions.[11,12]

Chemo-radiation results in both short-term and long-term toxicity, which can be reduced using risk-adapted therapy. Potential late-onset complications include secondary malignancies, cardiovascular diseases and diminished fertility, which are particularly problematic given the young age of the majority of cHL patients.[59] PET-adapted therapy has significantly improved outcomes, allowing for treatment intensification in patients with an incomplete response, and de-escalation of therapy by omitting radiotherapy or bleomycin in case of a CMR.[15,60] Noteworthy advances include substitution of bleomycin with BV.[61]

Treatment options in relapsed/refractory cHL

Approximately 15-30% of patients are primary refractory to first-line treatment or relapse after an initial response.[11,14,60,62] Standard salvage treatment consists of induction chemotherapy followed by high-dose myeloablative chemotherapy (HDCT) and ASCT. In transplant-eligible patients, about 40-60% of patients with R/R disease can be cured with this strategy.[63] Patients who are unfit for ASCT have a very poor prognosis and are generally treated with chemotherapy or in some cases radiotherapy. During the past decade, many advances were made in the R/R setting with the development of novel agents such as BV, the application of immune checkpoint

inhibitors and risk-adapted treatment strategies.[64-66] A more detailed description of treatment of R/R cHL can be found in **Chapter 2**.

^{18}F-FDG PET scans and Radiomics

^{18}F-FDG PET scans are the most commonly utilized PET scans for staging and response-assessment of lymphoma.[57] This procedure involves the intravenous administration of a tracer, namely radioactive-labeled glucose, ^{18}F-FDG, which disseminates throughout the body. Tissues with high glucose consumption readily take up ^{18}F-FDG. Cancer cells exhibit a distinct glucose metabolism deviation from normal cells, often referred to as the 'Warburg effect'.[67] This metabolic alteration involves a shift towards aerobic glycolysis instead of oxidative phosphorylation in mitochondria, resulting in increased glucose consumption. ^{18}F-FDG serves as a glucose analogue that enters the cell and undergoes phosphorylation akin to normal glucose. However, it cannot proceed with further metabolism and becomes trapped within the cell. This can be visualized using a PET scanner, which measures the gamma rays that result from emission of positrons released during the radioactive decay of ^{18}F-FDG. The PET system, composed of multiple detector rings encircling the patient, captures the gamma rays and converts them into electrical signals. These signals are then reconstructed into an image by the computer.

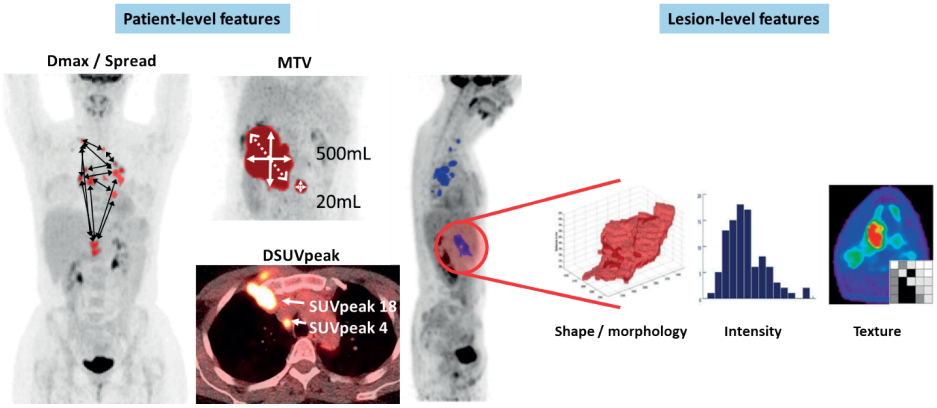

Figure 3. Examples of radiomics features. The metabolically active 'volume of interest' is delineated, from which radiomics features are extracted. Patient-level features represent total MTV, the maximum, mean or peak of SUV within the whole MTV, and dissemination features including distance parameters and differences in volume and intensity between lesions, such as Dmax, spread (sum of all distances) and DSUVpeak (difference in SUVpeak between lesions). Lesion-level features represent tumor shape, morphology, intensity and texture features and is based on individual voxels. Part of the images are adapted from Martens et al.[68]

Radiomics, the term originating from the fusion of 'radiology' and '-omics' like 'proteomics', represents an emerging field that employs quantitative analysis of radiographic images to extract intricate image features. In this thesis, our focus lies on PET-based radiomics, a method that extracts features based on the SUV of voxels (3-dimensional image pixels), thereby reflect-

ing the ^{18}F-FDG uptake of tissue. A PET scan essentially comprises a 3-dimensional grid of numerical data, on which mathematical computations can be performed to calculate various features. These features can be lesion-specific, including intensity, shape and texture within a volume-of-interest (VOI), as well as patient-level features such as total metabolic tumor volume (MTV), disease dissemination, and interlesional heterogeneity of intensity and volume **[Figure 3]**. These features are quantitative assessments of PET scans and can be used as biomarkers.[69,70] MTV is increasingly studied in cHL, demonstrating moderate prognostic value as a standalone biomarker.[71-77] Additionally, the largest distance between two lesions (Dmax) serves as another quantitative PET feature, and has shown prognostic value in newly diagnosed cHL.[78,79]

Challenges in cHL

Despite significant advances, contemporary management of cHL still faces significant challenges, particularly in the R/R setting. There is a high need to optimize treatment with innovative therapies to enhance outcomes, particularly for patients with primary refractory disease. The recent implementation of targeted antibodies and immunotherapy is promising in improving outcomes for R/R cHL patients. Prognostic models hold great potential in refining outcomes by identifying patients with a high risk of disease progression, enabling targeted intervention with novel therapies or intensified treatment. In addition, patients with a low risk of progression might benefit from less intense treatments, such as substituting HDCT/ASCT with maintenance treatment using BV or checkpoint inhibitors. The identification of biomarkers that predict treatment outcomes at baseline, and/or monitor responses during treatment, is critical to advance treatment strategies. The role of serum TARC as a response biomarker needs further validation before it can be fully applied in clinical practice. Additionally, while PET scans are currently utilized for staging and response assessment through visual interpretation, a shift towards quantitative assessment, so called radiomics, could unveil hidden patterns within imaging data, offering a non-invasive method to assess tumor characteristics and significantly enhance prognostic capabilities. The scarcity of large clinical trials in the relapsed/refractory setting emphasizes the importance of global collaboration among research groups. Collaborative efforts facilitate data pooling, augmenting the statistical power of individual studies and fostering a more comprehensive understanding of effective strategies for managing relapsed/refractory cHL.

OUTLINE OF THE THESIS

The primary objective of this thesis is to contribute to the development of personalized and efficient therapeutic strategies for patients diagnosed with cHL, particularly in the R/R setting. This is achieved by connecting information from various domains, including quantitative PET data, serum biomarkers, TME composition, and clinical data.

Epidemiology (chapter 3)

Advancements in the treatment for cHL in recent decades include the introduction of combination chemotherapy, novel therapies for R/R disease, incorporation of involved node radiotherapy, and the integration of PET-adapted treatment approaches. Furthermore, diagnostic capabilities have significantly improved with the introduction of PET-CT, alongside general improvements in supportive care. In **chapter 3**, temporal trends in primary treatment and relative survival among patients with cHL across various subgroups of age and stage are assessed. This analysis is performed by conducting a large, comprehensive, nationwide, population-based study in almost 10,000 adult cHL patients diagnosed in the Netherlands over a 29-year period.

Brentuximab vedotin treatment (chapter 2, 4 & 5)

There is an unmet need for innovative treatments in R/R cHL. In **chapter 2**, we delve into both current and emerging therapies tailored for R/R cHL patients, providing an overview of clinical trial findings exploring novel therapies and strategies. BV is an anti-CD30 antibody-drug conjugate, combined with the potent antimicrotubule agent monomethyl auristatin-E to specifically target HRS cells expressing CD30. **Chapter 4** presents the results from a prospective, multicenter, international Phase I/II study, investigating the efficacy and safety of BV in combination with dexamethasone, high-dose cytarabine and cisplatin (DHAP) for patients with R/R cHL. The study treatment included three BV-DHAP induction cycles, followed by HDCT/ASCT, assessing CMR rates prior to ASCT, as well as the progression free survival (PFS) and overall survival (OS).

Beyond our phase I/II study, several single-arm studies have shown promising efficacy of BV in the R/R salvage setting. In **Chapter 5** we gathered individual-patient data from 10 different single-arm studies and performed a propensity score matched analysis for patients treated with BV and chemotherapy versus patients treated with salvage chemotherapy alone. This extensive individual patient data analysis investigates the impact of adding BV to salvage chemotherapy compared to chemotherapy alone, evaluating pre-ASCT CMR rates, PFS and OS.

Quantitative PET analysis (chapter 6 & 7)

The ^{18}F-FDG PET-CT scan plays an important role in diagnosing cHL for staging at baseline and response assessment during treatment. Nowadays staging and response assessment are based on visual interpretation by a nuclear medicine physician, which is highly susceptible to interobserver variation and relies on the experience of the physician. Quantitative analysis of PET-CT scans, also known as radiomics, can provide large amounts of data from the PET images, which may reveal hidden patterns of the tumor distribution and FDG uptake. Analyzing PET-CT scans for radiomics involves an initial step of segmentation, where the volume of interest is selected to compute the MTV. However, consensus about a standard segmentation method to derive MTV in cHL is lacking, and it is unknown how different segmentation methods influence quantitative PET features. Therefore, in **chapter 6**, we aimed to evaluate the delineation and

completeness of lesion selection and the need for manual adaptation with different segmentation methods, and to assess the influence of segmentation methods on the prognostic value of MTV, intensity and dissemination radiomics features in cHL patients. We used the results of this study as input for **chapter 7**, in which we built a prognostic model using baseline quantitative ^{18}F-FDG PET radiomics features and clinical characteristics to predict PFS in R/R cHL patients. This model calculates a PET-based risk profile and can be applied to develop risk-stratified treatment strategies for R/R cHL patients.

Biomarkers (Chapter 8 & 9)

In addition to quantitative PET parameters, we investigated various blood-based biomarkers, including serum TARC, vitamin D and LDH, immunohistochemistry markers, and gene-expression signatures. This explorative study sought to discover patterns in the TME composition and to interpret PET results in relation to biological features. In **chapter 8**, the three-year follow-up results of the Transplant BRaVE study are described and we investigated the prognostic value of several biomarkers. The correlation between these biomarkers and quantitative PET features was explored to unveil biological associations. In **chapter 9**, we sought to establish the connection between the TME composition and FDG uptake on PET scans, unraveling which cells in the TME exhibit elevated glucose (FDG) uptake. This pioneering study marks the first attempt to correlate information on radiomics patterns in individual lesions with the TME composition obtained from tumor biopsies.

Discussion (chapter 10)

In **chapter 10**, we conclude with a general discussion on the key findings of the research presented in this thesis, followed by an English and Dutch summary.

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2

How to choose first salvage therapy in Hodgkin lymphoma: traditional chemotherapy vs novel agents

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ABSTRACT

Approximately 10 to 30% of patients with classical Hodgkin lymphoma (cHL) develop relapsed or refractory (R/R) disease. Of those patients, 50 to 60% show long-term progression-free survival after standard salvage chemotherapy followed by high-dose chemotherapy (HDCT) and autologous stem cell transplant (ASCT). In the past decade, novel therapies have been developed, such as the CD30-directed antibody-drug conjugate brentuximab vedotin and immune checkpoint inhibitors, which have greatly extended the treatment possibilities for patients with R/R cHL. Several phase 1/2 clinical trials have shown promising results of these new drugs as monotherapy or in combination with chemotherapy, but unfortunately, very few randomized phase 3 trials have been performed in this setting, making it difficult to give evidence-based recommendations for optimal treatment sequencing. Two important goals for the improvement in the treatment of R/R cHL can be identified: (1) increasing long-term progression-free and overall survival by optimizing risk-adapted treatment and (2) decreasing toxicity in patients with a low risk of relapse of disease by evaluating the need for HDCT/ASCT in these patients. In this review, we discuss treatment options for patients with R/R cHL in different settings: patients with a first relapse, primary refractory disease, and in patients who are ineligible or unfit for ASCT. Results of clinical trials investigating novel therapies or strategies published over the past 5 years will be summarized.

Learning objectives

1. Describe current and emerging therapies for patients with R/R Hodgkin lymphoma.
2. Understand the importance for patients with R/R Hodgkin lymphoma to achieve a CMR before HDCT/ASCT

CLINICAL CASE

A 30-year-old woman presented with a persistent painless lump in the neck without B-symptoms. A biopsy of a right supraclavicular node was performed, which showed a classical Hodgkin lymphoma (cHL). An ^{18}F-fluorodeoxyglucose positron emission tomography (PET)-computed tomography (CT) scan revealed lymphadenopathy bilaterally in the supraclavicular and infraclavicular region, retrosternally and in the mediastinum, hence cHL stage IIA unfavorable was diagnosed.

After oocyte preservation, treatment with adriamycin, bleomycin, vinblastine, and dacarbazine was initiated with the intention to administer a total 6 cycles in case of a complete metabolic response (CMR) after 2 cycles. However, the interim PET-scan showed only a partial metabolic response (PR) (Deauville score 4), and the treatment was intensified to escalated bleomycin, etoposide, adriamycin, cyclophosphamide, vincristine, procarbazine, and prednisone. After 2 cycles, a CMR was reached and the patient received consolidative involved node radiotherapy (30 Gy).

Unfortunately, 1 year later the patient presented with night sweats and severe itching. Imaging revealed extensive lymphadenopathy above and below the diaphragm, and a biopsy confirmed the relapse. Salvage chemotherapy with dexamethasone, high-dose cytarabine, and cisplatin (DHAP) was initiated, which resulted in a CMR after 2 cycles, and stem cells were mobilized and collected after a third cycle of DHAP with the intention to proceed to high-dose chemotherapy (HDCT) followed by autologous stem cell transplant (ASCT) rescue.

INTRODUCTION

Approximately 10 to 30% of patients with cHL will relapse or are primary refractory (R/R) to first-line treatment. Standard salvage chemotherapy and consolidation with HDCT/ASCT leads to long-term progression-free survival (PFS) in about 50 to 60% of patients. Until recently, patients who relapsed after ASCT or were ineligible for ASCT had limited treatment options, and 50% of those patients eventually died of the disease.[1] In the past decades, several novel therapeutic options for patients with R/R cHL have become available, including brentuximab vedotin (BV), and immune checkpoint inhibitors (CPIs), leading to high CMR rates pre-ASCT, especially when combined with chemotherapy.[2] Achieving a CMR prior to ASCT appears to be the most important prognostic factor for PFS.[3-10] Therefore, a risk- and PET-adapted treatment approach could probably lead to higher cure rates.[11] On the other hand, the burden of late toxicities related to HDCT, such as secondary malignancies and infertility, is considerable, especially as the disease typically affects patients early in life. For this reason, decreasing toxicity is one of the main goals in the treatment of R/R cHL.[12]

In this educational session, we discuss the results of studies that incorporated novel therapies and response-adapted treatment and how this could be implemented in standard practice to improve outcomes for patients with R/R cHL.

Table 1: Overview of first-salvage chemotherapy regimens since 2010

Study	N	Intervention	Refractory (%)	CR pre-ASCT	ORR pre-ASCT	PFS	OS
Josting et al (2010)[13] (RCT)	279	DHAP	0 (0%)	CT: 24%	CT: 71%	3 years: 69% (no sign. difference between arms)	3 years: 85% (no sign. difference between arms)
Moskowitz et al (2010)[14]	105	ICE	48 (46%)	PET/gallium: 61%. CT: 33%	CT: 59%	4 years: 56%	4 years: 72%
Moskowitz et al (2012)[11]	97	ICE + GVD (PET-adapted sequential)	41 (42%)	PET: 60% after ICE. 78% after GVD	-	51 months: 70%	51 months: 80%
Labrador et al (2014)[15] (retrospective)	82	ESHAP	41 (50%)	PET/gallium: 50%	PET/gallium: 67%	Median PFS: 56 months	5 years: 73%
Santoro et al (2016)[16]	58	BeGEV	27 (46%)	PET: 73%	PET: 83%	5 years: 59%	5 years: 78%

BeGEV, bendamustine, gemcitabine, and vinorelbine

Treatment for patients with a first relapse or primary refractory disease after first-line treatment

Conventional salvage chemotherapy results in pre-ASCT complete response (CR) rates of about 20 to 25%, and overall response rates (ORRs) of 60 to 70%, based on evaluation by CT scan.[13] More recent studies reporting response rates based on functional imaging using PET or gallium showed CMR rates of 50 to 60% after ifosfamide, carboplatin, and etoposide (ICE) or etoposide, methylprednisolone, high-dose cytarabine, and cisplatin (ESHAP). Even higher CMR rates were reported for the bendamustine, gemcitabine, and vinorelbine regimen (73%) and a sequential ICE-gemcitabine, vinorelbine, and liposomal doxorubicin (GVD) approach (78%) in which patients with no CR on ICE received additional chemotherapy with GVD before proceeding to ASCT.[11,14-16] PFS ranges between 50 to 60% with an overall survival (OS) of 70 to 80% at 5 years.[11,13-16] Overall, there seem to be no significant differences with regard to outcome between the most commonly used regimens (i.e. ICE/DHAP/ESHAP) (**Table 1**). However, randomized controlled trials (RCTs) comparing different salvage chemotherapy regimens or a PET-adapted approach are lacking.

BV and checkpoint inhibitors

cHL is characterized by the presence of a minority of bi- or multinucleated Hodgkin and Reed-Sternberg (HRS) cells that universally express CD30 in an inflammatory tumor microenvironment. BV is an anti-CD30 monoclonal antibody conjugated to the microtubule-disrupting agent monomethyl auristatin-E.[17] PD-L1 and PD-L2 are upregulated by HRS cells in about 90% of patients and induce T-cell exhaustion, which contributes to immune escape of HRS cells.[18] CPIs are monoclonal antibodies that block the interaction between inhibitory ligands such as PD-L1 and PD-L2 on the tumor cells and PD-1 receptors on immune effector cells.

Several studies have investigated the use of BV in combination with chemotherapy as first salvage regimen and showed high CMR rates prior to ASCT of up to 83%, with 2-year PFS rates ranging from 63 to 81% (**Table 2**).[3-10] In 5 studies, BV was combined with chemotherapy in 2 to 6 cycles followed by ASCT in patients with PR or CMR, whereas in 2 studies, patients were treated initially with 4 to 6 administrations of BV monotherapy; patients with a CMR could proceed directly to ASCT, whereas patients with a PR received additional salvage chemotherapy without BV.[4,5] This PET-adapted approach is interesting because approximately 30 to 50% of patients could proceed to ASCT after BV monotherapy only, thereby avoiding toxicity from salvage chemotherapy in these patients. Moreover, a trial investigating the combination of BV and the CPI nivolumab as pre-ASCT salvage regimen showed that 67 of 91 patients could proceed directly to ASCT after BV-nivolumab, without salvage chemotherapy. The study revealed low toxicity of this regimen compared with salvage chemotherapy.[10]

Thus far, in studies that incorporated a PET-adapted strategy, PFS seems to be similar for patients who proceeded to ASCT directly after having a CMR on BV, BV-nivolumab or ICE alone as for those patients who needed additional salvage chemotherapy to achieve a CMR.[4,5,10] This

Table 2: Overview of first-salvage regimens containing BV or CPI

Study	N	Intervention	Schedule	Refractory, n (%)	CMR pre-ASCT, n (%)	2-year PFS	2-year OS
Moskowitz et al (2017)[4]	65	BV + sequential ICE	BV 1.2 mg/kg d1, 8, 15 of 28d cycles, 2 cycles. ICE salvage in case of Deauville >3.	34 (52%)	54 (83%)	82%	97%
Herrer et al (2018)[5]	57	BV + sequential ICE/ GVD	BV 1.8mg/kg every 21d, 4 cycles. Last 2 cycles BV escalation to 2.4mg/kg in n=8 patients with PR/SD. Salvage chemotherapy at discretion of treating physician.	35 (61%)	37 (65%)	67%	93%
Cole et al (2018)[6]	45	BV + gemcitabine	BV 1.8 mg/kg on d1 and d8 every 21 days, 4 cycles. In combination with gemcitabine.	29 (64%)	28 (67%)	-	1 year: 95%
LaCasce et al (2018)[9]	55	BV + bendamustine	BV 1.8mg/kg every 21d, 2-6 cycles. In combination with bendamustine. Post-ASCT BV monotherapy maintenance up to 16 cycles.	28 (51%)	39 (74%)	63%	94%
Garcia-Sanz et al (2019)[8]	66	BV + ESHAP	BV 1.8mg/kg every 21 days, 4 cycles. In combination with 3 cycles of ESHAP.	40 (61%)	46 (70%)	71%	90%
Broccoli et al (2019)[7]	40	BV + bendamustine	BV 1.8mg/kg every 21 days, 4-6 cycles. In combination with bendamustine.	20 (50%)	30 (79%)	68%	97%
Abuelgasim et al (2019)[19]	28	BV + IGEV	BV 1.8 mg/kg every 21 days, 2-4 cycles. In combination with IGEV. 64% received BV consolidation after ASCT.	12 (43%). Incl n=14 with >1 line of therapy.	70%	73.5% (100% for patients with first relapse)	87.1% (100% for patients with first relapse)
Kersten et al (2021)[3]	67	BV + DHAP	BV 1.8 mg/kg every 21 days, 3 cycles. In combination with DHAP.	30 (45%)	53 (82%)	78%	96%
Advani et al (2021)[10]	91	BV + nivolumab	BV 1.8mg/kg and nivolumab 3.0mg/kg every 21 days, 4 cycles.	38 (42%)	61 (67%)	78%	93%
Moskowitz et al (2021)[20]	39	Pembrolizumab + GVD	Pembrolizumab 200mg every 21 days, 4 cycles. In combination with GVD.	16 (41%)	36 (95%)	1 year: 100%	1 year: 100%

d, day; IGEV, ifosfamide, gemcitabine, vinorelbine, and prednisolone; SD, stable disease.

confirms that the most important goal is to achieve a CMR before ASCT and that patients who do not respond initially can potentially be rescued with additional salvage chemotherapy and proceed to ASCT if they reach a CMR.

Figure 1 proposes a flowchart of PET-adapted treatment in patients with R/R cHL. Because BV and CPI are not yet available or reimbursed as first salvage treatment in many countries, salvage chemotherapy is often still the standard approach.

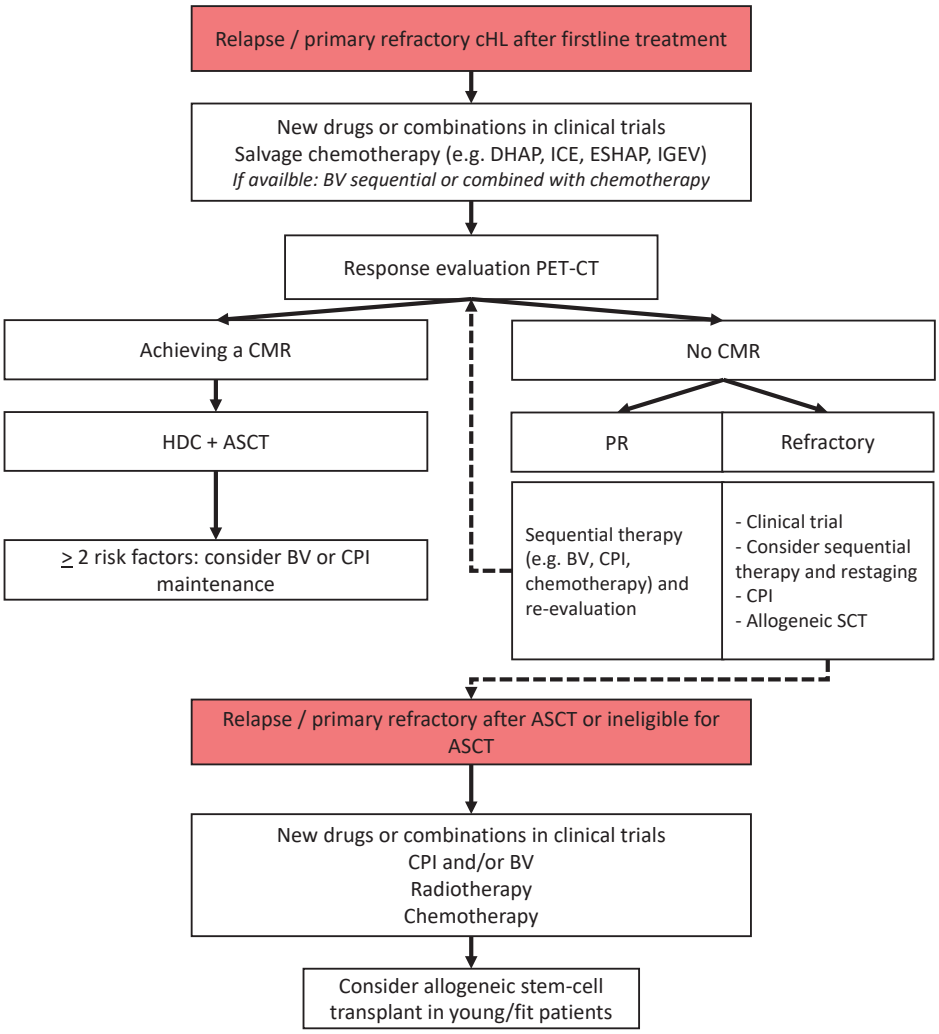

Figure 1. Flowchart of treatment for R/R cHL. IGEV, ifosfamide, gemcitabine, vinorelbine, and prednisolone; SCT, stem cell transplant.

High-dose chemotherapy and ASCT

With increasing CMR rates pre-ASCT, one might question the need for consolidation with HDCT/ASCT in all patients. HDCT/ASCT is associated with immediate toxicity such as cytopenias and mucositis and long-term toxicity such as infertility and secondary malignancies.[12] Superiority for HDCT/ASCT over mini-carmustine, etoposide, cytarabine, and melphalan (BEAM) (ie, reduced-dose BEAM without ASCT) in R/R cHL was shown in 2 RCTs in 1993 and 2002.[21,22] However, in these trials, patients did not receive any salvage chemotherapy before BEAM or mini-BEAM. In addition, with the advent of very effective drugs such as BV and CPIs, a subset of patients may be cured with salvage treatment alone.[17]

A recently published study using pembrolizumab and GVD chemotherapy followed by HDCT/ASCT showed a very high pre-ASCT CMR rate of 95%, and with a median follow-up of 1 year, no progressions have occurred.[20] This study has now started a second part in which patients with a CMR after 2 cycles of pembrolizumab-GVD continue with 2 additional cycles of pembrolizumab-GVD followed by pembrolizumab consolidation instead of HDCT/ASCT. Results of this revolutionary approach could change the treatment of patients with R/R cHL significantly.

There is a high unmet need for RCTs in the pre-ASCT R/R setting. Future studies should focus on the optimal sequence of using CPIs and BV in salvage treatment and consolidation strategies to induce high CMR rates while at the same time minimizing early and late toxicity. Eventually, an RCT should be performed to establish the role of risk- and PET-adapted treatment in R/R cHL, including the role of HDCT/ASCT.

Patients with primary refractory disease

Primary refractory disease and a short interval between first-line treatment and relapse are important poor prognostic factors for response to salvage therapy and PFS.[3,11,14] CMR rates to salvage therapy for refractory patients are usually lower compared with relapsed patients: 73% vs 86% after DHAP, 64% vs 84% after BV-bendamustine, and 53% vs 77% after BV-nivolumab, respectively.[3,9,10] However, in patients who do achieve CMR to salvage therapy and proceed to ASCT, post-ASCT PFS for primary refractory and relapsed patients is similar.[3,11] A retrospective analysis in 78 patients who progressed on one or more salvage regimens and who were treated with regimens containing CPI showed favorable results, with 59% of patients achieving a CMR and a post-ASCT PFS of 81% at 18 months.[23] This suggests that treatment with CPI may improve chemosensitivity of previously chemorefractory disease. Interestingly, pre-ASCT CMR status was not significantly prognostic for post-ASCT PFS in this cohort, suggesting a PR might be sufficient for proceeding to ASCT after CPI in this patient population.

Patients with a late relapse

Although most relapses after first-line treatment occur within the first 2 years, a minority of patients has a late relapse more than 5 years after first-line treatment.[24] A large retrospective

analysis showed that late relapses occur more frequently in patients with early-stage favorable disease compared with patients with unfavorable or advanced-stage disease. Whether these relapses represent true relapses or a new second cHL manifestation, possibly due to genetic or environmental risk factors in these patients, remains largely unknown. About half of these patients were treated with ASCT, which was associated with favorable PFS and OS compared with other salvage therapies; however, non-ASCT approaches, such as those using combination chemotherapy or occasionally radiation alone, could be considered depending upon the patient's initial treatment and underlying comorbidities.[24]

Maintenance treatment after ASCT

For patients with a high risk of relapse after ASCT, maintenance treatment with BV can be considered. In a study investigating BV maintenance, 329 patients with unfavorable risk R/R cHL (defined as primary refractory disease, relapse <1 year, or extranodal disease) received either up to 16 cycles of BV maintenance or placebo after ASCT.[25] The study showed improvement in PFS in patients receiving BV maintenance, with a 5-year PFS of 59% vs 41% for placebo. However, there was no difference in OS, probably because 87% of patients who relapsed in the placebo arm received BV at the subsequent relapse. Therefore, the use of BV maintenance after ASCT could potentially be restricted to patients with at least 2 risk factors, or alternatively, its use could be delayed until progression. With the increasing use of BV in the first-line setting, it is also important to investigate whether patients who relapse after BV in combination with chemotherapy will still show advantage of BV maintenance.[26] Alternatively, CPI could be used as maintenance treatment; a small phase 2 trial in 30 high-risk patients showed high post-ASCT PFS.[27] The combination of CPI and BV maintenance in 59 high-risk patients has also shown promising results, with 5 patients with PR converting to CR during maintenance.[28] Further studies should investigate the role of post-ASCT maintenance in high risk patients with CPI and/or BV vs reserving these treatments for a subsequent relapse.

BV and CPI treatment for patients who relapse after ASCT or are ineligible for ASCT

Patients who relapse after ASCT, or are ineligible for ASCT due to chemotherapy-resistant disease generally have a poor prognosis.[1] In **Table 3**, we summarize the most important recent studies in patients with R/R cHL who have progression after at least 1 line of salvage treatment. The first breakthrough in the post-ASCT setting was the application of monotherapy with BV in heavily pretreated R/R patients, which showed an ORR of 75% and a CMR rate of 34% with a median PFS of 20.5 months in those with a CMR. The PFS rate at 5-years, however, was only 22% with an OS of 41%, highlighting the need for additional treatment options (**Table 3**).[17]

A study that investigated pembrolizumab monotherapy showed an ORR of 69% and a CMR rate of 22%, with a 2-year PFS of 31% and OS of 91%.[2] Several different CPIs and also combinations of CPIs have been investigated in R/R cHL.[30,31,33,34] In a phase 1 trial, 64 patients were ran-

domized between ipilimumab-BV, nivolumab-BV, and triple therapy with ipilimumab-nivolumab-BV. The trial showed differences in toxicity profile and efficacy between the 3 regimens, with the highest percentage of grade 3/4 adverse events in the triplet and ipilimumab-BV group, whereas the highest ORR and CMR rates were found in the triplet and nivolumab-BV group.[34]

In a recently published head-to-head comparison of monotherapy with pembrolizumab vs monotherapy with BV, pembrolizumab showed a significantly higher median PFS of 13.2 months vs 8.3 months for BV.[35] The incidence of adverse events was comparable between the 2 groups, with immune-mediated adverse events in the pembrolizumab arm and neuropathy in the BV arm.

Importantly, in patients who received earlier treatment with BV or CPI, response rates seem to be similar to patients who have not received BV or CPI before. A retrospective study evaluated 18 patients with R/R cHL and 10 patients with R/R anaplastic large cell lymphoma who received treatment with BV in 2 lines and showed CMR and ORR that are comparable with patients who received BV for the first time.[37] Retreatment with CPI in 78 patients with R/R cHL who relapsed after nivolumab also showed comparable efficacy.[38]

Table 3. Overview of recently reported trial results incorporating BV or CPIs for patients after ≥1 line of salvage treatment

Study	N	Intervention	CMR	ORR	PFS	OS
Chen et al (2016)[17]	102	BV	34%	75%	5 years: 22%	5 years: 41%
O'Connor et al (2018)[29]	65	BV + bendamustine	32%	71%	2 years: 50%*	2 years: 65%*
Armand et al (2018)[18]	243	Nivolumab	16%	69%	1 year: 50%*	1 year: 92%
Chen et al (2019)[2]	210	Pembrolizumab	27.6%	71.9%	2 years: 31.3%	2 years: 90.9%
Shi et al (2019)[30]	92	Sintilimab	34%	80.4%	6 months: 77.6%	6 months: 100%
Song et al (2020)[31]	75	Camrelizumab	28%	76%	9 months: 76.6%	9 months: 96%*
Armand et al (2020)[32]	31	Pembrolizumab	19%	58%	2 years: 30%	2 years: 87%
Song et al (2020)[33]	70	Tislelizumab	63.9%	87.1%	9 months: 74.5%	9 months: 98.6%
Diefenbach et al (2020)[34] (including n=26 first relapse)	64	Ipilimumab+BV *versus* nivolumab+BV *versus* ipilimumab+nivolumab+BV	60% / 65% / 84%	80% / 94% / 95%	1 year: 59% / 77% / 87%	1 year: 90%* / 80%* / 95%* /
Kuruvilla et al (2021)[35]	304	Pembrolizumab (n=151) *vs* BV (n=152)	25% vs 24%	66% vs 54%	2 years: 35% vs 25%	-
Liu et al (2021)[36]	61	Camrelizumab monotherapy *vs* camrelizumab+ decitabine	79% vs 32% for cam+deci vs cam	95% vs 89%	2 years: 67% vs 42%	2 years: 100%

*data have been extracted from available Kaplan-Meier curves using WebPlot Digitizer.

Role of radiotherapy in the management of R/R cHL

The role of radiotherapy in the R/R setting has not been revisited well in this era of novel treatment options. Radiotherapy can be used pre-ASCT or post-ASCT on residual lesions or in patients with extranodal or bulky disease and as part of the conditioning regimen using total lymphoid irradiation, but comparative data about efficacy of radiotherapy in these settings are scarce and outdated.[39] Earlier studies have shown that patients who receive radiotherapy have decreased risk of local recurrence, and thus for patients with limited-stage disease at relapse, radiotherapy may be an effective option.[39] Using radiotherapy in patients who have a PR pre-ASCT would be an interesting strategy to increase the CMR rate, and studies investigating this approach are warranted. In addition, the synergistic effects of radiation with immunotherapy, as described in a few case reports, should be investigated more extensively and could be an option for patients who relapse after ASCT or are ineligible for ASCT.

GENERAL CONSIDERATIONS

In conclusion, the primary goal of treatment for patients with R/R cHL is to achieve a CMR before HDCT/ASCT as this significantly correlates with a favorable outcome after ASCT. Using a sequential approach, treatment intensity and toxicity can be reduced in a subset of fast-responding patients. Patients who are ineligible for ASCT or relapse after ASCT can be treated with BV or CPIs. There is a need to develop novel therapies to increase response rates without increasing toxicity. One of the next goals for clinical trials is to investigate which patients can possibly be cured without HDCT/ASCT. Risk-stratified and PET-adapted prospective studies could help achieve this goal. Optimized risk stratification and response evaluation will guide future treatment decisions and will help to find the right treatment for the right patient.

CLINICAL CASE (CONTINUED)

Despite the initial CMR after 2 cycles of DHAP, soon after the third and last cycle of DHAP, B-symptoms and itching returned and a relapse was confirmed by PET-CT and a biopsy. Given the poor prognosis in this patient with chemorefractory disease, combination treatment with BV and nivolumab was started based on the encouraging results of a phase 1/2 trial.[10] Already after 1 cycle of BV-nivolumab, her B-symptoms disappeared, and after 4 cycles, she reached a CMR. We decided to proceed to haploidentical allogeneic stem cell transplant, because the patient initially progressed during salvage chemotherapy. As mentioned above, though, emerging data on the role of ASCT after CPI for patients refractory to multiple prior lines of chemotherapy suggest that ASCT could be considered in this setting as well.[22]

CONFLICT-OF-INTEREST DISCLOSURE

Julia Driessen: travel support from Takeda.
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Marie José Kersten: honoraria for consulting or advisory boards: Kite/Gilead, Novartis, BMS/Celgene, Miltenyi Biotech, Research funding Takeda, Roche, Celgene, Kite/Gilead.

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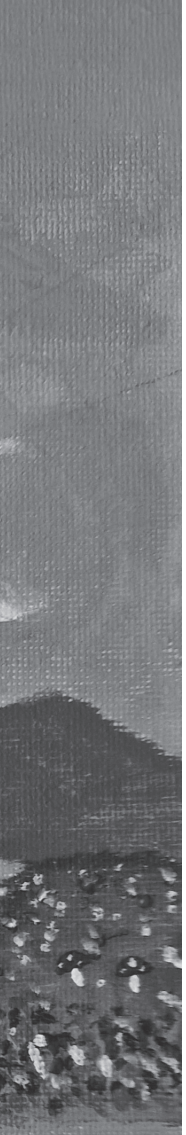

3

Primary therapy and relative survival in classical Hodgkin lymphoma: a nationwide population-based study in the Netherlands, 1989-2017

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ABSTRACT

Population-based studies of classical Hodgkin lymphoma (cHL) in contemporary clinical practice are scarce. The aim of this nationwide population-based study is to assess trends in primary therapy and relative survival (RS) during 1989-2017. We included 9,985 patients with cHL. Radiotherapy alone was virtually not applied as from 2000 among patients aged 18-69 years with stage I/II disease, following the broader application of chemotherapy combined with radiotherapy. Chemotherapy only was the preferred treatment for patients with stage III/IV disease. Throughout the entire study period, around 20% of patients aged ≥70 years across all disease stages received no anti-neoplastic therapy. The most considerable improvements in 5-year RS were confined to patients aged 18-59 years. Five-year RS for patients with stage I/II disease diagnosed during 2010-2017 was 99%, 98%, 100%, 93%, 84%, and 61% for patients aged 18-29, 30-39, 40-49, 50-59, 60-69, and ≥70 years, respectively. The corresponding estimates for stage III/IV disease were 96%, 92%, 90%, 80%, 58%, and 46%. Collectively, the improvements in survival likely relate to advances in cHL management. These achievements, however, do not seem to translate into significant benefits for patients ≥60 years. Therefore, novel therapies are urgently needed to reduce excess mortality in elderly cHL patients.

INTRODUCTION

Hodgkin lymphoma (HL) is a heterogeneous B-cell malignancy with an annual age-standardized incidence rate of 2 to 3 per 100 000 persons in Western countries.[1] The disease can broadly be categorized into two types: classical HL (cHL) and nodular lymphocyte-predominant HL (NLPHL).[2] This paper focuses on cHL because a comprehensive apprehension of the incidence, treatment, and survival of NLPHL in the Netherlands has been reported recently[3]

The early 1970s marked a critical milestone in the treatment of cHL with the introduction of polychemotherapy with the MOPP (mechlorethamine, vincristine, prednisone, and procarbazine) regimen. This regimen led to a relatively high response rate, with a 5-year overall survival (OS) rate of approximately 65%.[4,5] Thereafter, significant achievements have been accomplished in the management of cHL, in terms of higher response and OS rates, and less toxicity over the short- and long-term. These achievements include the widespread adoption of the ABVD (adriamycin, bleomycin, vinblastine, and dacarbazine) regimen in the early 1990s, the introduction of (escalated) BEACOPP (bleomycin, etoposide, adriamycin, cyclophosphamide, vincristine, procarbazine, and prednisone) in the late 1990s for patients with advanced-stage disease or unfavorable disease characteristics, and general improvements in supportive care.[5-10] More recently, the PET-CT scan has enabled tailoring of treatment strategies dynamically based on early response evaluation.[11-15)] Besides, new salvage treatment options for relapsed or refractory patients were recently introduced.[16-19] At present, depending on age and disease stage, long-term survival rates reported by clinical trials in cHL are around 90%.[9,10,12,13] As a result, the prevalence of cHL survivors is relatively high and continues to increase over time.

Therapeutic advances reported in clinical trials cannot always be readily translated into tangible benefits for patients managed in routine clinical practice. This issue relates to the strict inclusion and exclusion criteria of clinical trials that might hamper the extrapolation of trial results to a broader patient population.[20] In this regard, a population-based cancer registry is a useful instrument to investigate how pivotal findings of clinical trials are implemented in routine clinical practice and affect outcomes among the general patient population. At present, large population-based studies in cHL including patients managed in contemporary clinical practice are scarce and mostly lack comprehensive information on patient characteristics and therapy or report OS rates that do not account for the expected survival from the general population.[21-27] Therefore, it remains mostly unknown how contemporary advances in cHL management have impacted survival at the population-level.

Therefore, we conducted a large, comprehensive, nationwide, population-based study in almost 10,000 adult cHL patients diagnosed in the Netherlands over a 29-year period. This study aimed to assess temporal trends in primary treatment and relative survival among patients with cHL across various subgroups of age and stage.

PATIENTS AND METHODS

The Netherlands Cancer Registry

The Netherlands Cancer Registry (NCR) is maintained and hosted by the Netherlands Comprehensive Cancer Organisation (IKNL) and has a national coverage since 1989 with a completeness of more than 95% of all newly diagnosed malignancies in the Netherlands.[28] The NCR relies on comprehensive case notification through the Nationwide Network of Histopathology and Cytopathology, and the Nationwide Registry of Hospital Discharges (i.e. inpatient and outpatient discharges). Data on dates of birth and diagnosis, sex, disease stage, topography, and morphological subtype, and primary therapy are available in the NCR for individual patients. These data are collected by trained registrars of the IKNL through retrospective medical records review. Topography and morphology are coded according to the International Classification of Diseases for Oncology (ICD-O). Information on vital status (i.e., alive, death, or emigration) is obtained through annually linking the NCR to the Nationwide Population Registries Network that holds these data for all residents in the Netherlands.

Study population

We identified all patients diagnosed with histologically confirmed cHL between January 1, 1989 and December 31, 2017—with follow-up for survival until January 1, 2019—from the NCR using ICD-O morphology codes (details provided in **Supplemental Table S1**). The ICD-O enabled to classify patients into the following morphological subtypes: nodular sclerosis, mixed cellularity, lymphocyte rich, lymphocyte depleted, and cHL, not otherwise specified (NOS). Patients below age 18 at diagnosis (n=969; 8.8%) and patients diagnosed at autopsy (n=35; 0.3%) were excluded from the analysis of primary therapy and survival. However, these patients were not excluded from the analysis of the overall incidence rate of cHL. This approach allows for a comparison of the overall incidence rates with other international studies.

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 use of anonymous data for this study was approved by the Privacy Review Board of the NCR.

The data that support the findings of this study are available via IKNL. These data are not publicly available and restrictions apply to the availability of the data used for the current study. However, these data are available from the authors upon reasonable request and with permission of IKNL.

Primary therapy

Primary therapy was defined as chemotherapy, radiotherapy, chemotherapy with radiotherapy (hereafter referred to as combined modality therapy), no anti-neoplastic therapy, or other/unknown therapy. Data on the exact therapeutic regimens were recorded in the NCR for

patients diagnosed as of 2014. These regimens were defined as ABVD, escalated or baseline BEACOPP, CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone), or other, less common chemotherapeutic regimens.

Primary therapy is presented for three calendar periods (1989-1999, 2000-2009, and 2010-2017) and six age groups (18-29, 30-39, 40-49, 50-59, 60-69, and ≥70 years), and stratified according to disease stage as per the Ann Arbor classification—that is, stage I/II (limited-stage) and III/IV (advanced-stage). The calendar periods were based on changing treatment practices for cHL in the Netherlands. More specifically, the first calendar period represents the MOPP/ABVD era.[4,5] The second calendar period marks the era in which ABVD following involved node radiotherapy (INRT) was implemented for limited-stage disease.[8] Also, that era marks the implementation of ABVD or (escalated) BEACOPP for advanced-stage cHL, and high-dose chemotherapy followed by autologous stem-cell transplant for relapsed/refractory cHL.[6,29] Lastly, the most recent calendar period represents the era in which PET-guided treatment was gradually introduced into daily practice and new targeted therapies have become available for relapsed/refractory cHL.[11-13,30]

Statistical analysis

Patient and treatment characteristics were presented as descriptive statistics overall and according to disease stage (i.e. limited- and advanced-stage disease) across the three calendar periods. Differences among categorical variables were tested with the Pearson chi-square test or Fisher's exact test, whereas differences among continuous variables were tested with the Kruskal-Wallis test.

Overall and sex-specific incidence rates were calculated per 100,000 person-years using the annual mid-year population size as obtained from Statistics Netherlands and age-standardized as per the European standard population. Also, incidence rates were calculated according to the calendar period of diagnosis and stratified by disease stage. Age-specific incidence rates were calculated per 5-year age groupings of 0-4 years to ≥85 years.

Relative survival (RS) was calculated to estimate the disease-specific survival using the cohort methodology.[31] RS is the observed patient survival (i.e., OS) corrected for the expected survival of a comparable group in the general population, matched to the patients by age, sex, and year of diagnosis. Expected survival was estimated as per the Ederer II methodology using Dutch population life tables, stratified by age, sex, and calendar year.[32] The cohort-based methodology was employed since it enables us to assess the current survival outcomes of a well-defined patient cohort according to the calendar period of diagnosis. The main convenience of employing RS to estimate disease-specific survival is that it does not depend on the information on the cause of death. This information is not available in the NCR. Whenever this information is available in cancer registries, one might question whether the cause of death is accurately classified. Collectively, lack of information on the cause of death or its inaccuracy precludes or obscures the computation of mortality attributed to a specific cause (i.e. disease-

specific survival). Therefore, RS captures excess mortality—relative to the expected mortality in the general population—associated with a diagnosis of cHL, regardless of whether the excess mortality was directly or indirectly attributed to cHL.

RS was calculated up to ten years after diagnosis according to the calendar period of diagnosis and age at diagnosis and measured from the time of diagnosis to death, emigration, or end of follow-up (January 1, 2019), whichever occurred first. Although we aimed to compare outcomes from both a historical and contemporary perspective, RS was also calculated beyond ten years after diagnosis for patients diagnosed in the first calendar period (1989-1999).

Generalized linear models (GLMs) that assume a Poisson distribution for the observed number of deaths were applied to investigate linear trends in RS over the calendar periods studied.[31] GLMs were also applied to model excess mortality over the calendar periods studied during the first ten years after cHL diagnosis according to disease stage (i.e. limited- and advanced-stage disease), with simultaneous adjustment for sex, age at diagnosis, disease stage, and years of follow-up. Results from these models generate excess mortality rate ratios (EMRRs) with their associated 95% confidence intervals (CIs). The initial two years of follow-up were divided into 1-year time bands. The remaining eight years of follow-up were divided into 2-year time bands. The calendar period 2000-2009 was chosen as the reference as it was clinically relevant to estimate the excess mortality rate in the most recent calendar period (2010-2017).

A *P*-value <0.05 indicates statistical significance. All analyses were performed using STATA/SE 14.2 (StataCorp LP, College Station, Texas, USA).

RESULTS

Patient characteristics

A total of 9,985 adults (≥18 years) were diagnosed with cHL in the Netherlands between 1989 and 2017 and included in the study. The characteristics of these patients according to the calendar period of diagnosis are presented in **Table 1**. Patient characteristics according to the disease stage are presented in **Supplemental Tables S2 and S3**. Most patients were diagnosed with limited-stage disease (59%), 39% had advanced-stage disease, and for 2%, the disease stage was unknown. The proportion of patients with advanced-stage disease increased from 31% in the period 1989-1999 to 48% in 2010-2017, primarily owing to an increase in stage IV disease and patients aged ≥50 years. Patients with limited-stage disease were younger compared to those with advanced-stage disease (median age, 36 *versus* 44 years; *P*<0.001). Overall, nodular sclerosis was the most common morphological subtype across both disease stages. The proportion of patients with this subtype decreased over time, following an increased proportion of patients with unclassified cHL. The other morphological subtypes remained relatively stable over the calendar periods studied. Lastly, B symptoms were more often present in patients with advanced-stage disease compared to those with limited-stage disease (56% *versus*

26% in 2010-2017; *P*<0.001). Of note, the distribution of B symptoms in earlier periods could not be established adequately since the number of unknown values was high.

Table 1. Characteristics of adult patients diagnosed with classical Hodgkin lymphoma in the Netherlands between 1989 and 2017.

Characteristics	Calendar period						Total	
	1989-1999		2000-2009		2010-2017			
	No.	(%)	No.	(%)	No.	(%)	No.	(%)
Total No. of patients	3527	-	3463	-	2995	-	9985	-
Sex								
Male	2032	(58)	1980	(57)	1676	(56)	5688	(57)
Female	1495	(42)	1483	(43)	1319	(44)	4297	(43)
Age, years								
Median age (range)	37 (18-96)		40 (18-94)		41 (18-98)		39 (18-98)	
18-29	1147	(33)	1041	(30)	889	(30)	3077	(31)
30-39	740	(21)	687	(20)	544	(18)	1971	(20)
40-49	529	(15)	521	(15)	399	(13)	1449	(15)
50-59	343	(10)	446	(13)	372	(12)	1161	(12)
60-69	366	(10)	361	(10)	376	(13)	1103	(11)
≥70	402	(11)	407	(12)	415	(14)	1224	(12)
Ann Arbor stage								
I	722	(20)	509	(15)	304	(10)	1535	(15)
II	1539	(44)	1586	(46)	1249	(42)	4374	(44)
III	694	(20)	805	(23)	675	(23)	2174	(22)
IV	397	(11)	527	(15)	740	(25)	1664	(17)
Unknown	175	(5)	36	(1)	27	(1)	238	(2)
Median age, years (range)								
Stage I/II	35 (18-93)		37 (18-93)		37 (18-98)		36 (18-98)	
Stage III/IV	41 (18-96)		44 (18-94)		46 (18-91)		44 (18-96)	
Morphological subtype								
Nodular sclerosis	2569	(73)	2397	(69)	1500	(50)	6466	(65)
Mixed cellularity	488	(14)	342	(10)	324	(11)	1154	(12)
Lymphocyte rich	69	(2)	104	(3)	137	(5)	310	(3)
Lymphocyte depleted	66	(2)	21	(1)	13	(0)	100	(1)
Not otherwise specified	335	(9)	599	(17)	1021	(34)	1955	(20)
B symptoms								
No	1095	(31)	1581	(46)	1664	(56)	4340	(43)
Yes	812	(23)	1153	(33)	1199	(40)	3164	(32)
Unknown	1620	(46)	729	(21)	132	(4)	2481	(25)

Incidence

The incidence rate of cHL remained comparatively steady over time, irrespective of age and sex (**Supplemental Table S4**). Interestingly, however, there was an overall modest decrease in the incidence of limited-stage disease, following an increase of advanced-stage disease. The overall age-standardized incidence rate (ASR) was 2.44/100,000 in 2010-2017, with corresponding rates of 2.71/100,000 and 2.17/100,000 for males and females, respectively. Before the age of 25, the incidence rate was comparable between both sexes (**Figure 1A**). After that age, there was a consistent male predominance. The incidence showed a bimodal age distribution for both sexes with the highest peak in the incidence in young adults that was more pronounced for limited-stage disease compared to advanced-stage disease (**Figure 1B**).

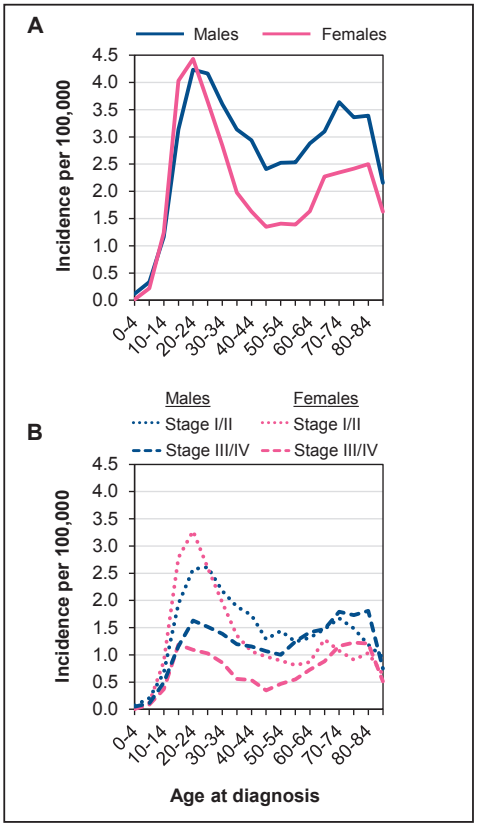


Figure 1. Age-specific incidence rates of patients with classical Hodgkin lymphoma in the Netherlands according to sex and disease stage, 1989–2017. The age-specific incidence rates are presented per 100,000 person-years. Panel **a** shows the age-specific incidence rates for the overall cohort according to sex, whereas Panel **b** is stratified according to sex and disease stage—that is, limited-stage (I/ II) and advanced-stage (III/IV).

Primary therapy of limited-stage cHL

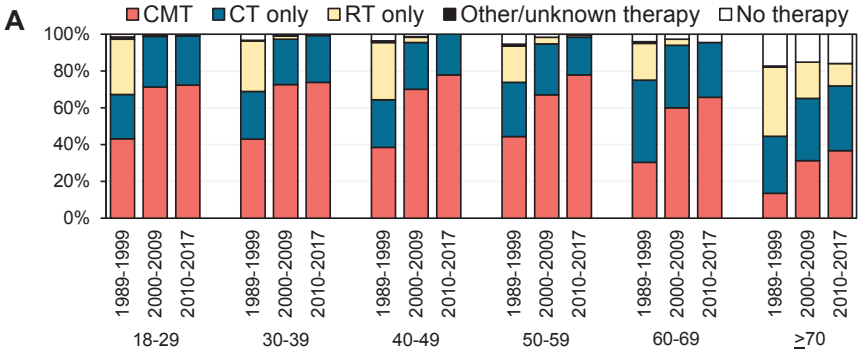
The distribution of primary therapy among adult patients with limited- and advanced-stage cHL is presented in **Figure 2A** and **Figure 2B**, respectively. Noteworthy is the application of radiotherapy alone in the first calendar period across all age groups in patients with limited-stage cHL. Its application, however, decreased dramatically over time, following an increased application of combined modality therapy. Moreover, radiotherapy alone was virtually not applied among patients aged 18-69 years since 2000, whereas 20% and 12% of patients aged ≥70 years diagnosed during 2000-2009 and 2010-2017 still received radiotherapy alone, respectively. The proportion of patients who received chemotherapy alone remained relatively stable over time across all age groups. Overall, the proportion of patients receiving no anti-neoplastic therapy was very low for patients aged 18-69 years compared to patients aged ≥70 years, of whom 16% of the latter group received no anti-neoplastic therapy in the calendar period 2010-2017.

Detailed data on primary therapy among 755 patients with limited-stage cHL, and 748 patients with advanced-stage, diagnosed between 2014-2017 is shown in **Figure 3A** and **Figure 3B**, respectively. For patients with limited-stage cHL, the vast majority of chemotherapy-treated patients were treated with ABVD, of whom most received it in combination with radiotherapy. Only a tiny proportion of patients up to age 60 received (escalated) BEACOPP. In contrast, treatment choices other than ABVD among patients aged ≥60 years included CHOP, a variety of less common chemotherapeutic regimens (**Supplemental Table S5**), and radiotherapy alone.

Primary therapy of advanced-stage cHL

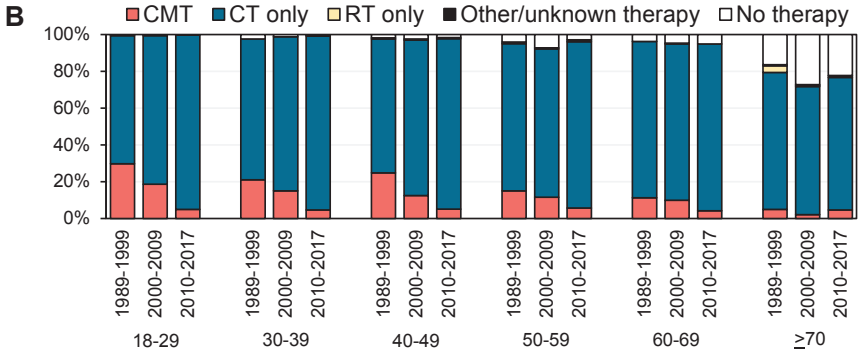
The vast majority of patients with advanced-stage cHL received chemotherapy only, of which its application gradually increased over time following the decreased application of combined modality therapy (**Figure 2B**). Similar to patients with limited-stage disease, patients aged ≥70 years more often received no anti-neoplastic therapy compared to their younger counterparts.

Almost 60% of patients with advanced-stage cHL aged 18-59 years received ABVD□of whom only a few received ABVD in combination with radiotherapy (**Figure 3B**). The majority of the remaining patients in that age group were initially treated with (escalated) BEACOPP. As expected, (escalated) BEACOPP was virtually not applied among patients aged ≥60 years. In line with patients aged ≥60 years with limited-stage disease, treatment choices other than ABVD included CHOP and a variety of less common chemotherapeutic regimens (**Supplemental Table S5**), but not radiotherapy alone.



Stage I/II **Age at diagnosis and calendar period of diagnosis**

Treatment	Column percentage																	
CMT	43	71	72	43	73	74	39	70	78	44	67	78	30	60	66	14	31	37
CT only	24	28	27	26	25	25	26	25	22	30	28	21	45	34	30	31	34	35
RT only	30	1	0	28	2	0	31	3	0	20	4	1	20	3	0	38	20	12
Other/unknown	1	0	1	0	0	0	1	0	0	1	0	0	1	0	0	1	0	0
No therapy	1	0	0	3	1	0	4	1	0	5	2	1	4	3	5	17	15	16



Stage III/IV **Age at diagnosis and calendar period of diagnosis**

Treatment	Column percentage																	
CMT	30	19	5	21	15	5	25	12	5	15	11	6	11	10	4	5	2	5
CT only	70	81	95	77	84	94	73	85	93	80	81	90	85	85	91	75	70	72
RT only	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	4	1	0
Other/unknown	0	0	0	0	0	0	1	0	1	0	1	1	0	0	0	1	1	1
No therapy	1	1	0	3	1	0	2	2	2	4	7	3	4	5	5	16	27	22

Figure 2. Primary therapy of adult patients with classical Hodgkin lymphoma in the Netherlands according to age at diagnosis and calendar period of diagnosis, 1989–2017. Panels **a** and **b** show the results for patients with limited-stage (I/II) and advanced-stage (III/IV) disease, respectively. The absolute number of patients within a specific stage and age group is shown in Supplemental Table S6 for limited-stage disease and Supplemental Table S7 for advanced-stage disease. *Abbreviations: CMT combined modality therapy (i.e., chemotherapy with radiotherapy), CT chemotherapy, and RT radiotherapy.*

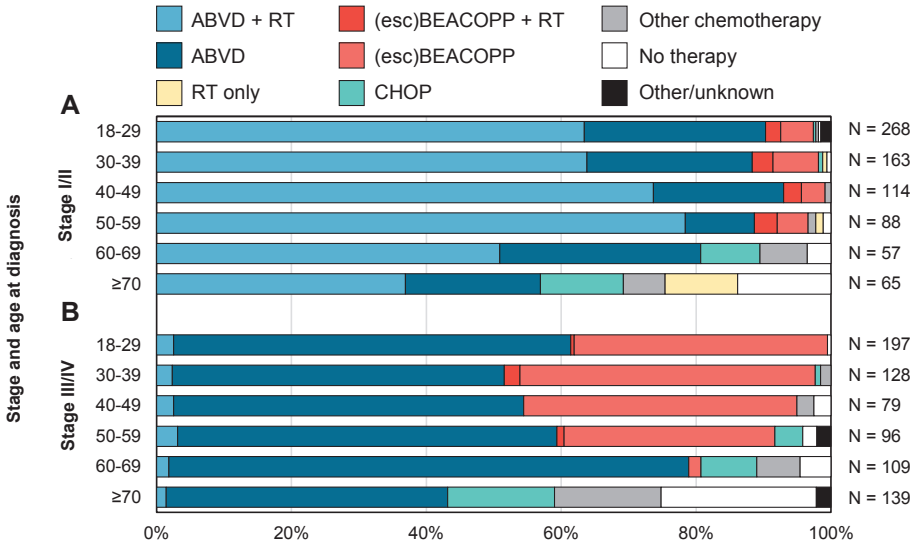
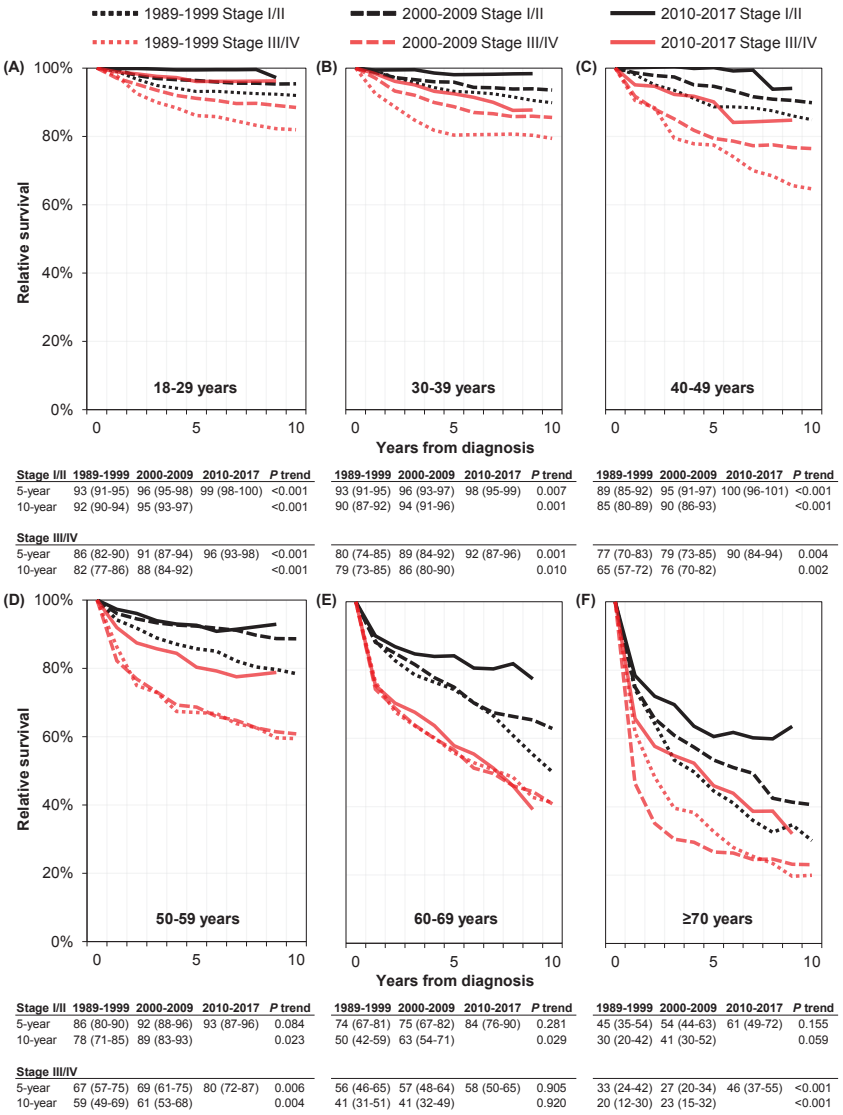


Figure 3. Primary therapy of adult patients with classical Hodgkin lymphoma in the Netherlands according to disease stage and age at diagnosis, 2014–2017. Panels **a** and **b** show the results for patients with limited-stage (I/II) and advanced-stage (III/IV) disease, respectively. The absolute number of patients within a specific stage and age group is shown in Supplemental Table S8. The group of other or unknown therapy (n=44) includes a variety of modalities and is delineated in Supplemental Table S5. *Abbreviations: ABVD adriamycin, bleomycin, vinblastine, and dacarbazine, RT radiotherapy, BEACOPP bleomycin, etoposide, adriamycin, cyclophosphamide, vincristine, procarbazine, and prednisone, and CHOP cyclophosphamide, adriamycin, vincristine, and prednisone.*

Relative survival of limited-stage cHL

As shown in **Figure 4**, RS rates (RSRs) up to ten years after diagnosis were relatively high over the calendar periods studied for patients up to age 60. However, patients aged ≥30 years diagnosed during the first calendar period (1989-1999), especially those aged ≥50 years, continued to experience considerable excess mortality after ten years since diagnosis (**Supplemental Figure S1**). Encouragingly enough, 5-year RSRs improved significantly over the three calendar periods studied (**Figure 4**). However, statistically significant improvements were restricted to patients up to age 50. Of note, patients up to age 50 diagnosed during 2010-2017 virtually experienced no excess mortality within the first five years after diagnosis. Furthermore, RS was substantially lower among patients aged ≥50 years, especially among patients aged ≥60 years, compared to their younger counterparts. As for 10-year RSRs, it improved between the calendar periods 1989-1999 and 2000-2009 for patients up to age 70.

Overall, when adjusted for age, sex, disease stage, and years of follow-up, patients diagnosed in 2010-2017 had 41% lower excess mortality compared to patients diagnosed in 2000-2009 (EMRR, 0.59; 95% CI, 0.45-0.77; $P<0.001$). Furthermore, there was an independent poor prognostic effect of male sex, older age, and stage II disease compared to stage I disease (**Table 2**).



Stage I/II	1989-1999	2000-2009	2010-2017	*P* trend	1989-1999	2000-2009	2010-2017	*P* trend	1989-1999	2000-2009	2010-2017	*P* trend
5-year	93 (91-95)	96 (95-98)	99 (98-100)	<0.001	93 (91-95)	96 (93-97)	98 (95-99)	0.007	89 (85-92)	95 (91-97)	100 (96-101)	<0.001
10-year	92 (90-94)	95 (93-97)		<0.001	90 (87-92)	94 (91-96)		0.001	85 (80-89)	90 (86-93)		<0.001
Stage III/IV												
5-year	86 (82-90)	91 (87-94)	96 (93-98)	<0.001	80 (74-85)	89 (84-92)	92 (87-96)	0.001	77 (70-83)	79 (73-85)	90 (84-94)	0.004
10-year	82 (77-86)	88 (84-92)		<0.001	79 (73-85)	86 (80-90)		0.010	65 (57-72)	76 (70-82)		0.002

Stage I/II	1989-1999	2000-2009	2010-2017	*P* trend	1989-1999	2000-2009	2010-2017	*P* trend	1989-1999	2000-2009	2010-2017	*P* trend
5-year	86 (80-90)	92 (88-96)	93 (87-96)	0.084	74 (67-81)	75 (67-82)	84 (76-90)	0.281	45 (35-54)	54 (44-63)	61 (49-72)	0.155
10-year	78 (71-85)	89 (83-93)		0.023	50 (42-59)	63 (54-71)		0.029	30 (20-42)	41 (30-52)		0.059
Stage III/IV												
5-year	67 (57-75)	69 (61-75)	80 (72-87)	0.006	56 (46-65)	57 (48-64)	58 (50-65)	0.905	33 (24-42)	27 (20-34)	46 (37-55)	<0.001
10-year	59 (49-69)	61 (53-68)		0.004	41 (31-51)	41 (32-49)		0.920	20 (12-30)	23 (15-32)		<0.001

Figure 4. Relative survival of patients with limited-stage (I/II) and advanced-stage (III/IV) classical Hodgkin lymphoma in the Netherlands according to age at diagnosis and calendar period of diagnosis, 1989–2017. Relative survival is shown for three calendar periods according to the following six age categories: (**a**) 18–29, (**b**) 30–39, (**c**) 40–49, (**d**) 50–59, (**e**) 60–69, and (**f**) ≥70 years. The tables present the projected 5- and 10-year relative survival rates (RSRs) with 95% confidence intervals (CIs) according to the calendar period of diagnosis for the six age categories. Relative survival is the observed patient survival (i.e., overall survival) corrected for the expected survival of a comparable group in the general population, matched to the patients by age, sex, and year of diagnosis. For readers interested in the dynamics of relative survival, we have plotted the relative survival, overall survival, and expected survival for the most recent calendar period (2010–2017) in Supplemental Fig. S2. *P value for likelihood ratio test assessing linear trends between the first and last calendar period.

Relative survival of advanced-stage cHL

RS was generally lower for patients with advanced-stage disease compared to patients with limited-stage disease (**Figure 4**). Nevertheless, 5- and 10-year RS increased over time for patients with advanced-stage disease across all age groups, except for patients aged 60-69 years. Similar to limited-stage disease, RS decreased with older age and was the lowest for the oldest age group. Interestingly, patients up to age 30 diagnosed during 1989-1999 virtually experienced no excess mortality after ten years since diagnosis, indicated by a plateau in RS. In contrast, patients aged ≥30 years, especially those aged ≥40 years, had ongoing excess mortality after ten years since diagnosis (**Supplemental Figure S1**).

Patients with advanced-stage disease diagnosed in 2010-2017 had 39% (EMRR, 0.61; 95% CI, 0.52-0.71; *P*<0.001) lower excess mortality compared to patients diagnosed in 2000-2009. Older age and stage IV disease compared to stage III disease were independent factors associated with inferior outcome. Of note, there was no indication that the EMRR was different between 1989-1999 and 2000-2009 (EMRR, 1.09; 95% CI, 0.94-1.26; *P*=0.253; **Table 2**). This finding indicates that there was less, if any, overall improvement in survival among patients with advanced-stage disease between 1989-1999 and 2000-2009.

Table 2. Excess mortality rate ratios (EMRRs), with associated 95% confidence intervals, during the first ten years after Hodgkin lymphoma diagnosis, stratified by limited- (i.e., stage I/II) and advanced-stage (i.e., stage III/IV) disease. EMRRs are presented according to years of follow-up, calendar period of diagnosis, sex, age at diagnosis, and disease stage.

Covariate	Limited-stage disease					Advanced-stage disease				
	EMR[a]	95% CI			*P*[b]	EMR[a]	95% CI			*P*[b]
Years from diagnosis										
0-1	1	(ref)				1	(ref)			
1-2	0.66	0.53	-	0.83	<0.001	0.43	0.36	-	0.52	<0.001
2-4	0.49	0.40	-	0.61	<0.001	0.28	0.24	-	0.34	<0.001
4-6	0.35	0.27	-	0.45	<0.001	0.22	0.18	-	0.28	<0.001
6-8	0.34	0.26	-	0.46	<0.001	0.16	0.12	-	0.21	<0.001
8-10	0.30	0.21	-	0.42	<0.001	0.14	0.10	-	0.20	<0.001
Period of diagnosis					**<0.001**[c]					**<0.001**[c]
1989-1999	1.49	1.26	-	1.77	<0.001	1.09	0.94	-	1.26	0.253
2000-2009	1	(ref)				1	(ref)			
2010-2017	0.59	0.45	-	0.77	<0.001	0.61	0.52	-	0.71	<0.001
Sex					**0.003**[c]					**0.399**[c]
Male	1	(ref)				1	(ref)			
Female	0.79	0.67	-	0.93	0.004	0.95	0.83	-	1.08	0.409
Age at diagnosis, years					**<0.001**[c]					**<0.001**[c]
18-29	1	(ref)				1	(ref)			
30-39	1.34	0.99	-	1.81	0.062	1.33	1.00	-	1.77	0.054
40-49	2.02	1.47	-	2.76	<0.001	2.30	1.76	-	3.02	<0.001

Table 2. Excess mortality rate ratios (EMRRs), with associated 95% confidence intervals, during the first ten years after Hodgkin lymphoma diagnosis, stratified by limited- (i.e., stage I/II) and advanced-stage (i.e., stage III/IV) disease. EMRRs are presented according to years of follow-up, calendar period of diagnosis, sex, age at diagnosis, and disease stage. (*continued*)

Covariate	Limited-stage disease					Advanced-stage disease				
	EMR[a]	95% CI			P[b]	EMR[a]	95% CI			P[b]
50-59	3.16	2.30	-	4.34	<0.001	3.59	2.77	-	4.65	<0.001
60-69	9.37	7.19	-	12.23	<0.001	6.70	5.28	-	8.50	<0.001
≥70	21.74	16.87	-	28.00	<0.001	13.24	10.54	-	16.62	<0.001
Stage					**<0.001[c]**					**<0.001[c]**
I	1	(ref)				-		-		-
II	1.41	1.18	-	1.69	<0.001	-		-		-
III	-		-		-	1	(ref)			
IV	-		-		-	1.56	1.37	-	1.77	<0.001

EMRR, excess mortality rate ratio; CI, confidence interval; ref, Reference

[a]All covariates are simultaneously adjusted.

[b]*P*-values are compared with the reference category.

[c]*P*-values denoted in bold are derived from the likelihood ratio test that compares the model without the specific covariate with the model containing all covariates

DISCUSSION

In this large, comprehensive, nationwide, population-based study among adult patients with cHL, we show changes in the application of different first-line treatment strategies over time and an improvement in RS for most, but not all, patients. Furthermore, this population-based study complements, but more importantly, extends on prior, relatively outdated population-based series,[21-23,25,33] because we included patients diagnosed in a contemporary era and had comprehensive information on primary therapy for individual patients.

The incidence rates of cHL in the Netherlands are mainly congruent with other epidemiological studies.[1,23,34,35] Interestingly though, we demonstrated that the peak in incidence for young adults was more profound among patients with limited-stage disease. The increase in incidence for advanced-stage, following a modest decrease for limited-stage disease, is probably related to the implementation of the PET-CT scan for staging. PET-CT can more accurately detect small extranodal lesions compared to CT only, which, in turn, may result in stage migration.[11]

We observed a substantial decline in the use of radiotherapy only, followed by an increased use of combined modality therapy for patients up to age 70 with limited-stage disease. This finding agrees with the notion that the combination of ABVD and radiotherapy is essential for proper disease control, since several studies have demonstrated that omitting radiotherapy increases the risk of relapse, even in patients with limited-stage disease that have a negative PET-CT scan after two cycles of ABVD.[12-14] For patients with advanced-stage disease, chemotherapy without radiotherapy was the preferred modality. Detailed data for patients diagnosed as from 2014 showed that ABVD was the preferred chemotherapeutic regimen for these patients, fol-

lowed by (escalated) BEACOPP for patients up to age 60. Nowadays, PET-guided treatment for advanced-stage or early-stage unfavorable disease is becoming the standard treatment strategy. This strategy is likely to provide an advantage for high-risk patients, who can escalate to BEACOPP in case of inadequate response on ABVD.[30]

Recent clinical trials that accrued patients with limited-stage disease treated with ABVD and radiotherapy reported OS rates exceeding 95% at 5 years.[12-14] In addition, a population-based study in Sweden and Norway also reported that there was no long-term excess mortality for limited-stage, favorable cHL patients diagnosed between 1999-2005.[27] For patients with advanced-stage disease who were treated with ABVD or (escalated) BEACOPP, 5-year OS rates approximate 90%.[9,10] We could confirm the excellent survival outcomes reported by clinical trials for patients up to age 60 diagnosed during 2010-2017. Significant improvements over time in RSRs were observed for all patients up to age 60, irrespective of stage. Interestingly, a plateau in RS was shortly observed after diagnosis in the most recent calendar period (2010-2017) among patients up to age 60. This finding suggests that these patients eventually do not experience excess mortality compared to the general population. Extended follow-up is, however, needed to evaluate long-term excess mortality (due to late treatment-related sequelae) in contemporary treated patients. Nevertheless, long-term excess mortality is expected to be low for patients with limited-stage disease because of the widespread application of INRT after chemotherapy.

Although cHL is often portrayed as a malignancy that can be successfully treated, this appears to only hold for patients up to age 60. More specifically, patients aged ≥60 years show little, if any, improvement in RS over time and continue to experience considerable excess mortality, especially patients with advanced-stage disease. Elderly patients were often underrepresented in the aforementioned clinical trials, especially in trials for patients with advanced-stage disease. Patients aged ≥60 years are often excluded from clinical trial participation due to concerns related to treatment-related sequelae associated with more intensive chemotherapeutic regimens, such as (escalated) BEACOPP.[9,10] Indeed, in line with treatment recommendations,[36] (escalated) BEACOPP was rarely applied among patients aged ≥60 years. Collectively, the vast majority of elderly patients did not seem to benefit from the advances in treatment to the same extent as their younger counterparts. Therefore, new effective and less toxic therapies are needed to reduce excess mortality in these patients. Recently, it has been reported that omitting bleomycin from ABVD after a good response on interim PET-CT may reduce toxicity without compromising efficacy.[37] Our study thus serves as a benchmark to assess the impact of a broader application of this strategy on population-level survival.

Approximately 15-30% of patients are primary refractory to first-line treatment or relapse after an initial response.[9,12,15,37] With high-dose chemotherapy and autologous stem-cell transplant, around 40-60% of these patients can be cured.[29,38] Novel treatment strategies for salvage treatment, such as targeted treatment with brentuximab vedotin or checkpoint inhibitors, could therefore have a significant impact on long-term survival of cHL.[16-19] Besides, these new agents

are currently investigated in clinical trials as first-line treatment for elderly patients, to prevent them from toxicity due to chemotherapy.[39]

Limitations of this population-based study are that detailed data on the type of chemotherapy regimen were only available from the year of diagnosis 2014 onwards and that there are no data on salvage treatment after relapsed or primary refractory disease. Therefore it is currently not known how advances in salvage treatment have contributed to the RS. Besides, some small improvements in RS were not statistically significant, which could be due to lower numbers of patients in certain subgroups.

The strengths of our study include the use of a nationwide population-based cancer registry with high coverage (i.e., >95%) of all newly diagnosed malignancies in the Netherlands. Therefore, our study represents the general population of cHL. Also, we had information on patient characteristics and primary therapy available for all individual patients. Besides, we used RS as a measure of disease-specific survival and had adequate survival follow-up for all patients.

In summary, in this large, nationwide population-based study, patients up to age 60 who were diagnosed with cHL between 2010-2017 have better RS compared to patients diagnosed before 2010, irrespective of stage. The improvements are likely related to advances in therapy across various lines of treatment and improved supportive care. These achievements, however, do not seem to translate into significant benefits for patients aged ≥60 years, as these patients still experience considerable excess mortality in contemporary clinical practice. Therefore, novel treatment strategies are urgently needed to reduce excess mortality in elderly patients.

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CONFLICT OF INTEREST

There is no financial support for this work that could have influenced the outcomes described in the manuscript. However, particular authors report a potential conflict of interest, which is described below.

Kersten: Millennium/Takeda: Honoraria, Research Funding; Celgene: Honoraria, Research Funding; Roche: Honoraria, Research Funding; Gilead: Honoraria; Kite Pharma: Honoraria; Novartis: Honoraria. **Lugtenburg:** Millennium/Takeda: Consultancy, Research Funding; Servier: Consultancy, Research Funding; Roche: Consultancy; BMS: Consultancy; Sandoz: Consultancy;

Genmab: Consultancy. **Zijlstra:** Consultant/Advisor: Gilead, Roche, Takeda; Honoraria: Gilead, Roche, Takeda, Janssen. **Dinmohamed:** BMS: Research funding. All remaining authors have declared no competing financial interests.

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SUPPLEMENTARY MATERIAL

Supplemental Table S1. ICD-O morphology codes used to select cases of classical Hodgkin lymphoma from the Netherlands Cancer Registry

Subtype	ICD-O code
HL, NOS	9650
Lymphocyte-rich cHL	9651
HL, lymphocytic predominance, NOS	9657
HL, lymphocytic predominance, diffuse	9658
Mixed cellularity cHL	9652
Lymphocytic depletion	9653
Lymphocyte depletion, diffuse fibrosis	9654
Lymphocyte depletion, reticular	9655
Nodular sclerosis	9656, 9663
Nodular sclerosis, cellular phase (1992-2009)	9664
Nodular sclerosis, grade I (2001-2009)	9665, 9667
Nodular sclerosis, mixed cellularity (1992-2000)	9666

Abbreviations: ICD-O, International Classification of Diseases for Oncology; HL, Hodgkin lymphoma; cHL; classical Hodgkin lymphoma; NOS, not otherwise specified

Supplemental Table S2. Characteristics of patients with limited-stage classical Hodgkin lymphoma

Characteristics	Calendar period						Total	
	1989-1999		2000-2009		2010-2017			
	No.	(%)	No.	(%)	No.	(%)	No.	(%)
Total No. of patients	2261	-	2095	-	1553	-	5909	-
Sex								
Male	1240	(55)	1130	(54)	797	(51)	3167	(54)
Female	1021	(45)	965	(46)	756	(49)	2742	(46)
Age, years								
Median (range)	35 (18-93)		37 (18-93)		37 (18-98)		36 (18-98)	
18-29	811	(36)	705	(34)	541	(35)	2057	(35)
30-39	515	(23)	446	(21)	306	(20)	1267	(21)
40-49	340	(15)	318	(15)	217	(14)	875	(15)
50-59	203	(9)	249	(12)	195	(13)	647	(11)
60-69	201	(9)	185	(9)	155	(10)	541	(9)
≥70	191	(8)	192	(9)	139	(9)	522	(9)
Ann Arbor stage								
I	722	(32)	509	(24)	304	(20)	1535	(26)
II	1539	(68)	1586	(76)	1249	(80)	4374	(74)
Morphological subtype								
Nodular sclerosis	1737	(77)	1501	(72)	848	(55)	4086	(69)
Mixed cellularity	292	(13)	185	(9)	150	(10)	627	(11)
Lymphocyte rich	54	(2)	81	(4)	81	(5)	216	(4)
Lymphocyte depleted	32	(1)	2	(0)	4	(0)	38	(1)
Not otherwise specified	146	(6)	326	(16)	470	(30)	942	(16)
B symptoms								
No	884	(39)	1206	(58)	1108	(71)	3198	(54)
Yes	422	(19)	465	(22)	397	(26)	1284	(22)
Unknown	955	(42)	424	(20)	48	(3)	1427	(24)

Supplemental Table S3. Characteristics of patients with advanced-stage classical Hodgkin lymphoma

Characteristics	Calendar period						Total	
	1989-1999		2000-2009		2010-2017			
	No.	(%)	No.	(%)	No.	(%)	No.	(%)
Total No. of patients	1091	-	1332	-	1415	-	3838	-
Sex								
Male	690	(63)	839	(63)	867	(61)	2396	(62)
Female	401	(37)	493	(37)	548	(39)	1442	(38)
Age, years								
Median (range)	41 (18-96)		44 (18-94)		46 (18-91)		44 (18-96)	
18-29	306	(28)	333	(25)	347	(25)	986	(26)
30-39	200	(18)	234	(18)	236	(17)	670	(17)
40-49	166	(15)	202	(15)	180	(13)	548	(14)
50-59	120	(11)	192	(14)	177	(13)	489	(13)
60-69	134	(12)	173	(13)	214	(15)	521	(14)
≥70	165	(15)	198	(15)	261	(18)	624	(16)
Ann Arbor stage								
III	694	(64)	805	(60)	675	(48)	2174	(57)
IV	397	(36)	527	(40)	740	(52)	1664	(43)
Morphological subtype								
Nodular sclerosis	748	(69)	884	(66)	643	(45)	2275	(59)
Mixed cellularity	158	(14)	155	(12)	173	(12)	486	(13)
Lymphocyte rich	12	(1)	22	(2)	56	(4)	90	(2)
Lymphocyte depleted	29	(3)	19	(1)	9	(1)	57	(1)
Not otherwise specified	144	(13)	252	(19)	534	(38)	930	(24)
B symptoms								
No	205	(19)	372	(28)	548	(39)	1125	(29)
Yes	385	(35)	686	(52)	799	(56)	1870	(49)
Unknown	501	(46)	274	(21)	68	(5)	843	(22)

Supplemental Table S4. Age-specific incidence rates according to sex, stage, and calendar period of diagnosis

		Age at diagnosis, years							Total
Sex	**Calendar period**	**0-14**	**15-29**	**30-39**	**40-49**	**50-59**	**60-69**	**≥70**	
Male (all stages)	1989-1999	0.57	3.52	3.27	2.69	2.54	3.15	3.52	2.65
	2000-2009	0.52	4.10	3.23	2.80	2.60	2.85	3.10	2.68
	2010-2017	0.53	4.03	3.67	2.51	2.40	2.89	3.45	2.71
Female (all stages)	1989-1999	0.46	3.58	2.14	1.60	1.24	1.99	2.35	1.99
	2000-2009	0.51	4.16	2.28	1.38	1.48	1.92	2.24	2.03
	2010-2017	0.52	4.44	2.92	1.47	1.52	1.87	2.18	2.17
Stage I/II	1989-1999	0.37	2.53	1.88	1.38	1.11	1.40	1.35	1.49
	2000-2009	0.32	2.78	1.78	1.28	1.14	1.22	1.24	1.43
	2010-2017	0.28	2.59	1.86	1.09	1.03	0.99	0.95	1.27
Stage III/ IV	1989-1999	0.14	0.93	0.73	0.67	0.66	0.94	1.16	0.70
	2000-2009	0.19	1.34	0.94	0.81	0.88	1.13	1.29	0.90
	2010-2017	0.25	1.65	1.43	0.89	0.93	1.35	1.75	1.14
Total	1989-1999	0.52	3.55	2.70	2.15	1.89	2.57	2.94	2.32
	2000-2009	0.51	4.13	2.75	2.09	2.04	2.39	2.67	2.35
	2010-2017	0.53	4.23	3.30	1.99	1.96	2.38	2.81	2.44

Incidence rates are presented per 100,000 person-years

Supplemental Table S5. Specification of other types of chemotherapy, 2014-2017

Therapy	**Stage I/II**	**Stage III/ IV**	**Total**
ChlVPP	1	9	10
LOPP	2	3	5
PECC	3	5	8
CHOP-like	2	4	6
DHAP	0	5	5
AVD	2	2	4
Etoposide	1	2	3
EBVP	0	1	1
CVP	0	1	1
Vinblastine	0	1	1
Total	**11**	**33**	**44**

Abbreviations: ChlVPP, chlorambucil, vinblastine, procarbazine and prednisone; LOPP, lomustine, vincristine, procarbazine and prednisone; PECC, prednisone, etoposide, lomustine, chlorambucil; CHOP, cyclophosphamide, doxorubicin, vincristine and prednisone; DHAP, dexamethason, cytarabine and cisplatin; AVD, adriamycin vinblastine, dacarbazine; EBVP, epirubicin, bleomycin, vinblastine and prednisone; and CVP, cyclophosphamide, vincristine, prednisone.

Supplemental Table S6. Primary therapy among adult patients with limited-stage disease, 1989-2017

Age at diagnosis, years	Calendar period of diagnosis	Primary therapy										Total
		CMT		CT only		RT only		No therapy		Other/ unknown		
		N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	N
18-29	1989-1999	350	(43)	196	(24)	243	(30)	12	(1)	10	(1)	811
	2000-2009	503	(71)	194	(28)	5	(1)	2	(0)	1	(0)	705
	2010-2017	392	(72)	144	(27)	0	(0)	1	(0)	4	(1)	541
	Total	**1245**	(61)	**534**	(26)	**248**	(12)	**15**	(1)	**15**	(1)	**2057**
30-39	1989-1999	222	(43)	133	(26)	142	(28)	17	(3)	1	(0)	515
	2000-2009	324	(73)	110	(25)	8	(2)	3	(1)	1	(0)	446
	2010-2017	226	(74)	78	(25)	1	(0)	1	(0)	0	(0)	306
	Total	**772**	(61)	**321**	(25)	**151**	(12)	**21**	(2)	**2**	(0)	**1267**
40-49	1989-1999	131	(39)	88	(26)	106	(31)	12	(4)	3	(1)	340
	2000-2009	223	(70)	81	(25)	9	(3)	4	(1)	1	(0)	318
	2010-2017	169	(78)	48	(22)	0	(0)	0	(0)	0	(0)	217
	Total	**523**	(60)	**217**	(25)	**115**	(13)	**16**	(2)	**4**	(0)	**875**
50-59	1989-1999	90	(44)	60	(30)	40	(20)	11	(5)	2	(1)	203
	2000-2009	167	(67)	69	(28)	9	(4)	4	(2)	0	(0)	249
	2010-2017	152	(78)	40	(21)	2	(1)	1	(1)	0	(0)	195
	Total	**409**	(63)	**169**	(26)	**51**	(8)	**16**	(2)	**2**	(0)	**647**
60-69	1989-1999	61	(30)	90	(45)	40	(20)	8	(4)	2	(1)	201
	2000-2009	111	(60)	63	(34)	6	(3)	5	(3)	0	(0)	185
	2010-2017	102	(66)	46	(30)	0	(0)	7	(5)	0	(0)	155
	Total	**274**	(51)	**199**	(37)	**46**	(9)	**20**	(4)	**2**	(0)	**541**
≥70	1989-1999	26	(14)	59	(31)	72	(38)	33	(17)	1	(1)	191
	2000-2009	60	(31)	65	(34)	38	(20)	29	(15)	0	(0)	192
	2010-2017	51	(37)	49	(35)	17	(12)	22	(16)	0	(0)	139
	Total	**137**	(26)	**173**	(33)	**127**	(24)	**84**	(16)	**1**	(0)	**522**
Total	1989-1999	880	(39)	626	(28)	643	(28)	93	(4)	19	(1)	2 261
	2000-2009	1,388	(66)	582	(28)	75	(4)	47	(2)	3	(0)	2 095
	2010-2017	1,092	(70)	405	(26)	20	(1)	32	(2)	4	(0)	1 553
	Total	**3 360**	(57)	**1 613**	(27)	**738**	(12)	**172**	(3)	**26**	(0)	**5909**

Abbreviations: CMT, combined modality therapy (i.e. chemotherapy with radiotherapy); CT, chemotherapy; and RT, radiotherapy.

Supplemental Table S7. Primary therapy among adult patients with advanced-stage disease, 1989-2017

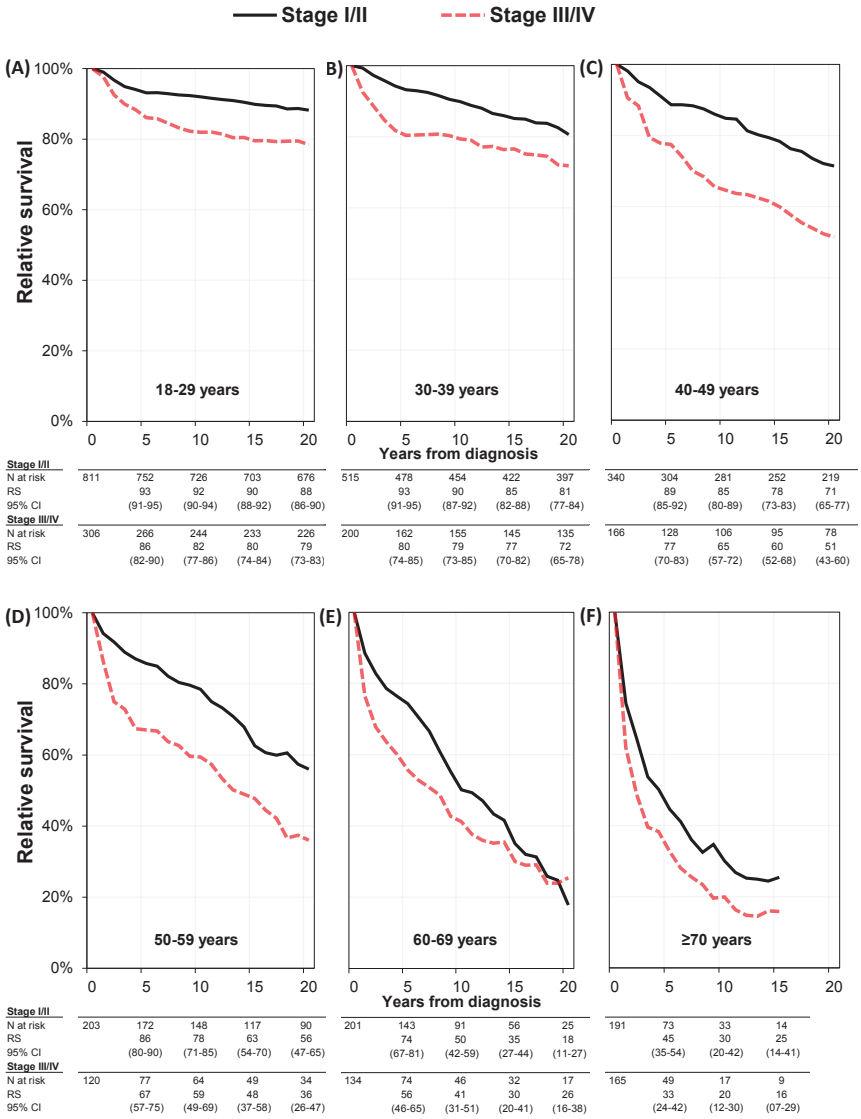
Age at diagnosis, years	Calendar period of diagnosis	Primary therapy										Total
		CMT		CT only		RT only		No therapy		Other/ unknown		
		N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	N
18-29	1989-1999	91	(30)	213	(70)	0	(0)	2	(1)	0	(0)	306
	2000-2009	62	(19)	269	(81)	0	(0)	2	(1)	0	(0)	333
	2010-2017	17	(5)	329	(95)	0	(0)	1	(0)	0	(0)	347
	Total	**170**	(17)	**811**	(82)	**0**	(0)	**5**	(1)	**0**	(0)	**986**
30-39	1989-1999	42	(21)	153	(77)	0	(0)	5	(3)	0	(0)	200
	2000-2009	35	(15)	196	(84)	0	(0)	3	(1)	0	(0)	234
	2010-2017	11	(5)	223	(94)	0	(0)	1	(0)	1	(0)	236
	Total	**88**	(13)	**572**	(85)	**0**	(0)	**9**	(1)	**1**	(0)	**670**
40-49	1989-1999	41	(25)	121	(73)	0	(0)	3	(2)	1	(1)	166
	2000-2009	25	(12)	171	(85)	0	(0)	5	(2)	1	(0)	202
	2010-2017	9	(5)	167	(93)	0	(0)	3	(2)	1	(1)	180
	Total	**75**	(14)	**459**	(84)	**0**	(0)	**11**	(2)	**3**	(1)	**548**
50-59	1989-1999	18	(15)	96	(80)	1	(1)	5	(4)	0	(0)	120
	2000-2009	22	(11)	155	(81)	0	(0)	14	(7)	1	(1)	192
	2010-2017	10	(6)	160	(90)	0	(0)	5	(3)	2	(1)	177
	Total	**50**	(10)	**411**	(84)	**1**	(0)	**24**	(5)	**3**	(1)	**489**
60-69	1989-1999	15	(11)	114	(85)	0	(0)	5	(4)	0	(0)	134
	2000-2009	17	(10)	147	(85)	1	(1)	8	(5)	0	(0)	173
	2010-2017	9	(4)	194	(91)	0	(0)	11	(5)	0	(0)	214
	Total	**41**	(8)	**455**	(87)	**1**	(0)	**24**	(5)	**0**	(0)	**521**
≥70	1989-1999	8	(5)	123	(75)	6	(4)	27	(16)	1	(1)	165
	2000-2009	4	(2)	138	(70)	1	(1)	54	(27)	1	(1)	198
	2010-2017	12	(5)	188	(72)	0	(0)	58	(22)	3	(1)	261
	Total	**24**	(4)	**449**	(72)	**7**	(1)	**139**	(22)	**5**	(1)	**624**
Total	1989-1999	215	(20)	820	(75)	7	(1)	47	(4)	2	(0)	1091
	2000-2009	165	(12)	1 076	(81)	2	(0)	86	(6)	3	(0)	1332
	2010-2017	68	(5)	1 261	(89)	0	(0)	79	(6)	7	(0)	1415
	Total	**448**	(12)	**3 157**	(82)	**9**	(0)	**212**	(6)	**12**	(0)	**3838**

Abbreviations: CMT, combined modality therapy (i.e. chemotherapy with radiotherapy); CT, chemotherapy; and RT, radiotherapy.

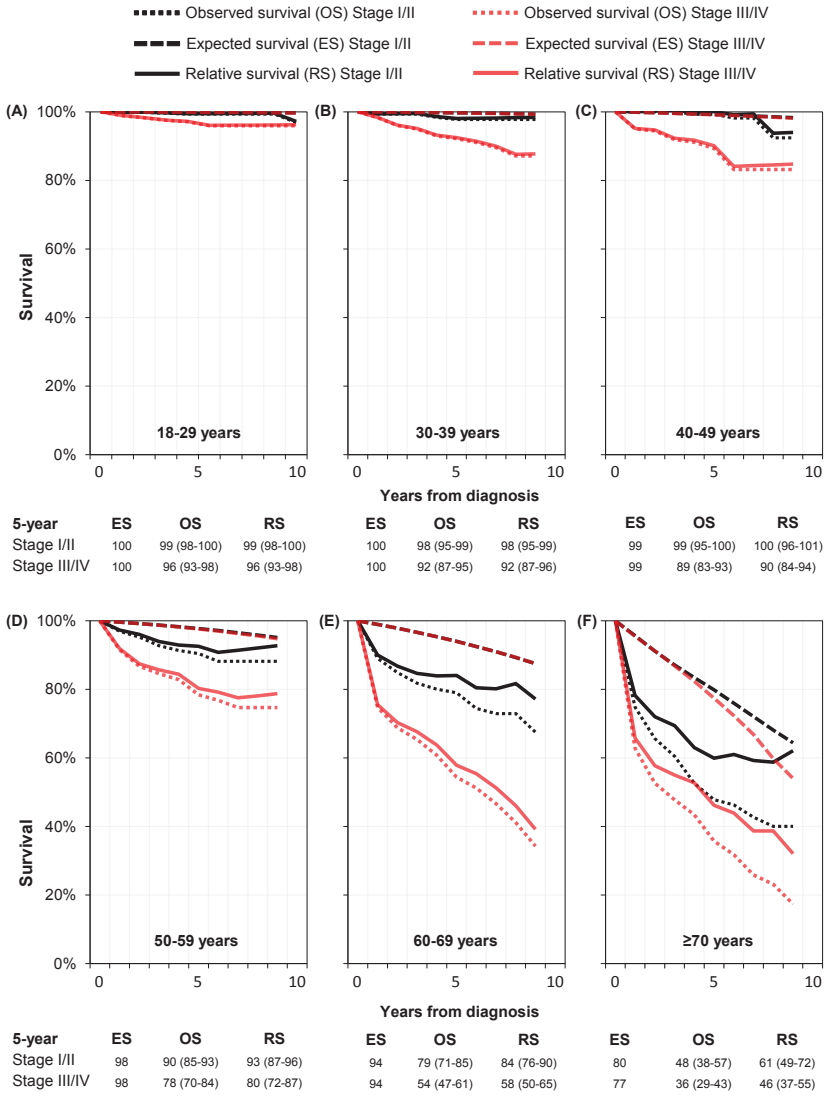
Supplemental Table S8. Primary therapy among adult patients with classical Hodgkin lymphoma, 2014-2017

		ABVD + RT		ABVD		(esc) BEACOPP + RT		(esc) BEACOPP		CHOP		Other CT		RT only		No therapy		Other/ unknown		Total
		N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	N
Stage I/II	18-29	170	(63)	72	(27)	6	(2)	13	(5)	1	(0)	1	(0)	0	(0)	1	(0)	4	(1)	**268**
	30-39	104	(64)	40	(25)	5	(3)	11	(7)	1	(1)	0	(0)	1	(1)	1	(1)	0	(0)	**163**
	40-49	84	(74)	22	(19)	3	(3)	4	(4)	0	(0)	1	(1)	0	(0)	0	(0)	0	(0)	**114**
	50-59	69	(78)	9	(10)	3	(3)	4	(5)	0	(0)	1	(1)	1	(1)	1	(1)	0	(0)	**88**
	60-69	29	(51)	17	(30)	0	(0)	0	(0)	5	(9)	4	(7)	0	(0)	2	(4)	0	(0)	**57**
	≥70	24	(37)	13	(20)	0	(0)	0	(0)	8	(12)	4	(6)	7	(11)	9	(14)	0	(0)	**65**
	Total	**480**	(64)	**173**	(23)	**17**	(2)	**32**	(4)	**15**	(2)	**11**	(1)	**9**	(1)	**14**	(2)	**4**	(1)	**755**
Stage III/IV	18-29	5	(3)	116	(59)	1	(1)	74	(38)	0	(0)	0	(0)	0	(0)	1	(1)	0	(0)	**197**
	30-39	3	(2)	63	(49)	3	(2)	56	(44)	1	(1)	2	(2)	0	(0)	0	(0)	0	(0)	**128**
	40-49	2	(3)	41	(52)	0	(0)	32	(41)	0	(0)	2	(3)	0	(0)	2	(3)	0	(0)	**79**
	50-59	3	(3)	54	(56)	1	(1)	30	(31)	4	(4)	0	(0)	0	(0)	2	(2)	2	(2)	**96**
	60-69	2	(2)	84	(77)	0	(0)	2	(2)	9	(8)	7	(6)	0	(0)	5	(5)	0	(0)	**109**
	≥70	2	(1)	58	(42)	0	(0)	0	(0)	22	(16)	22	(16)	0	(0)	32	(23)	3	(2)	**139**
	Total	**17**	(2)	**416**	(56)	**5**	(1)	**194**	(26)	**36**	(5)	**33**	(4)	**0**	(0)	**42**	(6)	**5**	(1)	**748**
All stages (I-IV)	18-29	175	(38)	188	(40)	7	(2)	87	(19)	1	(0)	1	(0)	0	(0)	2	(0)	4	(1)	**465**
	30-39	107	(37)	103	(35)	8	(3)	67	(23)	2	(1)	2	(1)	1	(0)	1	(0)	0	(0)	**291**
	40-49	86	(45)	63	(33)	3	(2)	36	(19)	0	(0)	3	(2)	0	(0)	2	(1)	0	(0)	**193**
	50-59	72	(39)	63	(34)	4	(2)	34	(18)	4	(2)	1	(1)	1	(1)	3	(2)	2	(1)	**184**
	60-69	31	(19)	101	(61)	0	(0)	2	(1)	14	(8)	11	(7)	0	(0)	7	(4)	0	(0)	**166**
	≥70	26	(13)	71	(35)	0	(0)	0	(0)	30	(15)	26	(13)	7	(3)	41	(20)	3	(1)	**204**
	Total	**497**	(33)	**589**	(39)	**22**	(1)	**226**	(15)	**51**	(3)	**44**	(3)	**9**	(1)	**56**	(4)	**9**	(1)	**1503**

Abbreviations: ABVD, adriamycin, bleomycin, vinblastine, and dacarbazine; RT, radiotherapy; esc, escalated; BEACOPP, bleomycin, etoposide, adriamycin, cyclophosphamide, vincristine, procarbazine, and prednisone; CHOP, cyclophosphamide, adriamycin, vincristine, and prednisone; and CT, chemotherapy



Supplemental Figure S1. Long-term relative survival of patients with limited stage (I/II) and advanced stage (III/IV) classical Hodgkin lymphoma in the Netherlands diagnosed between 1989 and 1999 according to age at diagnosis. Relative survival is shown according to the following six age categories: (A) 18-29, (B) 30-39, (C) 40-49, (D) 50-59, (E) 60-69, and (F) ≥70 years. The tables present the projected 20-year relative survival rates (RSRs) with 95% confidence intervals (CIs) and numbers at risk for the six age categories for every 5 years. For patients aged 70 years and older, RS is displayed up to 15 years, for other patients the RS is displayed up to 20 years.



5-year	ES	OS	RS	ES	OS	RS	ES	OS	RS
Stage I/II	100	99 (98-100)	99 (98-100)	100	98 (95-99)	98 (95-99)	99	99 (95-100)	100 (96-101)
Stage III/IV	100	96 (93-98)	96 (93-98)	100	92 (87-95)	92 (87-96)	99	89 (83-93)	90 (84-94)

5-year	ES	OS	RS	ES	OS	RS	ES	OS	RS
Stage I/II	98	90 (85-93)	93 (87-96)	94	79 (71-85)	84 (76-90)	80	48 (38-57)	61 (49-72)
Stage III/IV	98	78 (70-84)	80 (72-87)	94	54 (47-61)	58 (50-65)	77	36 (29-43)	46 (37-55)

Supplemental Figure S2. Relative survival (RS), expected survival (ES) and observed survival (OS) of patients with limited stage (I/II) and advanced stage (III/IV) classical Hodgkin lymphoma in the Netherlands diagnosed between 2010 and 2017 according to age at diagnosis: (A) 18-29, (B) 30-39, (C) 40-49, (D) 50-59, (E) 60-69, and (F) ≥70 years. The tables present the projected 5-year RS, ES and OS rates with 95% confidence intervals (CIs) for RS and OS.

4

Combining brentuximab vedotin with dexamethasone, high-dose cytarabine and cisplatin as salvage treatment in relapsed or refractory Hodgkin lymphoma: the phase II HOVON/LLPC Transplant BRaVE study.

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Marie José Kersten*, Julia Driessen*, Josée M. Zijlstra, Wouter J. Plattel, Franck Morschhauser, Pieternella J. Lugtenburg, Pauline Brice, Martin Hutchings, Thomas Gastinne, Roberto Liu, Coreline N. Burggraaff, Marcel Nijland, Sanne H. Tonino, Anne I.J. Arens, Roelf Valkema, Harm van Tinteren, Marta Lopez-Yurda, Arjan Diepstra, Daphne De Jong, Anton Hagenbeek

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ABSTRACT

Achieving a metabolic complete response (mCR) before high-dose chemotherapy (HDC) and autologous peripheral blood stem-cell transplant (auto-PBSCT) predicts progression free survival (PFS) in relapsed/refractory classical Hodgkin lymphoma (R/R cHL). We added brentuximab vedotin (BV) to DHAP to improve the mCR rate. In a Phase I dose-escalation part in 12 patients, we showed that BV-DHAP is feasible. This Phase II study included 55 R/R cHL patients (23 primary refractory). Treatment consisted of three 21-day cycles of BV 1.8 mg/kg on day 1, and DHAP (dexamethasone 40mg days 1-4, cisplatin 100mg/m² day 1 and cytarabine 2x2g/m² day 2). Patients with a metabolic partial response (mPR) or mCR proceeded to HDC/auto-PBSCT. Based on independent central FDG-PET-CT review, 42 of 52 evaluable patients (81% [95% CI: 67-90]) achieved an mCR before HDC/auto-PBSCT, five had an mPR and five had progressive disease (three were not evaluable). After HDC/auto-PBSCT, four patients with an mPR converted to an mCR. The 2-year PFS was 74% [95% CI: 63-86], and the overall survival 95% [95% CI: 90-100]. Toxicity was manageable and mainly consisted of grade 3/4 hematological toxicity, fever, nephrotoxicity, ototoxicity (grade 1/2) and transiently elevated liver enzymes during BV-DHAP. Eighteen patients developed new onset peripheral neuropathy (maximum grade 1/2) and all recovered. In conclusion, BV-DHAP is a very effective salvage regimen in R/R cHL patients, but patients should be monitored closely for toxicity. ClinicalTrials.gov identifier: NCT02280993.

INTRODUCTION

Salvage chemotherapy followed by high-dose chemotherapy (HDC) and autologous peripheral blood stem-cell transplant (auto-PBSCT) has been the standard of care for patients with relapsed or refractory classical Hodgkin Lymphoma (R/R cHL) for decades.[1,2] With this treatment, cure rates of 40% to 60% can be achieved. Patients failing this treatment and those relapsing after second line treatment generally have a very poor prognosis.[3-5]

Response to salvage treatment is one of the most important predictors of outcome after auto-PBSCT, with metabolic active residual disease, as assessed by [^{18}F]fluorodeoxyglucose (FDG) - positron emission tomography (PET) - computed tomography (CT) scan, before HDC/auto-PBSCT conferring an inferior prognosis.[6-8] Therefore, higher cure rates may be achieved by improving the metabolic complete response (mCR) rate before HDC/auto-PBSCT. Conventional salvage chemotherapy regimens result in mCR rates of about 50–60%.[6,9-11] DHAP (dexamethasone, high-dose cytarabine, cisplatin) is one of the most commonly used salvage regimens for R/R cHL in Europe.[12]

Brentuximab vedotin (BV) is targeted high-dose intracellular chemotherapy, consisting of an anti-CD30 antibody conjugated to the potent antimicrotubule agent monomethyl auristatin-E.[13,14] Several Phase II studies have shown promising clinical activity of BV in R/R cHL, both as monotherapy and combined with chemotherapy.[15-20] Toxicities of BV include infusion related reaction (IRR), myelosuppression and peripheral neuropathy, the latter being reversible in the majority of patients.[15,16,18,20,21]

In the current prospective, multicenter, international Phase I/II Transplant BRaVE study we investigated the efficacy and safety of BV-DHAP followed by HDC (BEAM) and auto-PBSCT in R/R cHL patients.

Results of the Phase I part of this study in 12 patients have been published previously and showed that the combination of BV-DHAP is feasible with acceptable toxicity.[22] The recommended dose level was established at full dose of all drugs with BV dosed at 1.8 mg/kg.[22]. The primary endpoints for the Phase II single arm part were the fraction of patients achieving an mCR as judged by independent review of PET-CT scan after the third cycle of BV-DHAP, and the rate of grade 3/4 non-hematological adverse events (AEs), including neurotoxicity, during BV-DHAP.

METHODS

Patients

The study enrolled patients aged ≥18 years with histologically confirmed CD30 positive cHL by local pathology assessment, either having primary refractory disease or a first relapse after first-line chemotherapy. **Supplemental Table 1** shows the complete list of inclusion and exclusion

criteria. Central pathology review was performed by two experienced hematopathologists (DDJ, AD).

All patients provided written informed consent. The study protocol was approved by the Ethical Review Committee (ERC) of all participating centers. The study was carried out in accordance with the principles of the Helsinki Declaration.

Study design and treatment

Transplant BRaVE (ClinicalTrials.gov identifier, NCT02280993) is a prospective, open-label study conducted at eight centers in the Netherlands (n=5), France (n=3) and Denmark (n=1). An independent Data Safety Monitoring Board (DSMB) evaluated the general progress and safety aspects of the study at predefined intervals.

Baseline assessment included a lymph node and bone marrow biopsy, and a PET-CT scan. Patients filled in a neurotoxicity questionnaire at study entry, prior to each cycle and at three months after auto-PBSCT. Patients were treated with three 21-day cycles of BV (1.8 mg/kg, i.v., day 1), dexamethasone (40 mg orally or i.v., days 1-4), cisplatin (100 mg/m^2, continuous i.v. (24hr), day 1) and cytarabine (2x2 g/m^2 q12hr, 3hr for each infusion, day 2). After cycle 2, stem cells were mobilized and harvested using granulocyte colony-stimulating factor (G-CSF). A PET-CT scan was performed after cycle 3. Patients with progressive disease (PD) went off study, whereas patients with a partial response (mPR) or mCR proceeded to BEAM (carmustine, 300 mg/m^2, day -7, etoposide, 100 mg/m^2 and cytarabine, 100 mg/m^2, 2x/day, days -6, -5, -4 and -3, and melphalan, 140 mg/m^2, day -2), followed by auto-PBSCT (on day 0). Six weeks after auto-PBSCT, response evaluation was performed by PET-CT. G-CSF was recommended to prevent long-lasting neutropenia.

Endpoints

All endpoints and their definitions are described in **Supplemental Table 2**. Responses were determined according to the 2014 Lugano criteria.[23] All PET-CT scans were centrally reviewed by two independent nuclear medicine physicians (AA, RV) and a third adjudicator (OH) in case of discrepancies. Visual assessment was performed using the Deauville score (DS), assessing DS1-3 as mCR. Toxicity was reported according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.03.

Statistical analysis

Details about the study design and statistical analysis are provided in **Appendix 1**. Efficacy analysis was performed among all evaluable patients. Primary safety analysis was performed among all patients who received at least one dose of study medication. Response rates and their corresponding 95% two-sided exact confidence intervals (CI) were calculated. AEs were analyzed descriptively. The Kaplan-Meier method was used for time-to-event analysis. An exploratory analysis with a Cox proportional hazards regression was performed on all Phase II patients, and

6 patients from the Phase I part of the study who were treated at the recommended dose level. The Kaplan-Meier method and log-rank test were used to analyze univariable associations with progression free survival (PFS). All statistical analyses were performed using R software version 3.6.1 and SAS software version 9.4.

RESULTS

Patients and treatment

Between May 2014 and July 2017, a total of 67 patients with R/R cHL were enrolled for the entire Transplant BRaVE Phase I/II study (n=12 in Phase I and n=55 in Phase II). Due to withdrawal of consent of two patients after one cycle of BV-DHAP and three patients not completing all BV-DHAP cycles, five more patients were enrolled in Phase II than planned according to the sample size calculations (n=50), to allow for sufficient evaluable patients in the primary analysis.

Patient characteristics for the Phase II patients are summarized in **Table 1**. The median age was 29 years, and 27 patients were female (49%). Twenty-three patients (43%) had primary refractory disease, and 16 patients (29%) had relapsed within one year of first-line treatment. Among the first 20 patients of Phase II (stage 1), enough responses were observed (16 mCR) with acceptable toxicity (seven patients experienced significant toxicity), which led to a positive advice of the DSMB to proceed to stage 2.

Of the 55 enrolled patients, 49 (89%) completed all three cycles of BV-DHAP, and 47 (85%) underwent BEAM and auto-PBSCT [**Figure 1**]. Two patients withdrew consent after cycle 1 due to psychological issues, and two patients had PD after cycle 2. In cycle 3, two patients did not receive BV due to toxicity. One of these patients received VIM (ifosfamide, mitoxantrone and etoposide) in cycle 3 because of hepatotoxicity and was not evaluable for response. However, this patient still proceeded successfully to BEAM and auto-PBSCT. The other patient received DHAP without BV because of an anaphylactic shock following BV infusion in cycle 2. This patient went off study thereafter because of toxicity and a mixed response by local PET-CT assessment (which was eventually considered mCR by central PET-CT review) and proceeded to auto-PBSCT after additional treatment with miniBEAM.

Besides the two patients who did not receive BV in cycle 3, dose reductions or delays included 3 delays of cycle 2 due to infection (n=1), venous thrombosis (n=1), or neutropenia (n=1), and 3 delays of BV infusion due to IRR (grade 1/2). Cycle 3 was delayed in 2 patients (malaise and neutropenia), and there were 2 delays of BV infusion (IRR: one grade 2 and one grade 3). Furthermore, eight patients switched from cisplatin to carboplatin due to ototoxicity (n=7; grade 1/2) or nephrotoxicity (n=1; grade 3, recovered completely), and one patient received no cisplatin and cytarabine in cycle 3 due to electrolyte disorder and sepsis.

Table 1. Patient Characteristics

Number of patients [n; (%)]	Phase II patients (n=55)
Age (years)	
Median [range]	29 [19 – 71]
Female	27 (49)
Ann Arbor stage at baseline	
I	8 (15)
II	16 (29)
III	10 (18)
IV	20 (36)
Unknown	1 (2)
ECOG PS at baseline	
0	35 (64)
1	17 (31)
Unknown	3 (5)
Baseline B-symptoms	20 (36)
Bone marrow involvement	2 (4)
First line treatment	
ABVD	40 (73)
BEACOPP baseline	2 (4)
Escalated BEACOPP	8 (15)
Other	5 (9)
Prior radiotherapy	9 (16)
Response to first line treatment	
CR	32 (58)
PR	10 (18)
SD	2 (4)
PD	11 (20)
Time from response to first line treatment to relapse	
Primary refractory disease*	23 (42)
Relapse within 1 year	16 (29)
Relapse after 1 year	16 (29)
Median time (months; [range])	5 [0 – 160]

Abbreviations: N, number; ECOG PS, Eastern Cooperative Oncology Group Performance Score; ABVD, adriamycin, bleomycin, vinblastine, and dacarbazine; BEACOPP, bleomycin, etoposide, adriamycin, cyclophosphamide, vincristine, procarbazine, and prednisone; CR, complete remission; PR, partial remission; SD, stable disease; PD, progressive disease.

* Primary refractory disease is defined as failure to obtain a complete remission with front-line therapy.

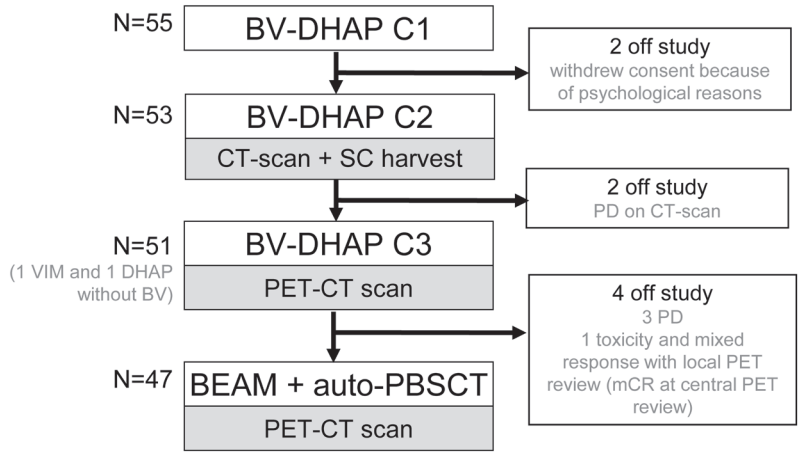

Figure 1. Consort diagram. Number of patients in the full analysis set going through the protocol treatment including reasons for exclusion. *BV: brentuximab vedotin; DHAP: dexamethasone, high-dose cytarabine, cisplatin; C: cycle; N: number; CT: computed tomography; SC: stem cell; PD: progressive disease; VIM: ifosfamide, mitoxantrone, etoposide; PET: positron emission tomography; mCR: metabolic complete response; BEAM: carmustine, etoposide, cytarabine, melphalan; auto-PBSCT: autologous peripheral blood stem-cell transplant.*

Efficacy and stem cell harvest

Three patients were not evaluable for response after three cycles of BV-DHAP: two patients withdrew consent after cycle 1, and one patient did not have a PET-CT scan after cycle 3. By independent central PET-CT review, 42 of 52 evaluable patients achieved an mCR (81% [95% CI: 67 – 90]) and five patients an mPR (10%), resulting in an overall response rate of 90% [95% CI: 79 – 97]. A total of five patients had PD (10%) and did not proceed to BEAM. Two of those patients showed PD on a CT scan after cycle 2 and three had PD on the PET-CT scan after cycle 3 [**Figure 1**]. After auto-PBSCT, four out of five patients with mPR converted to mCR. One patient had a persisting mPR and received additional radiotherapy according to the local physician's decision, and is still in mCR thereafter.

Baseline characteristics (i.e. age, time to relapse and first-line treatment) did not differ significantly between patients with mCR or mPR. The mCR rate was lower for patients with primary refractory disease compared to patients with a later relapse, but this was not statistically significant (mCR rate 73% [95% CI: 69 – 96] versus 86% [95% CI: 50 – 89]; p=0.29, respectively).

Stem cell harvest after cycle 2 was successful using G-CSF in all patients, with one apheresis session in 43 patients and two apheresis sessions in 9 patients, of whom two patients received plerixafor (three patients went off study before apheresis). The median yield was 5.3×10^6 CD34+/kg (range 1.8 – 22.7).

Table 2. Adverse Events grade ≥ 3 during BV-DHAP

	Cycle 1		Cycle 2		Cycle 3		Total*	
Adverse Event	(n=55)		(n=53)		(n=51)		(n=55)	
CTCAE grade (n)	**3**	**4**	**3**	**4**	**3**	**4**	**3**	**4**
Febrile neutropenia	7	1	2	0	3	1	12 (22%)	2 (4%)
Elevated liver enzymes	3	0	5	1	1	0	9 (16%)	1 (2%)
Electrolyte disorders	2	1	0	1	2	0	4 (7%)	2 (4%)
Nausea/vomiting	1	0	3	0	2	0	4 (7%)	0 (0%)
Fever	0	0	1	0	2	0	3 (5%)	0 (0%)
Renal function disorder	1	0	0	0	2	0	3 (5%)	0 (0%)
Sepsis	0	0	1	1	1	0	2 (4%)	1 (2%)
Bone pain	2	0	0	0	0	0	2 (4%)	0 (0%)
Diarrhea	1	0	1	0	0	0	2 (4%)	0 (0%)
Epistaxis	0	0	1	0	1	0	2 (4%)	0 (0%)
Infection	0	0	1	0	1	0	2 (4%)	0 (0%)
Infusion related reaction	0	0	2	0	0	0	2 (4%)	0 (0%)
Malaise	1	0	1	0	0	0	1 (2%)	0 (0%)
Abdominal pain	1	0	0	0	0	0	1 (2%)	0 (0%)
Back pain	0	0	0	0	1	0	1 (2%)	0 (0%)
Constipation	0	0	0	0	1	0	1 (2%)	0 (0%)
Headache	1	0	0	0	0	0	1 (2%)	0 (0%)
Myalgia shoulder	1	0	0	0	0	0	1 (2%)	0 (0%)
Periodic paralysis (hypokalemia)	1	0	0	0	0	0	1 (2%)	0 (0%)
Syncope	0	0	0	0	1	0	1 (2%)	0 (0%)
Total	**22**	**2**	**18**	**3**	**18**	**1**	**55**	**6**
Individual patients †	*17*	*2*	*14*	*2*	*11*	*1*	*29*	*5*
Individual patients total ‡	*18 (33%)*		*15 (28%)*		*11 (22%)*		*30 (55%)*	

Abbreviations: N, number; CTCAE, Common Terminology Criteria for Adverse Events.

* Patients with a specific toxicity in more than one cycle were only counted once in the column representing the total toxicity.

† Total of patients that experienced one or more grade 3 or 4 toxicity during the concerning cycle.

‡ Total of patients that experienced one or more grade 3 or 4 toxicity during the concerning cycle. Patients who experienced both a grade 3 and grade 4 toxicity were only counted once.

Safety

During BV-DHAP treatment, 20 patients (36%) experienced one or more AEs that met the dose limiting toxicity criteria (considered significant toxicity). Grade 3/4 neutropenia and thrombocytopenia were common [**Supplemental Table 3**]. After BEAM/auto-PBSCT, the median recovery time to an absolute neutrophil count (ANC) ≥0.5x10^9/L was 12 days [range: 8 – 29], and the median recovery time to platelets ≥20x10^9/L was 15 days [range: 6 – 46] [**Supplemental Table 3**].

During BV-DHAP, febrile neutropenia (n=14) was the most common non-hematological grade 3/4 toxicity, followed by elevated liver enzymes (n=10) and electrolyte disorders (n=6)

[**Table 2**]. After BEAM/auto-PBSCT, one patient developed veno-occlusive disease (VOD) that was fatal. This patient had elevated levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyl transferase (GGT) already during BV-DHAP and very high levels of AST (2400 Units/Liter (U/L)), ALT (970 U/L), lactate dehydrogenase (LDH; 1400 U/L), GGT (900 U/L) and direct bilirubin (660 µmol/L) during the VOD after BEAM/auto-PBSCT.

Peripheral neuropathy grade 1/2 was present before study entry in 11 patients (one grade 2) but did not worsen during BV-DHAP treatment. During BV-DHAP treatment, 15 (27%) and 3 (5%) patients developed novel onset grade 1 and 2 peripheral neuropathy, respectively, but all recovered. Of all patients, regardless of the presence of peripheral neuropathy at baseline, 12 patients reported transient muscle weakness (grade 1/2) in the neurotoxicity questionnaire, of whom 11 recovered without sequelae. No grade 3/4 neuropathy has occurred [**Supplemental table 4**].

In total, 7 patients experienced ototoxicity (three grade 1, four grade 2) and switched from cisplatin to carboplatin in cycle 2 or 3. Three patients recovered without sequelae, and three patients had continuing ototoxicity (hearing loss or tinnitus) 6 months after auto-PBSCT (one patient unknown).

Serious AEs (SAE)s grade 3/4 following BV-DHAP treatment are described in **Table 3**. In total, 18 (33%) patients experienced one or more SAEs during BV-DHAP. SAEs that occurred in more than one patient were febrile neutropenia (n=9), infections (n=3) and renal function disorder (n=2). Most SAEs recovered, except for the two renal function disorders which recovered with sequelae (persisting grade 1 or 2 nephrotoxicity, e.g. decreased glomerular filtration rate or persisting high levels of creatinine). One additional nephrotoxicity grade 3 was not considered an SAE because of rapid recovery without hospitalization.

Table 3. Serious adverse events grade ≥ 3 during BV-DHAP

Serious Adverse Event	Cycle 1		Cycle 2		Cycle 3		Total**		
	(n=55)		(n=53)		(n=51)		(n=55)		Recovered
CTCAE grade (n)	**3**	**4**	**3**	**4**	**3**	**4**	**3**	**4**	
Febrile neutropenia	5	1	0	0	3	0	8	1	All
Infection	0	0	1	0	1	0	2	0	All
Renal function disorder	0	0	0	0	2	0	2	0	With sequela*
Sepsis	0	0	0	1	1	0	1	1	All
Epistaxis	0	0	1	0	0	0	1	0	All
Fever	0	0	0	0	1	0	1	0	All
Elevated liver enzymes	0	0	0	1	0	0	0	1	All
Infusion related reaction	0	0	1	0	0	0	1	0	All
Malaise	1	0	1	0	0	0	1	0	All
Nausea/vomiting	1	0	0	0	1	0	1	0	All
Periodic paralysis (hypokalemia)	1	0	0	0	0	0	1	0	All
Total	**8**	**1**	**4**	**2**	**9**	**0**	**19**	**3**	
Individual patients†	*7*	*1*	*4*	*2*	*7*	*0*	*15*	*3*	
Individual patients total‡	*8 (15%)*		*6 (11%)*		*7 (14%)*		*18 (33%)*		

Abbreviations: N, number; CTCAE, Common Terminology Criteria for Adverse Events.

* persisting grade 1 or 2 nephrotoxicity (e.g. decreased glomerular filtration rate or persisting high levels of creatinine)

** Patients with a specific toxicity in more than one cycle were only counted once in the column representing the total toxicity.

Survival

After a median follow-up of 27 months, the 2-year PFS by intention-to-treat for all 55 patients was 73.5% [95% CI: 62.6 – 86.4]; (events=14/55), and the 2-year overall survival (OS) was 94.9% [95% CI: 89.5 – 100.0]; events=3/55), [**Figure 2A+B**]. Three patients died during the study period: one patient died of encephalitis (exact cause remained unknown despite a brain autopsy, the patient did not recover from seizures; brain autopsy did not show cerebral localization of lymphoma or infection), and one patient died due to VOD. Both occurred within four months after BEAM/auto-PBSCT. The third patient died of an unrelated head trauma, nine months after BEAM/auto-PBSCT while in mCR. One patient who withdrew consent after cycle 1 went off study and later died from PD and was censored at the time of withdrawal of consent.

Patients with progression after treatment in this study received salvage treatment according to the treating physician's choice. Four patients received BV monotherapy, two of whom had a complete remission, but all progressed again and needed a third salvage regimen.

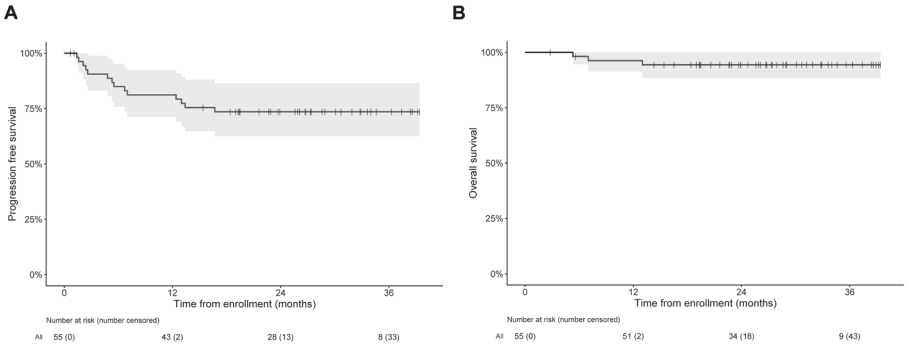

Figure 2. Kaplan-Meier survival analysis. Survival analysis for all 55 Phase II patients by intention-to-treat, including the number of patients at risk at 1, 2 and 3 years with regard to (**A**) progression free survival and (**B**) overall survival, measured from enrollment.

Exploratory analysis of survival

For an exploratory analysis of PFS, 6 patients from Phase I who were treated at the recommended dose level were added to the analysis to a total of 61 patients.[22]

Patients with mPR after 3 cycles showed a significantly lower PFS compared to patients with mCR. Two year PFS rates of patients with mPR (n=5) versus patients with mCR (n=48) were 40% (95% CI: 14 – 100) versus 87% (95% CI: 78 – 97), log-rank p=0.004, hazard ratio (HR): 6.02 (95% CI: 1.50 – 24.2; p=0.011), respectively [**Figure 3A** and **Supplemental Table 5**]. A multivariable Cox analysis showed that patients with an mPR had a significantly increased risk of progression, independently of primary refractory status. [**Supplemental table 5**]. Patients with relapsed disease (n=37) had a lower risk of progression compared to patients with primary

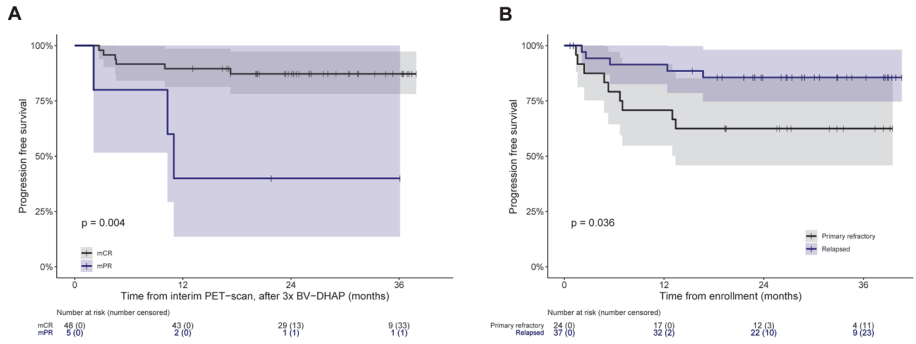

Figure 3. Kaplan-Meier exploratory analysis. Survival analysis for all 55 Phase II patients and 6 patients from Phase I who were treated at the same dose level, including the number of patients at risk at 1, 2 and 3 years with regard to (**A**) progression free survival stratified for patients with a metabolic complete response (mCR; n=48) or partial response (mPR; n=5) on the PET-CT scan after 3 cycles of BV-DHAP, measured from the time of that PET-CT scan, and (**B**) progression free survival stratified for relapsed patients (n=37; defined as recurrent disease after having reached a complete response on first line treatment) versus patients with primary refractory disease (n=24; no complete response on first line treatment), measured from enrollment.

refractory disease (n=24), with two year PFS rates of 86% (95% CI: 75 – 98) versus 63% (95% CI: 46 – 85), log-rank *p*=0.036, HR: 0.33 (95% CI: 0.11 – 0.98; *p*=0.046), respectively. [**Figure 3B** and **Supplemental Table 5**]. Univariable analysis did not show significant associations for other baseline risk factors (i.e. B-symptoms, age, stage and first line treatment regimen) [**Supplemental table 5**].

Central pathology review

Based on morphology, immunophenotype, and molecular clonality analysis if needed, central pathology review confirmed cHL (according to the WHO classification 2016[24]) in 59 of all 67 patients (88%) of the complete Phase I (cHL confirmed in 10 of 12 patients in total) and Phase II (cHL confirmed in 49 of 55 patients in total) part of the study. In all cases with equivocal morphological and/or immunohistochemical features, including cases with high numbers of EBER positive atypical large cells and/or small lymphocytes (n=16), extensive immunohistochemical and molecular T-cell receptor and immunoglobulin heavy and light chain gene rearrangement assays (BIOMED) were performed [**Supplemental table 6**]. In eight patients, cHL could not be confirmed. Of these, five patients were diagnosed with peripheral T-cell lymphoma (PTCL), not otherwise specified (NOS), one patient with angioimmunoblastic T-cell lymphoma (AITL) and one patient with immunodeficiency-associated B-lymphoproliferative disorder (IA-B-LPD).[25] In one patient a classifying diagnosis could not be made due to lack of representative material in the biopsy sample. Additionally, in one patient, a composite lymphoma of cHL and lymphoplasmacytic lymphoma (LPL) was diagnosed. In all cases high CD30 expression was present. Of the seven patients with PTCL, AITL or IA-B-LPD, six had an mCR after three cycles of BV-DHAP. One patient with PTCL had PD after cycle 2, one with AITL had PD after auto-PBSCT, and one patient with PTCL died due to unrelated head-trauma. When excluding the patient with unrelated death, the PFS was not significantly different for patients with confirmed cHL versus patients with another diagnosis (2-years PFS 81% versus 67%, log-rank *p*=0.36).

DISCUSSION

In this international, prospective Phase II study we investigated the efficacy and safety of BV-DHAP as first salvage treatment for patients with R/R cHL. This study is the first to investigate this combination. Treatment with BV-DHAP resulted in a high proportion of patients with an mCR prior to HDC/auto-PBSCT, and toxicity was mostly reversible.

Data on FDG-PET-CT results following treatment with DHAP are scarce, but generally only about 25% of patients achieved a CR as assessed by CT scan.[4,26]. Other trials have recently investigated BV in combination with other salvage chemotherapy combinations, such as bendamustine, ICE (ifosfamide, carboplatin, etoposide) or ESHAP (etoposide, methylprednisolone, high-dose cytarabine and cisplatin) and have shown mCR rates up to 76% prior to HDC/

auto-PBSCT.[15-18,27] The administration schedule of BV differed among these studies, and most studies used more than 3 administrations of BV in total.[15-18,27] In the current study, three cycles of BV-DHAP resulted in a high mCR rate with only 3 administrations of BV. This makes it a less 'financially toxic' therapy than using BV in first line for all patients or to use it as consolidation therapy after auto-PBSCT.

In R/R cHL patients treated with salvage chemotherapy followed by HDC and auto-PBSCT, historical studies demonstrate a 5-years PFS of approximately 50%.[1-4,26,28,29] In 97 patients treated with ICE the 2-year event free survival was 70%.[6] Another regimen consisting of bendamustine, gemcitabine and vinorelbine (in 59 patients) resulted in a 2-year PFS of 63%.[30] With the present treatment protocol, we have been able to achieve a high 2-year PFS rate of 74%. A total of 14 events occurred (including 3 deaths), and at the present median follow up of 27 months, no relapses have occurred beyond 18 months from enrollment. Longer follow-up is needed to confirm that the majority of patients in remission after 2 years are indeed cured.[3,28,31]

The unprecedented high response rate and prolonged PFS of this treatment regimen were achieved at the cost of higher toxicity in comparison to other salvage regimens. However, most of the observed toxicities, including neutropenia, thrombocytopenia, fever, nausea/vomiting, ototoxicity and nephrotoxicity are toxicities of specific concern during treatment with DHAP.[4,26,32,33]. Other regimens of BV with bendamustine, nivolumab, ICE or ESHAP seem to induce less AEs, with most toxicities consisting of hematological toxicity.[15,16,18,19,34] While the occurrence of grade 3/4 non-hematological toxicity was low with BV-bendamustine, a substantial part of the patients (25%) did not undergo auto-PBSCT, resulting in a lower 2-years PFS of 62.6%.[16] Another recent study with BV-bendamustine in 40 patients had a 3-years PFS of 67.3% and 82.5% of patients underwent auto-PBSCT.[19] The combination of BV with nivolumab resulted in an mCR rate of 61% with almost all patients experiencing grade 1/2 toxicity and 31% having grade 3/4 toxicity, however these AEs were also manageable.[34]

A sequential approach of BV monotherapy followed by chemotherapy in PET-positive patients is interesting, since some patients could be spared the toxicity of salvage chemotherapy without losing efficacy. However, only a minority of patients achieved a PET-negative response after BV monotherapy.[15] The ESHAP regimen is similar to DHAP, except for containing methylprednisolone instead of dexamethasone, and cisplatin being given over four days of 25 mg/m^2/day compared to 100 mg/m^2 in one day with the DHAP regimen.[18] Hematological AEs were comparable between BV-ESHAP and BV-DHAP with about 50% of patients experiencing grade 3/4 thrombocytopenia and neutropenia. For BV-ESHAP, grade 3 fever and mucositis were the most frequent non-hematological grade 3/4 toxicities whereas DHAP was also associated with fever, but not with mucositis. In contrast, only grade 1/2 renal dysfunction occurred with BV-ESHAP, and no cases of elevated liver enzymes or ototoxicity are described.[18]

In 10 patients, a transient grade 3/4 increase in liver enzymes was observed during BV-DHAP treatment (one grade 4), which was reversible in all patients. One patient developed a fatal VOD after BEAM/auto-PBSCT. Additionally, one patient treated in the phase I part of

this study also developed a grade 3 VOD, which however recovered without sequelae. Both patients already had elevated liver enzymes during BV-DHAP treatment. This complication has previously been described in patients receiving high-dose alkylating agents such as melphalan or cyclophosphamide.[35]

BV as consolidation therapy has been shown to prolong PFS in high-risk R/R cHL patients who have undergone HDC/auto-PBSCT.[36] Whether BV before auto-PBSCT in combination with chemotherapy, or as consolidation after auto-PBSCT will be more effective is unknown. Of note, with BV consolidation, peripheral neuropathy occurred in 67% of patients, including 13% (n=22) grade 3 peripheral neuropathy. With BV-DHAP, the incidence of peripheral neuropathy was lower, mostly reversible and no grade 3/4 occurred, probably because only three administrations of BV were given.

In depth pathology workup and reclassification was performed to exclude lymphomas that are known as cHL mimickers such as AITL and PTCL (with follicular helper T-cell immunophenotype with secondary cHL-like blasts), as well as IA-B-LPD.[37-39] In retrospect, seven cases were identified as cHL-mimickers with central pathology review. Awareness for cHL-mimickers is important because patients with T-cell lymphoma generally have a worse prognosis.[40] In this cohort of patients no significant differences in response rates or PFS were observed between patients with confirmed or unconfirmed cHL, although the number of patients is too small to validate this finding.

An exploratory analysis on PFS showed that patients with an mPR prior to BEAM/auto-PBSCT have a higher risk of relapse, despite conversion to an mCR after auto-PBSCT. This finding is in line with other trials investigating risk factors for relapse after auto-PBSCT.[5-7] PET-adapted therapy could probably further improve outcome by intensifying treatment for high-risk patients with new agents, such as checkpoint inhibitors in addition to BV. Moreover, a group of patients at low-risk for relapse, might possibly be cured with a combination of new drugs only, without the toxic consequences of HDC and auto-PBSCT. Risk stratification based on the PET-CT scan at relapse could also be further improved by quantitative analysis and the assessment of metabolic tumor volume.[41,42]

The addition of BV to salvage treatment has not yet been investigated in a randomized Phase III trial. However, several Phase II studies have now shown that BV in combination with chemotherapy results in high mCR rates prior to HDC/auto-PBSCT. A combined pooled analysis of all of these studies is planned to give more insight into the effect of BV on response rates and toxicity in this setting.

In conclusion, in R/R cHL, three cycles of BV-DHAP is a highly effective salvage regimen resulting in an mCR rate of 81% prior to HDC/auto-PBSCT as shown by independent central PET-CT review. Patients should be monitored closely for toxicity, especially hematological toxicity, nephrotoxicity and liver toxicity.

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CONFLICT OF INTEREST DISCLOSURES

The study drug (BV) was provided for the study and the study was funded by Takeda. Takeda did not have any influence on the analysis of the data or the interpretation of the results.

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SUPPLEMENTARY MATERIAL

Appendix 1. Study design and statistical analysis (extended methods)

A Bryant and Day two-stage design was used, with early stopping rules for poor response or toxicity.[1] An overall response rate (ORR) of 50% was considered unacceptable and an ORR of 70% was considered acceptable. The maximum rate of patients experiencing significant toxicity was defined as 55% to be unacceptable and 30% to be acceptable. Significant toxicity was defined as a grade 3/4 non-hematological adverse event (AE) according to the dose-limiting toxicity (DLT) criteria [**Supplemental Table 2**]. Error rates were set at 0.1 for both response and toxicity. The recommended sample size for stage 1 was 20 patients of whom at least 11 should have a response and a maximum of 9 could have significant toxicity. Subsequently, a further 30 evaluable patients would be recruited for stage 2, to a total of 50 patients for the entire Phase II study. If a participant were to withdraw from the study, he or she would be replaced by a new participant to reach the target number of participants. Progression free survival (PFS) was defined as time from study entry until progressive disease or death, whichever occurred first. Overall survival (OS) was defined as time from study entry until death from any cause.

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Supplemental Table 1. Patient selection criteria

Inclusion criteria
Histologically confirmed CD30+ classical HL (central pathology review; results not required to enroll the patient in the study), primarily refractory to first line chemotherapy or in first relapse after any polychemotherapy regimen (e.g. ABVD, baseline BEACOPP or escalated BEACOPP, or other induction regimens)
In case of relapse, the relapse must be histologically confirmed. In case histology is not possible, at least confirmation of the relapse by FNA is required.
Measurable disease, according to the definitions of response (Cheson 2014), i.e. CT scans showing at least 2 or more clearly demarcated lesions with a long axis ≥ 1.5 cm and a short axis diameter ≥ 1.0 cm, or 1 clearly demarcated lesion with a long axis ≥ 2.0 cm and a short axis diameter ≥ 1.0 cm. These lesions must be FDG-positive
Age ≥ 18 years (upper age limit for auto-PBSCT at the discretion of the participating center)
WHO Eastern Cooperative Oncology Group Performance Score ≤ 2
Life expectancy of > 3 months with treatment
No major organ dysfunction, unless HL-related
Total bilirubin < 1.5x ULN (unless due to lymphoma involvement of the liver or a known history of Gilbert's syndrome)
ALT/AST < 3x ULN (unless due to lymphoma involvement of the liver; in that case ALT/AST may be elevated up to 5 x ULN)
GFR > 60 ml/min as estimated by the Cockroft&Gault formula (1976)
Absolute neutrophil count ≥ 1.5x109/L, unless caused by diffuse bone marrow infiltration by the HL
Platelets ≥ 100x109/L, unless caused by diffuse bone marrow infiltration by the HL
Hemoglobin must be >8 g/dL
Written informed consent
Able to adhere to the study visit schedule and other protocol requirements
Female patient is either post-menopausal for at least 1 year before the screening visit or surgically sterile or if of childbearing potential, agrees to practice 2 effective methods of contraception, at the same time, from the time of signing the informed consent through 30 days after the last dose of study drug, or agrees to completely abstain from heterosexual intercourse.
Male patients, even if surgically sterilized, (i.e., status post vasectomy) agree to practice effective barrier contraception during the entire study period and through 6 months after the last dose of study drug, or agrees to completely abstain from heterosexual intercourse.
Eligible for high dose chemotherapy and autologous peripheral blood stem cell transplantation
Resolution of toxicities from first-line therapy
Exclusion criteria
Peripheral sensory or motor neuropathy grade ≥ 2
Known cerebral or meningeal disease (HL or any other etiology), including signs or symptoms of PML
Symptomatic neurologic disease compromising normal activities of daily living or requiring medications
Patients who have been using other investigational agents within at least 5 half lives of the most recent agent used prior to enrollment in the study
Patients who were treated with myelosuppressive chemotherapy or biological therapy ≤ 4 weeks before study inclusion
Female patients who are both lactating and breast feeding or have a positive serum pregnancy test during the screening period or a positive pregnancy test on Day 1 before first dose of study drug or adults of reproductive potential who are not using effective birth control methods.

Supplemental Table 1. Patient selection criteria (*continued*)

Inclusion criteria
Patients with any active systemic viral, bacterial, or fungal infection requiring systemic antibiotics within 2 weeks prior to first study drug dose
Patients who have a history of another primary malignancy less than 3 years before study inclusion or previously diagnosed with another malignancy and have evidence of residual disease, with the exception of non-melanoma skin cancer, completely resected melanoma TNMpT1 and carcinoma in situ of the uterine cervix
Patients with known hypersensitivity to recombinant proteins, murine proteins, or to any excipient contained in the drug formulation of brentuximab vedotin
Patients with known HIV seropositivity, known hepatitis B surface antigen-positivity, or known or suspected active hepatitis C infection
Patients receiving radiation therapy within 8 weeks prior to start of protocol treatment. Emergency radiation therapy is allowed, as long as measurable disease (at non-irradiated sites) persists.
Patients with a serious psychiatric disorder that could, in the investigator's opinion, potentially interfere with the completion of treatment according to the protocol
Patients who have any severe and/or uncontrolled medical condition or other conditions that could affect their participation in the study such as: Known history of symptomatic congestive heart failure (NYHA III, IV), myocardial infarction ≤ 6 months prior to first study drug
Evidence of current serious uncontrolled cardiac arrhythmia, angina pectoris, electrocardiographic evidence of acute ischemia or active conduction system abnormalities
Recent evidence (within 6 months before first dose of study drug) of a left-ventricular ejection fraction <50%
severely impaired pulmonary function as defined as spirometry and DLCO (diffusing capacity of the lung for carbon monoxide) that is 50% or less of the normal predicted value and/or O2 saturation that is 90% or less at rest on room air
Any active (acute or chronic) or uncontrolled infection/disorders that impair the ability to evaluate the patient or for the patient to complete the study
Nonmalignant medical illnesses that are uncontrolled or whose control may be jeopardized by this study drug, such as severe hypertension that is not controlled with medical management and thyroid abnormalities when thyroid function cannot be maintained in the normal range by medication

Abbreviations: Hodgkin lymphoma (HL); adriamycin, bleomycin, vinblastine, dacarbazine (ABVD); bleomycin, etoposide, adriamycin, cyclophosphamide, vincristine, procarbazine, prednisone (BEACOPP); fine-needle aspiration (FNA); computed tomography (CT); [^{18}F]fluorodeoxyglucose (FDG); upper limit of normal (ULN); alanine aminotransferase (ALT); aspartate aminotransferase (AST); glomerular filtration rate (GFR); Progessive Multifocal Leuko-encephalopathy (PML).

Supplemental Table 2. Study endpoints and definitions

Endpoint	Definition
Metabolic CR rate (PET-CT) after the third cycle of BV-DHAP reinduction therapy	According to the definitions of response (Cheson, 2014). Deauville 1-3 is considered a metabolic CR.
Rate of grade 3/4 non-hematological toxicity, including neurotoxicity after each cycle of BV-DHAP	Common Terminology Criteria of Adverse Events (CTCAE) version 4.01
The number of patients who experience significant toxicity during BV-DHAP	Significant toxicity is defined as a dose limiting toxicity (DLT); - grade ≥ 3 non-hematologic toxicity, including neurotoxicity# - death whatever the cause, except death due to Hodgkin lymphoma any of which must occur before day 22 of cycle I-III - postponement of course 2 or 3 of BV-DHAP– despite growth factor prophylaxis- due to neutropenia with more than 10 days and / or neutropenia grade 4 after course 1 , 2 or 3 lasting more than 10 days despite growth factor treatment. # Exceptions: 1. Laboratory abnormalities grade ≥ 3 are only considered to be DLT if they persist for > 2 weeks or if they do not return to ≤ grade 1 2. For nausea, vomiting, or diarrhea, subjects must have a grade 3 or 4 event that persists for at least 7 days at this level despite the use of optimal symptomatic treatment, in order for these events to be considered a DLT 3. Any infection/fever requiring iv antibiotics is not considered to be a DLT, only grade 4 infection is considered to be a DLT 4. Grade 3 thromboembolic events and grade 3 hypertension are not considered to be DLT 5. If a DLT is attributed to progressive disease, it will not be counted as DLT. 6. Alopecia.
Overall response rate (PR + CR) after the third cycle of BV-DHAP reinduction therapy (based on the results of the FDG-PET/CT scan)	
Overall response rate (PR + CR) after auto-PBSCT (based on the results of the FDG-PET/CT scan)	
Metabolic CR rate (PET-CT) after auto-PBSCT	
Fraction of patients (CR/PR) eligible for auto-PBSCT who actually undergo auto-PBSCT	
Progression free survival (PFS)	Disease progression or death from any cause, measured from study entry.
Event free survival (EFS)	Failure of treatment (no CR or PR, no stem cell harvest or auto-PBSCT possible or relapse), measured from study entry.
Overall survival (OS)	Death as a result of any cause, measured from study entry.

Supplemental Table 2. Study endpoints and definitions (*continued*)

Endpoint	Definition
Serious Adverse Events (SAE)s during the combination treatment	An SAE is any untoward medical occurrence or effect that: - Results in death; - Is life threatening (at the time of the event); - Requires hospitalization or prolongation of existing inpatients' hospitalization; - Results in persistent or significant disability or incapacity; - is a congenital anomaly or birth defect; - is a new event of the trial likely to affect the safety of the subjects, such as an unexpected outcome of an adverse reaction, lack of efficacy of an IMP used for the treatment of a life threatening disease, major safety finding from a newly completed animal study, etc.
Time to hematological recovery after each cycle of BV + DHAP	Absolute neutrophil count (ANC) recovery is defined as ≥0.5x10^9/L for three consecutive laboratory values obtained on different days. Platelet recovery is defined as ≥20x10^9/L for three untransfused platelet counts over 7 days with rising counts during the week.
Rate of successful PBSC collection (≥ 2x10^6 CD34+ cells/kg) after the second cycle of BV-DHAP	
Time to hematological recovery after auto-PBSCT	Absolute neutrophil count (ANC) recovery is defined as ≥0.5x10^9/L for three consecutive laboratory values obtained on different days. Platelet recovery is defined as ≥20x10^9/L for three untransfused platelet counts over 7 days with rising counts during the week.

Abbreviations: complete response (CR); positron emission tomography (PET); computed tomography (CT); brentuximab vedotin (BV); dexamethasone, high-dose cytarabine, cisplatin (DHAP); partial response (PR); autologous peripheral blood stem-cell transplant (auto-PBSCT); Investigational Medicinal Product (IMP).

Supplemental Table 3. Hematological toxicity and recovery

Grade (n(%)) Recovery (median days [range])		Cycle 1 (n=55)	Cycle 2 (n=53)	Cycle 3 (n=51)	BEAM + auto-PBSCT (n=47)
Neutropenia	**Grade 3**	5 (9)	7 (13)	10 (20)	
	Grade 4	29 (53)	24 (45)	23 (45)	
	Recovery†	13 [9 – 21]	15 [12 – 21]	17 [12 – 33]	12 [8 – 29]
Thrombocytopenia	**Grade 3**	16 (29)	15 (28)	11 (22)	
	Grade 4	21 (38)	27 (53)	31 (61)	
	Recovery‡	14 [11 – 22]	18 [12 – 26]	19 [13 – 37]	15 [6 – 46]
Anemia	**Grade 3**	1 (2)	9 (17)	13 (25)	
	Grade 4	0 (0)	0 (0)	0 (0)	
	No grade 4 anemia, so no recovery measured.				

Abbreviations: carmustine, etoposide, high-dose cytarabine, melphalan (BEAM); autologous peripheral blood stem-cell transplant (auto-PBSCT).

†Neutrophil recovery was defined as absolute neutrophil count (ANC) ≥0.5 x 10^9/L for three consecutive laboratory values obtained on different days and was measured from the start of BV-DHAP cycle 1-3 or from reinfusion of stem cells after BEAM, until the date of the first of three consecutive laboratory values where the ANC is ≥0.5 x 10^9/L in patients with grade 4 neutropenia.

‡Platelet recovery was defined as platelet count ≥20 x 10^9/L for three untransfused platelet counts over 7 days with rising counts during the week and was measured from the start of BV-DHAP cycle 1-3 or from reinfusion of stem cells after BEAM, until the date of the first of three consecutive laboratory values where the platelet count is ≥20 x 10^9/L in patients with grade 4 thrombocytopenia.

Supplemental Table 4. Neurotoxicity

	PNP not present at baseline (n=21)	Resolved	PNP present at baseline (n=11)	Resolved	Total (n=32)	Resolved
Highest CTCAE grade during BV-DHAP [n; (% resolved)]						
0	3	.	0	.	3	.
1	15	15 (100%)	10	7 (70%)	25	22 (88%)
2	3	3 (100%)	1	0 (0%)	4	3 (75%)
Highest CTCAE grade during BEAM/auto-PBSCT [n; (% resolved)]						
0	4	.	4	.	8	.
1	8	6 (75%)	1	0 (0%)	9	6 (67%)
2	7	5 (71%)	3	1 (33%)	10	6 (60%)
Unknown	2	.	3	.	5	.
Muscle weakness during BV-DHAP [n; (% resolved)]						
No	13	.	7	.	20	.
Yes	8	8 (100%)	4	3 (75%)	12	11 (92%)
Muscle weakness during BEAM/auto-PBSCT [n; (% resolved)]						
No	19	.	10	.	29	.
Yes	2	2 (100%)	1	0 (0%)	3	2 (67%)

Abbreviations: Peripheral neuropathy (PNP); number of patients (n); Common Terminology Criteria for Adverse Events (CTCAE); Brentuximab vedotin (BV); dexamethasone, high-dose cytarabine, cisplatin (DHAP); carmustine, etoposide, high-dose cytarabine, melphalan (BEAM); autologous peripheral blood stem-cell transplant (auto-PBSCT).

Supplemental Table 5. Cox proportional hazard regression on progression free survival

Univariable Cox models for PFS from enrollment						
Characteristic	**Events**	**N**	**HR**	**Lower 95% CI**	**Upper 95% CI**	**P-value**
Age						
Per unit	14	61	1.010	0.970	1.051	0.635
Age (grouped)						
< 45	10	45	1 (ref)			
≥ 45	4	16	1.634	0.512	5.215	0.407
Relapse 3 groups						
Primary refractory	9	24	1 (ref)			
Relapse < 1 year (not refractory)	3	17	0.424	0.115	1.567	0.198
Relapse ≥ 1 year	2	20	0.245	0.053	1.133	0.072
Relapse 2 groups						
Primary refractory	9	24	1 (ref)			
Relapse	5	37	0.328	0.110	0.980	0.046
B-symptoms						
No	8	38	1 (ref)			
Yes	6	23	1.392	0.483	4.016	0.540
Ann Arbor Stage at first diagnosis						
I / II	3	22	1 (ref)			
III / IV	10	36	2.025	0.557	7.366	0.284
(3 unknown)						
Ann Arbor Stage at relapse						
I / II	5	28	1 (ref)			
III / IV	9	32	1.756	0.588	5.244	0.313
(1 unknown)						
First line treatment						
ABVD	9	45	1 (ref)			
BEACOPP (escalated/baseline)	2	11	0.903	0.195	4.179	0.896
Other	3	5	3.878	1.039	14.474	0.044
Interim PET status*						
mCR	6	48	1 (ref)			
mPR	3	5	6.02	1.499	24.2	0.011
PD (censored from cox analysis)	5	5	-			
(3 not evaluable for response)	-	3	-			
Multivariable Cox model for PFS, measured from interim PET						
Characteristic	**Events**	**N**	**HR**	**Lower 95% CI**	**Upper 95% CI**	**P-value**
Interim PET status*						
mCR	6	48	1 (ref)			
mPR	3	5	4.785	1.167	19.628	0.030
Relapse 2 groups						
Primary refractory	6	20	1 (ref)			
Relapse	3	33	0.311	0.076	1.271	0.104

Abbreviations: Number of patients (N); Hazard Ratio (HR); Confidence Interval (CI); reference (ref); progression free survival (PFS); progressive disease (PD); metabolic complete response (mCR); metabolic partial response (mPR); adriamycin, bleomycin, vinblastine, dacarbazine (ABVD); bleomycin, etoposide, adriamycin, cyclophosphamide, vincristine, procarbazine, prednisone (BEACOPP); positron emission tomography (PET).

*For interim PET status analysis, the PFS was defined as time from interim PET-scan after 3 cycles of BV-DHAP, until progression or death, and patients with PD at time of the interim PET were excluded from this subanalysis.

Supplemental Table 6: Central pathology review

Table 6: Central pathology review of EBER positive cases (n=17)				
	IHC unequivocal	**IHC non-conclusive**	**(iatrogenic) immunodeficiency highly suggestive**	**No proof for (iatrogenic) immunodeficiency**
TcR monoclonal	46 – PTCL (PD1) 57 – AITL (PD1/ CD21)	44 – PTCL 53 – PTCL		
TcR equivocal (poor DNA quality)	45 – PTCL (T-cell marker loss)	6 – cHL 43 – cHL 18 – cHL		
TcR polyclonal	42 – PTCL (T-cell marker loss)	67 – cHL (PD1+ only)		
Ig-R monoclonal			11 – IA-B-LPD	21 – cHL 24 – cHL
Ig-R polyclonal				61 – cHL 64 – cHL
No Ig- and TcR information available				51 – cHL 10 – cHL

Abbreviations: Epstein-Barr virus encoded RNAs (EBER), in situ hybridization (ISH), Epstein-Barr virus (EBV), immunohistochemistry (IHC), T-cell receptor (TcR), immunoglobulin receptor (Ig-R), peripheral T-cell lymphoma, not otherwise specified (PTCL), angio-immunoblastic T-cell lymphoma (AITL), classical Hodgkin lymphoma (cHL), immunodeficiency-associated B-lymphoproliferative disorder (IA-B-LPD), programmed cell death protein1 (PD1).

Diagnostic biopsy samples at relapse were available for review for all of the 67 patients (100%) included in the phase I and/or phase II of this study. In 34 cases also the primary diagnostic biopsy sample was submitted for review (51%). At review, at least the following immunohistochemical stains were available in all cases: CD30, CD15, PAX5, CD20, CD3 as well as EBER-ISH. In one case cHL and synchronous lymphoplasmacytic lymphoma was diagnosed (case 60) and in another case the material was not diagnostic for cHL due to absence of tumor cells (case 50). In 4 of the cases, the cHL-cells expressed CD20, but lacked further arguments for a classification as "mediastinal grey zone lymphoma". All 17 cases with EBER positive Hodgkin-type cells and/or small lymphocytes were scrutinized to dissect the difficult differential diagnosis of cHL, T-cell lymphoma with secondary EBV+ Hodgkin-like blasts (either angio-immunoblastic T-cell lymphoma or peripheral T-cell lymphoma) and immunodeficiency-associated B-lymphoproliferative disorder (IA-B-LPD) (**Table 6**). T-cell receptor (TcR)- and immunoglobulin heavy (IgH) and kappa light chain (IgK) gene rearrangement studies according to standard methods (IgH, IgK, TcR beta and gamma standard BIOMED assays) and complementary immunohistochemistry was performed to include at least CD21 and PD1 and if sufficient material was available also CD79a, CD2, CD5, CD7, CD8, CD4, CD23. Of these 17 cases, 3 showed only EBER positivity in small cells and were considered fully consistent with cHL (cases 10, 24, 51). Only in case of unequivocal monoclonal TcR rearrangement (case 46 and 57) and/or immunohistochemical patterns (T-cell marker loss case 45 and 42), in the context of a fitting morphology, a diagnosis of T-cell lymphoma was rendered. Only in case of unequivocal (iatrogenic) immunodeficiency, the diagnosis IA-B-LPD was made (long history of steroid use). Equivocal cases were considered as cHL for this review. In conclusion, in 59/67 cases (88%), a diagnosis of cHL could be confirmed.

5

Brentuximab Vedotin and Chemotherapy in Relapsed/ Refractory Hodgkin Lymphoma: a Propensity Score Matched Analysis.

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ABSTRACT

Several single-arm studies have explored the inclusion of brentuximab vedotin (BV) in salvage chemotherapy followed by autologous stem-cell transplantation (ASCT) for relapsed/refractory (R/R) classical Hodgkin lymphoma (cHL). However, no head-to-head comparisons with standard salvage chemotherapy have been performed. This study presents a propensity score-matched analysis encompassing individual patient data from ten clinical trials to evaluate the impact of BV in transplant-eligible R/R cHL patients. We included 768 patients, of whom 386 were treated with BV +/- chemotherapy (BV-cohort), while 382 received chemotherapy alone (chemo-cohort). Propensity score matching resulted in balanced cohorts of 240 patients each. No significant differences were observed in pre-ASCT complete metabolic response (CMR) rates (p=0.69) or progression free survival (PFS) (p=0.14) between the BV- and chemo-cohorts. However, patients with relapsed disease had a significantly better 3-year PFS of 80% *versus* 70% in the BV- *versus* chemo-cohort (p=0.02), while there was no difference for primary refractory patients (56% *versus* 62%, respectively; p=0.67). Patients with stage IV disease achieved a significantly better 3-year PFS in the BV-cohort (p=0.015). Post-ASCT PFS was comparable for patients achieving a CMR after BV monotherapy and those receiving BV followed by sequential chemotherapy (p=0.24). While 3-year overall survival was higher in the BV-cohort (92% *versus* 80%, p<0.001, respectively), this is likely attributed to the use of other novel therapies in later lines for patients experiencing progression, given that studies in the BV-cohort were conducted more recently. In conclusion, BV +/- salvage chemotherapy appears to enhance PFS in relapsed but not primary refractory cHL patients.

KEY POINTS

- BV +/- chemotherapy does not increase CMR rates or PFS in R/R cHL, but seems to increase PFS in patients with relapsed or stage IV disease
- Sequential treatment with BV and chemotherapy is feasible and could spare salvage chemotherapy in a subset of fast responding patients

INTRODUCTION

For the past 30 years, standard treatment of patients with classical Hodgkin lymphoma (cHL) who are primary refractory or relapse (R/R) after first-line (primary) treatment, has been to test for chemosensitivity with salvage chemotherapy and, upon response, to treat with myeloablative high-dose chemotherapy (HDCT) followed by autologous stem-cell transplantation (ASCT).[1-3] With this strategy about 70-80% of patients respond to salvage chemotherapy of whom approximately 60% achieve a complete metabolic response (CMR) based on a negative ^{18}F-fluorodeoxyglucose (FDG)-positron emission tomography (PET) scan prior to ASCT.[1,4-6] However, 30-40% of patients will relapse within 5 years after ASCT and subsequently have a poor prognosis.[1,7] Importantly, it has been shown that patients who achieve a CMR pre-ASCT have a better prognosis with long-term post-ASCT progression free survival (PFS) of approximately 70-80%.[1,4,8]

In the past decade, new targeted treatment options such as brentuximab vedotin (BV) and checkpoint inhibitors have become available for patients with R/R cHL.[9-11] BV is an antibody-drug conjugate composed of an anti-CD30 monoclonal antibody with a cytotoxic payload of monomethyl auristatin E (MMAE).[12] In the first-line setting, BV in combination with adriamycin, vinblastine and dacarbazine (BV-AVD) has been shown to improve PFS and overall survival (OS) in advanced stage patients compared to standard adriamycin, bleomycin, vinblastine and dacarbazine (ABVD).[13,14] In the R/R setting, several phase II single arm clinical trials have investigated BV in combination with concomitant or sequential chemotherapy followed by ASCT.[15-24] These trials showed a high CMR rate prior to ASCT, and PFS and OS appear to be higher when compared to historical controls.[25] However, no randomized controlled trials (RCT) investigating the addition of BV to salvage chemotherapy compared to chemotherapy alone in R/R cHL have been published to this date. An individual patient-data analysis could provide more power for assessing the effect of novel treatments, and can also detect interactions between outcome parameters and patient characteristics outcomes, compared to standard meta-analyses.

Therefore, we aimed to perform a large, individual patient data analysis to investigate the effect of BV addition to salvage chemotherapy versus chemotherapy alone on pre-ASCT PET response, PFS and OS.

METHODS

Literature search and data collection

We performed a literature search on PubMed and clinicaltrials.gov to identify clinical trials investigating BV in combination with salvage chemotherapy (BV-cohort), or salvage chemotherapy alone (chemo-cohort) followed by ASCT in transplant-eligible cHL patients with a first relapse or primary refractory disease after first-line (primary) treatment **[Supplementary Extended**

Methods]. Ten studies were identified that met our inclusion criteria, the investigators of all ten studies provided the individual-patient data for inclusion in the analysis. Seven studies, published between 2017 and 2021, were included in the BV-cohort and three studies, published between 2010 and 2016, were included in the chemo-cohort [**Supplemental Figure 1** and **Supplemental Table 1**]. We gathered pseudonymized individual patient-data from case record forms or study databases. For secondary use of data for this analysis, a waiver for informed consent was obtained from the Ethics Committee of all participating centers.

Endpoints and definitions

The primary endpoint was the 3-year PFS. A cutoff of 3 years was chosen because most relapses occur within 2-3 years, and limited follow-up for several studies.[7] Secondary endpoints included event free survival (EFS), OS, and pre-ASCT CMR rate. PFS was defined as time from enrollment in the clinical trial to progressive disease (PD) or death from any cause, whichever occurs first. To eliminate bias in PFS occurring due to differences in study protocols, patients with stable disease (SD) after salvage treatment who did not proceed to ASCT were censored at time of going off study. Patients who did not undergo ASCT but received BV monotherapy instead were censored at time of end of salvage chemotherapy. EFS was defined as time from enrollment to PD or death, or until end of salvage therapy if patients could not proceed to ASCT due to toxicity or insufficient response (SD/PD) after salvage therapy. Patients with SD who received additional therapy before ASCT were counted as event. OS was defined as time from enrollment to death from any cause.

CMR was defined as Deauville score (DS) 1-3 according to the 2014 Lugano criteria.[26] A partial metabolic response (PMR) was defined as DS 4-5 without progression or development of new lesions. In the study of Moskowitz et al. in the chemo-cohort, the pre-ASCT PET-scans were evaluated according to the international working group criteria, in which a positive scan was defined as uptake greater than the mediastinal or abdominal aortic blood pool (comparable to ≥DS3).[4,27] To harmonize response assessment, all positive PET-scans from this study were re-assessed according to the Lugano criteria by a nuclear medicine physician (HS).[26]

The definition of primary refractory disease varied among studies, and not all collected relapse interval data. We defined primary refractory disease as 'not having achieved a complete response on first line treatment', encompassing partial response, SD or PD, irrespective of relapse interval. Bulky disease was defined as a tumor bulk ≥5 cm. Early relapse was defined as relapse interval <1 year. Stage was defined according to the Ann Arbor criteria. In the study of Santoro et al.[5] (n=59 patients), stage was not collected but information about the number of lymphatic and extralymphatic sites allowed to identify patients with stage I (one lymphatic site) or stage IV disease (≥1 lymphatic and ≥1 extralymphatic site, and the investigators confirmed that there were no patients with stage IE/IIE disease). However, stage II and III were combined for n=24 patients because the infra- or supradiaphragmatic distribution was unknown. Primary treatment was categorized into ABVD, escalated bleomycin, etoposide, adriamycin, cyclophos-

phamide, vincristine, procarbazine and prednisone (escBEACOPP) or other therapies. Patients initially treated with ABVD and later escalated to escBEACOPP were categorized under escBEACOPP.

Statistical analysis

Pearson's chi-squared or Fisher's exact test were used to compare categorical variables, and Kruskal–Wallis rank-sum test for assessing continuous variables. Survival outcomes were analyzed using the Kaplan-Meier method and pairwise log-rank tests. Univariable and multivariable Cox regression analyses were performed to assess the association between baseline characteristics and survival outcomes. Logistic regression was used to assess the association between baseline characteristics and binary response outcomes. Patients with missing data were only excluded from analyses when the missing variable was required for the specific analysis.

A 1:1 propensity score matching analysis was performed to adjust for the effects of unbalanced covariates between the BV- and chemo-cohort.[28] We conducted matching based on baseline patient characteristics significantly associated with PFS. To ensure a robust distribution of patients within the matched dataset, we repeated the matching process 2000 times as part of internal cross-validation. More detailed information about the matching procedure is provided in the **Supplementary Extended Methods**.

Statistical analysis was performed using R software version 4.0.3. A P-value of <0.05 was considered statistically significant.

Data sharing

Researchers may request access to certain de-identified data and related study documents by contacting the corresponding author.

RESULTS

Patient characteristics

Individual patient-data of ten clinical trials with a total of 832 transplant-eligible patients were collected.[4-6,15-21] Sixty-four patients were excluded (mainly because they had received >1 line of therapy). In total, 768 patients were included, with 386 in the BV-cohort (BV +/- salvage chemotherapy) and 382 in the chemo-cohort (salvage chemotherapy only) [**Figure 1** and **Table 1**]. There was an imbalance in primary refractory cases (55% versus 20% for the BV- and chemo-cohort, respectively) due to a substantial number of patients enrolled in the study of Josting et al.[6] (225 of 382; 59%) that specifically excluded primary refractory patients. Moreover, this study included more patients who were treated with escBEACOPP as primary treatment. An overview of study information including treatment regimens and summarized patient characteristics can be found in **Supplemental Table 1** and **2**.

Table 1. Baseline patient characteristics in the whole dataset

Patient characteristics (N; %)	BV-cohort (N=386)	Chemo-cohort (N=382)	*P-value*
Female sex	202 (52%)	168 (44%)	0.021
Age, median (range)	31 (5 - 68)	34 (18 - 72)	0.031
WHO PS			< 0.001
0	158 (64%)	256 (70%)	0.1030
1	85 (34%)	79 (22%)	0.0008
2	5 (2%)	29 (8%)	0.0029
Unknown	138	18	
Ann Arbor stage			< 0.001
I	29 (9%)	43 (11%)	0.3589
II	132 (41%)	135 (36%)	0.1861
III	53 (16%)	59 (16%)	0.8534
II or III[1]	0 (0%)	24 (6%)	*NA*
IV	109 (34%)	117 (31%)	0.4791
Unknown	63	4	
B symptoms	107 (28%)	74 (23%)	0.133
Unknown	2	59	
Extranodal disease	142 (38%)	134 (35%)	0.493
Unknown	8	1	
Bulky disease[2]	128 (37%)	101 (31%)	0.126
Unknown	40	60	
Primary refractory[3]	213 (55%)	78 (20%)	< 0.001
Relapse interval in days, median (range)	147 (0 - 4883)	250 (0 - 5258)	0.123
Unknown	*212*	*6*	
Early relapse <1 year	259 (76%)	230 (61%)	< 0.001
Unknown	43	5	
Response to primary treatment			< 0.001
Complete response	173 (59%)	304 (89%)	< 0.001
Partial response	55 (19%)	21 (6%)	< 0.001
Stable disease	18 (6%)	2 (1%)	< 0.001
Progressive disease	46 (16%)	14 (4%)	< 0.001
Unknown	94	41	
Primary treatment			< 0.001
ABVD	254 (90%)	259 (71%)	< 0.001
BEACOPP	16 (6%)	79 (22%)	< 0.001
Other	11 (4%)	25 (7%)	0.1455
Unknown	105	19	
BV maintenance post-ASCT	87 (24%)	*NA*	*NA*

Abbreviations: Chemo, chemotherapy; PS, performance status.

[1]For 24 patients in the chemo-cohort from the trial by Santoro et al, stage at relapse was not recorded but stage I and IV were deducted from the amount of involved lymph node sites, extranodal sites and bone marrow involvement. It was not possible to distinguish between stage II and III disease because no data was available on the spatial distribution of nodal sites (i.e. infra- and/or supradiaphragmatic location).

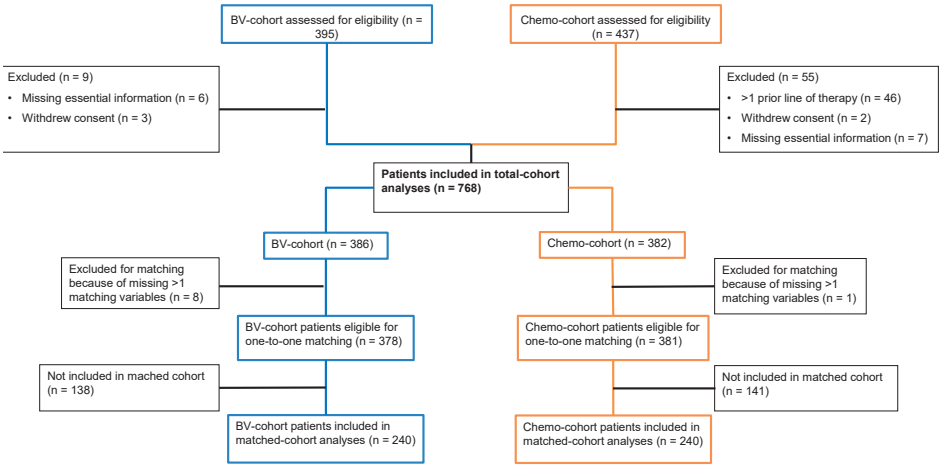

Figure 1. Consort diagram. *Abbreviations: Chemo, chemotherapy; n, number of patients.*

Survival outcomes in the whole cohort

The median follow-up time was 38 months (interquartile range, IQR, 24-50) for the BV-cohort and 47 months (IQR 31-68) for the chemo-cohort. Of 242 patients with PD, only 17 (7%) progressed beyond three years, supporting the 3-year cutoff for survival analysis [**Supplemental Table 3**]. The 3-year PFS, without matching for baseline characteristics, was not significantly different between the BV- and chemo-cohort: 66.7% (95% confidence interval (CI): 62-72) versus 67.4% (95% CI: 63-72) (p=0.61), respectively, and EFS was comparable to PFS [**Supplementary Figure 2**]. In the BV cohort, 40 (10.4%) patients died, of whom 9 patients died without having PD (n=2 toxicity, n=3 infection, n=1 other cause, n=3 unknown). In the chemo-cohort, a total of 76 (19.9%) patients died, of whom 14 patients died without PD (n=7 toxicity, n=1 infection, n=3 other cause, n=3 unknown). Three-year OS was significantly higher for the BV-cohort compared to the chemo-cohort: 91.0% (95% CI: 88-94) versus 80.4% (95% CI: 76-85) (p=0.002) [**Supplementary Figure 2** and **3**].

Survival outcomes in the matched dataset

The following variables were significantly related to PFS and were used for propensity score matching: R/R status, bulky disease, extranodal disease, stage IV, B symptoms and primary treatment with escBEACOPP [**Supplementary Extended methods Table 2**]. The matched dataset consists of a total of 480 patients with 240 patients each in the BV- and chemo-cohort in which the patient characteristics are now equally distributed, except for WHO performance status 2, but this was not significantly related to PFS (p=0.6) or OS (p=0.6) [**Table 2**, **Extended methods Table 2**].

Table 2. Patient characteristics in the matched dataset

	BV-cohort (N=240)	Chemo-cohort (N=240)	*P-value*
Female sex	132 (55%)	130 (54%)	0.855
Age, median (range)	30 (11 - 66)	33 (18 - 72)	0.118
Primary refractory	78 (32%)	78 (32%)	1.000
B symptoms at relapse	70 (29%)	42 (23%)	0.163
Unknown	*1*	*59*	
Stage at relapse[1]			
I	16 (8%)	23 (10%)	0.627
II	77 (38%)	84 (35%)	0.631
II or III	0 (0%)	24 (10%)	*NA*
III	37 (18%)	27 (11%)	0.112
IV	72 (36%)	79 (33%)	0.612
Unknown	*38*	*3*	
Extranodal disease at relapse	102 (42%)	94 (39%)	0.458
Bulky disease at relapse[2]	89 (41%)	71 (39%)	0.689
Unknown	*24*	*59*	
Primary treatment with escBEACOPP	14 (8%)	17 (7%)	0.985
Early relapse <1year	129 (65%)	162 (68%)	0.480
Unknown	42	3	
WHO PS			
0	98 (66%)	158 (70%)	0.505
1	49 (33%)	48 (21%)	1.000
2	2 (1%)	21 (9%)	0.0036
Unknown	91	13	
Response to primary treatment = PD	14 (7%)	14 (7%)	0.414
Unknown	*38*	*41*	

Abbreviations: PS, performance score; PD, progressive disease; N, number.

[1]For 24 patients in the chemo-cohort from the trial by Santoro et al, stage at relapse was not recorded but stage I and IV were deducted from the amount of involved lymph node sites, extranodal sites and bone marrow involvement. It was not possible to distinguish between stage II and III disease because no data was available on the spatial distribution of nodal sites (i.e. infra- and/or supradiaphragmatic).

In the matched dataset, 3-year PFS did not significantly differ between the BV- and chemo-cohort with a 3-year PFS of 72.2% (95% CI: 67-78) *versus* 67.1% (95% CI: 61-73) (p=0.14), respectively [**Figure 2A** and **Supplemental Table 4**]. The EFS was similar to PFS. However, there was a significant higher 3-year OS for patients treated within the BV-cohort of 91.9% (95% CI: 88-96) vs 79.5% (95% CI: 74-85) for the chemo-cohort, p=0.00043 [**Figure 2C**]. In patients with PD, significantly more patients died in the chemo-cohort (31/72; 43%) compared to the BV-cohort (19/65; 29%) (p=0.0011), while in patients without PD there was no significant difference in the number of deaths between the BV-cohort (5/175; 3%) versus the chemo-cohort (8/168; 5%)

(p=0.4), suggesting that advances in later lines of therapy are most likely the cause of improved OS in the BV-cohort.

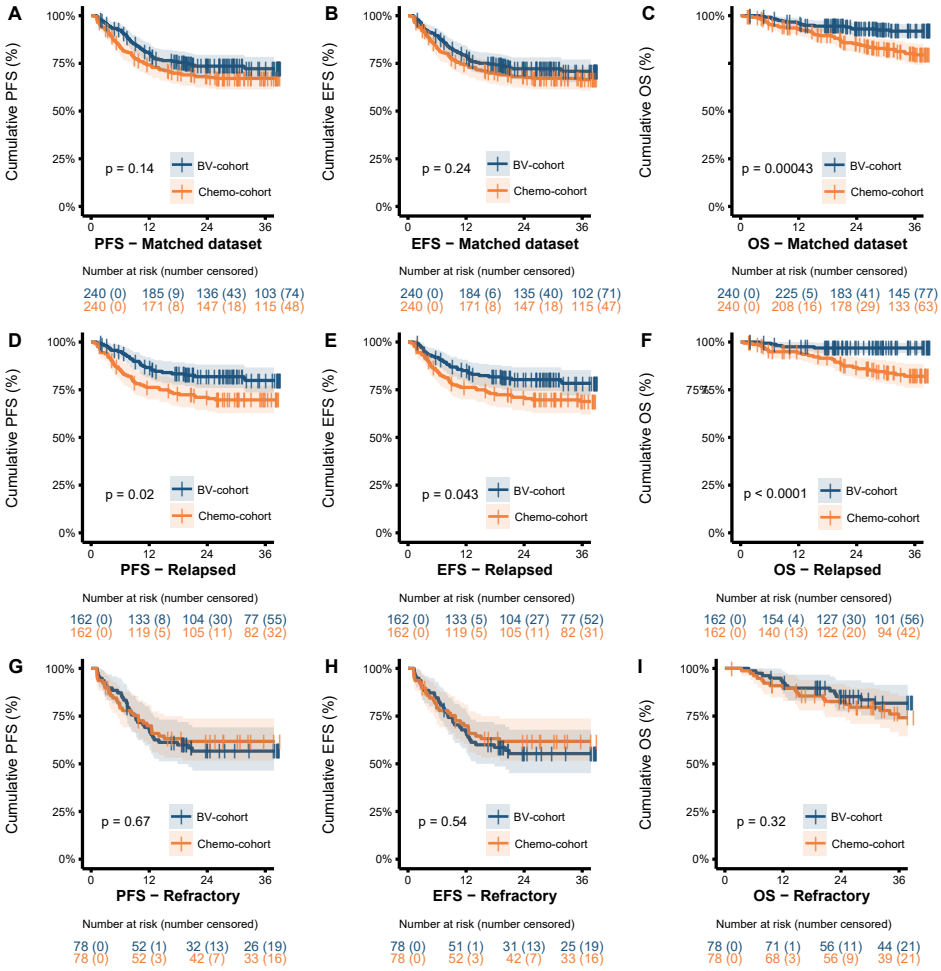

Figure 2: Kaplan-Meier survival analyses on the matched cohort. Kaplan-Meier curves showing the progression free survival (PFS), event free survival (EFS) and overall survival (OS) in the brentuximab vedotin (BV) and chemotherapy (chemo)-cohort in the matched dataset (**A, B, C**), and corresponding analyses stratified for patients with relapsed (**D, E, F**) or primary refractory disease (**G, H, I**).

In patients with relapsed disease, the BV-cohort showed a significantly better 3-year PFS compared to the chemo-cohort of 79.9% (95% CI: 74-87) versus 69.7% (95% CI: 63-77), respectively (p=0.02) [**Figure 2D**]. The EFS and OS for relapsed patients were also significantly better in the BV-cohort (p=0.043 and p<0.0001, respectively). However, for patients with primary refractory disease, there were no significant differences in 3-year PFS (p=0.67), EFS (p=0.54) and OS (p=0.32) between the BV- and chemo-cohorts [**Figure 2G-I**].

In the BV-cohort, 216 (90%) patients underwent ASCT compared to 199 (83%) patients in the chemo-cohort (p=0.023) [**Table 3**]. Post-ASCT survival outcomes were comparable between the BV- and chemo-cohorts [**Supplementary Figure 4**]. In patients with relapsed disease who underwent ASCT, the 3-year PFS (p=0.32) and EFS (p=0.32) were not significantly different, but the OS was significantly better for the BV-cohort (p=0.0097). Again, for primary

Table 3. Pre-ASCT response rates and patients who underwent ASCT

Outcome	Dataset	BV-cohort			Chemo-cohort			P chisq[1]	P multivar[2]
		N	Total	%	N	Total	%		
Underwent ASCT	Whole	335	386	87%	324	382	85%	0.38	0.064
Underwent ASCT	PET	335	386	87%	130	157	83%	0.20	0.23
Underwent ASCT	Matched	216	240	90%	199	240	83%	0.023	0.020
Underwent ASCT	Whole - Relapsed	156	173	90%	262	304	86%	0.20	0.012
Underwent ASCT	Whole - Refractory	179	213	84%	62	78	79%	0.32	0.40
Underwent ASCT	Whole - Stage IV	92	109	84%	91	117	78%	0.15	0.29
CMR	PET	292	386	76%	126	157	80%	0.30	0.23
CMR	Matched[4]	193	240	80%	108	137	79%	0.69	0.28
CMR	PET - Relapsed	148	173	86%	78	90	87%	0.72	0.75
CMR	PET - Refractory	144	213	68%	48	67	72%	0.67	0.11
CMR	PET - Stage IV	74	109	68%	46	60	77%	0.22	0.42
ORR (PET)	PET	343	386	89%	136	157	87%	0.46	0.51
ORR (PET)	PET - Relapsed	164	173	95%	81	90	90%	0.14	0.11
ORR (PET)	PET - Refractory	179	213	84%	55	67	82%	0.71	0.43
ORR (PET)	PET - Stage IV	90	109	83%	50	60	83%	0.90	0.97
ORR (CT)	Whole	343	386	89%	300	382	79%	<0.001	<0.001
ORR (CT)	Whole - Relapsed	164	173	95%	238	304	78%	<0.001	<0.001
ORR (CT)	Whole - Refractory	179	213	84%	62	78	79%	0.36	0.84
ORR (CT)	Whole - Stage IV	90	109	83%	88	117	75%	0.18	0.020
CMR ICE/BeGEV[5]	PET	292	386	76%	105	157	67%	0.025	0.0017
CMR ICE/BeGEV	Matched[4]	193	240	80%	93	137	68%	0.005	0.0040
CMR ICE/BeGEV	PET - Relapsed	148	173	86%	67	90	74%	0.030	0.007
CMR ICE/BeGEV	PET - Refractory	144	213	68%	38	67	57%	0.067	0.15
CMR ICE/BeGEV	PET - Stage IV	74	109	68%	39	60	65%	0.69	0.11

Abbreviations: chisq, chi-square test; multivar, multivariable logistic regression analysis.

[1]P values from chi-square comparison of BV- versus Chemo-cohort

[2]P values from multivariable logistic regression comparing BV- versus Chemo-cohort corrected for baseline characteristics: R/R status, stage, B symptoms, extranodal disease, bulky disease and primary treatment with escBEACOPP.

[3]The PET dataset is the whole dataset excluding patients from the study of Josting et al., in which response-assessment was done by conventional CT scan only.

[4]For CMR calculations in the matched dataset, patients from the study of Josting et al. have been removed from the chemo-cohort, resulting in a smaller chemo-cohort of n=137 patients instead of n=240.

[5]Comparison of pre-ASCT CMR rates measured after first sequential chemotherapy only. In the study of Moskowitz et al. patients received sequential ICE and GVD chemotherapy in case of no CMR. In this comparison the response after ICE only is used in the chemo cohort

refractory patients there was no difference in PFS (p=0.18), EFS (p=0.22) and OS (p=0.48) [**Supplemental Table 5** and **Supplementary Figure 4**].

Subgroup analysis for survival between BV- and chemo-cohort

In the matched dataset, we tested differences in 3-year PFS between the BV- and chemo-cohort for specific subgroups using univariable Cox regression [**Figure 3**]. Patients with relapsed disease in the BV-cohort had a significantly lower risk of PD compared to the chemo-cohort (HR 0.59; 95% CI: 0.37-0.93; p=0.022). Similarly, patients with stage IV disease had significantly lower

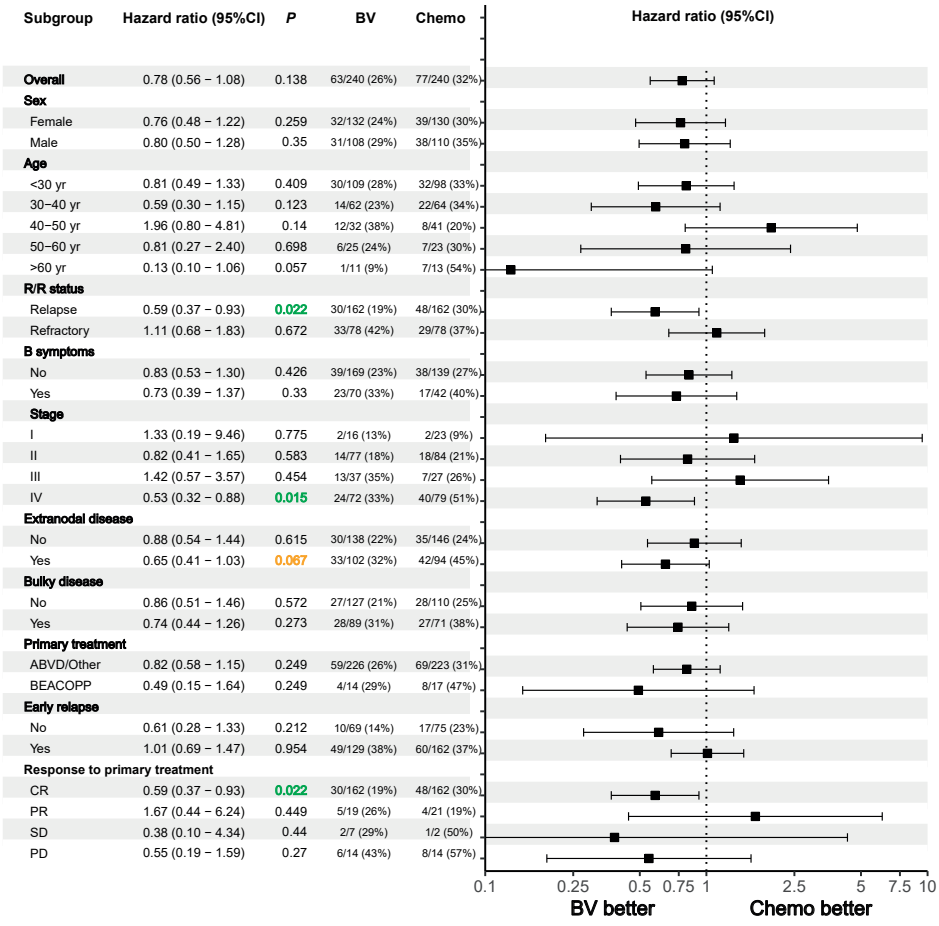

Figure 3: Forest plot of the association between baseline characteristics and differences in progression free survival between the BV- and chemo-cohorts. Hazard ratios are shown for univariable Cox regression on subgroup analyses of baseline characteristics for progression free survival (PFS) comparing the brentuximab vedotin (BV)- and chemo-cohorts. A hazard ratio lower than 1 corresponds to a higher PFS in the BV-cohort compared to the chemo-cohort.

Abbreviations: CI, confidence interval; yr, year; R/R status, relapsed or primary refractory disease status.

risk of PD in the BV-cohort (HR 0.53, 95% CI: 0.32-0.88; p=0.015). Patients with extranodal disease showed a trend for better PFS in the BV-cohort with a HR of 0.65 (95% CI: 0.41-1.03; p=0.067), but this was not significant. Exploratory multivariable subgroup analysis of R/R status and stage IV showed a trend for better PFS in the BV-cohort for patients who had both stage IV and relapsed disease (n=97) (HR 0.50; 0.25-1.02; p=0.058).

Pre-ASCT PET responses in the whole cohort

Nine out of ten studies had PET-CT data available. N=225 patients from the study of Josting et al. were excluded from the chemo-cohort because responses were assessed using conventional CT scan. Consequently, the chemo-cohort comprised 157 patients with available PET data. The CMR rate in the whole BV-cohort was 76% versus 80% in the chemo-cohort (p=0.30) [**Table 3**]. The ORR rates based on PET were not significantly different between the BV- and chemo cohorts. However, when including patients from the study of Josting et al. in which the ORR was based on conventional CT, the BV-cohort displayed a significantly higher ORR of 89%, compared to 79% in the chemo-cohort (p<0.001) [**Table 3**].

In subgroup analysis, patients with relapsed disease exhibited higher CMR rates compared to patients with primary refractory disease. However, no significant differences in CMR or ORR rates were observed between the BV- and chemo cohorts within these subgroups [**Table 3**].

In the study of Moskowitz et al. within the chemo-cohort, patients with a PMR or SD after ifosfamide, carboplatin, and etoposide (ICE) treatment underwent sequential gemcitabine, vinorelbine, and docxorubicin (GVD). This sequential therapy resulted in a conversion from PMR/SD to a CMR in n=21 patients (of whom n=15 were included in the matched cohort). To ensure a comprehensive assessment, we recalculated the CMR rate after ICE-only, excluding these patients from the CMR count. This adjustment yielded a CMR rate of 67% for the total matched chemo-cohort. Upon comparing the CMR rate of 76% in the BV-cohort to the CMR rate of 67% after ICE-only in the chemo-cohort, a notable significance emerged in both univariable (p=0.025) and multivariable analysis (p=0.0017) [**Table 3**]. This distinction was particularly pronounced among patients with relapsed disease, as in this subgroup the CMR rate was significantly higher in the BV-cohort compared to the chemo-cohort. Conversely, in primary refractory patients, no significant differences in CMR rates were observed between the two cohorts [**Table 3**].

Slightly more patients underwent ASCT in the BV-cohort (335/386; 87%) versus the chemo-cohort (324/382; 85%), but this was not significant in univariable (p=0.38) or multivariable analysis adjusted for baseline characteristics (p=0.06). For relapsed patients, a significant higher percentage of patients underwent ASCT in the BV-cohort compared to the chemo-cohort (90% versus 86%; p=0.012 multivariate) [**Table 3**]. Among patients who underwent ASCT, those achieving a CMR (n=398) pre-ASCT had a 3-year PFS of 78.3% (95% CI: 74-83), which was significantly higher than those transplanted after a PMR (n=57) with a 3-year PFS of 64.2% (95% CI: 53%-78%) (p=0.01), or SD (n=8) with a 3-year PFS of 37.5% (95% CI: 15-92; p=0.0004)

[**Figure 4A**]. Notably, post-ASCT there was a significantly lower OS for patients with SD compared to a CMR (p=0.0042), while no OS difference was observed for patients with a PMR versus CMR (p=0.286 [**Figure 4B**].

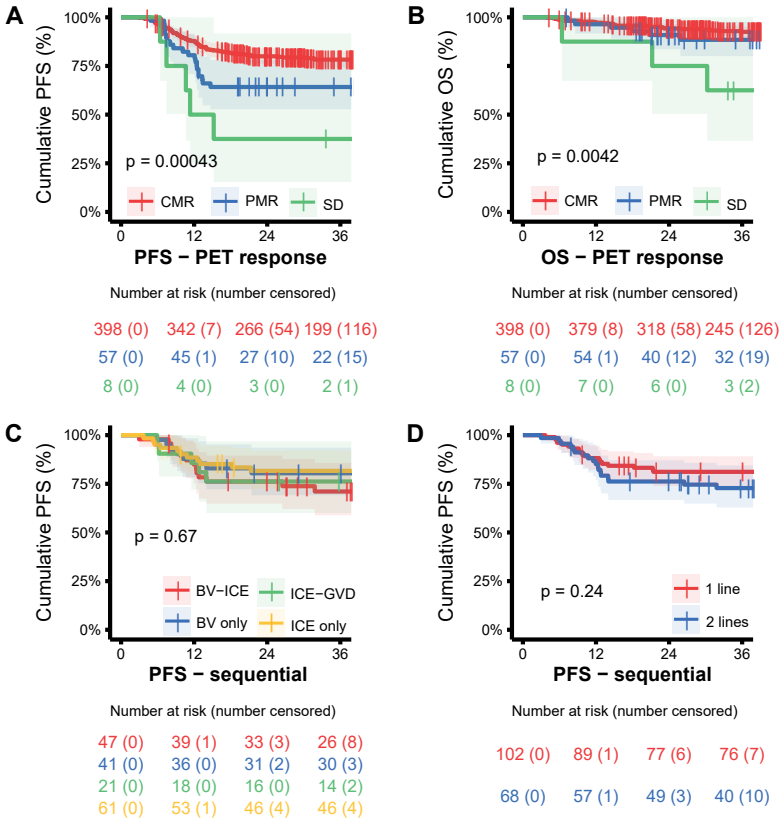

Figure 4: Kaplan-Meier subgroup survival analyses on the whole dataset. A) Progression free survival (PFS) and **B)** overall survival (OS) in patients who underwent ASCT stratified for pre-ASCT PET response in the whole dataset. **C-D)** PFS for patients who were treated in studies with a sequential approach and achieved a complete metabolic response (CMR) after one line of salvage treatment (BV or ICE only) *versus* patients who initially had no CMR but converted to a CMR after two lines of sequential treatment with additional chemotherapy (BV-ICE or ICE-GVD).

Influence of BV dose and salvage chemotherapy schedule

Within the whole BV-cohort (unmatched dataset, BV-cohort n=386), subgroup analysis shows a non-significant trend for a higher PFS (HR 0.72; 95% CI: 0.50 – 1.04; p=0.079) in studies that used BV with a combination of chemotherapeutic agents, e.g. dexamethasone, high-dose cytarabine, and cisplatin (DHAP), ICE, or etoposide, methylprednisolone, cisplatin and cytarabine (ESHAP), versus a single agent, e.g. bendamustine or gemcitabine [**Supplemental Table 6**].[16,17,21,24] The use of a sequential schedule (i.e. BV monotherapy followed by chemotherapy), the number of

BV cycles and the cumulative BV dose did not have an impact on 3-year PFS or pre-ASCT CMR rate between studies in the BV-cohort. This suggests that more cycles of BV does not improve CMR rates or PFS. Two studies applied BV maintenance after ASCT (11% of total number of patients).[17,19] However, not all patients received BV maintenance and many patients received less than the intended number of maintenance cycles due to toxicity or other reasons, which limits an analysis to assess the effect of BV maintenance [**Supplemental Table 2**].[17,19]

Outcomes of sequential treatment

Three studies followed a sequential approach: two studies in the BV-cohort used 2-4 cycles of BV monotherapy, allowing patients with a CMR to proceed directly to ASCT while PET-positive patients received additional ICE salvage chemotherapy before ASCT, and one study in the chemo-cohort used two cycles of ICE and patients without CMR received additional GVD chemotherapy before ASCT.[4,21,24] Subgroup analysis showed no significant differences in 3-year PFS between patients achieving CMR with one line of therapy (BV monotherapy or ICE only) and those requiring two lines (BV-ICE or ICE-GVD) to achieve a CMR (p=0.24) [**Figure 4C** and **4D**]. OS also showed no significant differences between these groups (p=0.62) [**Supplemental Table 7**].

DISCUSSION

In this matched analysis of individual patient-data from prospective single-arm clinical trials, we investigated the effect of BV addition to salvage chemotherapy followed by ASCT in transplant-eligible R/R cHL patients. We found no statistically significant differences in PFS, EFS and pre-ASCT CMR rate for patients treated with BV +/- chemotherapy compared to patients treated with salvage chemotherapy only. However, relapsed patients and patients with stage IV disease had a significantly better PFS and EFS when adding BV to the salvage treatment. While OS was significantly better in the BV-cohort, this may be influenced by the time in which the BV studies were conducted (2015-2021) compared to chemo-cohort studies (2010-2016). A recent retrospective study in R/R cHL patients who underwent ASCT showed an OS improvement over time, corresponding to the increased usage of immune-checkpoint inhibitors and BV.[29] Therefore, the observed OS difference in the BV cohort is probably driven by the availability of checkpoint inhibitors for patients who fail salvage therapy or relapse after ASCT.[9-11,30]

The disparity in survival outcomes between primary refractory and relapsed patients could potentially be explained by the antitumor mechanism of action of BV. BV elicits its antitumor effect through the cytotoxic warhead MMAE, a substrate for the multidrug resistance pump P-glycoprotein (PGP).[31] It has been shown that BV-resistant cell lines have elevated PGP, which is known to also occur after exposure to other cytotoxic agents such as doxorubicin.[32,33] Thus, tumor cells that are able to resist first-line chemotherapy might employ the same mechanism to

convey resistance to BV. Because patients with primary refractory disease are more likely to be resistant to chemotherapy, this might explain that they could also be resistant to BV. Therefore, in patients with primary refractory disease there is still an unmet need to improve outcomes, and other non-chemotherapeutic therapies such as immune checkpoint inhibitors should be considered.[34-36]

Patients with stage IV disease had improved PFS in the BV-cohort versus the chemo-cohort. This may be attributed to a larger total tumor volume, necessitating intensified treatment which could be achieved by augmenting standard chemotherapy with BV. In subgroup analyses of the Echelon-1 trial, stage IV was also associated with better PFS in patients treated with BV-AVD compared to ABVD, suggesting a similar effect in the R/R setting.[13,14]

We showed that patients who were treated with a sequential approach who achieved a PMR after BV or ICE only, yet converting to a CMR following salvage chemotherapy with ICE (after BV) or GVD (after ICE), exhibited comparable survival outcomes to those directly achieving CMR. This highlights the feasibility of a sequential approach, potentially sparing chemotherapy in rapid responders. Emphasizing the significance of attaining CMR pre-ASCT, our study suggests that improving survival in PMR patients could be accomplished by inducing CMR through additional salvage chemo- or immunotherapy before ASCT.[4,21,24]

Our analysis is limited by missing variables in certain studies, partially mitigated by our matching method. Consequently, not all patients could be included in specific (multivariable) analyses. Additionally, a large portion of patients in the chemo-cohort lacked response assessment using PET, restricting the comparison of pre-ASCT CMR rates between the BV- and chemo-cohorts. Unfortunately, we could not evaluate the impact of BV maintenance in our analysis as only a limited number of patients received BV maintenance in our cohort, and the number of BV maintenance cycles differed widely across patients due to various reasons, limiting a proper analysis. Additionally, assessing the impact of radiotherapy was hindered by varying protocols among the studies. While some universally applied pre-ASCT radiotherapy to patients with extranodal and bulky disease, others selectively used it on residual lesions either before or after ASCT.[4,16,24]

Generally, the PFS, OS and CMR rates in the chemo-cohort appear favorable compared to real-world data.[7,37] However, the studies in our analysis only included transplant-eligible patients, known for better outcomes compared to elderly or unfit patients. Furthermore, the study of Josting et al. specifically excluded primary refractory patients. While our analysis minimizes bias through matching and inclusion of prospective trials, caution is warranted in generalizing to real-world scenarios. Therefore, the observed results of our analysis should be interpreted with caution and cannot replace an RCT. Nonetheless, at the moment this is the largest matched analysis based on individual patient-data in R/R cHL, incorporating recent clinical trial data. Therefore, it serves as a benchmark for future (single-arm) studies exploring novel therapies or regimens that aim to replace HDCT/ASCT with novel drugs.

An ongoing phase IIb RCT is comparing BV-ESHAP versus ESHAP alone in 150 patients, but its limited sample size may hinder subgroup analyses for risk factors (e.g. primary refractory, B symptoms, stage IV disease).[38] This trial also lacks comparison with various other salvage regimens like ICE, DHAP, bendamustine or gemcitabine, and the inclusion of BV maintenance post-ASCT could obscure the primary effect of BV addition to salvage chemotherapy.[39] Additionally, emerging novel therapies, including immune-checkpoint inhibitors, are gaining attention in the relapsed/refractory setting.

In a phase III head-to-head comparison, single-agent pembrolizumab demonstrated superior median PFS and lower toxicity to BV.[40] Checkpoint inhibition, either alone or in combination with BV or chemotherapy, has proven effective in single-arm studies.[34-36] Exploring a similar individual patient-data analysis for studies combining chemotherapy with checkpoint inhibitors versus BV-chemo or chemotherapy alone could offer valuable insights. The evolving landscape, where BV is increasingly used in newly diagnosed patients, raises questions about its retreatment efficacy in the salvage setting.[13] However, retreatment with BV in patients with multiple relapses showed persistent efficacy.[41] Preliminary findings from an extensive ongoing RCT comparing nivolumab-AVD to BV-AVD demonstrated favorable outcomes for the nivolumab-AVD arm.[42] This outcome might potentially prompt a shift toward integrating checkpoint inhibitors as a first-line treatment, thereby reinstating the use of BV in the salvage setting. Consequently, our results remain pertinent for future treatment contexts. As novel therapeutic options shift to earlier lines of therapy, such as the use of checkpoint inhibitors in the first or second line, studying the sequencing effects of these agents becomes increasingly crucial, ideally through prospective clinical trials. However, it is essential to acknowledge the lack of universal global access to these novel (and often expensive) agents, a consideration that should also be addressed in guidelines outlining the optimal treatment for patients with R/R cHL.

In summary, our study indicates that the addition of BV to chemotherapy did not enhance CMR rates or PFS in the overall population of R/R cHL patients compared to standard salvage chemotherapy. However, notable PFS improvements were observed in patients with relapsed or stage IV disease undergoing salvage treatment that includes BV. Moreover, a sequential approach involving BV monotherapy followed by salvage chemotherapy is both viable and has the potential to reduce the need for salvage chemotherapy in certain patients. In the absence of RCTs, this propensity score matched analysis on individual patient-data, offers valuable insights in the treatment landscape for patients with R/R cHL. .e

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CONFLICT OF INTEREST

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SUPPLEMENTARY MATERIAL

EXTENDED METHODS

Part I: Literature search and inclusion of clinical trials

Inclusion criteria:

- Published after the year 2000
- Prospective design
- Included at least 10 patients
- Included only transplant eligible patients with Hodgkin' lymphoma
- Treatment with single agent or a combination of different chemotherapeutic agents followed by high-dose chemotherapy and autologous stem-cell transplantation
- Patients either relapsed after or were primary refractory on first-line chemotherapy treatment
- Biopsy proven relapse or refractory disease

Chemo-cohort

(((((((Hodgkin Disease[MeSH]) OR Hodgkin disease[tiab]) OR Hodgkin's disease[tiab]) OR ((Hodgkin lymphoma[tiab] NOT Non-Hodgkin lymphoma[tiab]))) OR ((Hodgkin's lymphoma[tiab] NOT Non-Hodgkin's lymphoma[tiab]))))) AND ((Refractory[tiab]) OR Relapse*[tiab]))) AND ((((((((((Chemotherapy, adjuvant[MeSH]) OR Consolidation chemotherapy[MeSH]) OR Chemoradiotherapy[MeSH]) OR Induction chemotherapy[MeSH]) OR Maintenance chemotherapy[MeSH]) OR Antineoplastic protocols[MeSH]) OR Chemo*[tiab]) OR Salvage therapy[MeSH]) OR Salvage Therapy[tiab]) OR Salvage chemotherapy[tiab]) OR Salvage regiment[tiab])*

The above search string for PubMed was performed on 01-06-2019 and yielded 2677 results and was checked regularly after this date for new published studies. Studies published before 2000 (n=1107) were excluded from the search. N=7 were selected as potentially includable (based on title/abstract screening and full text if inclusion was unsure) of which the authors were contacted. Three studies were assessed by the German Hodgkin Study Group (GHSG) and in contact with them we only included the most recent study from 2010 and therefore excluded the two older trials. Three studies were conducted by C. Moskowitz et al and as with the GHSG we decided to only include the most recent study from 2012 that also had PET data available. The third study of Santoro et al. was also included. This led to the inclusion of 3 prospective clinical trials of which the authors were contacted. All authors responded and were willing to collaborate.

BV-cohort

(((((((Hodgkin Disease[MeSH]) OR Hodgkin disease[tiab]) OR Hodgkin's disease[tiab]) OR ((Hodgkin lymphoma[tiab] NOT Non-Hodgkin lymphoma[tiab]))) OR ((Hodgkin's lymphoma[tiab] NOT Non-

Hodgkin's lymphoma[tiab])))) AND ((Refractory[tiab]) OR Relapse*[tiab]))) AND ((((Brentuximab vedotin[MeSH]) OR Brentuximab vedotin[tiab]) OR SGN-35[tiab]) OR Adcetris[tiab])*

The above search string for PubMed was performed on 01-06-2019 and yielded 329 results and was checked regularly after this date for new published studies. N=4 were selected as potentially includable. Two other studies were identified through abstract screening and were published shortly after the search date. Additionally, the clinicaltrials.gov database was searched on the following terms: Hodgkin lymphoma, brentuximab, SGN-35 or adcetris and identified 160 trials of which five trials were selected as potentially includable that were not yet identified on PubMed, including our own trial (Kersten et al., Haematologica 2021). The investigators of the remaining studies were contacted but eventually did not lead to inclusion since three of these trials were still recruiting and results were not expected on time for our analysis and another trial also included patients with multiple relapses and had less than 10 patients who were eligible according to our inclusion criteria. The authors of the six identified studies were contacted and were all willing to collaborate. Including our own study (Kersten et al., 2021) we included seven studies in the BV-cohort.

Part II: Matching of BV- and Chemo-cohorts

Matching was performed on a one-to-one base using propensity scores with the nearest neighbor method using the R package *MatchIt* (https://cran.r-project.org/web/packages/MatchIt/MatchIt.pdf) which has been validated for usage in relatively small cohorts.[1] As variables that are related to the outcome can influence outcomes of a propensity score analysis, the prognostic value of baseline characteristics on the whole dataset was determined using univariate cox regression and multivariate cox regression and variables that were independently related to the outcome were used as matching variables [**Extended methods Table 1** and **2**].[2] The following variables were independently associated with a significant higher risk of progressive disease: primary refractory disease, B symptoms, Ann Arbor stage IV disease, bulky disease, primary treatment with escBEACOPP, early relapse <1 year and progressive disease (PD) after primary treatment (i.e. no PR/SD). Extranodal disease was associated with a significant higher risk of progressive disease but was not dependent of stage IV disease. However, since one study missed Ann Arbor stage at relapse, but did have information about extranodal disease, we also used extranodal disease as matching variable. Early relapse and PD after primary treatment were not used as matching variable because of too many missing values, however after matching the distribution of these variables was checked between the cohorts. For each case in the BV-cohort exactly one case in the chemo-cohort will be matched. Because of an unequal number of patients with primary refractory disease in the BV- and chemo-cohorts (n=211 and n=78, respectively), we performed the matching separately for patients with relapsed disease or primary refractory disease. In addition, for some studies not all matching variables were known. For example, the study of LaCasce et al., did not have information about Ann Arbor stage at relapse and the study of Santoro et al. did not have information about bulky disease

and B symptoms [**Extended methods Table 3**]. Hence, we performed the matching in two steps: first, patients with all information available (Part 1) are matched separately from patients with a missing matching variable (Part 2) [**Extended methods Figure 1**]. In Part 1, patients are matched on all matching variables (i.e. primary refractory disease, bulky disease, extranodal disease, stage IV, presence of B-symptoms and primary treatment with escBEACOPP). In Part 2, patients are matched on primary refractory disease, extranodal disease and primary treatment with escBEACOPP. Second, patients with relapsed disease from the BV-cohort (*patient sample*) are matched one-to-one to a patient with relapsed disease from the chemo-cohort (*population sample*) while patients with primary refractory disease from the chemo-cohort (*patient sample*) are matched one-to-one to a patient with primary refractory disease from the BV-cohort (*population sample*). This is performed separately for Part 1 and 2. These two parts are merged afterwards and the spread of variables is checked in the final matched dataset [**Extended methods Figure 1**]. As sensitivity analysis, we also performed a one-stage matching in which patients with missing matching variables were excluded.

To reduce selection bias in the matched cohort, we performed an internal cross-validation by repeating the whole matching process 2000 times in which patients are randomly matched. For each matching iteration, we calculated the differences in progression free survival (PFS), event free survival (EFS) and overall survival (OS) for the BV- *versus* chemo-cohorts in the whole population and stratified for relapsed or primary refractory patients and took the iteration that produces the most median results as the final matched dataset [**Extended methods Table 4**].

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EXTENDED METHODS TABLES & FIGURES

Extended Methods Table 1. Univariable and multivariable Cox regression on the association between baseline characteristics and PFS

	Univariable			*Multivariable, corrected for R/R status*		
Covariates	HR	95% CI	*P-value*	HR	95% CI	*P-value*
Sex (Male)	1.08	0.83 - 1.39	0.5698	1.11	0.86 - 1.43	0.4180
Age (per unit)	1.00	0.99 - 1.01	0.3975	1.01	1.00 - 1.02	0.2473
Primary treatment with BEACOPP (ref = ABVD/Other)	1.31	0.92 - 1.86	0.139	1.57	1.09 - 2.27	0.0152
Ann Arbor stage	(ref = I)					
II	1.41	0.76 - 2.62	0.2723	1.33	0.72 - 2.48	0.3616
II or III	2.31	0.97 - 5.47	0.0581	1.99	0.84 - 4.75	0.1199
III	1.87	0.96 - 3.61	0.0644	1.85	0.96 - 3.59	0.0672
IV	3.15	1.73 - 5.73	0.0002	3.01	1.66 - 5.48	0.0003
B symptoms	1.78	1.61 - 2.74	0.0001	1.85	1.39 - 2.45	0.0000
Extranodal disease	1.74	1.34 - 2.36	0.0000	1.76	1.36 - 2.27	0.0000
Bulky disease	1.65	1.34 - 2.24	0.0004	1.65	1.25 - 2.18	0.0005
Primary refractory (ref = relapse)	1.69	1.25 - 2.18	0.0001	-	-	-
Early relapse <1 year	1.98	1.45 - 2.70	0.000	1.75	1.26 - 2.44	0.0010
Response_Dx=PD	2.51	1.69 - 3.71	0.000	2.20	1.32 - 3.66	0.0025
WHO PS (ref=0)						
1	1.48	1.09 - 2.02	0.011	1.43	1.05 - 1.94	0.0232
2	1.81	1.06 - 3.09	0.031	1.64	0.96 - 2.83	0.0718

Abbreviations: HR, hazard ratio; exp, exponential function; coef, coefficient; ref, reference group.

Univariable cox proportional hazard analysis was performed to assess the association between baseline patient characteristics and progression free survival. In multivariable analysis, the corresponding baseline characteristics were corrected for primary refractory *versus* relapsed disease (R/R status).

Extended Methods Table 2. Multivariable Cox regression on the association between baseline characteristics and PFS

		Multivariable	
Covariates	HR	95% CI	*P*
Primary treatment with BEACOPP	1.47	0.99 - 2.19	0.0559
Ann Arbor stage			
III	1.31	0.84 - 2.05	0.2364
IV	1.92	1.22 - 3.01	0.0045
B symptoms	1.89	1.35 - 2.65	0.0002
Extranodal disease	1.19	0.78 - 1.82	0.4147
Bulky disease	1.40	1.02 - 1.91	0.0364
Primary refractory	1.45	1.03 - 2.04	0.0331
Early relapse <1 year	1.70	1.12 - 2.58	0.0119
WHO PS			
1	0.96	0.67 - 1.37	0.8157
2	1.17	0.66 - 2.06	0.5950

Multivariate cox proportional hazard analysis for progression free survival (PFS) on baseline characteristics that were significant in univariable analysis.

Extended Methods Table 3. Missing values of matching variables

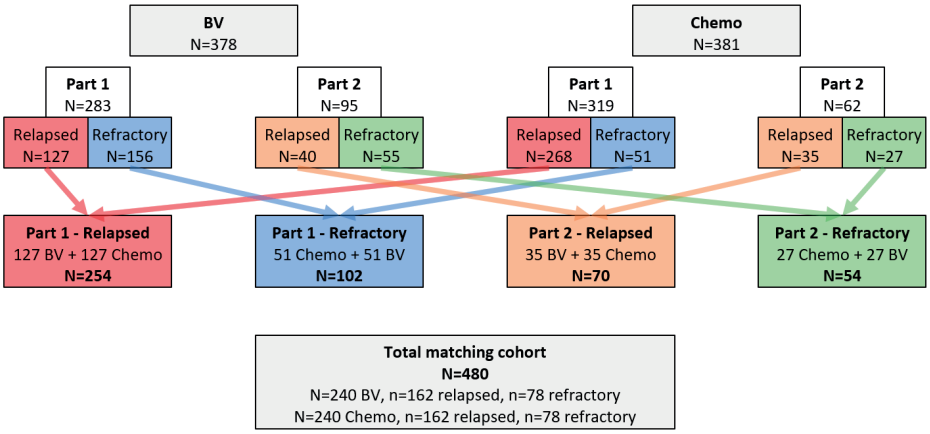
Variable	Missing	Patients with missing values per study (n)
R/R status	0	-
Extranodal	9	Garcia-Sanz (2), Herrera (6), Josting (1)
B symptoms	60	Santoro (59), LaCasce (1), Herrera (1)
Stage IV	61	Josting (4), LaCasce (55), Garcia-Sanz (2), Herrera (6)
Stage III	85	Josting (4), LaCasce (55), Garcia-Sanz (2), Herrera (6), Santoro (24)
Bulky	100	Cole (39), Herrera (1), Josting (1), Santoro (59)
BEACOPP	0	-
Early relapse	48	AMoskowitz (30), Broccoli (12), Herrera (1), Josting (5)
Response_Dx = PD	135	AMoskowitz (34), Broccoli (1), CMoskowitz (41), Cole (27), Herrera (31), LaCasce (1)
WHO-PS	156	AMoskowitz (64), CMoskowitz (10), Cole (16), Josting (1), Kersten (3), LaCasce (55), Santoro (7)

Number of patients per study with missing values in one or more matching variables.

Extended Methods Table 4. Progression free survival in the matched cohort for cross-validated matching repeats

	Two-stage matching (n=480)		One-stage matching (n=356)		Final matched dataset (n=480)	
Cohort	Median 3-year PFS of 2000 repeats (95% CI)	*P*	Median 3-year PFS of 2000 repeats (95% CI)	*P*	PFS (95% CI)	*P*
N per cohort *(refractory; %)*	N=240 *(n=78; 32%)*		N=178 *(n=51; 29%)*		N=240 *(n=78; 32%)*	
All patients						
BV	72.2% (66.5% - 78.4%)	0.1329	73.7% (67.2% - 80.7%)	0.1925	72.2% (66.5% - 78.3%)	0.1373
Chemo	66.9% (61.1% - 73.2%)		68.8% (62.3% - 76.1%)		67.1% (61.3% - 73.4%)	
HR	1.3 (0.9 - 1.8)	0.1326	1.3 (0.9 - 1.9)	0.1922	1.3 (0.9 - 1.8)	0.1370
Relapsed						
BV	79.7% (73.4% - 86.6%)	0.0196	80.6% (73.7% - 88.2%)	0.0335	79.9% (73.6% - 86.7%)	0.0203
Chemo	69.4% (62.5% - 77.0%)		70.7% (63.1% - 79.3%)		69.7% (62.8% - 77.2%)	
HR	1.7 (1.1 - 2.7)	0.0192	1.7 (1.0 - 2.9)	0.0331	1.7 (1.1 - 2.7)	0.0199
Refractory						
BV	56.9% (46.6% - 69.3%)	0.6388	56.3% (44.2% - 72.1%)	0.4580	56.6% (46.4% - 69.1%)	0.6716
Chemo	61.7% (51.7% - 73.8%)		64.4% (52.5% - 79.1%)		61.7% (51.7% - 73.8%)	
HR	0.9 (0.5 - 1.5)	0.6387	0.8 (0.4 - 1.5)	0.4578	0.9 (0.6 - 1.5)	0.6722

Matching was repeated 2000 times and for each iteration a log-rank comparison of 3-year PFS for the BV- vs chemo-cohort was performed on the whole dataset and stratified for relapsed and primary refractory status. Median results for all 2000 iterations are shown for the two-stage matching and for a one-stage matching sensitivity analysis in which patients with missing matching variables were excluded. *P*-values represent the median *P*-value for all iterations. The final matched dataset represents the iteration that approximates the median results the most and results for this single dataset are presented in the last column. *P*-values represent log-rank comparisons and hazard ratios of univariable cox regression between the BV- and chemo-cohort.



Extended methods Figure 1. Matching process of BV- and chemo-cohorts. Matching of BV- and chemo-cohorts in two steps. Part 1 includes patients with all information of matching variables available, Part 2 includes patients who have a missing variable in B-symptoms, Ann Arbor stage IV or bulky disease. The colored arrows indicate to which group patients are being matched.

Supplemental Table 1. Salvage regimen and BV dose per study

Study	Therapy	Salvage therapy schedule and dose
Moskowitz et al (2012)	ICE-GVD	Two treatment arms: 1. One cycle of etoposide (100mg/m^2) IV on day 1 and 3, carboplatin (5 AUC), and ifosfamide (5000mg/m^2) with equal dose MESNA. Followed by one cycle of ifosfamide (5000mg/m^2) mixed with equal dose MESNA IVCI 2 times starting on day 1, carboplatin (5 AUC) on day 3, etoposide (200mg/m^2) every 12 hours at 3 doses starting day 1. Second cycle was administered 14-21 days after cycle 1 dependent on platelet recovery. 2. Two cycles of ifosfamide (5000mg/m^2) mixed with equal dose mesna IVCI 2 times starting on day 1, carboplatin (5 AUC) on day 3, etoposide (200mg/m^2) every 12 hours at 3 doses starting day 1. Regimen was administered on a 17-21 day schedule. PET-positive patients after two cycles of (aug)ICE received two cycles of gemcitabine (1000mg/m^2), vinorelbine (20mg/m^2) and liposomal doxorubicin (15mg/m^2) every two weeks.
Josting et al (2010)	DHAP	Two cycles of dexamethasone (40 mg) IV on days 1 to 4, 2x cytarabine (2000 mg/m^2) over 3 hours on day 2, cisplatinum (100mg/m^2) IVCI for 24 hours on day 1. Second cycle was administered after platelet and white blood cell count recovery.
Santoro et al (2016)	BeGEV	Four cycles of gemcitabine (800mg/m^2) on days 1 to 4, vinorelbine (20mg/m^2) on day 1, bendamustine (90mg/m^2) on days 2 and 3, and prednisolone (100mg) on days 1 to 4. Regimen was administered every 21 days.
Herrera et al (2018)	BV-ICE (seq)	Two treatment arms: 1. A maximum of four 21-day cycles of BV (1.8mg/kg). Patients achieving CR or PR could proceed to ASCT after two cycles. 2. BV (1.8 mg/kg) every 21 days for a maximum of four cycles. Patients in PR or SD after two cycles received escalated BV (2.4 mg/kg) for two cycles. Patients with PR were given the option to receive salvage chemotherapy. Patients with PD or SD were required to undergo salvage chemotherapy. Therapy choice was at physicians discretion.
Moskowitz et al (2015)	BV-ICE (seq)	Two cycles of BV (1.2 mg/kg) on day 1, 8 and 15 of 28 day cycles. Patients with a Deauville score > 3 received two cycles of ifosfamide (5000 mg/m^2) combined with equal dose MESNA IVCI over 24 hours on days 1 and 2, 3x etoposide (200mg/m^2) IVCI over 60 min every 12 hours beginning on day 1, and carboplatin (5AUC) on day 3
LaCasce et al (2018)	BV-benda	Two to six cycles of BV (1.8 mg/kg) on day 1 and bendamustine (90mg/m^2) on days 1 and 2 of a 21 day cycle. Patients in CR may go off study to proceed to ASCT after at least two cycles. Patients who underwent ASCT are reregistered and may receive BV monotherapy until a total of 16 cycles has been reached (including pre-ASCT BV).
Cole et al (2018)	BV-gem	BV (1.8 mg/kg) on day 1 and gemcitabine (1000 mg/m^2) over 100 min on days 1 and 8 for a median of four cycles.
Broccoli et al (2019)	BV-benda	Up to six cycles of BV (1.8 mg/kg) on day 1 and bendamustine (90 mg/m^2) on days 1 and 2 of each 21 day cycle. Patients in response after two cycles were allowed to proceed to ASCT.
Garcia-Sanz et al (2019)	BV-ESHAP	Three 21-day cycles of BV (1.8 mg/kg) on day 1, etoposide (40 mg/m^2) on days 1 to 4, methylprednisolone (250mg/day) on days 1 to 4, cisplatin (25 mg/m^2) as 24h IVCI on days 1 to 4 and cytarabine (2g/m^2) on day 5. A fourth BV dose was given 21 days after the third dose.
Kersten et al (2021)	BV-DHAP	Three 21-day cycles of BV (1.8 mg/kg) on day 1, dexamethasone (40 mg) on days 1 to 4, cisplatin (100mg/m^2) as 24h IVCI on day 1 and cytarabine (2x 2 g/m^2) over a 3 hour infusion on day 2.

Abbreviations: IVCI, intravenous continuous infusion; IV, intravenous; AUC, area under the curve; seq, sequential

Supplemental Table 2. Study characteristics

Study	Year of publication	N	Salvage therapy	Primary refractory	Underwent ASCT	Received BV maintenance	Event PFS	Event EFS	Event OS	Median follow-up time (months) Median [Q1-Q3] (range)
Moskowitz et al (2015)	2015	64	BV-ICE (seq)	34 (53%)	62 (97%)	0 (0%)	13 (20%)	14 (22%)	3 (5%)	58 [40.8 - 66.8] (2 - 82.6)
Broccoli et al (2019)	2019	40	BV-benda	28 (70%)	32 (80%)	0 (0%)	13 (32%)	14 (35%)	2 (5%)	24.7 [21.1 - 29.5] (4.8 - 34)
Cole et al (2018)	2018	39	BV-gem	27 (69%)	32 (82%)	0 (0%)	16 (41%)	18 (46%)	7 (18%)	50.4 [39.1 - 54.6] (19.7 - 63.9)
Garcia-Sanz et al (2019)	2019	66	BV-ESHAP	40 (61%)	60 (91%)	56 (85%)	19 (29%)	20 (30%)	6 (9%)	22.1 [19.6 - 28.1] (3.1 - 34.5)
Herrera et al (2018)	2018	57	BV-ICE (seq)	31 (54%)	47 (84%)	0 (0%)	27 (47%)	28 (49%)	6 (11%)	61.4 [49.2 - 65.8] (17.4 - 89.5)
Kersten et al (2021)	2021	65	BV-DHAP	25 (38%)	60 (92%)	0 (0%)	15 (23%)	15 (23%)	3 (5%)	39.4 [38.4 - 40.7] (22.7 - 45.7)
LaCasce et al (2018)	2018	55	BV-benda	28 (51%)	42 (76%)	31 (56%)	19 (35%)	22 (40%)	4 (7%)	26.5 [12.4 - 38.8] (1.9 - 48)
Moskowitz et al (2012)	2012	98	ICE-GVD (seq)	41 (42%)	86 (88%)	0 (0%)	29 (30%)	29 (30%)	18 (18%)	75.6 [59.7 - 100] (9.8 - 146.5)
Josting et al (2010)	2010	225	DHAP	11 (5%)	194 (86%)	0 (0%)	69 (31%)	75 (33%)	35 (16%)	42 [27.3 - 60.7] (0.1 - 93.5)
Santoro et al (2016)	2016	59	BeGEV	26 (44%)	44 (75%)	0 (0%)	22 (37%)	22 (37%)	12 (20%)	36 [29.7 - 47.2] (3 - 59.4)
Total		768		291 (38%)	659 (86%)	87 (11%)	242 (32%)	257 (33%)	96 (12%)	39.8 [27.1 - 59.8] (0.1 - 146.5)

Overview of number of included patients, salvage regimens used in each study, number of patients receiving post-ASCT BV maintenance therapy, summarized patient characteristics and outcome parameters, and median follow-up time in patients without PFS event in months including interquartile ranges and min-max ranges.

Supplemental Table 3A. PFS per 6 months on the whole dataset before matching

	BV-cohort				Chemo-cohort			
Time (months)	N at risk	N events	N censor	PFS (95% CI)	N at risk	N events	N censor	PFS (95% CI)
0	386	0	0	100%	382	0	0	100%
6	333	44	9	88% (85% - 92%)	322	52	8	86% (83% - 90%)
12	281	46	6	76% (72% - 81%)	276	39	7	76% (71% - 80%)
18	247	24	10	70% (65% - 74%)	254	16	6	71% (67% - 76%)
24	200	4	43	68% (64% - 73%)	230	9	15	69% (64% - 74%)
30	164	1	35	68% (63% - 73%)	203	4	23	67% (63% - 72%)
36	144	3	17	67% (62% - 72%)	179	0	24	67% (63% - 72%)
42	88	1	56	66% (61% - 71%)	152	2	25	67% (62% - 72%)
48	75	1	11	65% (60% - 71%)	127	2	23	66% (61% - 71%)
60	43	2	30	63% (57% - 69%)	91	2	34	65% (60% - 70%)

Progression free survival (PFS) results per 6 months up to 10 years from enrollment. Number of patients at risk at given time point, number of events (i.e. progressive disease or death), number of patients censored and cumulative PFS at given time point, stratified for the BV and chemo-cohorts.

Supplemental Table 3B. Survival analysis on the whole dataset before matching

Group	N	3-year PFS (95% CI)	*P*	3-year EFS (95% CI)	*P*	3-year OS (95% CI)	*P*
Whole dataset							
BV	386	66.7% (62.0-71.8)	0.64	64.8% (60.1-69.9)	0.47	91.0% (88.0-94.1)	0.002
Chemo	382	67.4% (62.8-72.4)		66.0% (61.4-71.1)		80.4% (76.2-84.9)	
Relapsed							
BV	173	79.2% (73.0-85.9)	0.063	77.3% (71.1-84.1)	0.087	97.0% (94.5-99.6)	*<0.0001*
Chemo	304	68.9% (63.8-74.5)		67.1% (62.0-72.8)		82.1% (77.6-87.0)	
Refractory							
BV	213	56.9% (50.4-64.2)	0.53	54.9% (48.4-62.2)	0.33	85.9% (80.9-91.2)	0.19
Chemo	78	61.7% (51.7-73.8)		61.7% (51.7-73.8)		74.2% (64.4-85.4)	
BV studies							
Moskowitz et al (2015)	64	78.6% (69.0-89.6)		77.4% (67.6-88.6)		95.0% (89.6-100.0)	
Broccoli et al (2019)	40	66.2% (52.7-83.1)		64.2% (50.7-81.3)		96.8% (90.8-100.0)	
Cole et al (2018)	39	59.0% (45.4-76.6)		53.8% (40.3-72.0)		80.6% (68.6-94.6)	
Garcia-Sanz et al (2019)	66	70.5% (60.2-82.6)		69.4% (59.1-81.6)		90.9% (84.2-98.1)	
Herrera et al (2018)	57	52.4% (40.8-67.2)		50.6% (39.1-65.5)		89.2% (81.4-97.8)	
Kersten et al (2021)	65	76.7% (67.0-87.8)		76.7% (67.0-87.8)		95.4% (90.4-100.0)	
LaCasce et al (2018)	55	57.3% (43.9-74.8)		54.1% (41.1-71.1)		92.3% (85.3-99.9)	
Chemo studies							
Moskowitz et al (2012)	98	70.2% (61.7-79.9)		70.2% (61.7-79.9)		80.9% (73.4-89.3)	
Josting et al (2010)	225	67.9% (61.9-74.5)		65.5% (59.4-72.2)		81.0% (75.4-87.0)	
Santoro et al (2016)	59	60.7% (49.1-75.0)		60.8% (49.2-75.1)		78.8% (68.9-90.2)	

Log-rank comparison for 3-year PFS, EFS and OS of BV- versus chemo-cohorts in the whole dataset before matching and stratified for patients with relapsed or primary refractory disease. Survival outcomes for each BV and chemo study are provided.

Supplemental Table 4. Survival analysis on the matched dataset

Group	N	3-year PFS (95% CI)	*P*	3-year EFS (95% CI)	*P*	3-year OS (95% CI)	*P*
Matched dataset							
BV	240	72.2% (66.5-78.3)	0.140	70.8% (65.1-77.0)	0.240	91.9% (88.3-95.6)	0.00043
Chemo	240	67.1% (61.3-73.4)		66.5% (60.7-72.9)		79.5% (74.2-85.1)	
[HR; 95% CI]		1.287 (0.92-1.80)	0.137	1.214 (0.88-1.68)	0.244	2.583 (1.49-4.47)	<0.0001
Relapsed							
BV	162	79.9% (73.6-86.7)	0.020	78.4% (72.0-85.3)	0.043	96.9% (94.2-99.6)	<0.0001
Chemo	162	69.7% (62.8-77.2)		68.9% (62.0-76.5)		82.1% (76.0-88.6)	
[HR; 95% CI]		1.705 (1.08-2.69)	0.020	1.57 (1.01-2.44)	0.043	5.53 (2.12-14.39)	<0.0001
Refractory							
BV	78	56.6% (46.4-69.1)	0.670	55.3% (45.1-67.8)	0.540	81.7% (73.1-91.4)	0.320
Chemo	78	61.7% (51.7-73.8)		61.7% (51.7-73.8)		74.2% (64.4-85.4)	
[HR; 95% CI]		0.898 (0.55-1.48)	0.672	0.858 (0.52-1.41)	0.545	1.435 (0.7-2.93)	0.318

Log-rank comparison for 3-year PFS, EFS and OS of BV- versus chemo-cohorts in the matched dataset and stratified for patients with relapsed or primary refractory disease. Hazard ratios of cox proportional hazard regression are provided for each survival comparison.

Supplemental Table 5. Survival analysis in patients who underwent ASCT

Group	N	3-year PFS (95% CI)	*P*	3-year EFS (95% CI)	*P*	3-year OS (95% CI)	*P*
Matched dataset							
BV	216	77.6% (72.0-83.6)	0.920	72.2% (65.6-79.4)	0.930	93.6% (90.3-97.1)	0.018
Chemo	199	78.5% (72.9-84.5)		76.3% (70.1-82.9)		85.6% (80.6-90.9)	
[HR; 95% CI]		0.979 (0.64-1.49)	0.921	1.214 (0.88-1.68)	0.244	2.188 (1.12-4.26)	0.017
Relapsed							
BV	147	83.9% (77.9-90.4)	0.320	81.0% (74.1-88.6)	0.320	96.6% (93.7-99.6)	0.0097
Chemo	137	79.8% (73.3-86.9)		77.8% (70.6-85.6)		87.3% (81.7-93.4)	
[HR; 95% CI]		1.333 (0.76-2.34)	0.316	1.57 (1.01-2.44)	0.043	3.467 (1.27-9.47)	0.0083
Refractory							
BV	69	64.0% (53.4-76.8)	0.180	53.4% (41.3-69.0)	0.220	87.3% (79.4-96.0)	0.480
Chemo	62	75.5% (65.4-87.1)		72.8% (61.7-85.8)		81.5% (71.7-92.7)	
[HR; 95% CI]		0.647 (0.34-1.23)	0.181	0.858 (0.52-1.41)	0.545	1.40 (0.55-3.55)	0.476

Log-rank comparison for 3-year PFS, EFS and OS in patients who underwent ASCT of BV- versus chemo-cohorts in the matched dataset and stratified for patients with relapsed or primary refractory disease. Hazard ratios of cox proportional hazard regression are provided for each survival comparison.
Survival was measured from baseline because the date of stem-cell reinfusion was not known for all patients.

Supplemental Table 6. Influence of salvage regimen and BV dose

Table 6A: Summary of salvage therapy schedule and BV dose for each study in the BV-cohort

Study	N	Regimen	Chemo[1]	Sequential[2]	BV cycles[3]	BV dose[4]	Cumulative dose[5]
Moskowitz et al (2015)	64	BV-ICE	Multiple	Sequential	6	1.2mg/kg	7.2 mg
Herrera et al (2018)	57	BV-ICE	Multiple	Sequential	4	1.8mg/kg	7.2 mg
Garcia-Sanz et al (2019)	66	BV-ESHAP	Multiple	Concomitant	4	1.8mg/kg	7.2 mg
Kersten et al (2021)	65	BV-DHAP	Multiple	Concomitant	3	1.8mg/kg	5.4 mg
LaCasce et al (2018)	55	BV-benda	Mono	Concomitant	2-6 + 16	1.8mg/kg	36 mg
Broccoli et al (2019)	40	BV-benda	Mono	Concomitant	4-6	1.8mg/kg	10.8 mg
Cole et al (2018)	39	BV-gem	Mono	Concomitant	8	1.8mg/kg	14.4

Table 6B: Cox proportional hazard analysis of salvage therapy and BV schedule for 3-years PFS

	Univariable			*Corrected for R/R status*			*Multivariable*		
Covariate	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
Multiple chemo agents[1]	0.72	0.50 - 1.04	0.079	0.81	0.56 - 1.17	0.258	0.69	0.37 - 1.30	0.252
Sequential vs concomitant[2]	1.01	0.69 - 1.48	0.954	1.07	0.73 - 1.56	0.740	1.07	0.68 - 1.68	0.780
BV cycles[3]	1.02	0.99 - 1.05	0.285	1.01	0.98 - 1.05	0.372	0.99	0.82 - 1.19	0.922
Cumulative BV dose[5]	1.01	1.00 - 1.03	0.167	1.01	0.99 - 1.03	0.229	1.12	0.98 - 1.30	0.106

Cox proportional hazard analysis was done for each covariate in a univariate analysis, in a multivariate analysis corrected for R/R status (only results of the covariate shown) and in a multivariate analysis corrected for R/R status, B symptoms, stage IV disease, extranodal disease, primary treatment with escBEACOPP and bulky disease (only results of the covariate shown). A hazard ratio of >1 corresponds to a higher chance of having progressive disease within 3-years.

Table 6C: Cox proportional hazard analysis of salvage therapy and BV schedule for achieving a CMR

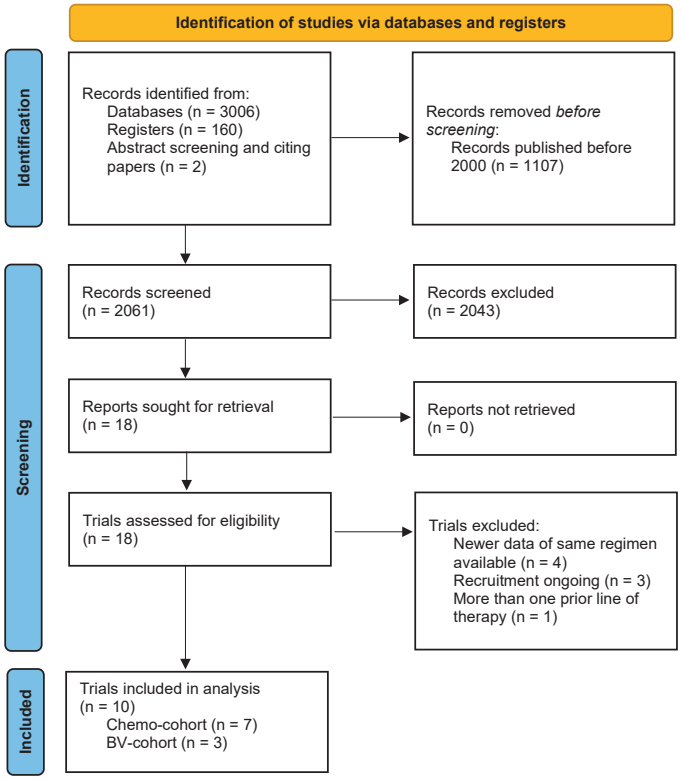
	Univariable			*Corrected for R/R status*			*Multivariable*		
Covariate	Est.	SE	*P*	Est.	SE	*P*	Est.	SE	*P*
Multiple chemo agents[1]	-0.20	0.26	0.436	-0.33	0.27	0.217	-0.15	0.43	0.732
Sequential vs concomitant[2]	-0.26	0.26	0.302	-0.30	0.26	0.254	-0.13	0.31	0.668
BV cycles[3]	-0.03	0.08	0.722	0.02	0.08	0.837	0.07	0.13	0.572
Cumulative BV dose[6]	-0.04	0.04	0.319	-0.02	0.05	0.679	-0.04	0.10	0.659

Logistic regression was done for each covariate in a univariate analysis, in a multivariate analysis corrected for R/R status (only results of the covariate shown) and in a multivariate analysis corrected for R/R status, B symptoms, stage IV disease, extranodal disease, primary treatment with escBEACOPP and bulky disease (only results of the covariate shown). A positive estimate corresponds to a higher chance of achieving a CMR on the pre-ASCT PET-scan.

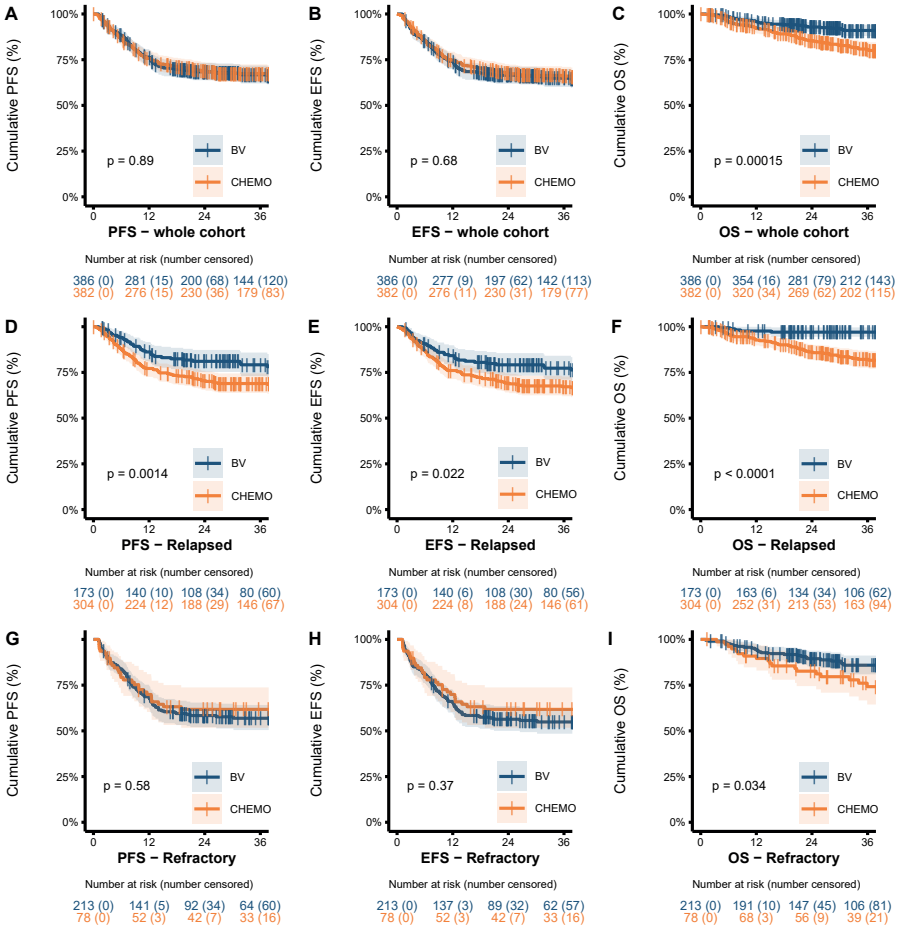
[1]Multiple chemotherapeutic agents versus a single chemotherapeutic agent. [2]Sequential treatment with BV monotherapy followed by salvage chemotherapy or concomitant BV plus salvage chemotherapy. [3]Total number of planned BV cycles including consolidation treatment, if a study provides an optional number of cycles the highest total number of cycles was used (for example in the study of LaCasce et al., patients could proceed to ASCT after 2-6 cycles of BV salvage treatment depending on the response and the local physicians discretion and patients could receive up to 16 cycles of BV consolidation monotherapy, the maximum number of cycles in 24 and was used in the analysis, despite not all patients having received the full number of cycles. [4]BV dose per cycle. [5]Cumulative dose of all BV cycles. [6]Cumulative dose of BV given during salvage treatment before ASCT.

Supplemental Table 7. Subgroup survival analyses on the whole dataset

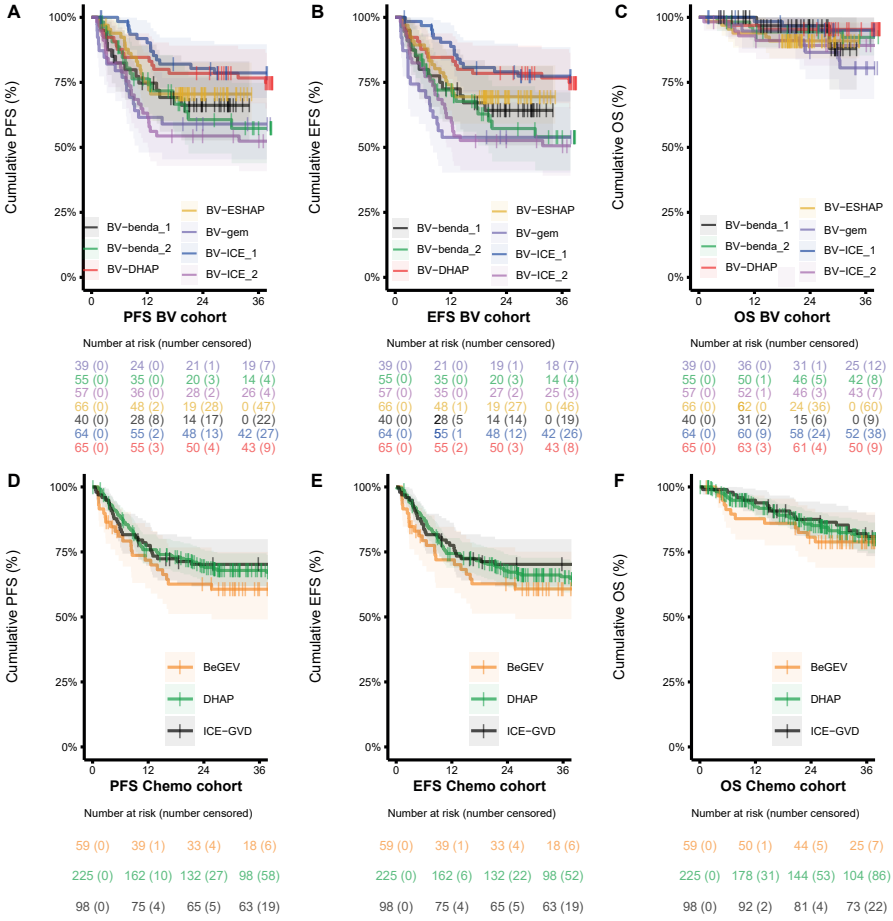
Subgroup	N	3-year survival	95% CI	*P*
Sequential salvage regimen in patients who underwent ASCT while in CMR		PFS		
CMR after BV-ICE (sequential)	47	71.0%	58.9%-85.8%	0.67
CMR after BV only	41	80.4%	69.1%-93.6%	
CMR after ICE-GVD (sequential)	21	76.2%	60.0%-96.8%	
CMR after ICE only	61	81.6%	72.4%-92.1%	
Sequential salvage regimen in patients who underwent ASCT while in CMR		PFS		
CMR after BV or ICE only	102	81.1%	73.8%-89.2%	0.24
CMR after BV-ICE or ICE-GVD	68	72.8%	62.8%-84.4%	
Sequential salvage regimen in patients who underwent ASCT while in CMR		OS		
CMR after BV or ICE only	102	92.8%	87.7%-98.1%	0.62
CMR after BV-ICE or ICE-GVD	68	90.8%	84.1%-98.1%	
Patients who underwent ASCT		PFS		
CMR pre-ASCT	398	78.3%	74.2%-82.5%	
PMR pre-ASCT	57	64.2%	52.8%-78.1%	0.0106
SD pre-ASCT	8	37.5%	15.3%-91.7%	0.00043
Patients who underwent ASCT		OS		
CMR pre-ASCT	398	92.5%	89.8%-95.3%	
PMR pre-ASCT	57	88.4%	80.0%-97.7%	0.286
SD pre-ASCT	8	62.5%	36.5%-100.0%	0.0042



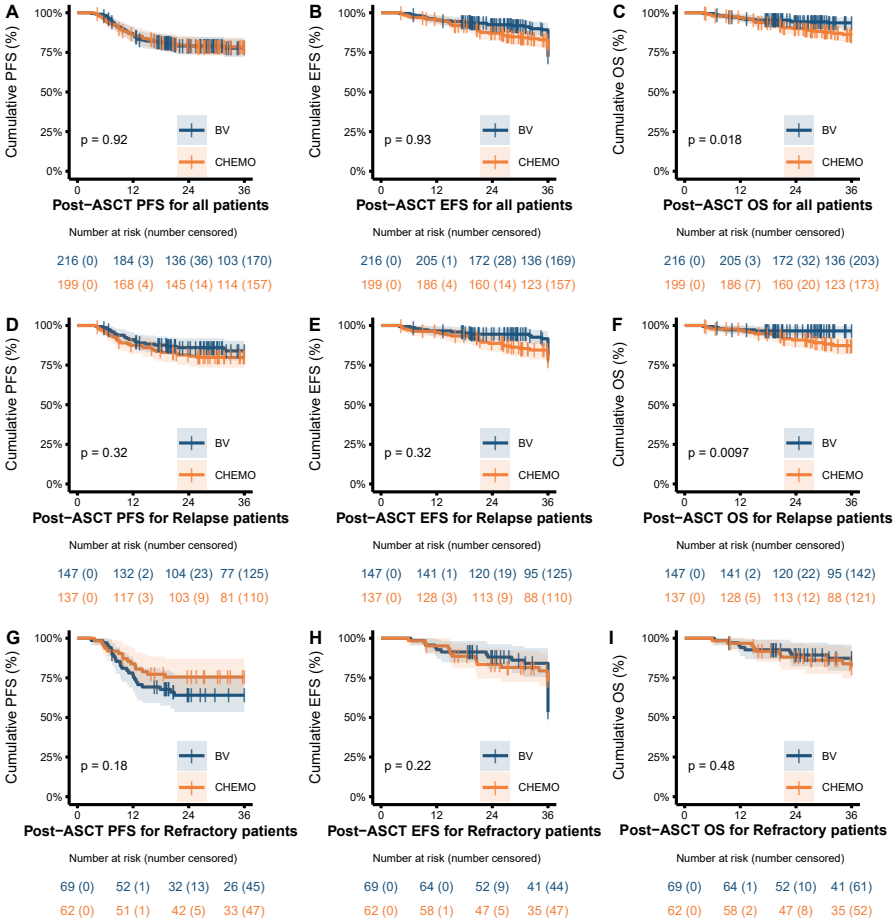
Supplemental Figure 1. PRISMA flowchart of included studies. Included articles were identified through the PubMed database and clinicaltrials.gov register.



Supplemental Figure 2. Kaplan-Meier survival curves in the whole, unmatched dataset comparing the chemo- and brentuximab vedotin (BV) cohorts. (A-C) progression free survival (PFS), event free survival (EFS) and overall survival (OS) in the whole cohort. **(D-F)** PFS, EFS and OS in all relapsed patients**. (G-I)** PFS, EFS and OS in all primary refractory patients.



Supplemental Figure 3. Kaplan-Meier survival analyses in all patients per study. (A-C) Progression free survival (PFS), event free survival (EFS) and overall survival (OS) per included study in the brentuximab vedotin (BV) cohort. BV-benda_1 is the study by Broccoli et al (2019), BV-benda_2 by LaCasce et al (2018), BV-ICE_1 by AMoskowitz et al (2015) and BV-ICE_2 by Herrera et al (2018). **(D-F)** PFS, EFS and OS per included study in the chemo-cohort.



Supplemental Figure 4. Kaplan-Meier survival analysis in patients who underwent ASCT in the matched dataset. (A-C) Post-ASCT progression free survival (PFS), event free survival (EFS) and overall survival (OS) for all matched patients who underwent ASCT. **(D-F)** Post-ASCT PFS, EFS and OS in all matched relapsed patients who underwent ASCT. **(G-I)** Post-ASCT PFS, EFS and OS in all matched refractory patients who underwent ASCT.

6

The Impact of Semiautomatic Segmentation Methods on Metabolic Tumor Volume, Intensity, and Dissemination Radiomics in ^{18}F-FDG PET Scans of Patients with Classical Hodgkin Lymphoma.

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ABSTRACT

Introduction: Consensus about a standard segmentation method to derive metabolic tumor volume (MTV) in classical Hodgkin lymphoma (cHL) is lacking, and it is unknown how different segmentation methods influence quantitative PET features. Therefore, we aimed to evaluate the delineation and completeness of lesion selection and the need for manual adaptation with different segmentation methods, and to assess the influence of segmentation methods on the prognostic value of MTV, intensity and dissemination radiomics features in cHL patients. **Methods:** We analyzed a total of 105 ^{18}F-FDG PET/CT scans from patients with newly diagnosed (n=35) and relapsed/refractory (n=70) cHL with 6 segmentation methods: 2 fixed thresholds on SUV4.0 and SUV2.5, 2 relative methods of 41% of SUVmax (41max), and a contrast-corrected 50% of SUVpeak (A50P) and 2 combination 'majority vote' methods (MV2, MV3). Segmentation quality was assessed by two reviewers on the basis of pre-defined quality criteria: completeness of selection, the need for manual adaptation, and delineation of lesion borders. Correlations and prognostic performance of resulting radiomics features were compared among the methods. **Results:** SUV4.0 required the least manual adaptation but tended to underestimate MTV and often missed small lesions with low ^{18}F-FDG uptake. SUV2.5 most frequently included all lesions but required minor manual adaptations and generally overestimated MTV. In contrast, few lesions were missed when using 41max, A50P, MV2 and MV3, but these segmentation methods required extensive manual adaptation and overestimated MTV in most cases. MTV and dissemination features significantly differed among the methods. However, correlations among methods were high for MTV and most intensity and dissemination features. There were no significant differences in prognostic performance for all features among the methods. **Conclusions:** A high correlation existed between MTV, intensity, and most dissemination features derived with the different segmentation methods, and the prognostic performance is similar. Despite frequently missing small lesions with low ^{18}F-FDG avidity, segmentation with a fixed threshold of SUV4.0 required the least manual adaptation, which is critical for future research and implementation in clinical practice. However, the importance of small, low ^{18}F-FDG-avidity lesions should be addressed in a larger cohort of cHL patients.

INTRODUCTION

The ^{18}F-FDG PET/CT scan is standard of care for staging and response evaluation in the treatment of classical Hodgkin lymphoma (cHL).[1] Optimizing baseline risk stratification contributes to the implementation of individualized treatment strategies aiming to lower toxicity in patients with favorable prognostic characteristics and identification of patients with unfavorable prognostic characteristics early for treatment with other therapies.[2-4] The use of quantitative PET features to improve risk stratification could be implemented in clinical practice if workflows are optimized.

Several studies have shown that metabolic tumor volume (MTV) is a potential prognostic marker in newly diagnosed (ND) and relapsed/refractory (R/R)-cHL.[4-11] However, there are different methods for assessing MTV, and there is no consensus which method performs best in cHL patients in terms of prognostic performance, ease of use, and interobserver variability.[12] MTV assessment is especially challenging in disseminated diseases such as lymphoma. cHL is a heterogeneous disease that is typically localized in the mediastinal and para-aortic regions, mainly affecting young patients who frequently show high physiological ^{18}F-FDG uptake in brown fat and muscles.[1] These regions with high physiological ^{18}F-FDG uptake impede accurate delineation of tumor lesions nearby. Therefore, it is important to evaluate different segmentation methods specifically for cHL.

Although manual segmentation is the current standard for determining MTV, it is very time-consuming and prone to interobserver variability[12] Semiautomatic segmentation includes algorithms that select regions with high ^{18}F-FDG uptake above the threshold of a certain SUV. Segmentation of the MTV can be performed by either pre-defining regions of interest in which lesions will be automatically selected, or by starting with automatic segmentation and deleting regions with high physiological ^{18}F-FDG uptake (e.g., brain, liver, kidneys) thereafter. Although the segmentation method applied can significantly impact the MTV, it is unknown how each method affects other quantitative PET radiomics features, such as patient-level dissemination parameters.[13-17] Besides, no comparative studies have been performed that address representativeness of the segmented MTV with the visual interpretation of the MTV in cHL patients.

The aim of our research was to evaluate the delineation and completeness of lesion selection, and the need for manual adaptation with 6 different semiautomatic segmentation methods, and to assess the influence of the segmentation method on the prognostic value of MTV, intensity and dissemination radiomics features in scans of cHL patients.

MATERIALS AND METHODS

Study Population

PET/CT scans from ND-cHL patients were collected from study cohorts of the Amsterdam UMC (n=35).[2,18] PET/CT scans of patients with RR-cHL were collected from 3 clinical trials conducted in Amsterdam UMC, the Netherlands (n=47) and Memorial Sloan Kettering Cancer Center, New York (n=23).[2-4] All patients had biopsy-proven cHL and the PET/CT scan was obtained before the start of therapy. All patients provided written informed consent for participation in the clinical trials (NCT02280993, NCT00255723, NCT01508312) or biobank cohort[18] of which the study protocols were approved by Institutional Review Boards and Ethics Committees of the centers that conducted the trials. For secondary use of data for this analysis, a waiver was obtained from the Ethics Committee.

^{18}F-FDG PET/CT Scans and Quality Control

The PET/CT systems used to acquire the scans were EANM Research GmbH (EARL, Europe)- or American College of Radiology (ACR, United States)-accredited.[19] PET/CT scans were deidentified at the participating centers and centrally collected. PET scans that did not meet the following 4 criteria, described by European Association of Nuclear Medicine guidelines,[19] were excluded from analysis: 1) plasma glucose <11mmol/L; 2) reconstruction of attenuation-corrected PET according to guidelines described by EARL or ACR; 3) total image activity (MBq) between 50-80% of the total injected ^{18}F-FDG activity or liver SUV_{mean} between 1.3-3.0; and 4) essential PET acquisition data and clinical data available.[19]

Segmentation of the Volume of Interest

Attenuation-corrected PET scans were analyzed using the ACCURATE tool.[20] Six different semiautomatic methods were used for each scan to select the VOI: 2 fixed thresholds of SUV4.0 and SUV2.5, 2 relative thresholds of 41% of SUVmax (41max) and a contrast corrected 50% of SUVpeak (A50P), and 2 'majority vote' (MV) methods selecting voxels that are selected with ≥2 (MV2) and ≥3 (MV3) of the previously mentioned fixed or relative methods, respectively. The VOI was delineated by automatic preselection of ^{18}F-FDG avid structures using the 6 different segmentation methods and a volume threshold of ≥3mL. Nontumor regions were deleted and lymphoma lesions <3mL were added with single mouse clicks. If tumor regions were adjacent to nontumor ^{18}F-FDG avid regions (e.g., heart, liver, bladder), nontumor regions were either removed manually or tumor segmentation was restricted by placing a border or mask, which prevented selection of lesions outside the border (**Figure 1A**). Only focal extranodal and splenic lesions were included in the VOI. A global increase in ^{18}F-FDG uptake of the spleen or bone marrow was not included in the VOI. Delineations were performed under supervision of a nuclear medicine physician.

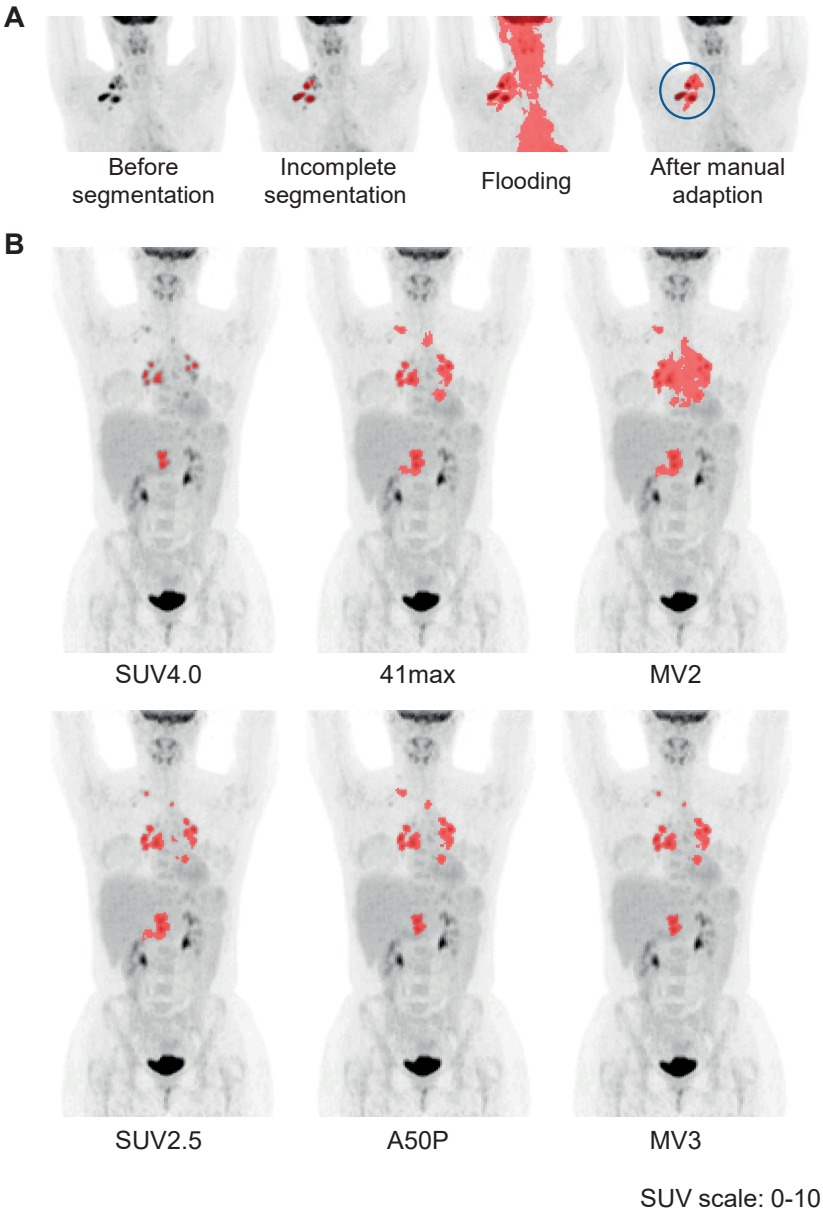

Figure 1. Examples of semiautomatic segmentation. (**A**) Minimal intensity projection (MIP) of the PET scan before segmentation; automatic selection with the 41max method missed multiple lesions; adding missing lesions resulted in flooding into the heart, tonsils, and brain; manual adaptation by placing a border around the volume of interest before segmentation resulted in complete selection. (B) Segmentation with SUV4.0 was scored as "missing minor lesions" and "representative delineation." Segmentation with SUV2.5, 41max, A50P, MV2, and MV3 were scored as "complete segmentation" with "overestimation of delineation." Segmentation with 41max flooded into the heart and required minor manual adaptation. Segmentation with MV2 flooded into the heart and liver and required major manual adaptations.

Quality Scores of Representativeness of Segmentations Compared with Visual Judgement

The quality of the segmentation by the 6 different methods was assessed using 3 quality score (QS) criteria (**Table 1**): completeness of selection of the VOI (i.e., were all tumor-lesions selected); requirement of manual adaptation after semiautomatic segmentation (i.e., manual removal of nontumor regions); and delineation quality of the VOI (i.e., does the VOI border reflect the visual interpretation of the ^{18}F-FDG avid tumor area on the PET scan?).

Two reviewers performed the QS assessment for each of the 6 segmentations for all scans, masked to patient outcome. Completeness of selection and delineation QS were assessed independently, followed by a consensus meeting in which the reviewers reached a consensus on all discrepancy scores and assigned a final QS to each segmentation. The manual adaptation QS was assessed in consensus between the reviewers during review of the segmentation of scans. An example of the QS assessment by the 6 segmentation methods is included in **Figure 1B**.

Table 1. Definitions of Quality Scores for visual assessment of segmentation quality

Quality score	Level	Definition
Completeness of selection	Complete	All visible tumor lesions are selected
	Missing minor lesions	Missing lesions are <3mL and within the selected VOI region (e.g., considered not to influence the Dmax)
	Missing major lesions	Lesions are missing that are either ≥3mL or outside of the selected VOI region (e.g., considered to influence the Dmax)
Manual adaptation	No adaptation	No manual adaptation is required. Adding lesions with single mouse clicks is not considered manual adaptation
	Minor adaptation	Manual adaptation is required in order to obtain a representative selection of the VOI by removing max 1 nontumor region
	Major adaptation	Extensive manual adaptation is required by removing >1 nontumor region
Delineation	Representive	Delineation of VOI borders is representative of the visual interpretation of the tumor
	Underestimation	Delineation of VOI borders is underestimated
	Overestimation	Delineation of VOI borders is overestimated

Radiomics Feature Extraction

RaCat software (developed by Professor Ronald Boelaard; Amsterdam UMC) was used to extract 18 patient-level dissemination features from the complete MTV at patient level.[21] Dissemination features included several novel features addressing interlesional heterogeneity based on distance, volume, SUV_{max} and SUV_{peak} (the 1 mL with the highest SUV within the VOI). In addition, MTV, SUV_{max}, SUV_{peak}, SUV_{mean} and total lesion glycolysis were extracted from the VOI. An overview of all features and its definitions are provided in **Supplemental Table 1** (supplemental materials are available at http://jnm.snmjournals.org).

Statistical Analysis

QS of segmentations were analyzed descriptively and compared using X^2 tests for the whole cohort and separately for ND-cHL and RR-cHL patients. MTV, intensity and dissemination radiomics features were compared between the ND-cHL and RR-cHL cohorts using Wilcoxon rank sum test for nonparametric data. Further analyses were performed on the whole cohort. Correlations of MTV, intensity and dissemination radiomics features among the 6 different segmentation methods were assessed using Spearman rank coefficients correlation. Receiver-operating-characteristics analysis was used to calculate the area under the curve (AUC) for each feature per segmentation method on the whole cohort. An event was defined as the occurrence of progressive disease within 3 y, and patients who died without progression were excluded. AUC curves were compared using a paired *t* test as described by DeLong *et al.*[22]

Statistical analysis was performed using R software (version 4.0.3; R Core Team). A *P*-value of <0.05 was considered statistically significant.

RESULTS

Patient Characteristics

A total of 105 PET/CT scans of patients with ND-cHL (n=35) and RR-cHL (n=70) were included in the analysis (**Supplemental Table 2**). A comparison of radiomics features between ND-cHL and RR-cHL showed no significant differences for most features, except for MTV, SUV_{peak} and Dvol (the maximum difference in volume between lesions), which were all higher in ND patients than RR patients (**Supplemental Table 3**).

Quality Scores of Segmentations

Agreement of QS assessment between the 2 reviewers was high (91% for segmentation quality and 82% for delineation quality). Segmentation resulted in complete selection of all lesions in most cases (**Figure 2A; Supplemental Table 4**). SUV2.5 showed the highest rate of complete selection, followed by 41max, MV2, A50P, and MV3, while SUV4.0 frequently missed minor (59%) and major (10%) lesions. When the SUV4.0 method was used, 91% of scans could be segmented without any manual adaptation (**Figure 2B**). The SUV2.5 method required minor adaptations in 37% of scans and 7% major adaptations. Using the 41max and MV2 methods, only 30% and 34% of scans could be segmented without manual adaptation, and in 47% and 33% of cases, major manual adaptations were required, respectively. When A50P and MV3 were used, about 50% of scans did not require manual adaptation. None of the methods resulted in a high percentage of representative delineation of tumor borders (**Figure 2C**). SUV4.0, SUV2.5, and MV3 resulted in representative delineation in about 50% of cases, whereas SUV4.0 tended to underestimate the MTV and SUV2.5 and MV3 tended to overestimate the MTV in the remaining cases. The

41max, A50P, and MV2 methods resulted in representative delineation in less than 30% and usually overestimated the MTV.

No significant differences were observed for QS between ND and RR patients, except for completeness of selection in which complete selection rates were higher in RR patients than in ND patients with 41max, A50P, or MV3 (**Supplemental Figure 1**).

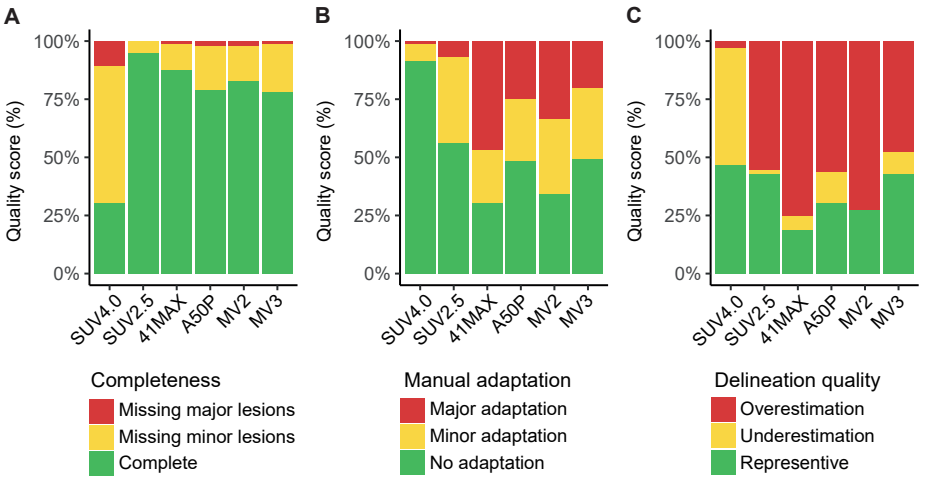

Figure 2. Quality scores (QS) of segmentation methods. (**A**) Completeness of selection. (**B**) Manual adaptations required for representative segmentation. (**C**) Delineation of tumor borders.

Comparison of Features

MTV differed significantly among the segmentation methods. The median MTV per method ranged between 44 and 143 mL (**Figure 3; Supplemental Table 5**). SUV4.0 resulted in a significantly lower MTV than all other segmentation methods (*P<0.001*). The number of lesions was significantly lower with 41max and MV2 than with SUV4.0 and SUV2.5 segmentation methods *(P<0.05*). Dmax (the maximum distance between 2 lesions) was not significantly different among the segmentation methods.

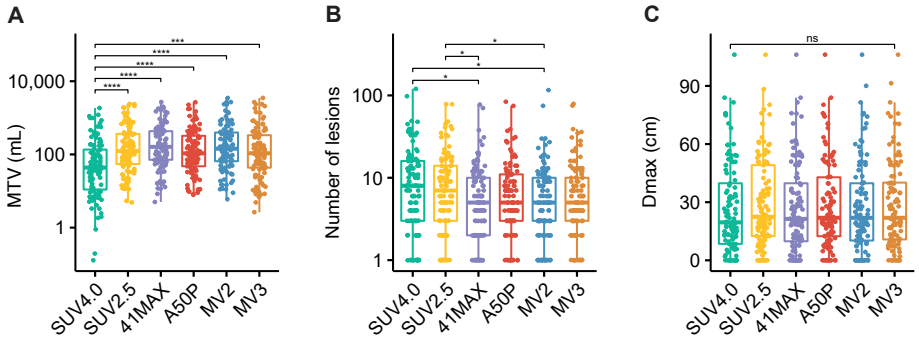

Figure 3. Radiomics features derived with 6 different semiautomatic segmentation methods. (**A**) MTV in mL. (**B**) Number of lesions. (**C**) Dmax in cm.
*P , 0.05. ***P , 0.001. ****P , 0.0001. ns 5 not significant.

MTV, the number of lesions and Dmax showed high correlations among most methods (**Figure 4; Supplemental Table 6).** For MTV and the number of lesions, the highest correlations were observed between the two fixed methods (SUV4.0 and SUV2.5), and between the relative and MV methods, with lower correlations between the fixed and relative or MV methods. SUV_{max} and SUV_{peak} had identical median values and were strongly correlated (R=1) across all methods. Dissemination features addressing differences in volume or SUV_{peak} among lesions showed lower correlations between SUV4.0 and the other 5 segmentation methods (**Supplemental Table 6**).

To assess the effect of incomplete selection of lesions, several features derived with SUV4.0 were plotted against SUV2.5 (**Supplemental Figure 2**). Scans that missed major lesions with SUV4.0 did not show large deviations in the correlation between SUV4.0 and SUV2.5 when compared with scans that had complete selection or missed only minor lesions.

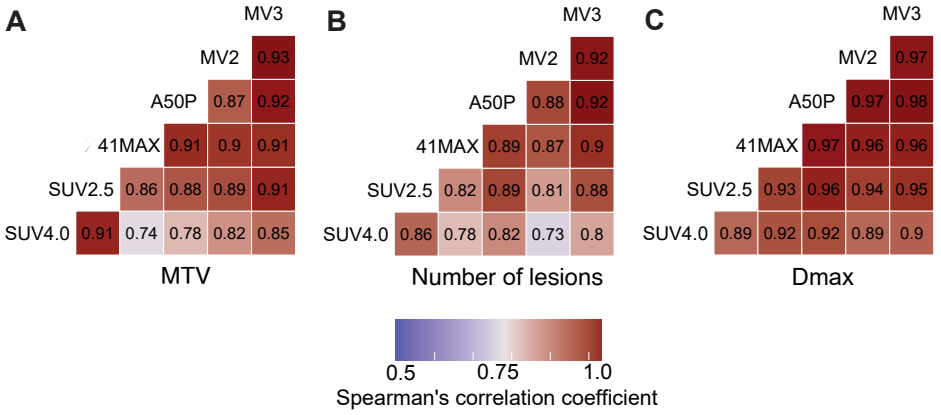

Figure 4. Spearman rank correlation coefficients for radiomics features among different segmentation methods. (**A**) MTV. (**B**) Number of lesions. (**C**) Dmax. All correlations assessed had a P value of <0.01.

Prognostic Performance per Method

Except for MV2, the AUC of the receiver-operating characteristics did not differ significantly among the segmentation methods for all features assessed (**Figure 5; Supplemental Table 7**). The highest AUCs were observed for MTV (range, 0.62-0.65), total lesion glycolysis (range, 0.63-0.65), number of lesions (range, 0.55-0.63), spread in volume (VolSpread) (range, 0.58-0.65), and the difference in SUV_{peak} between the hottest lesion and all other lesions (DSUVpeakSumHot) (range, 0.56-0.63). Of all methods MV2 showed the lowest AUC for the various features (median AUC of all variables, 0.55). The other 5 methods showed comparable median AUCs with the highest median AUC of all variables of 0.62 for SUV4.0.

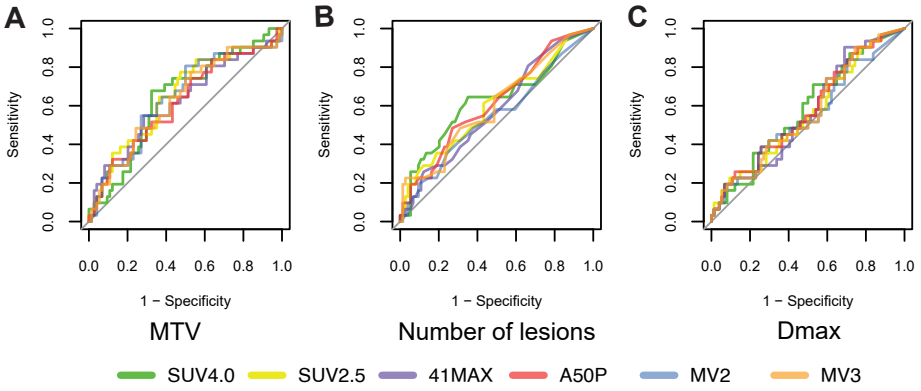

Figure 5. Prognostic performance of radiomics features per method assessed by area under the curve of receiver operating characteristics analysis. (**A**) MTV. (**B**) Number of lesions. (**C**) Dmax.

DISCUSSION

MTV has shown prognostic value in cHL, but the use of different segmentation methods hampers direct comparisons between studies[4-10] This is especially true if a cutoff for MTV is used to divide patients in low- and high-risk groups, since absolute MTV values significantly differ between methods. Harmonization of MTV assessment enables evaluating MTV as prognostic marker in cHL in multicohort setting. The same holds for other quantitative PET-features including dissemination features.

We evaluated the completeness of lesion selection, need for manual adaptations, and delineation quality of 6 semiautomatic segmentation methods to assess MTV and dissemination features in 105 cHL patients. Segmentation with SUV4.0 required the least manual adaptations because this method, in contrast to other methods, rarely floods into regions with high physiological ^{18}F-FDG uptake. SUV2.5 often required minor adaptations, but seldomly major adaptations. Although segmentation using SUV4.0 frequently did not include all lesions (missing those with a SUV<4.0), these lesions were often small and scans with major lesions missing

did not cause significant deviations in the correlation between SUV4.0 and SUV2.5, which was the most complete method. Additionally, the prognostic performance between all methods was similar, and SUV4.0 and SUV2.5 showed the highest AUCs for most variables.

The results of our evaluation suggest that small lesions with low SUV uptake, that are frequently not included with SUV4.0, probably do not contain critical prognostic information, which could be partly explained by the low contribution to total MTV of small lesions. However, small lesions could still influence dissemination features, of which the prognostic value needs to be established in a larger set of patients with more progression events. Additionally, small low-uptake lesions are potentially of higher importance in response-assessment, thus, SUV4.0 may be less suitable for quantitative interim PET analyses in cHL.[1]

All segmentation methods, except SUV4.0, frequently overestimated the MTV assessed by visual interpretation. This may be less relevant when using only patient-level features, as correlations among methods are high; however, lesion-based radiomics analysis involving texture features may be adversely affected by oversegmentation, that is, by selection of voxels that are not part of the tumor.[23] Methods that tended to overestimate the MTV also showed a lower number of lesions, because lesions close to each other were frequently clustered into 1 lesion, as illustrated in **Figure 1**. This explains the discrepancy that SUV4.0 often misses small or low-uptake lesions but still shows the highest number of lesions (**Figure 3**).

In a recent comparison of 6 segmentation methods in diffuse large B-cell lymphoma (DLBCL), a fixed threshold of SUV4.0 was considered the best method to derive MTV.[24] Similar to our findings, MTV significantly differed among the methods, but the prognostic performance was comparable. Interestingly, method performance in DLBCL at interim PET has been shown to depend on the lesional SUV_{max}, in which lesions with $SUV_{max}<10$ were delineated most successfully using MV3, while SUV4.0 was most successful in lesions with $SUV_{max}>10$.[25] Correlations for MTV were significantly higher in our cohort than previously described for DLBCL, possibly because our correlations were assessed after manual adaptation.[24,25] Additionally, and contrary to our findings, the 41max, A50P, and MV3 methods yielded lower exact MTV values than SUV4.0 in baseline DLBCL, showing that performance of different methods can be disease-dependent. In our cohort, 41max resulted in the highest MTV, which can be explained by the lower SUV in our cHL cohort (median SUV_{max} 11.3), compared with DLBCL patients (median SUV_{max} 22.6) [26]. Because SUV_{max} is a patient-level feature, and cHL shows heterogeneous ^{18}F-FDG uptake, other lesions within a patient may have a much lower SUV_{max}, resulting in overestimation of the MTV and flooding with relative methods such as 41max.

Methods based on relative thresholds (e.g., 41max and A50P) are less suitable for assessing MTV in diseases with heterogeneous ^{18}F-FDG uptake, such as cHL, because a high lesional SUV_{max} may exclude the lower avid voxels of the lesion, causing undersegmentation. A low lesional SUV_{max}, however, results in a low threshold, leading to flooding into regions with physiological ^{18}F-FDG uptake. The MV methods could not overcome this disadvantage of the relative methods. MV2 frequently uses voxels that are being selected with 41max and A50P, and although

MV3 needs a third method this did not result in better segmentation than methods with a fixed threshold.

Although the 41max method is recommended for MTV segmentation and has been used in several lymphoma studies, this method requires extensive manual adaptation, which is time-consuming and more susceptible to inter-observer variation.[13,15,19] Additionally, the recommendation for 41max is based on solid malignancies rather than disseminated diseases such as cHL, and 41max has not been compared directly to a fixed threshold of SUV4.0.[27-29] Therefore, this recommendation should be reconsidered for cHL.

CONCLUSION

For PET/CT segmentation in cHL, we showed a high correlation among MTV and most intensity and dissemination features derived with different segmentation methods, except for dissemination features addressing differences in volume and $SUV_{max/peak}$. The prognostic performance of all features is comparable among the methods. The SUV4.0 method required the least manual adaptation, which is critical for future research and implementation in clinical practice. Although segmentation with SUV4.0 often missed small lesions with low ^{18}F-FDG avidity, which may in particular affect dissemination features such as the Dmax, this seemed not to influence the prognostic performance of most features, including Dmax. However, to be conclusive about recommending SUV4.0 for cHL segmentation, the prognostic importance of small lesions with low uptake should be evaluated in a larger cohort of cHL patients with more progression events.

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DISCLOSURE

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Merck, Seatle Genetics. Research funding: Incyte, Merck, Seattle Genetics, ADC Therapeutics, Beigene, Miragen, Bristol-Myers Squibb. **JMZ:** Research funding: Takeda. All remaining authors have declared no conflict of interest.

KEY POINTS

QUESTIONS: Which segmentation method provides the best delineation and completeness of lesion selection with the least manual adaptation in scans of cHL patients, and what is the influence of the segmentation method on the prognostic value of MTV, intensity and dissemination radiomics features?

PERTINENT FINDINGS: Segmentation with a fixed threshold of SUV4.0 required the least manual adaptation, with SUV2.5 resulting in the most complete selection of all lesions. The prognostic performance of features was comparable per segmentation method, and there was a high correlation for MTV and intensity features, but not for all dissemination features, assessed with the different methods.

IMPLICATIONS FOR PATIENT CARE: Semiautomated estimation of MTV, intensity and dissemination radiomics features of cHL patients is feasible using a method with a fixed threshold.

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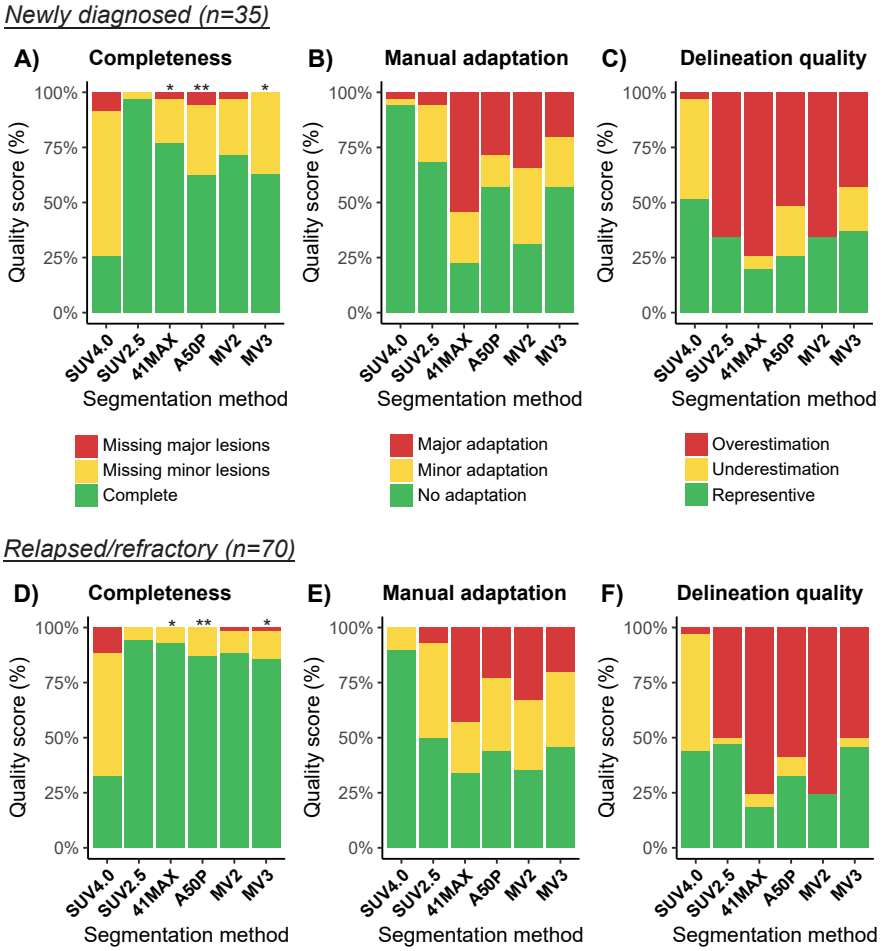
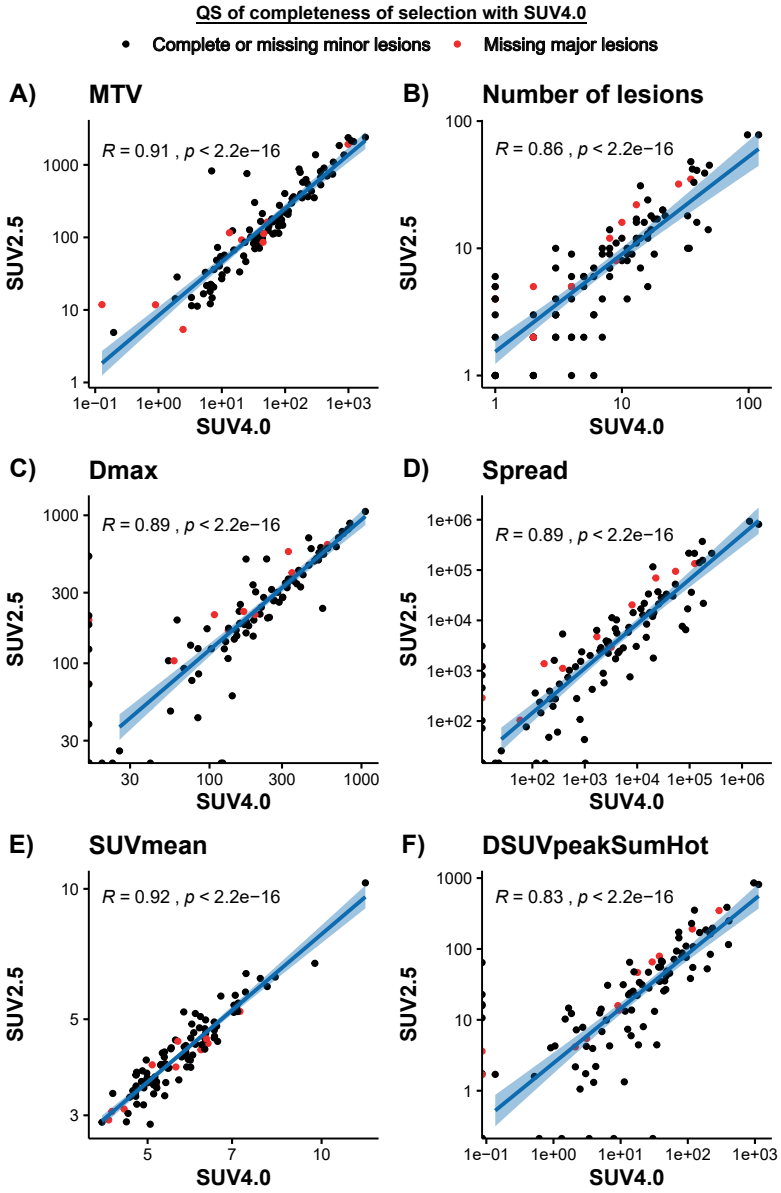
SUPPLEMENTARY MATERIAL



Supplemental Figure 1. Quality scores (QS) of segmentation methods, stratified for newly diagnosed (ND) and relapsed/refractory (RR) HL patients. A+D) QS of completeness of selection; **B+E)** Manual adaptations after semi-automatic segmentation; **C+F)** QS of delineation of the tumor borders; for ND (A,B,C) and RR (D,E,F) patients, respectively. P-values represent comparisons of QS=complete selection, no manual adaptations, or good delineation for ND vs RR. *P<0.05; **P<0.01.



Supplemental Figure 2. Scatter plots and Spearman rank correlation coefficients of radiomics features derived with the SUV4.0 method versus the SUV2.5 method. Black dots represent scans that had a complete selection of all lesions, or missed minor lesions with the SUV4.0 method. Red dots represent scans that missed major lesions with the SUV4.0 method. The SUV2.5 method resulted in complete selections of lesions in all cases.

Supplemental Table 1. Definitions of PET- and radiomics features

Variable	Definition
MTV	The FDG-avid tumor volume
TLG	MTV * SUVmean
SUVmean	The mean SUV value of the VOI
SUVmax	The SUV of the voxel with the highest SUV within the VOI
SUVpeak	The SUV of the 3mL with the highest SUV within the VOI (global peak)
Number of lesions	The number of separated lesion selections within the VOI
Dmax	The maximum distance between two lesions
DmaxBulk	The maximum distance between the largest lesion and any other lesion
Spread	The sum of the distance between all lesions
SpreadBulk	The sum of the distance between the largest lesion and all other lesions
Dvol	The difference in volume between the largest and the smallesfft lesion
VolSpread	The sum of the differences in volume between all lesions
VolSpreadBulk	The sum of the differences in volume between the largest lesion and all other lesions
DSUVmax	The difference in SUVmax between the lesion with the highest SUVmax and the lesion with the lowest SUVmax
DSUVmaxSum	The sum of the differences in SUVmax of all lesions
DSUVmaxBulk	The differences in SUVmax between the largest lesion and all other lesions
DSUVmaxSumBulk	The sum of the differences in SUVmax between the largest lesion and all other lesions
DSUVmaxSumHot	The sum of the differences in SUVmax between the lesion with the highest SUVmax and all other lesions
DSUVpeak	The difference in SUVpeak between the lesion with the highest SUVpeakmax and the lesion with the lowest SUVpeak
DSUVpeakSum	The sum of the differences in SUVpeak of all lesions
DSUVpeakBulk	The differences in SUVpeak between the largest lesion and all other lesions
DSUVpeakSumBulk	The sum of the differences in SUVpeak between the largest lesion and all other lesions
DSUVpeakSumHot	The sum of the differences in SUVpeak between the lesion with the highest SUVpeak and all other lesions

Supplemental Table 2. Patient Characteristics of included PET/CT scans

Variable [n; (%)]	Newly diagnosed *(N=35)*	Relapsed/refractory *(N=70)*	Total *(N=105)*
Sex			
• Female	21 (60%)	37 (53%)	58 (55%)
Age			
• Median (min, max)	34 (19, 66)	30 (13, 64)	30 (13, 66)
Relapse status*			
• Primary refractory	NA	32 (46%)	NA
• Relapse	NA	38 (54%)	NA
Ann Arbor stage			
• I	2 (6%)	6 (9%)	8 (8%)
• II	17 (49%)	25 (36%)	42 (40%)
• III	3 (8%)	14 (20%)	17 (16%)
• IV	13 (37%)	25 (36%)	38 (36%)
Extranodal disease			
• Yes	14 (40%)	26 (37%)	40 (39%)
Progression			
• Yes	17 (49%)**	14 (20%)	31 (30%)

*Primary refractory disease was defined as no complete response on first line treatment or relapse <3 months.

**This includes n=15 patients from the RR-cHL cohort of whom the PET-CT scans at primary diagnosis were retrospectively collected. Two other patients of the remaining n=20 newly diagnosed patients showed progression on first-line treatment but were not included in the RR cohort. Therefore, the percentage of patients with progression during or after first-line treatment is not representive for the general population of primary diagnosed cHL patients.

Supplemental Table 3. PET-features per patient group

Variable [ND – RR; p-value]	Method					
	SUV4.0	SUV2.5	41MAX	A50P	MV2	MV3
MTV	112.4 - 34.7; p=0.001	352.1 - 101.2; p<0.001	398.2 - 108.8; p<0.001	321.9 - 94.5; p=0.001	360.8 - 98.7; p<0.001	285.3 - 84.9; p=0.001
TLG	716.3 - 183.9; p=0.001	1436 - 401; p<0.001	1618 - 460.1; p<0.001	1300 - 382.6; p<0.001	1432 - 441.4; p<0.001	988.5 - 406.1; p<0.001
SUVmean	5.8 - 5.5; p=0.465	4.3 - 4; p=0.22	4 - 3.8; p=0.726	4.3 - 4; p=0.364	4.1 - 3.9; p=0.364	4.3 - 4.3; p=0.613
SUVmax	11.6 - 9.9; p=0.294	11.6 - 9.9; p=0.294	11.6 - 9.9; p=0.303	11.6 - 9.9; p=0.291	11.6 - 9.9; p=0.263	11.6 - 9.9; p=0.294
SUVpeak	9.4 - 7.4; p=0.016	9.4 - 7.4; p=0.017	9.4 - 7.4; p=0.017	9.4 - 7.4; p=0.017	9.4 - 7.4; p=0.017	9.4 - 7.4; p=0.017
Number of lesions	6 - 8; p=0.892	6 - 7.5; p=0.751	3 - 6; p=0.227	5 - 6.5; p=0.832	3 - 5.5; p=0.267	4 - 6; p=0.67
Dmax	18.1 - 20.8; p=0.477	21 - 24.2; p=0.716	19 - 22.2; p=0.374	19.4 - 22.9; p=0.582	18.4 - 23.4; p=0.372	19.4 - 24.4; p=0.605
DmaxBulk	15 - 17.8; p=0.516	17.9 - 20; p=0.696	18.1 - 18.4; p=0.401	18.3 - 19.6; p=0.596	17.3 - 17.8; p=0.414	18.3 - 19.5; p=0.624
Spread	2033 - 2821; p=0.916	1536 - 3221; p=0.729	497.7 - 2019; p=0.29	889.9 - 2163; p=0.913	575.1 - 1891; p=0.273	889.9 - 2209; p=0.541
SpreadBulk	58.3 - 69.4; p=0.9	50.3 - 98.8; p=0.634	29.7 - 62.2; p=0.335	39.9 - 64.7; p=0.865	30.4 - 63.3; p=0.273	32.2 - 63.9; p=0.663
Dvol	45.4 - 16.4; p=0.04	146.9 - 39.2; p=0.004	135.7 - 55.1; p=0.063	88.1 - 42.8; p=0.013	121 - 48.6; p=0.145	115.2 - 45.9; p=0.013
VolSpread	412.3 - 154.8; p=0.122	686 - 423.9; p=0.159	357.1 - 280.9; p=0.598	361.3 - 240.3; p=0.106	660.2 - 315.8; p=0.577	368.3 - 311; p=0.211
VolSpreadBulk	236.2 - 89.4; p=0.072	658 - 263.4; p=0.087	237.2 - 182.3; p=0.356	335.5 - 197.2; p=0.049	246.6 - 192.5; p=0.438	362.5 - 204; p=0.106
DSUVmax	6.1 - 5; p=0.793	7.5 - 6.5; p=0.654	5.2 - 5.4; p=0.49	7 - 5.4; p=0.536	5.6 - 5.7; p=0.698	6.9 - 6.3; p=0.77
DSUVmaxSum	38.2 - 49.6; p=0.731	54.1 - 72.7; p=0.86	10.5 - 32.8; p=0.207	39.4 - 36.7; p=0.916	19.3 - 26; p=0.344	25.1 - 42.9; p=0.754
DSUVmaxBulk	5.2 - 4.7; p=0.841	7.3 - 6.4; p=0.534	4.7 - 4.2; p=0.965	6.1 - 4.9; p=0.498	4.7 - 5; p=0.989	6.5 - 5.4; p=0.412
DSUVmaxSumBulk	26 - 18.6; p=0.788	35.4 - 28.6; p=0.822	8.3 - 13.3; p=0.408	17.9 - 14.5; p=0.545	14.3 - 16.2; p=0.426	19.1 - 15.7; p=0.708
DSUVmaxSumHot	27.6 - 27.3; p=0.804	35.4 - 31.2; p=0.775	8.3 - 12.6; p=0.685	28.2 - 12.7; p=0.369	13.8 - 12.9; p=0.653	19.9 - 15.9; p=0.713
DSUVpeak	4.1 - 3; p=0.284	5.2 - 4.2; p=0.187	4.4 - 4.2; p=0.783	5.6 - 4.2; p=0.292	4.7 - 4.2; p=0.843	5.5 - 4; p=0.167
DSUVpeakSum	26.5 - 26; p=0.46	36 - 39; p=0.754	8.8 - 27.1; p=0.293	31.7 - 25.3; p=0.61	18.6 - 20; p=0.505	21.9 - 32.1; p=0.854
DSUVpeakBulk	3.8 - 2.9; p=0.346	5.2 - 3.9; p=0.111	4.4 - 3.1; p=0.702	5.1 - 3.3; p=0.187	4.2 - 3.2; p=0.635	5.3 - 3.6; p=0.092
DSUVpeakSumBulk	20.9 - 11.4; p=0.424	28.1 - 16.3; p=0.45	8.3 - 12; p=0.701	19.5 - 12.3; p=0.308	12.8 - 12.6; p=0.648	18.7 - 12.7; p=0.426
DSUVpeakSumHot	20.9 - 14.2; p=0.416	28.1 - 19.3; p=0.501	8.3 - 12.2; p=0.69	23.7 - 11.3; p=0.392	13.9 - 12.5; p=0.624	19.5 - 12.7; p=0.45

PET and radiomics features per method, stratified for newly diagnosed (ND) and relapsed/refractory (RR) HL patients. Numbers represent median values for ND and RR patients. P-values are derived with wilcoxon rank sum test.

Supplemental Table 4. Quality Scores per method and per patient group

	Quality Score n (%)		SUV4.0	SUV2.5	41MAX	A50P	MV2	MV3
All patients (n=105)	Completeness	Complete	32 (30%)	100 (95%)	92 (88%)	83 (79%)	87 (83%)	82 (78%)
		Missing minor lesions	62 (59%)	5 (5%)	12 (11%)	20 (19%)	16 (15%)	22 (21%)
		Missing major lesions	11 (10%)	0 (0%)	1 (1%)	2 (2%)	2 (2%)	1 (1%)
	Manual adaptation	No adaptation	96 (91%)	59 (56%)	32 (30%)	51 (49%)	36 (34%)	52 (50%)
		Minor adaptation	8 (8%)	39 (37%)	24 (23%)	28 (27%)	34 (32%)	32 (30%)
		Major adaptation	1 (1%)	7 (7%)	49 (47%)	26 (25%)	35 (33%)	21 (20%)
	Delineation quality	Representive	49 (47%)	45 (43%)	20 (19%)	32 (30%)	29 (28%)	45 (43%)
		Underestimation	53 (50%)	2 (2%)	6 (6%)	14 (13%)	0 (0%)	10 (10%)
		Overestimation	3 (3%)	58 (55%)	79 (75%)	59 (56%)	76 (72%)	50 (48%)
Newly diagnosed (n=35)	Completeness	Complete	9 (26%)	34 (97%)	27 (77%)	22 (63%)	25 (71%)	22 (63%)
		Missing minor lesions	23 (66%)	1 (3%)	7 (20%)	11 (31%)	9 (26%)	13 (37%)
		Missing major lesions	3 (9%)	0 (0%)	1 (3%)	2 (6%)	1 (3%)	0 (0%)
	Manual adaptation	No adaptation	33 (94%)	24 (69%)	8 (23%)	20 (57%)	11 (31%)	20 (57%)
		Minor adaptation	1 (3%)	9 (26%)	8 (23%)	5 (14%)	12 (34%)	8 (23%)
		Major adaptation	1 (3%)	2 (6%)	19 (54%)	10 (29%)	12 (34%)	7 (20%)
	Delineation quality	Representive	18 (51%)	12 (34%)	7 (20%)	9 (26%)	12 (34%)	13 (37%)
		Underestimation	16 (46%)	0 (0%)	2 (6%)	8 (23%)	0 (0%)	7 (20%)
		Overestimation	1 (3%)	23 (66%)	26 (74%)	18 (51%)	23 (66%)	15 (43%)
Relapsed/refractory (n=70)	Completeness	Complete	23 (33%)	66 (94%)	65 (93%)	61 (87%)	62 (89%)	60 (86%)
		Missing minor lesions	39 (56%)	4 (6%)	5 (7%)	9 (13%)	7 (10%)	9 (13%)
		Missing major lesions	8 (11%)	0 (0%)	0 (0%)	0 (0%)	1 (1%)	1 (1%)
	Manual adaptation	No adaptation	63 (90%)	35 (50%)	24 (34%)	31 (44%)	25 (36%)	32 (46%)
		Minor adaptation	7 (10%)	30 (43%)	16 (23%)	23 (33%)	22 (31%)	24 (34%)
		Major adaptation	0 (0%)	5 (7%)	30 (43%)	16 (23%)	23 (33%)	14 (20%)
	Delineation quality	Representive	31 (44%)	33 (47%)	13 (19%)	23 (33%)	17 (24%)	32 (46%)
		Underestimation	37 (53%)	2 (3%)	4 (6%)	6 (9%)	0 (0%)	3 (4%)
		Overestimation	2 (3%)	35 (50%)	53 (76%)	41 (59%)	53 (76%)	35 (50%)

Quality scores (QS) of segmentation for 6 different segmentation methods in: all classical Hodgkin lymphoma (cHL) patients, complementary to Figure 2, newly-diagnosed cHL patients, complementary to Supplemental Figure 1A/B/C, and relapsed/refractory cHL patients complementary to Supplemental Figure 1D/E/F.

Supplemental Table 5. Summary statistics for radiomics features per method

Variable [Median (min – max)]	Method					
	SUV4.0	SUV2.5	41MAX	A50P	MV2	MV3
MTV	43.7 (0.1 - 1,853)	123.5 (4.9 - 2,402)	161.0 (5.0 - 2,694)	107.0 (8.0 - 2,655)	143.4 (6.0 - 3,481)	107.8 (2.6 - 3,489)
TLG	252.9 (0.5 - 10,704)	554.5 (14.1 - 12,481)	609.5 (33.3 - 11,463)	448.4 (28.5 - 13,055)	635.7 (24.1 - 13,159)	458.8 (14.1 - 13,205)
SUVmean	5.6 (4.2 - 11.9)	4.1 (2.9 - 10.3)	3.9 (1.8 - 16.4)	4.1 (2.1 - 16.8)	3.9 (1.8 - 10.1)	4.3 (2.2 - 13.6)
SUVmax	11.3 (4.2 - 28.3)	11.3 (4.2 - 28.3)	11.3 (4.2 - 28.3)	11.3 (4.2 - 28.3)	11.3 (4.2 - 28.3)	11.3 (4.2 - 28.3)
SUVpeak	8.0 (4.2 - 24.2)	8.0 (2.9 - 24.2)	8.0 (2.5 - 24.2)	8.0 (2.5 - 24.2)	8.0 (2.5 - 24.2)	8.0 (2.9 - 24.2)
Number of lesions	8 (1 - 120)	7 (1 - 78)	5 (1 - 77)	5 (1 - 84)	5 (1 - 116)	5 (1 -79)
Dmax	19.6 (0 - 106.1)	22.4 (0 - 106.1)	21.4 (0 - 106.1)	21.9 (0 - 106.1)	21.9 (0 - 106.1)	21.9 (0 - 106.1)
DmaxBulk	16.9 (0 - 94.0)	18.4 (0 - 88.6)	18.2 (0 - 70.6)	18.6 (0 - 88.6)	17.8 (0 - 88.6)	18.4 (0 - 70.6)
Spread	2,726 (0 - 2,075,620)	2,530 (0 - 931,350)	1,568 (0 - 884,185)	1,646 (0 - 1,053,960)	1,079 (0 - 1,450,100)	1,760 (0 - 915,957)
SpreadBulk	67.9 (0 - 3,755)	73.7 (0 - 2,543)	57.8 (0 - 2,357)	55.1 (0 - 1,924)	49.6 (0 - 2,046)	56.4 (0 - 2,587)
Dvol	19.9 (0 - 1,850)	63.9 (0 - 1,758)	74.4 (0 - 1,822)	52.5 (0 - 1,606)	53.3 (0 - 3,331)	55.7 (0 - 3,319)
VolSpread	196.8 (0 - 121,389)	456.4 (0 - 149,436)	282.9 (0 - 119,711)	274.0 (0 - 123,206)	353.4 (0 - 301,834)	317.5 (0 - 177,103)
VolSpreadBulk	124.7 (0 - 45,648)	301.2 (0 - 72,054)	211.9 (0 - 65,922)	226.0 (0 -57,759)	197.8 (0 - 264,307)	243.3 (0 - 160,844)
DSUVmax	5.3 (0 -20.9)	7.2 (0 -25.5)	5.3 (0 -25.2)	6.1 (0 -25.2)	5.7 (0 -25.2)	6.5 (0 -25.2)
DSUVmaxSum	45.7 (0 - 27,678)	69.3 (0 - 12,303)	26.3 (0 - 12,180)	39.4 (0 - 15,088)	25.5 (0 - 19,915)	32.7 (0 - 12,584)
DSUVmaxBulk	5.0 (0 -20.9)	6.6 (0 -25.5)	4.7 (0 -25.2)	5.1 (0 -25.2)	4.9 (0 -25.2)	5.6 (0 -25.2)
DSUVmaxSumBulk	25.4 (0 - 1,324)	30.8 (0 - 917.1)	13.1 (0 - 831.7)	15.7 (0 - 705.7)	14.8 (0 - 1,580)	17.4 (0 - 823.6)
DSUVmaxSumHot	27.6 (0 - 1,32)	31.2 (-1 - 963.3)	12.4 (-20.7 - 831.7)	13.7 (-16.8 - 739.8)	13.7 (-5.3 - 1,592)	17.6 (-3.7 - 1,031)
DSUVpeak	3.4 (0 -16.9)	4.8 (0 -21.5)	4.2 (0 -22.2)	4.7 (0 -22.2)	4.3 (0 -22.2)	4.3 (0 -21.5)
DSUVpeakSum	26.3 (0 -18,667)	36.0 (0 - 8,755)	22.2 (0 - 9,583)	28.8 (0 - 11,831)	18.6 (0 - 11,523)	24.3 (0 - 8,635)
DSUVpeakBulk	3.1 (0 - 16.9)	4.3 (0 - 21.5)	3.3 (0 - 22.2)	4.0 (0 - 22.2)	3.6 (0 - 22.2)	4.0 (0 - 21.5)
DSUVpeakSumBulk	13.1 (0 - 1,136)	22.5 (0 - 813)	11.4 (0 - 759)	12.6 (0 - 757)	12.7 (0 - 1,137)	14.5 (0 - 749)
DSUVpeakSumHot	15.3 (0 - 1,136)	22.8 (0 - 854.9)	11.9 (0 - 759.0)	12.6 (0 - 834.3)	12.8 (0 - 1,448)	14.2 (0 - 894.6)

Numbers represent median values and ranges. Volumes are in mL, distances are in cm.

Supplemental Table 6. Correlation coefficients for radiomics features between different methods

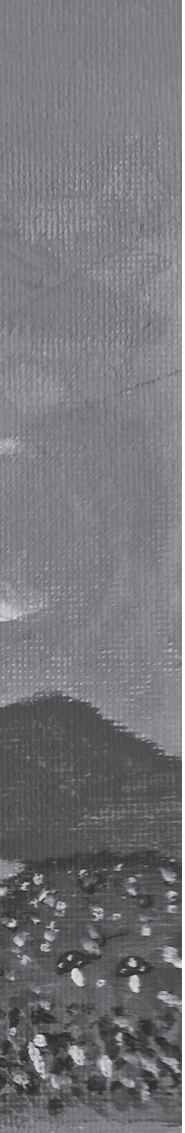
Variable [*R*]	Spearman's correlation between methods														
Method 1	SUV4.0	SUV4.0	SUV4.0	SUV4.0	SUV4.0	SUV2.5	SUV2.5	SUV2.5	SUV2.5	41MAX	41MAX	41MAX	A50P	A50P	MV2
Method 2	SUV2.5	41MAX	A50P	MV2	MV3	41MAX	A50P	MV2	MV3	A50P	MV2	MV3	MV2	MV3	MV3
MTV	0.91	0.74	0.78	0.82	0.85	0.86	0.88	0.89	0.91	0.91	0.90	0.91	0.87	0.92	0.93
TLG	0.94	0.86	0.88	0.89	0.92	0.94	0.95	0.94	0.96	0.96	0.96	0.95	0.93	0.96	0.96
SUVmean	0.92	0.69	0.71	0.66	0.62	0.77	0.77	0.71	0.71	0.89	0.85	0.81	0.81	0.81	0.86
SUVmax	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
SUVpeak	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
Number of lesions	0.86	0.78	0.82	0.73	0.80	0.82	0.89	0.81	0.88	0.89	0.87	0.90	0.88	0.92	0.92
Dmax	0.89	0.92	0.92	0.89	0.90	0.93	0.96	0.94	0.95	0.97	0.96	0.96	0.97	0.98	0.97
DmaxBulk	0.84	0.89	0.89	0.87	0.87	0.89	0.93	0.92	0.93	0.95	0.95	0.94	0.96	0.97	0.96
Spread	0.89	0.84	0.87	0.81	0.85	0.86	0.91	0.85	0.90	0.93	0.91	0.94	0.93	0.95	0.94
SpreadBulk	0.90	0.84	0.88	0.82	0.86	0.87	0.93	0.87	0.92	0.92	0.91	0.94	0.94	0.96	0.95
Dvol	0.70	0.64	0.61	0.63	0.67	0.84	0.87	0.84	0.89	0.82	0.85	0.87	0.80	0.85	0.86
VolSpread	0.83	0.81	0.83	0.77	0.82	0.90	0.96	0.89	0.94	0.92	0.89	0.93	0.90	0.94	0.92
VolSpreadBulk	0.80	0.79	0.80	0.73	0.79	0.89	0.94	0.86	0.92	0.90	0.87	0.91	0.87	0.92	0.89
DSUVmax	0.78	0.73	0.76	0.74	0.76	0.82	0.90	0.83	0.90	0.85	0.89	0.88	0.83	0.89	0.89
DSUVmaxSum	0.87	0.80	0.84	0.76	0.81	0.84	0.91	0.83	0.89	0.90	0.90	0.92	0.90	0.93	0.92
DSUVmaxBulk	0.73	0.68	0.70	0.69	0.69	0.78	0.85	0.79	0.86	0.83	0.86	0.81	0.79	0.83	0.84
DSUVmaxSumBulk	0.84	0.79	0.82	0.76	0.78	0.82	0.89	0.82	0.87	0.90	0.90	0.90	0.89	0.93	0.90
DSUVmaxSumHot	0.84	0.78	0.74	0.76	0.76	0.80	0.79	0.79	0.85	0.81	0.84	0.86	0.77	0.81	0.86
DSUVpeak	0.74	0.72	0.74	0.72	0.72	0.79	0.89	0.81	0.88	0.84	0.90	0.87	0.85	0.89	0.87
DSUVpeakSum	0.87	0.79	0.83	0.75	0.81	0.83	0.89	0.82	0.88	0.90	0.90	0.92	0.89	0.93	0.92
DSUVpeakBulk	0.69	0.65	0.66	0.65	0.66	0.77	0.85	0.79	0.86	0.83	0.86	0.81	0.80	0.85	0.84
DSUVpeakSumBulk	0.83	0.80	0.83	0.75	0.78	0.82	0.89	0.83	0.87	0.90	0.90	0.90	0.88	0.93	0.90
DSUVpeakSumHot	0.83	0.77	0.80	0.76	0.77	0.81	0.84	0.82	0.86	0.86	0.87	0.90	0.87	0.89	0.89
Color scale of *R*															
0.0-0.5	0.5-0.6			0.6-0.7			0.7-0.8			0.8-0.9			0.9-1.0		

Area under the curve (AUC) derived from receiver operating characteristic (ROC) analysis for each feature stratified per segmentation method. AUCs were compared between methods using a paired t-test as described by DeLong et al. A p-value of <0.05 was considered statistically significant.

$^{\alpha}$significantly lower compared to SUV4.0 $^{\beta}$significantly lower compared to SUV2.5

$^{\gamma}$significantly lower compared to 41max $^{\delta}$significantly lower compared to A50P

$^{\kappa}$significantly lower compared to MV2 $^{\lambda}$significantly lower compared to MV3



7

Prognostic model using ^{18}F-FDG PET radiomics predicts progression-free survival in relapsed/refractory Hodgkin lymphoma

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ABSTRACT

Investigating prognostic factors in patients with relapsed or primary refractory classical Hodgkin lymphoma (R/R cHL) is essential to optimize risk-adapted treatment strategies. We built a prognostic model using baseline quantitative 18F-fluorodeoxyglucose positron emission tomography (PET) radiomics features and clinical characteristics to predict the progression-free survival (PFS) among patients with R/R cHL treated with salvage chemotherapy followed by autologous stem cell transplantation. Metabolic tumor volume and several novel radiomics dissemination features, representing interlesional differences in distance, volume, and standard uptake value, were extracted from the baseline PET. Machine learning using backward selection and logistic regression were applied to develop and train the model on a total of 113 patients from 2 clinical trials. The model was validated on an independent external cohort of 69 patients. In addition, we validated 4 different PET segmentation methods to calculate radiomics features. We identified a subset of patients at high risk for progression with significant inferior 3-year PFS outcomes of 38.1% vs 88.4% for patients in the low-risk group in the training cohort ($P < .001$) and 38.5% vs 75.0% in the validation cohort ($P = .015$), respectively. The overall survival was also significantly better in the low-risk group ($P = .022$ and $P < .001$). We provide a formula to calculate a risk score for individual patients based on the model. In conclusion, we developed a prognostic model for PFS combining radiomics and clinical features in a large cohort of patients with R/R cHL. This model calculates a PET-based risk profile and can be applied to develop risk-stratified treatment strategies for patients with R/R cHL. These trials were registered at www.clinicaltrials.gov as #NCT02280993, #NCT00255723, and #NCT01508312.

KEY POINTS

- Quantitative PET radiomics and clinical features can be used to build a strong prognostic model for 3-year PFS in relapsed/refractory cHL.
- We identified a subgroup of high-risk patients with R/R cHL with inferior PFS and overall survival for whom novel therapies should be considered.

INTRODUCTION

Classical Hodgkin lymphoma (cHL) mainly affects young adults.[1] Treatment consists of chemotherapy and radiotherapy and is successful in most cases.[2] However, ~10 to 20% of patients still relapse or are primary refractory, of whom ~50 to 60% can be cured with salvage chemotherapy and autologous stem cell transplantation (ASCT). The remaining 40 to 50% generally have a very poor prognosis.[3,4] Risk profiling at baseline before starting second-line treatment could be used to identify patients with a high risk of progression, for whom novel (immune) therapies can be considered before the start of salvage chemotherapy, instead of adapting treatment to response assessment after reinduction therapy. ^{18}F-Fluorodeoxyglucose (FDG) positron emission tomography (PET) computed tomography (CT)-adapted treatment has improved outcomes for patients with newly diagnosed cHL.[5-7] Although the prognostic value of a complete metabolic response (CMR) before ASCT in patients with relapsed/refractory (R/R) cHL is well known, there is currently no PET-adapted treatment strategy that is widely applied in the salvage treatment setting.[8-10]

Metabolic tumor volume (MTV) is increasingly studied in cHL and has shown moderate prognostic value as a single biomarker.[9,11-16] In most studies a different cutoff for MTV is used without validation of results, which impedes the use of MTV as a prognostic marker. The Dmax, ie, the maximum distance between 2 lesions, provides another quantitative PET feature, which has shown prognostic value for newly diagnosed cHL.[17,18] Radiomics is an emerging field of research that uses high-throughput imaging-based data to extract quantitative image features from a predefined volume of interest (VOI), such as FDG-avid tumors on a PET. Differences in FDG intensity of the VOI (tumor), shape, volume, localization, texture, and intratumor and intertumor heterogeneity can be investigated and reinforced with available genomic and clinical data to develop prognostic models.[19,20-22]

Only a few studies have assessed radiomics in cHL, but most prognostic models lack validation in an independent cohort.[18,23,24] A prognostic radiomics model based on texture features in newly diagnosed cHL showed high prognostic value for predicting refractory disease, but results were not validated in an independend cohort.[23] In addition, texture features, which are calculations based on individual voxels, are susceptible to technical variations, especially in small lesions.[25] Many patients present with small lesions in the R/R setting because of early detection during follow-up after first-line treatment.[10] Therefore, radiomics dissemination and interlesion heterogeneity parameters (eg, the spread or the difference in distance, volume, and FDG uptake between lesions), which are less susceptible to technical variations, could be more suitable for use in disseminated diseases with smaller lesions such as lymphoma.[26] Most other prognostic models that have been developed to predict outcomes in the R/R setting, gene expression-based models,[27] have not yet been implemented in a prospective clinical trial or clinical practice, which can possibly be explained by high costs and time-consuming analyses. Because PETs are already used in clinical practice, information obtained through radiomics may contribute to

more accurately predict outcomes among patients with cHL and can be implemented in clinical practice to guide treatment decisions, which, in turn, may improve clinical outcome.[20-22]

MATERIALS AND METHODS

Study population

Patients treated within the following 3 clinical trials were included: (1) Kersten et al,[28] who investigated a combination of brentuximab vedotin (BV) and dexamethasone, high-dose cytarabine, and cisplatin (DHAP) followed by ASCT; (2) Moskowitz et al,[9,29] who investigated sequential BV and ifosfamide, carboplatin, and etoposide (ICE), followed by ASCT; and (3) Moskowitz et al,[30] who investigated ICE and optional sequential gemcitabine, vinorelbine, and doxorubicin (GVD) for patients with no CMR, followed by ASCT. A complete overview of treatment regimens is provided in **Supplemental Table 1**. All patients were transplant-eligible and had biopsy-proven cHL, and the PET-CT was performed at baseline, that is, before the start of salvage therapy. Patients were excluded if no PET was available or if the follow-up time was <2 years. An overview of reasons for patient exclusion is provided in **Supplemental Table 2**.

All patients provided written informed consent for participation in the clinical trials (NCT02280993, NCT00255723, and NCT01508312), of which the study protocols were approved by institutional review boards and ethics committees of the centers that conducted the trials. For secondary use of data for this analysis, a waiver was obtained from the ethics committee of Amsterdam University Medical Centers, The Netherlands, and the Memorial Sloan Kettering Cancer Center, NY.

^{18}F-FDG PET-CTs and quality control

The PET-CT systems used to perform the scans were accredited by the European Association of Nuclear Medicine Research Ltd. (EARL, Europe) or the American College of Radiology (ACR, United States).[31] PET-CTs were deidentified at the participating centers and centrally collected. Inclusion criteria were (1) plasma glucose <11mmol/L; (2) reconstruction of attenuation-corrected PET according to guidelines described by EARL or ACR; (3) total image activity (in megabecquerel) between 50 and 80% of the total injected FDG activity or liver standard uptake value (SUV) mean (SUV_{mean}) between 1.3 and 3.0; and (4) availability of essential PET acquisition data and clinical data.[31,32]

PET segmentation and radiomics feature extraction

Attenuation-corrected PETs were analyzed using the ACCURATE tool, as described before.[26,33] We published earlier that segmentation using a fixed threshold of an SUV of 4.0 (SUV4.0 method) is most suitable for application in clinical practice for patients with cHL.[26] However, because this method frequently does not include small lesions with low FDG uptake (SUV<4.0), we also

analyzed scans with a threshold of an SUV of 2.5 (SUV2.5 method) and a combination method ("combimethod") in which segmentation with an SUV of 4.0 is complemented with a threshold of an SUV of 2.5 for missing lesions with low uptake (ie, SUV<4.0). Additionally, we analyzed all scans with a relative threshold of 41% of the SUV_{max} (41max method) for comparison with those of other studies, because this method has also been used frequently in literature.[9,11]

Only focal extranodal and splenic lesions were included in the VOI. A global increase in FDG uptake of the spleen or bone marrow was not included in the VOI. Delineations were performed by J.D. under supervision of a nuclear medicine physician (G.J.C.Z. or H.S.). RaCat software was used to extract 18 patient-level dissemination, standard intensity-based, and volume-based features such as MTV, SUV parameters and total lesion glycolysis (ie, SUV_{mean} multiplied by MTV) from the complete VOI at patient-level.[34] An overview of all features and their definitions are provided in **Supplemental Table 3**, and examples are given in **Figure 1**. Dissemination features included several novel features addressing interlesional heterogeneity based on distance, volume, and intensity. Because of the multicenter aspect of this study and the use of different PET scanners, we only used robust radiomics features that are not susceptible to technical variations in PET acquisition, such as dissemination features and SUV_{peak} (ie, the average SUV of 1 mL with the highest FDG uptake) instead of SUV_{max} (which represents only the SUV of the highest single voxel, therefore being susceptible to image noise). Additionally, because the SUV_{mean} of the liver was used as a standard quality parameter to compare PETs and wa also the reference for a Deauville score of 3, we normalized the SUV_{mean} and SUV_{peak} (i.e. the 1mL with the highest SUV within the VOI) for the liver SUV_{mean} and used the tumor-to-liver ratio (TLR).[32,35-37] The liver SUV_{mean} was estimated on a 3 mL sphere in the right upper lobe of the liver.

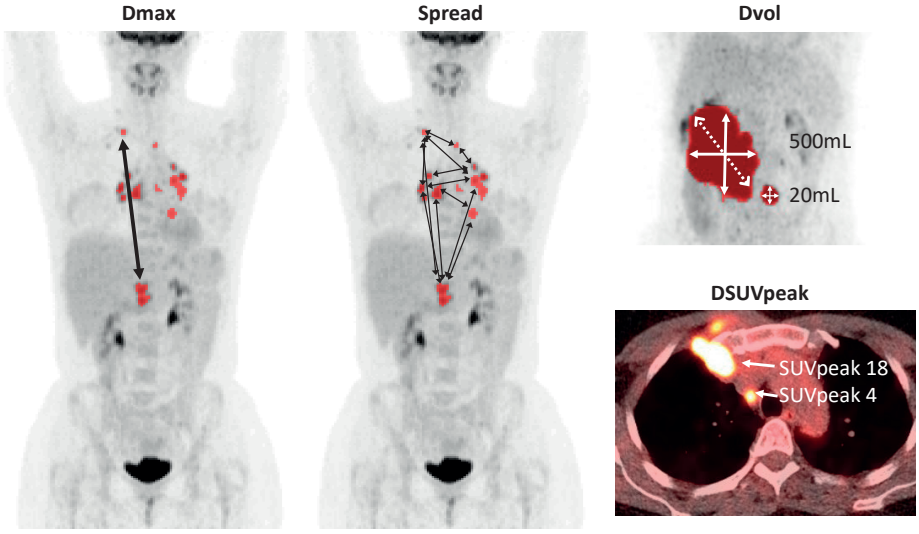


Figure 1. Examples of radiomics features. All definitions of radiomics features are listed in **Supplemental Table 3**. Dmax, maximum distance between 2 lesions; DSUVpeak, maximum difference in SUVpeak between 2 lesions; Dvol, maximum difference in volume between 2 lesions; spread, sum of the distances between all lesions.

Endpoint

The primary endpoint was to develop a prognostic model for 3-year progression-free survival (PFS) using clinical and radiomics features measured at baseline. PFS was defined as time from enrollment until progression or death from any cause (binary outcome: 1=progression or death and 0=no event at 3 years). The secondary endpoint was the 3-year overall survival (OS), defined as the time from enrollment until death from any cause.

Statistical analysis

We analyzed the 18 radiomics dissemination features as listed in **Supplemental Table 3**, and MTV, total lesion glycolysis, $TLR_{SUVmean}$ and $TLR_{SUVpeak}$, and 5 clinical features, that is, age, Ann Arbor stage, extranodal disease, primary refractory disease vs relapsed disease (R/R status), and B symptoms. Radiomics features were log transformed to obtain a linear relationship with the outcome variable. A clinical model was built using only clinical features, a radiomics model was built for each segmentation method, and the final model was built using both clinical and radiomics features using the segmentation method that showed the best performance. We applied backward feature selection using the stepAIC function of the R package "MASS" version 7.3-53 to select features for each training model and removed features with high multicollinearity. Backward selection was performed separately for each model and could, therefore, result in the selection of different features per model. Cook distance was calculated but identified no extreme outliers. The models were trained using logistic regression on the BV-DHAP and BV-ICE studies (n=113, training cohort) and validated on the ICE study (n=69, validation cohort), using the "glm" function of R package "stats" version 4.0.3. Model performance was assessed by calculating the area under the curve (AUC) of the receiver operating characteristics ROC curve on the training and validation cohort, which was also cross-validated on the training cohort using fivefold with 2000 repeats. The significance of the addition of radiomics features to clinical features was calculated using the deltaAUC test of R package "clinfun" version 1.0.15 for comparing the AUC from receiver operating characteristics curves from nested binary regression models.[38] The size of the high-risk group was predefined based on the prevalence of PFS events in the training cohort, which was 26 of 113 (23%). The high-risk group was identified by selecting the top 23% of patients with the highest prediction scores. Another cutoff based on the Youden Index of the cross-validation on the training cohort was also explored. The Kaplan-Meier method and log-rank test were used to analyze differences in PFS and OS for the high- and low-risk groups. Positive predictive value (PPV), negative predictive value (NPV), sensitivity, and specificity were calculated, and a Cox proportional hazards regression was performed for the high-risk vs low-risk groups. Statistical analysis was performed using R software version 4.0.3. A P-value <0.05 was considered statistically significant.

RESULTS

Patient characteristics

In total, 231 patients were treated in the 3 studies, of whom n=49 were excluded from the analysis. A total of 37 (16%) cases were excluded because the PET was of insufficient quality or not compatible with the analysis software [**Supplemental table 2**]. We included 182 patients in the analysis, of whom n=113 were included in the training cohort (n=58 treated with BV-DHAP and n=55 treated with BV-ICE) and n=69 in the validation cohort (treated with ICE). Patient characteristics are summarized in **Table 1**. Most clinical characteristics were well distributed across the training and validation cohorts. However, the training cohort consisted of a higher percentage of patients with B symptoms (p=0.011) and patients with stage II disease (p=0.004), whereas the validation set had more patients with stage III disease (p=0.004).

The median follow-up time was 42.4 months (range, 25.5 - 82.6 months) for the training cohort and 72.3 months (range, 25.5 - 146.5 months) for the validation cohort. In the training and validation cohort, 26 (23%) and 22 (32%) patients had a 3-year PFS event, and 9 (8%) and 15 patients (22%) died, of whom only 2 (2%) and 1 (1%) died without progressive disease, respectively.

Table 1. Patient characteristics of the training and validation cohorts

	Training (n=113)		Validation (n=69)		Total (n=182)		P value
	No.	%	No.	%	No.	%	
Study							< 0.001
BV-DHAP	58	51	0	0	58	32	
BV-ICE	55	49	0	0	55	30	
ICE-GVD	0	0	69	100	69	38	
Female sex	61	54	32	46	93	51	0.319
Median age, (range)	30 (13 - 65)		34 (18 - 66)		31 (13 - 66)		0.175
Primary refractory	55	50	25	37	80	45	0.062
Ann Arbor stage							0.002
I	10	9	1	1	11	6	0.042
II	46	41	43	62	89	49	0.004
III	19	17	2	3	21	12	0.004
IV	38	34	23	33	61	34	0.970
Extranodal disease	44	39	25	36	69	38	0.715
B symptoms	28	25	7	10	35	20	0.011

Clinical model

The following clinical patient characteristics were used at time of relapse: age, Ann Arbor stage, presence of extranodal disease, B symptoms, and R/R status. Backward feature selection

resulted in selection of 3 variables: stage, B symptoms, and R/R status. The clinical model yielded a cross-validated AUC of 0.729 on the training cohort and an AUC of 0.677 in the validation cohort [**Table 2**, **Supplemental Table 4**].

Table 2. Model performance

Model	Features[1]	AUC training cohort (95% CI)	CV-AUC training cohort (95% CI)	AUC validation cohort (95% CI)
Clinical	Ann Arbor Stage B symptoms R/R status	0.787 (0.692-0.883)	0.729 (0.724 - 0.734)	0.677 (0.535-0.819)
Radiomics SUV 4.0	Number of lesions VolSpread $TLR_{SUVmean}$	0.719 (0.605 - 0.833)	0.691 (0.685 – 0.696)	0.721 (0.58 - 0.863)
Final model	R/R status B symptoms MTV Spread $TLR_{SUVmean}$	0.837 (0.744 - 0.930)	0.810 (0.805 - 0.814)	0.750 (0.627 - 0.872)
P-value of clinical *versus* final model[2]		0.00094	0.0049	<0.0001

Spread, the sum of the distances between all lesions; $TLR_{SUVmean}$, tumor-to-liver ratio of lesion SUVmean and the liver SUVmean; VolSpread, the sum of differences in volume between all lesions.

[1]All radiomics variables are log transformed.

[2]P-values represent the added value of the radiomics features to the clinical model. P-value of the cross-validated (CV)-AUC represents the median p-value of 2000 repeats of fivefold of cross-validation.

Radiomics model with different segmentation methods

For each tumor segmentation method, that is, SUV4.0, SUV2.5, 41max, and combimethod, the backward feature selection was performed on all features as listed in **Supplemental Table 3**. This resulted in 4 prognostic models with different features for each method, of which the SUV4.0 method yielded a cross-validated AUC of 0.691 and highest validated AUC of 0.721 [**Table 2, Supplemental Figure 1B-E**]. The AUC values of the SUV2.5 method were comparable with those of the SUV4.0 method, whereas the 41max method yielded lower AUC values [**Supplemental Table 4**]. The model of the combimethod, in which segmentation using a threshold of SUV4.0 was combined with a threshold of SUV2.5 for missing lesions with low uptake, did not result in higher AUC values compared to the separate SUV4.0 or SUV2.5 models (validated AUC 0.712 vs 0.721 and 0.714, respectively). To rule out differences in model performance because of backward feature selection, the features of the SUV4.0 model selection were also tested on the other methods, but this did not increase the AUCs of these models [**Supplemental Table 4**]. Because of high AUC values and a technical validation in an earlier publication, the SUV4.0 method was chosen as the tumor segmentation method for the final model.[26]

Table 3. Logistic regression results of the model

Features	Estimate	Standard error	Z value	P-value
(Intercept)	-2.5	2.0	-1.2	0.219
R/R (Relapsed)	-2.5	0.7	-3.8	0.000
B symptoms	1.0	0.7	1.5	0.136
MTV	-0.4	0.2	-1.6	0.118
Spread	0.4	0.2	2.7	0.007
$TLR_{SUVmean}$	2.4	1.0	2.4	0.018

Logistic regression results of features in the baseline model. Formula of the model: -2.472 – [2.478 * (Relapsed=1, refractory=0)] + [1.010 * (B symptoms = 1, no B symptoms =0)] – [0.384 * log(MTV in uL)] + [0.413 * log(Spread)] + [2.409 * log(SUVmean / liverSUVmean)].

Combined prognostic model

For the final prognostic model, backward feature selection was performed using all radiomics features from segmentations with the SUV4.0 method in combination with clinical features. Backward selection resulted in selection of the following features: R/R status, B symptoms, MTV, sum of all distances between all lesions (Spread), and $TLR_{SUVmean}$, and yielded a high cross-validated AUC of 0.810 in the training cohort and an AUC of 0.750 in the validation cohort [**Tables 2** and **3**]. The addition of radiomics features (MTV, Spread, and $TLR_{SUVmean}$) to clinical features showed significant improvement of the AUC in the cross-validated training (p=0.0049) and validation (p<0.0001) cohorts [**Table 2**]. Ann Arbor stage was not part of the final prognostic model because it was being outperformed by the radiomics feature Spread. Replacing Spread for stage resulted in a lower prognostic value (data not shown). Logistic regression results of the models are shown in **Table 3**.

Based on the predefined cutoff (23% of PFS events), the high-risk group in the training cohort showed a significant inferior PFS compared to patients in the low-risk group, with a 3-year PFS of 38.1% (95% confidence interval (CI), 23-62) vs 88.4% (95% CI; 82-95; p<0.0001), respectively [**Figure 2A**; **Supplemental Table 5**]. Three-year PFS in the independent validation cohort was 38.5% (95% CI, 19-77) *vs* 75.0% (95% CI, 65-87; p=0.0153) for the high- and low-risk groups, respectively [**Figure 2B**]. The 3-year OS was also significantly different between the high- and low-risk groups in the training and validation cohorts [**Figure 2C-D**]. The PPV and NPV for prediction of 3-year PFS were 61.5% and 88.5%, respectively, in the training cohort, and 61.5% and 75.0%, respectively, in the validation cohort. The PPV and NPV were similar between the 2 studies in the training cohort, that is, the BV-DHAP and BV-ICE studies [**Table 4**]. Results using another exploratory cutoff based on the Youden Index on the cross-validation of the training cohort did not improve the PPV and NPV [**Supplemental Table 5**].

In the training cohort, the CMR rate before ASCT was significantly higher in the low-risk group compared with that of the high-risk group (86% vs 69%; p=0.049), but this was not the case in the validation cohort. Before ASCT, negative PET result rates seemed higher in the validation cohort because more patients with a positive PET result were excluded during the quality check of PETs. Furthermore, significantly more patients had progressive disease after ASCT in the high-risk groups of both training and validation cohorts [**Supplemental Table 6**].

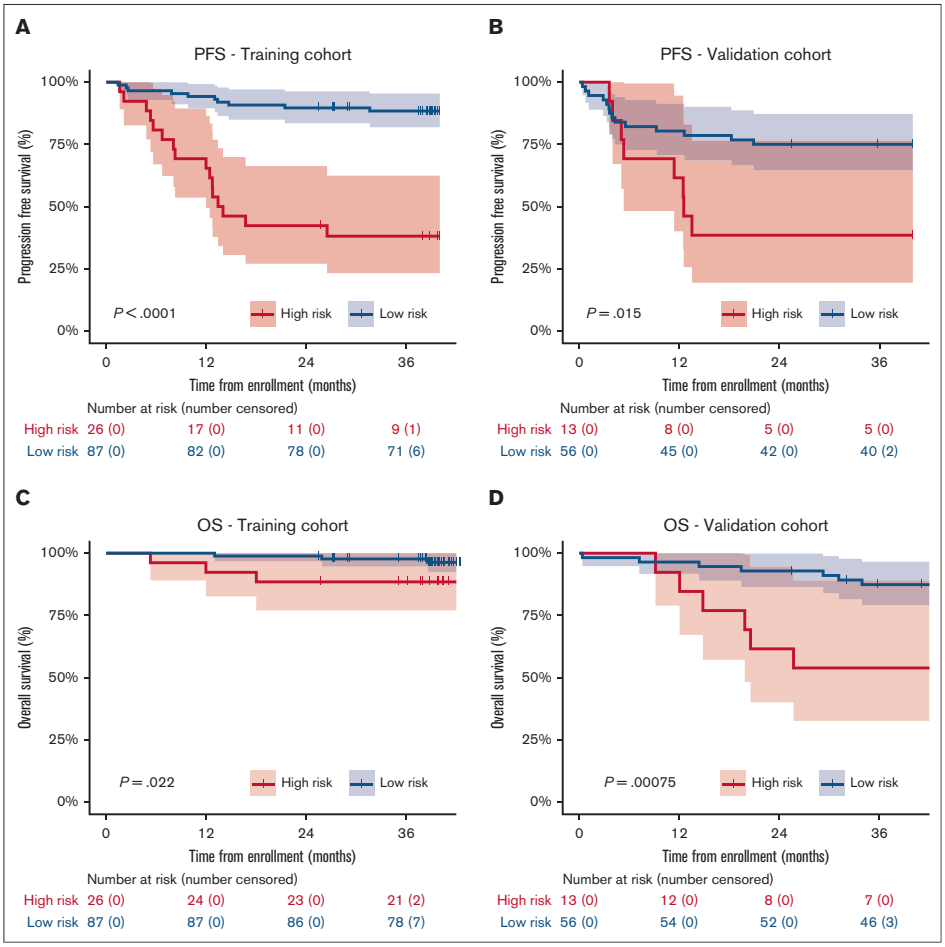


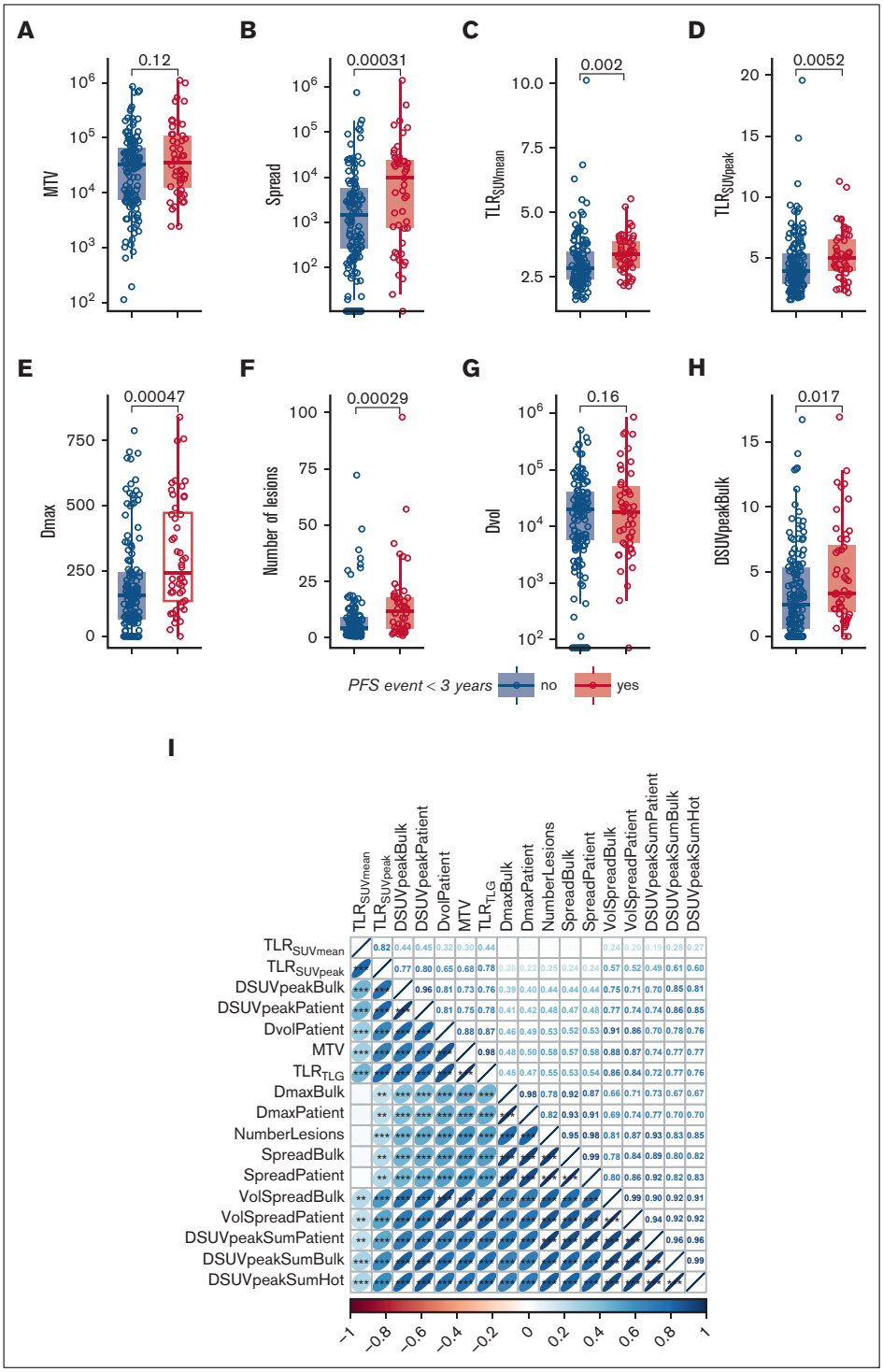
Figure 2. Kaplan-Meier curves of the prognostic model. PFS analysis in the training (A) and independent validation cohort (B). OS analysis in the training (C) and independent validation cohort (D). The size of the high- and low-risk groups were defined according to the percentage of patients with a PFS event in the training cohort, which was 23%. The respective percentage of patients with the highest prediction scores from the logistic regression was classified as high risk.

Table 4. Performance of the model

High- vs low-risk	Training	Validation	BV-DHAP	BV-ICE
Sensitivity	61.5	36.4	61.5	61.5
Specificity	88.5	89.4	93.3	83.3
PPV	61.5	61.5	72.7	53.3
NPV	88.5	75.0	89.4	87.5

NPV, negative predictive value; PPV, positive predictive value.

Performance of the model shown for the training and validation cohorts. The training cohort consists of the BV-DHAP and BV-ICE studies of which the model performance is also shown separately. The optimal cutoff for high- vs low-risk groups is based on the percentage of PFS events in the training cohort which was 23%.


A
B
C
D
E
F
G
H
I
MTV
Spread
TLRSUVmean
TLRSUVpeak
Dmax
Number of lesions
Dvol
DSUVpeakBulk
0.12
0.00031
0.002
0.0052
0.00047
0.00029
0.16
0.017
PFS event < 3 years
no
yes
TLRSUVmean
TLRSUVpeak
DSUVpeakBulk
DSUVpeakPatient
DvolPatient
MTV
TLRTLG
DmaxBulk
DmaxPatient
NumberLesions
SpreadBulk
SpreadPatient
VolSpreadBulk
VolSpreadPatient
DSUVpeakSumPatient
DSUVpeakSumBulk
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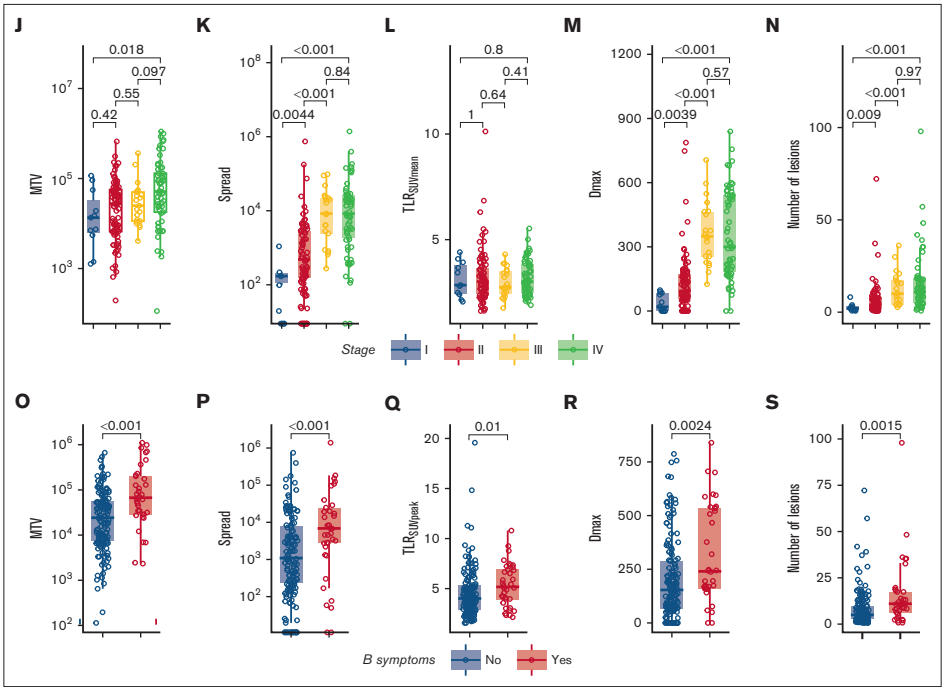
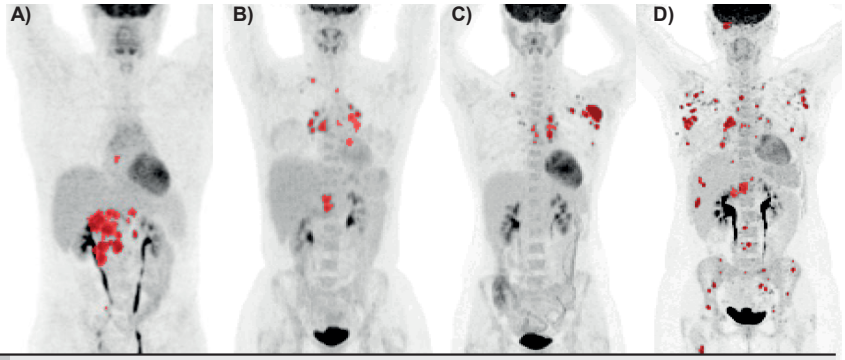


Figure 3. Correlations of radiomics features with PFS outcomes and clinical characteristics and intercorrelations of radiomics features. (A-H) Boxplots of logtransformed radiomics features stratified for patients with or without an event (progression and/or death) on the 3-years PFS. (I) Spearman rank correlation coefficient plot of all radiomics features. Asterisks indicate significance values (*P < .05; **P < .01; ***P < .001). (J-N) Boxplots of log-transformed radiomics features stratified for Ann Arbor stage. (O-S) Boxplots of log-transformed radiomics features stratified for the presence of B symptoms at baseline. Dmax, the largest distance between 2 lesions in mm; DSUVpeakBulk, difference of SUVpeak between the largest lesion and the lesion with the lowest SUVpeak in g/mL; Dvol, the difference in volume between the largest and the smallest lesion in mL; NumberLesions, number of lesions; TLRSUVmean, tumor-to-liver ratio of mean SUV corrected for the liver SUVmean; TLRSUVpeak, ratio of SUVpeak corrected for the liver SUVmean.

Correlations of clinical and radiomics features

In **Figure 3**, several radiomics features that were used in the models are stratified for patients with or without a PFS event. MTV was not significantly higher in patients with an event (p=0.12) [**Figure 3A**]. However, MTV still contributed to the prognostic value of the model because of a complex interaction term with Spread and $TLR_{SUVmean}$, in which Spread showed a higher prognostic value when MTV was high, and in contrast, $TLR_{SUVmean}$ showed a higher prognostic value when MTV was low [**Table 3; Supplemental Figure 2**]. A possible explanation for this interaction between Spread, MTV, and $TLR_{SUVmean}$ is that when MTV and Spread are low, a high $TLR_{SUVmean}$ indicates a more aggressive disease and has a worse prognosis, whereas when MTV and Spread are high, the $TLR_{SUVmean}$ is less relevant and the spread of the disease becomes more important to indicate a worse prognosis.

Most radiomics features show moderate to high correlations with other radiomics features. $TLR_{SUVmean}$, which is included in the model, shows the lowest correlations with other radiomics features [**Figure 3I**]. Ann Arbor stage was significantly correlated with MTV, Spread, Dmax,



	A	B	C	D
R/R status	Relapse	Relapse	Refractory	Relapse
B symptoms	No	No	Yes	No
Stage	IV	III	IV	IV
$TLR_{SUVmean}$	3.06	2.43	4.59	4.48
MTV (mL)	81.6	11.0	23.9	47.3
Spread (cm)	194	411	677	39576
Model score	**0.03**	**0.05**	**0.88**	**0.46**
Outcome	**No PD**	**No PD**	**PD 3m after ASCT**	**PD 12m after ASCT**

Figure 4. Examples of maximum intensity projections of baseline PETs in 4 different patients with R/R cHL. The model score was calculated based on the prognostic model using clinical and radiomics features. The outcome represents the clinical outcome of the patient. (A-B) Patients with a low prediction score with low risk of progressive disease. (C-D) Patients with a high prediction score with a high risk of progressive disease. *m, months; PD, progressive disease.*

and number of lesions but not with $TLR_{SUVmean}$ **[Figure 3J-N]**. Patients with B symptoms had significantly higher values of several radiomics features **[Figure 3O-S]**. R/R status did not correlate with any radiomics features (data not shown).

Patients with low and high prediction scores

Examples of patients with low and high prediction scores are provided in **Figure 4**. The formula for the prognostic model can be found in the description of **Table 3**. Additionally, we created a calculator in Excel format that can be used to calculate the predicted probability for individual patients (**Supplemental Appendix**). For example, patient B from **Figure 4** had relapsed disease with no B symptoms, an MTV of 11 mL, Spread of 411 cm and $TLR_{SUVmean}$ of 2.43 and the model calculated a risk score of 0.05 which is placed in the low-risk group. Correspondingly, this patients had a CMR before ASCT and is still in remission after 41 months of follow-up. In contrast, patient C had primary refractory disease with B symptoms, an MTV of 24 mL, Spread of 677 cm and $TLR_{SUVmean}$ of 4.59 corresponding to a risk score of 0.88, and relapsed 3 months after ASCT despite an initial CMR on the pre-ASCT PET.

DISCUSSION

There is an unmet need for better risk-stratification in the R/R setting for cHL patients receiving salvage therapy followed by ASCT.[4] Therefore, we have developed a novel prognostic model

in R/R cHL for 3-year PFS based on quantitative features from baseline PET scans and clinical characteristics that was validated on an independent dataset. The features that were included in our model are robust and not sensitive to technical variations, which makes it more feasible to implement in clinical practice because of the use of different quality of PET scanners across different countries or hospitals.

Several studies have developed prognostic models based on clinical characteristics and pre-ASCT response-assessment to predict post-ASCT outcomes, but models for risk profiling at baseline, before starting second-line treatment, are scarce.[8,39] A baseline model has the advantage of being able to preselect patients with high-risk disease and change therapy upfront, preventing these patients from not responding to salvage chemotherapy while being at risk for toxicity. The PPV was 61.5% in both the training and validation cohorts, which is similar or slightly higher than the PPV of pre-ASCT response assessment by PET, as described in literature (PPV ranges between 40 and 60% for individual studies), but with our model, this prediction can already be done at baseline.[9,10,30,40] Therefore, it is worthwhile to explore the model's applicability for changing salvage therapy in patients with R/R cHL with high-risk disease, such as treating these patients, who are most likely to be chemotherapy resistant, with checkpoint inhibitors.[41] In addition, our model showed a high NPV in both the training and validation cohorts, which means that the model is also suitable for selecting patients with a low risk of progression. This could be used to guide the selection of patients who can potentially be cured by replacing the ASCT for a less toxic consolidation with checkpoint inhibitors, as is currently being evaluated in several studies.[42].

A limitation of our analysis is that 32 PETs (16% of total cohort) had to be excluded from the analysis because of inefficient quality of the PET or because the PET format was not compatible with our analysis software. These scans mainly originated from the ICE study,[30] which enrolled patients between 2007 and 2010 when the use of PETs was just emerging in clinical practice. Because the quality of PETs has much improved over the years, it is expected that the percentage of excluded PETs will be much smaller in future trials.

Not all patient characteristics were balanced between the cohorts, which could have influenced the performance of the model on the validation cohort. Because the model was trained using the training cohort, the validation cohort showed lower AUC values. However, the cross-validated AUC of the training cohort closely resembled the AUC of the validation cohort, and the PPV was similar between the cohorts. This indicates that in both training and validation cohorts, patients with high-risk disease were well-identified. In addition, the ICE study had a higher number of events, possibly because this study did not treat patients with BV and the study was conducted ~10 years before the BV-ICE and BV-DHAP studies, so advances in supportive care could have improved over time. In the 3 patient cohorts that we used in this analysis, all patients were intended to receive salvage chemotherapy followed by ASCT, but the salvage chemotherapy schedules were different. Many different salvage regimens are used in R/R cHL across different countries but are generally comparable in terms of efficacy. Therefore, it

is also useful to validate the model with different treatment regimens so it can be extrapolated to other salvage regimens.

We used 3 different semiautomatic segmentation methods, which we have investigated earlier.[26] In our previous study, results with the SUV4.0 and SUV2.5 methods highly correlated and with these methods, there was the lowest need for manual adaptation during segmentation.[26] In the current analysis, the SUV4.0 yielded the highest validated AUC score for the prognostic model and was again also the least time-consuming method. The combimethod (SUV4.0 + SUV2.5) that we tested did not improve the prognostic value of radiomics features. A possible explanation for this may be that low FDG avid lesions (SUV<4.0) are reactive to the lymphoma and do not substantially contribute to the disease characteristics and, therefore, have no influence on the prognostic capabilities of the radiomics features. In this study, we have confirmed the findings of our previous analysis in a larger cohort of patients and propose to use SUV4.0 as a standard segmentation method for cHL at baseline assessment.[26]

Other studies investigating quantitative PET features in cHL mainly focused on only MTV and were performed in single cohorts without validation in external cohorts.[9,11-16] Besides, most studies used a cutoff for MTV instead of the continuous variable in a logistic regression. In our model, MTV was not the highest contributing factor; therefore we think that combining MTV with other quantitative PET features, for example, intensity and dissemination features, is important because this enables capturing differences between patients with localized bulky disease and those with disseminated disease.

Other biomarkers, such as circulating tumor DNA and thymus and activation regulated chemokine (TARC) have been shown to correlate with MTV.[43] Circulating tumor DNA seems a promising biomarker for detecting minimal residual disease, but its prognostic value at baseline is modest and comparable with studies that investigated MTV as a single biomarker.[44] We previously published an analysis of TARC in 65 patients with R/R cHL (who are also included in this analysis), in which we demonstrated that TARC has a high prognostic value after 1 cycle of chemotherapy, but it provides no prognostic value at baseline.[10] Combination of TARC and pre-ASCT SUV_{peak} increased the accuracy of predicting progression; therefore, combining biomarkers could possibly enhance the prognostic capacities of biomarker models.

PET-CT is already being performed as part of standard clinical practice in most countries.[37] We showed earlier that semiautomatic segmentation using the SUV4.0 method requires the least manual adaptation by a nuclear medicine physician and is, thus, less observer dependent. Therefore, quantitative analysis of PETs can be used in clinical practice at low extra costs and will probably not be very time consuming. With upcoming technological advances, such as automated segmentation, it is expected that PET radiomics analysis can be performed much easier in the future.[45] Our model consists of robust quantitative PET features, which prevents a high variability of features between different PET scanners, hospitals, and observers. Therefore, quantitative PET analysis provides a promising method for prognostication, which is feasible to be implemented in prospective baseline risk-adapted clinical trials.

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CONFLICT OF INTEREST

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SUPPLEMENTARY MATERIAL

Supplemental Table 1. Overview of treatment regimens and response assessment in the included studies

Study	Salvage treatment regimen	High-dose chemotherapy	Response assessment
BV-DHAP, Kersten et al., 2021	Patients were treated with three 21-day cycles of BV (1.8 mg/kg, i.v., day 1), dexamethasone (40 mg orally or i.v., days 1-4), cisplatin (100 mg/m2, continuous i.v. (24hr), day 1) and cytarabine (2x2 g/m2 q12hr, 3hr for each infusion, day 2).	BEAM: carmustine, 300 mg/m2, day -7, etoposide, 100 mg/m2 and cytarabine, 100mg/m2, 2x/day, days -6, -5, -4 and -3, and melphalan, 140 mg/m2, day -2), followed by auto-PBSCT (on day 0).	A PET-CT scan was performed after cycle 3. Patients with progressive disease (PD) went off study, whereas patients with a partial response (mPR; i.e. Deauville score 4-5) or mCR (i.e. Deauville score 1-3) proceeded to BEAM.
BV-ICE, Moskowitz et al., 2017	**BV** 1.2 mg/kg on days 1, 8, and 15 every 28 days for 2 cycles. **AugICE**: same dose as in Moskowitz 2012.	The choice of conditioning regimens and use of pretransplantation radiation for HDT/ASCT was at the discretion of the treating physician. Patients with localized, nodal-based disease who had not previously received radiation were treated with involved field radiation therapy (IFRT) before ASCT.	1 week after 2 cycles of BV: those who achieved PET normalization (defined by Deauville score of #2) proceeded directly to ASCT; those with persistent abnormalities on PET received 2 cycles of augICE before being considered for ASCT.
ICE, Moskowitz et al., 2012	Arm A: 0 or 1 risk factor (remission duration <1year, B symptoms or extranodal localization. 1 cycle of ICE followed by 1 cycle of augmented ICE. Arm B: >1 risk factors: 2 cycles of aICE (administered on a 17- to 21-day schedule. **AugICE**: 2 doses of ifosfamide 5000 mg/m² combined with 5000 mg/m² of the uroprotective agent mesna (which were given as a continuous infusion over 24 h on days 1 and 2); three doses of etoposide (200 mg/m² by intravenous infusion over 60 min every 12 h beginning on day 1); and carboplatin (dosed at an area-under-the-curve of 5 [maximum dose 800 mg] on day 3). **GVD:** gemcitabine 1000 mg/m2, vinorelbine 20 mg/m2, and liposomal doxorubicin 15 mg/m2) administered every 2 weeks for 4 doses	Patients with residual radiographic disease or initially bulky sites were eligible for accelerated involved field radiation (IFRT). Two options: **Total lymphoid irradiation** (TLI) or STLI (subtotal lymphoid irradiation) administered in 1.8-Gy twice-a-day fractions (total dose, 18 Gy) for 5 days, cyclophosphamide 900 mg/m2 every 12 hours for 8 doses (total dose, 7200 mg/m2), and etoposide 500 mg/m2 administered as a 24-hour infusion, for 4 doses (total dose, 2000 mg/m2). **No TLI**: Cyclophosphamide 900 mg/m2 every 12 hours for 8 doses (total dose, 7200 mg/m2), Etoposide 500 mg/m2 administered as a 24-hour infusion, for 4 doses (total dose, 2000 mg/m2), and Carmustine 360 mg/m2 administered on day 2.	All patients with repeat scans that were abnormal were presented by the reference nuclear medicine physician at a multidisciplinary lymphoma staging conference, and the decision to administer gemcitabine, vinorelbine, and liposomal doxorubicin (GVD) was made at that time. Patients with a positive FDG-PET received GVD; those with a negative scan underwent HDT/ASCT.

Supplemental Table 2. Reasons for exclusion of patients

	BV-DHAP	BV-ICE	ICE	Total
Total population	**67**	**65**	**99**	**231**
Withdrew consent	2	1	0	3
No PET available	1	0	0	1
PET not compatible[1]	5	2	21	28
Quality check PET not compliant[2]	0	3	6	9
Follow-up time <2 years (without event)	1	4	3	8
Total included	**58**	**55**	**69**	**182**

[1]PET or DICOM not compatible to ACCURATE software

[2]PET scans that did not meet the following criteria: 1) plasma glucose <11mmol/L; 2) reconstruction of attenuation-corrected PET according to guidelines described by EARL or ACR; 3) total image activity (MBq) between 50-80% of the total injected FDG activity or liver SUVmean between 1.3-3.0;

Supplemental Table 3. Definitions of PET- and radiomics features

Variable	Definition
MTV	Metabolic tumor volume; The FDG-avid tumor volume
TLG	Total lesion glycolysis; MTV multiplied by SUVmean
SUVmean	The mean SUV value of the VOI
SUVmax	The SUV of the voxel with the highest SUV within the VOI
SUVpeak	The SUV of the 3mL with the highest SUV within the VOI (global peak)
$TLR_{SUVmean}$	Tumor to liver ratio of the lesional SUVmean and the liver SUVmean
$TLR_{SUVpeak}$	Tumor to liver ratio of the lesional SUVpeak and the liver SUVmean
Number of lesions	The number of separated lesion selections within the VOI
Dmax	The maximum distance between two lesions
DmaxBulk	The maximum distance between the largest lesion and any other lesion
Spread	The sum of the distance between all lesions
SpreadBulk	The sum of the distance between the largest lesion and all other lesions
Dvol	The difference in volume between the largest and the smalles lesion
VolSpread	The sum of the differences in volume between all lesions
VolSpreadBulk	The sum of the differences in volume between the largest lesion and all other lesions
DSUVmax	The difference in SUVmax between the lesion with the highest SUVmax and the lesion with the lowest SUVmax
DSUVmaxSum	The sum of the differences in SUVmax of all lesions
DSUVmaxBulk	The differences in SUVmax between the largest lesion and all other lesions
DSUVmaxSumBulk	The sum of the differences in SUVmax between the largest lesion and all other lesions
DSUVmaxSumHot	The sum of the differences in SUVmax between the lesion with the highest SUVmax and all other lesions
DSUVpeak	The difference in SUVpeak between the lesion with the highest SUVpeakmax and the lesion with the lowest SUVpeak
DSUVpeakSum	The sum of the differences in SUVpeak of all lesions
DSUVpeakBulk	The differences in SUVpeak between the largest lesion and all other lesions
DSUVpeakSumBulk	The sum of the differences in SUVpeak between the largest lesion and all other lesions
DSUVpeakSumHot	The sum of the differences in SUVpeak between the lesion with the highest SUVpeak and all other lesions

Supplemental Table 4. Model performance for radiomics models per segmentation method

Model	Features	AUC train	CV-AUC train	AUC val
SUV4.0	Number of lesions VolSpreadPatient TLR_SUVmean	0.719 (0.605 - 0.833)	0.691 (0.685 – 0.696)	0.721 (0.58 - 0.863)
SUV2.5	DmaxBulk SpreadPatient DSUVpeakSumBulk DSUVpeakSumHot TLR_SUVmean	0.746 (0.635 - 0.857)	0.692 (0.687 - 0.698)	0.714 (0.569 - 0.858)
SUV2.5 with features from SUV4.0 model	Number of lesions VolSpreadPatient TLRSUVmean	0.697 (0.580 - 0.814)	0.673 (0.667 - 0.678)	0.715 (0.572 – 0.857)
41MAX	DmaxBulk SpreadBulk	0.633 (0.475 - 0.791)	0.607 (0.6 - 0.615)	0.617 (0.456 - 0.778)
41MAX with features from SUV4.0 model	Number of lesions VolSpreadPatient TLRSUVmean	0.628 (0.485 – 0.772)	0.566 (0.559 - 0.572)	0.712 (0.588 - 0.835)
Combimethod	SpreadPatient VolSpreadPatient TLR_SUVpeak	0.672 (0.553 - 0.791)	0.628 (0.622 - 0.634)	0.712 (0.57 - 0.853)
Combimethod with features from SUV4.0 model	Number of lesions VolSpreadPatient TLRSUVmean	0.676 (0.552 - 0.799)	0.641 (0.635 - 0.647)	0.730 (0.592 - 0.869)

Supplemental Table 5. Final model results and exploration of high-risk group cutoff based on Youden-index on cross-validation in training cohort

	PFS	PFS	OS	OS
Pre-defined cutoff (0.408)	**Training**	**Validation**	**Training**	**Validation**
Low risk (N; %)	87	56	87	56
Low risk event (N; %)	10 (11%)	14 (25%)	4 (5%)	8 (14%)
High risk (N; %)	26	13	26	13
High risk event (N; %)	16 (62%)	8 (62%)	5 (19%)	7 (54%)
Sensitivity	61.5	36.4	55.6	46.7
Specificity	88.5	89.4	79.8	88.9
PPV	61.5	61.5	19.2	53.8
NPV	88.5	75.0	95.4	85.7
PFS Low risk (3yr%; 95% CI)	88.4 (81.9 - 95.4)	75.0 (64.5 - 87.2)	97.7 (94.6 - 100)	87.4 (79 - 96.6)
PFS High risk (3yr%; 95% CI)	38.1 (23.2 - 62.4)	38.5 (19.3 - 76.5)	88.5 (77 - 100)	53.8 (32.6 - 89.1)
P-value log-rank	0.0000	0.0153	0.0217	0.0008
HR (95% CI)	7.7 (3.48 - 17.12)	2.8 (1.18 - 6.76)	0.2 (0.06 - 0.9)	0.2 (0.07 - 0.57)
P-value HR	0.0000	0.0290	0.0370	0.0041
Youden cutoff (0.292)	**Training**	**Validation**	**Training**	**Validation**
Low risk (N; %)	77	53	77	53
Low risk event (N; %)	7 (9%)	13 (25%)	4 (5%)	7 (13%)
High risk (N; %)	36	16	36	16
High risk event (N; %)	19 (53%)	9 (56%)	5 (14%)	8 (50%)
Sensitivity	73.1	40.9	55.6	53.3
Specificity	80.5	85.1	70.2	85.2
PPV	52.8	56.3	13.9	50.0
NPV	90.9	75.5	94.8	86.8
PFS Low risk (3yr%; 95% CI)	90.8 (84.5 - 97.5)	75.5 (64.7 - 88)	97.4 (93.9 - 100)	86.6 (77.9 - 96.4)
PFS High risk (3yr%; 95% CI)	47.1 (33.2 - 66.6)	43.8 (25.1 - 76.3)	91.7 (83.1 - 100)	62.5 (42.8 - 91.4)
P-value log-rank	0.0000	0.0216	0.1476	0.0012
HR (95%CI)	8.1 (3.38 - 19.26)	2.6 (1.12 - 6.17)	0.4 (0.1 - 1.46)	0.2 (0.08 - 0.6)
P-value HR	0.0000	0.0343	0.1627	0.0043

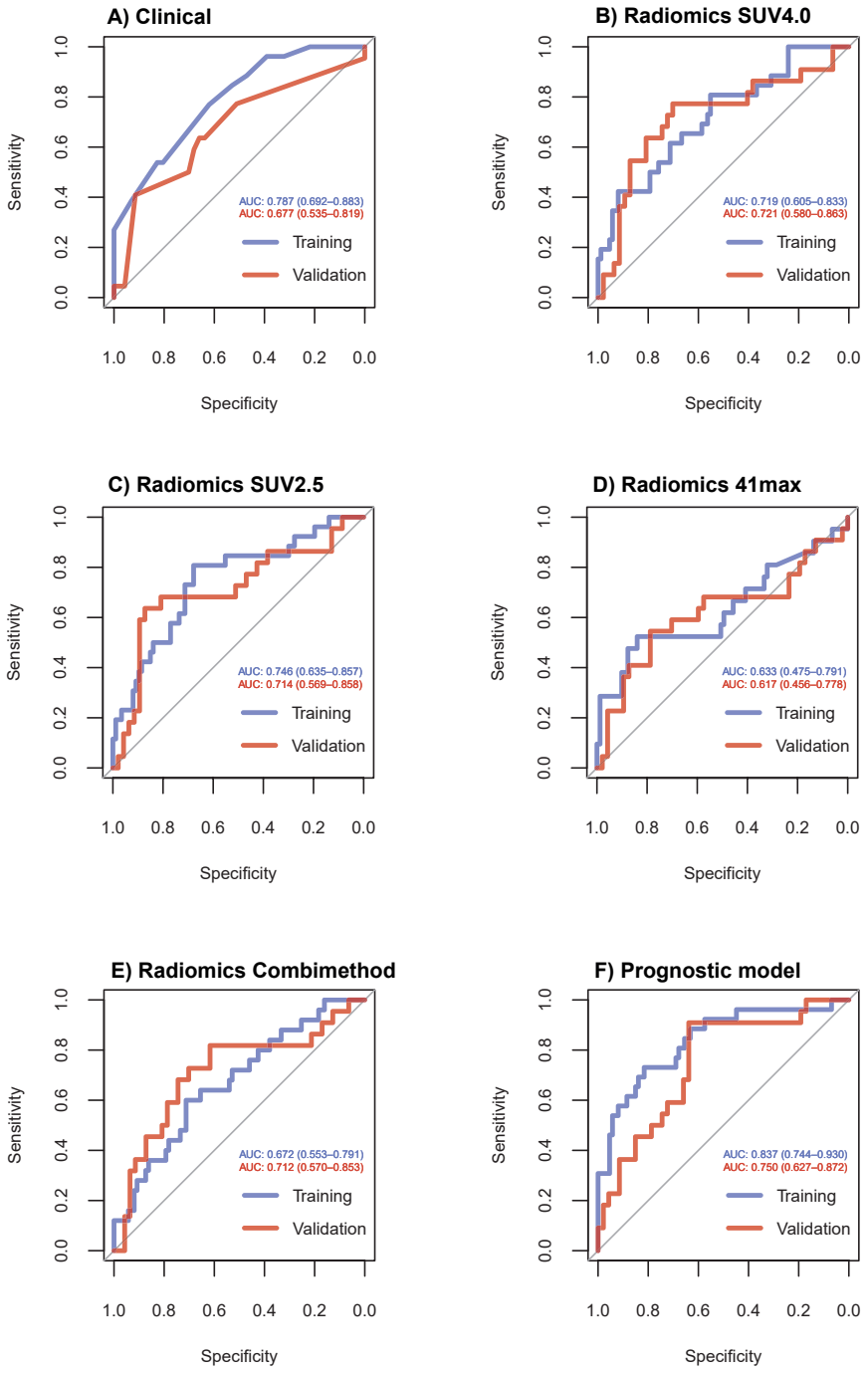
The pre-defined cutoff is based on the percentage of patients with a PFS event in the training cohort, which was 23% (26/113), corresponding to a prediction score of 0.408. The optimal cutoff based on Youden-index was calculated for each iteration in the 5-fold cross-validation with 2000 repeats on the training cohort and the median cutoff value was used, which was a prediction score of 0.292.

Abbreviations: n, number; PPV, positive predictive value; NPV, negative predictive value; yr, year; HR, hazard ratio; CI, confidence interval.

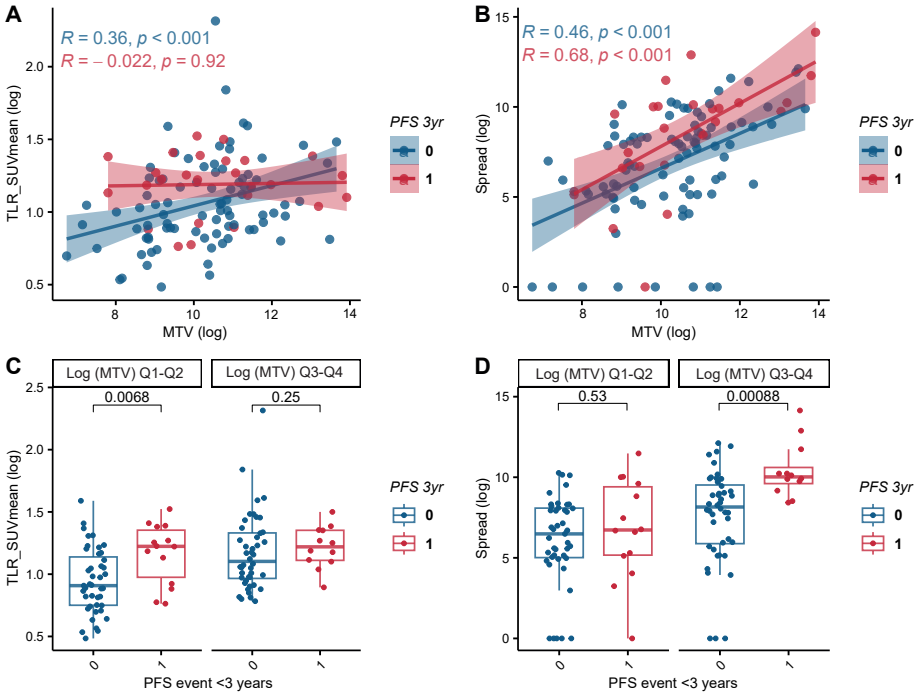
Supplemental Table 6. Final model response and ASCT results

N (%)	Training	Training		Validation	Validation	
Pre-defined cutoff (0.408)	**Low-risk**	**High-risk**	**P**	**Low-risk**	**High-risk**	**P**
Pre-ASCT CMR	75 (86%)	18 (69%)	0.0494	45 (80%)	11 (85%)	0.5660
Underwent ASCT	84 (97%)	24 (92%)	0.3558	46 (82%)	12 (92%)	0.3671
PD before ASCT	4 (5%)	2 (8%)	0.0494	9 (16%)	1 (8%)	0.5660
PD after ASCT	7 (8%)	14 (54%)	0.0000	5 (9%)	7 (54%)	0.0001
Youden cutoff (0.292)	**Low-risk**	**High-risk**	**P**	**Low-risk**	**High-risk**	**P**
Pre-ASCT CMR	66 (86%)	27 (75%)	0.2964	43 (81%)	13 (81%)	0.6881
Underwent ASCT	75 (97%)	33 (92%)	0.1671	44 (83%)	14 (88%)	0.6678
PD before ASCT	3 (4%)	3 (8%)	0.2964	8 (15%)	2 (13%)	0.6881
PD after ASCT	5 (6%)	16 (44%)	0.0000	5 (9%)	7 (44%)	0.0015

The pre-defined cutoff is based on the percentage of patients with a PFS event in the training cohort, which was 23% (26/113), corresponding to a prediction score of 0.408. The optimal cutoff based on Youden-index was calculated for each iteration in the 5-fold cross-validation with 2000 repeats on the training cohort and the median cutoff value was used, which was a prediction score of 0.292.



Supplemental Figure 1. AUC curves of different models.



Supplemental Figure 2. Spearman rank correlation coefficients scatter plots of MTV and **A)** $TLR_{SUVmean}$ and **B)** SpreadPatient, stratified for patients with or without progressive disease <3 years from enrollment. Boxplots of **C)** $TLR_{SUVmean}$ and **D)** SpreadPatient stratified stratified for patients with or without progressive disease <3 years from enrollment and separated for patients with a low (interquartile range Q1-Q2 of 6.74-10.5) or high (interquartile range Q3-Q4 of 10.5-13.8) of log(MTV), p-values represent Wilcoxon rank sum test. *Q, interquartile range; R, correlation coefficient.*

8

Prognostic value of TARC and quantitative PET parameters in relapsed or refractory Hodgkin lymphoma patients treated with brentuximab vedotin and DHAP.

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ABSTRACT

Risk-stratified treatment strategies have the potential to increase survival and lower toxicity in relapsed/refractory classical Hodgkin lymphoma (R/R cHL) patients. This study investigated the prognostic value of serum (s)TARC, vitamin D and lactate dehydrogenase (LDH), TARC immunohistochemistry and quantitative PET parameters in 65 R/R cHL patients who were treated with brentuximab vedotin (BV) and DHAP followed by autologous stem-cell transplantation (ASCT) within the Transplant BRaVE study (NCT02280993). At a median follow-up of 40 months, the 3-year progression free survival (PFS) was 77% (95% CI: 67–88%) and the overall survival was 95% (90–100%). Significant adverse prognostic markers for progression were weak/negative TARC staining of Hodgkin Reed-Sternberg cells in the baseline biopsy, and a high standard uptake value (SUV)mean or SUVpeak on the baseline PET scan. After one cycle of BV-DHAP, sTARC levels were strongly associated with the risk of progression using a cutoff of 500 pg/ml. On the pre-ASCT PET scan, SUVpeak was highly prognostic for progression post-ASCT. Vitamin D, LDH and metabolic tumor volume had low prognostic value. In conclusion, we established the prognostic impact of sTARC, TARC staining, and quantitative PET parameters for R/R cHL, allowing the use of these parameters in prospective risk-stratified clinical trials. Trial registration: NCT02280993.

INTRODUCTION

Approximately 50-60% of relapsed or primary refractory classical Hodgkin lymphoma (R/R cHL) patients can be cured with standard salvage chemotherapy followed by high-dose chemotherapy (HDC) and autologous stem-cell transplantation (ASCT).[1-5] With the advent of novel therapies for R/R cHL, optimizing baseline risk stratification and early response assessment are becoming increasingly important to guide treatment decisions.[6-8]

We and others have shown that brentuximab vedotin (BV), an anti-CD30 antibody-drug conjugate, can be safely added to standard salvage chemotherapy.[6,8-14] In the prospective, multicenter, international Phase I-II Transplant BRaVE study, we investigated the safety and efficacy of BV in combination with dexamethasone, cisplatin and high-dose cytarabine (DHAP) followed by ASCT.[8] The complete metabolic response (CMR) rate after three cycles of BV-DHAP was 100% in the Phase I part (n=12) of the study and 81% in the Phase II part (n=55).

To enable broader application of risk-stratified treatment, it is important to identify biomarkers that are associated with response to salvage treatment and the risk of relapse thereafter. Achieving a CMR, i.e., Deauville score (DS) 1-3, assessed by an ^{18}F-fluorodeoxyglucose (FDG)-positron emission tomography (PET)-computed tomography (CT) scan after salvage chemotherapy prior to ASCT, is an important predictor of progression free survival (PFS).[15-17] The DS is determined by visual comparison of the FDG uptake in tumor localizations compared to the liver and mediastinal blood pool. However, visual assessment of DS inevitably leads to inter-observer disagreement.[18] Quantitative PET analysis leads to standardized interpretation and could provide prognostic information beyond staging and DS alone, such as metabolic tumor volume (MTV) and FDG uptake of lymphoma lesions.[9,19-21]

Besides imaging biomarkers, several blood-based and immunohistochemistry (IHC)-based markers have been investigated in newly diagnosed cHL.[22,23,24] Thymus and activation regulated chemokine (TARC, CCL17) is secreted by Hodgkin Reed-Sternberg (HRS) cells and can be visualized by IHC. Serum (s)TARC levels correlate with disease activity during treatment in newly diagnosed cHL.[22,24,25] Furthermore, serum 25-hydroxyvitamin D deficiency has been shown to correlate with poor PFS in newly diagnosed cHL.[26,27] However, studies that have prospectively investigated biomarkers in the R/R setting are scarce.

Combination of blood-based, IHC-based and imaging-based biomarkers could provide prognostic information already at baseline and could complement treatment response-evaluation with visual assessment of PET-CT before ASCT. Additionally, blood-based biomarkers have the advantage that they can be assessed at multiple time points and are less invasive compared to PET-CT scans.

Here we present the 3-year follow-up results of the Transplant BRaVE study. We investigated the correlation between sTARC, tumoral TARC IHC, lactate dehydrogenase (LDH), vitamin D, quantitative PET parameters and clinical characteristics, and the prognostic value of these variables to predict progression of disease during or after BV-DHAP.

METHODS

Patients and study design

This multicenter, single-arm, Phase I-II trial (NCT02280993) enrolled adults with histologically confirmed cHL either having primary refractory disease (i.e., no complete response (CR) or progression <3 months after first-line treatment) or a first relapse after first-line chemotherapy (i.e., progression ≥3 months after CR). The complete list of inclusion and exclusion criteria has been published before.[8] Patients were treated with three cycles of BV-DHAP, followed by PET-CT response assessment. Patients with a CMR or partial metabolic response (PR) proceeded to ASCT.[8]

All patients provided written informed consent. The study protocol was approved by the Ethical Review Committee of all participating centers. The study was carried out in accordance with the principles of the Helsinki Declaration.

Serum biomarker assessment

Serum samples were centrally collected at baseline, after each cycle of BV-DHAP, after ASCT and during follow-up until 3-years post-ASCT. sTARC (ELISA, R&D systems, Minneapolis, MN, USA) and 25-hydroxyvitamin D (PromoCell, Heidelberg, Germany) levels were measured in serum by enzyme-linked immunosorbent assay, and analyzed blinded for patient outcome. LDH was not centrally analyzed but results of local laboratory assessments were collected and divided by the laboratory-specific upper limit of normal (ULN).

Tissues and immunohistochemistry

A lymph node biopsy was done at baseline, i.e., before start of BV-DHAP. For n=21 patients for whom insufficient material was available for additional IHC staining, the primary diagnostic biopsy was used. Central pathology review was performed by two experienced hemato-pathologists (AD, DdJ). All cases were stained for TARC in an automated setting. Paraffin tissue sections (3um) were incubated with polyclonal goat-anti-human TARC antibody (1:800 R&D Systems, Minneapolis, MN) on the automated Benchmark ULTRA platform (Ultra CC1, 52 minutes, Roche, Ventana Medical Systems). For each TARC stain, a section of cHL tissue was applied on the same slide as an external positive control. Intensity of TARC staining (i.e., negative, weak, positive) was scored by an experienced hemato-pathologist (AD), blinded for patient outcome. Positive TARC staining was defined as cytoplasmic staining visible at a magnification of x20 or less, weak staining was defined as cytoplasmic staining only discernable at higher magnification (x200).

PET-CT scan analysis

PET acquisition was performed according to the EANM guidelines and EARL standards in eight medical centers (**Supplemental Table 1**).[28,29] PET-CT scans were performed at baseline, prior

to ASCT (4-6 weeks after the third cycle of BV-DHAP) and 6 weeks after ASCT. Central PET review for response assessment according to the Lugano classification was performed by two nuclear medicine physicians (AA, RV).[8] Discrepancies were adjudicated by a third reviewer (GJCZ).

Segmentation of baseline PET scans was performed semi-automatically using the ACCURATE tool, by automatic selection of regions with FDG uptake above a threshold of standard uptake value (SUV) ≥4.0 g/mL, followed by manually adding tumor regions or removing non-tumor regions with high physiological uptake if necessary, as described earlier.[30,31] Pre-ASCT PET scans were analyzed manually if metabolic active disease was present. In patients without measurable metabolic active lesions (DS-1), SUV was set to 0 and deltaSUV to 100%. Regarding extranodal and splenic lesions, only focal lesions were included. PET segmentation was performed by JD under supervision of a nuclear medicine physician (GJCZ). The following quantitative features were extracted at the patient-level: SUVmean, SUVpeak, total MTV, total lesion glycolysis (TLG; i.e. MTV multiplied by SUVmean), and number of lesions.[32,33] Because of the multicenter aspect of this study and the use of different PET scanners, only PET parameters that are not too sensitive to technical variations were used, such as SUVpeak (i.e., the average SUV of the 1 mL with the highest FDG uptake) instead of SUVmax (which represents only the highest single voxel). Additionally, as the SUVmean of the liver is used as a standard quality parameter to compare PET scans and is also the reference for a DS-3, we normalized the SUV for the liver SUVmean and used the tumor-to-liver ratio (TLR).[28,34-36] The liver SUVmean was estimated on a 3 mL sphere in the right upper lobe of the liver. In addition, we calculated the tumor-ratio for the mediastinal blood-pool (MBP), which is the reference for a DS-2.

Endpoints

The efficacy and safety endpoints of the Transplant BRaVE phase I-II study have been reported earlier.[8] The endpoint of the clinical follow-up analysis is the 3-year PFS and OS. PFS was defined as time from study entry until progressive disease or death, whichever came first. OS was defined as time from study entry until death from any cause. The primary endpoint for biomarker analysis is the 3-year freedom from progression (FFP), defined as time from study entry until progressive disease, and patients who died without progression were censored at the time of death. This provides a more biologically meaningful analysis of the correlation between the biomarkers and disease activity.

Statistical analysis

The Kaplan-Meier method and log-rank test were used to analyze univariable associations with PFS and OS and a Cox proportional hazards regression was performed.

Biomarker values were compared for patients who showed progressive disease during or after BV-DHAP vs patients in remission using the Wilcoxon rank sum test for non-parametric data. Correlations between biomarkers were assessed using Spearman's Rank correlation coef-

ficients. The prognostic value of biomarkers for FFP was assessed by calculating the area under the curve (AUC) of the receiver operating characteristics curve and log-rank survival analysis. Added prognostic value of combining two biomarkers was assessed using logistic regression and Wald test. Pre-specified cutoffs were used to calculate sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). The pre-specified cutoff for response-assessment with sTARC was 1000 pg/mL, based on a study in newly diagnosed cHL and levels in healthy controls.[22] Patients who had sTARC-baseline levels below the cutoff were excluded from subsequent analysis. The cutoff for sufficient vitamin D levels was 50 ng/mL.[26,27] The cutoff for baseline $TLR_{SUVmean}$ and $TLR_{SUVpeak}$ was 3.0, aligning with a DS-5 (uptake markedly higher than the liver), and for pre-ASCT $TLR_{SUVmean}$ and $TLR_{SUVpeak}$, a cutoff of 1.0 was used, aligning with DS-3.[36] Missing values of sTARC-1 were replaced by sTARC levels after cycle 2 or 3 for eight respective patients. Missing values of sTARC-3 were replaced by sTARC levels after cycle 1 or 2 for six respective patients. Sensitivity analyses were performed with and without replacement for missing values. For other variables, no missing values were replaced. Clinical data were collected using OpenClinica version 3.6,[37] and the statistical analysis was performed in R software version 4.0.

RESULTS

Patients and treatment

Between May 2014 and July 2017, 67 patients with R/R cHL were enrolled in the Transplant BRaVE study (n=12 in Phase I, and n=55 in Phase II) [**Table 1**]. Two patients withdrew consent after one cycle of BV-DHAP due to psychological issues and were excluded from further analyses. Seven patients were reclassified as non-Hodgkin lymphoma (e.g., peripheral T-cell lymphoma) according to central pathology review and were excluded from biomarker analyses, but not from evaluation of clinical endpoints per intention to treat.[8]

Long-term follow-up results

The median follow-up time was 40 months (range 23-65) in patients still alive at time of the analysis. The 3-year PFS by intention-to-treat for all 65 patients was 77% (95% confidence interval; CI: 67-88%) and the overall survival (OS) was 95% (95% CI: 90-100%) [**Figure 1A**]. In total, three patients died (n=2 cHL, n=1 peripheral T-cell lymphoma), all without signs of progressive disease.[8] The 3-year FFP in patients with confirmed cHL diagnosis who were included in the biomarker analyses was 82% (95% CI: 73-93) [**Figure 1B**].

Table 1. Baseline demographics and disease characteristics

	cHL (N=58)	Other (N=7)	Total (N=65)
Age at relapse (years)			
Median (Min - Max)	29 (19 - 64)	30 (20 - 63)	29 (19 - 64)
Disease status			
Refractory	26 (45%)	4 (57%)	30 (46%)
Relapse < 1 year	16 (28%)	-	16 (25%)
Relapse ≥ year	16 (28%)	3 (43%)	19 (29%)
Stage at relapse			
I/II	27 (47%)	3 (43%)	30 (46%)
III/IV	31 (53%)	4 (57%)	35 (54%)
B-symptoms			
Yes	18 (31%)	5 (71%)	23 (35%)
Extranodal Disease			
Yes	25 (43%)	1 (14%)	26 (40%)
Splenic focal lesions			
Yes	8 (14%)	1 (14%)	9 (14%)
Morphological subtype			
NS	38 (66%)	-	38 (58%)
MC	13 (22%)	-	13 (20%)
NOS	7 (12%)	-	7 (11%)
AITL	-	1 (14%)	1 (2%)
IA-B-LPD	-	1 (14%)	1 (2%)
PTCL	-	5 (71%)	5 (7%)
EBV positive			
Yes	7 (13%)	7 (100%)	14 (23%)
Missing	*3*	-	*3*
TARC staining			
Positive	43 (86%)	4 (57%)	47 (83%)
Weak	4 (8%)	2 (29%)	6 (11%)
Negative	3 (6%)	1 (14%)	4 (7%)
Missing	*8*	-	*8*
Events			
Progression	11 (19%)	2 (29%)	13 (20%)
Death	2 (3%)	1 (14%)	3 (5%)

cHL, classical Hodgkin lymphoma; N, number of patients; NS, nodular sclerosis; MC, mixed cellularity; NOS, not otherwise specified; AITL, angioimmunoblastic T-cell lymphoma; IA-B-LPD, immunodeficiency-associated B-lymphoproliferative disorder; PTCL, peripheral T-cell lymphoma not otherwise specified; EBV, Epstein-Barr virus; TARC, thymus and activation regulated chemokine.

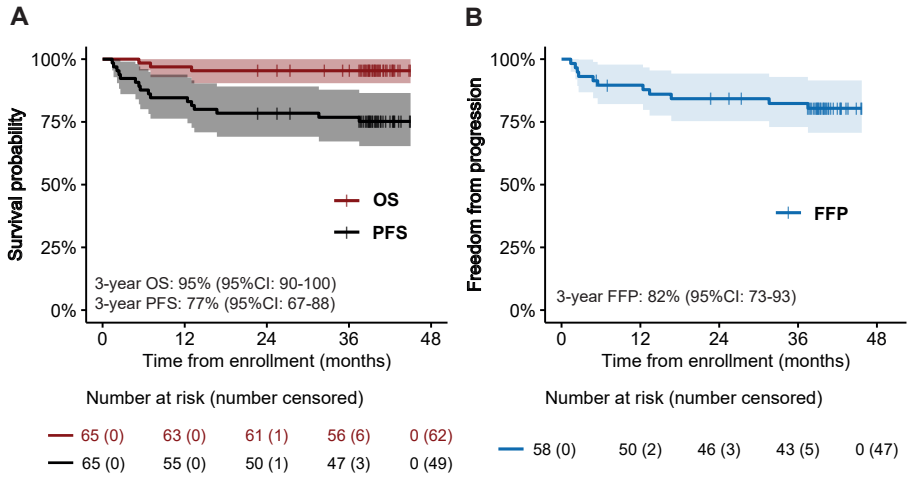

Figure 1. Progression free survival, overall survival, and freedom from progression in patients with R/R cHL. A Three-year progression free survival (PFS) and overall survival (OS) in all patients enrolled in the study. B Freedom from progression (FFP) in all patients with confirmed classical Hodgkin lymphoma diagnosis during central pathology who were included in the biomarker analyses. Patients who had a diagnosis other than cHL on central pathology review (n = 7) were excluded. Patients who died without progression (n = 2) were censored at time of death. OS overall survival, PFS progression free survival, FFP freedom from progression.

Serum TARC

The median sTARC-baseline level was 4885 pg/mL (range 282–120,654) and significantly decreased to 384 pg/ml (113–28,448) after cycle 1 (p<0.0001) [**Figure 2A**]. sTARC-baseline did not differ significantly between patients who relapsed and patients in remission after BV-DHAP (median 5204 vs 3600 pg/mL; p=0.9), and was not prognostic for FFP (AUC 0.49) [**Supplemental Table 2**]. The percentual drop in sTARC levels after cycle 1 (deltaTARC-1) was larger in patients with favorable outcomes but showed only moderate prognostic value (AUC 0.663) [**Figure 2B** and **Supplemental Table 2**].

sTARC after cycle 1 (sTARC-1) was significantly higher in patients who relapsed during or after BV-DHAP in comparison to patients in remission (median 889 vs 338 pg/mL; p=0.008) [**Figure 2C**]. This was also the case for sTARC after cycle 2 (sTARC-2) (p=0.017) and sTARC after cycle 3 (sTARC-3) (p=0.009). sTARC-1 had strong prognostic value for FFP (AUC 0.76) [**Supplemental Table 2**]. Sensitivity analysis showed no differences in prognostic value of sTARC-1 when patients with missing values were excluded [**Supplemental Table 2 and 3**].

A predefined cutoff of 1000 pg/mL was used based on levels in healthy controls and use in a clinical setting to have high specificity for newly diagnosed cHL patients.[22] However, compared to sTARC levels described in newly diagnosed cHL patients, sTARC-baseline levels were much lower in our R/R cHL cohort (median serum TARC 28,013 versus 4885 pg/mL, respectively),[22] and n=14 patients had sTARC-baseline levels <1000 pg/mL. Therefore, we decided to use a lower sTARC cutoff of 500 pg/mL for response evaluation in addition to the pre-specified cutoff of

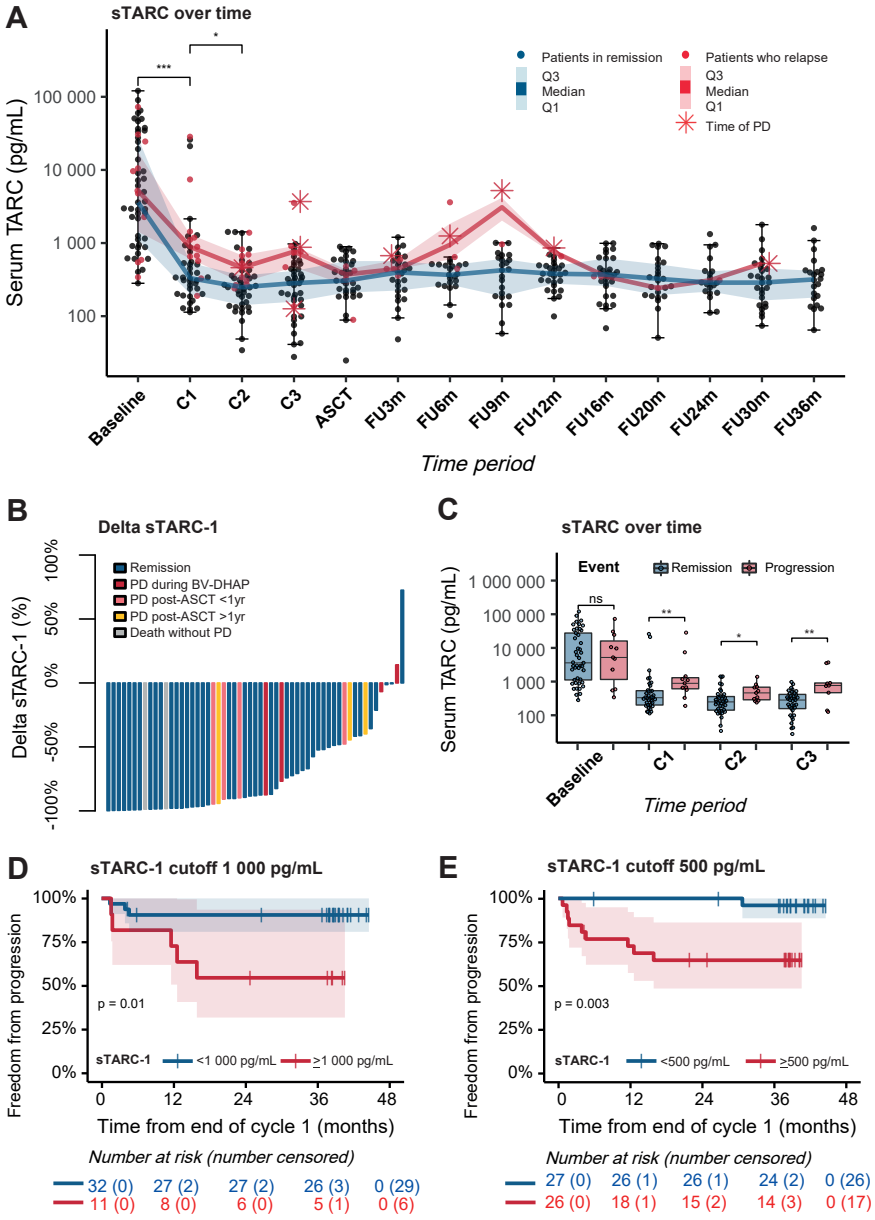

Figure 2. Serum TARC levels during BV-DHAP treatment in R/R cHL. A sTARC at baseline, during treatment and follow-up, stratified for patients in remission (blue) and patients who developed progressive disease during or after BV-DHAP treatment (red). Dots represent sTARC values at indicated time points. The red stars represent the specific time point of progressive disease of an individual patient. Lines represent median values, bands indicate interquartile ranges (Q1–Q3). **B** Delta sTARC (%) after cycle 1 stratified for patients with and without progression during or after treatment. **C** sTARC stratified for patients in remission vs. progression on BV-DHAP at baseline, and after each cycle of BV-DHAP. Freedom from progression (FFP) Kaplan–Meier analysis for sTARC levels after cycle 1 with a cutoff of 1000 (**D**) and 500 (**E**) pg/ml. *p < 0.05; **p < 0.01; ***p < 0.001; *ns not significant. s serum, TARC thymus and activation regulated chemokine, C cycle, ASCT autologous stem-cell transplantation, FU follow-up, m months, Q1 first interquartile, Q3 third interquartile, PD progressive disease, Tx treatment, yr year.*

1000 pg/mL. The pre-specified cutoff of 1000 pg/mL could significantly discriminate patients with a favorable FFP (3-year FFP 90% vs 55%; p=0.01) [**Figure 2D**]. Only four patients had sTARC-baseline levels <500 pg/mL. The cutoff of 500 pg/mL for sTARC-1 provided strong significant discrimination between patients with favorable and unfavorable FFP (3-year FFP 96% vs 64%; p=0.003) [**Figure 2E**]. Additionally, when excluding the four patients with an sTARC-baseline <500 pg/mL (n=4), the AUC of sTARC-1 increased from 0.76 to 0.81 [**Supplemental Table 2**].

sTARC-3 levels were higher in patients with a PR (DS 4-5) or progressive disease on the pre-ASCT PET scan, but this was not statistically significant. [**Supplemental figure 1A**]. For patients with progressive disease during follow-up, sTARC levels at time of progression were ≥500 pg/mL in 7/9 patients (78%) [**Supplemental figure 1B-C**]. Taking sTARC levels of all time points of patients with a CMR during follow-up (n=278 time points) compared to sTARC levels from patients at time of progression (n=9), sTARC showed a PPV of only 8% for detecting progressive disease and an NPV of 99% for excluding progressive disease using a cutoff of 500 pg/mL [**Supplemental figure 1C**].

25-hydroxyvitamin D and LDH

Baseline serum 25-hydroxyvitamin D levels indicated deficiency (<30 ng/mL) in four patients (7%) and insufficiency (30-50 ng/mL) in 16 patients (29%). Patients with primary refractory disease had lower vitamin D levels compared to relapsed patients (p=0.018). Vitamin D levels as a continuous variable had low prognostic value for FFP (AUC 0.57), and there were no significant difference in vitamin D levels between patients with or without progression (p=0.52) or patients with a CMR vs PMR (p=0.92) or progression (p=0.62) on the pre-ASCT PET scan [**Supplemental Figure 2; Supplemental Tables 2 and 3**].

LDH was elevated (≥1 ULN) at baseline in 13 patients, but these patients did not show a higher incidence of progression (p=0.5). LDH levels were not significantly higher in patients who progressed compared to patients without progression (p=0.13) and there were no differences in pre-ASCT LDH levels for patients with a CMR vs PMR (p=0.16) or progression (p=0.54) on the pre-ASCT PET scan. LDH significantly increased during BV-DHAP treatment, and after ASCT decreased to normal levels for most patients, probably coinciding with administration of granulocyte colony-stimulating factor [**Supplemental Figure 3**].

TARC immunohistochemistry

In total, 50 out of 58 confirmed cHL patients had a lymph node biopsy available for additional IHC staining. All patients stained positive for CD30 in HRS cells. Forty-three patients (86%) stained positive for TARC in the HRS cells [**Figure 3A**]. Patients with negative or weak TARC staining (n=7) showed significantly lower sTARC-baseline levels compared to patients with positive TARC staining in the HRS cells (median 608 vs 3701 pg/mL, respectively; p=0.04) [**Figure 3B**]. More importantly, these patients showed significant lower 3-year FFP compared to patients with positive TARC staining in the HRS cells (3-year FFP 89% vs 48%; p=0.0004) [**Figure 3C**].

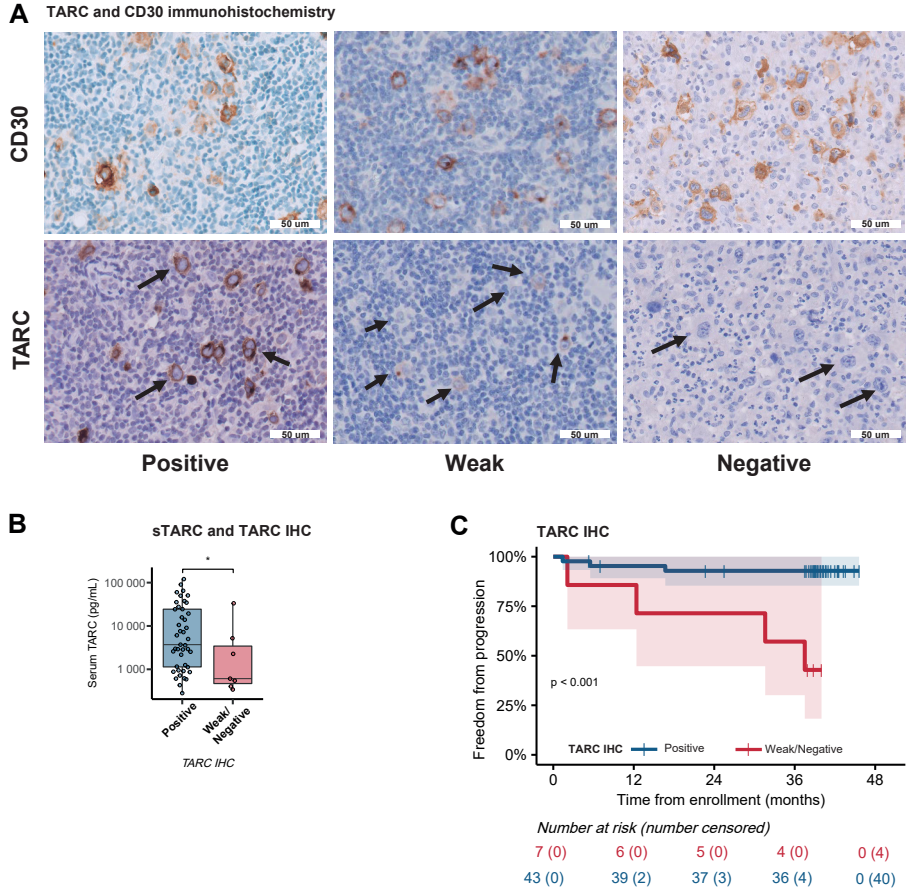

Figure 3. TARC immunohistochemistry of lymph node biopsies at baseline. A TARC and CD30 immunohistochemistry in the lymph node biopsy of a patient with positive TARC staining (left), weak TARC staining (middle), and a patient with negative TARC staining (right). Arrows indicate HRS cells with positive, weak or negative TARC staining. Images were captured at ×20 magnification. **B** Baseline sTARC levels for patients with weak or negative TARC staining in the HRS cells vs. patients with positive TARC staining. **C** Freedom from progression (FFP) Kaplan–Meier analysis for patients with negative/weak TARC staining compared to positive TARC staining in the HRS cells of baseline lymph node biopsies. *p < 0.05; **p < 0.01; ***p < 0.001; *ns not significant. TARC thymus and activation regulated chemokine.*

Quantitative PET scan analysis

Baseline $TLR_{SUVmean}$ and $TLR_{SUVpeak}$ were higher in patients who progressed during or after BV-DHAP compared to patients in remission (p<0.001 and p=0.04, respectively) [**Figure 4A, B**]. Similar differences were observed for $TLR_{SUVmean}$ and $TLR_{SUVpeak}$ after three cycles of BV-DHAP prior to ASCT (p<0.001 and p<0.001) and after ASCT (p=0.01 and p=0.03), respectively [**Figure 4A, B**]. Patients who progressed during or after treatment also showed a lower $deltaTLR_{SUVmean}$ and $deltaTLR_{SUVpeak}$ [**Figure 4C, D**]. Only one patient who relapsed after 3 years had a $deltaTLR_{SUVmean}$ of -100%. Prognostic value as estimated by AUC was low for MTV and TLG

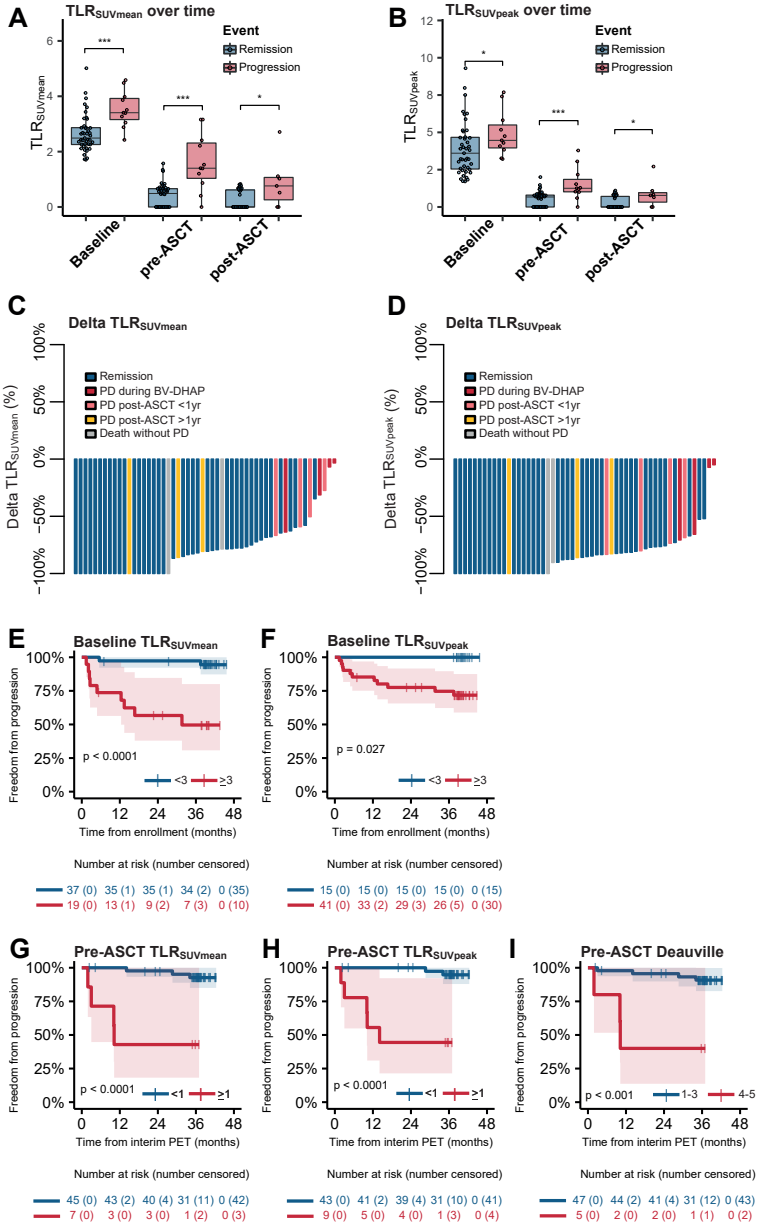

Figure 4. Quantitative baseline and pre-ASCT PET parameters. A TLRSUVmean and **B** TLRSUVpeak, on the PET-CT at baseline, pre-ASCT and post- ASCT, stratified for patients who are in remission after 3-years of follow-up compared to patients who had progressive disease during or after treatment. **C** DeltaTLRSUVmean and **D** deltaTLRSUVpeak pre-ASCT, ranked by deltaTLRSUV. **E** Kaplan–Meier FFP analysis for TLRSUVmean at baseline. **F** Kaplan–Meier FFP analysis for TLRSUVpeak at baseline. **G** Kaplan–Meier FFP analysis for pre-ASCT TLRSUVmean. **H** Kaplan–Meier FFP analysis for pre-ASCT TLRSUVpeak. **I** Kaplan-Meier FFP analysis for pre-ASCT Deauville scores with a cutoff of DS1-3 for CMR. All SUVs represent ratios of tumor-to-liver SUV, corrected for the liver SUVmean. *p < 0.05; **p < 0.01; ***p < 0.001; *ns not significant. TLR tumor-to-liver ratio, SUV standard uptake value, ASCT autologous stem-cell transplantation, Tx treatment, yr year, DS Deauville score, FFP freedom from progression.*

(0.47 and 0.54, respectively), and highest in baseline $TLR_{SUVmean}$ and $TLR_{SUVpeak}$ (AUC 0.85 and 0.70, respectively) [**Supplemental Table 2**]. The predefined cutoffs of $TLR_{SUVmean}$ and $TLR_{SUVpeak}$ of ≥3.0 at baseline could significantly discriminate patients in low and high-risk groups for FFP (p<0.0001 and p=0.027, respectively), with an NPV of 94% and a PPV of 50% for $TLR_{SUVmean}$, and an NPV of 100% and a PPV of 28% for $TLR_{SUVpeak}$ [**Figure 4E, F and Supplemental Table 3**]. Prognostic value of $TLR_{SUVmean}$ (AUC 0.73) and $TLR_{SUVpeak}$ (AUC 0.76) at the pre-ASCT PET-CT was higher compared to visual DS (AUC 0.69), but was comparable in terms of NPV/PPV when using a cutoff of TLR≥1 [**Figure 4G-I and Supplemental Table 2 and 3**]. Results for $MBP_{SUVmean}$ and $MBP_{SUVpeak}$ showed similar results compared to $TLR_{SUVmean}$ and $TLR_{SUVpeak}$ and are summarized in **Supplemental Table 2 and 3**.

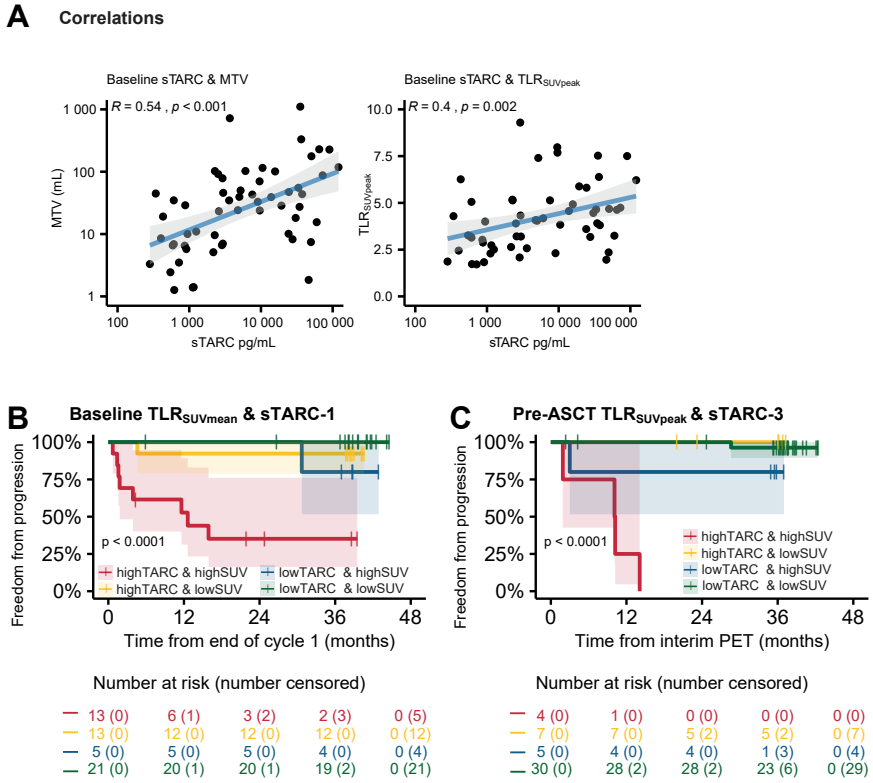

Figure 5. Correlations and combinations of several biomarkers. A Spearman's rank correlations of sTARC and MTV and TLRSUVpeak at baseline. **B** Kaplan–Meier analysis for FFP stratified for patients with high (≥3.0) vs. low (<3.0) TLRSUVmean at baseline and high (≥500 pg/ml) vs. low (<500 pg/ml) sTARC-1. **C** Kaplan–Meier analysis for post-ASCT FFP stratified for patients with high (≥500 pg/ml) vs. low (<500 pg/ml) pre-ASCT sTARC and high (≥1.0) vs. low (<1.0) pre-ASCT TLRSUVpeak. *p < 0.05; **p < 0.01; ***p < 0.001; *ns not significant. MTV metabolic tumor volume, sTARC serum thymus and activation regulated chemokine, TLR tumor liver ratio, SUV standard uptake value, sTARC-1 sTARC after cycle 1, sTARC-3 sTARC after cycle 3, ASCT autologous stem-cell transplantation.*

Correlations and combinations of biomarkers

There was a significant moderate to high correlation between sTARC-baseline and several PET parameters, such as MTV (r=0.54) and $TLR_{SUVpeak}$ (r=0.4) [**Figure 5A**]. Serum vitamin D did not show any correlation with PET parameters. LDH showed moderate correlation with $TLR_{SUVpeak}$ (r=0.36). Hemoglobin showed a negative correlation with MTV (r=-0.26) and number of lesions (r=-0.31) [**Supplemental figure 4**]. Patients with B symptoms had significantly higher baseline MTV (p=0.014), $TLR_{SUVpeak}$ (p=0.006) and LDH (p=0.013), but there were no differences in sTARC-baseline levels (p=0.95) [**Supplemental figure 5**].

An explorative multivariable analysis showed an increased AUC for the combinations of sTARC-1 and baseline $TLR_{SUVmean}$ (AUC 0.85) or $TLR_{SUVpeak}$ (AUC 0.77), with both variables showing an independent prognostic value (p<0.05) [**Supplemental Figure 6**]. Patients who had both a high baseline $TLR_{SUVmean}$ (≥3.0), and high sTARC-1 (≥500pg/mL) (n=13) showed significantly lower 3-year FFP compared to patients who had either low $TLR_{SUVmean}$ or low sTARC-1 (35% vs 95%; p<0.0001), with an NPV of 95% and a PPV of 67% [**Figure 5B, Supplemental Table 4**]. Similarly, patients who showed both a high pre-ASCT $TLR_{SUVpeak}$ (≥1.0) and high sTARC-3 (≥500pg/mL) (n=4) showed the highest risk of progression with a 3-year FFP of 0% vs 95% for other patients (p<0.0001), with an NPV of 95% and a PPV of 100% [**Figure 5C, Supplemental Table 4**].

DISCUSSION

This long-term follow-up analysis of the Transplant BRaVE study, investigating the addition of BV to DHAP followed by ASCT in patients with R/R cHL, showed a high 3-year PFS and OS. The 3-year PFS of 77% is higher compared to historical controls in patients treated with DHAP only, or other chemotherapy-based salvage regimens, in which the 2-5 year PFS is ~50-60%.[15,38-40] Because the vast majority of progressions occur within 2-3 years of follow-up, the PFS rate after 3 years is a good surrogate for cure.[3] OS appears to be higher than previously reported, but this may be partially explained by the use of other novel therapies in patients who failed BV-DHAP/ASCT (e.g., checkpoint inhibition).[41] We show that sTARC-1 is a strong prognostic biomarker in R/R cHL. Additionally, we identified several baseline and response-assessment biomarkers with prognostic value for 3-year FFP, including TARC IHC of HRS cells in tissue, and baseline and pre-ASCT $TLR_{SUVmean}$ and $TLR_{SUVpeak}$.

Strong points of this study are the prospective design regarding sample and data collection, the use of predefined cutoffs for sTARC based on results in healthy controls, and cutoffs for the SUV ratio to the liver SUVmean based on response-assessment by DS.[22,36] The latter also justifies the comparison of quantitative PET parameters over time and between patients in different hospitals.[22,35,36] Limitations of this study are the small sample size and low number of events, which precluded cross-validation of results. Therefore, the possibly more optimal cutoff

for sTARC-1 of 500 pg/mL instead of 1000 pg/mL, TARC immunohistochemistry in tissue, and prognostic PET parameters need validation in other R/R cHL cohorts.

The high prognostic value of baseline and pre-ASCT $TLR_{SUVmean}$ and $TLR_{SUVpeak}$ warrants further exploration of using quantitative PET parameters for response-assessment and baseline risk stratification in R/R cHL. This can easily be implemented in clinical practice since the PET scan is performed at baseline and pre-ASCT as standard of care. Regarding to baseline PET measurements, $TLR_{SUVmean}$ showed higher prognostic value compared to $TLR_{SUVpeak}$, while $TLR_{SUVmean}$ and $TLR_{SUVpeak}$ had comparable prognostic value in the pre-ASCT setting. Considering low metabolic residual disease on the pre-ASCT PET in most patients, SUVpeak of the most FDG-avid lesion is easier to measure compared to patient-level SUVmean which requires segmentation of the total MTV.

In newly diagnosed cHL patients, sTARC is a strong, early response marker.[22] We showed that in R/R patients, sTARC can be used as a response marker already after one cycle of BV-DHAP. Moreover, the combination of sTARC-1 and $TLR_{SUVmean}$ or $TLR_{SUVpeak}$ provides complementary prognostic information, and identified the majority of patients who progressed. Therefore, patients having both a high baseline (≥3.0) $TLR_{SUVmean}$ and high sTARC-1 (≥500pg/mL), or a high pre-ASCT $TLR_{SUVpeak}$ (≥1.0) and high sTARC-3 (≥500), could be regarded as high-risk for progressive disease. These patients potentially would benefit from additional treatment, for example with post-ASCT consolidation or maintenance treatment with BV or checkpoint inhibitors, which should be studied in prospective clinical trials.[10,42,43]

Despite the strong prognostic value of sTARC, it still shows a low PPV for detecting progressive disease during follow-up.[22,23] Therefore, sTARC may be less suitable as follow-up marker in the R/R setting. It should be noted, however, that in a small study in patients who relapsed after allogeneic transplant, all seven patients showed sTARC levels ≥1000 pg/mL at time of progression.[44] In our cohort, sTARC-baseline levels (median 4885 pg/mL) were increased compared to healthy controls (median 118 pg/mL), but less pronounced as compared to earlier published data of newly diagnosed cHL patients (median 28,013 pg/mL).[22] This may in part be explained by the generally lower tumor load as per MTV in the R/R setting, which correlates with lower sTARC-baseline levels.[23] Therefore we used a lower cutoff of 500 pg/mL in addition to the pre-specified cutoff of 1000 pg/mL. However, this cutoff should be validated in other prospective studies in R/R cHL patients.

Despite the prognostic value in newly diagnosed patients, vitamin D levels did not show prognostic value in our cohort.[26,27] We found that patients with primary refractory disease had lower vitamin D levels compared to relapsed patients, which could be explained by either a shorter time to first-line treatment and hospital admission and thus lack of sun exposure in primary refractory patients, or by an increased chance of being primary refractory after first-line treatment when patients already have low vitamin D levels. This can however not be concluded from our data and should be investigated in other prospective studies.

Analysis of cell-free DNA (cfDNA) is emerging as a measure for minimal residual disease. It was recently shown that individual mutational fingerprints correlate with response in newly diagnosed cHL patients.[45] However, cfDNA is an expensive technique and requires complex analysis as compared to measuring sTARC. Combination of sTARC and cfDNA might provide additional prognostic information and studies combining these biomarkers are needed.

This is the first study to show prognostic value of TARC expression in HRS cells as measured by IHC in tissue biopsy samples. The mechanism behind this association is not clear and may be related to characteristics of the HRS cells, or the influence of TARC on the composition of the tumor micro-environment.[46]

With the advent of highly effective novel therapies such as BV and checkpoint inhibition, one of the next goals for clinical trials is to investigate whether some R/R cHL patients can possibly be cured without HDCT/ASCT. Risk-stratified and PET-adapted prospective studies could help to identify patients who have low-risk of relapse and can be cured with salvage therapy alone, and on the other hand, identify patients who are chemotherapy-refractory early, so they can receive alternative therapies such as checkpoint inhibition.

In conclusion, we have shown a high 3-year PFS and OS with three cycles of BV-DHAP followed by ASCT in R/R cHL. sTARC can be used as an early response marker already after one cycle of BV-DHAP, and combination with $TLR_{SUVmean}$ and $TLR_{SUVpeak}$ at baseline and pre-ASCT provides strong prognostic information which can help to identify patients with high risk of progression early in the treatment course.

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CONFLICT OF INTEREST DISCLOSURES

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SUPPLEMENTARY MATERIAL

Supplemental Table 1. Number of patients per medical center.

Medical center	N patients
Amsterdam UMC, location Academic Medical Center (AMC), Amsterdam, The Netherlands	**17**
Amsterdam UMC, location Vrije Universiteit medical center (Vumc), Amsterdam, The Netherlands	**11**
Erasmus MC Cancer Institute, University Medical Center, Rotterdam, The Netherlands	**8**
University Medical Center Groningen, Groningen, The Netherlands	**7**
Rigshospitalet, Copenhagen, Denmark	**5**
Centre Hospitalier Universitaire, Lille, France	**8**
Hopital Saint Louis, Paris, France	**7**
Centre Hospitalier Universitaire, Nantes, France	**2**

Supplemental Table 2. Prognostic value of several serum-, PET- and immunohistochemistry-based biomarkers – ROC analysis and univariable Cox regression on continuous values.

Parameter	N	AUC for 3-year FFP	HR univariable (continuous)	HR 95% CI	P-value
Baseline parameters (at relapse)					
sTARC-baseline	56	0.487	0.99	0.71 - 1.4	0.97
Vitamin D	53	0.565	1.63	0.53 - 5.06	0.39
LDH	54	0.650	4.55	0.75 - 27.67	0.11
TARC IHC staining	48	0.753		-	-
MTV	55	0.469	1.04	0.7 - 1.55	0.84
TLG	55	0.543	1.06	0.73 - 1.54	0.75
$TLR_{SUVpeak}$	54	0.704	4.27	0.99 - 18.42	0.046
$MBP_{SUVpeak}$	54	0.660	3.46	0.8 - 15.02	0.088
$TLR_{SUVmean}$	54	0.846	48.21	5.03 - 462.24	<0.001
$MBP_{SUVmean}$	54	0.827	52.59	5.1 - 542.06	0.001
Parameters after 1 cycle of BV-DHAP					
deltaTARC-1	55	0.663	1.01	0.99 - 1.02	0.30
sTARC-1, no replacement*	47	0.768	1.46	1.05 - 2.03	0.043
sTARC-1	55	0.756	1.51	1.07 - 2.12	0.034
sTARC-1, excl baseline<500	51	0.805	1.55	1.09 - 2.2	0.027
sTARC-1, excl baseline<750	45	0.855	1.63	1.12 - 2.39	0.020
sTARC-1, excl baseline<1000	41	0.837	1.58	1.08 - 2.33	0.030
sTARC-1, FFP post-ASCT	49	0.704	1.39	0.88 - 2.19	0.20
Parameters after 3 cycles of BV-DHAP (pre-ASCT)					
sTARC-3, no replacement	41	0.814	3.54	1.5 - 8.39	0.005
sTARC-3	47	0.764	3.13	1.38 - 7.07	0.007
Deauville score (DS1-3 vs 4-5)	50	0.691	-	-	-
$TLR_{SUVpeak}$	50	0.764	6.4	0.47 - 87.81	0.19
$MBP_{SUVpeak}$	50	0.671	11.42	1.16 - 112.69	0.049
$TLR_{SUVmean}$	50	0.731	14.99	1.73 - 129.65	0.015
$MBP_{SUVmean}$	50	0.748	11.12	2.59 - 47.82	0.005

For each variable, the AUC was calculated for 3-year FFP. For baseline variables, FFP was measured from enrollment, for parameters after 1 cycle of BV-DHAP, FFP was measured from start of cycle 2, for pre-ASCT variables, the FFP was measured from ASCT. Hazard ratio's represent cox regression on continuous variables. DeltaTARC was calculated as the percentual drop of sTARC levels after 1 cycle of BV-DHAP compared to baseline levels.

*patients with missing sTARC-1 values were excluded. **for patients with missing sTARC-1 values, the missing values were replaced by the serum TARC levels after 2 or 3 cycles.

Abbreviations: N, number; AUC, area under the curve; FFP, freedom from progression; HR, hazard ratio; CI, confidence interval; DS, deauville score; MBP, mediastinal blood pool.

Supplemental Table 3. Prognostic value of several serum-, PET- and immunohistochemistry-based biomarkers – Univariable Cox regression based on cutoffs or subgroups.

Variable	N	Cutoff	N (events) group 1 *vs* group 2			HR	95%CI	P-val	Sens	Spec	NPV	PPV
Baseline parameters (at relapse)												
Vitamin D	53	50ng/mL	32 (6)	*vs*	21 (5)	1.31	0.4 - 4.29	0.66	-	-	-	-
TARC IHC staining	48	Pos *vs* weak/neg	41 (3)	*vs*	7 (4)	9.19	2.05 - 41.24	0.005	57%	93%	93%	57%
Number of lesions	52	12	37 (5)	*vs*	15 (5)	2.82	0.81 - 9.77	0.11	-	-	-	-
$TLR_{SUVpeak}$	54	3	15 (0)	*vs*	39 (11)	$3x10^8$	0 - Inf	0.004	100%	35%	100%	28%
$MBP_{SUVpeak}$	54	4.2	18 (1)	*vs*	36 (10)	5.83	0.75 - 45.56	0.03	91%	40%	94%	28%
$TLR_{SUVmean}$	54	3	36 (2)	*vs*	18 (9)	12.90	2.76 - 60.24	<0.001	82%	79%	94%	50%
$MBP_{SUVmean}$	54	4.2	37 (2)	*vs*	17 (9)	13.18	2.84 - 61.23	<0.001	82%	81%	95%	53%
Parameters after 1 cycle of BV-DHAP												
sTARC-1*, no replacement	43	500	22 (1)	*vs*	21 (9)	12.57	1.59 - 99.61	0.001	90%	64%	96%	43%
sTARC-1**	51	500	26 (1)	*vs*	25 (9)	11.76	1.49 - 93.05	0.002	90%	61%	96%	36%
sTARC-1	45	750	30 (2)	*vs*	15 (6)	6.85	1.38 - 34.01	0.010	75%	76%	93%	40%
sTARC-1	41	1000	30 (3)	*vs*	11 (5)	5.15	1.23 - 21.58	0.023	63%	82%	90%	46%
sTARC-1, FFP post-ASCT	46	500	26 (1)	*vs*	20 (5)	7.34	0.86 - 62.99	0.031	83%	63%	96%	25%
Parameters after 3 cycles of BV-DHAP (pre-ASCT)												
sTARC-3*, no replacement	39	500	29 (2)	*vs*	10 (4)	7.57	1.36 - 42.02	0.017	67%	82%	93%	40%
sTARC-3	44	500	33 (2)	*vs*	11 (4)	7.33	1.33 - 40.53	0.018	67%	82%	94%	36%
sTARC-3	39	750	33 (2)	*vs*	6 (3)	9.38	1.56 - 56.3	0.017	60%	91%	94%	50%
sTARC-3	36	1000	34 (4)	*vs*	2 (1)	4.39	0.49 - 39.63	0.26	-	-	-	-
Deauville score	50	1-3 *vs* 4-5	45 (4)	*vs*	5 (3)	10.60	2.33 - 48.29	0.007	43%	95%	91%	60%
$TLR_{SUVpeak}$	50	1	41 (2)	*vs*	9 (5)	16.84	3.23 - 87.80	<0.001	71%	91%	95%	56%
$MBP_{SUVpeak}$	50	1.4	38 (3)	vs	12 (4)	5.25	1.17 - 23.54	0.03	57%	81%	92%	33%
$TLR_{SUVmean}$	50	1	43 (3)	*vs*	7 (4)	13.03	2.87 - 59.17	0.002	57%	93%	93%	57%
$MBP_{SUVmean}$	50	1.4	42 (3)	vs	8 (4)	10.41	2.31 - 46.97	0.004	57%	91%	93%	50%

Pre-specified cutoffs were used to calculate univariable hazard ratio's (HR), sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV).

*patients with missing sTARC-1 values were excluded. **for patients with missing sTARC-1 values, the missing values were replaced by the serum TARC levels after 2 or 3 cycles.

Abbreviations: N, number; AUC, area under the curve; FFP, freedom from progression; HR, hazard ratio; CI, confidence interval; DS, deauville score; MBP, mediastinal blood pool.

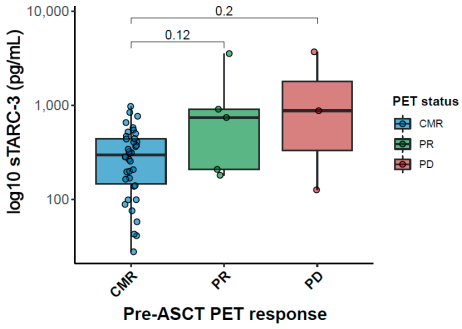
Supplemental Table 4. Combination of serum TARC and TLRSUV as prognostic markers

Variables (cutoff)	N (events) low-risk vs high-risk	3-year FFP (95% CI)	p log-rank	HR (95%CI); p-value	Sensisitivy	Specificity	PPV	NPV
Baseline $TLR_{SUVmean}$ (3) & sTARC-1 (500)	38 (2) vs 12 (8)	95% (88-100) vs 35% (16-76)	p<0.001	22.3 (4.5-109); p<0.001	80%	90%	67%	95%
Baseline $TLR_{SUVpeak}$ (3) & sTARC-1 (500)	31 (1) vs 19 (9)	97% (90-100) vs 54% (35-81)	p=0.001	20.3 (2.6-162); p<0.001	90%	75%	47%	97%
Pre-ASCT $TLR_{SUVmean}$ (1) & sTARC-3 (500)	41 (3) vs 3 (3)	93% (85-100) vs 0% (NA-NA)	<0.001	68.7 (6.9-682); p<0.001	50%	100%	100%	93%
Pre-ASCT $TLR_{SUVpeak}$ (1) & sTARC-3 (500)	40 (2) vs 4 (4)	95% (88-100) vs 0% (NA-NA)	<0.001	72.4 (7.7-678); p<0.001	67%	100%	100%	95%

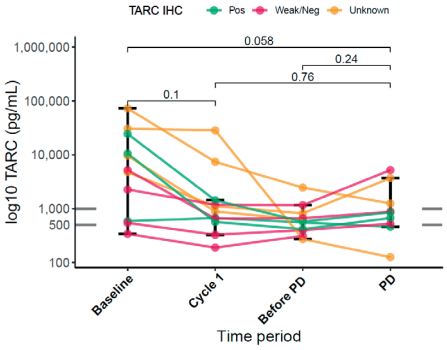
Patients with both a high $TLR_{SUVmean}$/$TLR_{SUVpeak}$ and a high serum TARC are considered 'high-risk'. Patients with either low $TLR_{SUVmean}$/$TLR_{SUVpeak}$ or low serum TARC are considered 'low-risk'. For baseline analysis, the cutoff for TLR_{SUV} is 3 and serum TARC after 1 cycle (sTARC-1) was used with a cutoff of 500 pg/mL. For pre-ASCT analysis, the cutoff for TLR_{SUV} is 1 and serum TARC after 3 cycles (sTARC-3) was used with a cutoff of 500 pg/mL. Patients who died without progression were censored in time-to-event analysis, and excluded in the calculation of sensitivity/specificity/PPV/NPV.

Abbreviations: N, number; FFP, freedom from progression; CI, confidence interval; HR, hazard ratio; PPV, positive predictive value; NPV, negative predictive value; TLR, tumor-liver-ratio; SUV, standard uptake value; sTARC-1, serum TARC levels after cycle 1; ASCT, autologous stem-cell transplant; sTARC-3, serum TARC levels after cycle 3; NA, not applicable.

A) Serum TARC and pre-ASCT PET response



B) Serum TARC in patients with progression

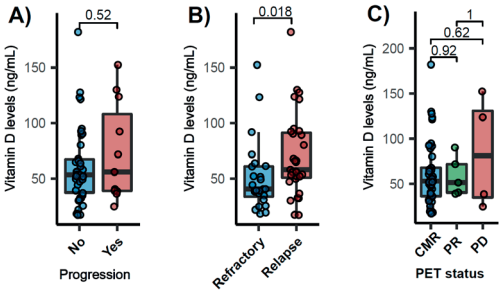


**No significant differences for patients with positive versus weak/negative TARC IHC expression in the biopsy (p=0.4)*

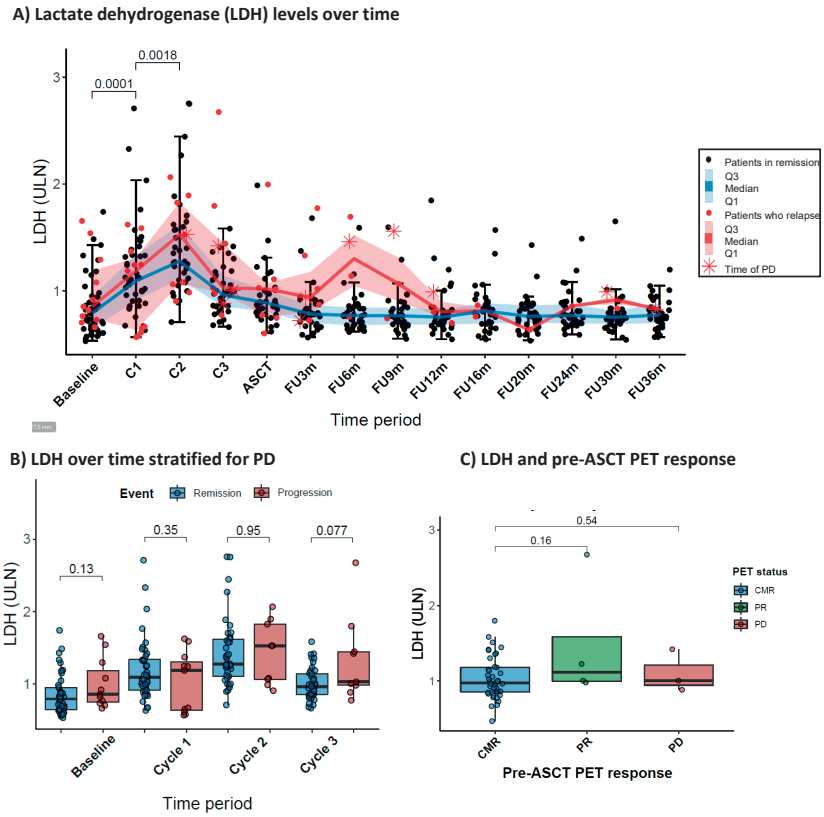
C) Serum TARC at time of progression

Cutoff	Time-points		Sensitivity	Specificity	PPV	NPV
	CMR	PD				
<500	201 (72%)	2 (22%)	78%	72%	8%	99%
>500	77 (28%)	7 (78%)				

Supplemental Figure 1. serum TARC analyses complementary to Figure 1. **A)** Serum TARC stratified for PET response after 3 cycles. **B)** Serum TARC at baseline, after 1 cycle, the timepoint before progression during or after BV-DHAP was confirmed, and the timepoint of progressive disease in patients who progress during or after BV-DHAP. Colors indicate TARC expression in the baseline biopsy. **C)** Sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) of serum TARC during follow-up for detecting progressive disease. sTARC levels from all time points of patients with a CMR pre-ASCT and/or post-ASCT (n=278 time points) were compared to the sTARC levels at time of progression in n=9 patients. A cutoff of 500 pg/mL was used to define the test as positive or negative. Patients with baseline serum TARC lower than 500 pg/mL (n=4) were excluded. *Abbreviations: TARC, thymus and activation chemokine; CMR, complete metabolic response; PR, partial response; PD, progressive disease; C1, cycle 1; s, serum.*

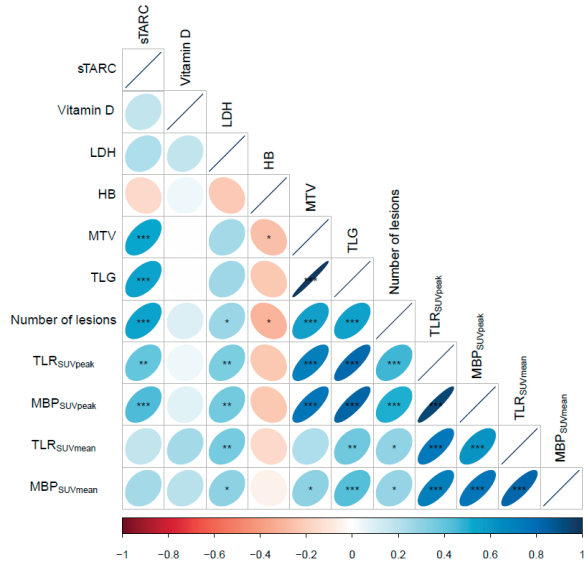


Supplemental Figure 2. Baseline serum vitamin D levels stratified for **A)** patients who develop progressive disease during or after BV-DHAP treatment; **B)** patients with primary refractory or relapsed disease after first-line treatment; **C)** patients with a complete metabolic response (CMR), partial response (PR) or progressive disease (PD) on the pre-ASCT PET-scan.



Supplemental Figure 3. A) LDH at baseline, during treatment and follow-up for patients who do not respond to or relapse after BV-DHAP (red) and patients who respond and are still in remission. Lines represent median values, bands indicate interquartile range (Q1-Q3). **B)** LDH stratified for patients in remission versus progression on BV-DHAP at baseline, and after each cycle of BV-DHAP. **C)** LDHstratified for PET response after 3 cycles. *Abbreviations: LDH, lactate dehydrogenase; ULN, upper limit of normal; CMR, complete metabolic response; PR, partial response; PD, progressive disease; C1, cycle 1; s, serum; C, cycle; ASCT, autologous stem-cell transplant; FU follow-up; m, months.*

A)

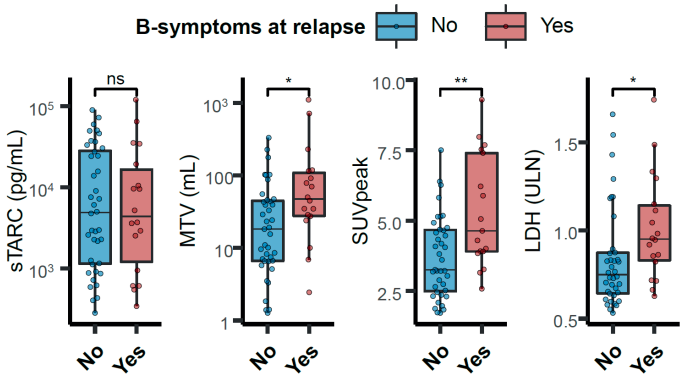


B)

	sTARC	VitD	LDH	HB	MTV	TLG	N lesions	$TLR_{SUVpeak}$	$TLR_{SUVpeak}$	$TLR_{SUVmean}$	$TLR_{SUVmean}$	P-value
sTARC		0.228	0.094	0.248	<0.001	<0.001	<0.001	0.002	<0.001	0.192	0.063	
VitD	0.17		0.215	0.733	0.986	0.988	0.532	0.741	0.605	0.069	0.162	
LDH	0.23	0.18		0.100	0.058	0.055	0.046	0.010	0.007	0.008	0.027	
HB	-0.15	0.05	-0.22		0.048	0.080	0.025	0.083	0.082	0.253	0.665	
MTV	0.54	0.00	0.26	-0.26		<0.001	<0.001	<0.001	<0.001	0.089	0.024	
TLG	0.54	0.00	0.26	-0.24	0.98		<0.001	<0.001	<0.001	0.004	<0.001	
N lesions	0.54	0.09	0.28	-0.31	0.57	0.55		<0.001	<0.001	0.034	0.041	
$TLR_{SUVpeak}$	0.40	0.05	0.35	-0.23	0.72	0.82	0.47		<0.001	<0.001	<0.001	
$MBP_{SUVpeak}$	0.45	0.07	0.36	-0.23	0.77	0.83	0.50	0.94		<0.001	<0.001	
$TLR_{SUVmean}$	0.18	0.26	0.36	-0.16	0.23	0.38	0.29	0.74	0.62		<0.001	
$MBP_{SUVmean}$	0.25	0.20	0.30	-0.06	0.30	0.43	0.28	0.72	0.75	0.83		
	Spearman rank correlation coefficients (*R*)											

Supplemental Figure 4. A) Correlation matrix of several serum and PET-parameters. Blue color and angle to right indicates positive correlation, red color and angle to left indicates negative correlation. *P<0.05; **P<0.01; ***P<0.001; **B)** Correlation matrix with correlation coëfficients and p-values.

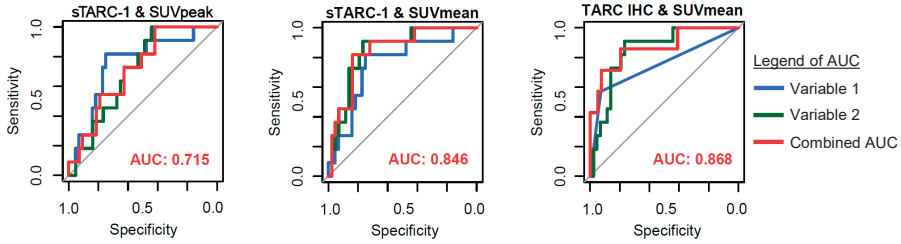
Abbreviations: s, serum; TARC, thymus and activation chemokine; LDH, lactate dehydrogenase; HB, hemoglobin; MTV, metabolic tumor volume; TLG, total lesion glycolysis; N, number; TLR, tumor-to-liver-ratio; SUV, standard uptake value; MBP, mediastinal blood pool.



Supplemental Figure 5. Boxplots of sTARC-baseline, MTV, $TLR_{SUVpeak}$, and LDH stratified for patients with and without B-symptoms at baseline.

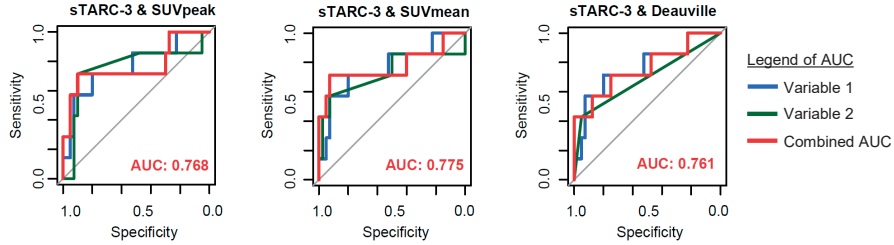
Abbreviations: sTARC, serum thymus and activation regulated chemokine; MTV, metabolic tumor volume; TLR, tumor liver ratio; SUV, standard uptake value; LDH, lactate dehydrogenase; ULN, upper limit of normal.

A)



N patients	Variable 1	AUC	P-val	Variable 2	AUC	P-val	AUC of combination
54	sTARC-1	0.756	0.035	$TLR_{SUVpeak}$	0.704	0.047	0.765
54	sTARC-1	0.756	0.052	$TLR_{SUVmean}$	0.846	0.005	0.852
46	TARC IHC	0.749	0.020	$TLR_{SUVmean}$	0.846	0.038	0.872

B)



N patients	Variable 1	AUC	P-val	Variable 2	AUC	P-val	AUC of combination
47	sTARC-3	0.764	0.037	$TLR_{SUVpeak}$	0.764	0.310	0.768
47	sTARC-3	0.764	0.069	$TLR_{SUVmean}$	0.731	0.165	0.775
47	sTARC-3	0.764	0.095	Deauville	0.691	0.077	0.761

Supplemental Figure 6. Multivariable analysis of **A)** baseline variables and serum TARC after cycle 1 (sTARC-1) for prediction of time to progression (TTP) measured from time after the first cycle. **B)** pre-ASCT variables and serum TARC after cycle 3 (sTARC-3) for prediction of TTP measured from time after the third cycle. For each variable, first the AUC of the respective variable is calculated. Then, a multivariable logistic regression is done in which patients with missing values in one of the two variables are excluded. A Wald-test was performed to assess indepencence of variables from each other. If the p-value is <0.05, the variable is independently prognostic of the other variable. Lastly, the AUC of the combined model was calculated. N patients: number of patients in the combined model. *Abbreviations: N, number; sTARC-1, serum Thymus and Activation Regulated Chemokine levels after cycle 1; IHC, immunohistochemistry; SUV, standard uptake value; AUC, area under the curve; sTARC-3, serum TARC levels after cycle 3, before ASCT.*

9

Tumor Microenvironment Composition Correlates with Quantitative ^{18}F-FDG PET-CT Features in classical Hodgkin Lymphoma.

Manuscript in preparation

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ABSTRACT

BACKGROUND: Classical Hodgkin lymphoma (cHL) is characterized by a limited number of Hodgkin Reed-Sternberg (HRS) cells in a complex tumor microenvironment (TME). HRS cells secrete Thymus and Activation Regulated Chemokine (TARC) in high quantities, which attracts CD4+ T cells to the TME. This study aims to explore correlations between gene-expression-based cell composition in tissue, quantitative PET features and serum TARC levels in cHL patients.

METHODS: Lymph node biopsies, PET scans and serum samples from 59 cHL cases from a clinical trial (NCT02280993) were analyzed, including 33 cases at primary diagnosis and 26 at time of relapse or primary refractory disease (R/R). The NanoString platform was used to measure expression of 141 genes linked to 26 TME-signatures in lymph node biopsies and immunohistochemistry was applied to determine TARC expression. For 34 cases, PET scans were analyzed at the patient-level with additional single-lesion analysis of the biopsied lymph nodes. Serum TARC was analyzed in samples of R/R cases.

RESULTS: Significant positive correlations were observed between metabolic tumor volume (MTV), standard uptake value (SUV)mean and SUVpeak, and TME-signatures of HRS cells, T-synapse, Tregs and NK cells. In addition, the T-synapse signature, representing genes involved in costimulation and immune checkpoints, and the Treg signature, showed significant correlations with SUVmean/peak in the biopsied single-lesion. TARC immunohistochemical staining and serum TARC correlated significantly with the HRS signature.

CONCLUSIONS: Our findings suggest that TME-signatures of HRS cells, T-synapse, Tregs and NK cells correlate with a higher glucose metabolism as determined by PET in cHL. These insights may aid in understanding the biological relevance of quantitative PET scan features in clinical practice.

INTRODUCTION

Classical Hodgkin lymphoma (cHL) is a heterogeneous disease characterized by the presence of Hodgkin Reed-Sternberg (HRS) cells, that represent approximately 1-5% of the total tumor mass. HRS cells are surrounded by an abundance of immune cells that form a complex tumor microenvironment (TME).[1] HRS cells arise from germinal center B cells, but show loss of typical B-lineage markers such as CD20, CD79a and the B-cell receptor, with aberrant expression of CD30, MUM1 (*IRF4*), CD15, weak expression of PAX5, and lack of CD45.[1-3] HRS cells secrete various cytokines and chemokines that recruit reactive immune cells to the TME.[4] The TME is primarily composed of CD4+ T helper (Th) cells, alongside regulatory T cells (Tregs), CD8+ cytotoxic T cells, eosinophils, macrophages, plasma cells, neutrophils, natural killer (NK) cells, B cells and mast cells.[5,6] The crosstalk between HRS cells and these immune cells is thought to play an important role in HRS cell survival and immune evasion.[3,5]

One of the most abundant chemokines in cHL is CC chemokine 17 (CCL17), also known as Thymus and Activation Regulated Chemokine (TARC), which is secreted by HRS cells at highly elevated levels compared to physiological secretion by normal dendritic and thymic epithelial cells.[7] TARC binds to CCR4 on CD4+ T cells and thereby attracts these cells to the TME.[6] In a previous analysis, we found that 86% of relapsed or primary refractory (R/R) cHL patients showed positive TARC staining in the lymph node biopsy and that 93% had elevated serum TARC levels before start of salvage therapy.[8] We showed that serum TARC correlates with metabolic tumor volume (MTV) and standard uptake value (SUV)peak measured by ^{18}F-fluorodeoxyglucose (FDG)-positron emission tomography (PET) scan and that serum TARC levels after just one cycle of salvage chemotherapy exhibit strong prognostic value for progression free survival.[8] Another study has shown that serum TARC is elevated in the majority of cHL patients already up to 6 years before diagnosis.[9]

cHL is a metabolically active disease, represented by high uptake of glucose in affected lymph nodes and in extranodal manifestations of the disease, as measured by ^{18}F-FDG PET.[10] PET-computed tomography (CT) scans are used in standard clinical practice in cHL for staging, response-assessment and monitoring of disease recurrence.[11] Quantitative PET scan features, such as MTV, intensity-based features (SUV) and dissemination features (e.g. Dmax, the largest distance between two lesions within a patient), are a novel class of prognostic markers in cHL.[8,12] We previously showed that R/R cHL patients with a high SUVpeak (i.e. the 1 mL with the highest FDG uptake) or SUVmean before start of salvage treatment have worse progression free survival compared to patients with a lower SUVpeak/SUVmean.[8,12] However, the low percentage of HRS cells in the TME, combined with the relatively high percentage of false-positive interim PET scans (as indicated by a low positive predictive value) suggests that other factors than the mere presence of HRS cells contribute to the high FDG uptake in cHL.[5,8,12]

It is currently unknown which cells within the TME are driving high glucose metabolism or whether glucose uptake correlates with the number of HRS cells. A clear link between HRS/

TME composition and glucose metabolism measured by PET is thusfar lacking. This study aims to explore correlations between gene-expression-based TME cell composition in cHL lymph node biopsies and quantitative PET features.

MATERIALS AND METHODS

Study population

We included samples from patients with histologically confirmed R/R cHL who were enrolled in the Transplant BRaVE study.[8,13] Patients were treated with 3 cycles of brentuximab vedotin (BV) combined with dexamethasone, high-dose cytarabine and cisplatin (DHAP) followed by high-dose chemotherapy and autologous stem-cell transplant (ASCT). Material from lymph node biopsies and PET-CT scans at both first diagnosis, and at relapse or detection of primary refractory disease were collected. All patients provided written informed consent. The study protocol was approved by the Ethical Review Committee of all participating centers. The study was carried out in accordance with the principles of the Helsinki Declaration.

^{18}F-FDG PET-CT scan segmentation and radiomics features

The PET-CT systems used to perform the scans were accredited in accordance with the quality guidelines of the European Association of Nuclear Medicine.[14] Attenuation-corrected PET scans were analyzed using the ACCURATE tool, as described before.[15,16] We published earlier that segmentation using a fixed threshold of SUV4.0 is most suitable for application in clinical practice in cHL patients and therefore used this method to analyze the PET scans.[16] Delineations were performed by JD under supervision of a nuclear medicine physician (GJCZ). For each scan, the total MTV was segmented. In addition, a separate segmentation was performed for the lymph node that was biopsied and used for gene-expression and immunohistochemistry analysis. Location data from pathology reports were used to pinpoint the biopsied lymph node on the PET scan. In cases where the pathology report lacked specificity regarding a single lymph node, such as mentioning a 'right cervical node' when multiple cervical nodes were present, analysis was performed on the lymph node exhibiting the highest FDG uptake within that region. If the PET scan was conducted after the biopsy, segmentation of the biopsied lymph node was only performed if the biopsy was performed by fine needle biopsy and the (residual) lesion was still visually present, for example in a bulky lesion. In case the lymph node was surgically excised, only the total MTV was segmentated.

RaCat software was used to extract 4 patient-level dissemination features and standard intensity and volume-based features such as MTV, SUV parameters and total lesion glycolysis (TLG, i.e. SUVmean multiplied by MTV) from the complete volume of interest at the patient-level.[17] These features were selected because they are most commonly used in the literature and have been shown to exhibit prognostic value in a previous analysis.[12] We normalized the

SUVmean and SUVpeak for the liver SUVmean and used the tumor-to-liver ratio (TLR).[11,18-20] For the single-lesion analysis, standard intensity and volume-based features were analyzed on the biopsied lymph node.[17] An overview of all PET features and their definitions is provided in **Supplemental Table 1**.

Serum samples

Serum samples were centrally collected at time of enrolment in the study, which was at relapse or at confirmation of primary refractory disease. There were no serum samples available at the time of primary diagnosis. Serum TARC (ELISA, R&D systems, Minneapolis, MN, USA) levels were measured in serum by enzyme-linked immunosorbent assay, and analyzed blinded for patient outcome.

Immunohistochemistry

Lymph node biopsies were collected at primary diagnosis, and at inclusion in the study, i.e. at time of R/R disease. Central pathology review was performed as described before.[13] All cases were stained for TARC in an automated setting. Paraffin tissue sections (3µm) were incubated with polyclonal goat-anti-human TARC antibody (1:800 R&D Systems, Minneapolis, MN) on the automated Benchmark ULTRA platform (Ultra CC1, 52 minutes, Roche, Ventana Medical Systems). For each TARC stain, a section of cHL tissue was applied on the same slide as an external positive control. Intensity of TARC staining (i.e. negative, weak, positive) was scored by an experienced hematopathologist (AD), blinded for patient outcome, serum TARC measurements and gene-expression results. Positive TARC staining was defined as cytoplasmic staining visible at a magnification of 20x or less, weak staining was defined as cytoplasmic staining only discernable at higher magnification (200x).[8]

Gene-expression profiling and TME-signature scores

We designed a gene panel for NanoString analysis which was based on the RHL800 Nanostring panel that was created by Chan et al.[21] who developed a prognostic model for post-ASCT outcomes in R/R cHL. The RHL800 panel constitutes 800 genes that were assigned to various 'TME-signatures' according to the literature by the authors. We selected 141 genes from 26 different signatures of TME components and cell types for our analysis [**Supplemental Table 3**]. In addition, we added the category 'antigen-presenting cells' including HLA-related genes, and a second 'limited'-HRS signature containing only the 3 most relevant genes from the original HRS signature (i.e. *CCL17*, *TNFRSF8* and *IRF4*).[1]

Total RNA was isolated from paraffin embedded lymph node biopsies using the RNeasy Micro Kit from Qiagen (Venlo, The Netherlands) according to the manufacturer's recommendation. Initial quality check and RNA quantification of the samples were performed by automated gel electrophoresis on the 2200 TapeStation System (Agilent Technologies). The set of probes was hybridized to 100-200ng of RNA for 16h at 65 ºC. Samples were loaded on an nCounter

SPRINT Cartridge and processed on the nCounter SPRINT™ Profiler. The expression data were analyzed using NanoString's nSolver analysis software (version 4.0). Data was normalized on the geometric mean of 10 housekeeping genes, which was based on the analysis of Chan et al.: *ACTB, ALAS1, CLTC, POLR2A, RPL19, RPLP0, SDHA, TBP, TUBB, POLR1B*.[21] Samples with a low expression of housekeeping genes (i.e. geometric mean count <35) were excluded. The normalized data were scaled and transformed to log2. TME-signature scores were calculated by taking the geometric mean of its constituent genes.

Statistical analysis

Expression levels of TME-signatures between different groups of cases were assessed by calculating the log2 fold change of absolute counts. The Wilcoxon rank-sum test was used for comparing continuous variables between subgroups. Correlations were assessed using Spearman's rank correlation coefficients. Correlation plots were sorted by unsupervised hierarchical clustering using complete linkage clustering on the computed distance matrix. Heatmap analysis was performed by unsupervised hierarchical clustering of the z-value of PET features using the *ComplexHeatmap* package in R. P-values were adjusted for multiple comparisons using the Benjamini-Hochberg procedure with a 5% false discovery rate. A P-value of <0.05 was considered statistically significant. Statistical analysis was performed using R software version 4.0.3.

RESULTS

Patient characteristics

Sixty-seven patients with transplant-eligible R/R cHL were enrolled in the Transplant BRaVE study for treatment with BV-DHAP followed by ASCT. Seven patients were excluded because central pathology review showed other lymphoma subtypes such as peripheral T-cell lymphoma with HRS-like cells. A sufficient amount of material and adequate RNA quality for Nanostring analyses were available for 59 biopsies from 42 patients, with matched diagnostic and R/R samples being available for 17 patients. Patient characteristics are documented at the time of the biopsy. The median age was 29 years (range 18-72), and 46% were female [**Table 1**]. Most patients had Ann Arbor stage III or IV disease (60%), 41% had extranodal disease and 37% had B symptoms. In primary diagnosis cases (n=33), all showed progressive disease or relapse because they were retrospectively included from the Transplant BRaVE study in R/R cHL. In R/R cases (n=26), 5 (19%) had progressive disease or died after salvage treatment and ASCT.[13]

TARC immunohistochemistry

For 17 patients, both primary and R/R biopsies were available. Interestingly, TARC staining patterns were not consistent for 3 of these paired biopsies (18%). In one patient, who had a 10-year interval between primary diagnosis and relapse, the primary diagnostic biopsy showed

Table 1. Patient characteristics

	Primary diagnosis N=33	%	Relapsed/ refractory N=26	%	Total N=59	%
Female	15	45%	12	46%	27	46%
Median age (range)	28 (18 - 68)		32 (20 - 72)		29 (18 - 72)	
Ann Arbor Stage						
I	0	0%	3	12%	3	5%
II	13	39%	8	31%	21	36%
III	8	24%	6	23%	14	24%
IV	12	36%	9	35%	21	36%
Extranodal disease	12	36%	12	46%	24	41%
B symptoms	14	42%	8	31%	22	37%
Primary refractory	8	24%	7	27%	15	25%
Morphologic subtype						
NS	21	64%	12	46%	33	56%
MC	6	18%	9	35%	15	25%
NOS	6	18%	5	19%	11	19%
EBER positive	5	15%	3	12%	8	14%
TARC staining						
Positive	28	85%	22	85%	50	85%
Weak	4	12%	2	8%	6	10%
Negative	1	3%	2	8%	3	5%
Event PFS	33	100%	5	19%	38	64%
PET data	12	36%	22	85%	34	58%
PET single lesion data	12	36%	21	81%	33	56%

N, number; NS, nodular sclerosis; MC, mixed cellularity; NOS, not-otherwise-specified; EBER, Epstein-Barr virus-encoded small RNA;

positive TARC staining, while the biopsy at relapse was negative. In the remaining 2 cases, the paired biopsies showed either a change from weak to positive or from weak to negative staining.

Overall, TARC staining was positive in 50 (85%) cases (28 primary and 22 R/R), weak in 6 (10%) (4 primary and 2 R/R) cases and negative in 3 (5%) (1 primary and 2 R/R) cases [**Table 2**]. Weak or negative TARC staining was significantly associated with the mixed cellularity (MC) subtype (p=0.007), positive Epstein-Barr virus-encoded small RNA (EBER) staining (p=0.010), and the presence of B symptoms (p=0.048) [**Table 2**].

TME-signatures and clinical characteristics

We observed significant correlations between several TME-signatures [**Supplemental Figure 1**]. The original HRS signature was significantly correlated to the limited-HRS signature (with only the 3 most relevant genes). The HRS signature correlated positively with the macrophage, myeloid-derived suppressor cell (MDSC), neutrophil, eosinophil, NK, T-synapse, Th1, Th17, germinal center B cell (GCB), plasma cell and Th2 signatures, while the limited-HRS signature only

Table 2. Immunohistochemistry staining of TARC

	Positive (N=50)	Weak (n=6)	Negative (N=3)	Total (N=59)	*P* value (pos vs weak/neg)
Patient					0.637
Primary	28 (56%)	4 (67%)	1 (33%)	33 (56%)	
Relapsed/refractory	22 (44%)	2 (33%)	2 (67%)	26 (44%)	
Morphological subtype					0.012
MC	9 (18%)	3 (50%)	3 (100%)	15 (25%)	0.00757
NS	30 (60%)	3 (50%)	0 (0%)	33 (56%)	
NOS	11 (22%)	0 (0%)	0 (0%)	11 (19%)	
EBER	4 (8%)	3 (50%)	1 (33%)	8 (14%)	0.010
Ann Arbor Stage					0.805
I	3 (6%)	0 (0%)	0 (0%)	3 (5%)	
II	18 (36%)	2 (33%)	1 (33%)	21 (36%)	
III	11 (22%)	3 (50%)	0 (0%)	14 (24%)	
IV	18 (36%)	1 (17%)	2 (67%)	21 (36%)	
Extranodal disease	20 (40%)	2 (33%)	2 (67%)	24 (41%)	0.803
B symptoms	16 (32%)	5 (83%)	1 (33%)	22 (37%)	0.048

P-values are based on chi-square tests on positive versus weak or negative cases.
pos, positive; neg, negative.

correlated with the T-synapse, GCB, plasma cell and Th2 signatures. The follicular dendritic cell (FDC), B cell, GCB, and stroma signatures showed weak correlations with other TME-signatures and were inversely correlated with the macrophage, myeloid-lineage-1 and -2, and neutrophil signatures

There were no significant differences for primary versus R/R cases in expression of any of the TME-signatures (data not shown). Therefore, we combined the data of primary and R/R cases in further analyses. We compared TME-signature expression stratified for several patient characteristics, including morphological subtype, EBER and TARC immunohistochemistry. Results are presented in **Supplemental Table 4** with significant results highlighted in **Figure 1**. Compared to the nodular sclerosis (NS) or cHL-not otherwise specified (NOS) subtypes, cases with MC showed significantly lower expression of the fibroblast-extracellular matrix (ECM) signature (fold change -1.6; p<0.0001; p-value adjusted for multiple comparisons (p_adj)=0.0022) and limited-HRS signature (fold change -1.0; p=0.0023; p_adj=0.032) [**Figure 1A-B**]. Interestingly, cases with MC also showed lower expression of *CCL17* (fold change -1.6, p<0.001; p_adj=0.0015) [**Figure 1C**]. Positive EBER staining was associated with higher expression of the viral signature (Fold change 4.9, p<0.0001, p_adj<0.0001), but showed no significant differences in other signatures [**Figure 1D**]. The presence of B symptoms, stage, extranodal disease, age and sex were not associated with any significant differences in TME-signatures (data not shown).

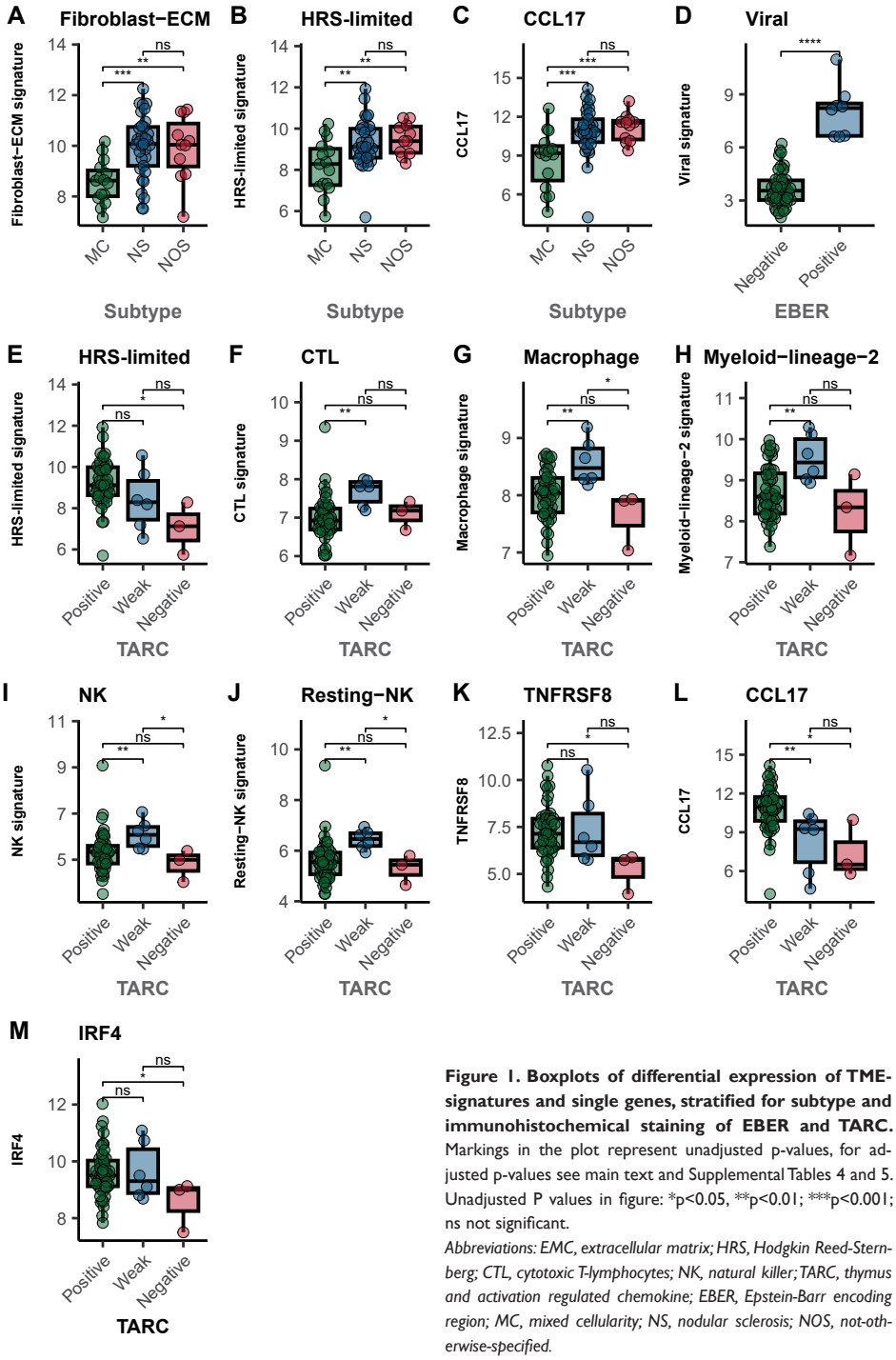

Figure 1. Boxplots of differential expression of TME-signatures and single genes, stratified for subtype and immunohistochemical staining of EBER and TARC. Markings in the plot represent unadjusted p-values, for adjusted p-values see main text and Supplemental Tables 4 and 5. Unadjusted P values in figure: *p<0.05, **p<0.01; ***p<0.001; ns not significant.

Abbreviations: EMC, extracellular matrix; HRS, Hodgkin Reed-Sternberg; CTL, cytotoxic T-lymphocytes; NK, natural killer; TARC, thymus and activation regulated chemokine; EBER, Epstein-Barr encoding region; MC, mixed cellularity; NS, nodular sclerosis; NOS, not-otherwise-specified.

TME-signatures and TARC immunohistochemistry

Cases with positive TARC immunohistochemistry had a trend for higher expression of the limited-HRS signature compared to cases with weak or negative staining (fold change 0.93; p=0.009; p_adj=0.08) [**Figure 1E** and **Supplemental Table 5**]. Cases with weak TARC staining showed significantly higher expression of several TME-signatures compared to cases with positive or negative TARC staining, including the cytotoxic T cell (CTL), macrophage, myeloid-lineage-2, NK and resting-NK signatures [**Figure 1F-J**]. Within the HRS signature, cases with positive TARC staining showed significant higher expression of *CCL17* (fold change 2.5; p_adj=0.0099) compared to weak/negative cases, while after correcting for multiple comparisons there were no significant differences for *TNFRSF8* and *IRF4* [**Figure 1K-M**].

Correlations between TME-signatures and PET features

Matched PET scans and biopsies were available for 34 cases, including 12 primary and 22 R/R cases. For one patient, the single-lesion analysis could not be conducted because the PET scan was performed after excision of the lymph node. Four patients had paired biopsies and PET scans available and both primary and R/R samples of these patients are included in the analysis. Intensity and volume-based PET features at the patient-level showed high correlations with PET features at the single-lesion level, while dissemination PET features showed low correlations with volume- and intensity-based features [**Supplemental Figure 2**]. There were no significant differences in PET features between cases with positive, weak or negative TARC staining (data not shown).

The HRS, T-synapse, Treg and NK signatures showed significant positive correlations with one or more PET features [**Figure 2A** and **Figure 3**]. For TME-signatures that showed at least one significant correlation with one or more PET-features, we also analyzed correlations between single genes within the signatures and PET features [**Figure 2B**].

The HRS signature was significantly correlated with the maximum difference in SUVpeak within a patient (DSUVpeak) (p_adj=0.02), MTV (p_adj=0.04), TLR_{TLG} (p_adj=0.02) and $TLR_{SUVpeak}$ (p_adj=0.009) at patient-level, while the limited-HRS signature only showed a significant correlation with DSUVpeak (p_adj=0.02) [**Figure 2A** and **3A-C**]. The HRS signature did not show significant correlations with PET-features at single-lesion level. Within the HRS signature, the majority of genes showed a positive correlation with intensity- and volume-based PET features of which *TNFRSF8* (CD30) correlated significantly with DSUVpeak [**Figure 2B**]. *IL4* correlated significantly with $TLR_{SUVmean}$ and $TLR_{SUVpeak}$. *IL4* promotes a Th2-type immune response and most cHL cell lines show high surface expression of the IL4-receptor, which upon activation by IL4 induces *STAT6* activation.[22] *SYTL3* correlated significantly with TLR_{TLG}, $TLR_{SUVmean}$ and $TLR_{SUVpeak}$. It encodes for a tumor necrosis factor alpha-induced protein involved in regulating trafficking of vesicles and its role in cHL is related to cytokine secretion.[23,24]

The T-synapse signature showed high correlations with intensity- and volume- based PET-features at the patient-level, for example with MTV (p_adj=0.03; **Figure 3D**) and $TLR_{SUVpeak}$ (p_

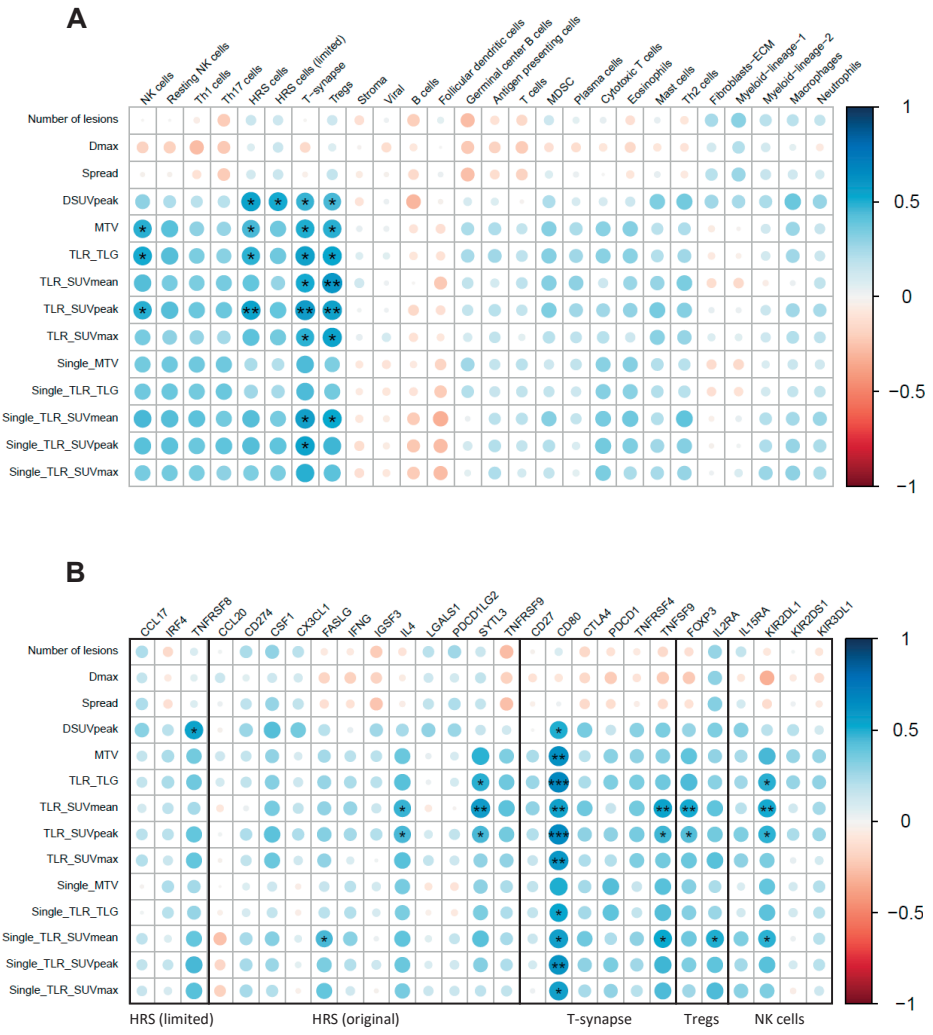

Figure 2. Spearman's rank correlation coefficients of PET features and TME-signatures. Markings in the plots represent p-values that are corrected for multiple comparisons. **A)** TME-signatures and PET features at the patient-level and single-lesion level (features that start with Single). **B)** Genes from TME-signatures that showed a significant correlation with one or more PET features.

P values adjusted for multiple comparisons: *p<0.05, **p<0.01; ***p<0.001;

Abbreviations: Dmax, the largest distance between two lesions; Spread, the sum of all distances between al lesions; DSUVpeak, the largest difference in SUVpeak between two lesions; MTV, metabolic tumor volume; TLR, tumor-to-liver ratio; TLG, total lesion glycolysis; SUV, standard-uptake-value; ECM, extracellular matrix; HRS, Hodgkin-Reed Sternberg; MDSC, myeloid derived suppressor cells; NK cells, natural killer cells.

adj=0.007; **Figure 3E**) at the patient-level. In addition, the T-synapse signature was significantly correlated with $TLR_{SUVmean}$ and $TLR_{SUVpeak}$ at the single-lesion level (p_adj=0.02 and p_adj=0.03, respectively), thus with FDG uptake measured directly in the corresponding biopsied lesion [**Figure 2A** and **Figure 3FG**]. The T-synapse signature comprises genes encoding for costimu-

latory molecules and immune checkpoints involved in the interaction between HRS cells and T cells. Within the T-synapse signature, *CD80*, which encodes a costimulatory molecule expressed on activated B cells and HRS cells and serves as the ligand for CD28 on T cells, showed strong and significant correlations with all intensity- and volume-based PET features, with the highest correlations observed for TLR_{TLG} and $TLR_{SUVpeak}$ [**Figure 2B**].[25] *TNFSF9* (CD137L) showed significant correlations with $TLR_{SUVmean}$ and $TLR_{SUVpeak}$ [**Figure 2B**]. HRS cells express CD137 (*TNFRSF9*), which is transferred to nearby HRS and antigen-presenting cells expressing CD137L (*TNFSF9*) through trogocytosis.[26] This results in the internalization of the CD137-CD137L complex, causing disappearance of CD137L on the surface of these cells, diminishing costimulation of T cells and reduces IFN-γ release. *PDCD1*, the gene for programmed cell death protein 1 (PD-1), and its ligand *CD274* (PD-L1) which is often expressed by HRS cells,[27] also showed trends for positive correlations with several PET features, but these correlations were not significant after correction for multiple comparisons [**Figure 2B**].

The Treg signature correlated significantly with all volume- and intensity-based PET features at the patient-level, with the strongest correlations observed for $TLR_{SUVmean}$ (p_adj=0.004) and $TLR_{SUVpeak}$ (p_adj=0.009) at the patient-level, and with $TLR_{SUVmean}$ at the single-lesion level (p_adj=0.02) [**Figure 2A** and **3H-J**]. This signature includes the transcription factor *FOXP3*, which is highly expressed in Tregs, and showed significant correlations with $TLR_{SUVmean}$ and $TLR_{SUVpeak}$ [**Figure 2B**].[28] *IL2RA* (CD25) correlated only with $TLR_{SUVmean}$ at the single-lesion level (p_adj=0.02). CD25 is primarily expressed by Tregs and serves the receptor for IL2, which plays a crucial role in the development and activation of Tregs.[29]

The NK signature showed significant correlations with MTV (p_adj=0.03), TLR_{TLG} (p_adj=0.02), and $TLR_{SUVpeak}$ (p_adj=0.02) at the patient-level [**Figure 2A and 3KL**]. Within this signature, the gene *KIR2DL1* (CD158a) correlated with TLR_{TLG}, $TLR_{SUVmean}$ and $TLR_{SUVpeak}$ at patient-level and $TLR_{SUVmean}$ at single-lesion level [**Figure 2B**]. *KIR2DL1* is a marker for (resting) NK cells and inhibits NK cell activation and cytotoxicity upon interaction with its ligands, such as HLA-C on HRS cells, which may contribute to immune evasion of HRS cells.[30,31]

In general, TME-signatures showed low correlations with distance-based dissemination PET features (number of lesions, Dmax, Spread). The B cell, stroma and FDC signatures showed a trend for low to negative correlations with PET features.

Heatmap of PET features and TME-signatures

Unsupervised hierarchical clustering of z-transformed PET features divided patients into two groups, exhibiting significant differences in all PET features except for Dmax. The 'high-PET' group comprised cases with elevated PET features and the 'low-PET' group comprised cases with a low value of PET features [**Figure 4**]. Between these groups, there was a significant difference in the expression of the Treg, T-synapse, HRS and NK signatures, which all showed higher expression in the 'high-PET' group. After correcting for multiple comparisons, only the T-synapse signature showed a significant difference between the high- and low-PET groups (fold

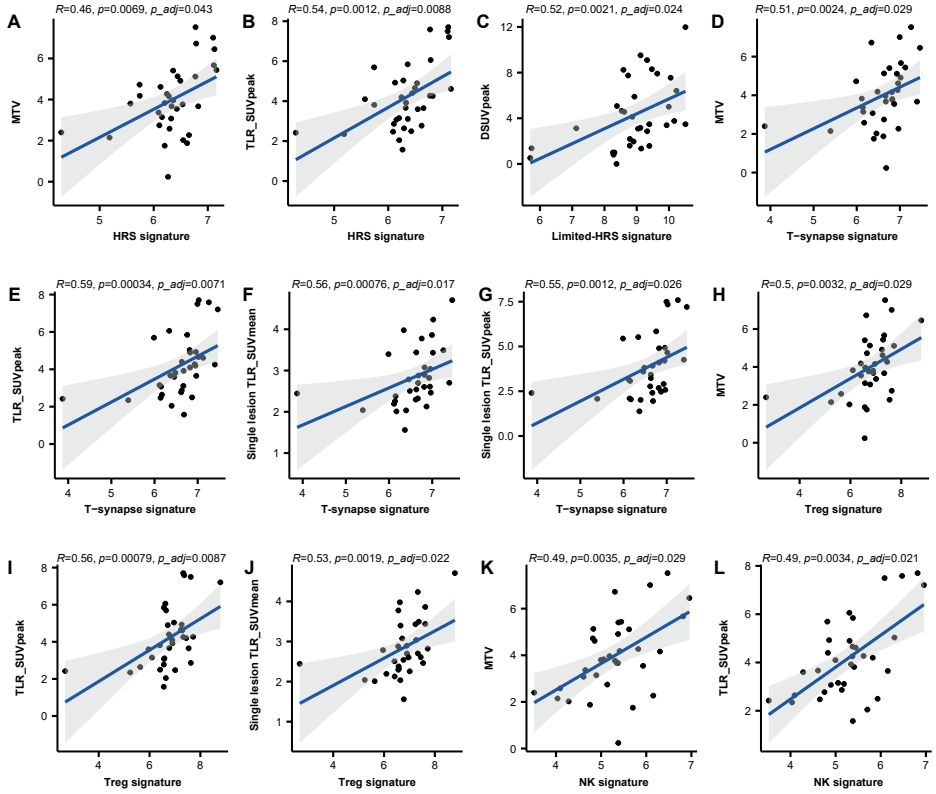

Figure 3. Spearman's rank correlation coefficients of PET features and selected TME-signatures.
Abbreviations: R, correlation coefficient; DSUVpeak, the largest difference in SUVpeak between two lesions; MTV, metabolic tumor volume; TLR, tumor-to-liver ratio; TLG, total lesion glycolysis; SUV, standard-uptake-value; HRS, Hodgkin-Reed Sternberg; NK cells, natural killer cells.

change 1.09; p_adj=0.043). Overall, the antigen presenting cell, fibroblast-ECM, and limited-HRS signatures showed the highest expression for all cases, while the viral, Th17 and Th1 signatures showed low expression.

Serum TARC

Serum TARC levels measured in 26 R/R cases before start of salvage treatment[8] showed a significant positive correlation with the limited-HRS signature (R=0.61, p=0.001, adjusted p=0.027) [**Supplemental Figure 3A**]. All genes of the limited-HRS signature, were significantly correlated to serum TARC, with the highest correlation for *IRF4* (*R*=0.51, p_adj=0.008), followed by *CCL17* (*R*=0.49, p_adj=0.01) and *TNFRSF8* (R=0.49, p_adj=0.01), [**Supplemental Figure 3B-D**].

Figure 4

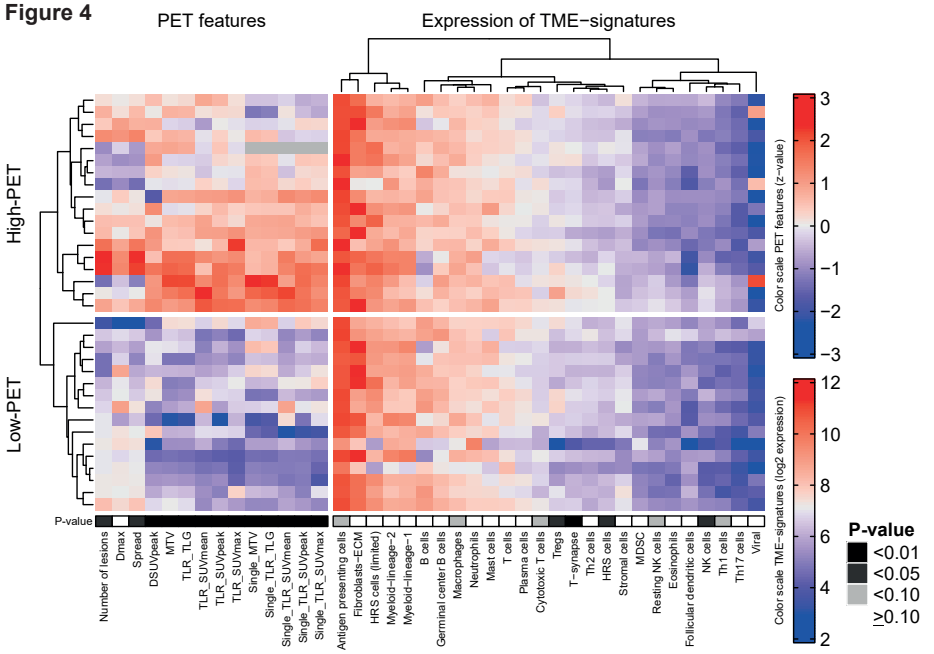

Figure 4. Unsupervised clustering of PET features and TME-signatures. PET data are z-transformed, gene-expression data is log2 transformed. Each row represents PET and TME-signature gene-expression data of a single case. Unsupervised hierarchical clustering was performed on all PET features and the TME-signatures were added to the heatmap. The high- and low-PET groups were created by the first dendogram group of the unsupervised clustering. P-values represent unadjusted p-values of Wilcoxon rank sum tests between the high- and low-PET groups. TME-signatures were sorted by unsupervised clustering. For one case the single-lesion PET analysis was not available, which is represented in grey values.

Abbreviations: PET, positron emission tomography; TME, tumor micro-environment; Dmax, the largest distance between two lesions; Spread, the sum of all distances between al lesions; DSUVpeak, the largest difference in SUVpeak between two lesions; MTV, metabolic tumor volume; TLR, tumor-to-liver ratio; TLG, total lesion glycolysis; SUV, standard-uptake-value; ECM, extracellular matrix; HRS, Hodgkin-Reed Sternberg; MDSC, myeloid derived suppressor cells; NK cells, natural killer cells.

DISCUSSION

In this study, we aimed to explore correlations between the TME composition and FDG uptake measured by quantitative PET scan analysis, TARC immunohistochemistry, serum TARC levels and clinical characteristics. The integration of PET imaging data with molecular analyses provides insights into the biological processes driving glucose metabolism in cHL. We found significant correlations between various TME-signatures and volume- and intensity-based PET features, while dissemination features showed no correlation with TME-signatures. Particularly the T-synapse signature, which contains genes related to costimulation of T cells and immune checkpoints such as CD80, TNFSF9 and PDCD1, showed high correlations with FDG uptake on both the patient-level and single-lesion level. In addition, the NK signature was significantly correlated with several PET features, involving mainly genes inhibiting NK cell function. The B

cell, FDC and fibroblast-ECM signatures showed no correlation with PET features. This suggests that immune activation is mainly responsible for the high glucose uptake measured by PET scans.

As we have shown in earlier studies, a high SUVpeak/SUVmean on baseline PET is associated with poor prognosis.[8,12] Therefore, the immune response that correlates with high FDG uptake on the PET scan probably does not constitute an effective anti-HRS cell response, but rather a pro-inflammatory reaction that is triggered by high cytokine excretion of HRS cells. Immune evasion mechanisms of HRS cells are well-documented and include 1) the recruitment of CD4+ T helper cells, Tregs and macrophages, establishing an immunosuppressive TME, 2) secretion of cytokines that suppress T cell activation, induce apoptosis of cytotoxic T cells, and promote Treg differentiation, 3) upregulation of PD-L1 on HRS cells, inhibiting cytotoxic T cell activity and inducing T cell exhaustion and thereby impairing an effective Th1 response, and 4) polarization of macrophages to an M2 phenotype, characterized by the expression of PD-L1, thereby preventing costimulation of cytotoxic T cells.[3,5,27,32,33] Insights in the correlation between immune evasion by HRS cells and FDG uptake in cHL may have implications for treatment with immune checkpoint inhibitors, which have demonstrated efficacy in cHL by releasing the blockage on an effective immune response against tumor cells.[34-36] Furthermore, given the risk of pseudoprogression following treatment with immune checkpoint inhibitors, the utility of PET scans in this context requires reassessment.[37] Therefore, explorative studies investigating TME-signatures and PET features carried out in the immune-checkpoint inhibitor setting are warranted. This study lays the groundwork for further research in this population.

We observed differences in TME-signatures between cHL subtypes, with the MC subtype showing distinct patterns of gene-expression compared to other subtypes. The MC subtype showed more often weak TARC expression, and was characterized by a higher percentage of EBV positivity. Although subgroups were too small for multivariable analysis, this suggests that there is a subgroup of cHL with a MC subtype that is often EBV+ and shows weak TARC expression, which is consistent with findings from another recent analysis on cHL cases.[38]

Limitations of this analysis are the small sample size and the lack of enough material for validation of TME-signatures with immunohistochemistry in tissue. In addition, our results need to be validated on other cohorts, preferably combined with spatial gene-expression or immunohistochemistry analysis in tissue. Most correlations that we found are positive, which raises the question what the TME of patients with low FDG uptake looks like. A possible explanation for this phenomenon is that cases with low FDG uptake have higher amounts of fibrosis in the biopsies, which are not fully reflected in higher expression in the TME-signatures. It is also possible that activated cells have higher expression levels of all genes compared to latent cells. Unfortunately, there were not enough progression events in the R/R cohort to validate findings from a prognostic gene-expression panel.[21]

Overall, our findings underscore the complexity of interactions within the TME and its impact on glucose metabolism, as assessed by FDG-PET. By exploring these relationships, our study lays the groundwork for further investigation into targeted therapeutic strategies aimed

at specific immune cell populations and metabolic pathways implicated in cHL progression and treatment response.

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CONFLICT OF INTEREST

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SUPPLEMENTARY MATERIAL

Supplemental Table 1. Overview of PET features and their definitions

Variable	Definition
MTV	Metabolic tumor volume; The FDG-avid tumor volume
TLG	Total lesion glycolysis; MTV multiplied by SUVmean
SUVmean	The mean SUV value of the VOI
SUVmax	The SUV of the voxel with the highest SUV within the VOI
SUVpeak	The SUV of the 3mL with the highest SUV within the VOI (global peak)
$TLR_{SUVmean}$	Tumor to liver ratio of the lesional SUVmean and the liver SUVmean
$TLR_{SUVpeak}$	Tumor to liver ratio of the lesional SUVpeak and the liver SUVmean
Number of lesions	The number of separated lesion selections within the VOI
Dmax	The maximum distance between two lesions
Spread	The sum of the distance between all lesions
DSUVpeak	The difference in SUVpeak between the lesion with the highest SUVpeakmax and the lesion with the lowest SUVpeak

Abbreviations: SUV, standard uptake value; VOI, volume of interest.

Supplemental Table 2: List of genes in Nanostring panel, probes and signatures

HUGO Gene	Probe NSID	Signature1	Signature2	Signature3	Signature4
ACTB	NM_001101.2:1010	Housekeeping			
ALAS1	NM_000688.4:1615	Housekeeping			
ALDH1A1	NM_000689.3:11	Macrophage			
APOE	NM_000041.2:96	Macrophage			
B2M	NM_004048.2:235	Antigen presenting cells			
BACH2	NM_021813.2:3395	B-cell			
BCL11A	NM_018014.2:3780	B-cell			
BCL2	NM_000633.2:1525	GCB			
BCL6	NM_138931.1:505	Neutrophils			
C1QB	NM_000491.3:819	Cytotoxic T cells			
C1QC	NM_001114101.1:608	Cytotoxic T cells			
CCL17	NM_002987.2:229	HRS	HRS_short		
CCL20	NM_004591.1:35	HRS	Macrophage		
CCL3	NM_002983.2:681	Eosinophils			
CCR2	NM_001123041.2:20	Plasma cells			
CCR4	NM_005508.4:672	TH2			
CD19	NM_001770.4:1770	B-cell			
CD1C	NM_001765.2:750	Follicular dendritic cells			
CD22	NM_001771.2:2515	B-cell			
CD27	NM_001242.4:330	Plasma cells	T-synapse		
CD274	NM_014143.2:684	HRS	T-synapse		
CD33	NM_001177608.1:730	MDSC			

HUGO Gene	Probe NSID	Signature1	Signature2	Signature3	Signature4
CD3D	NM_000732.4:110	T-cell			
CD3E	NM_000733.2:75	T-cell			
CD3G	NM_000073.2:515	T-cell			
CD40	NM_001250.4:196	Antigen presenting cells			
CD47	NM_001777.3:897	T-cell			
CD68	NM_001251.2:1140	Macrophage			
CD72	NM_001782.2:1044	B-cell			
CD79A	NM_001783.3:695	B-cell			
CD8A	NM_001768.5:1320	Cytotoxic T cells	T-cell		
CD8B	NM_004931.3:440	Cytotoxic T cells	T-cell		
CD93	NM_012072.3:4270	Myeloid-lineage-1			
CEACAM8	NM_001816.3:825	MDSC	Neutrophils		
CHUK	NM_001278.3:860	Macrophage			
CLTC	NM_004859.2:290	Housekeeping			
COL1A2	NM_000089.3:2635	Fibroblast-ECM			
COL6A1	NM_001848.2:3665	Fibroblast-ECM			
CPA3	NM_001870.2:220	Mast cell			
CR2	NM_001006658.1:485	B-cell	Stroma		
CSF1	NM_000757.4:823	HRS			
CSF1R	NM_005211.2:3775	Cytotoxic T cells	Macrophage		
CTLA4	NM_005214.3:405	T-cell	T-synapse		
CX3CL1	NM_002996.3:140	HRS	Macrophage		
CXCL12	NM_199168.2:505	Fibroblast-ECM			
CXCL13	NM_006419.2:0	Follicular dendritic cells			
DEFA1	NM_004084.2:346	Neutrophils			
EBER1	HHV4_000057.1:41	Viral			
EBER2	HHV4_000080.1:39	Viral			
EBNA-2	HHV4_000068.1:47	Viral			
ELANE	NM_001972.2:195	Neutrophils			
FAS	NM_000043.3:90	Cytotoxic T cells			
FASLG	NM_000639.1:625	HRS			
FCER2	NM_002002.4:106	B-cell	Stroma		
FCGRT	NM_004107.4:1276	Neutrophils			
FLT1	NM_002019.2:5615	Macrophage	MDSC		
FOXP3	NM_014009.3:1230	Tregs			
FUT4	NM_002033.2:1345	Eosinophils	MDSC		
GATA1	NM_002049.2:1001	Eosinophils			
GNB2	NM_005273.3:884	Neutrophils			
GNS	NM_002076.3:1340	Myeloid-lineage-1			
GPNMB	NM_001005340.1:535	Mast cell			
HK3	NM_002115.1:495	Myeloid-lineage-1			

HUGO Gene	Probe NSID	Signature1	Signature2	Signature3	Signature4
HLA-A	NM_002116.7:397	Antigen presenting cells			
HLA-B	NM_005514.6:1247	Antigen presenting cells			
HLA-C	NM_002117.4:898	Antigen presenting cells			
HLA-DRA	NM_019111.3:335	Antigen presenting cells			
HSD17B8	NM_014234.3:875	Plasma cells	T-cell		
HSP90AA1	NM_001017963.2:1655	Macrophage			
ICAM1	NM_000201.2:2253	MDSC			
ICAM3	NM_002162.3:1225	Neutrophils			
IFNG	NM_000619.2:970	HRS	Th1		
IGSF3	NM_001542.2:6865	HRS			
IL10	NM_000572.2:230	TH2			
IL13RA1	NM_001560.2:1230	Neutrophils			
IL15RA	NM_002189.2:505	NK cells			
IL17A	NM_002190.2:240	Th17 cells			
IL17F	NM_052872.3:210	Th17 cells			
IL2	NM_000586.2:300	Th1			
IL21	NM_021803.2:65	Th17 cells			
IL22	NM_020525.4:319	Th17 cells			
IL2RA	NM_000417.1:1000	NK cells	Tregs		
IL3RA	NM_002183.2:745	Stroma			
IL4	NM_000589.2:625	HRS	TH2		
ITGB2	NM_000211.2:520	Myeloid-lineage-2	Neutrophils		
ITM2A	NM_004867.4:988	T-cell			
KIR2DL1	NM_014218.2:149	NK cells	Resting-NK		
KIR2DS1	NM_014512.1:698	NK cells	Resting-NK		
KIR3DL1	NM_013289.2:1626	NK cells	Resting-NK		
LGALS1	NM_002305.3:60	HRS			
LILRB2	NM_005874.1:595	Myeloid-lineage-1			
LMP-1	HHV4_000020.1:248	Viral			
LMP-2B	HHV4_000043.1:23	Viral			
LYZ	NM_000239.2:305	Macrophage			
MARCO	NM_006770.3:1434	Macrophage			
MATK	NM_139354.1:1365	Cytotoxic T cells	T-cell		
MIF	NM_002415.1:319	Macrophage			
MS4A1	NM_152866.2:620	B-cell			
PDCD1	NM_005018.1:175	T-synapse	TH1		
PDCD1LG2	NM_025239.3:235	HRS	T-synapse		
PEA15	NM_003768.2:1050	Myeloid-lineage-1			
PECAM1	NM_000442.3:1365	Myeloid-lineage-1	Plasma cells		
POLR1B	NM_019014.3:3320	Housekeeping			
POLR2A	NM_000937.2:3775	Housekeeping			

HUGO Gene	Probe NSID	Signature1	Signature2	Signature3	Signature4
PRF1	NM_005041.3:2120	Cytotoxic T cells	Resting-NK		
PTGDR2	NM_004778.1:1835	Eosinophils			
RPL19	NM_000981.3:315	Housekeeping			
RPL31	NM_000993.4:140	B-cell			
RPLP0	NM_001002.3:250	Housekeeping			
SCARB2	NM_005506.2:1825	Cytotoxic T cells			
SDHA	NM_004168.1:230	Housekeeping			
SERPINA1	NM_000295.4:760	Myeloid-lineage-1	Myeloid-lineage-2		
SLC31A2	NM_001860.2:105	Myeloid-lineage-2			
SOD2	NM_000636.2:640	Neutrophils			
STAT1	NM_007315.2:205	Macrophage			
SYTL3	NM_001009991.2:1615	HRS			
TBP	NM_001172085.1:587	housekeeping			
TGFB1	NM_000660.3:1260	TH2			
TIA1	NM_022037.1:1245	Cytotoxic T cells			
TLR8	NM_016610.2:2310	Stroma			
TNFRSF4	NM_003327.2:200	T-synapse			
TNFRSF8	NM_001243.3:3355	HRS	HRS_short		
TNFRSF9	NM_001561.4:255	HRS	T-synapse		
TNFSF8	NM_001244.2:1630	Eosinophils	Mast cell		
TNFSF9	NM_003811.3:398	T-synapse			
TPP1	NM_000391.3:746	Myeloid-lineage-1			
TPSAB1	NM_003294.3:579	Mast			
TUBB	NM_178014.2:320	Housekeeping			
VCAN	NM_004385.3:9915	Myeloid-lineage-1			
VNN2	NM_004665.2:1829	Myeloid-lineage-2	Neutrophils		
VPREB3	NM_013378.2:350	B-cell			
IRF4	NM_002460.1:325	GCB	HRS	HRS_short	Plasma cells
CD163	NM_004244.4:1630	Macrophage	Myeloid-lineage-1	Myeloid-lineage-2	
CTSB	NM_001908.3:595	Macrophage	Myeloid-lineage-1	Myeloid-lineage-2	Neutrophils
CCL4	NM_002984.2:35	Cytotoxic T cells	Eosinophils	Resting-NK	
TLR2	NM_003264.3:180	Macrophage	Myeloid-lineage-1	Stroma	
CD300A	NM_007261.2:0	Cytotoxic T cells	Resting-NK	T-cell	
FCGR3B	NM_000570.4:255	Neutrophils	Resting-NK	T-cell	
CD80	NM_005191.3:1288	Antigen presenting cells	Macrophage	T-synapse	
TBX21	NM_013351.1:890	Cytotoxic T cells	Resting-NK	TH1	
CD4	NM_000616.3:835	T-cell	Th1	Th17 cells	Th2

Abbreviations: GCB, germinal center B cell; HRS, Hodgkin Reed-Sternberg; Th, T helper; MDSC, myeloid derived suppressor cell; HRS_short, the limited HRS cell TME-signature (see supplemental Table 3); Tregs, regulatory T cells.

Supplemental Table 3. Genes per TME-signature

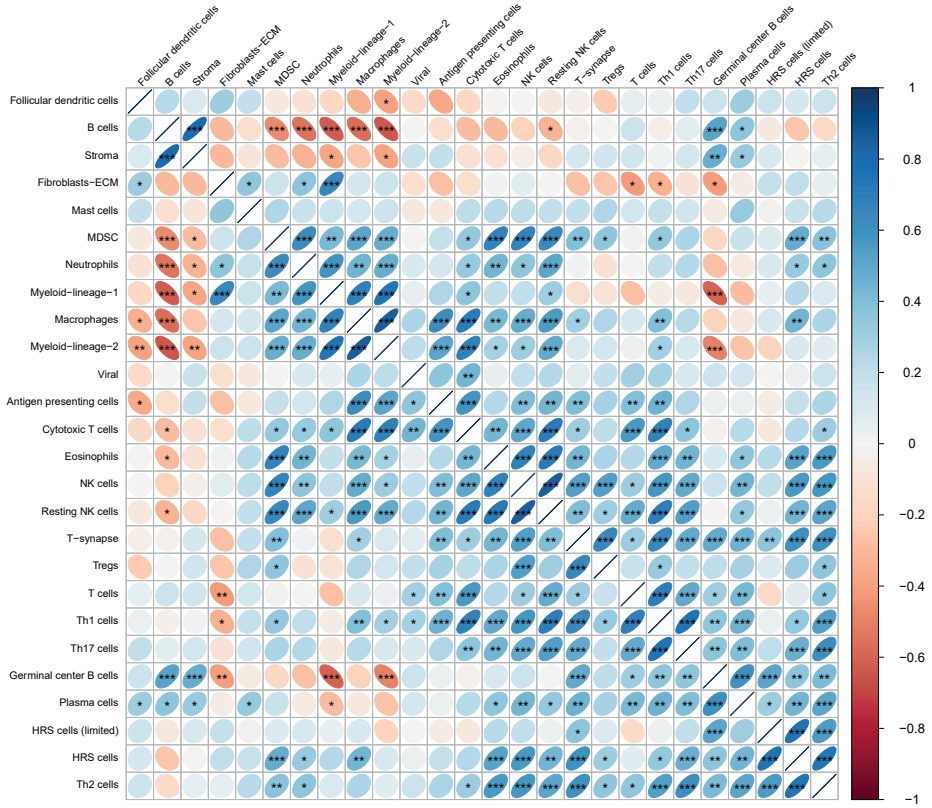
Signature	Genes
Antigen presenting cells	B2M, CD40, CD80, HLA-A, HLA-B, HLA-C, HLA-DRA
B cells	BACH2, BCL11A, CD19, CD22, CD72, CD79A, CR2, FCER2, MS4A1, RPL31, VPREB3
Cytotoxic T cells	C1QB, C1QC, CCL4, CD300A, CD8A, CD8B, CSF1R, FAS, MATK, PRF1, SCARB2, TBX21, TIA1
Eosinophils	CCL3, CCL4, FUT4, GATA1, PTGDR2, TNFSF8
Follicular dendritic cells	CD1C, CXCL13
Fibroblasts-ECM	COL1A2, COL6A1, CXCL12
Germinal center B cells	BCL2, IRF4
HRS cells	CCL17, CCL20, CD274, CSF1, CX3CL1, FASLG, IFNG, IGSF3, IL4, IRF4, LGALS1, PDCD1LG2, SYTL3, TNFRSF8, TNFRSF9
HRS cells (limited)	CCL17, IRF4, TNFRSF8
Macrophages	ALDH1A1, APOE, CCL20, CD163, CD68, CD80, CHUK, CSF1R, CTSB, CX3CL1, FLT1, HSP90AA1, LYZ, MARCO, MIF, STAT1, TLR2
Mast cells	CPA3, GPNMB, TNFSF8, TPSAB1
Myeloid derived suppressor cells	CD33, CEACAM8, FLT1, FUT4, ICAM1
Myeloid-lineage-1	CD163, CD93, CTSB, GNS, HK3, LILRB2, PEA15, PECAM1, SERPINA1, TLR2, TPP1, VCAN
Myeloid-lineage-2	CD163, CTSB, ITGB2, SERPINA1, SLC31A2, VNN2
Neutrophils	BCL6, CEACAM8, CTSB, DEFA1, ELANE, FCGR3B, FCGRT, GNB2, ICAM3, IL13RA1, ITGB2, SOD2, VNN2
NK cells	IL15RA, IL2RA, KIR2DL1, KIR2DS1, KIR3DL1
Plasma cells	CCR2, CD27, HSD17B8, IRF4, PECAM1
Resting NK cells	CCL4, CD300A, FCGR3B, KIR2DL1, KIR2DS1, KIR3DL1, PRF1, TBX21
Stroma	CR2, FCER2, IL3RA, TLR2, TLR2, TLR8
T cells	CD300A, CD3D, CD3E, CD3G, CD4, CD47, CD8A, CD8B, CTLA4, FCGR3B, HSD17B8, ITM2A, MATK
T-synapse	CD27, CD274, CD80, CTLA4, PDCD1, PDCD1LG2, TNFRSF4, TNFRSF9, TNFSF9
Th1 cells	CD4, IFNG, IL2, PDCD1, TBX21
Th17 cells	CD4, IL17A, IL17F, IL21, IL22
Th2 cells	CCR4, CD4, IL10, IL4, TGFB1
Tregs	FOXP3, IL2RA
Viral	EBER1, EBER2, EBNA2, LMP1, LMP2B
Housekeeping genes	ALAS1, POLR1B, SDHA, TBP, CLTC, POLR2A, TUBB, RPLP0, RPL19, ACTB

Supplemental Table 4. Fold change expression of TME-signatures for EBER IHC and morphological subtype

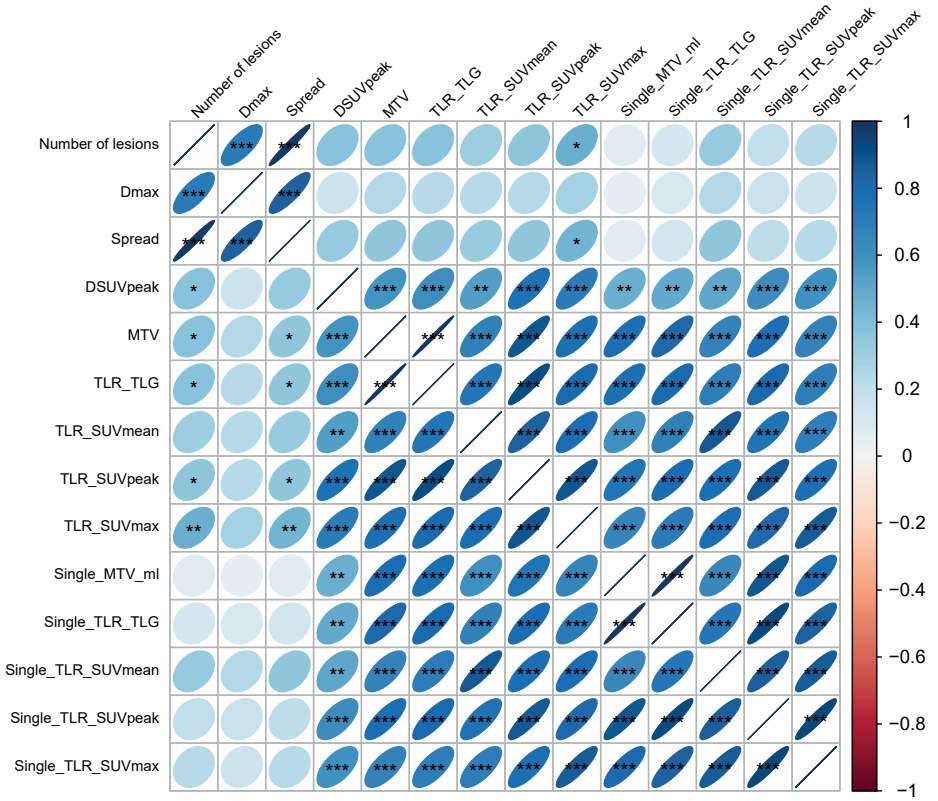
	EBER Positive vs negative			Mixed cellularity vs NS/ NOS		
	Fold log2	**Wilcox p**	**Adjusted P**	**Fold log2**	**Wilcox p**	**Adjusted P**
APC	0.37	0.012	0.075	0.10	0.116	0.274
B cell	-0.10	0.886	0.921	0.24	0.236	0.472
CTL	0.30	0.014	0.075	0.52	0.027	0.115
Eosinophil	-0.26	0.715	0.808	0.73	0.776	0.807
FDC	-0.81	0.020	0.085	-0.41	0.112	0.274
Fibroblast-ECM	-1.11	0.026	0.097	-1.60	0.000	0.002
GCB	-0.20	0.528	0.723	0.11	1.000	1.000
HRS	-0.29	0.108	0.256	-0.23	0.047	0.176
HRS-limited	-1.08	0.013	0.075	-1.05	0.002	0.031
Macrophage	0.15	0.347	0.644	0.04	0.673	0.784
Mast	-0.21	0.765	0.829	0.29	0.304	0.555
MDSC	-0.09	0.472	0.722	0.24	0.711	0.784
Myeloid lineage-1	-0.21	0.618	0.804	-0.48	0.027	0.115
Myeloid lineage-2	0.50	0.061	0.160	0.25	0.507	0.724
Neutrophil	-0.14	0.698	0.808	-0.26	0.074	0.214
NK cells	-0.07	0.129	0.259	0.76	0.648	0.784
Plasma	-0.13	0.472	0.722	-0.05	0.724	0.784
Resting NK	0.00	0.129	0.259	0.87	0.529	0.724
Stroma	0.13	0.650	0.805	0.21	0.147	0.319
T cell	0.28	0.033	0.106	0.29	0.006	0.051
T-synapse	0.09	0.432	0.722	0.07	0.529	0.724
TH1	0.14	0.058	0.160	0.59	0.320	0.555
TH17	-0.28	0.991	0.991	0.26	0.724	0.784
TH2	-0.18	0.528	0.723	-0.14	0.347	0.563
Tregs	0.37	0.005	0.071	0.50	0.014	0.091
Viral	4.85	0.000	0.000	2.60	0.069	0.214

Supplemental Table 5. Fold change expression of TME-signatures for TARC IHC staining

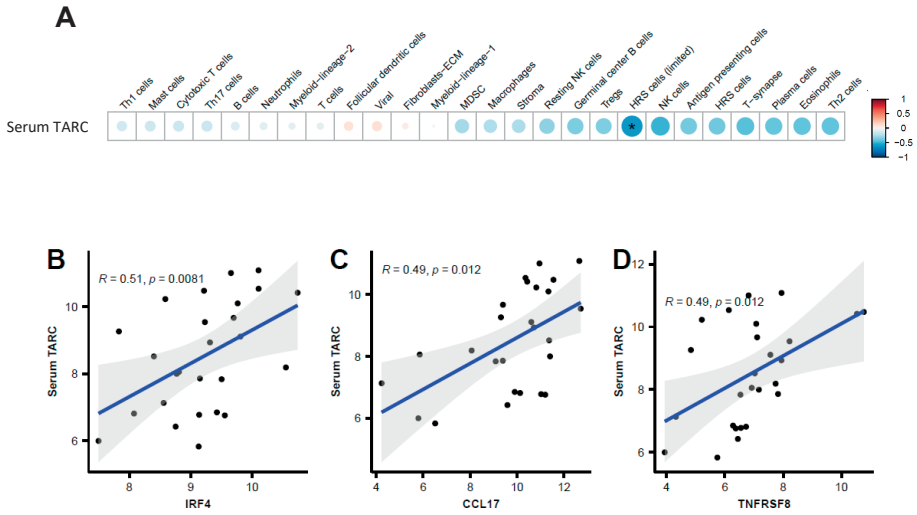
	Positive			Weak			Negative		
Signature	**Fold log2**	**Wilcox p**	**Adjusted P**	**Fold log2**	**Wilcox p**	**Adjusted P**	**Fold log2**	**Wilcox p**	**Adjusted P**
APC	-0.30	0.026	0.168	0.45	0.012	0.051	-0.09	0.877	0.979
B cell	0.42	0.242	0.449	-0.97	0.036	0.105	0.35	0.343	0.521
CTL	-0.42	0.007	0.080	0.59	0.003	0.033	-0.07	0.823	0.979
Eosinophil	0.20	0.728	0.879	0.05	0.095	0.201	-0.76	0.088	0.253
FDC	0.26	0.405	0.585	-0.38	0.298	0.408	0.00	0.959	0.986
Fibroblast-ECM	0.71	0.082	0.355	-0.32	0.506	0.572	-1.69	0.056	0.211
GCB	0.34	0.181	0.375	-0.23	0.475	0.572	-0.50	0.234	0.468
HRS	0.22	0.337	0.516	0.01	0.831	0.831	-0.73	0.060	0.211
HRS-limited	0.93	0.009	0.080	-0.50	0.197	0.319	-2.17	0.014	0.192
Macrophage	-0.29	0.174	0.375	0.57	0.006	0.036	-0.47	0.116	0.303
Mast	0.42	0.167	0.375	-0.38	0.264	0.404	-0.41	0.479	0.623
MDSC	0.09	1.000	1.000	0.22	0.100	0.201	-0.91	0.024	0.192
Myeloid lineage-1	-0.15	0.891	0.927	0.53	0.172	0.319	-1.04	0.034	0.192
Myeloid lineage-2	-0.49	0.116	0.375	0.81	0.009	0.046	-0.53	0.309	0.521
Neutrophil	-0.04	0.744	0.879	0.28	0.041	0.107	-0.57	0.024	0.192
NK cells	-0.19	0.116	0.375	0.55	0.006	0.036	-0.87	0.221	0.468
Plasma	0.17	0.337	0.516	-0.10	0.661	0.716	-0.27	0.343	0.521
Resting-NK	-0.27	0.032	0.168	0.58	0.001	0.033	-0.63	0.361	0.521
Stroma	0.23	0.278	0.481	-0.30	0.188	0.319	-0.06	0.986	0.986
T cell	-0.38	0.002	0.056	0.32	0.034	0.105	0.39	0.037	0.192
T-synapse	0.09	0.480	0.657	0.03	0.792	0.824	-0.34	0.438	0.599
TH1	-0.07	0.143	0.375	0.24	0.058	0.138	-0.33	0.849	0.979
TH17	0.03	0.620	0.806	0.16	0.490	0.572	-0.45	0.904	0.979
TH2	-0.04	0.808	0.911	0.30	0.298	0.408	-0.61	0.065	0.211
Tregs	0.19	0.841	0.911	0.04	0.430	0.558	-0.71	0.152	0.360
Viral	-3.44	0.188	0.375	3.71	0.023	0.086	0.36	0.343	0.521



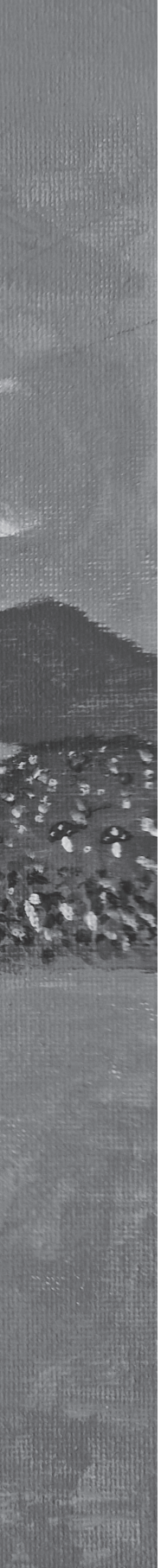
Supplemental Figure 1. Correlations between TME-signatures. Stars indicate significant p-values adjusted for multiple comparisons: *p<0.05, **p<0.01, ***p<0.001.



Supplemental Figure 2: Correlations between PET features on patient- and single-lesion level. Stars indicate significant p-values adjusted for multiple comparisons: *p<0.05, **p<0.01, ***p<0.001.



Supplemental Figure 3. Correlation plots of serum TARC. **A)** Spearman's rank correlation matrix of serum TARC and gene-signatures. Stars indicate significant p-values adjusted for multiple comparisons: *p<0.05, **p<0.01, ***p<0.001. **B-D)** Spearman's rank correlation plots of serum TARC and individual genes from the limited HRS cell signature. *Abbreviations: R, correlation coefficient; p_adj, p-value adjusted for multiple comparisons.*



10

GENERAL DISCUSSION AND FUTURE PERSPECTIVES

DISCUSSION

The primary objective of this thesis was to contribute to the development of personalized and efficient therapeutic strategies for patients diagnosed with classical Hodgkin lymphoma (cHL), particularly in the relapsed/refractory (R/R) setting. To achieve this, we connected information from various domains, including quantitative positron emission tomography (PET) data, serum biomarkers, tumor microenvironment (TME) composition, and clinical data.

Treatment of cHL

Over the past decades, the treatment landscape for cHL has changed drastically. Historically, cHL has been characterized by a high cure rate with standard frontline chemotherapy regimens such as ABVD (doxorubicin, bleomycin, vinblastine, dacarbazine) and escBEACOPP (escalated bleomycin, etoposide, adriamycin, cyclophosphamide, vincristine, procarbazine, prednisone).[1-8] However, a subset of patients experience relapse or have primary refractory disease, posing significant therapeutic challenges.[9] In recent years, novel therapeutic agents, including the CD30 antibody-drug conjugate brentuximab vedotin (BV) and immune checkpoint inhibitors, have emerged as promising therapeutic options for these patients.[10-14] In **chapter 3**, our population-based study showed that relative survival improved over the last decades, which is likely attributed to improvements in chemotherapy treatment schedules, the more precise application of involved node radiotherapy, and improved supportive care.[15] Our contemporary population-based study included comprehensive information on primary therapy for individual patients, which is unique for population-based studies. In addition, our study extends on prior, relatively outdated population-based studies.[16,17] Population-based studies are important to evaluate advances in treatment because most clinical trials have strict inclusion and exclusion criteria which hamper extrapolation to the real-world setting. This is underlined by our findings in the elderly cHL population (i.e. >60 years of age), who showed only minimal improvement in relative survival over the years, indicating that more intensive chemotherapy schedules are too toxic for this population and that there is an unmet need for more targeted interventions.[18] Aside from improvements in primary treatment, a large part of the improved relative survival can probably be contributed to advances in later lines of therapies. Since about 15-30% of patients are primary refractory to first-line treatment or relapse after an initial response, and only 40-60% of those can be cured with high-dose chemotherapy and autologous stem-cell transplant, the biggest unmet needs for improving overall survival (OS) are certainly in the R/R setting. With more and more options available for this population, median OS has improved, and more patients are cured.[10-14]

One of these options involves BV. We have shown in the clinical trial described in **chapter 4** that BV in combination with chemotherapy followed by high-dose chemotherapy (HDCT) and autologous stem-cell transplant (ASCT) leads to high complete metabolic response (CMR) rates and high progression free survival (PFS) rates after 3 years.[19] Importantly, the 3 deaths in

our study were caused by toxicity or were unrelated to cHL. Our trial is especially important because it is the first trial that combined BV with dexamethasone, high-dose cytarabine and cisplatin (DHAP), the most commonly used salvage regimen in the Netherlands and Germany and led to the reimbursement of using BV in the second line in the Netherlands.[20] In addition, in this salvage regimen we only used 3 doses of BV, in contrast to other studies that used up to 16 cycles of BV, which comes with high percentages of peripheral neuropathy and also financial toxicity.[21,22]

Another remarkable finding in this study was that, with central pathology review, seven cases were found not to be cHL. Among these cases, five were classified as peripheral T-cell lymphoma with secondary HRS-like blasts, one as angioimmunoblastic T cell lymphoma, and one case as with immunodeficiency-associated B-lymphoproliferative disorder. All cases had CD30+ HRS-like cells, which probably led to the initial misdiagnosis. The diagnosis of T-cell lymphoma is important because this lymphoma subtype usually carries a poor prognosis.[19,23] However, in our analysis the PFS was comparable to that of the cHL cases. Another series of cHL mimickers was recently described, showing cHL relapses, especially late relapses (>2years), sometimes represent a T cell lymphoma mimicking cHL. This suggests that molecular testing in cHL relapse cases should be performed more often.[24]

The clinical trial described in chapter 3, the Transplant BRaVE study, is the basis for much of the research conducted in this thesis.[13,25] Because it involved a relatively large international phase II study in the R/R setting, it provided a firm basis for translational research. We collected serum samples at baseline and after each cycle up to 3 years of follow-up. PET-computed tomography (CT) scans were performed according to the EANM guidelines on EARL accredited scanners and were performed at baseline, pre-ASCT and 6 weeks post-ASCT and were centrally collected and reviewed.[26] All patients had biopsy-proven relapse or primary refractory disease and also biopsies from the primary diagnosis were collected for central pathology review, ensuring that all patients had cHL. Although the diagnosis of cHL is usually straightforward, distinction from Hodgkin-mimickers is especially relevant in the R/R setting.[24]

The Transplant BRaVE clinical trial is not the only study that evaluated the use of BV in the salvage setting, either combined with salvage chemotherapy or in a sequential approach.[21,22,27-29] However, there are no randomized controlled trials that compare the addition of BV to salvage chemotherapy versus salvage chemotherapy alone. A phase IIb randomized trial in 150 R/R cHL patients is currently being conducted that compares BV-ESHAP versus ESHAP.[30] Preliminary results show higher CMR rates for the BV-ESHAP arm, however PFS data have not been published thus far and the study is probably too small for extensive subgroup analyses. In addition, this study investigates the possibility to replace HDCT/ASCT for BV maintenance in patients with a CMR, which is currently being investigated in several trials.[31] Because it is unlikely that a large phase III study will be conducted that compares BV addition to salvage therapy versus salvage therapy alone, in **Chapter 5** we have performed an individual patient-level meta-analysis of all phase II single arm studies that investigated BV in R/R cHL and compared the data to those

from studies that used chemotherapy only.[21,22,27-29,32-34] We matched data of the BV-studies to the chemo-studies by propensity scores and in this way statistically 'simulated' an RCT. This led to important conclusions, namely that BV+chemo does not seem to increase PFS in patients with primary refractory disease, while this patient population has the highest unmet need for improved therapies, but that it does increase PFS in patients with relapsed disease. In addition, OS was improved, but we have attributed this to improved supportive care and advances in later lines of treatment over time. We also showed that a sequential approach is feasible in the salvage setting, in which patients with no CMR after salvage treatment receive additional chemotherapy before ASCT, with the goal to transplant more patients while in CMR, which has been shown to improve PFS.[28,34,35] By using a sequential approach, salvage chemotherapy could be spared in a subset of fast-responding patients.

In addition to BV, immune checkpoint inhibitors are increasingly being studied in cHL.[10,36,37] In a phase 3 head-to-head comparison of monotherapy with BV versus the checkpoint inhibitor pembrolizumab in patients with R/R cHL who have relapsed after ASCT or were ineligible for ASCT, the median PFS was higher for pembrolizumab versus BV (13.2 vs 8.3 months; p=0.0027) and the treatment with pembrolizumab led to less toxicity and improved quality of life.[36] Another recent study investigating the combination of pembrolizumab with gemcitabine, vinorelbine, and docxorubicin (GVD) salvage chemotherapy in transplant-eligible patients showed a very high pre-ASCT CMR rate of 95% and preliminary PFS data showed no progressive disease events after 1 year of follow-up.[38]

The high CMR rates that are now achieved with regimens such as pembrolizumab-GVD and BV-salvage chemotherapy raise the question whether consolidation with HDCT/ASCT is still necessary in all patients.[14,31,39] The efficacy of HDCT/ASCT is based on two small RCTs performed more than 2 decades ago,[40,41] while nowadays PET-adapted treatment strategies might be more effective in selecting patients who might not need HDCT/ASCT and can be cured with maintenance therapy with checkpoint inhibitors or BV.[31,42] To achieve this, more effective methods of selecting low-risk patients are needed.

Prognostication

The International Prognostic Score (IPS) for cHL, which was developed about 25 years ago by Hasenclever et al.,[43] is the most widely used risk stratification index for predicting outcomes in patients with advanced-stage HL at primary diagnosis. However, it is based on outdated clinical trial data in which long-term PFS and OS were much lower compared to contemporary prognosis in cHL.[15] Recent studies show that the Hasenclever-IPS performs poorly among contemporarily treated patients.[44,45] A novel IPS score, called A-HIPI, has recently been published by the HoLISTIC consortium, a collaborative group of cHL researchers that have combined real-world and clinical-trial data of over 5,000 advanced-stage cHL patients at primary diagnosis.[46] The A-HIPI score, based on age, sex, stage, bulky disease, lymphocyte count, hemoglobin and albumin, showed a 5-year PFS of 87% for patients in the lowest risk group, compared to

71.7% for patients in the highest risk group. Compared to the 5-year PFS of 77% in the whole population, the difference with the highest-risk group is only modest and this score still fails to identify a high-risk group that has poor PFS of <50%. This is important because with a 71.7% 5-year PFS, it is improbable that significant upfront treatment decisions will be based on this risk score. The high PFS indicates a low positive predictive value, and thus a high number needed to treat, thereby challenging the practical utility of the risk score in guiding treatment strategies. Therefore, there is still a need for risk scores that combine clinical data with biomarkers and PET scan data to improve the positive predictive value of risk score systems.

The IPS and A-HIPI scores have both been developed for newly diagnosed advanced-stage cHL patients. A validated risk score for R/R patients is still lacking. Established risk factors in the R/R setting include the presence of B symptoms, extranodal disease, time to relapse <1 year or primary refractory disease after first-line treatment, and not achieving a CMR after salvage treatment prior to ASCT.[47-49] However, no validated prognostic model exists for R/R cHL on which treatment decisions can be based. Outside of clinical trials, virtually all transplant-eligible patients are treated the same way with several cycles of salvage chemotherapy (most commonly used regimens DHAP, ifosfamide, carboplatin, etoposide; ICE), and consolidation with HDCT/ASCT in patients with a CMR or partial response, while patients with stable disease or progression receive other salvage chemotherapy regimens or allogenic stem-cell transplant. In the era of novel treatment options with BV and checkpoint inhibitors, prognostic tools at baseline would be helpful to select patients with high-risk of progression or chemotherapy resistant disease upfront for treatment with novel drugs. Since clinical risk factors are probably not strong enough to create prognostic models with high positive and negative predictive value, it needs to be combined with biomarkers, such as serum TARC, circulating tumor (ct)DNA and quantitative PET features. Interestingly, the first clinical trial incorporating MTV and ctDNA as risk-assessment tools is currently enrolling (NCT04866654).

In **chapters 6-9** we aimed to find prognostic biomarkers and develop prognostic models in R/R cHL patients and to explore biological correlations between several different biomarkers.

Prognostic value of quantitative PET features

Interim response assessment using ^{18}F-fluorodeoxyglucose (FDG) PET-CT is now the standard way of selecting patients for treatment intensification in the primary treatment setting, and is also being used in the salvage setting to select patients with a sufficient response to continue to ASCT.[28,34,50,51] While the negative predictive value of achieving a CMR on long-term PFS in generally high, the positive predictive value is low.[19,27] This is thought to be caused by false-positive PET scans, in which there are no tumor cells in the lymph nodes anymore but there is still an ongoing immune response. To improve prognostication of patients, it is important to not only look at the interim PET, but also at the baseline PET at relapse or primary refractory disease, and the PET scan at primary diagnosis. This can be achieved by visual assessment of PET-CT scans, which is standard in contemporary clinical practice by staging according to the

Ann Arbor system, and response-assessment using the Lugano criteria and Deauville scores.[50,51] Quantitative analysis of PET scans may reveal FDG uptake patterns between patients that give more information than only looking at the lymph node with the highest uptake.

However, analyzing all these PET scans is time-consuming and there was no consensus on a standard segmentation method for quantitative analysis of PET scans in cHL.[52,53] It is important to compare the performance of different segmentation methods before radiomics will be used in more clinical trials, so that results can be compared between trials.[54] Therefore, in **chapter 6**, we analyzed 105 FDG PET-scans with 6 different semi-automatic segmentation methods, and showed that a fixed cutoff of standard uptake value (SUV)4.0 requires the least manual adaptations, and despite the fact that this cutoff sometimes tends to underestimate the metabolic tumor volume (MTV), this did not influence the prognostic value of PET features compared to analysis with other methods.[55] This is consistent with findings in diffuse large B-cell lymphoma (DLBCL), in which the SUV4.0 method was also considered the best method to derive MTV.[53] Although the 41max method has been used in several lymphoma studies, we found that this method requires extensive manual adaptation, which is time-consuming and more susceptible to inter-observer variation.[26,56,57] We also validated the use of 3 methods in a larger cohort in **chapter 7**, in which we developed a prognostic model based on clinical characteristics and quantitative PET features.[58] We analyzed all scans with 3 different segmentation methods and showed that the 41max method had significantly lower prognostic value of PET features compared to SUV4.0 and SUV2.5. Therefore we propose to use SUV4.0 as the standard segmentation method for baseline PET scans in cHL.

In DLBCL, a prognostic model of radiomics features that combined clinical and radiomics features outperformed the international-prognostic-index (IPI) score.[59] This model also used the SUV4.0 method for segmentation to extract the most commonly used radiomics features such as MTV, SUV parameters and dissemination features. The model using all radiomics features including single-lesion radiomics analysis did not outperform the more simple and robust radiomics model. Indicating that intensity-, volume- and dissemination based features capture disease characteristics in a more detailed way than Ann Arbor stage alone.[59] The model that we developed in **chapter 7** is similar to the model in DLBCL. Our model comprised of the clinical features primary refractory vs relapsed disease and B symptoms and the radiomics features MTV, spread (i.e. the sum of all distances between all lesion0 and the tumor-to-liver-ratio (TLR) of SUVmean and yielded a high area under the curve in the validation cohort of 0.75. Our model is the first radiomics model in R/R cHL that was trained in a relatively large R/R cHL population of 110 patients and validated in an external cohort. In addition, we used robust PET features that limit the chance of differences in PET features between different PET scanners and hospitals. This ensures a proper robust analysis and increases the chance of reliable extrapolation to other populations. Furthermore, the integration of both clinical and radiomics features is pivotal in clinical practice, as it allows for a comprehensive evaluation of patients beyond mere PET scan or biomarker assessments. Our model can be used for upfront

clinical decision making and should be investigated in a prospective clinical trial. For example, R/R patients in the high-risk group could be treated with a checkpoint inhibitor, as they are likely to fail on salvage chemotherapy, and patients in the low-risk group could be treated with a sequential approach with BV monotherapy and salvage chemotherapy in patients who do not achieve a CMR.[14,28,34,35] Based on interim PET response and serum TARC assessment, it could be considered for patients with favorable characteristics and a CMR to not consolidate with ASCT but rather treat with checkpoint inhibitor maintenance treatment.[31]

Prognostic value of serum TARC and other biomarkers

Preferably, reinforcing the radiomics model with other biomarker data such as serum TARC and ctDNA would enable to create a model that incorporates insights of various domains. We initiated this approach in **chapter 8** by analyzing serum TARC in R/R cHL patients and integrating these results with quantitative PET features.[25] Serum TARC has been investigated in several studies as a response biomarker and has shown promising results.[60-65] The study by Plattel et al. suggests that TARC exhibits a higher positive predictive value compared to interim PET.[66] This is in line with our findings in the R/R setting in which we demonstrate that serum TARC levels, assessed after just one cycle of BV-DHAP salvage therapy, exhibit high prognostic value comparable to interim PET scans performed after three cycles of BV-DHAP.[25] This suggests that early assessment of serum TARC levels enables risk-adapted treatment strategies, where patients with elevated serum TARC levels may benefit from treatment intensification. In addition, we showed that combining serum TARC and quantitative PET assessment such as $TLR_{SUVpeak}$ and $TLR_{SUVmean}$ on the interim PET scan may increase the positive predictive value of 3-year freedom from progression and thus may limit false-positive findings. However, not all patients displayed elevated serum TARC levels at baseline. Approximately 24% of patients had serum TARC levels below 1,000 pg/mL, and 7% had levels below 500 pg/mL. Since serum TARC at baseline correlates with MTV, which tends to be lower in the R/R setting due to early detection of relapse or primary refractory disease, it follows that in patients without an elevated baseline serum TARC, it cannot serve as a reliable response marker.

ctDNA is another biomarker that has gained attention in cHL as a measure for minimal residual disease. It was recently shown that individual mutational fingerprints correlate with response in newly diagnosed cHL patients.[67] However, ctDNA is an expensive technique and requires complex analysis as compared to measuring serum TARC. Combination of serum TARC and ctDNA might provide additional prognostic information and studies combining these biomarkers are needed.

The high secretion of cytokines by HRS cells is coupled with a high secretion of noncoding microRNAs (miRNAs) in extracellular vesicles (EV), which can potentially be used as diagnostic and prognostic cancer biomarkers. Several miRNAs have been detected in the plasma of cHL patients that correlate with disease activity and treatment response on the interim PET.[68-70] In an exploratory analysis of blood-circulating EV-miRNAs, serum TARC and quantitative PET

features in cHL patients, several HL related miRNAs showed a weak correlation with MTV and dissemination PET features, but not with intensity features.[68] Further analysis is needed to investigate the prognostic value of EV-miRNAs in cHL.

Biological explanation of FDG uptake in cHL

As previously noted, the positive predictive value of the interim PET is often low, and there have been reports of biopsy-confirmed false-positive assessments. These cases reveal a disparity where PET scans indicate high FDG uptake in a lymph node despite absence of HRS cells in the biopsy sample.[71,72] Therefore, it remains important to confirm relapsed or primary refractory disease through biopsy of affected lymph nodes or extranodal sites. In many cancers FDG uptake measured by PET scans typically corresponds to tumor cell infiltration, with higher FDG uptake indicating more aggressive disease. However, this relationship is not as straightforward in cHL due to the fact that HRS cells only comprise 1-5% of tumor mass, which makes it unlikely that HRS cells alone account for the elevated FDG uptake observed in PET scans of cHL patients. In **chapter 9** we sought to explore correlations between FDG uptake patterns on baseline PET scans and gene-expression based cell composition of the TME in cHL. While the TME-signatures reflecting antigen-presenting cells and fibroblasts-extracellular matrix cells showed the highest expression in all patients, the differences in FDG uptake correlated significantly with TME-signatures reflecting regulatory T cells (Tregs), T-synapse (representing genes involved in co-stimulation and immune checkpoints), HRS cells and NK cells. This suggests that an active immune response, driven by immune evasion mechanisms by HRS cells, may primarily account for the high glucose uptake detected in PET scans.[73,74] This study is unique as it is the first study that explores correlations between TME composition and quantitative PET features in cHL. Insights from this study have the potential to deepen our understanding of the biological significance of quantitative PET scan features in clinical practice. However, it is important to acknowledge the study's limitations, including a relatively small sample size and a lack of validation in other cohorts or with alternative methods of assessing TME composition, such as immunohistochemistry staining. Therefore, further research should preferably be done in a larger cohort and integrate gene-expression based TME composition analysis with validation through immunohistochemistry staining or spatial proteomics.[75] We plan, however, to validate the main findings with immunohistochemistry staining in biopsies. This approach would provide a more comprehensive understanding of the relationship between TME composition and quantitative PET features in cHL.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

In conclusion, with the research described in this thesis significant progress towards advancing personalized therapeutic strategies for patients with cHL has been made, particularly in the

R/R setting. By integrating data from various domains such as quantitative PET scans, serum biomarkers, TME composition, and clinical data, we have shed light on important aspects of cHL management.

The treatment landscape for cHL has evolved considerably over recent decades, with novel therapeutic agents like antibody-drug conjugates and immune checkpoint inhibitors offering promising options for patients with R/R disease. Studies conducted within the context of this thesis, including the Transplant BRaVE trial, have demonstrated the efficacy of treatments like BV in combination with chemotherapy followed by ASCT, leading to high CMR rates and improved PFS and OS.

Prognostication in cHL, particularly in the R/R setting, remains a challenge. While established risk scores like the IPS have limitations, exploration of novel biomarkers like serum TARC and quantitative PET scan analysis show promise in refining risk assessment and guiding treatment decisions. Quantitative analysis of PET scans has emerged as a valuable tool in prognostication, with studies in this thesis highlighting the importance of standardized segmentation methods and the integration of PET features with clinical data for improved risk stratification. Additionally, investigations into the biological basis of FDG uptake patterns in cHL, particularly their correlation with TME composition, offer insights into the underlying mechanisms driving PET scan findings.

Looking ahead, future research should focus on further investigating the role of checkpoint inhibitors in the R/R setting, validating prognostic models incorporating PET features, serum biomarkers, and genetic data, as well as exploring novel therapeutic avenues such as immune checkpoint inhibitors and targeted therapies.

A matched individual patient-level meta-analysis akin to our study on BV would be valuable to perform on studies that investigated checkpoint inhibitors in the salvage setting. Such an analysis is planned in collaboration with the HoLISTIC consortium, that developed the earlier mentioned A-HIPI score in a large cohort of 5,000 cHL patients. This also emphasizes the importance of international collaboration in relatively rare diseases like R/R cHL. Such collaboration not only facilitates the pooling of diverse datasets but also presents a unique opportunity to identify and establish clinical risk factors within a large and comprehensive dataset of R/R cHL patients. In addition, clinical trials that investigate the possibility of replacing HDCT/ASCT with maintenance treatment, with checkpoint inhibitors or BV, is a very interesting and revolutionary development and will significantly change the treatment landscape of cHL. The incorporation of risk factors based on clinical data, biomarkers and quantitative PET features in these trials will further contribute to clinical decision making in the R/R setting.

Larger-scale studies investigating quantitative PET features are warranted to implement quantitative PET in clinical practice. First, refinement and standardization of radiomics features and segmentation methods will be crucial. We set an important step in this direction by evaluation different segmentation methods for baseline segmentation. Such an analysis would also be

valuable in the interim PET setting, which generally comes with lesions that show lower FDG uptake. Advances in artificial intelligence and machine learning algorithms will further simplify PET segmentation and possibly lead to fully automated PET segmentation. Our prognostic model using clinical and radiomics features in R/R cHL can be used in a clinical trial to guide treatment decisions. In addition, there is a high need for prognostic radiomics models for newly diagnosed cHL, which could possibly even replace Ann Arbor staging in upfront decision making. A large radiomics analysis in advanced-stage cHL patients is currently ongoing in collaboration with the German Hodgkin Study Group.

Several biomarkers, including serum TARC and ctDNA have the potential to revolutionize response assessment in cHL and could possibly be used in combination with quantitative PET analysis to increase both positive and negative predictive value of response assessment. Moreover, there is a pressing need for additional biological studies in cHL to deepen our understanding of the disease's pathophysiology. Larger studies investigating the relationship between PET features and TME composition could increase our knowledge on glucose metabolism in the TME. Furthermore, delving into the fundamental pathophysiology of cHL, including the transition of precursor mononucleated Hodgkin cells into HRS cells will be crucial in the development of novel targeted therapies. This includes unraveling the mechanisms underlying the high cytokine release observed in HRS cells, which can possibly be explained by the senescence associated secretory phenotype (SASP).[76] This opens up the possibility of employing senolytic drugs in cHL.

Last but not least, more focus is needed on psychological effects of cHL diagnosis and treatment, particularly in the predominantly young population affected by this disease. A cancer diagnosis at a young age can significantly impact psychological well-being, compounded by concerns regarding the potential late-effects of chemotherapy on fertility, cardiovascular health and the risk of secondary malignancies. Thus, optimizing lifestyle and providing psychological support during and after treatment holds immense potential in helping patients navigate this challenging period of their life. Future research efforts should not only focus on assessing the quality of life but also explore interventions aimed at actively improving it, such as implementing lifestyle modifications. By addressing the holistic needs of patients, including their psychological and emotional well-being, we can enhance their overall resilience and improve their long-term outcomes.

Overall, this thesis contributes significantly to the ongoing efforts to personalize and optimize therapeutic approaches for patients with cHL, paving the way for more effective management strategies in the future.

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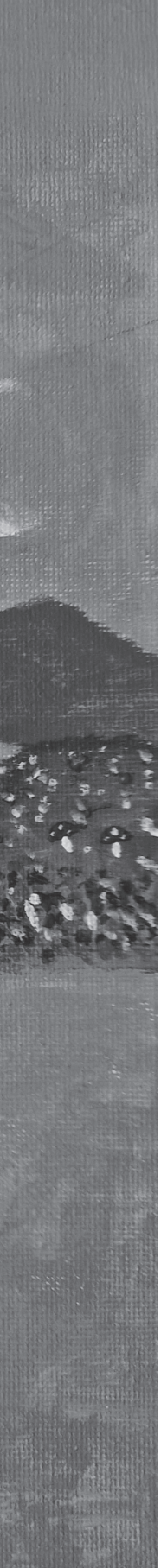


II

English Summary & Nederlandse Samenvatting

ENGLISH SUMMARY

Connecting the dots in classical Hodgkin lymphoma

Classical Hodgkin Lymphoma (cHL) is as the most prevalent lymphoma subtype among adolescents in the Western world. Central to the pathology of cHL are the malignant Hodgkin and Reed-Sternberg (HRS) cells, that originate from germinal center B cells but are often referred to as 'crippled' B cells due to their lack of typical B-lineage markers and aberrant expression of CD30. HRS cells constitute only a minority of the tumor mass, surrounded by a reactive tumor-microenvironment (TME) that is rich in immune cells, particularly CD4+ T helper cells. Despite this abundance of immune cells around the tumor cells, the TME fails to mount an effective response against HRS cells due to their sophisticated immune evasion mechanisms. The diagnosis of cHL relies on biopsy of suspected lymph nodes and staging is performed using ^{18}F-fluorodeoxyglucose (FDG)-positron emission tomography (PET)-computed tomography (CT) scans. Treatment for newly diagnosed cHL is risk-stratified and consists of mainly chemotherapy and radiotherapy. PET-adapted therapy has improved outcomes by allowing treatment intensification or de-escalation based on response. Approximately 15-30% of patients are primary refractory to first-line treatment or relapse (R/R) after an initial response. Salvage treatment may involve high-dose chemotherapy and autologous stem-cell transplantation (ASCT), with novel agents like brentuximab vedotin (BV) and immune checkpoint inhibitors (CPI) offering promising options. Despite notable progress, managing R/R cHL remains challenging, urging the optimization of innovative therapies for better outcomes. Prognostic models can refine outcomes by identifying high-risk patients for treatment intensification, while low-risk patients may benefit from less intense treatment regimens. Biomarkers predicting treatment responses, such as serum Thymus and Activation Regulated Chemokine (TARC) are crucial for advancing strategies. In addition, shifting towards quantitative PET assessment (radiomics) could reveal hidden tumor characteristics, enhancing prognostic capabilities.

The aim of this thesis was to advance towards more personalized and effective therapeutic strategies for patients with cHL, with a focus on the relapsed/refractory setting. This is achieved by connecting information from various domains, including quantitative PET data, serum biomarkers, TME composition, and clinical data.

In **chapter 2**, we review treatment options for R/R cHL patients in various treatment settings, such as first relapse, primary refractory disease and patients who relapse after or are ineligible for ASCT. We emphasize the significance of achieving a complete metabolic response (CMR) before ASCT and discuss the emergence of novel therapies like BV and CPI and the need for risk- and PET-adapted treatment approaches to improve outcomes while minimizing toxicity. Overall, we provided a comprehensive review of current challenges, advancements and future directions in the management of R/R cHL, incorporating recent clinical trial data and highlighting the evolving treatment landscape.

The translation of therapeutic advancements from clinical trials to routine clinical practice is often hindered by strict inclusion and exclusion criteria. Therefore, in **chapter 3**, we conducted a large-scale, nationwide, population-based study in almost 10,000 adult cHL patients diagnosed in the Netherlands over a 29-year period. Our aim was to explore real world treatment trends and relative survival outcomes from 1989 to 2017. We observed a decline in the use of radiotherapy alone, replaced by an increase of the use of combined chemotherapy and radiotherapy, while chemotherapy alone became the preferred treatment for patients with advanced-stage disease. Notably, a substantial number of elderly patients (>60 years) received no anti-neoplastic therapy at all. We showed significant survival improvements for patients up to age 60, attributed to therapeutic advancements and enhanced supportive care. However, elderly patients, aged 60 and above, showed only limited improvement in survival, with notable excess mortality. These findings underscore the need for innovative treatment approaches, particularly for elderly patients, to address the persistent excess mortality in this population.

Chapter 4 and **5** focus on the use of BV in R/R cHL. BV is an anti-CD30 antibody-drug conjugate that specifically targets HRS cells which universally express CD30. In **chapter 4** we present the results from the Transplant BRaVE study, a prospective, multicenter, international Phase I/II study, investigating the efficacy and safety of BV in combination with dexamethasone, high-dose cytarabine and cisplatin (DHAP) for patients with R/R cHL. In a Phase I dose-escalation part in 12 patients, BV-DHAP was shown to be feasible. The Phase II study included 55 R/R cHL patients, with treatment consisting of three cycles of BV-DHAP followed by HDCT/ASCT. Results showed that 81% of evaluable patients achieved a CMR pre-ASCT. The 2-year progression free survival (PFS) was 74% with an overall survival (OS) of 95%. Toxicity was manageable and consisted primarily of hematological adverse events. Notably, patients with a partial metabolic response pre-ASCT had a significantly lower PFS compared to those in whom a CMR was achieved. This study showed that BV-DHAP is a highly effective salvage regimen for R/R cHL patients, but close monitoring for toxicity remains necessary.

Further investigation in randomized controlled Phase III trials (RCT) would be needed to confirm the efficacy of BV over standard salvage chemotherapy alone. However, there are currently no RCTs that compare the addition of BV to salvage chemotherapy alone, except for a small phase IIb RCT that has not been published yet. In **chapter 5** we have performed an individual patient-data analysis of all phase II single arm studies that investigated BV in R/R cHL and compared this to studies that used chemotherapy only. Using propensity score matching, data from ten clinical trials were analyzed, comprising a total of 768 R/R cHL patients. While no significant differences were found in pre-ASCT CMR rates or PFS between the total BV and chemotherapy cohorts, subgroup analysis revealed significant improvements in 3-year PFS in patients with relapsed or stage IV disease in the BV-cohort. Moreover, OS was higher in the BV cohort, but this was likely due to treatment advances in later therapy lines, i.e. the emergence of checkpoint inhibitors as a therapeutic option. Unfortunately, patients with primary refractory disease did not experience improvements in PFS or OS with BV. We showed that sequential

treatment with BV and chemotherapy is feasible and shows potential benefits, sparing salvage chemotherapy in fast-responding patients. These findings, provide valuable insights into optimizing treatment approaches for R/R cHL patients.

Chapter 6 and 7 delve into the quantitative analysis of PET scans, also known as radiomics. Analyzing PET-CT scans for radiomics involves an initial step of segmentation to compute the metabolic tumor volume (MTV). However, a standard segmentation method to derive MTV in cHL was lacking. In **chapter 6**, we aimed to address this gap by investigating six different semi-automatic segmentation methods. We evaluated these methods on their delineation, completeness of lesion selection, and the need for manual adaptation, and tested the correlations and prognostic value of the resulting radiomics features. Our findings from 105 ^{18}F-FDG PET-scans revealed that standard uptake value (SUV)4.0, despite its limitation in detecting small lesions, was the most suitable segmentation method for future research and clinical implementation due to its minimal need for manual adaptation. In **chapter 7**, we validated the applicability of the SUV4.0 method in a larger cohort of 172 R/R cHL patients. Based on our findings, we advocate for the adoption of SUV4.0 as the standard segmentation method for baseline PET scans in cHL. Using the SUV4.0 method, we analyzed MTV and several dissemination radiomics features. We developed a prognostic model using machine learning, encompassing robust radiomics and clinical features. The model was able to identify a subset of high-risk patients with significantly inferior 3-year PFS and OS outcomes. Importantly, we validated our results in an external patient cohort. The model calculates a PET-based risk profile and can be applied to develop risk-stratified treatment strategies for R/R cHL patients.

Preferably, reinforcing the radiomics model with other biomarker data such as serum TARC would enable to create a model that incorporates insights into various domains. We embarked on this approach in **chapter 8** by analyzing serum TARC levels in R/R cHL patients and integrating these results with quantitative PET features. We showed updated results of the Transplant BRaVE study, as introduced in chapter 4, with consistently high 3-year PFS and OS. Immunohistochemistry staining of TARC revealed that weak or negative TARC expression was a significant adverse prognostic marker for disease progression. Moreover, elevated serum TARC levels after one cycle of BV-DHAP correlated strongly with an increased risk of progression. Additionally, both baseline and pre-ASCT SUVmean and SUVpeak (corrected for liver SUVmean) were strong prognostic factors, consistent with our findings in chapter 7. Combining serum TARC levels after 1 cycle with baseline SUVmean/peak, or the combination of pre-ASCT serum TARC and SUVmean/peak, offered complementary prognostic insights. This integration appeared to enhance the positive predictive value of response assessment, aiding in the identification of patients at high risk of progression. While our findings necessitate validation in a larger cohort, they underscore the importance of further exploring the combination of biomarkers across different domains to guide treatment decisions in R/R cHL.

In **chapter 9**, we aimed to explore correlations between FDG uptake patterns on baseline PET scans and gene-expression based cell composition of the TME in corresponding lymph

node biopsies. Analysis was performed on both primary diagnostic and R/R samples, with corresponding clinical data and PET scan features. Moreover, single-lesion radiomics analysis was performed on the specific biopsied lesion. Expression of genes related to the TME was measured using NanoString probes, and certain TME-signatures were calculated that represented various cell types from the TME. Significant correlations were found between several PET features and the HRS cell, T-synapse, Tregs and NK cell signatures. The T-synapse signature, representing costimulatory molecules and immune checkpoints, showed strong correlations with PET features, suggesting an active immune response driving glucose metabolism. Further research in this area, especially in the context of immune checkpoint inhibitors, is warranted. Connecting data from various domains provides interesting insights into the complex interplay between tumor biology, immune response and metabolic activity in cHL.

The findings of this thesis and future perspectives of cHL research are extensively discussed in **chapter 10**.

NEDERLANDSE SAMENVATTING

Klassiek Hodgkin lymfoom (cHL) is een van de meest voorkomende maligniteiten onder jongvolwassenen in de Westerse wereld. cHL wordt gekenmerkt door de aanwezigheid van Hodgkin Reed-Sternberg (HRS) cellen, die ontstaan zijn uit B cellen uit het kiemcentrum maar maligne zijn ontaard. Deze cellen worden ook wel kreupele B cellen genoemd, vanwege het missen van typische B cel markers en een abnormale expressie van markers zoals CD30. HRS cellen vormen slechts een minderheid van de tumor massa, ongeveer 1-5%. Het grootste deel van de cellen bestaat uit een reactief infiltraat van leukocyten, met name CD4+ T helper cellen. Ondanks de aanwezigheid van veel immuun cellen in de tumor-micro omgeving (TME) is er geen effectieve afweerrespons tegen de maligne HRS cellen vanwege allerlei geavanceerde immuun-ontwijkingsmechanismen.

Diagnostiek van cHL berust op een biopt van de verdachte lymfeklier. Stadiëring wordt uitgevoerd middels een ^{18}F-fluorodeoxyglucose (FDG)-positronemissietomografie (PET)-computertomografie (CT) scan. De behandeling bij patiënten met nieuw gediagnosticeerde cHL is risico-gestratificeerd en bestaat voornamelijk uit een combinatie van chemotherapie en radiotherapie. Individuele aanpassing van de behandeling op basis van de tumor respons op de PET heeft de uitkomsten van patiënten significant verbeterd, doordat bij een goede respons de behandeling gede-escaleerd kan worden, en bij een matige respons deze kan worden geïntensiveerd. Ondanks significante verbetering de afgelopen jaren, is ongeveer 15-30% van de patiënten primair refractair op eerstelijnsbehandeling, of er treedt een recidief op na een initiële respons (recidief/refractair; R/R). Tweedelijnsbehandeling bestaat meestal uit hoog gedoseerde chemotherapie en een autologe stamceltransplantatie (ASCT) voor patiënten die daar fit genoeg voor zijn. Voor deze patiëntengroep zijn nieuwe middelen zoals brentuximab vedotin (BV), een anti-CD30 antibody-drug conjugate, en immunotherapie met checkpoint remmers (CPI) veelbelovende opties. Ondanks significante verbetering van overleving en vermindering van toxiciteit door de jaren heen blijft de behandeling van R/R cHL patiënten een uitdaging. Voor deze patiënten is optimalisatie van nieuwe therapieën nodig voor betere resultaten. Prognostische modellen kunnen hierbij helpen doordat ze hoog-risicopatiënten kunnen identificeren, waardoor een betere risico-inschatting mogelijk is en behandeling hierop aangepast kan worden. Biomarkers die prognostische waarde hebben zijn bijvoorbeeld serum Thymus en Activation Regulated chemokine (TARC), welke door HRS cellen geproduceerd wordt en een tumor-specifieke marker is. Daarnaast kan de kwantitatieve analyse van PET-scans, ook wel radiomics genoemd, inter- en intra-tumorale patronen herkennen van de tumor, welke ook als prognostische markers gebruikt kunnen worden.

Het doel van dit proefschrift was om meer gepersonaliseerde en effectieve therapeutische behandelstrategieën te ontwikkelen voor patiënten met cHL, met een focus op de R/R setting. Dit is onderzocht door data van verschillende domeinen te integreren en te verbinden, waar-

onder kwantitatieve PET data, serum biomarkers, genexpressie gebaseerde TME-samenstelling en klinische gegevens.

In **hoofdstuk 2** vatten we behandelopties voor R/R cHL patiënten samen in verschillende behandel settings, waaronder het eerste recidief of de primair refractaire setting en voor patiënten die recidiveren na ASCT of niet in aanmerking komen voor ASCT. We benadrukken het belang van het bereiken van een complete metabole response (CMR) voor ASCT en discussiëren de opkomst van nieuwe middelen zoals BV en CPI en de noodzaak voor risico- en PET-scan gebaseerde behandelstrategieën om uitkomsten te verbeteren en toxiciteit te verlagen. We geven een beknopte review van hedendaagse uitdagingen, ontwikkelingen en toekomstige perspectieven in de behandeling van R/R cHL, waarin we data van recente klinische studies en het snel evoluerende behandellandschap meenemen.

De extrapolatie van therapeutische vooruitgang van studies naar de klinische praktijk wordt vaak belemmerd door strikte inclusie- en exclusie criteria. Daarom hebben we in **hoofdstuk 3** een grootschalige, landelijke studie uitgevoerd op populatieniveau waarin we bijna 10.000 volwassenen met cHL hebben geïncludeerd die tussen 1989 en 2017 zijn gediagnosticeerd in Nederland. Het doel was om patronen in behandelstrategieën en relatieve overlevingsresultaten te onderzoeken door de jaren heen. We constateerden een afname van het gebruik van alleen radiotherapie, waarbij we een toename zagen van het gebruik van gecombineerde chemotherapie en radiotherapie. Voor patiënten met een gevorderd ziektestadium was chemotherapie alleen de voorkeursbehandeling. Opmerkelijk is dat een aanzienlijk deel van de patiënten van 60 jaar en ouder geen antineoplastische behandeling kreeg. Dit valt te verklaren omdat dit vaak veel toxiciteit met zich meebrengt. Er was een significante verbetering in de overleving voor patiënten tot 60 jaar, wat toegeschreven wordt aan therapeutische vooruitgang en verbeterde ondersteunende zorg. Echter was er voor patiënten van 60 jaar en ouder slechts beperkte verbetering in de overleving te zien, met opmerkelijke oversterfte ten opzichte van de Nederlands populatie. Deze bevindingen benadrukken de noodzaak voor het ontwikkelen van innovatieve behandelstrategieën voor ouderen om de overleving in deze groep te verbeteren.

In **hoofdstuk 4** en **5** wordt verder ingegaan op het gebruik van BV in R/R cHL. BV is een anti-CD30 antibody-drug conjugaat dat specifiek gericht is op HRS cellen die altijd CD30 tot expressie brengen. In **hoofdstuk 4** presenteren we de resultaten van de Transplant BRaVE studie: een prospectieve, multicentrische, internationale fase I/II-studie, waarin de werkzaamheid en veiligheid van BV in combinatie met dexamethason, hoge dosis cytarabine en cisplatin (DHAP) werd onderzocht bij patiënten met R/R cHL. In een fase I-dosisescalatiestudie in 12 patiënten werd aangetoond dat behandeling met BV-DHAP haalbaar was. De fase II-studie omvatte 55 R/R cHL-patiënten, waarbij de behandeling bestond uit drie cycli van BV-DHAP gevolgd door ASCT. De resultaten toonden aan dat 81% van de evalueerbare patiënten een CMR vóór ASCT bereikte. De progressievrije overleving (PFS) na 2 jaar was 74% voor de hele groep met een algehele overleving (OS) van 95%. De toxiciteit was controleerbaar en bestond voornamelijk uit hematologische bijwerkingen. Opmerkelijk is dat patiënten met een partiële metabole respons

vóór ASCT een aanzienlijk lagere PFS hadden vergeleken met diegenen die een CMR hadden bereikt. Deze studie toonde aan dat BV-DHAP een zeer effectief tweedelijns behandelregime is voor R/R cHL-patiënten, maar dat nauwlettende monitoring van toxiciteit noodzakelijk blijft.

Verdere onderzoek in gerandomiseerde gecontroleerde fase III-onderzoeken (RCT's) is vereist om de werkzaamheid van BV ten opzichte van standaard tweedelijns chemotherapie te bevestigen. Er zijn echter momenteel geen RCT's die de toevoeging van BV aan tweedelijns chemotherapie vergelijken met alleen chemotherapie, op een kleine fase IIb RCT na die momenteel nog niet is gepubliceerd. In **hoofdstuk 5** hebben we een analyse op individuele patiëntgegeven uitgevoerd van alle fase II studies die BV hebben onderzocht bij R/R cHL. Dit hebben we vergeleken met onderzoeken die alleen chemotherapie gebruikten. Met behulp van propensity score-matching werden gegevens van tien klinische onderzoeken geanalyseerd, bestaande uit in totaal 768 R/R cHL patiënten. Hoewel er geen significante verschillen werden gevonden in CMR percentages vóór ASCT en PFS tussen de totale BV- en chemotherapiecohorten, liet subgroep analyse significante verbeteringen zien in 3-jaars PFS bij patiënten met een recidief of stadium IV-ziekte in het BV-cohort. Bovendien was de OS hoger in het BV-cohort, maar dit is waarschijnlijk toe te schrijven aan vooruitgang in behandeling in latere lijnen van therapie. Helaas was er geen verbetering voor patiënten met primaire refractaire ziekte ten aanzien van de PFS of OS met BV. Daarnaast laten de data zien dat sequentiële behandeling met BV en chemotherapie haalbaar is en potentiële voordelen laat zien, waarbij chemotherapie kan worden bespaard bij een deel van de snel reagerende patiënten. Deze bevindingen bieden waardevolle inzichten in het optimaliseren van behandelstrategieën voor R/R cHL-patiënten.

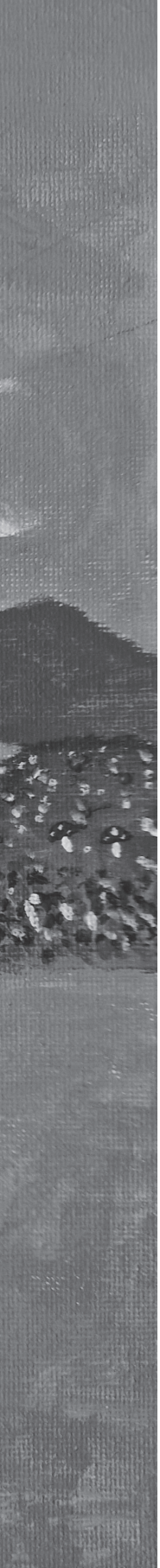
Hoofdstuk 6 en **7** zijn studies naar de kwantitatieve analyse van PET-scans, ook wel bekend als radiomics. De eerste stap voor het analyseren van PET-CT-scans voor radiomics analyse is de segmentatie van het metabool tumorvolume (MTV). Echter, ontbrak er nog een standaard segmentatiemethode om MTV te berekenen bij cHL. In **hoofdstuk 6** hebben we zes verschillende semi-automatische segmentatiemethoden onderzocht met als doel een standaard methode voor segmentatie te vinden. We hebben deze methoden geëvalueerd op de tumor omlijning, volledigheid van laesieselectie, en de noodzaak voor handmatige aanpassing. Vervolgens hebben we de correlaties en prognostische waarde van de resulterende radiomics-kenmerken getest en vergeleken. Onze bevindingen uit 105 ^{18}F-FDG PET-scans lieten zien dat standard uptake value (SUV)4.0, ondanks enige beperkingen bij het detecteren van kleine laesies, de meest geschikte segmentatiemethode was voor toekomstig onderzoek en klinische implementatie vanwege de minimale noodzaak tot handmatige aanpassing. In **hoofdstuk 7** hebben we de toepasbaarheid van de SUV4.0-methode gevalideerd in een groter cohort van 172 R/R cHL-patiënten. Op basis van onze bevindingen pleiten we voor het aanstellen van SUV4.0 als de standaard segmentatiemethode voor PET-scans bij cHL. Met behulp van de SUV4.0-methode hebben we MTV en verschillende disseminatie-kenmerken geanalyseerd. We hebben een prognostisch model ontwikkeld met behulp van machine learning, dat robuuste radiomics- en klinische kenmerken omvat. Het model was in staat om een subset van hoogrisico patiënten te identificeren met

significant slechtere 3-jaars PFS- en OS-resultaten. We hebben onze resultaten gevalideerd in een extern patiëntencohort. Het model berekent een op PET gebaseerd risicoprofiel en kan worden toegepast om risico-gerichte behandelingsstrategieën te ontwikkelen voor R/R cHL-patiënten.

Het radiomics model zou versterkt kunnen worden door andere biomarkers, zoals serum TARC, te integreren in het model. Op die manier worden inzichten uit verschillende domeinen samengebracht en kan een goed risicoprofiel van een patiënt gemaakt worden. In **hoofdstuk 8** hebben we hier een eerste analyse naar gedaan door de analyse van serum TARC waardes bij R/R cHL-patiënten en het integreren van deze resultaten met kwantitatieve PET-kenmerken. Daarnaast geven we een update van de resultaten van de Transplant BRaVE-studie, zoals geïntroduceerd in hoofdstuk 4, die hoge 3-jaars PFS en OS laat zien. Immunohistochemische kleuring van TARC liet zien dat zwakke of negatieve TARC-expressie een significante prognostische marker was voor ziekteprogressie. Bovendien correleerden verhoogde serum TARC waardes na één cyclus van BV-DHAP sterk met een verhoogd risico op progressie. Daarnaast waren zowel de baseline als pre-ASCT SUVmean en SUVpeak (gecorrigeerd voor lever-SUVmean) sterke prognostische factoren, wat in lijn ligt met onze bevindingen in hoofdstuk 7. Het combineren van serum TARC waardes na 1 cyclus met baseline SUVmean/peak, of de combinatie van pre-ASCT serum TARC en SUVmean/peak, had complementaire prognostische waarde. Deze integratie leek de positieve voorspellende waarde van responsevaluatie te verbeteren, wat helpt bij het identificeren van patiënten met een hoog risico op progressie. Hoewel onze bevindingen validatie in een grotere cohort vereisen, benadrukken ze het belang van verdere verkenning van de combinatie van biomarkers over verschillende domeinen om klinsche besluitvorming bij R/R cHL te verbeteren.

Het doel van **hoofdstuk 9** was om correlaties te exploreren tussen de patronen van FDG-opname op baseline PET-scans en genexpressie-gebaseerde celcompositie van de TME. De analyse werd uitgevoerd op zowel primaire diagnostische als R/R-biopten, met bijbehorende klinische gegevens en PET-scan parameters. Daarnaast werd een aparte radiomics-analyse uitgevoerd op de specifieke lymfeklier die gebiopteerd was. De expressie van genen gerelateerd aan de TME werd gemeten met NanoString-probes, en TME-profielen werden berekend die verschillende celtypen van de TME vertegenwoordigden. Er werden significante correlaties gevonden tussen verschillende PET-parameters en de HRS-cel, T-synaps, Tregs en NK-cell TME-profielen. Het T-synaps profiel, dat bestaat uit genen met betrekking tot co-stimulatie van T-cellen en immuun-checkpunten, vertoonde sterke correlaties met PET-parameters, wat suggereert dat een actieve immuunrespons het glucosemetabolisme aandrijft in cHL. Verder onderzoek op dit gebied, vooral in de context van immunotherapie met checkpoint inhibitors, is noodzakelijk om de rol van PET bij immunotherapie beter te begrijpen. Het verbinden van gegevens uit verschillende domeinen biedt interessante inzichten in de complexe interactie tussen tumorbiologie, immuunrespons en metabole activiteit bij cHL.

De bevindingen van dit proefschrift en de toekomstperspectieven van cHL onderzoek worden uitgebreid besproken in **hoofdstuk 10.**



A

PhD portfolio
List of publications
About the author / Curriculum Vitae
Acknowledgements / Dankwoord

PHD PORTFOLIO

Name PhD student: **Julia Driessen**
PhD period: **2019-2024**
PhD supervisors: **Prof. dr. M.J. Kersten & prof. dr. J.M. Zijlstra-Baalbergen**
Co-supervisors: **Dr. A. Diepstra & Dr. A.G. Dinmohamed**

1. PhD training

General courses	**Date/year**	**Workload (ECTS)**
- Practical Biostatistics	09-01-2019	1.1
- Bioinformatics Sequence Analysis	11-03-2019	1.1
- Genetic epidemiology	19-03-2019	1.1
- Epigenetics and epitranscriptomics (OOA)	20-05-2019	1.5
- AMC World of science	20-06-2019	0.7
- eBROK	19-09-2019	1.5
- Peer to peer	2019	0.5
- Using R for Data Analysis	28-01-2019	1.5
- ImageJ	31-01-2020	0.6
- Advanced biostatistics	19-01-2021	2.1
Subtotal		**11.7**
Specific courses	**Date**	**Workload (ECTS)**
-		
Subtotal		**0**
Seminars, workshops and master classes	**Date**	**Workload (ECTS)**
- Workshop PET-CT imaging malignant lymphomas (8hr)	17-01-2019	0.4
- Radiomics journal club (1x/2 weeks = 24 times/yr)	2019	2.0
- Journal club hemato-onco epidemiology (1x/month)	2019	1.0
- Symposium: Waldenstrom / IgM (3hr)	07-02-2019	0.1
- Symposium: HL + DLBCL UMCG (6hr)	16-10-2019	0.2
- LYMMCARE journal club (1x/week)	2021	0.4
- AUMC research meeting (1x/2 weken)	2022-2023	0.2
- Hodgkin liquid biopsy symposium (6hr)	24-03-2022	0.2
- Hodgkin symposium Takeda (6hr)	09-03-2023	0.2
- EORTC lymphoma meeting (8hr)	10-03-2023	0.3
Subtotal		**5.0**
Presentations	**Date**	**Workload (ECTS)**
- Dutch Hematology Congress (oral presentation Transplant Brave study)	23-01-2019	0.5
- Cancer Center Amsterdam (CCA) retreat (oral presentation lenalidomide in CLL)	14-02-2019	0.5
- European Hematology Association congress (2 posters: lenalidomide in CLL and HL epidemiology)	13-06-2019	1.0

- EANM congress (oral presentation MTV segmentation)	12-10-2019	0.5
- American Society of Hematology annual meeting (poster presentation MTV segmentation)	06-12-2019	0.5
- Dutch Hematology Congress (oral presentation HL epidemiology)	22-01-2020	0.5
- Cancer Center Amsterdam retreat (poster quantitative PET analysis)	05-03-2020	0.5
- European Hematology Association congress (2 oral presentations: elderly HL patients and conditional relative survival)	12-06-2020	1.0
- European Hematology Association congress (oral presentation prognostic radiomics model; poster presentation biomarkers in HL)	09-06-2021	1.0
- International Conference on Malignant Lymphoma (oral presentation prognostic radiomics model; poster presentation biomarkers in HL)	19-06-2020	1.0
- 8th international workshop on PET in lymphoma and myeloma (oral presentation prognostic radiomics model)	10-09-2021	0.5
- American Society of Hematology annual meeting (oral presentation brentuximab in HL IPD study)	11-12-2021	0.5
- Dutch Hematology Congress (oral presentation HL radiomics)	20-01-2022	0.5
- International Symposium on Hodgkin Lymphoma (poster brentuximab in HL IPD study)	22-10-2022	0.5
- American Society of Hematology annual meeting (poster Nanostring and PET)	9-12-2022	0.5
- Dutch Hematology Congress (oral presentation Nanostring and PET)	25-01-2023	0.5
Subtotal		**10**
(Inter)national conferences	**Date**	**Workload (ECTS)**
- Dutch Hematology Congress, Papendal, NL (3 days)	23-01-2019	0.75
- Cancer Center Amsterdam retreat, Noordwijkerhout, NL (2 days)	14-02-2019	0.5
- European Hematology Association congress, Amsterdam, NL (3 days)	13-06-2019	0.75
- EANM congress, Barcelona, Spain (4 days)	12-10-2019	1.0
- American Society of Hematology annual meeting, Orlando, FL, USA (4 days)	06-12-2019	1.0
- Dutch Hematology Congress, Papendal NL (2 days)	22-01-2020	0.5
- Cancer Center Amsterdam retreat, Noordwijkerhout, NL (2 days)	05-03-2020	0.5
- European Hematology Association congress, virtual (3 days)	11-06-2020	0.75
- American Society of Hematology annual meeting, virtual (4 days)	02-12-2020	1.0
- European Hematology Association congress, virtual (3 days)	09-06-2021	0.75
- International Conference on Malignant Lymphoma, virtual (3 days)	19-06-2020	0.75
- 8th international workshop on PET in lymphoma and myeloma, virtual (2 days)	10-09-2021	0.5
- American Society of Hematology annual meeting, virtual (4 days)	11-12-2021	1.0
- Dutch Hematology Congress, virtual (1 day)	19-01-2022	0.25
- European Hematology Association congress, Vienna, Austria (3 days)	10-06-2022	0.75
- International Symposium on Hodgkin Lymphoma, Cologne, Germany (3 days)	22-10-2022	0.75
- American Society of Hematology annual meeting, New Orleans, LA, USA (4 days)	09-12-2022	1.0
- Dutch Hematology Congress, Papendal, NL (1 day)	25-01-2023	0.25

-	
Subtotal	**12.75**
Total *(20-30 ECTS points recommended)*	**39.45**

2. Teaching

Lecturing	**Year**	**Workload (Hours/ ECTS)**
Supervising		
- Third year medical student for Bachelor thesis	2020	1-2h/week for 6 months: 1.5 ECTS
- Supervising MD/PhD student	2021-2024	2h/week for 3 years: +/- 7 ECTS
- Amsterdam Medical Student Journal staff reviewer Hematology/ Oncology	2020-2024	1h/month for 3 years: 1.3 ECTS

3. Parameters of Esteem

Grants and awards	**Year**
Travel grant for the 24th congress of the European Hematology Association	2019
ASH Abstract Achievement Award for the 2019 ASH Annual Meeting	2019
Travel grant for the 25th congress of the European Hematology Association	2020
Clinical Trainee Award, Young EHA Best Abstracts Awards Oral presentation	2020
Swiss Cancer Research Foundation educational grants for the 16th ICML lymphoma conference.	2021
ASH Abstract Achievement Award for the 2021 ASH Annual Meeting	2021
Abstract award of the International Symposium on Hodgkin Lymphoma (ISHL)	2022
ASH Abstract Achievement Award for the 2022 ASH Annual Meeting	2022

LIST OF PUBLICATIONS

Inside this thesis

Kersten MJ*, **Driessen J***, Zijlstra JM, Plattel WJ, Morschhauser F, Lugtenburg PJ, Brice P, Hutchings M, Gastinne T, Liu R, Burggraaff CN, Nijland M, Tonino SH, Arens AIJ, Valkema R, van Tinteren H, Lopez-Yurda M, Diepstra A, De Jong D, Hagenbeek A. Combining brentuximab vedotin with dexamethasone, high-dose cytarabine and cisplatin as salvage treatment in relapsed or refractory Hodgkin lymphoma: the phase II HOVON/LLPC Transplant BRaVE study. Haematologica. 2021 Apr 1;106(4):1129-1137. doi: 10.3324/haematol.2019.243238. PMID: 32273476; PMCID: PMC8018114.

Driessen J, Visser O, Zijlstra JM, Lugtenburg PJ, Plattel WJ, Kersten MJ, Dinmohamed AG. Primary therapy and relative survival in classical Hodgkin lymphoma: a nationwide population-based study in the Netherlands, 1989-2017. Leukemia. 2021 Feb;35(2):494-505. doi: 10.1038/s41375-020-0875-0. Epub 2020 May 28. PMID: 32461630.

Driessen J, Tonino SH, Moskowitz AJ, Kersten MJ. How to choose first salvage therapy in Hodgkin lymphoma: traditional chemotherapy vs novel agents. Hematology Am Soc Hematol Educ Program. 2021 Dec 10;2021(1):240-246. doi: 10.1182/hematology.2021000311. PMID: 34889399; PMCID: PMC8791111.

Driessen J*, Zwezerijnen GJC*, Schöder H, Drees EEE, Kersten MJ, Moskowitz AJ, Moskowitz CH, Eertink JJ, Vet HCW, Hoekstra OS, Zijlstra JM, Boellaard R. The Impact of Semiautomatic Segmentation Methods on Metabolic Tumor Volume, Intensity, and Dissemination Radiomics in ^{18}F-FDG PET Scans of Patients with Classical Hodgkin Lymphoma. J Nucl Med. 2022 Sep;63(9):1424-1430. doi: 10.2967/jnumed.121.263067. Epub 2022 Jan 6. PMID: 34992152; PMCID: PMC9454468.

Driessen J*, Kersten MJ*, Visser L, van den Berg A, Tonino SH, Zijlstra JM, Lugtenburg PJ, Morschhauser F, Hutchings M, Amorim S, Gastinne T, Nijland M, Zwezerijnen GJC, Boellaard R, de Vet HCW, Arens AIJ, Valkema R, Liu RDK, Drees EEE, de Jong D, Plattel WJ, Diepstra A; HOVON Lunenburg Lymphoma Phase I/II Consortium (LLPC). Prognostic value of TARC and quantitative PET parameters in relapsed or refractory Hodgkin lymphoma patients treated with brentuximab vedotin and DHAP. Leukemia. 2022 Dec;36(12):2853-2862. doi: 10.1038/s41375-022-01717-8. Epub 2022 Oct 14. PMID: 36241696.

Driessen J, Zwezerijnen GJC, Schöder H, Kersten MJ, Moskowitz AJ, Moskowitz CH, Eertink JJ, Heymans MW, Boellaard R, Zijlstra JM. Prognostic model using ^{18}F-FDG PET radiomics predicts progression-free survival in relapsed/refractory Hodgkin lymphoma. Blood Adv. 2023

Nov 14;7(21):6732-6743. doi: 10.1182/bloodadvances.2023010404. PMID: 37722357; PMCID: PMC10651466.

Driessen J, de Wit F, Herrera AF, Zinzani PL, LaCasce AS, Cole PD, Moskowitz CH, García-Sanz R, Fuchs M, Mueller H, Borchmann P, Santoro A, Schöder H, Zijlstra JM, Hutten BA, Moskowitz AJ, Kersten MJ. Brentuximab Vedotin and Chemotherapy in Relapsed/Refractory Hodgkin Lymphoma: a Propensity Score Matched Analysis. Blood Adv. 2024 Mar 19:bloodadvances.2023012145. doi: 10.1182/bloodadvances.2023012145. Epub ahead of print. PMID: 38502227.

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Outside this thesis

Drees EEE*, **Driessen J***, Zwezerijnen GJC, Verkuijlen SAWM, Eertink JJ, van Eijndhoven MAJ, Groenewegen NJ, Vallés-Martí A, de Jong D, Boellaard R, de Vet HCW, Pegtel DM, Zijlstra JM. Blood-circulating EV-miRNAs, serum TARC, and quantitative FDG-PET features in classical Hodgkin lymphoma. EJHaem. 2022 Apr 28;3(3):908-912. doi: 10.1002/jha2.432. PMID: 36051072; PMCID: PMC9422001.

Drees EEE, Roemer MGM, Groenewegen NJ, Perez-Boza J, van Eijndhoven MAJ, Prins LI, Verkuijlen SAWM, Tran XM, **Driessen J**, Zwezerijnen GJC, Stathi P, Mol K, Karregat JJJP, Kalantidou A, Vallés-Martí A, Molenaar TJ, Aparicio-Puerta E, van Dijk E, Ylstra B, Groothuis-Oudshoorn CGM, Hackenberg M, de Jong D, Zijlstra JM, Pegtel DM. Extracellular vesicle miRNA predict FDG-PET status in patients with classical Hodgkin Lymphoma. J Extracell Vesicles. 2021 Jul;10(9):e12121. doi: 10.1002/jev2.12121. Epub 2021 Jul 15. PMID: 34295456; PMCID: PMC8282992.

Kater AP, van Oers MHJ, van Norden Y, van der Straten L, **Driessen J,** Posthuma WFM, Schipperus M, Chamuleau MED, Nijland M, Doorduijn JK, Van Gelder M, Hoogendoorn M, De Croon F, Wittebol S, Kerst JM, Marijt EWA, Raymakers RAP, Schaafsma MR, Dobber JA, Kersting S, Levin MD; HOVON CLL study group. Feasibility and efficacy of addition of individualized-dose lenalidomide to chlorambucil and rituximab as first-line treatment in elderly and FCR-unfit patients with advanced chronic lymphocytic leukemia. Haematologica. 2019 Jan;104(1):147-154. doi: 10.3324/haematol.2018.193854. Epub 2018 Aug 16. PMID: 30115656; PMCID: PMC6312018.

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AUTHOR CONTRIBUTIONS PER CHAPTER

How to choose first salvage therapy in Hodgkin lymphoma: traditional chemotherapy vs novel agents.
Hematology Am Soc Hematol Educ Program, 2021

All authors designed the study, collected, analyzed and interpreted the data, and wrote and approved the final version of the manuscript.

Primary therapy and relative survival in classical Hodgkin lymphoma: a nationwide population-based study in the Netherlands, 1989-2017.
Leukemia, 2021

AGD designed the study; JD analyzed the data; AGD provided statistical support; OV collected the data; JD wrote the manuscript with contributions from all authors, who also interpreted the data, and read, commented on, and approved the final version of the manuscript.

Combining brentuximab vedotin with dexamethasone, high-dose cytarabine and cisplatin as salvage treatment in relapsed or refractory Hodgkin lymphoma: the phase II HOVON/LLPC Transplant BRaVE study.
Haematologica, 2021

MJK and AH designed the study; all authors collected the data; JD, MLY and HvT analyzed the data; JD and MJK wrote the manuscript with contributions from all authors, who also interpreted the data, read, commented on, and approved the final version of the manuscript; DdJ and AD performed the central pathology review; JZ, CB, AA and RV organized and performed the central FDG-PET-CT review; MJK and AH supervised the study.

Brentuximab Vedotin and Chemotherapy in Relapsed/Refractory Hodgkin Lymphoma: a Propensity Score Matched Analysis.
Blood Advances, 2024

JD and MJK designed the study. All authors collected the data. FdW and JD performed the database harmonization. HS performed the PET revision. JD performed the statistical analysis under supervision of BAH. JD and FdW drafted the manuscript with contributions from all authors.
All authors interpreted the data, read, commented on, and approved the final version of the Manuscript.

The Impact of Semiautomatic Segmentation Methods on Metabolic Tumor Volume, Intensity, and Dissemination Radiomics in 18F-FDG PET Scans of Patients with Classical Hodgkin Lymphoma.
The Journal of Nuclear Medicine, 2022

JD, GJCZ, RB and JMZ designed the study. JD, EEED, MJK, JMZ, HS, AJM and CHM collected the data. JD and GJCZ performed the MTV segmentation analysis. GJCZ and HS reviewed the PET scans and supervised the segmentation analysis. JD performed the statistical analysis. JD drafted the manuscript with contributions from all authors. All authors interpreted the data, read, commented on, and approved the final version of the manuscript.

Prognostic model using 18F-FDG PET radiomics predicts progression-free survival in relapsed/refractory Hodgkin lymphoma.
Blood Advances, 2023

Contribution: J.D., R.B., and J.M.Z. designed the study; J.D. performed the PET segmentation under supervision of G.J.C.Z. and H.S.; G.J.C.Z. and H.S. reviewed the staging and response assessment of the PETs; J.D. performed the statistical analysis and drafted the manuscript, with contributions from all authors; and all authors collected and interpreted the data, and read, commented on, and approved the final version of the manuscript.

Prognostic value of TARC and quantitative PET parameters in relapsed or refractory Hodgkin lymphoma patients treated with brentuximab vedotin and DHAP.
Leukemia, 2022

MJK and Anton Hagenbeek designed and supervised the clinical study. AD supervised the biomarker study. All authors collected the data. JD and LV performed biomarker analysis. JD performed the PET segmentation. GJCZ and RB supervised the PET segmentation. DdJ and AD performed the central pathology review. AA, RV and GJCZ performed the central PET-CT review. JD performed the statistical analysis. JD wrote the manuscript with contributions from all authors. All authors interpreted the
data, read, commented on, and approved the final version of the manuscript.

Tumor Microenvironment Composition Correlates with Quantitative 18F-FDG PET-CT Features in classical Hodgkin Lymphoma.
Manuscript in preparation

JD and AD designed the study. All authors collected the data. JD and LV performed biomarker analysis. JD performed the PET segmentation. GJCZ and RB supervised the PET segmentation.

JD performed the statistical analysis. JD drafted the manuscript with contributions from all authors. All authors interpreted the data, read, commented on, and approved the final version of the manuscript.

ABOUT THE AUTHOR

Julia Driessen was born on July 7th 1997 in Amsterdam, the Netherlands. By chance this was in the AMC hospital. She graduated cum laude from the A. Roland Holst College in Hilversum in 2015, after which she started her study Medicine in 2015 at the University of Amsterdam in the Amsterdam UMC, location AMC. During a nursing internship at the Hematology department in the first year of her studies, Julia's fascination with Hematology blossomed and she started working at the clinical Hematology department as a nursing assistant during the holidays. She also started doing research at the Oncology department in addition to her studies because she wanted to know more about research, and she wrote her bachelor thesis within the Hematology domain. Her passion for research led her to decide to pursue a PhD before undertaking her medical internships. In 2018, at the age of 21, she started her PhD research described in this thesis under the supervision of prof. dr. Marie José Kersten and prof. dr. Josée Zijlstra. Throughout her doctoral tenure, Julia's curiosity propelled her into diverse research projects spanning the spectrum of classical Hodgkin lymphoma. Her goal was to to enhance risk-stratification and refine clinical decision-making by connecting data from various domains of research. During the final two years of her PhD journey, she concurrently completed her medical internships and obtained her medical degree in 2024.

In addition to her dedication to medicine and research, Julia leads a vibrant artistic life as a musician known by the stage name 'Juulz'. Proficient in piano and Hammond organ, she has performed on many stages across Europe, touring with various bands. Notably, in 2019, she embarked on a tour with the renowned Australian hard-rock band Wolfmother throughout Europe and performed at a major festival in Australia. Julia's creative spirit extends to songwriting, and she has penned numerous original compositions. Upon completing her PhD, she harbors ambitions of assembling a new band to embark on the exciting journey of recording an album.

In the future, Julia aspires to integrate her passions for medicine and music, envisioning a more holistic approach to patient care. Her goal is to consider not only traditional medical treatments but also lifestyle and environmental factors that influence diseases. By adopting this comprehensive perspective, she aims to enhance health outcomes by addressing the holistic well-being of each patient.

DANKWOORD

De artikelen in dit proefschrift zijn tot stand gekomen op de afdeling Hematologie van het Amsterdam UMC. Het was een bijzondere en zeer leerzame tijd. Ik wil graag iedereen bedanken met wie ik in deze periode heb mogen samenwerken. Mijn hartelijke dank gaat uit naar de patiënten die deelnamen aan de studies, hun toestemming gaven en wiens data en materiaal ik mocht gebruiken. Een aantal personen wil ik hieronder in het bijzonder noemen.

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Beste Arjan, graag wil ik je hartelijk bedanken voor jouw begeleiding bij de TARC- en nanostring-projecten en voor de kans om de samples in Groningen te analyseren. De periode in Groningen was voor mij een waardevolle ervaring, waar ik veel van heb geleerd. Het was mijn eerste echte laboratoriumervaring en ik haal er nog steeds veel waarde uit. Jouw uitmuntende kennis over Hodgkin lymfoom heeft geleid tot extra diepgang in de projecten van mijn PhD. Ik waardeer jouw deskundigheid enorm en ben dankbaar voor de mogelijkheid om met jou te werken.

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geleerd over statistiek en epidemiologie, wat vaardigheden zijn die ik later met succes heb toegepast in andere projecten.

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I would like to extend a special thank you to dr. Alison Moskowitz and dr. Heiko Schöder for their invaluable collaboration on the PET radiomics project and for facilitating my research internship at Memorial Sloan Kettering Cancer Center in New York. During my time there, I had the opportunity to expand my knowledge on radiomics, where you generously provided access to analyse PET-CT scans to enrich my database. Alison, I am grateful for the insightful tour of the outpatient clinic, which provided invaluable insights into patient care at MSKCC. Heiko, thank you for your assistance with the PET scan review. Your guidance and support have been instrumental to my research journey.

Building upon this, I would like to express my sincere gratitude to all the collaborators of the BV-chemo individual patient study, who graciously contributed their data for our analysis. It has been a pleasure to meet and interact with each of you at various congresses, with a special mention to the meeting in Cologne. Your participation and shared data have been invaluable in advancing our research efforts. A special thank to Barbara Hutten for helping with the complex statistics in this paper!

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