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RUXOLITINIB IN ACUTE AND CHRONIC GRAFT-VERSUS-HOST DISEASE

EXTENDED FOLLOW-UP OF A COMBINED NAMED PATIENT AND IN-LABEL USE COHORT INCLUDING OVERLAP- AND DONOR LYMPHOCYTE INDUCED GVHD

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EXTENDED RETROSPECTIVE MONOCENTRIC STUDY OF RUXOLITINIB FOR REFRACTORY ACUTE AND CHRONIC (INCLUDING OVERLAP) GRAFT VERSUS HOST DISEASE (GVHD), AND INCLUDING DONOR LYMPHOCYTE INDUCED GVHD (INCLUDING NAMED PATIENT USE AND IN-LABEL USE)

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1. Abstract

Acute and chronic GvHD are frequent and serious complications after allogeneic haematopoietic stem cell transplantation. Ruxolitinib (RUX) is the only agent approved by EMA for treatment of steroid refractory (SR) acute and chronic GvHD.

We retrospectively evaluated 118 patients with acute (61 patients), classical chronic (43 patients) and overlap chronic GvHD (14 patients) who received RUX as second line (2L) therapy or beyond 2L. In 22 patients, GvHD occurred after donor lymphocyte infusion (DLI). The cohort included heavily pretreated patients, with 26,2% of acute GvHD in 3rd line or beyond and 29,8% of chronic GvHD in 4th line or beyond.

Best overall response rate to RUX was 68,9% in acute GvHD, 62,8% in classical cGvHD and 78,6% in overlap cGvHD. The response increased to 78,7% in acute GvHD, 74,4% in classical cGvHD and 85,7 % in overlap cGvHD upon the addition of other agents to RUX in a proportion of patients. Steroid dose was successfully tapered to a median of 0 mg/kg of body weight in all three GvHD types. Under RUX treatment, grade 3/4 infections occurred in 39,8%, and CMV reactivation in 22,0%. In 9 patients (7,6%) relapse occurred under or after RUX treatment. With a median survivors' follow up of 46,2 (range, 13,8 – 85,8) months from initiation of RUX, the 2-year probability of survival was 57,0% in aGvHD, 86,0% in classical cGvHD, and 78,6% in overlap cGvHD. Only 22 patients (18,6%) met all in-/exclusion criteria of the respective pivotal phase III studies. The most frequent incompatibilities were unmet SR criteria, excessive pretreatment lines, DLI-associated GvHD, overlap GvHD, and medical and/or haematological exclusion criteria.

Our findings confirm the favourable efficacy profile of RUX established in the prospective studies. Real world use of RUX may differ from the use in the prospective studies in terms of an earlier and more liberal initiation, particularly with regard to SR criteria. On the other hand, our cohort also demonstrated efficacy in patients with excessive pretreatment, and in patients with DLI-induced and/or overlap GvHD.

2. Zusammenfassung

Die akute und chronische Graft-versus-Host Erkrankung sind häufige und gravierende Komplikationen nach allogener hämatopoetischer Stammzelltransplantation. Ruxolitinib (RUX) ist die einzige von der EMA zugelassene Therapie für Steroid-refraktäre (SR) akute und chronische GvHD.

Wir untersuchten 118 Patienten mit akuter (61 Patienten), klassischer chronischer (43 Patienten) und overlap chronischer GvHD (14 Patienten) retrospektiv, die RUX in zweiter oder höherer Linie erhielten. Bei 22 Patienten trat die GvHD im Zusammenhang mit einer Spenderlymphozyten-Infusion auf. Unsere Kohorte inkludiert stark vortherapierte Patienten, wobei 26,2% der Patienten mit akuter GvHD Ruxolitinib als dritte oder höhere Linie und bei 29,8 % der cGvHD Patienten als vierte oder höhere Linie erhielten.

Das beste Gesamtansprechen auf Ruxolitinib war 68,9 % bei akuter GvHD, 62,8 % bei klassischer cGvHD and 78,6 % bei overlap cGvHD. Durch das Hinzufügen weiterer Wirkstoffe in einem Teil der Patienten konnte die Ansprechrate auf 78,7 % in aGvHD, 74,4 % in klassischer cGvHD and 85,7 % in overlap cGvHD verbessert werden. Die Steroiddosis konnte in allen drei GvHD-Typen erfolgreich auf 0 mg/kg Körpergewicht im Median ausgeschlichen werden.

Während der RUX-Behandlung kam es in 39,8 % zu Grad 3/4 Infektionen und in 22,0% CMV-Reaktivierungen. Bei 9 Patienten (7,6%) kam es zu einem Relapse während oder nach der RUX-Behandlung. Mit einem Follow-Up von Überlebenden von 46,2 (range, 13,8 – 85,8) Monaten nach RUX-Beginn ergibt sich eine 2-Jahres-Überlebenswahrscheinlichkeit von 57,0% in aGvHD, 86,0% in klassischer cGvHD und 78,6% in overlap cGvHD. Nur 22 Patienten (18,6%) erfüllten alle Ein-/Ausschlusskriterien der jeweiligen Phase-III-Studien. Die häufigsten Ausschlussgründe waren nicht erfüllte SR-Kriterien, exzessive Anzahl der Vor-Therapielinien, DLI-assoziierte GvHD, overlap GvHD und andere medizinische oder hämatologische Ausschlussgründe.

Unsere Ergebnisse bestätigen das günstige Wirkungsprofil von RUX aus den prospektiven REACH-Studien. Real-World-Anwendung von Ruxolitinib kann sich von der in den prospektiven Studien im Hinblick auf den früheren und liberaleren Beginn unterscheiden, insbesondere in Bezug auf die SR-Kriterien. Andererseits demonstriert unsere Kohorte die Wirksamkeit in exzessiv vortherapierten Patienten und in jenen mit DLI-assoziierter und/oder overlap GvHD.

3. List of abbreviations

2L	second (2nd) line
8-MOP	8-methoxypsoalen
AB0	major human blood group including erythrocyte antigens, A, B, 0
ADL	activities of daily living
aGvHD	acute GvHD
AKI	acute kidney injury
ALL	acute lympoblastic leukaemia
ALT	alanine transaminase
AML	acute myeloid leukaemia
AP	alkaline phosphatase
APC	antigen presenting cells
AraC	cytosine arabinoside
ASH	American Society of Hematology
ATG	anti-thymocyte globulin
ATLG	anti-T-lymphocyte globulin (= ATG)
BAL	broncho-alveolary lavage
BCR	B-cell receptor
BM	bone marrow
BOS	bronchiolitis obliterans syndrome
BSA	body surface area
BTK	Bruton's tyrosine kinase
CAR-T	chimeric antigen receptor T cell
CB	cord blood
CD	cluster of differentiation
cGvHD	chronic GvHD
CMV	cytomegalovirus
CNI	calcineurin inhibitor
COP	cryptogenic organizing pneumonia
COPD	chronic obstructive pulmonary disease
CR	complete response
CSA	cyclosporine A
CT	computer tomography
CTL	cytotoxic T-cell lymphocyte
CYP	cytochromes P450, superfamily of enzymes
DAMP	damage-associated molecular pattern
DC	dendritic cell
DLCO	diffusing capacity of the lungs for carbon monoxide
DLI	donor lymphocyte infusion
DLT	donor lymphocyte transfusion
DNA	desoxy-ribo-nuclein acid
EBMT	European Group for Blood and Marrow Transplantation
EBMT-ADT	EBMT – Alternating Decistion Tree
EBV	Epstein-Barr-Virus
ECOG	Eastern Cooperative Oncology
ECP	extracorporeal photopheresis

EMA	european medicines agency
ES	engraftment syndrome
ES	engraftment syndrome
ET	essential thrombocytaemia
FDA	food and drugs administration (U.S.)
FEV1	forced expiratory volume in 1 second
FMT	faecal microbiota transplantation
FOXP3	transcription factor forkhead box P3
G-CSF	granulocyte colony stimulating factor
GI	gastrointestinal
GIST	gastrointestinal stromal tumor
GLP-2	glucagon-like-peptide-2
GM-Dexa	Glandomed®-Dexamethasone
GvHD	graft-versus-host disease
GvL	graft versus leukaemia effect
HCT	Hematopoietic Cell Transplantation
HCT-CI	Hematopoietic Cell Transplantation Comorbidity Index
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HSCT	hematopoietic stem cell transplantation
i.v.	intravenous
IgG	immune globulin G
IL	interleukin
ISCT	international Society for Cell & Gene Therapy
JACIE	joint accreditation committee – ISCT and EBMT
JACIE	Joint Accreditation Committee of ISCT and EBMT
JAK	janus kinase
KIR-L	killer-cell Ig-like receptor- ligand
KPS	Karnofsky Performance status
MAC	myeloablative conditioning regimen
MAGIC	Mount Sinai Acute GvHD International Consortium
MDS	myelodysplastic syndrome
mEBMT	modified European Group for Blood and Marrow Transplantation
mg/kg	milligrams per kilogram of body weight
MHC	major histocompatibility complex
ml	millilitre
MM	multiple myeloma
MMF	mycophenolate mofetil
MMUD	mismatched unrelated donor
MPD	myeloproliferative disease
MPN	myeloproliferative neoplasia
MRD	minimal residual disease
mRNA	messenger ribo-nuclein acid
MRT	matched related donor
mTOR	mammalian target of rapamycin
MTX	methotrexate
MUD	matched unrelated donor

NFkB	nuclear factor-kappa B
NHL	non-Hodgkin lymphoma
NIH	national institute of health
NK	natural killer cells
NMA	non-myeloablative conditioning regimen
NPU	named patient use
NRM	non-relapse mortality
NSAID	non-steroidal anti-inflammatory drug
O_2	oxygen
ORR	overall response rate
ORR	overall response rate
OS	overall survival
PAMP	pathogen-associated molecular pattern
PBSC	peripheral blood stem cells
PCR	polymerase chain reaction
PDGF	platelet-derived growth factor
PD-L1	programmed cell death-ligand 1
PFT	pulmonary function testing
PMF	primary myelofibrosis
PR	partial response
PTCY	post-transplant cyclophosphamide
PV	polycythaemia vera
RA	rheumatoid arthritis
RDS	Ruxolitinib discontinuation syndrome
REACH	trials that ultimately led to approval of RUX in GvHD
REG3α	regenerating islet-derived protein 3α
RIC	reduced intensity conditioning regimen
ROCK2	rho-associated coiled-coil protein kinase 2
ROM	range of motion
ROM	range of motion
ROS	reactive oxygen species
RUX	Ruxolitinib
RV	residual volume
s.c.	subcutaneous
SARS	severe acute respiratory syndrome
SCID	severe combined immunodeficiency
SCT	stem cell transplantation
SJS/TEN	Stevens-Johnson-syndrome/toxic epidermal necrolysis
SOS	sinusoidal obstruction syndrome
SR	steroid refractory
STAT	signal transducer of activators of Transcription
TAC	tacrolimus
TBI	total body irradiation
TCD	T-cell depletion
TCR	T-cell receptor
TGF	transforming growth factor
Th	helper T cell

TLC	total lung capacity
TLS	tumor lysis syndrome
TMA	thrombotic microangiopathy
TNF	tissue necrosis factor
Treg	regulatory T cell
TRM	treatment-related mortality
UCB	umbilical cord blood
VC	vital capacity
VOD	veno-occlusive disease
VZV	varicella zoster virus

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4. Introduction

The history of allogeneic stem cell transplantation is inextricably linked to the emergence of graft-versus host disease.

In the 19th century, John Bennet and Rudolf Virchow first described “white blood” as newly discovered disease, and it has kept doctors occupied ever since.

But despite all efforts it had taken still another century until Sidney Farber first achieved a temporary remission in children with acute lymphatic leukaemia using aminopterin in Boston 1948. (1)

Being the best measurable neoplasm in an era prior to cross-sectional imaging, leukaemia has always been a field of progressive cancer research.

The concept of allogeneic stem cell transplantation is to eradicate the malignantly degenerated bone marrow of the patient and repopulate it with the healthy bone marrow of a fitting donor.

Although the outcome of haematopoietic stem cell transplantation has improved over the last decades, it is still associated with significant transplant-related morbidity, mortality and long-term health issues. (2)

Replacement of bone marrow allows a significantly higher intensity of chemotherapy, further the donor’s immune cells act as immune therapy, attacking potentially remaining malign cells.

In allogeneic HSCT, the infused donor cells evoke a immune response, a favoured effect against tumor cells (graft-versus-leukaemia effect), but also inducing potentially life-threatening graft-versus-host disease. (3)

The donor’s immune system does not only trigger this desired graft-versus-leukaemia effect, but it also recognizes healthy tissue of the recipient as foreign and starts an immune response called graft-versus-host disease which affects around 50 % of transplant recipients. The donor immune cells attack host tissue, resulting in inflammation, apoptosis, and fibrosis in multiple organs. Thus, GvHD is a significant contributor to non-relapse mortality and deterioration in quality of life and adverse long-term prognostic outcomes.

Depending on the clinical presentation and time of occurrence, GvHD is classified as acute or chronic GvHD, with overlap GvHD being the simultaneous presence of acute and chronic GvHD features.

Steroids are generally used as the first line therapy. When response is being insufficient or the dose of steroids cannot be tapered, there has been no established therapy approved for second line treatment of glucocorticoid refractory GvHD until recently.

Ruxolitinib has been already approved for MPN and a promising candidate in targeting pathophysiology of GvHD (4), so in 2015 the first patients at our centre could initiate RUX in a named-patient access programme.

In both acute and chronic GvHD Ruxolitinib has demonstrated safety and efficacy, which has led to the official FDA approval for acute GvHD (May 2019, (5)) and chronic GvHD (September 2021, (6)). The official approval by the EMA followed in May 2022.1

However, overlap GvHD and GvHD induced by interventional donor lymphocyte infusion (DLI), including both pre-emptive DLI for molecular relapse or mixed chimerism and DLI given for

[1] EMA Jakavi: https://www.ema.europa.eu/en/medicines/human/EPAR/jakavi

clinical/haematological relapse of the underlying disease were excluded from the prospective trials leading to approval.

All patients that received RUX for GvHD treatment at our centre were evaluated in this study, independently from either as NPP or in-label use after official approval.

It is the intention of this master thesis to add real-word evidence about these common clinical scenarios.

5. Allogeneic haematopoietic stem cell transplantation

Allogeneic stem cell transplantation is a potentially curative treatment strategy for many haemato-oncological conditions, but also for selected benign immunological and hereditary diseases through replacing the deranged haematopoiesis by generating a new haematopoietic and immune system from a healthy donor.

In contrast to the more frequently used autologous stem cell transplantation, where the stem cells are collected from the recipient beforehand and later reinfused, in allogeneic stem cell transplantation the cells originate from a different person. (2)

Thanks to great advances in this field, the number of stem cell transplants are rising, expanding the potential indications and has permitted consideration of older patients or those with preexisting comorbidities. (7)

Some of the ground-breaking advances are the progress in reduced-intensity conditioning regimens and more donors being available due to PTCY, which made haplo-transplant possible. (7)

An enhanced GvHD-prophylaxis with PTCY allows the utilization of haploidentical donors, thereby expanding the pool of potential donors and increasing the likelihood of identifying a suitable donor.

Despite the great advances, allogeneic stem cell transplantation is still a procedure that carries a significant risk of non-relapse mortality. Transplant-related mortality is up to 30 % at 1 year post transplant, and even among survivors of allogeneic HSCT organ dysfunctions, infertility and secondary cancers is significantly more prevalent than the general population. (2)

Therefore, the advent of new targeted therapies are pushing back the allogeneic HSCT as rescue therapy into later lines. (7)

5.1. Indication

The most common indications for allogeneic stem cell transplantation according to the EBMT survey published 2017 are acute myeloid leukaemia, myeloproliferative disorders, acute lymphatic leukaemia and non-Hodgkin leukaemia. (8)

For best results, patients should enter transplantation in complete response of their underlying malignancy.

In AML, which represents the most frequent indication for HSCT in adults, the sequencing of treatment lines is influenced by risk stratification of cytogenetics or molecular markers. Transplant is recommended in 1st CR of AML in adverse-risk genetics or persistence of minimal residual disease (MRD). In intermediate-risk AML achieving MRD-negative CR, HSCT should be

discussed. In case the cytogenetic risk is considered “favourable”, a HSCT is usually not advised in CR1. (9) (10)

In other diseases, there are less precise recommendations regarding the remission, age of recipient or comorbidities and depend on the individual transplant centre.

In borderline cases, the individual preference of the patient is included in the recommendation.

In the event that the patient’s preference is to decline HSCT, regardless of the medical recommendation, this must be respected.

5.2. Pre-transplant evaluation and donor matching

The decision to initiate the transplantation process is preceded by an intensive risk-benefit analysis to determine eligibility for allogeneic HSCT. This procedure should include individual patient preferences and evaluation of health care structures.

Clinical judgement should be employed to assess a comprehensive medical history, evaluate performance status, consider comorbidities, ascertain biological age and compliance, and determine the extent and status of disease. Additionally, it is also essential to consider the sensitivity of the underlying diagnosis to standard therapeutic interventions.

Multiple factors regarding the patient’s physical condition need to be taken into consideration, many of which are not modifiable, such as patient age, type of disease, prior therapies, or comorbidities.

Rather the physiologic age than the chronological age should be used to determine transplant-eligibility. (11)

There are various scoring systems available for estimating the risk of mortality in patients receiving allogeneic HSCT. A common assessment to evaluate the comorbidities before HSCT and asses the associated risk of non-relapse mortality is the HCT-CI score (haematopoietic cell transplantation-specific comorbidity index) (12), which includes single organ dysfunctions as well as systemic disorders. It scores from 0 to 29 and considers cardiovascular, gastrointestinal, hepatic, pulmonary, and renal dysfunction, amongst others. The most heavily weighted factors in the HCT-CI score include prior solid cancer, heart valve disease and severe pulmonary or hepatic dysfunction. (12) A higher HCT-CI score is associated with increased mortality. (13)

Other scores assessing pre-transplant outcomes include the Modified EBMT (European Group for Blood and Marrow Transplantation) risk score (14) or the EBMT-ADT (EBMT- Alternating Decision Tree) score (13).

To collect important information of the medical history that include data about the current and past illnesses following examinations must be performed within 30, better 15 days before HSCT. (15)

Laboratory studies should include complete blood count with differential, complete biochemistries with liver and renal function parameters, and electrolytes, basic coagulation, and AB0 blood type with Rhesus and irregular antibodies. In addition, a recheck of HLA typing should be performed. (15)

An assessment of prior exposure to various infectious agents is needed because of potential reactivation after transplant, which means taking serology for HIV, hepatitis B and C virus, CMV,

EBV, tuberculosis, herpes simplex virus, toxoplasma, syphilis, Varicella zoster virus, and human T-cell lymphotropic virus I and II. (15) (11)

Restaging of the underlying disease and evaluating the remission status is usually performed by bone marrow aspiration. (15) (11)

Additionally, dental evaluation, gynaecological evaluation, psychological/psychiatric evaluation and nutrition assessment should be performed. (15)

From urine, a micro- and macroanalysis and pregnancy test should be done.

Finally, a chest radiograph, electrocardiogram, echocardiogram and pulmonary function test including DLCO should be done. (15) (11)

Additional evaluation should be performed as clinically indicated, this may include lumbar puncture for cerebrospinal fluid analysis and further evaluation considering physical therapy, nutritional status or geriatric assessment. (11)

Additional laboratory tests may include thyroid-stimulating hormone (TSH) and iron profile with ferritin level. (11)

Options for fertility-preserving measures for children, men and women with childbearing potential should be discussed before any gonadotoxic cancer treatment. There are different existing options existing, the first line option is oocyte vitrification for female patients and cryopreservation of sperm for male patients. (16) (17)

The psychosocial situation should be assessed to secure the patient has adequate social and financial support for the time after discharge from hospital. Adequate medication adherence and caregiver availability must be ensured. (11)

The patient needs extensive education about the procedure, the prospect of success, possible complications, and long-term sequelae. The patient should fully understand the potential complications, that there is currently no way to predict cGvHD and the effect on quality of life.

Donor selection

If the patient seems eligible for transplant at the time of examination, they should undergo HLA typing as soon as possible. If there are healthy, potentially suitable family members for donation, analysing their tissue type should be encouraged.

Depending on the availability of suitable family donors, a request is usually sent to initiate a search for a matched unrelated donor in the international register, which can take several months. This may be more difficult for patients from certain ethnic or racial backgrounds due to underrepresentation in the data bank. (18)

Major advantages in GvHD prophylaxis have enabled haploidentical HSCT and therefore amplified the pool of potential donors. Haploidentical donors, who share exactly one HLA haplotype with the recipient through common inheritance, can be biological parents, siblings, children, aunts or uncles, nieces, grandchildren and cousins. (18)

The HLA markers of potential donors are compared those of the patient, which may result in HLA identical, haploidentical or mismatched statuses.

There are numerous criteria the selection of donor is based on, such as degree of HLA match of the recipient and donor, age, and performance status of the donor, AB0 blood type,

comorbidities of the donor, viral serology with special attention to CMV serostatus matching, donor-recipient gender matching and stem cell source.

Exclusion criteria are any unsuitable health or psychological conditions of the donor, and antibodies against donor present in the recipient, which would result in a positive crossmatch.

An HLA-identical sibling donor is generally the first choice, because of lower GvHD-rates compared to unrelated or mismatched donors, and because access to this stem cells is faster. If required after HSCT, donor-derived cellular therapies can be easy accessed from related donors. (18)

Allografts from HLA-identical siblings were thought to produce the best overall and progression-free survival but are unavailable for a major part of patients. In these cases, unrelated donors or umbilical blood were an alternative source of stem cells. In an era before PTCY, HLA-haploidentical HSCT was associated with a significant risk of graft rejection and severe GvHD due to excessive alloreactivity. (19)

An eligible HLA-haploidentical donor can be identified rapidly, as every patient shares exactly one HLA haplotype with each biologic parent, child, and 50% of siblings. (19)

Logistic challenges concerning availability of donor may make it necessary to take measures to avoid complications for the patient. This may lead to selection of a backup-donor in case the first donor is unable to donate.

Umbilical cord blood (UCB) is used more rarely nowadays, as related donors are easier to find in times of haploidentical transplantation and the major limitations of UCB is that the donor is not available for further donor-derived cellular therapies. (20)

5.3. Procedure

The individual strategy of conditioning and prophylaxis regimen should be determined before initiation and be adapted to the patient’s disease and comorbidities, the chosen donor of the stem cells, and the way of harvesting stem cells.

Choice of transplant technique does depend on centre policies, survival is determined by patients pre-transplantation risk factors. (21)

Patients undergo intense chemotherapy, total-body irradiation, or both to eradicate the patient’s own hematopoietic system, with the potential to also eliminate underlying disease. This also prepares the bone marrow for being repopulated with the donor’s stem cells.

Donor stem cells can be collected from bone marrow via aspiration, peripheral blood via apheresis, or umbilical blood. (2)

In donors for allogeneic stem cell transplantation, the most common procedure is to collect (“harvest”) the haematopoietic stem cells via apheresis from peripheral blood 5-6 days after mobilization (“priming”) with granulocyte-colony stimulating factor (G-CSF). (2)

Alternatively, haematopoietic stem cells can also be harvested from the donor by bone marrow aspiration, performed under general anaesthesia.

To avoid loss of quality and the number of viable cells due to freezing and thawing, the collected stem cells are ideally infused fresh, which means that donor and recipient need to be prepared simultaneously. This requires a precise coordination of timing. In case of unrelated donation, it is sometimes logistically impossible to avoid freezing the stem cell graft.

Conditioning regime

Pre-transplant conditioning is a procedure designed to suppress or eradicate the recipient's immune system, thereby facilitating the engraftment of the donor's haematopoietic stem cells. There are several conditioning regimes which consist of great variation of combination, timing, and dosing of chemotherapy agents with or without radiotherapy (TBI, total body irradiation) and vary in intensity. These are categorized as myeloablative conditioning (MAC, high intensity), reduced intensity conditioning (RIC, intermediate intensity) and non-myeloablative conditioning (NMA, low intensity), based on the duration and degree of cytopenia induced and on the requirement for stem cell support. (22)

When selecting the individually optimal conditioning regimen, various patient- and disease-related factors need to be considered, such as age, donor availability, comorbidities, diagnosis and remission status, previous therapies, and risk of recurrence. Therefore, comparing those **regimen intensities in trials can hardly eliminate selection bias. (23)**

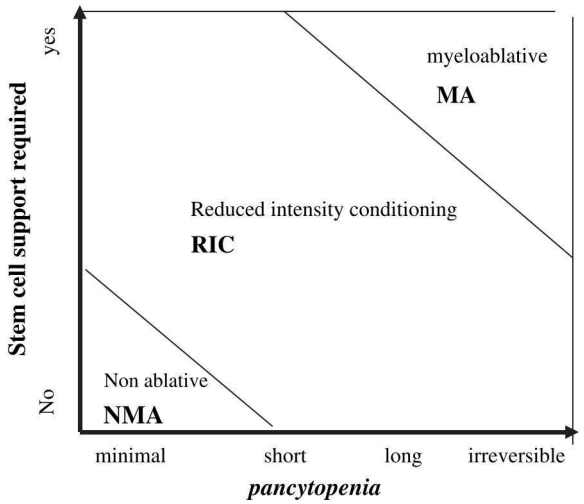

Figure 1: Classification of conditioning regimens in 3 categories, based on duration of pancytopenia and requirement for stem cell support. (22)

Best disease control is achieved by myeloablative conditioning (MAC) with alkylating agents with or without TBI, which is associated with a significant toxicity and treatment-related mortality (TRM) needs to be considered. Pancytopenia caused by MAC is long lasting and usually irreversible without haematopoietic stem cell infusion. (22) The concomitant tissue damage due to the high intensity of conditioning regimen predisposes patients for developing aGvHD. (24)

Non-myeloablative (NMA) regimens cause minimal cytopenia that does not require stem cell support but are suitable to enable full engraftment of donor stem cells. This necessitates a large number of donor T lymphocytes and donor CD34+ cells infused to facilitate engraftment. Donor T lymphocytes will eliminate host hematopoietic cells to establish donor hematopoiesis. (22) In NMA conditioning, aGvHD onset is delayed and may occur after day +100. (22)

Reduced intensity conditioning regimens are all other regimens allocated in between MAC and NMA regimen that fit neither of the definitions mentioned above. Alkylating agents or TBI are at least 30% reduced in dosage compared to MAC, yet still causing pancytopenia of such duration to cause significant morbidity and TRM. (22)

In RIC and NMA regimens, higher relapse rates have been observed. (23)

The less intense approaches of non-myeloablative and reduced intensity conditioning have expanded the potential for HSCT in older patients and those with comorbidities. These groups,

which would have been contraindicated for MAC, now achieve a risk-benefit ratio to favor undergoing HSCT. These developments also open up the possibility of using HSCT for non-malignant indications. (2) (23)

The sensitivity to GvL-effect can vary between different haematologic malignancies and may be impacted by the tumour burden and proliferation rate. This phenomenon is not fully understood yet. (23)

In conditioning regimens, some of the most used alkylating agents are busulfan, cyclophosphamide, and melphalan, but also thiotepa, carmustine or treosulfan. Other agents used are the topoisomerase inhibitor etoposide and the antimetabolite AraC (cytosine arabinoside). (23) Busulfan has extensive toxic effect on non-proliferating marrow cells including myeloid precursor cells, but limited toxicity to mature lymphocytes. (23) Fludarabine is a purine analogue acting synergistic with alkylators by inhibiting DNA repair. (23)

In RIC and NMA conditioning regimen, the same agents or drug classes are used as in MAC, but in reduced dosage and other combinations. In individual cases, personalized targeted therapies can be added to reduce the risk of post-HSCT relapse.

Donor stem cells are typically suspended in a sterile solution of about 200 - 1500 ml with additives that help the infused cells to survive the process.

Supportive care includes antimicrobial prophylaxis and the use of growth factor, which depends on the institutional practice at each center. (25)

Supportive care at our centre includes the use of granulocyte colony-stimulating factor (G-CSF) if leukocytes drop below < 1.0 g/l to accelerate haematopoietic recovery in bone marrow transplantation. When PBSC are used, G-CSF is used when neutrophil recovery is overdue beyond day 21. Antimicrobial prophylaxis consists of trimethoprim-sulfamethoxazole for pneumocystis jirovecii pneumonia prophylaxis, valaciclovir for herpes simplex and varicella zoster virus prophylaxis and posaconazole for antifungal prophylaxis. Irradiated leukocyte-depleted platelet units from single donors and red cells are given when haemoglobin levels drop below 8.0 g/dl and platelet counts decrease to < 10 G/l. In presence of risk factors or signs of bleeding, platelet transfusion threshold is raised to 20 G/l or higher.

5.4. Prophylaxis of GvHD

Immunosuppressive treatment is established before engraftment to prevent two distinct forms of rejection reactions: Firstly, donor immune cells may target recipient tissue, referred to as graft-versus-host disease. Secondly, residual immune cells of the recipient may initiate transplant rejection, subsequently leading to graft loss.
Typically, the dosage of these immunosuppressive drugs can be tapered within the first months after HSCT. (25)

In the context of HSCT there is an increased risk of infections for several months, before immune reconstitution develops and the dosage of immunosuppressants can be reduced in absence of GvHD-signs. (2)

There are several drugs used in variable combinations, in HLA-matched HSCT most commonly including a calcineurin inhibitor (CNI), cyclosporine or tacrolimus plus one antimetabolite agent such as methotrexate (MTX) or mycophenolate mofetil (MMF). (2) (26) (27)

Nonetheless, all immunosuppressive drugs are known to promote new occurrence of malignancies, additionally intensive prophylaxis impairs GvL-effect and therefore leads to higher relapse rates.

The prophylaxis regimen should ideally be determined considering numerous factors, affecting the individual GvHD risk:

- remission status of the underlying disease
- estimated risk of leukaemia recurrence
- virological status of donor and recipient
- HLA-match status
- sex match
- age of donor and recipient
- general health condition of the recipient
- the applied conditioning regimen, especially if TBI is included
- side-effect profile of the contemplated immunosuppressive substances

Particularly during the first 2-3 weeks after HSCT, CNI trough levels need to be closely monitored to ensure sufficient exposure for prevention of severe aGvHD. (28)

Subsequently, CNI are tapered off by month 3-6, depending on the relapse risk of the underlying malignancy.

There are many promising pre-clinical advances in understanding and targeting GvHD pathogenesis with potential to translate into prophylactic therapies. Promising targets are cytokine cascades in modulating adaptive T-cell mediated immunity, endothelial damage, or immune checkpoints. (26)

5.4.1. Substances used in GvHD-prophylaxis

Cyclosporine A (CSA)

CSA is a calcineurin-inhibitor that primarily inhibits gene expression at the level of mRNA transcription, therefore blocking biosynthesis of lymphokines in T-cells, especially interleukin-2 (IL-2), which would be the signal to proliferate. (29)

CSA is metabolized by hepatic cytochrome P450 enzymes, therefore it has great potential to interact with other agents. (29)

Considerable intra- and interindividual differences in pharmacokinetics necessitates therapeutic drug monitoring to ensure adequate immunosuppression while avoiding toxic side effects. (30)

The most important side effects of CSA are an increased risk of infection, hypertension, and nephrotoxicity with elevated serum creatinine. Additionally, neurotoxic symptoms as tremor or paraesthesia (29) and cyclosporine-associated thrombotic microangiopathy (31) are seen.

Tacrolimus (TAC)

Tacrolimus is another calcineurin-Inhibitor with many pharmacodynamical similarities with CSA, but is a macrolide lactone immunosuppressant. (32)

TAC is also metabolized via the CYP3A4 system that is part of the cytochrome P450 superfamily, which leads to great interaction potential. (30)

In contrast to CSA, TAC allows proliferation of human T-regulatory cells, which may provide a better basis for tolerance. (33)

The choice between CSA and TAC in the setting for sibling or MUD transplants is based on centre experience. (27)

Mycophenolate Mofetil (MMF)

MMF is the prodrug to mycophenolic acid, a selective inhibitor of de novo purine synthesis in T- and B- lymphocytes, and therefore acts as antimetabolite. (32) (34)

In patients receiving RIC or NMA conditioning, MMF is the recommended antimetabolite. (27)

MMF is mainly used in GvHD prophylaxis combined with CNI, as adverse events are less common than in combinations of CNI with MTX, although CNI+MMF is not as effective in preventing severe aGvHD. (32)

As described in chapter 7.1., Nikoloudis et al. reported that a high CD4/CD8 T cell ratio in the graft is associated with increased rates of GvHD and NRM in patients receiving MMF as part of their prophylaxis regimen. (35)

Methotrexate (MTX)

Methotrexate is a folic acid antagonist, acting as antimetabolite by blocking de novo synthesis of purines and pyrimidines. It is also used in inflammatory diseases such as psoriasis for the promotion of adenosine release resulting in suppression of T-cell activation and inflammation. There are various administration routes available, but bioavailability varies intra- and interindividual and depends on applied dosage. (36)

In patients receiving MAC, MTX is the recommended antimetabolite. (27)

Adverse outcomes associated with MTX usage are severe mucositis, delayed neutrophil and platelet recovery and renal, pulmonary and hepatic toxicity. (32)

In contrast to MMF, the CD4/CD8 ratio in the allograft had no significant impact on GvHD incidence or NRM in patients receiving MTX as antimetabolite in GvHD prophylaxis. (35)

Sirolimus / Rapamycin

Sirolimus / Rapamycin is an mTORC1 inhibitor, influencing the PI3K/AKT/mTOR (phosphatidylinositol 3-kinase/AKT/mammalian target of rapamycin) signalling cascade, which is important for the regulation of T-cell survival, proliferation, cell cycle progression, differentiation and metabolism. (3) CSA and sirolimus act synergistically in the inhibition of T- and B-cell proliferation. (37)

Important adverse events include increased serum lipids, anaemia, a higher risk of infection, pneumonitis with lung fibrosis, and haemolytic uremic syndrome. (38)

In the context of allogeneic hematopoietic stem cell transplantation, Sirolimus can be used as prophylaxis or treatment of GvHD. (3) (15)

Post-Transplantation Cyclophosphamide (PTCY)

Cyclophosphamide is an alkylating agent, also used in conditioning regimes before being replaced by fludarabine. (23)

Administration of cyclophosphamide is usually done on day 3 and 4 and causes apoptosis of alloreactive donor T cells, which are rapidly proliferating during early expansion, while it spares resting, unactivated T cells due to short exposure. (39)

Cyclophosphamide added post-transplant to the prophylaxis regimen using tacrolimus and mycophenolate mofetil was a major improvement in transplant technology, as it opened the possibility of HLA-haploidentical HSCT by selective and time-specific elimination of alloreactive T-cells. (25) This facilitates identifying an eligible donor for nearly every patient. (19)

PTCY is not only a method of in vivo alloreactive T-cell depletion, it also allows the rapid, preferential recovery of regulatory T cells. (40)
In haploidentical transplants, PTCY- based prophylaxis has become the standard in many centres worldwide, but indication for the use of PTCY-based GvHD prophylaxis is gradually expanded to other transplant settings, such as HLA-matching donors. (26) (39) (41)

TAC + MMF + PTCY

Bolaños-Meade et al. showed that patients receiving TAC+MMF+PTCY appeared to have less severe acute GvHD or chronic GvHD, compared to TAC-MTX. Despite lower incidence of GvHD, relapse, overall survival, engraftment, hematopoietic recovery, transplant related mortality and severe infections remained unchanged. (25)

This contrasts to historical data, in which extensive suppressing GvHD has been associated with worse overall outcomes, especially higher relapse rates. (42)

Antihuman T-Lymphocyte immunoglobulin (ATG/ATLG)

ATG is a polyclonal immune globulin product derived from the sera of rabbits or horses. (26)

For instance, Grafalon® (former ATG-Fresenius®) is derived from rabbits after immunization with the Jurkat human T-cell line, which is further purified and finally results in polyclonal antihuman T-lymphocyte antibodies. In vivo, these exhibit a direct effect on T cells via opsonization and lysis through complement system. (43)

There are other polyclonal T-cell directed serotherapy preparations available, but also the CD52 antibody alemtuzumab (44) has been successfully used in this strategy. The potency of those preparations can differ due to other production methods and therefore cannot be compared directly.

Antigens that are targeted by ATLG may also be expressed on certain B-cell cancers and myeloid cancers, in which antitumor effects have been observed. (45)

ATLG can be added to GvHD prophylaxis and causes in-vivo T-cell depletion, which results in decreased incidence of acute and chronic GvHD.

The administration and dosage of ATLG is handled very differently in different centres.

ATLG dosage should ideally take the estimated GvHD risk into account, based on the presence of risk factors such as unrelated donor, peripheral blood stem cells, and type of post-grafting immunosuppression. (46) (47)

In an Austrian study by Clausen et al. in 2017, ATLG usage was significantly associated with a reduced incidence of aGvHD grade 3-4 and moderate/severe cGvHD. This was especially emphasized in patients with HLA-C KIR-L status C1/1, previously reported as a risk factor for severe aGvHD. (48)

A higher relapse risk due to ATLG similarly to other approaches of T-cell depletion cannot be ruled out on the basis of current data, although ATLG seems to have no impact on relapse or infection rates. (49) This may be an dose-dependent effect. (48)

Kröger et al. reported a significant reduction in cumulative incidence of chronic GvHD without differences in the rates of acute GvHD, relapse, infectious complications or adverse events. (43)

Further, 91% of patients that received ATG had discontinued cyclosporine as immunosuppressive medication within the first year after HSCT, compared to only 39% of patients in the non-ATG group. The relapse rates were similar in the two groups after a follow-up of two years. (43)

5.5. Complications of haematopoietic stem cell transplantation

Even if HSCT has the potential to permanently cure the patient's underlying disease, patients often cannot achieve full recovery of health due to early or late complications.

Apart from relapse of the underlying disease, there are several complications that can occur when undergoing HSCT, the individual risk depends on the patient's comorbidities, organ dysfunctions, toxicities of prior cancer therapies, the stage of underlying disease at transplantation, the intensity of the conditioning regimen, that determines the duration and degree of cytopenia, and the presence and severity of GvHD.

As one of the major complications of HSCT and central topic of this thesis, GvHD is mentioned below.

5.5.1. Hematologic complications

Cytopenia

The degree of neutropenia, anaemia, and thrombocytopenia early after transplantation is determined by the conditioning regimen used. (22)

Supportive treatment includes transfusion of erythrocyte and thrombocyte transfusions, strict isolation of the patient during neutropenia and usage of hematopoietic growth factors. (15)

On the longer term, cytopenia may also be caused immune-mediated, but this may only be diagnosed after exclusion of other causes like medications, infection, GvHD, disease relapse or mismatched transplantation. (50)

Transplantation-associated thrombotic microangiopathy (TMA)

Caused by endothelial injury, coagulation activation and microvascular thrombosis, this disorder manifests by heterogeneous organ impairment presenting as renal dysfunction or unexplained neurologic dysfunction combined with intravascular haemolysis. It typically arises 20 to 100 days after transplantation. (15)

Risk factors female sex of the patient, prior autologous HCT, underlying acute lymphoblastic leukaemia or severe aplastic anaemia, mismatched or unrelated donor, myeloablative conditioning regimen with TBI, acute GvHD grade II-IV, and pretransplant kidney dysfunction were identified as independent risk factors for TMA. (51) (52)

There is an increasing association between severe gastrointestinal aGvHD and TMA. This may be due to the progressive endothelial injury associated with severe gastrointestinal GvHD. (39)

Bleeding

25,7% of allogeneic HSCT recipients experienced a haemorrhagic event, 9,4% developed a life-threatening bleeding episode. (53)

Life-threatening haemorrhagic events are associated with severe thrombocytopenia, grade III-IV acute GvHD and thrombotic microangiopathy. (53)

5.5.2. Complications during engraftment

Engraftment after HSCT is defined as an absolute neutrophil count greater than 500 cells per microlitre on the first day of three consecutive days. Platelet recovery is defined as platelet count greater than 20.000 cells per microlitre on the first day of seven consecutive days without requiring transfusion support.

Engraftment syndrome

Engraftment syndrome (ES) is a non-infectious complication of both autologous and allogeneic HSCT associated with the process of neutrophil recovery. It is characterized by the presence of non-infectious fever, diarrhea, skin rash, pulmonary infiltration or non-cardiogenic oedema, and deranged liver or renal function tests. Less common symptoms include transient encephalopathy of unknown origin and weight gain. (54)

The exact pathophysiological mechanism behind ES is still unclear, but seems to be a dysregulation of pro-inflammatory cytokines such as IL-1, IL-2, IL-6, IL-8, interferons and TNF-alpha and subsequent immune response. (54)

Despite common features, ES is distinguishable from aGvHD based on cytokine levels in plasma being higher in ES than in patients with aGvHD. High-dose intravenous methylprednisolone tapered after symptoms ameliorate is the most common treatment of choice in ES complemented by supportive care, but only to be initiated after exclusion of infectious causes. (54)

Graft failure or rejection

Graft failure is a rare, but life-threatening complication of allogeneic HSCT, that remains a major obstacle to the success of allogeneic HSCT. (55)

Graft failure is defined as either lack of engraftment of donor cells (primary graft failure) by day 28 in the absence of relapse or loss of donor cells after initial engraftment (secondary graft failure). (55)

Risk factors associated with graft failure include HLA-mismatched grafts, type of underlying disease and disease status at transplantation, intensity and type of conditioning regimen, the stem cell source employed, low stem cell dose, major AB0 incompatibility and sex mismatched female donor grafts. (55)

Particularly in the NMA-conditioning-setting, a high number of grafted T-cell monocyte and CD34+ cells reduce the risk of graft rejection. (23)

Graft failure needs to be distinguished from poor graft function, which is defined as severe cytopenia of at least two cell lines with or without transfusion requirement, but in the presence of hypo-/ or aplastic bone marrow with full donor chimerism. (55)

Relapse, hematotoxic drugs, viral infections, and severe GvHD need to be ruled out to establish the diagnosis. (55)

5.5.3. Infections

Pancytopenic patients are at high risk for bacterial, viral, and fungal infections. The risk for infections differs by type of HSCT, conditioning regimen and GvHD prophylaxis regimen used, and whether complications such as GvHD occur. (56)

During neutropenia before engraftment, the infectious risks are comparable to those of neutropenic cancer patients without HSCT. (56)

After engraftment, the cell-mediated immunity recovers, which drastically reduces the threat of infections. (56)

In case of fever without further symptoms, blood cultures should be obtained, a CT scan of the chest and abdomen and an urine analysis should be performed, and blood samples should be monitored via PCR for CMV viremia. As many bacterial infections are associated with the central venous catheter, its removal should be considered, if it is deliberated to be the focus. (56)

In autologous HSCT, immune reconstitution occurs within 2 to 9 months. This is much faster than in allogeneic HSCT, because no immunosuppression is given. In allogeneic HSCT, it may takes a year or longer, especially if GvHD occurs. (56)

Preventive measures

Preventive measures include maximized isolation in an protective environment, infection control practices, antibiotics, antiviral and antifungal drugs. (15)

Close monitoring for reactivation of CMV and antiviral management strategies are part of the routine after allogeneic HSCT as described in chapter 5.3. (56)

5.5.4. Liver dysfunction

Liver dysfunction is common in the setting of HSCT and can range from asymptomatic increased serum bilirubin and transaminases to fatal fulminant liver failure.

Laboratory findings may show two different patterns. Firstly, cholestasis, which means elevated bilirubin and alkaline phosphatase. And secondly, hepatitis, which is the equivalent of elevated hepatic transaminases. (56)

Hepatic sinusoidal obstruction syndrome (SOS), also referred to as veno-occlusive disease (VOD), is a potentially life-threatening complication of HSCT. It is characterized by painful hepatomegaly, jaundice, ascites, and weight gain greater than 5 % of body weight with fluid overload. (57) SOS/VOD typically develops within the first 3 weeks after HSCT. (58)

Initiated by damage to sinusoidal endothelial and hepatic cells by the conditioning regimen, SOS/VOD is then amplified by a complex pathogenesis, involving local inflammatory response and activation of coagulation and fibrinolytic pathways. (57) (59)

The lumen progressively narrows, resulting in post-sinusoidal portal hypertension until reversal of portal venous flow, this can be examined in abdominal ultrasound. (58)

Risk factors for SOS/VOD include pre-existent liver or lung disease, higher-intensity conditioning regimens, patient-related factors like impaired Karnofsky status (< 90), a higher degree of alloreactivity and certain GvHD prophylaxis regimens. (57)

To confirm diagnosis, daily clinical examination and weight monitoring should be supplemented by serial ultrasound measurements to detect signs suggestive of SOS/VOD early. (58)

Thrombocytopenia refractory to platelet transfusions is usually the earliest laboratory abnormality, maybe even before clinical signs. (57)

Treatment depends on disease severity, starting from supportive care measures like maintaining euvolemia, minimizing hepatotoxic drugs and paracentesis to relieve pain. In severe SOS, defibrotide, a sodium salt of single-stranded oligodeoxyribonucleotides derived from DNA of porcine intestinal mucosa, is suggested. (57)

In severe cases, multi-organ failure, characterized by pulmonary and renal dysfunction, but also encephalopathy can occur. (58)

These severe forms are associated with a mortality rate of > 80 %, most frequently associated with hepatorenal syndrome and multi organ failure. (59)

In clinically severe SOS/VOD, associated histological features were hepatocyte necrosis and sinusoidal fibrosis. (60)

It is important to rule out cholestasis due to biliary obstruction by a stone or cholecystitis before diagnosing SOS/VOD. (61) Budd-Chiari syndrome, defined as obstruction of hepatic veins and inferior vena cava, may mirror hepatic SOS. (62)

Despite all efforts to avoid infectious complications including prophylactic drugs, viral hepatitis and hepatosplenic candidiasis may also occur. (15)

Any medications that raise the suspicion for drug toxicity should be reevaluated and discontinued, if possible. Cholestasis due to medications is generally not associated with refractory thrombocytopenia as seen in VOD/SOS. (15)

Although the interpretation of serum iron studies may be impeded by ongoing inflammation, iron overload syndrome should be considered if patients have a history of multiple erythrocyte transfusions. (56)

Hepatic graft-versus-host disease is a strong consideration after engraftment and is mentioned in chapter 7.2.1.4.

If the aetiology remains uncertain and crucial differential diagnosis is required, a liver biopsy is indicated. One must bear in mind that biopsies are fraught with the risk of haemorrhagic complications, and may bring false-negative results. (15)

One must keep in mind that these conditions may coexist with others.

5.5.5. Oral mucositis

Oral mucositis is a painful source of morbidity adversely affecting the quality of life. The complex pathophysiology involves direct tissue damage induced by chemotherapy and/or radiation, generation of reactive oxygen species inflammatory cytokines and alterations in microbiome. (63) It usually develops between day +6 and 12 and takes around 7-14 days to resolve. (64)

Impaired nutritional intake due to pain may be supported by parenteral nutrition until adequate oral nutrition can be resumed. (65)

Local alleviation of symptoms and averting progression may be achieved by local cryotherapy, low level laser therapy (photobiomodulation), professional oral hygiene, antimicrobial agents and benzydamine. (63)

Systemic pain medication such as NSAIDs or opioids may be added as needed.

5.5.6. Diarrhea

Diarrhea is a frequent symptom in the setting of allogeneic HSCT that may has multiple possible etiologies.

The most common cause attributed to diarrhea in patients who have recently undergone allogeneic HSCT is GvHD, but exclusion of other causes before initiation of glucocorticoids is crucial to avoid treatment-related complications. (66)

The gastrointestinal tract is affected in around the half of cases of aGvHD, coexistence of other typical symptoms of GvHD increases the probability of diagnosis. Clinical manifestation of diarrhea associated with aGvHD is initially watery diarrhea with mucous. (66)

The epithelial cells of the GI tract do have a physiological intense proliferative activity, which makes them especially vulnerable to cytostatic drugs and radiation. (66) So especially within the first two weeks after HSCT, mucosal damage by the toxicity of the individual conditioning regimen is the most probable cause for diarrhea, especially related to TBI. (66) This predisposes the patient for other complications, especially during neutropenia. (66)

It is not unusual for conditioning-therapy-induced gastrointestinal symptoms and aGvHD to coexist or for one to transition into the other. (67)

Infectious enterocolitis may be caused by Clostridium difficile, but also invasion of the damaged bowel wall by Candida species has been reported. (56) To identify the pathogen, the method of choice is a stool sample culture on selective growth media. (66)

Pathogenic bacteria causing infectious gastroenteritis after HSCT besides C. difficile are Salmonella, Shigella, Campylobacter or Yersinia. (68)

Just as in the healthy population, viral infection can induce diarrhea, but after HSCT, patients are even more vulnerable due to immunosuppression. This is especially common after hospital discharge. (66) For early diagnosis, real-time PCR of a stool sample should be performed. (66) While CMV is the most common pathogen, other viruses should be considered, such as adenoviruses, rotaviruses or noroviruses. (66) (67)

Parasitic infections with Cryptosporidium spp. or Giardia lamblia and other pathogens should be considered but are hard to detect and results may be false-negative. (66)

Transplant-associated TMA may also damage the gastrointestinal tract. (66)

First diagnostic measures should be stool specimen analysed for C. difficile toxin and culture, the most common viruses as mentioned above and parasites G. lamblia and Cryptosporidium. CMV-DNA in serum should be determined by quantitative PCR. If these tests are negative and other clinical features of GvHD are present, this is the most probable diagnosis. (66)

In uncertain cases, CT scans and colonoscopy with histopathological verification, if required, should be performed. (56)

5.5.7. Acute kidney injury

The majority of patients undergoing allogeneic HSCT has some degree of renal dysfunction, mostly being mild. Severe nephrotoxicity is associated with higher frequencies of other organ toxicities and increased non-relapse mortality. (69) (52)

During allogeneic HSCT, multiple factors may contribute to AKI simultaneously, such as nephrotoxic drugs, infections, TMA or SOS/VOD. (52)

Tumor lysis syndrome (TLS) can provoke AKI by release of intracellular contents that leads to hyperkalaemia, hypocalcaemia, hyperphosphatemia, and hyperuricemia, that can cause crystal-induced kidney injury. TLS rarely occurs in patients when undergoing HSCT, because previous treatment lines usually reduced tumor burden dramatically before initiation of HSCT. (52)

Risk factors for AKI associated with HSCT include TBI conditioning and usage of calcineurin inhibitors and MTX for GvHD prophylaxis. (52)

The greatest risk of AKI is with myeloablative conditioning in allogeneic HSCT. (52)

Although the kidney is not considered a target organ of aGvHD, severe diarrhea can indirectly cause dehydration leading to prerenal AKI. (52)

Complications associated with severe nephrotoxicity are higher frequencies of sepsis, hepatic toxicity, SOS/VOD, and pulmonary toxicity. (69)

Strategies to prevent AKI during the allogeneic HSCT includes prevention of other HSCT-related complications, reduce the use of nephrotoxic agents when possible, and adequate hydration while avoiding fluid overload, and early intervention at first signs of renal dysfunction. (52)

Treatment should focus on correcting the cause of AKI, as soon as it is determined. (52)

5.6. Long-term care

The number of survivors of allogeneic HSCT continues to increase, but they suffer from significant long-term morbidity and TRM. The leading risk factors for late non-relapse death in patients that previously survived at least two years after allogeneic HSCT include older age and cGvHD, but relapse was the most common cause of death. (70) In 2-year survivors without relapse, the probability of being alive 10 years after allogeneic HSCT was 85% in a 2011 study. Cumulative incidence of relapse at 10 years after HSCT in relapse-free patients for 2 years after HSCT ranges from 6-10 %, depending on the underlying malignancy. (70)

Multidisciplinary coordination of care from transplant centres and community healthcare providers is essential to ensure best possible long-term outcome. (71)

After discharge from hospital, patients require constant surveillance for transplant-related complications such as GvHD, infection or disease relapse. In the course, the required frequency of outpatient visits decreases, presupposed the absence of complications. (11)

But absence of relapse of the underlying disease after HSCT is not necessarily synonymous with full restoration of health. Physical, psychological and social sequalae may have an adverse effect on survivors for many years after HSCT. (72)

In allogeneic HSCT, adequate reconstitution of the cellular and humoral immune system may take up 2 years or more, especially further delayed in patients who develop GvHD. (71)

Patients after undergoing HSCT need long-term support in a care plan according to the biopsychosocial model, with preventive and screening practices focused on individual patient exposures and risk factors. (71)

Chronical health conditions occurring after HSCT include diseases of the cardiovascular, pulmonary, and endocrine system, renal and hepatic dysfunction, infertility, iron overload, osteoporosis, metabolic problems, infections, and secondary malignancies. Subjective well-being may be impaired by chronic pain, fatigue, sexual dysfunction, and cognitive impairment. (72) (71)

These health conditions may not be directly related to the allogeneic HSCT itself but may result from the sum of antitumour treatments before and during the HSCT.

Apart from regularly monitoring for relapse and post-transplant complications, generally recommended age- and gender-appropriate preventive medical checkups according to established guidelines should also be performed. (72)

Patients should be provided with continued medical surveillance and psychosocial support, encouraging healthy lifestyle behaviours and preventive care should be continued as recommended for the general population. (72)

Treatment-related complications may contribute to late non-relapse mortality (e.g., cardiovascular diseases, end-stage renal disease, cGvHD), or impair quality of life (e.g., dry eyes, xerostomia). Chronic GvHD and its treatment is a major contributor to complications and late TRM. (71)

Psychosocial sequelae (e.g., depression, anxiety, adverse coping strategies, social isolation, financial burden, inability to return to work) may also affect patients and their caregivers. (72)

Vaccinations are an especially important component in preventive care and should be caught up after checking a possibly remaining immunity status. (72)

Vaccinations should begin at 6-12 months after HSCT. (71)

In a recent study, Nikoloudis, Neumann et al. showed on the example of SARS-CoV-2 mRNA vaccinations that as early as three months post-HSCT, most recipients seroconverted after four doses. As risk factors for non-response, MMF usage and low B-cell counts were identified. (73)

Late complications include radiation-related toxicities such as cataract and hypothyroidism, late chemotherapy-related toxicities (e.g., heart failure), various organ dysfunctions, and secondary malignancies. (11)

There are several endocrine disorders reported after HSCT, such as thyroid dysfunction, diabetes or metabolic syndrome. (74) Thyroid dysfunction from radiation and high-dose chemotherapy or autoimmune-like thyroid complications such as Hashimoto’s disease may occur months to years after HSCT. (50)

Iron overload is common in patients after undergoing HSCT and may deteriorate clinical outcomes due to iron-induced toxicity by producing reactive oxygen species (ROS). ROS impairs haematopoietic stem cells and stem cell niche and causes oxidative DNA damage. Therefore, serum ferritin levels should be monitored, and iron removal therapy initiated as soon as possible. This may be done by iron chelating therapy, but also phlebotomy in non-anaemic patients. (75)

Infertility is common after HSCT and especially after MAC, but nowadays there are good possibilities for fertility preservation that should be discussed before any gonadotoxic treatments. Although a low number of pregnancies is observed after HSCT compared to a general population in childbearing age, there is no evidence for a higher rate of congenital abnormalities. (17)

Second malignancies after HSCT are responsible for 5-10% of late deaths after HSCT, especially increased in patients of advanced age at transplantation and those who received TBI conditioning. (70) (76) Typical manifestations are lymphomas, secondary myelodysplasia or AML, epithelial cancers often occur 10 or more years after allogeneic HSCT. (70) Apart from

chemoradiotherapy, GvHD, immunodeficiency and genetic susceptibility may contribute to the risk for secondary cancers. (70)

As the rate is higher than expected than in a matched general population, all transplantation survivors require lifelong cancer screening and preventive interventions. (76)

Osteoporosis is a common condition especially in patients with a history of chronic glucocorticoid use, so screening for osteoporosis should be done by regularly routine densitometry.

6. Graft-versus-Malignancy effect

In patients receiving MAC, the high intensity of chemotherapy with or without TBI is aimed at eliminating remaining malignant cells together with the whole haematopoietic system. However, the impact of this intense therapy fades over time, and concepts of lower intensity conditioning are executed successfully, suggesting the presence of another mechanism of action in allogeneic HSCT.

The graft-versus-leukaemia (GvL) effect, also referred to as graft-versus-tumor effect or graft-versus-malignancy effect, describes the removal of residual cancer cells through immune cells of the donor. This is the central immunological mechanism of allogeneic stem cell transplantation.

The two major remaining challenges in HSCT are relapse and GvHD, and while GvHD is an outcome of immunological dysregulation, relapse is a consequence of malignant cells evading toxicity of the conditioning regimen and failure to utilise the desired immunological GvL-effect.

In 1965, Mathé et al. first described adoptive immunotherapy by allogeneic marrow transplantation. (77)

GvL effect seems to be closely interrelated with GvHD. The immune cells of the donor do not only recognize the cancer cells as foreign, but also the healthy tissue of the recipient.

Horowitz et al. described in 1990 the correlation between the lowest relapse rates in patients with present cGvHD, and the highest probability of relapse in patients without GvHD and patients that received T-cell-depleted grafts. (78)

Allogeneic HSCT is a heterogeneous multistep treatment with various individual adaptations to numerous factors explained in chapter 5.2.

Still, GvHD is the main cause of non-relapse mortality, and comes along with considerable morbidity even in the group of survivors. Additionally, the current GvHD treatment relies on immunosuppressants, which may potentially diminish the GvL effect.

Most relapses occur early after HSCT when the immune system is still compromised to avoid acute GvHD. (79)

Optimal GvL activity would evolve within the first three months after allogeneic HSCT in an MRD-negative state, target haematopoietic-restricted antigens present on leukaemia cells, and in the absence of GvHD. (80)

The efficacy of GvL effect depends on various factors, such as disease characteristics (immunogenicity of the tumor, proliferation rate), donor histocompatibility, degree of chimerism and ongoing treatment. (81) (82)

GvL effects were observed to work best in patients with slow growing tumors and relatively low tumor burden. Large tumor burden, faster proliferation rates and lower sensitivity of malignant

cells to donor immune cells present at the time of allogeneic HSCT have adversely affect the outcome. (82)

Storb et al. subsumed that preventing chronic GvHD would reduce NRM, but increase mortality related to relapse due to the restricted GvL effect. (79)

Storb hypothesized that only after immunosuppressive drugs were tapered and later discontinued, donor immune cells begin to engage in GvL effects. (79)

Preclinical murine models of immune responses in GvHD and GvL effects often lack transferability to the clinical scenario. Observing well-characterized tumour cell lines transplanted into healthy, young and lean inbred laboratory mice housed within specific-pathogen-free facilities does not reflect the immune response of cancer patients' immune systems. (80)

Neither GvHD nor GvL-effect require a specific antigen presenting cell (APC) subset to initiate immune response, however both are initiated by recipient APCs. (80)

Non-haematopoietic APCs are associated with T-cell exhaustion and thereby impair GvL activity. (80)

In contrast to GvHD, which is governed by naïve T cells, GvL effects can be mediated by both naïve and memory T cells. (80)

Target antigens on leukaemia cells recognized by donor T cells are generally alloantigens, only sporadically haematopoietic or leukaemia-specific antigens. (80)

Natural killer (NK) cells also contribute to GvL effects, though considerable variability has been noted across haematological malignancies. Strongest evidence of NK cell mediated GvL activity exists in AML. (80)

In HLA-haploidentical HSCT, the antileukemic activity of NK cells is utilized deliberately. The usual inhibition by self-HLA-antigens does not take place, as the patients cells express a different group than the donors' immune cells. Lacking inhibition, the allogeneic NK cells exert a strong GvL effect, though without initiating GvHD. (81)

Storb et al. assessed GvL effect in patients that received minimal-intensity conditioning, which minimizes regimen-related toxicities that may augment GvHD. (79)

In this study, presence of chronic GvHD is also associated with enhanced GvL effects, but acute GvHD was not. It is assumed that GvT-effects are present event without GvHD. (79)

There was no difference in graft-versus-tumour effect between related or unrelated grafts. (79)

Potential immune escape mechanisms of leukaemia cells that lead to failure of GvL effect and disease relapse include loss of MHC expression, increased expression of immune checkpoint ligands (e.g., programmed cell death-ligand 1 (PD-L1)), secretion of inhibitory cytokines and upregulation of immunosuppressive enzymes. (80)

Concomitant GvHD may even amplifies immune evasion, as T cells are chronically exposed to alloantigens and therefore increase the expression of immune checkpoint molecules. (80)

MRD prior to allogeneic HSCT is associated with higher rate of relapse, remaining leukaemia cells probably bypass GvL effects by the pathways detailed above before GvL effects are full-fledged. (80)

To bridge the timespan until the grafted immune system has recovered, Storb et al. hypothesize to delay disease progression with targeted drugs or antibodies to allow establishing curative GvT effects. (79)

Augmenting donor T cell alloreactivity to modulate GvL activity is hindered by T cell-depleting agents used in GvHD prophylaxis. A first step in case of relapse while still receiving immunosuppression is to intentionally reduce dosage of calcineurin inhibitors early or more rapidly, or alternatively consider donor lymphocyte infusion, although this comes along with increased risk of GvHD. (80)

Clausen et al. reported increased relapse rates if higher ATG doses were administered, and upon stratification for HLA-C KIR-L status, in the C2/2 cohort any ATG dosage significantly increased relapse risk while in the C1/2 cohort the relapse risk was unchanged irrespective of the ATG dosage. In the C1/1 cohort, ATG seemed to increase the relapse risk especially if the higher ATG dose levels were applied. The HLA-C KIR-L status was shown to influence both, the risk for developing GvHD and the risk of relapse. Recipients with C1/1 KIR-L status have lower relapse rates compared to recipients with at least one C2 allele, but also have an increased risk for severe aGvHD. (48)

These findings suggest that there is a link between GvHD and GvL effects in the C1/1 recipients. (48)

Maintenance therapies targeting proteins upregulated or mutated in malignancies (e.g., FLT3 in AML, or BCR-ABL1 in Philadelphia chromosome-positive CML or ALL) can sensitize malignant cells to GvL effects by inhibiting growth signals or disrupting pathways of immune evasion. (80)

However, the effect of these agents can hardly be separated into contributing to GvL effect or direct antitumoral activity. (80)

GvL does not eliminate cancer cells in sanctuary sites like the central nervous system, which results in relapses at extramedullary sites, potentially without any blasts detectable in marrow or blood. (81)

Novel approaches would be to further illuminate the pathophysiologic differences between GvL and GvHD and therefore develop therapeutic strategies to specifically inhibit GvHD, while minimizing the restriction of GvL-effect. (3)

On ASH 2022, Baron et al. presented a study suggesting dissociation of GvL effects from GvHD in patients receiving haploidentical HSCT for active AML with PTCy-based GvHD prophylaxis. Grade III-IV aGvHD and severe cGvHD correlated with higher NRM, but there were no associations between aGvHD or cGvHD of any grade and lower relapse incidence. Two-year leukaemia-free survival was 32%, two-year relapse mortality incidence was 49%, two-year NRM was 19%. (83)

6.1. Donor-Lymphocyte Infusion (DLI)

Donor lymphocyte infusion (DLI) or -transfusion (DLT) use the GvL effect to control leukemic relapse after allogeneic HSCT. (84) DLI is given for mixed chimerism, as preemptive DLI in case of minimal residual disease (MRD) or scheduled after T-cell depleted allogeneic HSCT. (85) Mixed chimerism can be converted to complete chimerism by DLI. (81)

This procedure carries the risk of (re-)introduction of severe acute and chronic GvHD. (86)

The outcome after DLI in terms of GvHD incidence and prolonged survival are influenced by donor type, gender of donor, disease phase at transplantation, T cell depletion, interval from transplantation to DLI, GvHD prior to relapse, and relapse type. (85)

The GvL effect may take up to 4-8 weeks after DLI to be observed. To achieve molecular remission, it can take up to 4-6 months. (81) The mechanism behind this delayed response may result from an ongoing immune reaction of donor T cells against malignant cells or the elimination of an early leukemic stem cell, whose progeny may persist for months. (81)

However, this may be outpaced by progression of acute leukaemia, therefore often chemotherapy is used to permit time for the development of an efficient GvL effect. (81)

To separate the GvL effect of DLI from provoking GvHD in patients with haematological relapse, transfusion of donor cells should be started at low cell numbers followed by escalating doses until response of leukaemia or induction of GvHD. (87)

Escalation of the dose can be delayed for 2 months, considering the delayed response possible after DLI. In urgent cases due to rapid progression of relapse, the interval can be shortened to 4 weeks, as GvHD would occur in most cases within this timespan, if occurring. (81)

If remission is achieved by DLI, it is mostly long-lasting. (81)

Viral infections or reactivations of viruses are a major factor in the generation of GvHD after DLI, which reinforces the role antiviral and antimicrobial prophylaxis in preventing infections and therefore improving the response to DLI. (88)

In approximately 10 % of patients, myelosuppression is observed, especially in patients with hematologic relapse. This could be due to the elimination of recipient’s haematopoiesis without sufficient replacement by donor-type haematopoiesis. In some cases, re-transfusion of donor marrow without prior conditioning treatment could restore haematopoiesis. (81)

7. Graft-versus-Host Disease (GvHD)

Graft-versus-Host disease belongs to the most important complications and the leading causes of non-relapse mortality after allogeneic HSCT. (89)

GvHD is the manifestation of donor T- and B- cells recognizing the recipient's tissue as foreign, potentially leading to severe inflammation and organ damage. Typical target organs are included in the staging and grading of acute and chronic GvHD, although essentially any organ can potentially be affected. (3)

Despite standard prophylaxis, up to 50% of the patients undergoing allogeneic stem cell transplantation develop aGvHD. (90) , up to 27 % have severe acute GvHD (grades 3-4). (48)

The distinction between acute and chronic GvHD is now solely based on clinical features, rather than the temporal relationship to transplantation. (91)

Features of acute and chronic GvHD can occur simultaneously, called overlap cGvHD, classified as subcategory of chronic GvHD in the NIH Consensus Conference. (91)

The difference between clinical presentation of acute or chronic GvHD suggests differences in the immunological mechanisms causing the observed organ damage.

Nevertheless, the prior used day 100 after transplantation to discriminate between acute and chronic GvHD is now used to further subdivide acute GvHD regarding its development, as illustrated by Lee et al. (92)

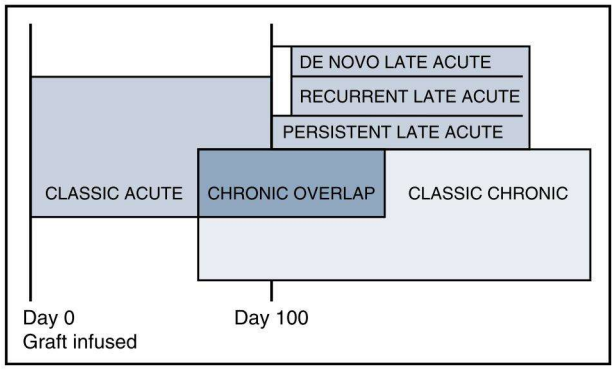

Figure 2: Lee 2017 - Acute, late acute, chronic overlap, and classic chronic GvHD. The box sizes do not reflect prevalence. (92)

Signs and symptoms of acute GvHD present after day 100 without chronic GvHD are called late acute GvHD, which is further subclassified into "persistent", "recurrent" or "de novo". (92)

In all cases, infection, drug toxicity, malignancy, or other complications in the setting of HSCT need to be ruled out before diagnosing GvHD and initiation of treatment.

Since most currently available treatments of GvHD are based on non-specific immunosuppression, an increasing the risk of non-relapse mortality secondary to infection and higher rates of relapse may be expected. (93)

It must be pointed out that GvHD itself also impairs immune defence by dysregulation of immune response and impaired mucosal barriers, such as the oral or gastrointestinal mucosa.

7.1. Risk factors

HLA-incompatibility / disparity between donor and recipient

Histoincompatibility includes HLA mismatches and ‘minor’ non-HLA antigens. (94)

The use of HLA-mismatched donors has been associated with higher rates of GvHD. (95) (96)

The role of mismatches at individual loci needs to be reevaluated in times of haploidentical transplantations and PTCy usage. (97)

Some mismatches may enhance the GvL response, closer matching could potentially increase the risk of relapse. (98)

A recent field of research is the HLA-C-KIR ligands status, HLA-C1 homozygous (C1/1) KIR-L status is reported as risk factor for both relapse and GvHD. (48) (99) (100) (101) (102)

Intensity of the conditioning regimen

A higher intensity of the conditioning regimen is associated with higher rates of acute and chronic GvHD, especially in TBI-based conditioning, as described in chapter 5.3. (24) (94)

Reduced intensity conditioning is accordingly reported to be associated with a lower risk of acute GvHD. (33)

Patients who received RIC have shifts in GvHD timing with increased rate of late acute GvHD. (103)

Source of graft /hematopoietic stem cells

The usage of G-CSF-mobilized peripheral blood stem cells (PBSC) is associated with faster neutrophil and platelet engraftment, but also increased risk for development of severe aGvHD (grade 3-4) (104) and chronic GvHD. (33) (94) (96) The higher risk of GvHD may be associated to PBSC grafts contain a higher T-cell number than in those harvested from bone marrow. (105)

Donor-recipient relationship

The use of unrelated donors is also an established risk factor for developing GvHD. (94)

Advanced age of donors and recipients

The advanced age of the recipient or donor is a risk factor for development of GvHD and a worse outcome. (96) (94) (106)

Younger donors are associated with lower aGvHD-incidence. (97)

This phenomenon is challenging to investigate in HSCT from HLA-matched or haploidentical siblings due to the high correlation between the age of the donor and recipient. However, in unrelated HSCT and in haploidentical HSCT from another family generation (e.g., parents or children), the age effect of donor versus recipient can be differentiated.

Sex mismatched female donor

Regarding the constellation of gender of recipient and donor, the highest risk for development of severe GvHD comes for male recipients receiving stem cells of a female donor, this was markedly increased if those female donors were previously pregnant. (94) (96) (107)

This effect is observed in cGvHD and is further discussed in chapter 7.4.1.

Type of GvHD prophylaxis

The agents used in GvHD-prophylaxis and the subsequent immunosuppression are essential for the prevention of excessive alloreactivity and the subsequent development of severe aGvHD. Achieving this balance is crucial to avoid prolonged immunodepletion, which may increase the risk of disease relapse and opportunistic infections. (28)

In prophylaxis combinations with calcineurin inhibitors, tacrolimus-based prophylaxis is reported to be associated with lower incidence of acute GvHD grade 2-4 compared to cyclosporin-based prophylaxis. (108)

Further risk factors

In addition to the aforementioned factors, the use of donor lymphocyte infusion, AB0-incompatible transplantation (109), and active malignancy at the time of HSCT are also associated with an increased risk of developing GvHD.

The cellular composition of the graft is an early predictor of outcome of HCST, especially a high CD4/CD8 ratio in the graft increased the risk of aGvHD and mortality. (110) Nikoloudis et al. reported that this adverse impact of a high CD4/CD8 ratio may be overcome by the use of CSA/MTX, but not by CSA/MMF or PTCy/TAC/MMF for GvHD prophylaxis. (35)

In patients undergoing MAC, a low number of Treg in the graft is associated with increased incidence of aGvHD. This observation was not made in patients after RIC, and there were no differences in relapse rates in patients receiving grafts with different number of Treg in the graft. (111) Treg cells are defined by expression of CD4, CD25 and FOXP3, transcription factor forkhead box P3. They account for 5-10 % of CD4+ T cells in circulation and control innate and adaptive immune responses, especially suppressing autoreactive lymphocytes. (112)

Prior acute GvHD makes development of cGvHD development more probable. (3) (94) (91) (96) Better prevention of aGvHD appears to lower the incidence of cGvHD. (94)

Chronic GvHD that arises directly from aGvHD (progressive onset) is known to be associated with an increased risk of NRM. (95)

The impact of GvHD risk factors can be considerably reduced by serotherapy with ATG in patients who are expected to have a high-risk constellation for severe GvHD. (48)

In ATG-treated PBSC recipients, Clausen et al. reported that the risk factors C1 homozygosity and sex mismatched female donor both lost their entire impact on the risk for severe aGvHD (grade 3-4). (48) By contrast, even in ATG treated recipients, HLA mismatching remained a significant risk factor for severe aGvHD. (48)

Despite the knowledge of numerous risk factors for developing severe GvHD, it is still not possible to precisely predict the individual risk of developing severe GvHD at the time of HSCT.

7.2. Acute GvHD

Acute GvHD belongs to the main causes of morbidity and non-relapse mortality after allogeneic HSCT, it affects up 50 % of the patients receiving hematopoietic stem cell transplant. (113)

It characteristically appears within the first 100 days after allogeneic hematopoietic stem cell transplantation. (114) Particularly in patients with non-myeloablative conditioning, acute GvHD onset can be delayed even after day +100. (22)

Acute GvHD is defined as a clinical diagnosis, biopsy can only confirm the diagnosis, and may help rule out or point to differential diagnoses such as infections or toxic effects of pharmacotherapy.

The clinical presentation may be challenging to record as standardized staging and grading system, which lead to a plethora of definitions. In this study, we employed the definition of the Mount Sinai Acute GvHD International Consortium 2016 (MAGIC criteria, (115)), which is more commonly employed in clinical practice and outlined in chapter 7.2.1.

Currently, aGvHD is thought to affect the classical target organs skin, gastrointestinal tract, and liver, but there is emerging evidence that other organs might also be damaged. This damage is often more difficult to distinguish from other causes such as drug toxicity. Nonclassical target organs of aGvHD that need further research include the central nervous system, thymus, lungs, kidneys, ovaries and testes, bone marrow, and endothelium. (116)

Belated diagnosis and treatment initiation may lead to worse and delayed overall response, which affects patients quality of life. (113)

The involvement of the gastrointestinal tract fundamentally determines the outcome of aGvHD. (117)

Biomarkers

There are currently no biomarkers in clinical use at our centre, although there is a great deal of research is being conducted on this topic.

Immune cell-derived biomarkers to predict patients at risk for developing aGvHD, their response to corticosteroids and monitor patients' response to treatment are currently under investigation. Promising biomarkers are pro-inflammatory interleukins, interleukin receptors, anti-inflammatory cytokines and their dysregulation and chemokines as mediators of leukocyte chemotaxis. (118)

MicroRNAs are potent regulators the transcription of multiple target genes and can be measured in patient serum. (118)

For intestinal GvHD, REG3α (regenerating islet-derived protein 3α, a C-type lectin secreted by Paneth cells) or cleavage fragments from cytokeratin-18, suggesting epithelial cell death, may be used as biomarkers. (118)

Other potential biomarkers derive from target organ tissue injury, such as elafin, an elastase specific protease inhibitor, being associated with higher incidence of skin GvHD and lower overall survival. (118)

7.2.1. Pathophysiology of acute GvHD

Acute GvHD is mediated by host-reactive, donor derived T-cells, that recognize the recipient (the host) as foreign and initiate an immune response. Activated donor T cells gain cytolytic capacity to attack tissue of the recipient. (98)

Typically, acute GvHD develops in organs incurring tissue damage from a variety of possible mechanisms, such as transplant conditioning, infection, pre-transplant damage from underlying disease or the respective treatment. (114)

Radiotherapy and chemotherapy induce tissue damage, which results in the release of endogenous alarmin proteins and damage-associated molecular pattern (DAMP), including nucleic acids, intracellular proteins, heat shock proteins, histone and others. (24)

These are inflammatory triggers in the early phase of acute GvHD, divided into sterile damage-associated molecular pattern (DAMP) molecules and pathogen-associated molecular pattern (PAMP) molecules, both can drive innate and adaptive immune responses. (98)

Pathogen-derived molecules translocate through the damaged mucosal barrier, particularly intestinal flora from the lumen of the gastrointestinal tract. (24)

The gut microbiota produce bioactive metabolites from undigested food eaten by the patient, which directly or indirectly influences the physiological function of digestion, metabolism, and immune regulation. (119)

Injured cells release pro-inflammatory cytokines hence activating host and donor antigen-presenting cells (APCs). Those pro-inflammatory cytokines include tissue necrosis factor (TNF) alpha, interleukin-1 (IL-1), IL-6 and DAMPs/alarmins. (24)

No single APC subset (e.g., dendritic cells (DC), macrophages or B-cells) is accountable. (80)

It has been demonstrated that increased conditioning intensity leads to premature activation of APCs and accelerated stimulation of donor T cells (81), which is why this is associated with higher rates of aGvHD. (93)

Dendritic cells are dependent on JAK1/2 activation during differentiation and maturation. (120)

Some cytokines promoting GvHD also act as survival signals for malignant cells, such as GM-CSF in AML (121) and IL-6 in ALL (122), which makes them attractive targets to neutralize.

Intestinal microbiota regulate the antigen presentation by GvHD-initiating APC, arising from observations that both, gut decontamination with broad spectrum antibiotics, but also microbiota components may act protective against GvHD. (80)

Reduced gut microbial diversity after allogeneic HSCT is associated with an increased risk of GvHD, increased GvHD-related mortality and shortened overall survival. (119)

While the APCs present the alloantigen to donor T-cells, they also release cytokines such as IL-12, IL-23, IL-6, IL-27, IL-10, and transforming growth factor-beta, which leads to activation, expansion and differentiation of T cells. (24)

DCs derived from the intestinal tract presenting alloantigens within the mesenteric lymph nodes initiate differentiation of donor T-cells and their emigration to the GI-tract, where they mediate fulminant GvHD. (117)

Haematopoietic antigen-presenting cells (APCs) of the recipient activate and induce the differentiation of naïve T cells of the donor that leads to MHC-I-dependent, CD8+ T-cell mediated GvHD, donor-derived APCs play a minor role. (80) (123)

Non-haematopoietic APCs (e.g., epithelial, or stromal cells) play an important role in initiating MHC-II-dependent GvHD, and maintaining and amplifying GvHD in addition to GM-CSF-dependent DCs in the GI-tract. (80)

Activated DCs are capable of activating cytotoxic CD8+ effector T cells. (124)

Th cells and cytotoxic cells mediate damage to aGvHD target organs by secreting a variety of cytokines. (119) This damage is done by multiple cytotoxicity pathways, recruiting neutrophils to inflammatory sites and further production of proinflammatory cytokines. (123)

Neutrophils were shown to amplify tissue damage by production of ROS and thereby promoting T-cell activation, which is expressed by a strong correlation of the severity of intestinal GvHD with the number of neutrophils present in GvHD lesions. (125)

Activated T cells release cytokines themselves, which can subsequently result in the development of a cytokine storm. These cytokines include interferon-gamma, TNF, IL-2 and IL-17 and may be released by differentiated effector T cells, but also phagocytes. (24)

Therapeutic interventions intended to decelerate this cytokine storm are agents such as a TNF-alpha antibody, mentioned in chapter 7.5.

Currently, there are no clinically relevant pharmacologic ways to induce tissue tolerance and mitigate severity of aGvHD by contributing to target tissue resilience, repair and regeneration without altering the function of the alloreactive immune cells. (126)

In biopsies from patients with severe forms of gastrointestinal GvHD, shortened enterocyte telomeres suggest excessive proliferation leading to eventual exhaustion of tissue-resident adult stem cell populations. (93)

Another observation suggests that the intestinal microbiota and metabolites may influence severity of aGvHD. Distortion of the intestinal microbiome by parenteral nutrition or antibiotics was shown to be correlated with aGvHD severity. (93) (127)

Enteral nutrition seems to be associated to milder GvHD, while parenteral nutrition promotes cytokine imbalance leading to increased enterocyte apoptosis. (93)

The JAK/STAT signaling pathway (STAT1 and STAT3) is activated early after disease onset in acute GvHD. The signaling is of great significance in mediating T-cell activation and the alteration of the T-cell phenotype. (3)

The JAK/STAT pathway also exerts influence over the APC compartment as dendritic cells (DC) are affected in their development, maturation, activation and migration into GvHD target organs. (3)

Glucocorticoids used as first line therapy can mitigate epithelial injury and promote tight junction integrity, but also hinder epithelial proliferation and migration and generation of IL-22 and ILC3. (93)
Ruxolitinib seems to preserve adult stem cells in the skin and gut better than glucocorticoids, as it directly inhibits JAK1-dependend apoptosis of intestinal stem cells. (93)

7.2.1. Staging and Grading of acute graft-versus-host disease

For a standardized approach of staging and grading of acute graft-versus-host disease the guidelines of the Mount Sinai Acute GvHD International Consortium, published by Harris et al. in 2016 were used. (115)

Principally staging of acute GvHD is done by quantification of symptoms in the target organs: extent of skin rash, total bilirubin level, volume of diarrhea and presence of nausea and/or vomiting.

Onset of GvHD is defined as the date of clinical diagnosis and therefore date of initiation of therapy, not antedated to onset of symptoms.

The diagnosis of acute GvHD should be assigned a confidence level. GvHD can only be confirmed by unequivocal evidence on a biopsy, but a confidence level of probable is considered

sufficient to initiate first line GvHD therapy. If the clinical suspicion of GvHD is not high enough to initiate treatment, but the diagnosis of GvHD is being considered, it is deemed possible.

Pathologic findings in biopsies are often inconclusive, therefore aGvHD remains a clinical diagnosis. When multiple target organs are symptomatic, but only one has been biopsied and the result confirms the diagnosis of GvHD, this may increase the confidence level in another symptomatic organ, but it cannot confirm the presence of GvHD there.

The assessment becomes more consistent through repeated examination by the same diagnostician.

If there are several possible causes that may lead to the symptoms described, but symptoms originate at least partially from GvHD, no downstaging of the severity of symptoms is done.

GvHD rarely occurs prior to day 14 post-transplant, however, it is much less likely than other differential diagnoses and needs to be biopsy-proven in this time interval.

Table 1: Organ staging of acute GvHD

Staging	0	1	2	3	4
skin	no active (erythematous) GvHD rash	maculopapular rash < 25% BSA	maculopapular rash 25 - 50% BSA	maculopapular rash > 50% BSA	Erythrodermie Bullae Desquamation > 5% BSA
liver (direct bilirubin)	< 2 mg/dl	2-3 mg/dl	3,1 - 6 mg/dl	6,1 - 15 mg/dl	> 15 mg/dl
upper GI	asymptomatic	nausea, vomiting, anorexia, dyspepsia	-	-	-
lower GI	< 500 ml/d < 3 episodes/d	500 - 999 ml/d 3-4 episodes/d	1.000 - 1.500 ml/d 5-7 episodes/d	> 1.500 ml/d > 7 episodes/d	severe abdominal pain ileus grossly bloody stool

7.2.1.1. Acute GvHD of the skin

Being the most prevalently involved and often the first manifestation of acute GvHD (113), acute GvHD of the skin presents as inflammatory erythema or erythematous maculopapular or morbilliform rash. It is staged by the affected body surface area (BSA) and the presence of desquamation or fluid-filled bullae, which are the hallmarks of stage 4 skin aGvHD.

Only active inflammatory erythema should be considered as affected area of acute GvHD, inactive hyperpigmentation or other changes (petechiae, non-GvHD skin lesions) should be excluded from calculation. Dissolved active inflammatory erythema appears as hyperpigmentation or “browned over”. (115)

For determination of affected BSA the “rule of nines” should be used. (128)

Stage 4 skin GvHD is a massive inflammation and is defined as both generalized erythema plus > 5 % BSA with desquamation and/or blisters. Minor blisters or smaller patches of desquamation (less than 5 % of BSA) without other hints of severe inflammation do not encourage the diagnosis of stage 4 skin GvHD. (115)

Typically, skin aGvHD starts on the face, palms, and soles. Patients may describe pruritis or burning sensations in the affected areas. Over time, these rashes may spread to the trunk, up to generalized erythroderma. Severe cases can show SJS/TEN-like features like fluid-filled bullae or desquamation, being a byword for stage 4. (113)

Typical skin rash caused by acute GvHD of the skin can be difficult to differentiate from numerous of potential non-GvHD causes, like medication-induced or viral exanthema. (115) Even pathologic findings in skin biopsies overlap between those causes and GvHD. In the case of isolated skin rash corresponding to acute GvHD stage 1-2 (overall severity grade 1) often topical steroid treatment is started for limited rashes of any cause without further diagnostics. (115)

7.2.1.2. Acute GvHD of the upper gastrointestinal tract

Staging of upper GI acute GvHD is defined by subjective presence (stage 1) or absence (stage 0) of following possible symptoms: anorexia, nausea, vomiting or dyspepsia. There is no higher stage in this category. To be downgraded, these symptoms need to be fully resolved. So if weight loss occurred before, the patients weight needs to be stable or increasing to be downgraded.

Differential diagnosis can be difficult again, as these symptoms can also occur with infection, mucositis, conditioning regimen toxicity, or as side effect of medication. GvHD is not considered as etiological factor when nausea lasts fewer than 3 days, or with fewer than 2 vomiting episodes per day for at least 2 days, or anorexia without weight loss.

The MAGIC Criteria allow diagnosis without upper GI endoscopy to avoid underreporting, although it is encouraged to obtain whenever possible.

7.2.1.3. Acute GvHD of the lower gastrointestinal tract

Staging of lower GI acute GvHD is based on measurement of daily stool volumes (stage 0-3) in case of diarrhea and presence of grossly bloody stool, ileus or severe abdominal pain as hallmarks for defining stage 4 independently from stool volume.

To alleviate this for outpatient setting, an average volume of 200 ml per diarrhea episode is presumed and converted into the number of diarrhea episodes per day. Formed or mostly-formed stools should be excluded from calculation, also diarrhea volumes attributable to bowel preps for endoscopy procedures.

Stool volumes can vary widely from day to day, so over time it can be more expressive to assess the average of 2-3 days.

Lower GI involvement in acute GvHD is the most distinctive associated target organ with non-relapse mortality.

7.2.1.4. Acute GvHD of the liver

Staging of liver acute GvHD is based solely on total serum bilirubin levels (not conjugated / direct) compared to the bilirubin levels before the diagnosis of GvHD.

In case of doubt, liver biopsy can be performed.

Transaminitis is entirely excluded from staging, as non-GvHD causes are common after hematopoietic stem cell transplantation. But even if GvHD of the liver is confirmed by biopsy, isolated transaminitis without concomitant elevation in serum bilirubin would be scored as stage 0.

Involvement of the liver is the least frequent feature of acute GvHD and often develops over time during present GvHD, it indicates a poorer prognosis.

Many patients have hyperbilirubinemia from other causes prior to the onset of GvHD, so sole liver GvHD needs biopsy confirmation. Alternative causes of hyperbilirubinemia in the context of hematopoietic stem cell transplantation are e.g. chemotherapy toxicity, sinusoidal obstructive syndrome, and parenteral nutrition-associated cholestasis.

If hyperbilirubinemia newly develops concurrently or after onset of GvHD in another target organ, it is presumed that liver aGvHD is present.

7.2.1.5. Overall severity: Grading of acute GvHD

Table 2: Grading of overall severity in acute GvHD

Grading of acute GvHD	
0	every organ stage 0
1	stage 1-2 skin **WITHOUT liver, upper GI, lower GI**
2	stage 3 skin *and/or* stage 1 liver *and/or* stage 1 upper GI *and/or* stage 1 lower GI
3	stage 0-3 skin *and/or* stage 2-3 liver *and/or* stage 1 upper GI *and/or* stage 2-3 lower GI
4	**every stage 4: skin** *and/or* **liver** *and/or* **lower GI** involvement *and/or* stage 0-1 upper GI

Acute GvHD of the skin up to stage 2 is defined as grade 1. Any involvement of target organs other than skin is graded as stage 2 or higher. Every stage 4 organ involvement is defined as overall severity of grade 4.

7.3. Differential diagnosis

Interpreting the symptoms and assigning them to GvHD should always be based on exclusion of alternative causes. Depending on the organ in question, other possible complications of HSCT should be systematically evaluated. Any infectious causes need to be ruled out before initiation of immunosuppressive GvHD treatment.

Using the example of skin involvement, differential diagnoses that should be considered are drug induced skin alterations like hypersensitivity reactions, Stevens-Johnson-syndrome/toxic epidermal necrolysis (SJS/TEN), photosensitivity, toxic erythema from chemotherapy or infectious diseases like bacterial infections, viral exanthems or other HSCT-associated afflictions like engraftment syndrome. (113)

Helpful in assigning skin manifestations to aGvHD or differential diagnoses can be interpreting the clinical context, including the appearance and distribution of the rash, extracutaneous symptoms of other typical target organs of aGvHD (such as diarrhea, or rising serum bilirubin), timing from transplantation and histologic samples. The classical maculopapular rash of aGvHD affects especially the palms and soles, but may spread to the entire body. (56)

Onset of skin aGvHD usually occurs after neutrophil engraftment, thus, during pre-engraftment period other causes are more likely, but still hyperacute GvHD (defined as aGvHD developing before day 14 post-HSCT) does exist. Hyperacute GvHD shows skin involvement more frequently and more severe than aGvHD diagnosed after day 14. (129)

Cutaneous involvement in engraftment syndrome is accompanied by non-infectious fever and hypoxia due to non-cardiogenic pulmonary oedema, caused by proinflammatory cytokines within 4 days of neutrophil engraftment. (113)

Findings of skin biopsy are often non-specific and inconclusive, so it can be a useful tool to narrow down the differential diagnoses, but is not recommended for routine use. (113)

7.4. Chronic GvHD

Chronic GvHD develops in 30-70 % of patients receiving allogeneic HSCT (3) (96) and is one of the major causes of late treatment-related mortality (TRM) after allogeneic HSCT. (91) (130)

Chronic GvHD poses a risk of long-term morbidity and impaired quality of life. (96)

Nearly all clinical manifestations of cGvHD present within the first year after transplantation. (91)

Prior occurrence of acute GvHD is an important risk factor for developing chronic GvHD. (113)

Thrombocytopenia (< 100,000/µl) at time of diagnosis and progressive onset of chronic GvHD from prior aGvHD are characteristics that are associated with an increased risk of late NRM. (91) (95) Other risk factors predicting increased mortality in cGvHD patients are lichenoid skin changes, skin involvement of > 50% of body surface area, and absence of early response to immunosuppression. (106)

In severe cases or inadequate treatment, cGvHD can lead to major disability related to organ manifestations such as keratoconjunctivitis sicca, pulmonary insufficiency due to bronchiolitis obliterans, restrictive lung disease related to scleroderma, joint contractures, or oesophageal stenosis. (94)

NRM in patients with present cGvHD does not plateau but rises over time. This may be associated with ongoing immunocompromised status, as most deaths are attributable to GvHD itself or infection. (130)
Lung involvement in cGvHD is associated with the poorest prognosis due to the irreversibility of damage and fibrosis, once it occurred. (130) (131)

The incidence of cGvHD is increasing, associated with more liberal donor selection other than matched sibling, the use of DLI, an older age of recipient at transplantation, the predominant use of PBSC and the better supportive care improving early NRM, such that more patients are at risk to develop cGvHD. (132)

The clinical presentation of chronic GvHD can be composed of variable features mirroring autoimmune and other immunological disorders. (91) Chronic GvHD can involve any other organ system. (96)

The extent of chronic GvHD may vary from self-limiting manifestations in a single organ or site to widespread manifestation with profound impact to quality of life. (91)

Simultaneous presence of signs and symptoms of acute and chronic GvHD are classified as a subtype of chronic GvHD, called overlap cGvHD. (92)

Chronic GvHD can resolve by emerging of immunological tolerance without immunosuppressive treatment, so the lowest amount of treatment to control the disease should be used in self-limited cases. (91)

The first line treatment are glucocorticoids, but in about 50 % the disease will become glucocorticoid-dependent or glucocorticoid-refractory, which necessitates an effective second line treatment. This topic is discussed in chapter 7.5. (3, 130)

Treatment of cGvHD remains challenging, in many patients fibrotic sequelae are seen. The persisting immune dysfunction comes along with the risk of serious infectious complications, whether caused by cGvHD itself or as medication side effect. (133)

Patients suffering from cGvHD should receive infection preventive measures, as cGvHD itself and the entailed immunosuppressive therapy both impair immune defence. (91)

For this study, we employed the staging and grading of cGvHD as defined by Jagasia et al., which is predominantly based on the clinical features that can be grasped by anamnesis and clinical examination and extensively explained in chapter 7.4.4. Further examination is only necessary when symptoms occur (e.g. ophthalmologist, gynaecologist, or urologist).

7.4.1. Pathophysiology of chronic GvHD

There is only limited understanding of the pathophysiology of chronic GvHD. However, it is not an evolution of previous acute GvHD. (91)

In cGvHD, disease manifestations are more heterogeneous than in aGvHD. Chronic GvHD often shows fibrosis with only little inflammation, although there can also be highly inflammatory manifestations such as polyserositis or polymyositis. (96)

Chronic GvHD is a disorder that involves cell-mediated and humoral immunity in inflammation and is often accompanied by organ fibrosis. Chronic GvHD often mirrors features of autoimmune diseases. (3) (91)

A conceptual model classifies the pathophysiology of cGvHD into three phases. In phase 1, tissue injury causes early inflammation, that is followed by chronic inflammation, dysregulated B- and T-cell immunity, and thymic injury in phase 2. This ends up in phase 3 by tissue repair with fibrosis. (96)

In the early phase of cGvHD, soluble inflammatory mediators are released into the extracellular space and circulation by cytotoxic therapy, infections or acute GvHD. This leads to increased antigen presentation by APCs such as inflammatory monocytes, macrophages, dendritic cells, and B cells. (96)

Tissue damage is aggravated by innate immune cells through ROS, release of matrix metalloproteinases, activation of inflammasomes and subsequent inflammation. (96)

In mouse models, this involves rapid activation of innate immune cells, endothelial cells, and fibroblasts. Simultaneously, T cells are activated by APCs that up-regulate costimulatory molecules from conditioning-related tissue damage. (96)

In a mouse model of BOS, Th17 cells and CD4+ T-cell subset that is TH17-cell-prone and CD146 expressing, are required for the development of cGvHD. (96)

In the second phase, effector cells with a suitable T- or B-cell receptor are activated by recognizing peptides presented by APCs. (96)

After germinal-center formation, B cells are undergoing somatic hypermutation there, producing immunoglobulin isotype-switched antibodies, which augments cutaneous cGvHD, BOS and liver damage. (96)

Antigen targets of B- and T-cell responses in cGvHD remain largely unknown and are therefore difficult to study. (134)

Despite the presence of B-cells and allo- and autoreactive antibodies in GvHD, it is associated with a lack of cells important for response to pathogens. (134)

Immune responses are seen against host MHC proteins in cGvHD patients, and especially antibody generation against neoantigens, such as those encoded by the Y chromosome in male recipients of female donor cells. (96)

Thymic injury is caused by toxic effects of conditioning regimen, CNI, or alloreactive T cells, among others. (96)

In patients with cGvHD, the thymic production of mature T cells is severely impaired. (96)

The loss of thymic dendritic cells, medullary and cortical thymic epithelial cells by being targeted from alloreactive T cells leads to loss of central tolerance, which allows the release of autoreactive CD4+ T cells to the periphery. (96)

Paradoxically, calcineurin inhibitors used in GvHD prophylaxis and treatment may facilitate development of cGvHD by blocking thymic central tolerance and peripheral Treg-cell function. (96)

In the setting of donor B-cell reconstitution after allogeneic HSCT, instead of deletion of autoreactive B-cells, the microenvironment supports their survival. (134) These cells do not take part in the germinal center reaction. (134)

To achieve B-cell tolerance, deletion of donor-derived B cells that react with the recipients tissue would be necessary. (134)

In cGvHD the B-cell homeostasis shows marked abnormalities like heightened B-cell responses, that result in inability to establish B-cell tolerance. (134)

Observations after total B-cell ablation with anti-CD20 antibody rituximab suggest that both a microenvironment that facilitates the survival of autoreactive B-cells and limited capacity to generate sufficient numbers of naïve and transitional B-cells contribute to abnormal B-cell homeostasis in cGvHD. In the latter setting, global B cell depletion may have only limited efficacy in treating or preventing of cGvHD. (134)

By generation of large numbers of naïve and transitional B-cells early after HSCT, these may outcompete themselves for pro-survival factors for autoreactive cells and thereby promote the deletion of alloreactive and autoreactive B cells. (134)

Usually after tissue damage, monocytes and macrophages transition acute inflammation towards tissue repair and enable restoration of integrity of tissue with limited scarring. (93)

In cGvHD, accumulated activated macrophages produce TGF-beta and PDGF-alpha (platelet-derived growth factor alpha), which leads to fibroblast activation. These fibroblasts produce extracellular matrix components including collagen and biglycan, which contribute to tissue stiffness by cross-linking collagen. This process can be conceptualized as extracellular matrix remodelling towards fibrotic changes. (96)

In preclinical models of sclerodermatous GvHD and bronchiolitis obliterans, the responsible anti-inflammatory epigenetic programs for healthy tissue repair with limited scarring are inoperative. (93)

In pulmonary cGvHD (or bronchiolitis obliterans syndrome, BOS) the immunological attack of the small airways leads to fibrotic occlusion of bronchioles and consecutive obliteration. (131)

In fact, localized, cutaneous cGvHD can be triggered by restricted areas of tissue damage, provoked by local pressure, exposure to sunlight, radiation therapy, or reactivation of VZV. (96)

7.4.2. Chronic GvHD of the skin

Chronic GvHD of the skin can present itself very typical like lichen-planus-like or sclerotic manifestation, but also share features with variable other skin diseases, so collaboration with dermatologists are crucial elements of diagnosis and treatment. (113)

Lichen planus-like lesions are similar to true lichen planus, with erythematous to purplish flat papules or plaques, often attended with pruritus. Commonly affected areas are dorsal hands, feet, wrists, ankles, inner forearm and trunk. (113)

Keratosis pilaris appears as similar skin lesions as lichen planus, but in a follicular distribution. (113)

Sclerotic cutaneous lesions are considered more advanced lesions and can be categorized by the affected layers of skin. Lichen-sclerosus like (superficial dermis), morpheaform (dermis) or deep sclerosis (fibrosis of subcutaneous layer). These lesions can develop from previously inconspicuous skin or as resolving lichen-planus-like lesions. (113)

Poikiloderma presents itself by skin atrophy, hyper-/hypopigmentation, dilation of blood vessels with or without lichen planus-like or sclerotic features. (113)

The aforementioned characteristics are determined as diagnostic features, thereby rendering their presence is sufficient to confirm the diagnosis of cutaneous cGvHD without a biopsy. (113)

Diagnostic probability	Explanation	Skin lesions
Diagnostic	Sufficient to conclude chronic GVHD diagnosis	■ Lichen-planus like features
		■ Sclerotic features
		■ Poikiloderma
		■ Morphea-like features
		■ Lichen sclerosus-like features
Distinctive	Observed in chronic GVHD but insufficient alone to conclude	■ Depigmentation
		■ Papulosquamous lesions
Others unclassifiable	Can be regarded as part of chronic GVHD manifestation only if diagnosis is confirmed by other criteria	■ Keratosis pilaris
		■ Hypo- or hyper-pigmentation
		■ Ichthyosis
		■ Sweat impairment
Common	Shared features by both acute and chronic GVHD	■ Erythematous maculopapular rash
		■ Pruritus

Figure 3: Features and their diagnostic probability of cutaneous chronic GvHD (113)

Photosensitivity is a common result after stem cell transplantation, although this is not attributable to GvHD. Patients should endeavour to avoid prolonged exposure to sunlight, or alternatively, apply a sunscreen with a sun protection factor of at least 50.

Superficial forms of skin involvement may often be manageable by topical corticosteroids alone.

7.4.3. Chronic GvHD of the lung

Lung manifestations of cGvHD come with poor prognosis as they are associated with a higher risk of NRM.

Early diagnosis of bronchiolitis obliterans syndrome (BOS), prior to the onset of symptoms can be permitted by frequent screening pulmonary function tests (PFT) for 2 years after HSCT. (131)

PFT should also be performed at the time of cGvHD diagnosis, any cGvHD flare or organ progression. (131)

Hypothetically, earlier detection may result in relative stabilization due to interventions and that a plateau may be reached at a higher FEV1. (131)

The symptoms of BOS typically manifest at a late stage in the disease process, with nonproductive cough, dyspnoea on exertion, wheezing, a reduction in exercise tolerance, or pneumomediastinum being among the most commonly observed. (131)

In order to exclude other potential diagnoses, such as idiopathic pneumonia syndrome, cryptogenic-organizing pneumonia (COP), broncho-obliterans syndrome, pulmonary fibrosis, late radiation effects, infection, asthma, or chronic obstructive pulmonary disease (COPD) it is necessary to conduct thorough diagnostics. Rare disorders that may present with similar symptoms to BOS include tracheomegaly, tracheobronchomalacia, and alpha-antitrypsin deficiency. (131)

Due to the trapped air, chest radiograph may not show infections reliably, it can conceal even larger infiltrates. (131)

BAL may reveal the pathogens causing infection, so it should be considered in patients with acute decline in PFT.

In smokers or patients with preexisting COPD airflow obstruction may progress without presence of lung cGvHD, although this should not outpace the diagnostic criteria of BOS. (131)

Broncho-obliterans syndrome describes the obliteration of large airways in contrast to small airways in bronchiolitis-obliterans syndrome (BOS). This may be observable in bronchoscopy. (131)

In patients with new onset of BOS after HSCT, initiation of azithromycin, montelukast and inhalation of fluticasone twice daily, with an initial steroid burst with rapid taper (1 mg/kg/day prednisone, taper 0,25 mg/kg per week) has showed stabilization or improvement and was well tolerated. (135) (27)

Azithromycin should be discontinued after resolution of BOS, as there is a potential for an increased risk of relapse. (27)

In patients that are sufficiently immunocompetent, immunizations are an important measure for the prevention of lung infections, particularly influenza. (131)

7.4.4. Staging and Grading of chronic graft-versus-host disease

In chronic graft-versus-host disease the standardized criteria for staging and grading of the National Institutes of Health as published by Jagasia et al.in 2015 was applied. (91)

Overlap GvHD is a subcategory of chronic GvHD. This means that at least one diagnostic feature of cGvHD is present, along with concurrent symptoms of aGvHD.

7.4.4.1. Classification of signs and symptoms for initial diagnosis

Table 3: Level of diagnostic relevance for establishing diagnosis of chronic GvHD

	description	examples
diagnostic	establish the presence of chronic GvHD, no need for further testing or evidence	lichen planus-like features of the skin, oral or genital mucosa, Fasciitis
distinctive	uncommon in acute GvHD, support cGvHD as tentative diagnosis, but if solely occurring, additional testing is needed to establish cGvHD diagnosis	Nail dystrophy, xerostomia, new onset dry eyes
common	found in both acute and chronic GvHD, cannot be used to establish the diagnosis of cGvHD	maculopapular rash, pruritus, nausea, diarrhea
other / unclassified	rare, controversial or nonspecific features, cannot be used to establish the diagnosis of cGvHD	periorbital hyperpigmentation, haematopoietic disorders, serositis, sweat impairment, ichthyosis

The demanded mode of additional testing depends on the questionable target organ and may be biopsy, laboratory or other specialized tests, evaluation by a specialist (ophthalmologist, gynaecologist) or radiographic imaging.

As in acute GvHD, other causes can generate similar signs and symptoms that may complicate differential diagnosis. Nevertheless, drug reaction, infection, recurrent or new malignancy and other potential causes of present symptoms need to be excluded before diagnosing chronic GvHD.

Symptoms that are attributable to multiple factors and cannot be viewed in isolation should be scored as if the entire deficit is attributable to GvHD.

If symptoms or changes in one organ can are fully allegeable through a non-GvHD-cause, these should be documented, but the affected organ must be excluded from calculation of overall severity of cGvHD.

If there is a diagnostic feature present, taking biopsy of a distinctive feature is not mandatory.

Clinical features establishing the diagnosis of chronic GvHD may not be the best for assessing severity or being sufficiently sensitive for evaluating response after treatment.

7.4.4.2. Onset of chronic GvHD

De novo onset of chronic GvHD is defined as new symptoms of chronic GvHD in patients – without a previous episode of acute GvHD. Quiescent onset of chronic GvHD applies to patients

with symptoms of chronic GvHD, that had a previous episode of acute GvHD that has had fully responded to therapy. (92)

Progressive onset of chronic GvHD describes a situation in which symptoms of chronic GvHD developed directly from an episode of acute GvHD. This does not necessarily simultaneously mean overlap GvHD, but every overlap GvHD is progressive onset by definition. (92)

7.4.4.3. Chronic GvHD of the skin and appendages

There are two components of staging skin cGvHD: the affected body surface area (BSA) or specific features, the higher of the two scores is used for global scoring. (91)

Skin cGvHD can present itself with following diagnostic features:

- Poikiloderma
- Lichen-planus-like eruption
- deep sclerotic features
  - These are characterized by diffusely thickened, tight and fragile skin, often associated with poor wound-healing, inadequate lymphatic drainage and skin ulcers from minor trauma.
- Morphea-like localized superficial sclerotic features
- Lichen-sclerosus like lesions

Distinctive features of chronic skin GvHD are depigmentation (vitiligo) or papulosquamous lesions. Common features of acute and chronic skin GvHD include erythema, maculopapular rash, and pruritus.

First, the affected body surface area of following features is estimated: maculopapular rash, lichen-planus like features, sclerotic features, papulosquamous lesions, ichthyosis or keratosis pilaris-like GvHD. Areas with solely pigmentary chances should not be included in the determination of BSA. Second, the presence of sclerotic features is scored. If there are superficial features, scoring is 2, and a score of 3 if any of the following is present: deep sclerotic features, “hidebound”, impaired mobility, or ulcerations. The higher of these two scores is used in global grading.

Nail dystrophy is a distinctive sign of cGvHD. It can present itself as longitudinal ridging, nail splitting or brittleness, onycholysis, or pterygium unguis up to nail loss.

Distinctive features of hair involvement in cGvHD include new scalp alopecia (scarring or non-scarring) and loss of body hair, that are not due to chemotherapy or radiotherapy. Premature greying, thinning or brittleness can be categorized as other features. (91)

7.4.4.4. Chronic GvHD of mouth

Often the diagnostic sign of Lichen-planus like changes of the mouth, occurring frequently buccal or on the tongue are the first diagnostic sign of cGvHD. The characteristic hyperkeratotic white lines on the oral mucosa can be observed with or without erythema or ulcerations.

If the patient is completely asymptomatic, the staging is zero despite the visual presentation.

Distinctive features of oral cGvHD are xerostomia or ulcers, mucoceles, mucosal atrophy and pseudomembranes, these can be difficult to separate from infections or secondary malignancy. Common oral manifestations of acute and chronic GvHD include gingivitis, mucositis, erythema, and pain.

Isolated hyperkeratotic plaques without lichen planus-like changes (leukoplakia) should be considered as a separate entity and may imply malignant potential.

Localized skin sclerosis can result in secondary decreased range of motion in the jaw should be evaluated according to the skin criteria.

7.4.4.5. Chronic GvHD of the eyes

Every ocular involvement is a distinctive feature of chronic GvHD.

It presents as new onset of dry, “gritty” or painful eyes, cicatricial conjunctivitis, keratoconjunctivitis sicca (KCS) and confluent areas of punctate keratopathy. Other features of eye involvement are photophobia, periorbital hyperpigmentation, and blepharitis.

Keratoconjunctivitis should be confirmed by ophthalmologist.

The staging of ocular involvement is based on clinical symptoms and on the frequency the patient requires lubricant eyedrops each day, in comparison to the baseline evaluation pre-transplant and post-transplant around day 100, which should be performed as standard.

Any new ocular symptoms or vision impairment should be assessed by an ophthalmologist by Schirmer’s test and slit lamp exam.

The follow-up of ocular GvHD is based solely on symptomatic use of lubricant eye drops. Again, if the patient is fully asymptomatic, the staging is zero.

7.4.4.6. Chronic GvHD of the gastrointestinal tract

The only diagnostic feature of the involvement of the gastrointestinal tract in chronic GvHD is the oesophageal involvement, may presenting clinically as dysphagia. The diagnostic features oesophageal web, stricture or concentric rings and may be documented by endoscopic or barium contrast radiograph examination.

Common features of acute and chronic GvHD are anorexia, nausea, and vomiting, diarrhea, weight loss and failure to thrive in paediatric patients. Staging of severity in chronic GvHD is mainly based on weight loss in the previous three months.

Wasting syndrome in terms of unintentional weight loss as result of chronic GvHD is often multifactorial. These symptoms can also result from a variety of non-GvHD causes like medication side effect, motility disorders or infections. But also decreased caloric intake or hypercatabolism due to increased resting energy expenditure should be contemplable.

Scoring gastrointestinal chronic GvHD is based on the extent of weight loss within the last three months, but also severity of diarrhea and the presence of oesophageal dilation.

Pancreatic atrophy leading to exocrine insufficiency and subsequent poor intestinal absorption of macronutrients that can be correlated with chronic GvHD may improve with oral pancreatic enzyme supplementation. (91)

7.4.4.7. Chronic GvHD of the liver

Hepatic cGvHD can present itself in two clinical variants.
One is akin to acute hepatitis and generally occurs after tapering immunosuppressive prophylaxis or after donor lymphocyte infusion. It arises as surging serum-ALT (transaminitis) with or without jaundice and needs immediate diagnosis and treatment initiation, including liver biopsy when necessary. The second possible clinical presentation is a slowly progressive cholestasis with elevated AP and gamma-GT concentration and potential jaundice.

Hepatic cGvHD is classified as common feature of GvHD, offers neither diagnostic nor distinctive criteria. Additionally, liver GvHD comes with various potential differential diagnoses, i.e., viral infections, biliary obstruction, drug toxicity or non-alcoholic steatohepatitis.

Liver function tests are the only laboratory testing required in staging of chronic GvHD. Scoring is based on total bilirubin, ALT, and AP.

7.4.4.8. Chronic GvHD of the lungs

The pulmonary manifestation of chronic GvHD is bronchiolitis obliterans syndrome (BOS), which is characterized by new onset of an obstructive lung defect. The clinical presentation of exertional dyspnoea, cough or wheezing often arise late in disease development.

Therefore, pulmonary function testing is established as screening pre-transplant, on day 100 post-transplant, at first diagnosis of chronic GvHD or in 6-month intervals for the first two years post-transplant. Time intervals may be contracted when BOS is maybe currently developing without meeting diagnostic criteria yet.

The required examination for establishing diagnosis of pulmonary involvement in chronic GvHD depends on the constellation of other involved organs in cGvHD.

If BOS is the only clinical symptom of chronic GvHD, lung biopsy is necessary to establish the diagnosis of cGvHD. In the case of a distinctive manifestation of chronic GvHD in another organ, following criteria need to be met to establish the diagnosis:

1. FEV1/VC < 70 % of predicted
2. FEV1 < 75 % of predicted with ≥ 10 % decline over < 2 years
   FEV1 should not correct to > 75 % of predicted with albuterol
3. absence of infection in the respiratory tract
   chest radiograph, CT-scan, microbiologic cultures
4. one of the following supporting features of BOS:
    a. evidence of air trapping by expiratory CT
    b. small airway thickening
    c. bronchiectasis by high-resolution chest CT
    d. evidence of air trapping by pulmonary function tests
        i. residual volume > 120 % of predicted
        ii. RV/TLC elevated outside the 90 % confidence interval

If the diagnosis of chronic GvHD is already established by diagnostic features in another organ, only criteria 1-3 are necessary.

In advanced disease, pneumothorax, pneumomediastinum or subcutaneous emphysema may rarely occur.

Restrictive pulmonary function abnormalities may reflect extra-pulmonary sclerotic GvHD or non-GvHD intrapulmonary changes like pneumonia or pulmonary fibrosis.

Whenever possible, lung scoring should be concordant by using FEV1 scores and symptoms. Severity of symptoms is assessed as the amount of activity leading to shortness of breath. In case of discrepancy between FEV1 scores and symptoms, FEV1 scores should be used for final scoring.

Abbreviations used: FEV1 = Forced expiratory volume in 1 second, VC = Vital Capacity, CT = computer tomography, RV = residual volume, PFT = pulmonary function test

7.4.4.9. Musculoskeletal chronic GvHD, cGvHD of joints and/or fascia

Fascial involvement is a diagnostic feature, and often is associated with deep sclerotic features of overlying skin/subcutis. It often affects forearms or legs, presenting as oedema of extremities or peau d'orange. It can also lead to joint stiffness and contractures, which presents as restricted range of motion.

Clinical myositis with accordingly elevated muscle enzymes is a distinctive feature of cGvHD, it may present as proximal myopathy. Clinical examination should be affiliated by electromyography and creatinine-phosphokinase or aldolase. To rule out differential diagnoses, biopsies should be considered.

Arthralgia and manifested arthritis are uncommon.

Scoring of musculoskeletal chronic GvHD is rated by the amount of tightness of arms or legs, decreased range of motion (ROM) as pictured in the original paper and therefore impaired ADL.

7.4.4.10. Chronic GvHD of genital tract

The involvement of the genital tract (female and male) is often associated with oral cGvHD.

Genital cGvHD presents numerous features overlapping with oral and dermal involvement in chronic GvHD. Diagnostic features include lichen planus-like features, lichen sclerosus-like features, vaginal scarring, clitoral/labial agglutination (in females), phimosis and scarring/stenosis of urethral or meatus (in males). As distinctive features of genital cGvHD erosion, fissures and ulcers are listed.

The signs and symptoms of cGvHD of genital tract may lead to sexual dysfunction, which can severely affect the patient's quality of life.

Even in asymptomatic patients, genital examination by a specialist is recommended, specifically if oral GvHD is present. New genital symptoms should be assessed by a gynaecologist.

When unable to examine the patient, genital GvHD is not scored.

7.4.4.11. Other symptoms of chronic GvHD

Chronic GvHD can hypothetically imitate every autoimmune disorder. However, attributing these findings to chronic GvHD is often diagnosis of exclusion.

Following findings are described occasionally:

- Serositis: pericardial or pleural effusions, ascites
- Peripheral neuropathy
- Myasthenia gravis
- Polymyositis
- Nephrotic syndrome
- Membranous glomeronephritis
- Raynaud's phenomenon
- Eosinophilia > 500/µl
- Platelets < 100,000/µl

Heterogenous haematopoietic and immunological abnormalities may occur, especially thrombocytopenia with < 100.000/µl is associated with worse outcome. Autoimmune processes or stromal damage may lead to cytopenia, hypo- or hypergammaglobulinemia or idiopathic thrombocytopenic purpura.

7.4.4.12. Performance Status in chronic GvHD

General condition is evaluated by established criteria, using ECOG performance status (136) and Karnofsky performance status (137).

Karnofsky performance status (KPS) is scored from 0 to 100 in 10-point increments. Higher scores represent better well-being and performance status.

ECOG performance status is valued in scores from 0 to 5, with lower scores representing better performance status.

7.4.4.13. Overview and Grading of chronic GvHD

Table 4: Organ staging of chronic GvHD

Staging	0 = none	1 = mild	2 = moderate	3 = severe
performance status	Karnofsky 100 ECOG 0 asymptomatic	KPS 80-90 ECOG 1	KPS 60-70 ECOG 2	KPS < 60 ECOG 3-4
skin	no exanthema	1-18 % BSA	19-50 % BSA	> 50 % BSA
	no sclerotic features	-	superficial sclerotic features, “not hidebound”, “able to pinch”	deep sclerotic features “hidebound” / “unable to pinch” Impaired mobility, ulceration
mouth	asymptomatic	mild symptoms, disease signs, but **not limiting oral intake significantly**	moderate symptoms, partial limitation of oral intake	Severe symptoms with disease signs with major limitation or oral intake
eyes	asymptomatic	mild dry eye symptoms, requirement of lubricant eye drops ≤ 3 x per day	moderate dry eye symptoms, requirement of lubricant eye drops > 3 x per day, **without new vision impairment**	severe dry eye symptoms, significantly affecting ADL, unable to work because of ocular symptoms, loss of vision
GI-tract	asymptomatic	mild symptoms, without significant weight loss (< 5 %)	symptoms associated with weight loss (5-15 %), moderate diarrhea	symptoms associated with significant weight loss > 15%, esophageal dilatation, severe diarrhea, significant interference with daily living
liver	normal total serum bilirubin, and ALT/AP < 3 x of upper normal limit	normal total serum bilirubin, ALT ≥ 3-5 x of upper normal limit or AP ≥ 3 x of upper normal limit	elevated total serum bilirubin, but ≤ 3 mg/dl or ALT > 5 x of upper normal limit	elevated total serum bilirubin > 3 mg/dl
lungs	asymptomatic	mild symptoms (dyspnoea after climbing one flight of steps)	moderate symptoms (dyspnoea after walking on flat ground)	Severe dyspnea, at rest, requiring O_2
	FEV1 ≥ 80 %	FEV1 60-79 %	FEV 1 40-59 %	FEV ≤ 39 %
joints & fascia	asymptomatic	mild tightness of arms or legs, normal/ mild decreased ROM, ADL not affected	mild tightness of arms or legs OR joint contractures, moderate decreased ROM, mild-moderate limitation of ADL	contractures with significant decrease of ROM and significant limitation of ADL
genital tract	no signs	mild signs	moderate signs, may discomfort on exam	severe signs, even if asymptomatic

Abbreviations used: KPS = Karnofsky Performance Status, ECOG = Eastern Cooperative Oncology Group, BSA = body surface area, GI-tract = gastrointestinal tract, ALT = alanine transaminase, AP = alkaline phosphatase, FEV1 = forced expiratory volume in 1 second, O2 = Oxygen, ROM = range of motion, ADL = activities of daily living

This staging system does not distinguish between active inflammatory disease or fixed deficits from past tissue injury.

For calculating global scoring, eight organ systems are considered: skin, mouth, eyes, gastrointestinal tract, liver, lungs, joints and fascia, and genital tract.

Table 5: Grading of overall severity in chronic GvHD

Grading / Global Scoring	
mild cGvHD	1-2 organs, max. stage 1 AND **lung 0**
moderate cGvHD	**≥ 3 organs** max. stage 1 or mind. 1 organ with **stage 2** or **lung 1**
severe cGvHD	at least 1 organ with stage 3 or **lunge 2-3**

For grading overall severity of chronic GvHD, the number of organs/sites involved, and the severity are taken into account.

Excluded from global scoring are performance status, the genital tract if not examined by a specialist, and any symptoms and abnormalities fully attributable to non-GvHD-causes.

Asymptomatic, yet detectable abnormalities have no effect on global scoring, as they are assessed in organ staging as zero.

Global Scoring can only be applied if the diagnosis of chronic GvHD is confirmed by the criteria mentioned above.

7.5. Treatment of GvHD

Early diagnosis and initiation of systemic treatment can prevent GvHD from progressing into severe GvHD, which becomes apparent in quality of life and possibly survival benefit. (91)

General skin precautions include the regular use of topical moisturisers, applied gently in a thin layer in the direction of hair growth, which can relieve itching and prevent skin cracking. Persisting pruritus may be controlled with topical or oral antihistamines. (113)

Systemic corticosteroids remain first line treatment for both acute and chronic GvHD, although response may be short-termed, and approximately 50 % of patients require second line treatment, as they are unresponsive to or dependent on systemic corticosteroids.. (138)(139)(140)

The decision to initiate glucocorticoids for treatment of GvHD is based on clinical signs and symptoms. Although biopsies are recommended before initiation, particularly to rule out or detect other (co)etiologies such as viral infections, histology reporting should not be awaited before treatment initiation. (27)

Mild cutaneous GvHD (aGvHD skin stages 1-2 / cGvHD skin mild) without other organ manifestations can be effectively treated with topical corticosteroids or topical tacrolimus (CNI). (27) (113)

But even in topical use, corticosteroids are associated with side effects as they inhibit keratinocyte proliferation, induce skin atrophy and delay wound healing. (141)

High-dose corticosteroids are concomitant with severe complications and toxicities. In case of only temporary response or steroid-resistant GvHD, second-line treatment is recommended. (27) (142)

Steroids may have numerous well-recognized side effects, which include the suppression of the hypothalamic-pituitary-adrenal axis, an increased risk of infection, glucose intolerance, increased appetite, weight gain, sleep disturbances, psychiatric disturbances, cushingoid changes (moon facies, buffalo hump, central trunk obesity), muscle wasting, myopathy, electrolyte disorders, osteoporosis and osteonecrosis, oedema, poor wound healing, increased bruisability, atrophy of skin, hirsutism, acne, and an increased risk of cataracts and glaucoma. The risk for adverse effects is related to the dose and duration of therapy, so especially patients requiring moderate to high dosage for longer periods of time are affected. (143, 144) (145)

7.5.1. Treatment of acute GvHD

The recommended initial doses of systemic corticosteroids for acute GvHD are 1-2 mg methylprednisolone per kilogram body weight per day. Over time, it is possible to replace methylprednisolone with an equivalent dose of prednisone. (113) (27)

In patients with lower-grade acute GvHD with predominantly cutaneous involvement, lower dose systemic prednisone (0.5 mg/kg of body weight) has been demonstrated to be non-inferior to higher doses. (146)

The response to glucocorticoids in the first line of treatment for aGvHD depends on the grading at initiation and ranges from 30 – 40% in patients with grade IV disease to approximately 60% in patients with grade II disease. (138)

The duration of steroid taper varies widely in clinical practice and must be tailored to the individual situation. In responsive acute GvHD, the steroid dose can be tapered gradually about every 5-7 days, as tolerated. (113)

In the REACH-2 trial (138), acute GvHD was considered glucocorticoid-refractory if one of the following definitions applied:

- progressive disease on the base of organ assessment after at least three days of high-dose systemic glucocorticoid therapy (2.0 mg of methylprednisolone equivalent per kilogram of body weight per day), with or without calcineurin inhibitors
- absence of partial response or better after seven days of high-dose systemic glucocorticoid therapy
- treatment failure during glucocorticoid taper, defined as one of the following:
  - reescalation of systemic glucocorticoid dose to ≥ 2.0 mg/kg methylprednisolone per day
  - failure to taper the methylprednisolone dose to < 0.5 mg/kg per day for at least seven days

In clinical practice, particularly since the availability of established and approved second-line treatments, GvHD is judged more liberally as steroid-refractory or steroid-dependent, and second-line therapies are often initiated before these criteria are met.

The sole agent approved by FDA and EMA for second line treatment of acute GvHD so far is Ruxolitinib, based on the REACH-2 trial. (138)

Other commonly used agents used in SR-aGvHD, such as used as control therapy in the REACH-2 trial, include ATG, ECP, mesenchymal stromal cells, low-dose MTX, MMF, everolimus or sirolimus, etanercept, and infliximab. (138)

7.5.2. Treatment of chronic GvHD

In cGvHD, the intensity of treatment should be geared to the severity of disease manifestation. In asymptomatic or mild manifestations, watchful waiting, topical treatment or decelerating the taper of GvHD-prophylaxis may be sufficient. (91)

The recommended initial doses of systemic corticosteroids are 0.5 – 1 mg prednisolone per kilogram of body weight per day for moderate – severe chronic GvHD. It is possible to substitute prednisone with an equivalent dose of methylprednisolone. (113) (27)

The dose of methyl-/prednisone should be rapidly adjusted to the minimal amount needed to control symptoms, and can be tapered by 25 percent per week. (147) At low doses, cGvHD often requires delicate reductions of the steroid dose to avoid GvHD flares or adrenal insufficiency. (113)

The REACH-3 trial applied the definition of glucocorticoid-refractory classical chronic GvHD as defined by Martin et al. in 2015 (148).

- progressive manifestations after at least 7 days of ≥ 1 mg prednisone equivalent per kilogram of body weight per day
- lack of partial response or better despite ≥ 0.5 mg prednisone equivalent per kilogram of body weight per day for at least four weeks
- treatment failure during glucocorticoid taper, defined as one of the following:
  - doses of > 0.25 mg prednisone equivalent per kilogram of body weight per day required to avoid reappearance of GvHD manifestation.
  - progression of chronic GvHD manifestations in two failed attempts to taper glucocorticoids in an interval of at least eight weeks, requiring increasing the glucocorticoid dose to ≥ 0.25 mg prednisone equivalent per kilogram of body weight per day.

As in aGvHD (see above), in clinical trials GvHD is judged more liberally as steroid-refractory or steroid-dependent, and second-line therapies are often initiated before these criteria are met.

In chronic GvHD, there are wider variety of FDA-approved agents, including ibrutinib, belumosudil and RUX. Ruxolitinib is the sole agent that has been approved by the EMA based on the results of the REACH-3 trial. (139)

Other commonly used agents used in SR-cGvHD, such as used as control therapy in REACH-3 trial, include ECP, MMF, ibrutinib, low-dose MTX, everolimus or sirolimus, infliximab, rituximab, and imatinib. (139)

As described in chapter 7.4.1, cGvHD is a disease with heterogeneous disease biology. To avoid a “trial and error” approach, biomarkers are urgently needed and a major topic of research to predict response to different classes of agents, to prevent progression of cGvHD towards irreversible changes. (149)

7.5.3. Second line treatment or beyond

Many agents used in second line treatment, such as CNI, antimetabolites (MMF, MTX) or ATG, target the alloreactive T cell activation and proliferation, as this is a key element in the pathophysiology of both acute and chronic GvHD. (150)

In patients who are already taking or are still taking immunosuppressive drugs, the dose administered may be increased or another agent with a different mechanism of action may be added. (91)

Ruxolitinib is the key topic in the next chapter, as it is an approved second line treatment and the primary focus of this thesis.

In patients with SR-cGvHD after RUX failure, RUX intolerance or contraindications for RUX, inclusion in clinical trials is the preferred option whenever possible. (27)

Agents used in prophylaxis regimens such as calcineurin-inhibitors (CNI), antimetabolites or mTOR-inhibitors (mammalian target of rapamycin) can be used in addition to corticosteroids to permit lower doses or rapid taper and therefore decrease steroid associated toxicity. (4) (113)

When choosing the agent for refractory GvHD, it needs to be considered that the absorption of oral drugs might be reduced in patient with severe malabsorption or diarrhea. (27)

7.5.4. Agents used in further treatment of GvHD

The following agents represent examples of those that were employed as a second line treatment prior to the advent of RUX and continue to be utilized in further line treatment.

Anti-TNF: Etanercept, Infliximab

TNF-alpha was considered as target in GvHD treatment early, considering its central role as proinflammatory mediator in induction and maintenance of GvHD. Both etanercept and infliximab are primarily employed in the treatment of acute GvHD.

Etanercept is a subcutaneously given recombinant human soluble TNF-alpha-receptor fusion protein, binding and acting as inhibitor for TNF-alpha. It showed response in both SR acute and chronic GvHD, with response most commonly seen in gastrointestinal aGvHD. It was generally well tolerated, the major adverse events were bacterial (14%) and fungal infections (19%) and CMV reactivation in 48%. (151)

Infliximab is a chimeric monoclonal antibody that binds TNF-alpha, which results in an inhibitory effect. In the treatment of SR-aGvHD, Infliximab has shown an overall response rate of 40% within 4 weeks but decreasing down to 17% at 12 weeks from initiation. Additionally, this was associated with infectious complications in 83% of patients within 12 weeks of initiation of Infliximab. (152)

Extracorporeal photopheresis (ECP)

This leukapheresis based procedure is an immunomodulatory treatment frequently applied in T-cell mediated immune disease, which exposes the collected leukocytes ex vivo to ultraviolet-A light irradiation along with a photosensitizer (8-methoxypsoalen, 8-MOP). This inhibits DNA synthesis by causing cross-linking when exposed to UV-A light, hence the lymphocytes undergo apoptosis. When reinfused, these apoptotic lymphocytes induce immunomodulatory effects that mitigate the alloreactive T-cell effects. (113) (133)

Different cell types show varying susceptibility to ECP-induced apoptosis. T lymphocytes are highly susceptible to 8-MOP/UVA exposure, with Tregs being more resilient. (153)

Apoptotic cells are phagocytized by nonexposed APCs, which leads to secretion of anti-inflammatory cytokines and chemokines. (153)

The mechanism involves antigen-presenting cells and affects T-cell subset expression, which results in balanced immune reconstitution and induces immune tolerance. (154)

It is an effective treatment in both acute and chronic GvHD with low treatment associated toxicity with an ORR of 56,0 % in chronic GvHD in a randomized controlled study. (154)(155)

ECP does not result in general immunosuppression, on the contrary, patients have normal response to vaccination and opportunistic infections are rarely seen. (133) (153)

In a recent retrospective study by the EBMT transplant complications working party, Penack et al. compared ECP and RUX in SR-cGvHD. The odds ratio in the RUX group to achieve overall response vs. the ECP group was 1,35 without statistically significant differences in OS, PFS, NRM and relapse incidence, which further emphasises ECP as effective treatment option in SR-cGvHD. (156)

In both REACH-2 and REACH3 trials, ECP was the most common BAT used. (138) (139) Combination of RUX and ECP should be considered and further evaluated in prospective trials to aim to improve response rates and reduce time under high-dose glucocorticoids and avoid cytopenia observed under RUX. (133)

Tocilizumab (Anti-IL-6R)

This humanized monoclonal antibody targets the IL-6 receptor (IL-6R), which is approved by the EMA for the use in rheumatologic conditions and cytokine-release syndrome induced by CAR-T-cell treatment. (26) IL-6 drives Th17 differentiation, which in turn promotes production of other pro-inflammatory cytokines by Th1 cells. (39)

Although IL-6 has been shown to be one of the fundamental mediators of aGvHD in murine models, inhibiting IL-6 signaling with tocilizumab has shown no significant improvement as part of GvHD prophylaxis in a placebo-controlled phase III study in preventing grades II-IV or III-IV aGvHD. (157)

In aGvHD, there are several small case series that reporting mixed results, with most of the responses observed in isolated cGvHD of the gastrointestinal tract. (158) (159)

Abatacept (CTLA-4-IgG)

Abatacept is a CTLA-4-IgG1 recombinant soluble fusion protein. It binds to CD80/86 on APCs and inhibits CD28 costimulatory signaling required for T-cell activation and inhibits complement fixation and antibody-dependent, cell-mediated cytotoxicity, which is usually mediated by costimulatory molecule CTL4 (cytotoxic T-cell lymphocyte-4) and co-stimulation CTLA4. Thereby the T-cell costimulation is blocked. (160) (161) This prevents T-cell activation during phase II of aGvHD pathophysiology. (39)

Addition of Abatacept to standard aGvHD-prophylaxis regimen in a phase 2 study lowered the risk of grade 3/4 aGvHD and showed a significantly better overall survival at day 180 compared to standard prophylaxis without. (161) (162)

It has been demonstrated that higher level of exposure than that employed in the phase 2 trial results in a further reduction in the risk of grade 2-4 aGvHD without an increase in adverse safety outcomes, including relapse or CMV/EBV viremia. (160)

Abatacept has been approved by the FDA for GvHD prophylaxis.

In SR-cGvHD, abatacept was evaluated in a phase 2 trial. Koshy et al reported an ORR of 58% (21/36 patients) at 1 month after 6 doses of abatacept, with all respondents achieving a partial

response and greatest improvement observed in the lungs, liver, GI tract, and mouth. Regarding cGvHD of the lungs being a difficult-to-treat organ, 57% response rate was observed, including 4 patients who improved from moderate to mild severity, and one patient who improved from severe to moderate symptom lung score. The patient cohort included heavily pretreated patients with a median number of prior lines of treatment of 3. Adverse events included neutropenia (2/36 grade 3/4), fatigue, headache, and serious infectious complications. (163)

Faecal microbiota transplantation

Faecal microbiota transplantation (FMT) is intended to change the composition of the gut microbiota of the recipient by transplanting functional bacteria found in the faeces of healthy donors into the intestinal tract of the patient. (119)

As gut microbiota influences the pathogenesis of gastrointestinal GvHD, several studies have confirmed clinical efficacy and safety in SR GvHD. (119)

The precise mechanism by which GvHD is affected by FMT remains unclear. (119)

As an additional effect, some patients carrying multidrug-resistant bacteria were successfully decolonized after FMT. (119)

Liu et al. confirmed the combination of FMT and RUX is an effective treatment for SR intestinal aGvHD, with declining levels of inflammatory cytokines and higher diversity of gut microbiota. (164)

The most common grade 3/4 adverse events in patients undergoing FMT were infections. Safety of FMT needs to be further evaluated, particularly in patients with mucosal barrier injury. The transfer of microbiome from the donor to the recipient carries a risk of infection. Therefore it is essential to implement rigorous donor screening procedures. (119)

Anti-CD20: Rituximab

When it was first indicated that B-cells play a role in the pathophysiology of cGvHD, the hypothesis emerged that anti-B-cell therapy may be an effective treatment in cGvHD.

Rituximab is an anti-CD20 chimeric monoclonal antibody and was evaluated in phase 2 studies for prophylaxis and treatment of SR cGvHD.

In the phase 2 trial for rituximab used in GvHD prophylaxis, rituximab was given to ensure B lymphopenia during the first year after transplantation. No excess infections were observed, B-cell recovery was observed between month 18 and 24 after HSCT in the majority of patients. (165)

In the context of SR-cGvHD, Rituximab demonstrated a response rate of 70%, but limited to patients with cutaneous and musculoskeletal manifestations. The majority of adverse events were of an infectious nature, with no instances of adverse haematologic events. Levels of circulating immunoglobulin fell after rituximab therapy, with an decrease of 36.5% in median level of circulating IgG and a decrease of 76% of IgM by week 16 after Rituximab therapy compared to baseline. (143, 143)

Hypogammaglobulinemia is a frequently observed adverse effect of rituximab across all indications, particularly in patients who have undergone HSCT. (166) (167) (168) In general, this is transient and recovers slowly after approximately 12 months (169), although severe cases have been reported with persisting hypogammaglobulinemia for more than 7 and 10 years. (170)

Imatinib

Imatinib is a multiple-target tyrosine kinase inhibitor that is approved by the EMA for the use in CML and gastrointestinal stroma tumors (GIST), amongst others.

In cGvHD, Imatinib inhibits both, PDGF- and TGF-beta signaling pathways, that were shown to play an important role in the pathogenesis of cGvHD.

Based on reports of varying responses in refractory fibrotic cGvHD (171–175) , Ibrutinib was evaluated in a phase 2 trial for SR-cGvHD, in which it demonstrated efficacy and safety with tolerable toxicity within the known side effect profile. (176)

Baek et al. report an overall response rate of 58.3%, with organ response of 34.8% in cutaneous and 25% in lung cGvHD. (176)

These side effects include gastrointestinal symptoms such as nausea, vomiting and diarrhea, haematologic toxicity, and muscle pain, headache, liver function test elevation, fatigue, skin rash, fluid retention, infections and anaemia. (171, 173–176)

Ibrutinib

Ibrutinib is an oral selective and irreversible inhibitor of Bruton's tyrosine kinase (BTK) and interleukin-2 inducible T-cell kinase (ITK). BTK is part of the BCR signalling complex, it regulates survival, migration and proliferation of B-cells. (3) ITK facilitates T-cell activation and enhances proliferation and cytokine production.

Ibrutinib is FDA approved for the use in B-cell cancers. In 2017 the FDA approved Ibrutinib as the first agent for glucocorticoid-refractory cGvHD[2]. (3)

In cGvHD, Ibrutinib reduces both T-cell and B-cell activation. (96)

Ibrutinib showed 67,0 % ORR in SR-cGvHD in a multicenter, open-label study, the predominant part of responders showed sustained response for ≥ 20 weeks. (177) In first line treatment of cGvHD, ibrutinib showed no benefit when added to corticosteroid therapy in a randomized controlled trial (iNTEGRATE study). (178)
Overall, Ibrutinib showed a tolerable safety profile, adverse events most commonly reported were fatigue, diarrhea, muscle spasms, nausea, infectious complications, bruising due to platelet dysfunction, and hair and nail changes, corresponding to previous observations. (177) (113)

More rarely, atrial fibrillation was observed. (177)

Ibrutinib is the only agent also approved for use in paediatric patients from the age of one year with SR-cGvHD as a result of the iMAGINE trial. (179)

Belumosudil

Belumosudil is an oral selective inhibitor of ROCK2 (rho-associated coiled-coil protein kinase 2), which is a serine-threonine kinase activated by Rho GTPases. ROCK2 is part of the TCR signalling pathway, leading to phosphorylation of STAT molecules. The ROCK2 signalling pathway has been shown to intervene in the balance between Th17 cells and Tregs. (3)

By inhibiting ROCK2, Belumosudil shifts the Th17/Treg balance towards regulatory T-cells through a STAT5-dependent mechanism. (180)

[2] https://www.fda.gov/drugs/resources-information-approved-drugs/fda-expands-ibrutinib-indications-chronic-gvhd

The downregulation of STAT3 phosphorylation leads to decreased expression of inflammatory Th17 cell-specific transcription factors, while upregulation of STAT5 enhances regulatory T cells. (180) As explained above, Tregs do have a high potency in reducing the GvHD severity.

ROCK signalling is a crucial part of numerous fibrotic pathways by promoting the differentiation of fibroblasts into myofibroblasts and increasing the production of collagen. (180)

The ROCKstar study (NCT03640481) evaluated Belumosudil for cGvHD in 3rd or higher line for patients aged 12 years or older, and led to FDA approval[3] for the use in SR-cGvHD. (180)

Even in pulmonary cGvHD, usually difficult to treat, belumosudil was associated with clinical response, especially in patients with less advanced disease. (180) (181)

Belumosudil appears safe and well tolerated, adverse events occurring in ≥ 20 % of patients include fatigue, diarrhea, nausea and vomiting, hypertension, upper respiratory tract infections, and headache. 24 % of patients had increased liver function tests. Worth mentioning are the low rates of ≥ 3 cytopenia and CMV infections and -reactivations. (180)

Encouraging overall response rates and the low drug-induced toxicity profile led to the EBMT classifying Belumosudil as potential therapeutic option in SR-cGvHD, although not approved by the EMA in this indication. (27)

In murine allo-HCST models, ROCK1/2 inhibition combined with Ruxolitinib indicated a synergistic effect against aGvHD without impairing GvL effects. (150)

Proteasome inhibitors: Bortezomib, Ixazomib

Inhibition of the 20S proteasome activity has been demonstrated to have diverse immune modulatory, anti-inflammatory and direct tumouricidal effects. This is achieved by inhibiting the NFkB (nuclear factor-kappa B) signaling pathway which is a regulator of T- and B-cell development, activation, differentiation, and survival, and promoting apoptosis via various mechanisms. In the context of GvHD, it is postulated that the cellular processes of DCs, B cells and T cells are influenced in order to prevent or ameliorate GvHD. (182–185)

Bortezomib has been approved by the FDA and EMA for the i.v./s.c. treatment of multiple myeloma and mantle cell lymphoma.

Based on promising results of murine models, Pai et al. initiated a small pilot clinical trial for evaluating bortezomib in SR-cGvHD in 6 patients, and observed response in 5/6 patients, with reduction of dose and/or number of other immunosuppressive drug therapies. (183)

In first line in cGvHD with bortezomib in addition to corticosteroids, there was observed an overall response rate of 80% at week 15. (185)

Bortezomib was also evaluated as addition to GvHD-prophylaxis (TAC/MTX/BOR), but was inferior to prophylaxis regimen consisting of TAC/MMF/PTCy. (186)

Ixazomib is also a proteasome inhibitor, that inhibits a subunit of the 20S-proteasome, but is available for oral intake. Additionally, the peripheral neuropathy described as side effect of bortezomib is more rarely in Ixazomib.

Ixazomib was evaluated in a phase 2 trial for the treatment of SR-cGvHD, in a patient population with 84% severe cGvHD, and 52% of patients with 4 or more organ involvement. 78% of the 50

[3] https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-belumosudil-chronic-graft-versus-host-disease

patients enrolled in this study were heavily pretreated with 3 or more prior lines of systemic therapy for cGvHD. In this cohort the overall response rate at 6 months was 40%. (184)

Side effects for Ixazomib include nausea, vomiting, diarrhea, fatigue, infectious adverse events, and haematologic toxicity with thrombocytopenia, and lymphopenia. In Bortezomib, peripheral neuropathy is observed(184)

Itacitinib

Itacitinib is a selective JAK1 inhibitor that seemed promising in phase I study.

The GRAVITAS-301 study evaluated Itacitinib in addition to glucocorticoids for the initial treatment of aGvHD, which did not improve outcomes significantly. (187)

The GRAVITAS-309 trial (NCT03584516, (188)) which investigated Itacitinib and glucocorticoids as a first line treatment in cGvHD was terminated due to an insufficient efficacy outcome.

Low-dose IL-2

IL-2 is critical for expansion, activity and survival of Treg-cells, and is usually produced by effector T cells. (96) In patients with cGvHD, a relative deficiency of Treg is observed, as the proliferation of Treg cannot sufficiently compensate for the increased susceptibility to apoptosis, while conventional CD4+ T cells do not appear to be impaired. (189)

Reconstitution of Treg may create or maintain a favourable immunologic milieu, preventing or ameliorating cGvHD.

In a phase 1 trial, the subcutaneous administration of low-dose IL-2 for 8 weeks generated increased proliferation of CD4+ Treg-cells, without a significant increase in conventional CD4+ T cells or CD8+ T cells. (112)

12 of 23 patients had partial response, with 11 of them including improving skin involvement. No GvHD progression was observed. Continued treatment for 14 months led to CR in a patient with prior extensive sclerodermatous cGvHD. (112)

There were numerous adverse events reported, such as injection-site induration (3 of 28 patients), TMA-associated renal failure (in 2 of 28 patients, both receiving TAC and SIR), and severe infections in 3 of 28 patients. (112)

Local reactions and constitutional symptoms may resolve with dose reduction and supportive measures, but further improvements are needed to simplify compounding and storage need to be made to further improve patient compliance. (190)

A murine study suggests that despite the amelioration of GvHD in a mild inflammatory state, IL-2 therapy may provide opposite effects, even exacerbating GvHD, when administered in an intense inflammatory state. This phenomenon can be attributed to the constitutive expression of high-affinity IL-2 receptors in Tregs, whereas conventional T cells (Tcons) do not express CD25, which is part of the IL-2 receptor, in the steady state. When activated in an intense inflammatory state, Tcons do express CD25, rendering them responsive to IL-2. (191)

Phototherapy

In both cutaneous acute and chronic GvHD, responses are seen for Psoralen and UV-A phototherapy (PUVA) and narrowband UV-B therapy. Even in sclerodermoid cutaneous cGvHD, which is characteristically challenging to treat, responses are seen, particularly with PUVA-bath. (113)

Other approaches to target GvHD

The activation of physiological tolerance mechanisms would be an desirable alternative strategy in GvHD therapy.

One potential target could be an interferon-lambda dependent improvement of intestinal barrier and intestinal stem cell function, which protects key tissue targets from GvHD. (192)

Another potential avenue for the protection and regeneration of cells within the gastrointestinal tract could be to substitute glucagon-like-peptide-2 (GLP-2). GLP-2 has been demonstrated to enhance the regeneration of Paneth cells and intestinal stem cells, which in turn promotes the production of antimicrobial peptides and causes microbiome changes. (193)

In cGvHD, targeting fibrosis and inciting mechanisms are promising fields of research.

Targeting macrophage proliferation may reduce the fibrosis classical for cGvHD. (140)

A phase I study of pirfenidone in BOS, which has already been approved for the treatment of lung fibrosis, has demonstrated the drug's safety and suggests potential in BOS treatment. (194)

8. Ruxolitinib

Ruxolitinib (RUX, Jakavi®) is a potent selective inhibitor of JAK (Janus kinase) 1 and JAK 2, which are essential components of signaling pathways of haematopoiesis through gene transcription, production and secretion of hematologic growth factors and inflammatory cytokines. (195) (196) (142)

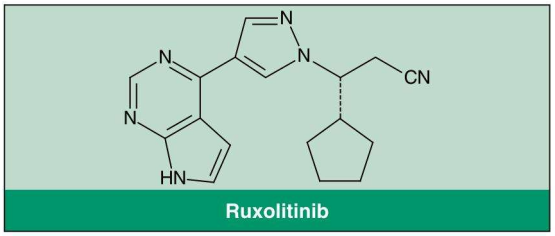

Figure 4: Ruxolitinib (196)

JAK1 and JAK2 are inherent parts in the development of myelofibrosis and other haematological malignancies. (196)

RUX was originally approved for the treatment of intermediate- and high-risk myelofibrosis (COMFORT-I and II) in 2011, which typically arises because of mutations in the JAK2 gene. (196) (197) (198) (195)

In this study, myelofibrosis included primary myelofibrosis (PMF), post-polycythaemia vera (PV) myelofibrosis and post-essential thrombocythemia (ET) myelofibrosis. (196) These are collectively known as Philadelphia-negative classical myeloproliferative neoplasms (MPD). (199)

RUX has demonstrated efficacy and was the first drug to be approved for the treatment of corticosteroid-refractory acute and chronic GvHD by the EMA in May 2022. (138)

Clinical trials for topical use of Ruxolitinib in cutaneous cGvHD are currently underway, as topical Ruxolitinib has shown efficacy in other autoimmune dermatologic conditions such as vitiligo. (200) (201)

There are numerous trials currently underway, extending the area of application of RUX to other haematologic, but also dermatologic-immunological diseases.

8.1. JAK-STAT Pathway

The Janus kinase (JAK) and signal transducer of activators of transcription (STAT) pathway represents a connection by which extracellular factors can regulate gene expression. (202)

Janus kinases (JAKs) are a group of multidomain non-receptor tyrosine kinases that have pivotal roles in cellular signal transduction. (203)

Four JAKs and seven mammalian STAT family members have been identified. (202)

JAKs are employed selectively by specific receptors to fulfil distinct in vivo roles. (202)

Extracellular factors that employ these mechanisms include a wide variety of cytokines, interferons, and growth factors. (202)

Pathological changes in the JAK-STAT pathway are essential in the pathogenesis of multiple diseases, especially haematological, malignant and immune-related conditions. (202)

Mutations in JAKs or STATs are associated with or are proven to cause diseases such as severe combined immunodeficiency (SCID) or myeloproliferative diseases (MPDs: PV, ET, PMF). Aberrant activation of JAKs and STATs were described in various haematologic and solid-organ malignancies. (202)

In MPDs, the signal transduction of receptors targeted by erythropoietin and thrombopoietin is pathologically changed by activating mutations of JAK2, which leads to increased erythrocyte and megakaryocyte expansion. (202)

Polymorphisms of STATs are associated with autoimmune diseases such as allergies, asthma, Crohn’s disease, psoriasis-arthritis, rheumatoid arthritis (RA), or systemic lupus erythematosus. (202)

Agents acting as JAK-inhibitors have been approved by the EMA for use in a heterogeneous group of numerous disorders, including MPDs and multiple immune-driven diseases such as rheumatoid arthritis, psoriasis-arthritis, ankylosing spondylitis, juvenile idiopathic arthritis, ulcerative colitis, atopic dermatitis, and alopecia areata. Several agents are currently undergoing trials, both before and after approval.

8.2. JAK/STAT signaling pathway in GvHD

The stimulation of inflammatory cytokine production leads to the activation of immune cells.

During phase II of aGvHD, the JAK/STAT signaling pathway is the key to T-cell activation. (39)

Ruxolitinib blocks the common gamma chain downstream effects in T-cells, reduces the migration of neutrophil granulocytes into GvHD target organs.

Potential target cells in which signaling is mediated by JAK1/2 also include neutrophils, macrophages, and dendritic cells. (204)

The production of Interferon-γ and IL-2Rγc cytokine requires consecutive activation of cytokine receptors by JAK1/2, which is blocked by RUX. (96)

In phase III of aGvHD in the gastrointestinal tract, despite the presence of inflammatory cytokines the target destruction is prevented by the inhibition of the JAK/STAT pathway and thus preserving the intestinal stem cell compartment. (39)

8.3. Retrospective and prospective evaluation of RUX in acute and chronic GvHD

In GvHD, RUX has been demonstrated to reduce the release of proinflammatory cytokines, block the maturation and activation of APCs, and thereby limit the allogeneic T-cell proliferation and reduce the infiltration of donor T-cell into GvHD target organs. In the intestine, a greater number of regulatory T-cells (Tregs) was observed. (3)

In a first retrospective study conducted by Zeiser et al. in 2015, the overall response rate was of 81.5 % in steroid-refractory aGvHD and 85.4 % in SR-cGvHD in both heavily pretreated patient cohorts. This represented a significant advance in this field of pharmacological therapy. (4)

These findings led to the initiation of numerous prospective and retrospective trials, but also to off-label use of RUX at our centre.

REACH-1

In the REACH1 trial, a prospective, multicentre, open-label phase II study (NCT02953678), Ruxolitinib was evaluated in 71 patients with SR-aGvHD. At the time of enrolment, 67.6% of patients had grade III/IV SR-aGvHD, a patient group that had demonstrated poor outcomes in historical data. (205) At day 28, 54.9% of the patients exhibited an overall response, including 26.8% with a complete response. (205)

In patients with grade III/IV aGvHD who responded well (CR, very good PR) to RUX, the 12-month OS probability was 82%. This finding has led to a re-evaluation of the potential for salvaging patients with advanced SR-aGvHD who are RUX-responsive. (205)

The observations made in the REACH1 study indicate that patients may benefit from the early initiation of 2L treatment at the first signs of steroid-refractoriness and before development of severe aGvHD, which can result in significant damage to the patients organs and tissues. (205)

REACH-2

The REACH2 trial was a randomized, open label, phase 3 trial comparing the efficacy and safety of RUX (10 mg twice daily) with the investigators choice of therapy in steroid-refractory (SR) aGvHD. Overall response on day 28 was 62% in the RUX group, with 40% durable response at day 56. 34% of patients receiving RUX achieved CR on day 28. (138)

The most common adverse events were thrombocytopenia and anaemia, in 26% CMV infection was observed. (138) In 38% of patients, RUX dose had to be modified up to day 28 due to adverse events, but only 11% discontinued RUX because of adverse events. (138) Median overall survival was 11,1 months in median in the RUX group, compared to 6,5 months in the control group. (138)

In order to facilitate comparison, the SR-criteria of the REACH2 trials for patients with aGvHD, as outlined in chapter 7.5.1 were applied. Discrepancies of our cohort to the inclusion and exclusion criteria of REACH-2 are depicted in **Table 11**.
Exclusion criteria included unmet SR-criteria, more than one previous treatment line for SR-aGvHD, relapsed underlying malignancy after any allogeneic HSCT, or if patients had received any JAK inhibitor for any indication after allogeneic HSCT. (138)

Control therapy was selected from a range of commonly used options, including ATG, ECP, mesenchymal stromal cells, low-dose MTX, MMF, mTOR-inhibitors (everolimus or sirolimus), etanercept or infliximab. A crossover from control therapy to RUX was permitted in the event of a

lack of response after 28 days of treatment, a subsequent loss of response, and the absence of signs of cGvHD. A total of 32% of the patients in the control group underwent a crossover to receive RUX. (138)

Regarding the severity of aGvHD at treatment initiation, 34% of patients had grade 2 aGvHD, 44% had grade 3 aGvHD, and 20% had grade 4 aGvHD in both treatment groups. (138)

In an Post-Hoc analysis it was shown that response to RUX was more durable when initiated early in SR-aGvHD. (206)

REACH-3

The REACH-3 study was a phase 3 clinical trial to assess the efficacy and safety of RUX in moderate to severe SR-cGvHD. Patients in the RUX group received 10 mg of RUX twice daily, while those in the control group received the investigator's choice of therapy. At week 24, the overall response rate in the RUX group was 49.7%, with 6.7% achieving a complete response, regardless of the organs involved. This represents a significantly superior overall response and failure free survival compared to the best available therapy. (139)

Best overall response at any time was observed in 76,4% of patients up to week 24.

The most common adverse effects were thrombocytopenia and anaemia, with a similar prevalence observed for CMV-infections and reactivations when compared to the control group. (139)

In the control arm, the "best available treatment" (BAT) could be chosen from a list of 10 commonly used options and included ECP, low-dose MTX, MMF, mTOR inhibitor (everolimus or sirolimus), infliximab, rituximab, pentostatin, imatinib, or ibrutinib. Crossover from control group was allowed in patients that had disease progression, mixed response or unacceptable side effects from control therapy. (139)

Of the 165 patients receiving RUX, 56,5% of patients had severe cGvHD, and 71,4% had glucocorticoid-refractory cGvHD, 28,6% had glucocorticoid-dependent disease. In the control group, ECP was the most frequently employed treatment in 34.8% of cases, followed by MMF (22.2%) and ibrutinib (17.1%). In 61 patients that crossed over from control group to RUX, a best overall response was observed in 78.7% of cases at the data cutoff. (139)

In 97.6% of patients in the RUX group adverse events of any grade occurred, with 57,0% of these events being of grade 3 or higher. The most prevalent AE of grade 3 or higher was thrombocytopenia (15,2%), followed by anaemia (in 12,7%), neutropenia (8,5%), and pneumonia (8,5%). Both thrombocytopenia and anaemia are reversible and manageable through dose adjustments and supportive treatment. In 16.4% of cases, adverse events led to the discontinuation of RUX, while in 37.6% of cases, AE necessitated dose adjustments or interruptions. Infections of any type were observed in 63.6% of the RUX group, with 19.4% of these classified as grade 3 infections. A total of 5.5% of patients exhibited evidence of CMV-reactivation. (139)

In a post hoc analysis, response rate and duration to RUX was independent from the time elapsing between diagnosis of steroid-refractoriness and RUX initiation. (207)

Further trials

The subsequent REACH-4 trial evaluated the efficacy of RUX in the treatment of aGvHD in paediatric patients. RUX demonstrated comparable ORR to those reported in REACH-2, but should not be gone into details in this thesis. (208)

Greinix et al. hypothesized that the mechanisms of action of ECP and RUX would complement each other and thereby improve response rates and durability of response in SR GvHD, but further studies are needed. (133)

A Spanish trial conducted by Escamilla Gomez et al. reported real live experience of RUX in acute and chronic GvHD, with outcomes comparable to clinical trials. In aGvHD, the ORR was 57.6 % of patients, with 30.7% achieving CR. The median overall survival was 4.1 months, with an OS at 2 years of 28.8%. (209) In cGvHD, Escamilla Gomez et al. observed an ORR of 65.6%, with an OS at 2 years of 78.9%. (209)

Yang et al. observed comparable outcomes in children with acute and chronic GvHD to those observed in patients aged 12 years and older included in the REACH-2 and 3 trials. (210)

In a small, but heavily pretreated cohort (41 patients with a median of 3 previous agents, range 1-6) of cGvHD patients, Wu et al. observed an overall response rate of 70.7% after 6 months of RUX treatment. (211)

In a meta-analysis in 2022, Fan et al. reported an overall response rate at any time of 77% in SR-aGvHD and 78% in SR-cGvHD. The complete response rate was 49% in aGvHD and 15% in cGvHD. The probability rate of overall survival at 2 years were 75.3% in cGvHD, and not reported in aGvHD. In aGvHD, the probability rate of overall survival at 6 months was 63,9%. (212)

8.4. Adverse events

In general, Ruxolitinib has an acceptable tolerability profile. (196)

The safety profile of RUX in GvHD patients is consistent with that previously described in myeloproliferative neoplasms with pronounced drug-induced cytopenia. (3) Haematologic toxicity is observed more frequent and more severe in acute GvHD compared to cGvHD. (139, 138)

The most common adverse event is dose-related anaemia, even in the population receiving Ruxolitinib because of polycythaemia vera. Other hematologic adverse events are thrombocytopenia and lymphopenia, but also less common leukopenia and neutropenia. (198),(213) These haematological adverse events were manageable with dosage modification or supportive measures such as transfusions of packed red blood cells. (196) The dose-limiting toxicity is usually thrombocytopenia. (214)

Nonhematologic adverse events include headache, diarrhea, nausea/vomiting, fatigue, peripheral oedema, hypertension pruritus, dizziness, muscle spasms, dyspnoea, abdominal pain and asthenia. Laboratory abnormalities were also observed, such as increased ALT, creatinine, AST, hypertriglyceridemia, hyperglycaemia, amongst others. (138) (139) (213)

Due to the immunosuppressive effects of Ruxolitinib, there is an increased risk of opportunistic infections and reactivation of tuberculosis, hepatitis B, herpes zoster and EBV. Furthermore, there is also an increased risk of upper respiratory tract infections, pneumonia, urinary tract infections, BK virus and JC virus, the latter manifesting as progressive multifocal

leukoencephalopathy. (195) (113) (213) In several real-life studies a greater incidence of CMV reactivation was reported, which makes monitoring of the viral load necessary. (215)

Ruxolitinib is metabolized by hepatic enzymes, predominantly by CYP3A4 which leads to great interaction potential.

8.5. Dosage

Ruxolitinib (Jakavi / Jakafi) is orally administered and currently available in tablets of 5, 10, 15, 20 and 25 mg. (195)

The recommended dosage depends on indication, clinical status and thrombocyte count. (195)

In Myelofibrosis, recommended starting dose ranges from 15 – 20 mg twice daily, depending on platelet count. (196) A dosage of 25 mg twice daily should not be exceeded. (196)

The dosage of Ruxolitinib may be adjusted according to efficacy and tolerability, e.g. to manage anaemia or thrombocytopenia. A reduced dosage of Ruxolitinib should be considered if combined to a treatment with a strong CYP3A4 inhibitor (e.g. posaconazole) or a platelet count of < 150 G/L.

Isberner et al. found that median serum concentrations of patients who had required dose reductions for adverse events did not differ significantly from serum concentration of patients receiving 20 mg daily without need for AE-associated dose reduction. (142)

The dosage of Ruxolitinib used in GvHD is comparably low, considering the dosage used in myeloproliferative diseases. (213) In patients receiving RUX for GvHD, the exposure is higher compared to myelofibrosis patients as a result of concomitant medication with CYP3A4 or CYP2C9 inhibitors and reduced clearance. (142)

Sudden withdrawal of Ruxolitinib can lead to an Ruxolitinib-discontinuation-syndrome (RDS), which includes rapid relapse of symptoms like symptomatic spleen increase and constitutional symptoms and can lead to symptoms of a cytokine-storm like fever, acute respiratory distress syndrome (ARDS), anaemia, systemic inflammatory response syndrome / septic-like shock and disseminated intravascular coagulation (DIC)-like symptoms. RDS may even occur when rapidly tapering the RUX dose. (214)

In their review Palandri et al. showed that 34 of 251 patients (13,5%) who discontinued Ruxolitinib developed RDS. Although symptoms were mild in 21 patients (61,8%) and moderate in 10 patients out of 34 (29,4%), three patients (8,8%; <1% of patients who stopped RUX therapy) showed severe symptoms of Ruxolitinib-discontinuation-syndrome which required ICU admission. In those three cases the symptoms of severe RDS occurred within 48 hours of RUX discontinuation and improved promptly after resuming Ruxolitinib. (216)

9. Retrospective monocentric study of RUX for acute and chronic GvHD

9.1. Methods

The objective of this study is to evaluate the safety, tolerability, and effectiveness of RUX.

This single centre retrospective study evaluated 118 patients who received RUX as therapy of graft-versus-host-disease.

As RUX was used as therapy for treatment-refractory GvHD in an early access program (named patient use / NPU) before its EMA-approval in May 2022, we could retrospectively investigate patients back to May 2015.

The evaluated patients were divided into three subgroups based on clinical type of GvHD at RUX initiation (steroid refractory acute GvHD, chronic GvHD and the simultaneous occurrence of acute and chronic GvHD, described as overlap GvHD), which were treated with RUX as second or subsequent line.

Staging and grading of GvHD were evaluated according to MAGIC-criteria in acute GvHD and the NIH consensus criteria in chronic GvHD, as detailed in the chapter 7.

We evaluated the indication (staging of GvHD, earlier therapies) and the clinically observable response until March 31st, 2023 (including tolerability, effectiveness, duration of therapy, subsequent therapies), follow up regarding the GvHD severity was censored at RUX discontinuation.

Adverse events of all patients who received at least one dose of Ruxolitinib was documented. Response was analysed in patients who received RUX for at least 14 days or until death if the latter occurred before day 14 on RUX.

Adverse events were graded according to the Clinical Terminology for Adverse Events Criteria (CTCAE) Version 5.0. (217)

Supportive care was performed according to centre-specific standards.

Data were collected retrospectively from electronic patient records of the hospital using the hospital information system. The clinical data were first collected, processed, and graphically displayed in Microsoft Excel and R.

The study was approved by the Ethics Commission of the Johannes Kepler University (EK Nr: 1061/2023). In accordance with the Declaration of Helsinki, all patients provided written informed consent to the treatment, and to data collection and analysis.

Survival events were assessed for an extended observation period, with database lock on December 31st, 2023. Overall survival was calculated from the date of first RUX administration until death from any cause or last follow up.

9.1.1. Inclusion and exclusion criteria

Patients were included if they received Ruxolitinib as second or further treatment line at any dosing regimen for acute, classical chronic and overlap chronic GvHD of any organ.

We explicitly included patients with overlap GvHD and those who developed GvHD after donor lymphocyte infusion (DLI), as these groups were excluded in many other trials even though they represent a relevant number of patients in daily clinical routine and are associated with a worse prognosis. (218)

The first patient started RUX in May 2015 in an early access program (named patient use / NPU), we subsequently included those who started RUX up to September 30th, 2022, to allow sufficient follow-up time for response. The development of GvHD was evaluated until March 31st, 2023.

The minimum age was 18 years, but as in OKL Linz is a centre without paediatric HSCT, no patient was excluded for this reason. Both genders were included.

Patients without symptoms of GvHD at the start of RUX were excluded, as RUX was also used as part of prophylaxis in individual cases where other drugs were contraindicated. Three patients were excluded for this reason, as they started Ruxolitinib as replacement for CNI in prophylaxis because of acute kidney injury without any present symptoms of GvHD. In all three patients, prior GvHD was in CR after first line therapy with steroids.

Patients that previously received Ruxolitinib prior to HSCT as therapy of underlying disease were not evaluated separately.

In addition to the majority (n=108) of patients who received RUX within the NPU program prior to EMA approval for GvHD, a smaller proportion of patients (n=10) was included who received RUX after its approval for usage in GvHD in May 2022.

9.2. Patient characteristics

A total of 118 adult patients who developed GvHD after receiving an allogeneic hematopoietic stem cell transplantation received Ruxolitinib as second or further treatment line at the Ordensklinikum Linz Elisabethinen, Linz, Austria.

Baseline characteristics of patient demography and transplantation of the overall cohort and the subgroups by GvHD type are shown in **Table 6.**

GvHD characteristics are shown in **Table 7 and 8**.

A total of 4 patients were recruited for REACH-trials. Two patients were included in REACH-2 trial (aGvHD), and 2 patients were included in the REACH-3 trial (cGvHD). All four of them initially received the BAT but lacked response, and eventually received RUX on a compassionate use basis, and were therefore included in the present retrospective analysis.

Table 6: Baseline characteristics of patients and transplantation

	overall	acute	classical chronic	overlap chronic
patient characteristics				
number of patients, n (%)	118	61	43	14
recipient age (years) at transplant median [range]	55,1 [18,1 to 73,6]	56,8 [22,2 to 70,7]	51,9 [18,1 to 73,5]	55,1 [24,4 to 68,8]
sex, n (%) male / female	75/43 (64/36)	40/21 (66/34)	24/19 (56/44)	11/3 (79/21)
recipient CMV serostatus IgG positive, n (%)	59 (50)	32 (52)	22 (51)	5 (36)
Primary disease / underlying diagnosis / transplant indication, n (%)				
ALL	14 (11,9)	6 (9,8)	7 (16,3)	1 (7,1)
AML	56 (47,5)	30 (49,2)	22 (51,2)	4 (28,6)
MDS/MPN	28 (23,7)	12 (19,7)	8 (18,6)	8 (57,1)
Lymphoma, Myeloma, other	20 (16,9)	13 (21,3)	6 (14,0)	1 (7,1)
disease stage at transplant, n (%)				
early	37 (31,4)	15 (24,6)	19 (44,2)	3 (21,4)
intermediate	38 (32,2)	20 (32,8)	15 (34,9)	3 (21,4)
advanced	43 (36,4)	26 (42,6)	9 (20,9)	8 (57,1)
transplant characteristics / donor type, n (%)				
matched related	31 (26,3)	13 (21,3)	12 (27,9)	6 (42,9)
matched unrelated	32 (27,1)	11 (18,0)	19 (44,2)	2 (14,3)
MMUD	6 (5,1)	4 (6,6)	2 (4,7)	0 (0)
UCB (unrelated)	1 (0,9)	1 (1,6)	0 (0)	0 (0)
haploidentical	48 (40,7)	32 (52,5)	10 (23,3)	6 (42,9)
donor characteristics, n (%)				
sex mismatched female donor	31 (26,3)	14 (23,0)	13 (30,2)	4 (28,6)
donor age (years, median [range])	36,9 [19,7 to 67,1]	42,7 [19,7 to 67,1]	33,3 [21,1;64,0]	39,5 [22,5;60,4]
donor CMV serostatus IgG positive	43 (36,4)	22 (36,1)	16 (37,2)	5 (35,7)
graft source, n (%)				
BM	9 (7,6)	3 (4,9)	5 (11,6)	1 (7,1)

	overall	acute	classical chronic	overlap chronic
PBSC	108 (91,6)	57 (93,4)	38 (88,4)	13 (92,9)
CB	1 (0,9)	1 (1,6)	0 (0)	0 (0)
conditioning regimen, n (%)				
MAC	80 (67,8)	46 (75,4)	23 (53,5)	11 (78,6)
RIC	38 (32,2)	15 (24,6)	20 (46,5)	3 (21,4)
GvHD prophylaxis, n (%)				
CSA-MMF	32 (27,1)	13 (20,3)	15 (34,9)	4 (28,6)
CSA-MTX	33 (28,0)	12 (19,7)	17 (39,5)	4 (28,6)
PTCY-TAC-MMF	53 (44,9)	36 (59,0)	11 (25,6)	6 (42,9)
ATLG-based prophylaxis	42 (35,6)	23 (37,7)	16 (37,2)	3 (21,4)

Criteria not other specified are given in absolute numbers and percentage of overall cohort (number of patients as mentioned above). Donor age excluded cord blood.

The intensity of conditioning regimen (MAC versus RIC) was classified as the criteria suggested by the Center of International Blood and Marrow Transplant Research in 2009. (22)

Percentages may not total 100 because of rounding.

Abbreviations used: CMV = cytomegalovirus, IgG = immune globulin G, ALL = acute lymphoblastic leukaemia, AML = acute myeloid leukaemia, MDS/MPN = myelodysplastic syndrome / myeloproliferative neoplasia, MMUD = mismatched unrelated donor, UCB = umbilical cord blood, BM = bone marrow, PBSC = peripheral blood stem cells, CB = cord blood, MAC = myeloablative conditioning, RIC = reduced intensity conditioning, CSA = cyclosporine A, MMF = mycophenolate mofetil, MTX = methotrexate, PTCY = post-transplant cyclophosphamide, TAC = tacrolimus, ATLG = antihuman T-lymphocyte globuline

Date of stem cell transplantations for the patients included were between May 2003 and September 1st, 2023.

About half of the acute GvHD patients (52,5 %) received stem cells from a haploidentical donor. In patients receiving RUX for chronic GvHD, 44,2 % had a matched unrelated donor.

Most stem cell grafts (91,6 % of overall cohort) were obtained from the peripheral blood.

Table 7: Maximum GvHD characteristics prior to RUX initiation

Maximum GvHD characteristics prior to RUX initiation				
	overall	**acute**	**classical chronic**	**overlap chronic**
	118	**61**	**43**	**14**
GvHD onset following DLI				
n (%)	22 (18,6)	7 (11,5)	12 (27,9)	3 (21,4)
maximum severity of acute GvHD any time prior to RUX initiation, n (%)				
no aGvHD	6 (5,1)	0 (0)	6 (14,0)	0 (0)
aGvHD grade 1	8 (6,8)	0 (0)	6 (14,0)	2 (14,3)
aGvHD grade 2	38 (32,2)	10 (16,4)	20 (46,5)	8 (57,1)
aGvHD grade 3	59 (50,0)	46 (75,4)	10 (23,3)	3 (21,4)
aGvHD grade 4	7 (5,9)	5 (8,2)	1 (2,3)	1 (7,1)
maximum severity of chronic GvHD any time prior to RUX initiation, n (%)				
no cGvHD	60 (50,9)	60 (98,4)	0 (0)	0 (0)
cGvHD mild	3 (2,5)	0 (0)	2 (4,7)	1 (7,1)
cGvHD moderate	28 (23,7)	0 (0)	18 (41,9)	10 (71,4)
cGvHD severe	27 (22,9)	1* (1,6)	23 (53,5)	3 (21,4)
maximum steroid dose any time before RUX initiation for the respective GvHD type in mg methylprednisolone equivalent per kilogram body weight per day				
median [range]	1,1 [0,0 to 6,7]	1,9 [0,07 to 6,7]	0,8 [0,0 to 4,7]	1,1 [0,08 to 3,2]
treatment lines prior to RUX for the respective GvHD type, n (%)				
1 (= steroids only)	69 (58,5)	45 (73,8)	15 (34,9)	9 (64,3)
2	27 (22,9)	11 (18,0)	13 (30,2)	3 (21,4)
3	14 (11,9)	4 (6,6)	9 (20,9)	1 (7,1)
4	3 (2,5)	0 (0)	3 (7,0)	0 (0)
5	2 (1,7)	0 (0)	1 (2,3)	1 (7,1)
6	2 (1,7)	1 (1,6)	1 (2,3)	0 (0)
> 6	1 (0,8)	0 (0)	1 (2,3)	0 (0)
maximum organ staging of GvHD prior to RUX initiation, n (%)				
acute GvHD				
no aGvHD	6 (5,1)	0 (0)	6 (14,0)	0 (0)
aGvHD upperGI	60 (50,9)	35 (57,4)	18 (41,9)	7 (50,0)
aGvHD lowerGI	88 (74,6)	58 (95,1)	21 (48,8)	9 (64,3)
loGI stage 1	22 (18,6)	6 (9,8)	12 (27,9)	4 (28,6)
loGI stage 2	33 (28,0)	23 (37,7)	8 (18,6)	2 (14,3)
loGI stage 3	26 (22,0)	23 (37,7)	1 (2,3)	2 (14,3)

Maximum GvHD characteristics prior to RUX initiation				
	overall	**acute**	**classical chronic**	**overlap chronic**
	118	**61**	**43**	**14**
loGI stage 4	7 (5,9)	6 (9,8)	0 (0)	1 (7,1)
aGvHD skin	66 (55,9)	22 (36,1)	31 (72,1)	13 (92,9)
skin stage 1	25 (21,2)	10 (16,4)	9 (20,9)	6 (42,9)
skin stage 2	20 (17,0)	4 (6,6)	13 (30,2)	3 (21,4)
skin stage 3	19 (16,1)	8 (13,1)	8 (18,6)	3 (21,4)
skin stage 4	2 (1,7)	0 (0)	1 (2,3)	1 (7,1)
aGvHD liver	5 (4,2)	3 (4,9)	2 (4,7)	0 (0)
liver stage 1	2 (1,7)	2 (3,3)	0 (0)	0 (0)
liver stage 2	2 (1,7)	1 (1,6)	1 (2,3)	0 (0)
liver stage 3	1 (0,9)	0 (0)	1 (2,3)	0 (0)
liver stage 4	0 (0)	0 (0)	0 (0)	0 (0)
chronic GvHD				
no cGvHD	59 (50,0)	60 (98,4)	0 (0)	0 (0)
cGvHD skin	30 (25,4)	0 (0)	26 (60,5)	4 (28,6)
skin mild	10 (8,5)	0 (0)	8 (18,6)	2 (14,3)
skin moderate	6 (5,1)	0 (0)	5 (11,6)	1 (7,1)
skin severe	14 (11,9)	0 (0)	13 (30,2)	1 (7,1)
cGvHD GI	8 (6,8)	1 (1,6)	5 (11,6)	2 (14,3)
GI mild	4 (3,4)	1 (1,6)	2 (4,7)	1 (7,1)
GI moderate	4 (3,4)	0 (0)	3 (7,0)	1 (7,1)
GI severe	0 (0)	0 (0)	0 (0)	0 (0)
cGvHD eyes	29 (24,6)	1 (1,6)	21 (48,8)	7 (50,0)
eyes mild	15 (12,7)	1 (1,6)	11 (25,6)	3 (21,4)
eyes moderate	10 (8,5)	0 (0)	7 (16,3)	3 (21,4)
eyes severe	4 (3,4)	0 (0)	3 (7,0)	1 (7,1)
cGvHD mouth	42 (35,6)	0 (0)	32 (74,4)	10 (71,4)
mouth mild	26 (22,0)	0 (0)	20 (46,5)	6 (42,9)
mouth moderate	16 (13,6)	0 (0)	12 (27,9)	4 (28,6)
mouth severe	0 (0)	0 (0)	0 (0)	0 (0)
cGvHD joints & muscles	16 (13,6)	1 (1,6)	12 (27,9)	3 (21,4)
joints mild	9 (7,6)	1 (1,6)	7 (16,3)	1 (7,1)
joints moderate	4 (3,4)	0 (0)	3 (7,0)	1 (7,1)
joints severe	3 (2,5)	0 (0)	2 (4,7)	1 (7,1)
cGvHD lung	16 (13,6)	1 (1,6)	14 (32,6)	1 (7,1)
lung mild	6 (5,1)	0 (0)	6 (14,0)	0 (0)
lung moderate	8 (6,8)	1 (1,6)	6 (14,0)	1 (7,1)

Maximum GvHD characteristics prior to RUX initiation				
	overall	**acute**	**classical chronic**	**overlap chronic**
	118	**61**	**43**	**14**
lung severe	2 (1,7)	0 (0)	2 (4,7)	0 (0)
cGvHD liver	17 (14,4)	0 (0)	13 (30,2)	4 (28,6)
liver mild	5 (4,2)	0 (0)	5 (11,6)	0 (0)
liver moderate	11 (9,3)	0 (0)	7 (16,3)	4 (28,6)
liver severe	1 (0,9)	0 (0)	1 (2,3)	0 (0)
cGvHD other organs	16 (13,6)	0 (0)	14 (32,6)	2 (14,3)

All data are absolute numbers and percentage of overall cohort (number of patients as mentioned above). Staging and grading was done according to criteria explained in chapter 7. The steroid dose is given in milligrams methylprednisolone equivalent per kilogram of body weight per day. The pharmaceutic agents most predominantly employed were methylprednisolone or prednisone (1 mg prednisone is the equivalent to 0.8 mg of methylprednisolone). In individual cases, dexamethasone was used according to clinical presentation, the glucocorticoid equivalent dosage converted to methylprednisolone.

*One patient had had cGvHD before Ruxolitinib initiation for DLI-induced aGvHD, 72 months after HSCT.

Abbreviations used: DLI = donor lymphocyte infusion, upperGI = upper gastrointestinal tract, lowerGI / loGI = lower gastrointestinal tract, GI = gastrointestinal tract

Manifestations not classified elsewhere attributed to chronic GvHD (referred to as “other organs”) included neuropathic symptoms in two patients (one cGvHD, one overlap cGvHD), hypothyroidism in one patient, pleural effusion in two patients and eosinophilia in one patient. Additionally, impaired performance status was subclassified as “other organ”, which included nine patients with classical cGvHD and one patient with overlap GvHD. Two patients with classical cGvHD and one patient with overlap GvHD exhibited genital involvement.

86 % of cGvHD cases had signs of acute GvHD prior to chronic GvHD.

Overall, 22 (18,6%) of the investigated GvHD episodes occurred after administration of interventional (unscheduled) donor lymphocyte infusion (DLI), performed either preemptively for molecular residual disease, relapse or decreasing chimerism, or for overt haematological relapse, with 7 patients (11,5%) of aGvHD, 12 patients (27,9%) of classical cGvHD and 3 patients (21,4%) of overlap GvHD cases (**Table 7**).

Table 7 provides details regarding the history of prior GvHD episodes, while **Table 8** represents the current GvHD status of patients at the initiation of RUX. It is possible that patients with cGvHD may have a history of aGvHD, although the indication for RUX was classical cGvHD. Conversely, one patient exhibited cGvHD prior to DLI-induced aGvHD.

Table 8: GvHD characteristics at RUX initiation

	overall	**acute**	**classical chronic**	**overlap chronic**
	118	61	43	14
time from transplantation to RUX initiation in months				
median [range]	7,0 [0,3 to 170,2]	1,5 [0,3 to 72,0]	23,4 [5,5 to 170,2]	9,1 [2,4 to 64,0]
acute GvHD overall severity at RUX initiation, n (%)				
no aGvHD	45 (38,1)	0 (0)	43 (100)	2 (14,3)
aGvHD grade 1	7 (5,9)	0 (0)	0 (0)	7 (50,0)
aGvHD grade 2	15 (12,7)	13 (21,3)	0 (0)	2 (14,3)
aGvHD grade 3	44 (37,3)	42 (68,9)	0 (0)	2 (14,3)
aGvHD grade 4	7 (5,9)	6 (9,8)	0 (0)	1 (7,1)
chronic GvHD overall severity at RUX initiation, n (%)				
no cGvHD	61 (51,7)	61 (100)	0 (0)	0 (0)
cGvHD mild	6 (5,1)	0 (0)	3 (7,0)	3 (21,4)
cGvHD moderate	27 (22,9)	0 (0)	19 (44,2)	8 (57,1)
cGvHD severe	24 (20,3)	0 (0)	21 (48,8)	3 (21,4)
steroid status, n (% of overall cohort)				
primary steroid refractory	21 (17,8)	17 (27,9)	2 (4,7)	2 (14,3)
steroid dependent (failed taper)	12 (10,2)	5 (8,2)	4 (9,3)	3 (21,4)
steroid-refractory criteria not met	52 (44,1)	23 (37,7)	22 (51,2)	7 (50,0)
RUX beyond second/third line*	33 (28,0)	16 (26,2)	15 (34,9)	2 (14,3)

GvHD Grading was performed according to criteria mentioned below.

*Steroid status was only interpreted in patients receiving Ruxolitinib as second line treatment in aGvHD or as second- or third-line treatment in cGvHD. Evaluation was done according to the criteria for definition of glucocorticoid-refractory disease in the corresponding REACH trial, as mentioned in chapter 7.5. In case of overlap chronic GvHD, criteria of REACH 3 trials defined by Martin et al (148) were used.

Abbreviations used: SR = steroid refractory

The one patient with > 6 prior GvHD therapy lines in chronic GvHD had RUX as his 9th line 48,0 months after initial steroid therapy because of chronic GvHD.

In particular, patients with classical chronic GvHD had undergone extensive prior treatment, with a median time from transplantation to RUX initiation of 23.4 months. Furthermore, 34.1% of patients had undergone three or more previous treatment lines.

Table 9: Chronic GvHD onset as classified by Lee in Blood, 2017

Onset of classical chronic GvHD (43 patients) according to Lee (92)	
new onset	7 (16,3)
quiescent	23 (53,5)
progressive	13 (30,2)

New onset of chronic GvHD applies to a patient without history of acute GvHD, quiescent onset chronic GvHD indicates cGvHD onset after full resolution of acute GvHD and progressive onset cGvHD directly emerges from acute GvHD without resolution. (92)

By definition, overlap GvHD has a progressive onset. One patient with overlap GvHD had overlap GvHD as overall first GvHD presentation. One other patient classified as overlap chronic GvHD had a flare of acute GvHD while persisting classical chronic GvHD, RUX was begun for overlap cGvHD. (92)

Table 10: Organ staging of GvHD at RUX initiation

	overall	**acute**	**classical chronic**	**overlap chronic**
	118	**61**	**43**	**14**
acute GvHD organ staging at RUX initiation, n (%)				
no aGvHD	44 (37,3)	0 (0)	42	2 (14,3)
aGvHD upperGI stage 1	34 (28,8)	31 (50,8)	0 (0)	3 (21,4)
aGvHD lowerGI	61 (51,7)	56 (91,8)	0 (0)	5 (35,7)
loGI stage 1	12 (10,2)	10 (16,4)	0 (0)	2 (14,3)
loGI stage 2	23 (19,5)	22 (36,1)	0 (0)	1 (7,1)
loGI stage 3	19 (16,1)	18 (29,5)	0 (0)	1 (7,1)
loGI stage 4	7 (5,9)	6 (9,8)	0 (0)	1 (7,1)
aGvHD skin	28 (23,7)	19 (31,2)	0 (0)	9 (64,3)
skin stage 1	13 (11,0)	8 (13,1)	0 (0)	5 (35,7)
skin stage 2	6 (5,1)	4 (6,6)	0 (0)	2 (14,3)
skin stage 3	8 (6,8)	7 (11,5)	0 (0)	1 (7,1)
skin stage 4	1 (0,9)	0 (0)	0 (0)	1 (7,1)
aGvHD liver	2 (1,7)	2 (3,3)	0 (0)	0 (0)
liver stage 1	0 (0)	0 (0)	0 (0)	0 (0)
liver stage 2	1 (0,9)	1 (1,6)	0 (0)	0 (0)
liver stage 3	1 (0,9)	1 (1,6)	0 (0)	0 (0)
liver stage 4	0 (0)	0 (0)	0 (0)	0 (0)
chronic GvHD organ staging, n (%)				
no cGvHD	61 (51,7)	61 (100)	0 (0)	0 (0)
cGvHD skin	26 (22,0)	0 (0)	23 (53,5)	3 (21,4)
skin mild	6 (5,1)	0 (0)	5 (11,6)	1 (7,1)

	overall	acute	classical chronic	overlap chronic
	118	**61**	**43**	**14**
skin moderate	6 (5,1)	0 (0)	5 (11,6)	1 (7,1)
skin severe	14 (11,9)	0 (0)	13 (30,2)	1 (7,1)
cGvHD GI	7 (5,9)	0 (0)	7 (16,3)	0 (0)
GI mild	4 (3,4)	0 (0)	4 (9,3)	0 (0)
GI moderate	3 (2,5)	0 (0)	3 (7,0)	0 (0)
GI severe	0 (0)	0 (0)	0 (0)	0 (0)
cGvHD eyes	29 (24,6)	0 (0)	22 (51,2)	7 (50,0)
eyes mild	14 (11,9)	0 (0)	10 (23,3)	4 (28,6)
eyes moderate	11 (9,3)	0 (0)	9 (20,9)	2 (14,3)
eyes severe	4 (3,4)	0 (0)	3 (7,0)	1 (7,1)
cGvHD mouth	38 (32,2)	0 (0)	28 (65,1)	10 (71,4)
mouth mild	22 (18,6)	0 (0)	15 (34,9)	7 (50,0)
mouth moderate	16 (13,6)	0 (0)	13 (30,2)	3 (21,4)
mouth severe	0 (0)	0 (0)	0 (0)	0 (0)
cGvHD joints & muscles	12 (10,2)	0 (0)	11 (25,6)	1 (7,1)
joints mild	6 (5,1)	0 (0)	6 (14,0)	0 (0)
joints moderate	3 (2,5)	0 (0)	3 (7,0)	0 (0)
joints severe	3 (2,5)	0 (0)	2 (4,7)	1 (7,1)
cGvHD lung	15 (12,7)	0 (0)	14 (32,6)	1 (7,1)
lung mild	6 (5,1)	0 (0)	6 (14,0)	0 (0)
lung moderate	7 (5,9)	0 (0)	6 (14,0)	1 (7,1)
lung severe	2 (1,7)	0 (0)	2 (4,7)	0 (0)
cGvHD liver	14 (11,9)	0 (0)	10 (23,3)	4 (28,6)
liver mild	5 (4,2)	0 (0)	5 (11,6)	0 (0)
liver moderate	9 (7,6)	0 (0)	5 (11,6)	4 (28,6)
liver severe	0 (0)	0 (0)	0 (0)	0 (0)
cGvHD other organs	16 (13,6)	0 (0)	14 (32,6)	2 (14,3)

Abbreviations used: upperGI = upper gastrointestinal tract, lowerGI / loGI = lower gastrointestinal tract, GI = gastrointestinal tract

Not elsewhere classified manifestations attributed to chronic GvHD (referred to as “other organs”) were neuropathic symptoms in two patients, hypothyroidism in one patient, pleural effusion in two patients and nephrotic syndrome in one patient. Impaired performance status was also subclassified as “other organ”. This included 11 patients with classical cGvHD and one patient with overlap GvHD. Genital involvement was observed in one patient with classical cGvHD and one patient with overlap GvHD.

The most common manifestations of aGvHD were lower gastrointestinal tract in 91,8 % and upper gastrointestinal tract in 50,8%. In cGvHD, the most common organ manifestations were oral cGvHD in 65,1 %, skin involvement in 53,5 % and ocular symptoms in 51,2 %.

In overlap cGvHD, manifestations of acute GvHD were most commonly lower gastrointestinal and skin involvement, while the most common signs of cGvHD were oral and ocular symptoms.

In overlap GvHD, laboratory findings suggesting liver involvement were evaluated as liver cGvHD, being present in 28,6 % of this cohort.

Table 11: Characterization of the cohort according to in- and exclusion criteria of the respective Phase 3 trial (REACH-2 or REACH-3): reasons for not being eligible

	overall	acute	classical + overlap chronic
n	118	61	57 (43 + 14)
start NPU outside the recruitment period	58 (49,2)	21 (34,4)	37 (64,9)
reasons for not being eligible for the respective phase 3 trial, n (%)			
prior participation in REACH-trial	4 (3,4)	2 (3,3)	2 (3,5)
prior participation in other study	3 (2,5)	0 (0)	3 (5,3)
steroid refractory/dependency criteria not met*	52 (44,1)	23 (37,7)	29 (50,9)
RUX beyond second/third line**	33 (28,0)	16 (26,2)	17 (29,8)
DLI-induced GVHD	22 (18,6)	7 (11,5)	15 (26,3)
relapse after allogeneic HSCT	14 (11,9)	6 (9,8)	8 (14,0)
lack of haematological regeneration	5 (4,2)	4 (6,6)	1 (1,8)
overlap GvHD	14 (11,9)	0 (0)	14 (24,6)
serious illness or other medical indication for exclusion	11 (9,3)	8 (13,1)	3 (5,3)

Abbreviations used: DLI = donor lymphocyte infusion, HSCT = haematopoietic stem cell transplantation, NPU = named patient use

* The criteria for steroid-refractory or dependency were only met when RUX was used in the 1st line (aGvHD) or 2nd line (cGvHD) of treatment.

** more than 1 line (steroids) in aGvHD or more than 2 lines (steroids plus other) for cGvHD

Several patients showed more than one exclusion criteria. Steroid-refractory/-dependency criteria not met includes using Ruxolitinib in higher line than second/third.

Three patients with chronic GvHD were participants in other trials, one each in iNTEGRATE-study (PCYC, 1140 Ibrutinib in cGvHD), Gravitas 301 (Itacitinib vs. Placebo in aGvHD (187), but indication for RUX was cGvHD), and Gravitas 309 (Gravitas_INCB 39110-309, randomized to BAT).

Serious illness or other medical indication for exclusion were uncontrolled infections (present in 6 cases), but also severe obesity (1 patient), acute kidney injury (infection + AKI in 1 patient), acute neurologic disorders (PRES, 1 patient), reduced general condition with swallowing disorder (1 patient) or questionable resorption (1 patient) or questionable compliance (1 patient).

9.3. Endpoints and Definitions

The primary endpoint of the evaluation was best overall response at any time, defined as the patients who achieved complete response (CR) or partial response (PR) while on RUX treatment during the observation period. This was evaluated across all affected organs.

Major secondary endpoints were the current response or latest response while RUX is taken at last follow up, the feasibility of steroid taper, duration of RUX treatment, overall survival, and the incidence of non-relapse mortality (NRM) after initiation of RUX treatment, cytomegalovirus (CMV) reactivation during RUX and the description and quantification of treatment lines before and while RUX treatment.

Staging and grading of acute GvHD was done according to the MAGIC criteria (115), staging and grading of chronic GvHD was evaluated as defined in the 2005 NIH consensus criteria revised and amended in 2014 (91), both mentioned in chapter 7.

Response was evaluated at day 28 and three months for aGvHD, and 3 months and 6 months for cGvHD, and at the last RUX dose in patients who discontinued RUX or last follow up in patients with ongoing RUX treatment until March 31st, 2023.

Since RUX was frequently continued even if an additional treatment line was initiated, response was separately evaluated as best response separately for RUX monotherapy and the combination treatment.

Response was classified according to published criteria for acute (145) and chronic (219) GvHD.

Response categories in acute GvHD were complete response (CR), partial response (PR) and no response (including stable / unchanged or progressive GvHD). In cGvHD, response categories were CR, PR, mixed response (response in one organ, progression in another organ), stable disease and progressive disease.

Complete remission (CR) is defined as complete response in all organs (= stage 0) and fully resolved symptoms. Partial remission (PR) is defined as response in at least one organ, without worsening or recurrence in any other organ or a new onset of GvHD in a previously unaffected organ, but without fully resolved symptoms. The group of “no response” in acute GvHD equals treatment failure and includes all patients with stable or progressive disease. (145)

If Ruxolitinib treatment was discontinued due to lack of efficacy or progression, patients were also reported as “no response/treatment failure”.

Patients who discontinued RUX within less than 10 days due to adverse effects or toxicity were excluded from this study. This applies to only one patient; another patient was able to tolerate the second attempt and was included in the study at a later point in time.

In chronic GvHD, we additionally subclassified “no response/treatment failure” into following criteria: stable disease (SD) describes a stable situation without objective improvement, but also no worsening of GvHD. Mixed response (MR) is defined as improvement in one affected organ, but also simultaneous worsening in another organ or new onset of cGvHD in a previously unaffected organ. Progressive disease (PD) is defined as worsening of GvHD. (219)

In absence of a separate classification system for overlap GvHD, staging, response and SR criteria were assessed according to the respective component (aGvHD, cGvHD), respectively.

Conditioning regimens were classified as myeloablative (MAC) or reduced intensity (RIC) as defined by the Centre of International Blood and Marrow Transplant Research in 2009. (22)

Disease stage (disease risk) at transplant was categorized as early (low risk), intermediate or advanced (high risk).

Early (low risk) disease stage included non-malignant disease, acute leukaemia in first CR and low-risk MDS or MPN. Transplant indications categorized as intermediate risk were malignancy in second CR or intermediate-risk MDS or MPN. Advanced risk (high risk) disease stage included active disease at the time of HSCT, high/very high-risk MDS or MPN, and relapse of malignancy after a previous allogeneic hematopoietic stem cell transplantation. (48)

Lymphomas and plasma cell disorders were categorized according to the CIBMTR disease risk index (220), with the high- and the very high-risk group summarized as high-risk.

Treatment lines prior to RUX included all immunosuppressive drugs including steroids and/or calcineurin inhibitors (CNI) as first-line therapy, which were initiated to improve the symptoms of present GvHD. This excludes ongoing drugs used for GvHD prophylaxis.

Treatment lines added after RUX initiation included all immunosuppressive drugs intended for the treatment of GvHD that were initiated after the first dose of RUX. This includes any medication either added to RUX during the observation period or used as a substitute for it when RUX was discontinued.

Adverse events (AE) occurring during RUX therapy were evaluated as defined in the most current version of the Common Terminology Criteria for Adverse Events (CTCAE), dated November 27, 2017. (217)

GvHD flare was defined as significant worsening while on ongoing RUX in one organ by at least one stage in organ grading based on MAGIC criteria for acute GvHD or NIH consensus criteria for chronic GvHD after previous response.

New onset of GvHD organs not affected prior to RUX initiation was evaluated during observation period or until RUX was discontinued.

Time to best response was calculated from the first RUX dose to the date of an event or censored at last follow up. GvHD data were updated as of March 31st, 2023.

Overall survival was calculated as the time from first RUX administration until death from any cause, or until last follow up in an extended observation period until December 31st, 2023.

Non-relapse mortality was referred to as mortality without prior or active relapse or progression of the underlying disease.

9.4. Statistical analysis

Data were collected and processed using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA). Medians and ranges were calculated using descriptive statistics.

All data were evaluated, processed, and analysed graphically using Microsoft Excel and the statistical software R version 4.2.2 (R Foundation for Statistical Computing, Vienna, Austria).

Overall survival was plotted using Kaplan-Meier curve, survival curves were compared using log-rank test. For best response and response at last follow-up, and visualization of death causes a stacked bar plot was created for each GvHD group.

10. Results

A total of 118 patients were included in the retrospective data analysis who initiated RUX treatment from May 2015 to September 2022 with a follow-up period until March 31st, 2023. During this period, 118 patients received at least one dose of RUX second or further treatment line for GvHD.

Out of the overall cohort of 118 patients, 61 patients (51,7%) were treated with RUX for acute GvHD, 43 patients (36,4%) received RUX for classical chronic GvHD, and 14 patients (11.9%) were treated with RUX for overlap GvHD.

The median time from transplantation to initiation of therapy with Ruxolitinib was 7,0 months (range, 0,3 – 170,2) for the entire cohort. It was 1,5 months (range, 0,3 – 72,0) for aGvHD, 23,4 months (range, 5,5 – 170,2) for classical chronic GvHD, and 9,1 months (range, 2,4 – 64,0) for overlap chronic GvHD (**Table 8**).

Median time from first onset of steroid requiring (later RUX-triggering) GvHD-type to initiation of RUX was 1,2 months (range, 0 – 165,7) for the total cohort, 0,5 months (range, 0 – 10,4) for aGvHD, 7,7 months (range 0 – 165,7) for classical chronic GvHD, and 0,7 months (range, 0 – 38,3) for overlap chronic GvHD (**Table 12**).

Our cohort of patients with GvHD received a median daily dose of 10 mg (range, 5 – 40) RUX at initiation, which could be reduced over the course as mentioned in **Table 12**.

RUX was used as a 3rd or higher line in 16/61 patients (26,2%) with aGvHD, and after 4 or more prior lines in 17/57 patients (29,8%) with cGvHD or overlap GvHD. Consequently, these patients were excluded from the evaluation of steroid status, as at least one agent was refractory in addition to first-line therapy with steroids.

The steroid dose could be tapered to a median of zero milligrams of methylprednisolone per kilogram of body weight per day in all subgroups (range; 0,0 – 2,7; **Table 23**).

The median follow-up of survivors of the entire cohort was 46,2 months (range 13,8 – 85,8). The median duration of RUX treatment was 15,6 months (range from 0,1 to 74,4) in the overall cohort.

Table 12: Ruxolitinib treatment details

	overall	acute	classical chronic	overlap chronic
	118	**61**	**43**	**14**
time from transplantation to RUX initiation in months				
median [range]	7,0 [0,3 to 170,2]	1,5 [0,3 to 72,0]	23,4 [5,5 to 170,2]	9,1 [2,4 to 64,0]
time from start systemic steroid requiring GvHD to RUX initiation in months				
median [range]	1,2 [0,0 to 165,7]	0,5 [0,0 to 10,4]	7,7 [0,0 to 165,7]	0,7 [0,0 to 38,3]
topical steroid use at RUX initiation				
GM-Dexa usage number of patients, n (%)	22 (18,6)	3 (4,9)	13 (30,2)	6 (42,9)
usage of GM-Dexa per day, median [range]	2 [1 to 4]	3 [2 to 3]	2 [1 to 3]	2 [2 to 4]
Budesonide capsules usage number of patients, n (%)	60 (50,8)	51 (83,6)	5 (11,6)	4 (28,6)
Budesonide capsules per day, median [range]	3 [0,5 to 3]	3 [1 to 3]	1 [0,5 to 3]	2,5 [1 to 3]
RUX dosage in mg, median [range]				
at initiation	10 [5 to 40]	10 [5 to 20]	10 [5 to 40]	10 [5 to 20]
maximum dosage	16,1 [4 to 40]	20 [7,9 to 30]	15 [4 to 40]	20 [10 to 20]
at last follow up	2,1 [0,0 to 30]	1,4 [0,0 to 20]	5 [0,0 to 30]	1,7 [0,0 to 20]
RUX treatment duration in months				
median [range]	15,6 [0,1 to 74,4]	6,8 [0,1 to 64,7]	33,9 [2,1 to 74,4]	27,9 [0,2 to 66,2]

Dosage is assessed milligrams (mg) for Ruxolitinib and milligrams methylprednisolone equivalent per kilogram body weight per day for steroids. Ruxolitinib dose at last follow up was assessed as zero, if Ruxolitinib treatment was discontinued and not restarted.

The dosage of systemic steroids is detailed in **Table 23**.

In acute GvHD, more than half of the patients had discontinued Ruxolitinib after 7 months of treatment. Median treatment duration in cGvHD was 33,9 months at last follow up, matching the chronic course of this disease.

Topical steroid use

Additional information is provided by observing the topical use of steroids as mouthwash with Glandomed-Dexamethason or capsules with budesonide (Entocort®).

Glandomed® is a mouthwash commonly used, especially to relieve symptoms of oral mucositis. As centre-specific standard in oral GvHD, 100 mg dexamethasone are added to 490 ml of Glandomed®, resulting in an 0,02 % solution, called Glandomed-Dexa (abbr. GM-Dexa). In case of painful mucositis, local anaesthetics (lidocaine) may be added. This drug compounding tailored for the patients' needs is initially used 2-3 times per day and slowly tapered.

Budesonide 3 mg capsules (Entocort®) are approved in inflammatory bowel disease and used off-label as non-absorbable oral steroid in the gastrointestinal tract with little systemic side effects due to a high first pass metabolism. (27) (221)

Due to high local steroid application, comparatively low systemic steroid is needed to achieve response in gastrointestinal and oral GvHD, therefore preventing long-term side effects of high-dosed systemic steroids.

10.1. Response evaluation

Best overall response rate to RUX was 68.9% in acute GvHD, 62.8% in classical cGvHD and 78.6% in overlap cGvHD. The response increased to 78.7% in acute GvHD, 74.4% in classical cGvHD and 85.7 % in overlap cGvHD upon the addition of other agents to RUX in a proportion of patients.

In aGvHD, response on day 28 was observed in 68.9% with 44.2% CR-rate, which improved to 72.2% ORR three months after RUX initiation with a CR-rate of 57.4%, and 67.2% ORR at last follow up.

In cGvHD, after three months of RUX treatment an ORR of 53.5% was observed, which improved to 58.1% after 6 months and further to 65.1% at last follow up, after a median treatment duration of 33.9 months.

Overall, 40 patients (33,9 % of the entire cohort) had neither acute nor chronic GvHD at the time of last Follow up or last RUX dose. This includes 32 patients (52,5 %) of aGvHD group, 5 patients (11,6 %) of classical cGvHD group and 3 patients (21,4 %) of overlap cGvHD group being fully free of GvHD.

Figure 5 depicts the course of GvHD staging at RUX initiation and at last follow up or last RUX dose. At last follow up, 52,5 % of patients who initially had aGvHD were free of GvHD signs.

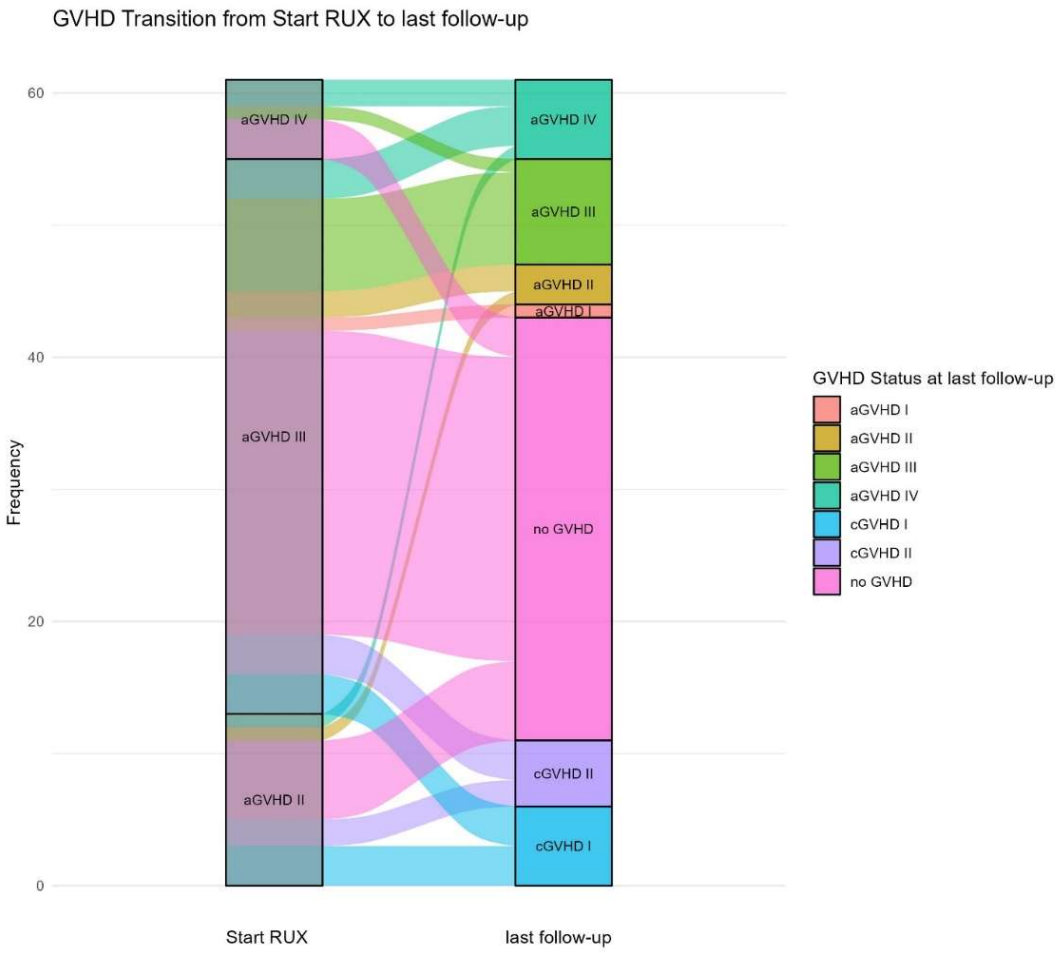

Figure 5: GvHD transition in patients with acute GvHD

Table 13: GvHD overall severity at last follow up

	overall	acute	classical chronic	overlap chronic
	118	61	43	14
acute GvHD overall severity at last follow up or final intake of RUX, n (%)				
no aGvHD	96 (81,4)	42 (68,9)	42 (97,7)	12 (85,7)
aGvHD grade 1	2 (1,7)	2 (3,3)	0 (0)	0 (0)
aGvHD grade 2	4 (3,4)	3 (4,9)	1 (2,3)	0 (0)
aGvHD grade 3	9 (7,6)	8 (13,1)	0 (0)	1 (7,1)
aGvHD grade 4	7 (5,9)	6 (9,8)	0 (0)	1 (7,1)
chronic GvHD overall severity at last follow up or final intake of RUX, n (%)				
no cGvHD	58 (49,2)	50 (82,0)	5 (11,6)	3 (21,4)
cGvHD mild	19 (16,1)	6 (9,8)	8 (18,6)	5 (35,7)
cGvHD moderate	22 (18,6)	5 (8,2)	14 (32,6)	3 (21,4)
cGvHD severe	19 (16,1)	0 (0)	16 (37,2)	3 (21,4)

Figures 5 and 6 are based on the data of GvHD overall severity provided in **Table 3** and **Table 13**. More than two thirds of patients receiving RUX for aGvHD were in CR of acute GvHD, with 52,5 % being fully free of any GvHD signs.

In 18,0 % of patients receiving RUX for aGvHD, cGvHD developed and was persistent at last follow up with 9,8 % of mild cGvHD and 8,2 % of moderate cGvHD.

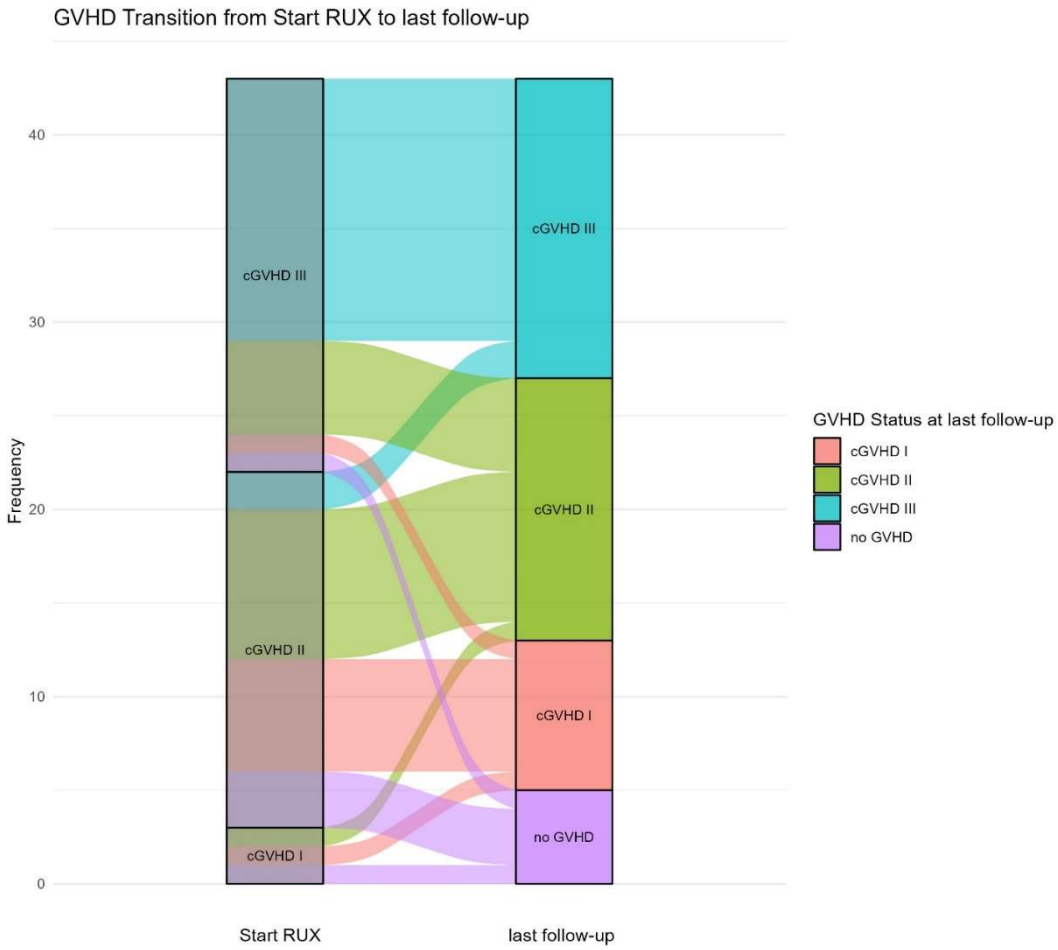

Figure 6: GvHD transition in patients with classical chronic GvHD

In classical chronic GvHD, 11,6 % of patients achieved complete response.

The ongoing grading of severity in cGvHD must consider the permanent sequelae that persist despite the absence of active inflammation, and the fact that improvements in sclerosis the current NIH-grading system does not capture improvements in cases of ongoing deep sclerosis.

Table 14: Best response to RUX therapy with or without combination with additional lines

	overall	**acute**	**classical chronic**	**overlap chronic**
	118	61	43	14
overall response rate, (CR + PR)				
n (%)	92 (78,0)	48 (78,7)	32 (74,4)	12 (85,7)
best response while RUX therapy (with or without combination), n (%)				
CR	58 (49,2)	45 (73,8)	8 (18,6)	5 (35,7)
PR	34 (28,8)	3 (4,9)	24 (55,8)	7 (50,0)
no response	26 (22,0)	13 (21,3)	11 (25,6)	2 (14,3)
time to best response in months				
median [range]	1,6 [0,2 to 48,4]	1,1 [0,2 to 6,0]	5,5 [0,4 to 48,4]	0,9 [0,5 to 19,5]

In this table the best response of RUX therapy is reported, irrespective of being achieved with or without added treatment lines.

In the following tables we report both, response to Ruxolitinib mono and in combination with following treatment lines, because although the efficacy of Ruxolitinib without further treatment is an important finding, in real world clinical practice it is much more relevant if RUX therapy can profit from addition of new lines.

10.1.1. Response in acute GvHD

Table 15: Best response in acute GvHD

Best Response in acute GvHD, n (%)		
61 patients	best response RUX monotherapy	best response RUX in combination
ORR (CR + PR)	42 (68,9)	48 (78,7)
CR	38 (62,3)	45 (73,8)
PR	4 (6,6)	3 (4,9)
no response	19 (31,1)	13 (21,3)

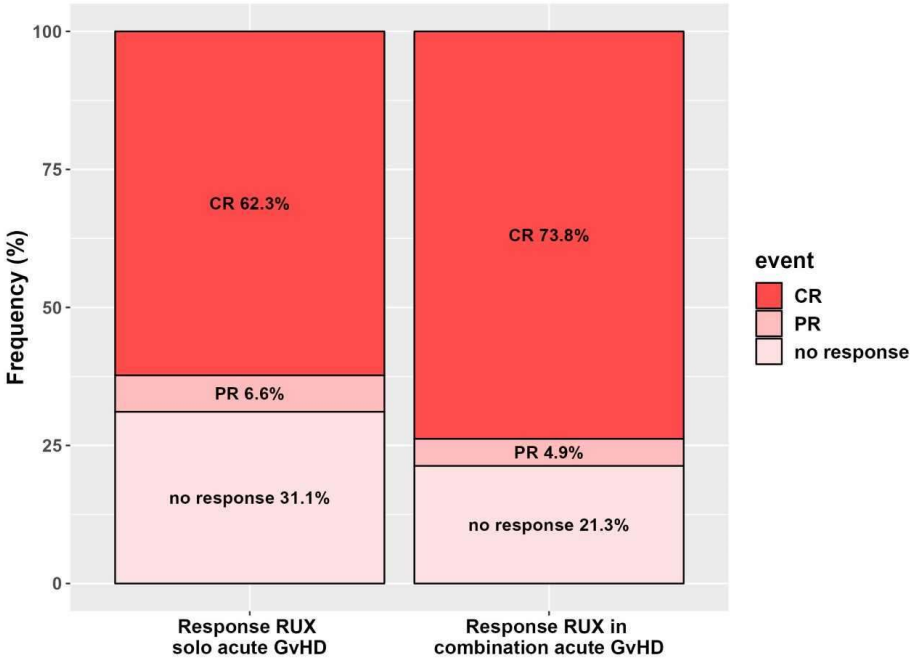

Figure 7: Best response in acute GvHD

Table 16: Response in acute GvHD over the course

Response in aGvHD over the course, n (%)			
61 patients	day 28	3 months	last follow up
ORR (CR + PR)	42 (68,9)	44 (72,2)	41 (67,2)
CR	27 (44,2)	35 (57,4)	34 (55,7)
PR	15 (24,6)	9 (14,8)	7 (11,5)
no response	19 (31,1)	17 (27,8)	20 (32,8)

One patient died from sepsis and cardiac amyloidosis but was in CR of aGvHD on day 13 of RUX treatment. This patient was classified as no response at reference dates but was considered as CR in best response evaluation.

9 patients classified as “no response” at 3 months had discontinued RUX after less than 3 months treatment duration, they are composed as follows:
In 3 patients, RUX was discontinued due to the full resolution of GvHD before the three months treatment period had elapsed. However, these patients died before the three-month-mark.

Two patients were switched to intravenous Infliximab before day 14 because of uncertain resorption of Ruxolitinib as they suffered from emesis respectively severe diarrhea. In both cases, Infliximab was chosen as intravenous alternative treatment. Three patients died from GvHD with (1 patient) or without (2 patients) signs of infection before three-month-reference date.

Although the precise date of response evaluation was not always possible in accordance with the specifications of phase 3 trials, the control intervals were closely aligned, with the closest visit being assigned.

At the aforementioned reference date evaluation, any cause of death was considered to be a non-response.

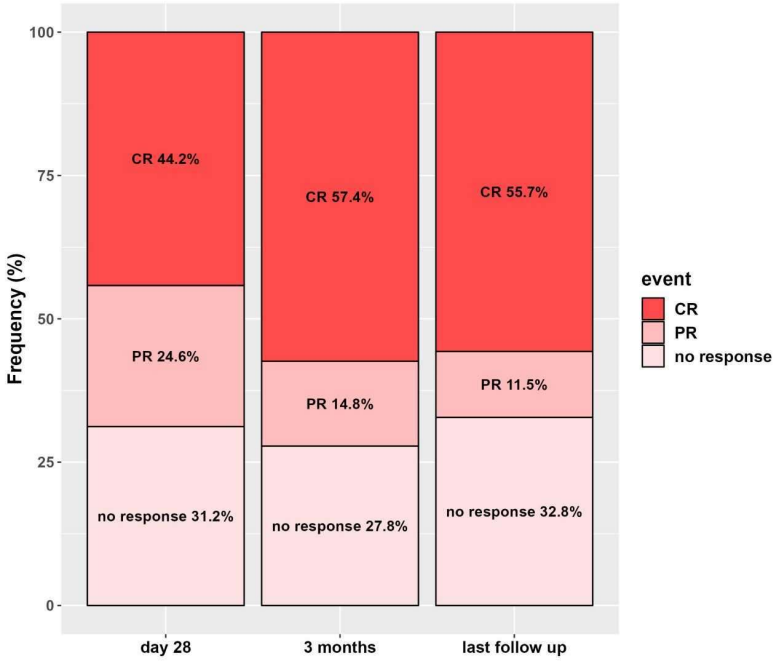

Figure 8: Response in aGvHD over the course

We provide a further drill-down in response for patients with DLI-induced acute GvHD.

Table 17: Response in DLI-induced acute GvHD

acute GvHD onset following DLI, n (%)					
61 patients	overall 61 (100)	DLI received 7 (11,5)		no DLI received 54 (88,5)	
Response reached with		RUX mono	RUX ± combi	RUX mono	RUX ± combi
ORR (CR + PR)	48 (78,7)	5 (71,4)	7 (100)	37 (68,5)	41 (75,9)
CR	45 (73,8)	4 (57,1)	6 (85,7)	34 (63,0)	39 (72,2)
PR	3 (4,9)	1 (14,3)	1 (14,3)	3 (5,6)	2 (3,7)
no response	13 (21,3)	2 (28,6)	0 (0)	17 (31,5)	13 (24,1)

In 2 patients, Ruxolitinib was discontinued due to questionable resorption because of severe emesis (1 patient) and diarrhea (1 patient). In both cases, Infliximab was chosen as intravenous alternative treatment.

Often flares of known GvHD manifestations occur when tapering the dose of immunosuppressants or GvHD treatment, but also new manifestations can occur. These cases are allegorized in **Table 24**.

10.1.2. Response in chronic GvHD

Table 18: Best response in chronic GvHD

best response in chronic GvHD, n (%)				
classic 43 patients overlap 14	best response RUX mono classical cGvHD	best response RUX ± combi classical cGvHD	best response RUX mono overlap cGvHD	best response RUX ± combi overlap cGvHD
ORR (CR+PR)	27 (62,8)	32 (74,4)	11 (78,6)	12 (85,7)
CR	8 (18,6)	8 (18,6)	5 (35,7)	5 (35,7)
PR	19 (44,2)	24 (55,8)	6 (42,9)	7 (50,0)
no response	16 (37,2)	11 (25,6)	3 (21,4)	2 (14,3)
SD	12 (27,9)	6 (14,0)	1 (7,1)	0 (0)
MR	3 (7,0)	4 (9,3)	0 (0)	0 (0)
PD	1 (2,3)	1 (2,3)	2 (14,3)	2 (14,3)

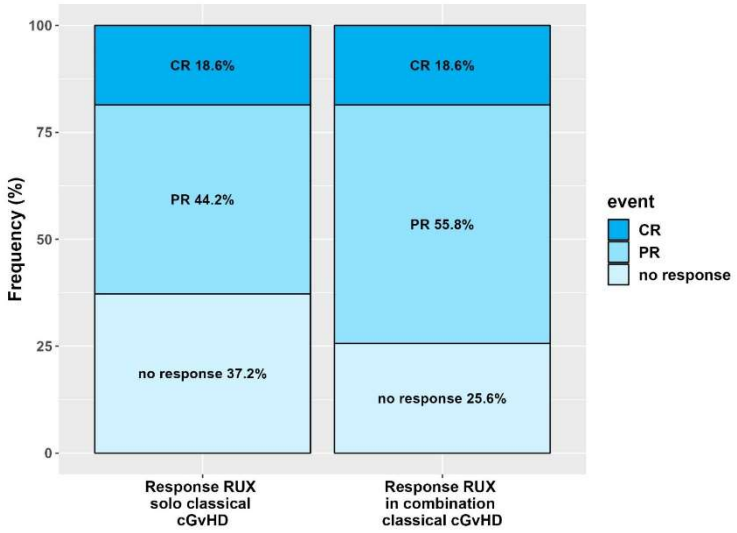

Figure 9: Best response in classical chronic GvHD

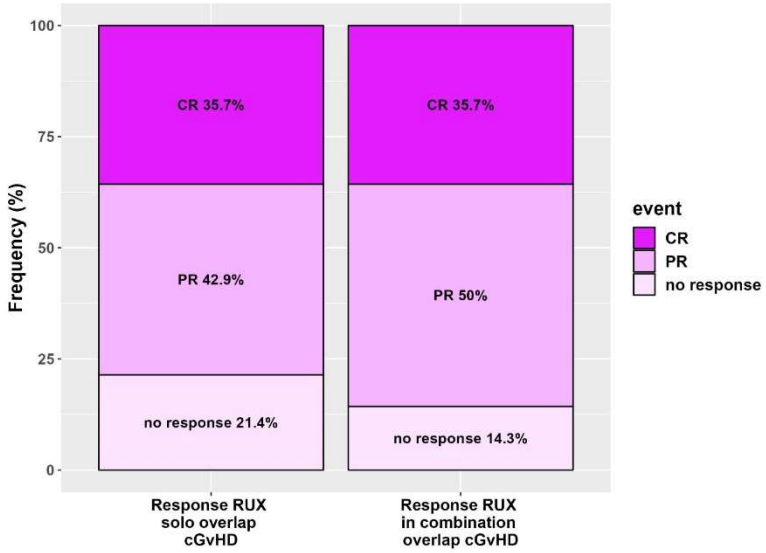

Figure 10: Best response in overlap chronic GvHD

The patient who received RUX as 9th therapy line for classical chronic GvHD reached stable disease without flares but died from sepsis after 4,5 months of RUX treatment.

Table 19: Response in classical chronic GvHD over the course

Response in classical chronic GvHD over the course, n (%)			
43 patients	3 months	6 months	last follow up
ORR (CR+PR)	23 (53,5)	25 (58,1)	28 (65,1)
CR	5 (11,6)	4 (9,3)	5 (11,6)
PR	18 (41,9)	21 (48,8)	23 (53,5)
no response	20 (46,5)	18 (41,9)	15 (34,9)

The development depicted below suggests that continuous treatment with RUX in cGvHD can improve response even after several months. This applies to overlap cGvHD too, as depicted in **Figure 12**.

Although the precise date of response evaluation was not always possible in accordance with the specifications of phase 3 trials, the control intervals were closely aligned, with the closest visit being assigned.

At the aforementioned reference date evaluation, any cause of death was considered to be a non-response.

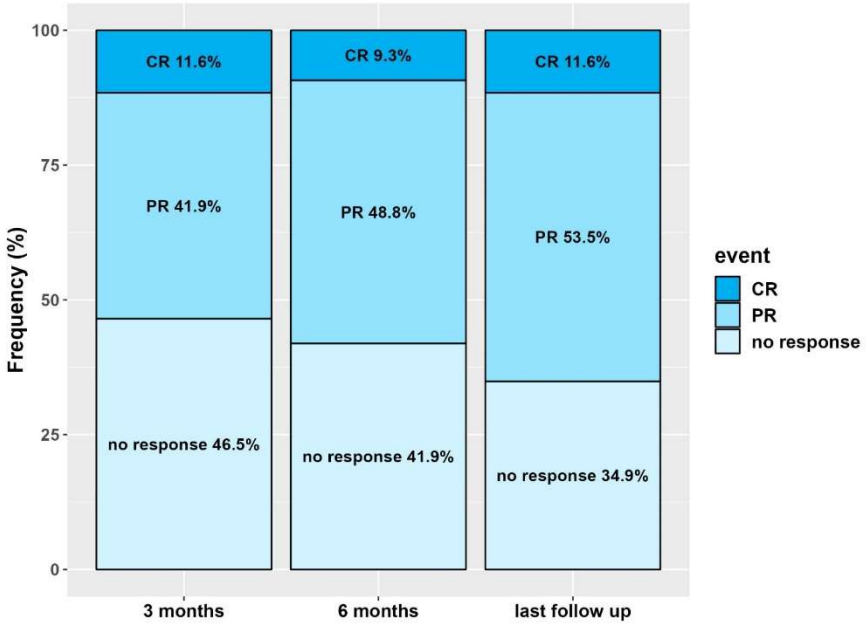

Figure 11: Response to classical chronic GvHD over time

Table 20: Response in overlap chronic GvHD over the course

Response in overlap chronic GvHD over the course, n (%)				
14 patients	day 28	3 months	6 months	last follow up
CR	3 (21,4)	6 (42,9)	6 (42,9)	3 (21,4)
PR	7 (50,0)	5 (35,7)	6 (42,9)	6 (42,9)
no response	4 (28,6)	3 (21,4)	2 (14,3)	5 (35,7)

Two overlap cGvHD patients had died and three patients developed new cGvHD manifestations over the course of this study. These patients are therefore classified as having no response at the time of the last follow up.

Figure 12: Response in overlap chronic GvHD over time

Table 21: Response in DLI-associated classical chronic GvHD

classical chronic GvHD onset following DLI, n (%)				
43 patients	DLI received 12 (27,9)		no DLI received 31 (72,1)	
	RUX mono	RUX ± combi	RUX mono	RUX ± combi
ORR (CR+PR)	7 (58,3)	10 (83,3)	20 (64,5)	22 (71,0)
CR	3 (25,0)	3 (25,0)	5 (16,1)	5 (16,1)
PR	4 (33,3)	7 (58,3)	15 (48,4)	17 (54,8)
no response (less than PR)	5 (41,7)	2 (16,7)	11 (35,5)	9 (29,0)
SD	3 (25,0)	0 (0)	9 (29,0)	6 (19,4)
MR	1 (8,3)	1 (8,3)	2 (6,5)	3 (9,7)
PD	1 (8,3)	1 (8,3)	0 (0)	0 (0)

Table 22: Response in DLI-associated overlap chronic GvHD

overlap chronic GvHD onset following DLI, n (%)				
14 patients	DLI received 3 (21,4)		no DLI received 11 (78,6)	
	RUX mono	RUX combi	RUX mono	RUX combi
ORR (CR+PR)	2 (66,7)	2 (66,7)	9 (81,8)	10 (90,9)
CR	0 (0)	0 (0)	5 (45,5)	5 (45,5)
PR	2 (66,7)	2 (66,7)	4 (36,4)	5 (45,5)
no response (less than PR)	1 (33,3)	1 (33,3)	2 (18,2)	1 (9,1)
SD	0 (0)	0 (0)	1 (9,1)	0 (0)
MR	0 (0)	0 (0)	0 (0)	0 (0)
PD	1 (33,3)	1 (33,3)	1 (9,1)	1 (9,1)

Organ specific Response in cGvHD lung and sclerotic skin

As previously outlined in chapter 7.4.3., the presence of lung manifestations of cGvHD is associated with a poor prognosis. In our cohort, 15 patients exhibited lung cGvHD (14 classical cGvHD, 1 overlap cGvHD) at the initiation of RUX therapy. Of these, four patients were classified as having stage 1 lung cGvHD, three patients were classified as having stage 2 lung cGvHD, and two patients were classified as having stage 3 lung cGvHD, as detailed in **Table 10.**

In total, 4/15 patients (26.7%) exhibited an organ-specific response for lung cGvHD.

In two patients with stage 2 lung cGvHD, lung cGvHD has been completely resolved following the treatment with RUX. Nevertheless, one patient developed ocular cGvHD stage 1 despite continued RUX therapy.

In two patients, partial remission was observed, both of whom had stage 2 lung cGvHD at the initiation of RUX.

In 9/15 patients, the disease remained stable. The 9 patients were initially classified as follows: Four patients were classified as having stage 1 lung cGvHD, three patients were classified as having stage 2 lung cGvHD (including the one patient with overlap cGvHD), and two patients were classified as having stage 3 lung cGvHD. One patient, who had been classified as having stable disease stage 3 lung cGvHD, nevertheless reported subjective improvement, although he still fulfilled the criteria for lung stage 3.

In two patients who initially presented with stage 1 lung cGvHD, the disease progressed to higher stages.

The sclerotic features of the skin in cGvHD are also considered to be difficult to treat. In our cohort, two out of eight patients with sclerotic skin features exhibited complete resolution of skin involvement cGvHD, corresponding to a CR rate of 25%. In the remaining six patients, the sclerotic features persisted. But despite the persistence of sclerotic features, the patients reported improvements in skin flexibility and complete resolution of restricted range of motion.

10.2. Longitudinal characterisation of GvHD and GvHD treatments following RUX initiation

10.2.1. Steroid dose over the course

Table 23: Steroid dose over the course

	overall	acute	classical chronic	overlap chronic
n (%)	118	61	43	14
steroid dose over the course for the respective GvHD type, median [range] indicated as mg methylprednisolone per kilogram of body weight per day				
maximum dose (any time before RUX initiation)	1,1 [0,0 to 6,7]	1,9 [0,07 to 6,7]	0,8 [0,0 to 4,7]	1,1 [0,08 to 3,2]
at RUX initiation	0,7 [0,0 to 2,6]	1,9 [0,0 to 2,3]	0,1 [0,0 to 2,1]	0,2 [0,0 to 2,6]
at end of RUX treatment or last follow up	0,0 [0,0 to 2,7]	0,0 [0,0 to 2,1]	0,0 [0,0 to 0,3]	0,0 [0,0 to 2,7]

Steroid dose is represented as methylprednisolone dose equivalent. Maximum dose is evaluated for the GvHD type that was indication for RUX.

Most patients in any GvHD type could discontinue glucocorticoids, being a major goal in GvHD therapy, considering the various side effects like Cushing syndrome.

All 81 survivors at last follow up (35 aGvHD, 35 classical cGvHD, 11 overlap cGvHD) had steroid dosage ≤ 0,1 mg methylprednisolone per kilogram body weight, 52 patients without any steroid medication (20 acute, 24 classical chronic, 8 overlap chronic). 46 patients alive at last follow up (13 acute, 25 classical chronic, 8 overlap chronic GvHD) had ongoing treatment with RUX.

10.2.2. Flares of GvHD

Table 24: Flares of GvHD

	overall	acute	classical chronic	overlap chronic
n (%)	118	61	43	14
flare of known GvHD while RUX treatment				
yes	50 (42,4)	30 (49,2)	14 (32,6)	6 (42,9)
chronic GvHD intermittently present under RUX treatment				
	56 (47,5)	21 (34,4)	25 (58,1)	10 (71,4)
new onset of GvHD organs not affected prior RUX initiation				
no new onset	73 (61,9)	35 (57,4)	31 (72,1)	7 (50,0)
overall	45 (38,1)	26 (42,6)	12 (27,9)	7 (50,0)
aGvHD	8 (6,8)	8 (13,1)	0 (0)	0 (0)

aGvHD upperGI	0 (0)	0 (0)	0 (0)	0 (0)
aGvHD lowerGI	3 (2,5)	3 (4,9)	0 (0)	0 (0)
aGvHD skin	6 (5,1)	6 (9,8)	0 (0)	0 (0)
aGvHD liver	0 (0)	0 (0)	0 (0)	0 (0)
cGvHD	40 (33,9)	21 (34,4)	12 (27,9)	7 (50,0)
cGvHD skin	17 (14,4)	8 (13,1)	3 (7,0)	6
cGvHD GI	3 (2,5)	2 (3,3)	1 (2,3)	0 (0)
cGvHD eyes	17 (14,4)	7 (11,5)	8 (18,6)	2 (14,3)
cGvHD mouth	18 (15,3)	14 (23,0)	2 (4,7)	2 (14,3)
cGvHD joints	7 (5,9)	4 (6,6)	1 (2,3)	2 (14,3)
cGvHD lung	1 (0,9)	0 (0)	1 (2,3)	0 (0)
cGvHD liver	4 (3,4)	4 (6,6)	0 (0)	0 (0)
cGvHD other	0 (0)	0 (0)	0 (0)	0 (0)

In this table, reoccurrence of GvHD in organs described at first RUX dose that had responded in the meantime and new presentation of GvHD in prior unaffected organs are mentioned separately.

In 21 patients (34,4 %) initially treated with RUX for aGvHD, cGvHD occurred before RUX was discontinued. RUX was then proceeded for cGvHD, which leads to longer RUX treatment duration, but was not evaluated separately.

Table 25: RUX treatment discontinuation

	overall	acute	classical chronic	overlap chronic
	118	61	43	14
RUX still used at last follow up	49 (41,5)	16 (26,2)	25 (58,1)	8 (57,1)
overall treatment discontinuation, n (%)				
RUX discontinued	69 (58,5)	45 (73,8)	18 (41,9)	6 (42,9)
reasons for RUX discontinuation, n (%)				
CR reached	30 (25,4)	22 (36,1)	7 (16,3)	1 (7,1)
Toxicity, intolerance	6 (5,1)	4 (6,6)	2 (4,7)	0 (0)
Lack of efficacy, GvHD progression	12 (10,2)	8 (13,1)	3 (7,0)	1 (7,1)
Progression of GvHD with infection	8 (6,8)	4 (6,6)	3 (7,0)	1 (7,1)
Progression of underlying disease with persisting GvHD	2 (1,7)	1 (1,6)	1 (2,3)	0 (0)
Progression of underlying disease without infection or GvHD	7 (5,9)	3 (4,9)	1 (2,3)	3 (21,4)
Infection	4 (3,4)	3 (4,9)	1 (2,3)	0 (0)

In patients that discontinued RUX, the most frequent reason was the complete response of GvHD. However, about half of patients who discontinued RUX did so because of toxicity, progression of GvHD, progression of underlying disease or infectious complications.

Furthermore, it must be noted that in classical and overlap cGvHD, more than half of the patients still have taken RUX at last follow up.

Table 26: GvHD treatment at last follow up

	overall	**acute**	**classical chronic**	**overlap chronic**
n (%)	118	61	43	14
RUX discontinued	69 (58,5)	45 (73,8)	18 (41,9)	6 (42,9)
RUX ongoing at last follow up	49 (41,5)	16 (26,2)	25 (58,1)	8 (57,1)
ongoing GvHD treatment at last follow up, n (% of overall cohort)				
RUX monotherapy	24 (20,3)	9 (14,8)	10 (23,3)	5 (35,7)
RUX + steroids	8 (6,8)	3 (4,9)	4 (9,3)	1 (7,1)
RUX + agent(s) other than steroids	17 (14,4)	4 (6,6)	11 (25,6)	2 (14,3)

Patients using only topical steroids (budesonide capsules, dexamethasone mouthwash as described in chapter 9.2, inhalative corticosteroids, skin lotion) were assigned to the group of “RUX monotherapy” (2 patients).

The median of the systemic steroid-dose is outlined in **Table 23**.

The patients receiving ongoing treatment with Ruxolitinib and immunosuppressive agents other than steroids were further subclassified. In all patients with acute GvHD (n=4, all TAC) and overlap chronic GvHD (n=2, both CSA), the immunosuppressive agents in addition to RUX with or without steroids were a single agent ongoing from initial GvHD prophylaxis. In classical chronic GvHD, 11 patients received another immunosuppressive agent apart from steroid, these were CSA in 5 patients, TAC in 2 patients, MMF in 2 patients, imatinib in one patient, and MTX in one patient. In overlap cGvHD, both patients had ongoing CSA.

Table 27: Added treatment lines after RUX initiation

	overall	**acute**	**classical chronic**	**overlap chronic**
	118	61	43	14
number of added GvHD treatment lines after RUX initiation, n (%)				
0	69 (58,5)	33 (54,1)	27 (62,8)	9 (64,3)
1	22 (18,6)	12 (19,7)	6 (14,0)	4 (28,6)
2	12 (10,2)	8 (13,1)	3 (7,0)	1 (7,1)
3 or > 3	15 (12,7)	8 (13,1)	7 (16,3)	0 (0)

49 patients (41,5%) received one or more GVHD treatment lines, either in addition to RUX, or replacing RUX. We observed the patients until RUX was tapered and the last dose was taken.

Table 28: Agents added to RUX for treatment of GvHD

	overall	acute	classical chronic	overlap chronic
n (%)	118	61	43	14
agents added to RUX for treatment of GvHD				
CSA	8 (6,8)	1 (1,6)	5 (11,6)	2 (14,3)
TAC	6 (5,1)	3 (4,9)	2 (4,7)	1 (7,1)
MMF	8 (6,8)	5 (8,2)	3 (7,0)	0 (0)
MTX	6 (5,1)	1 (1,6)	5 (11,6)	0 (0)
Sirolimus	3 (2,5)	1 (1,6)	1 (2,3)	1 (7,1)
ATG	1 (0,9)	1 (1,6)	0 (0)	0 (0)
ECP	19 (16,1)	12 (19,7)	6 (14,0)	1 (7,1)
UVA-therapy	1 (0,9)	0 (0)	1 (2,3)	0 (0)
Etanercept	22 (18,6)	18 (29,5)	4 (9,3)	0 (0)
Infliximab	8 (6,8)	8 (13,1)	0 (0)	0 (0)
Rituximab	12 (10,2)	8 (13,1)	4 (9,3)	0 (0)
Tocilizumab	2 (1,7)	2 (3,3)	0 (0)	0 (0)
Abatacept	1 (0,9)	0 (0)	1 (2,3)	0 (0)
Fecal microbiota transplantation	2 (1,7)	2 (3,3)	0 (0)	0 (0)
Imatinib	3 (2,5)	0 (0)	3 (7,0)	0 (0)
Ibrutinib	2 (1,7)	0 (0)	0 (0)	0 (0)
Ixazomib	2 (1,7)	0 (0)	2 (4,7)	0 (0)
Fedratinib	1 (0,9)	0 (0)	0 (0)	1 (7,1)
Belumosudil	1 (0,9)	0 (0)	1 (2,3)	0 (0)

As a significant part of patients received more than one agent added, these numbers do not sum up to 100 %. In this table, restart of drugs prior used in prophylaxis but were fully tapered intermittently are also included.

Abbreviations used: CSA = cyclosporine A, TAC = tacrolimus, MMF = mycophenolate mofetil, MTS = methotrexate, ATG = anti-thymocyte globulin, ECP = extracorporeal photopheresis

It shows the broad variety of agents used at our centre.

11 patients had received at least one session of ECP prior to commencing Ruxolitinib, with the treatment being restarted after the initiation of RUX. In 8 patients, ECP was initiated after the first dose of RUX as an additional therapy.

Table 29: Staging of GvHD at last follow up or final intake of RUX

	overall	acute	classical chronic	overlap chronic
	118	61	43	14
Staging of GvHD at last follow up or final intake of RUX				
acute GvHD				
no aGvHD	97 (82,2)	43 (70,5)	42 (97,7)	12 (85,7)
aGvHD upperGI stage 1	8 (6,8)	7 (11,5)	0 (0)	1 (7,1)
aGvHD lowerGI	20 (17,0)	17 (27,9)	1 (2,3)	2 (14,3)
loGI stage 1	4 (3,4)	3 (4,9)	1 (2,3)	0 (0)
loGI stage 2	3 (2,5)	3 (4,9)	0 (0)	0 (0)
loGI stage 3	5 (4,2)	4 (6,6)	0 (0)	1 (7,1)
loGI stage 4	8 (6,8)	7 (11,5)	0 (0)	1 (7,1)
aGvHD skin	3 (2,5)	2 (3,3)	0 (0)	1 (7,1)
Skin stage 1	1 (0,9)	1 (1,6)	0 (0)	0 (0)
Skin stage 2	0 (0)	0 (0)	0 (0)	0 (0)
Skin stage 3	1 (0,9)	1 (1,6)	0 (0)	0 (0)
Skin stage 4	1 (0,9)	0 (0)	0 (0)	1 (7,1)
aGvHD liver	1 (0,9)	1 (1,6)	0 (0)	0 (0)
Liver stage 1	0 (0)	0 (0)	0 (0)	0 (0)
Liver stage 2	1 (0,9)	1 (1,6)	0 (0)	0 (0)
Liver stage 3	0 (0)	0 (0)	0 (0)	0 (0)
Liver stage 4	0 (0)	0 (0)	0 (0)	0 (0)
chronic GvHD				
no cGvHD	59 (50,0)	52 (85,3)	5 (11,6)	3 (21,4)
cGvHD skin	20 (17,0)	3 (4,9)	13 (30,2)	4 (28,6)
skin mild	9 (7,6)	3 (4,9)	4 (9,3)	2 (14,3)
skin moderate	2 (1,7)	0 (0)	1 (2,3)	1 (7,1)
skin severe	9 (7,6)	0 (0)	8 (18,6)	1 (7,1)
cGvHD GI	3 (2,5)	1 (1,6)	2 (4,7)	0 (0)
GI mild	2 (1,7)	1 (1,6)	1 (2,3)	0 (0)
GI moderate	1 (0,9)	0 (0)	1 (2,3)	0 (0)
GI severe	0 (0)	0 (0)	0 (0)	0 (0)
cGvHD eyes	38 (32,2)	8 (13,1)	22 (51,2)	8 (57,1)
eyes mild	19 (16,1)	5 (8,2)	8 (18,6)	6 (42,9)
eyes moderate	17 (14,4)	3 (4,9)	13 (30,2)	1 (7,1)
eyes severe	2 (1,7)	0 (0)	1 (2,3)	1 (7,1)
cGvHD mouth	16 (13,6)	2 (3,3)	11 (25,6)	3 (21,4)

	overall	acute	classical chronic	overlap chronic
mouth mild	16 (13,6)	2 (3,3)	11 (25,6)	3 (21,4)
mouth moderate	0 (0)	0 (0)	0 (0)	0 (0)
mouth severe	0 (0)	0 (0)	0 (0)	0 (0)
cGvHD joints & muscles	15 (12,7)	3 (4,9)	10 (23,3)	2 (14,3)
joints mild	14 (11,9)	3 (4,9)	9 (20,9)	2 (14,3)
joints moderate	0 (0)	0 (0)	0 (0)	0 (0)
joints severe	1 (0,9)	0 (0)	1 (2,3)	0 (0)
cGvHD lung	15 (12,7)	0 (0)	14 (32,6)	1 (7,1)
lung mild	6 (5,1)	0 (0)	6 (14,0)	0 (0)
lung moderate	5 (4,2)	0 (0)	4 (9,3)	1 (7,1)
lung severe	4 (3,4)	0 (0)	4 (9,3)	0 (0)
cGvHD liver	2 (1,7)	1 (1,6)	0 (0)	1 (7,1)
liver mild	0 (0)	0 (0)	0 (0)	0 (0)
liver moderate	2 (1,7)	1 (1,6)	0 (0)	1 (7,1)
liver severe	0 (0)	0 (0)	0 (0)	0 (0)
cGvHD other organs	11 (9,3)	2 (3,3)	8 (18,6)	1 (7,1)

Organ manifestations attributed to cGvHD, but not specifically classified (referred to as “other organs”) at last follow up included neuropathic symptoms, hypothyroidism, genital cGvHD, and impaired performance status.

10.3. Adverse events after RUX initiation

Overall, 58,5 % of patients (69/118) have discontinued RUX, but adverse events (including infection and relapse) were the reason in only 22,9% (27/118) patients. In general, adverse events and especially cytopenia were manageable by dose adjustments.

Cytopenia recorded under RUX treatment include preexisting cytopenia before initiation, in contrast to the reported cytopenia in pivotal studies.

Table 30: Cytopenia observed under RUX treatment (including pre-existing cytopenia)

n (%)	**overall**	**acute**	**classical chronic**	**overlap**
	118	61	43	14
anaemia				
any grade	93 (78,8)	58 (95,1)	24 (55,8)	11 (78,6)
grade 3/4	45 (38,1)	38 (62,3)	5 (11,6)	2 (14,3)
leukopenia				
any grade	82 (69,5)	55 (90,2)	18 (41,9)	9 (64,3)
grade 3/4	45 (38,1)	39 (63,9)	2 (4,7)	4 (28,6)
thrombocytopenia				
any grade	67 (56,8)	55 (90,2)	6 (14,0)	6 (42,9)
grade 3/4	49 (41,5)	43 (70,5)	3 (7,0)	3 (21,4)

Table 31: Adverse events under RUX treatment

n (%)	**overall**	**acute**	**classical chronic**	**overlap**
	118	61	43	14
Infections under RUX treatment				
any grade	90 (76,3)	47 (77,1)	33 (76,7)	10 (71,4)
grade ¾	47 (39,8)	30 (49,2)	13 (30,2)	4 (28,6)
sepsis	15 (12,7)	9 (14,8)	5 (11,6)	1 (7,1)
relapse under RUX treatment				
	9 (7,6)	4 (6,6)	3 (7,0)	2 (14,3)
Treatment emergent CMV-reactivation* under RUX treatment among patients at risk				
% of patients at risk**	26/69 (37,7)	22/37 (59,5)	3/26 (11,5)	1/6 (16,7)

Relapse was defined as clinical/haematological relapse (i.e., excluding molecular relapse).

*Only significant CMV-reactivation were considered, i.e. reactivations for which antiviral therapy was initiated.

**Patients at risk were defined as CMV IgG positive in recipient and/or donor before HSCT.
Grading of adverse events was done as defined in the Clinical Terminology for Adverse Events Criteria (CTCAE) Version 5.0. (217)

Supportive care was done according to centre-specific standards.

The higher infection rate in cGvHD needs to be interpreted in the context of the extended observation period, with more than two thirds being followed-up for more than 40 months.

10.4. Overall survival and non-relapse mortality

The median follow-up of survivors was 46,2 months (n=81, range 13,8 – 85,8) from the day of RUX initiation. Median overall survival was not reached in any GvHD type.

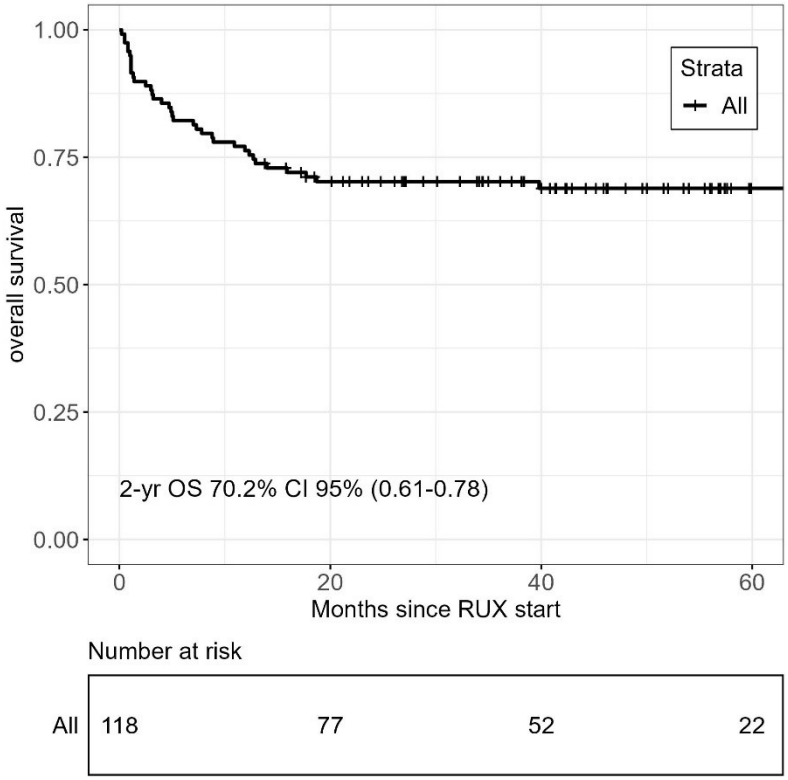

Figure 13: Overall Survival

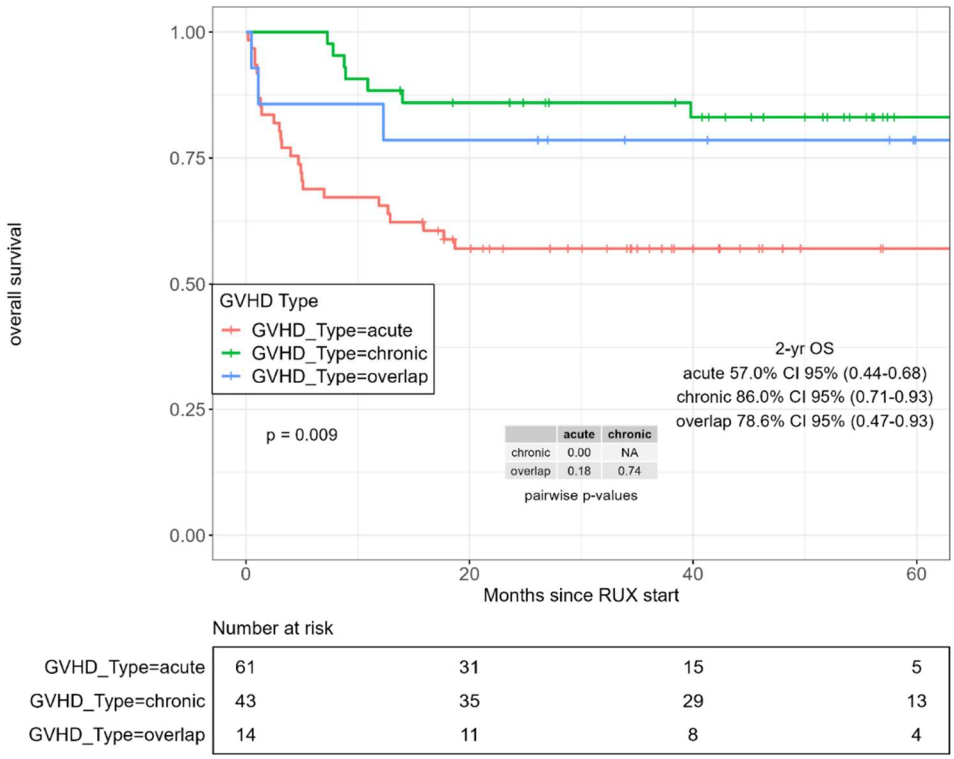

Figure 14: Overall Survival from initiation of RUX according to GvHD type

Table 32: Causes of death

n (%*)	**overall**	**acute**	**classical chronic**	**overlap**
	118	61	43	14
dead	37 (31,4)	26 (42,6)	8 (18,6)	3 (21,3)
causes of death				
relapse	8 (21,6)	4 (15,4)	3 (37,5)	1 (33,3)
relapse + GvHD	1 (2,7)	1 (3,8)	0 (0)	0 (0)
GvHD without infection	7 (18,9)	5 (19,2)	1 (12,5)	1 (33,3)
GvHD with infection	9 (24,3)	7 (26,9)	2 (25)	0 (0)
infection without GvHD	8 (21,6)	5 (19,2)	2 (25)	1 (33,3)
secondary malignancy	1 (2,7)	1 (3,8)	0 (0)	0 (0)
other non-relapse causes	3 (8,1)	3 (11,5)	0 (0)	0 (0)

*Percentage of causes of death was calculated in relation to overall death rate.

Survival events were assessed for an extended observation period, with database lock on December 31st, 2023.

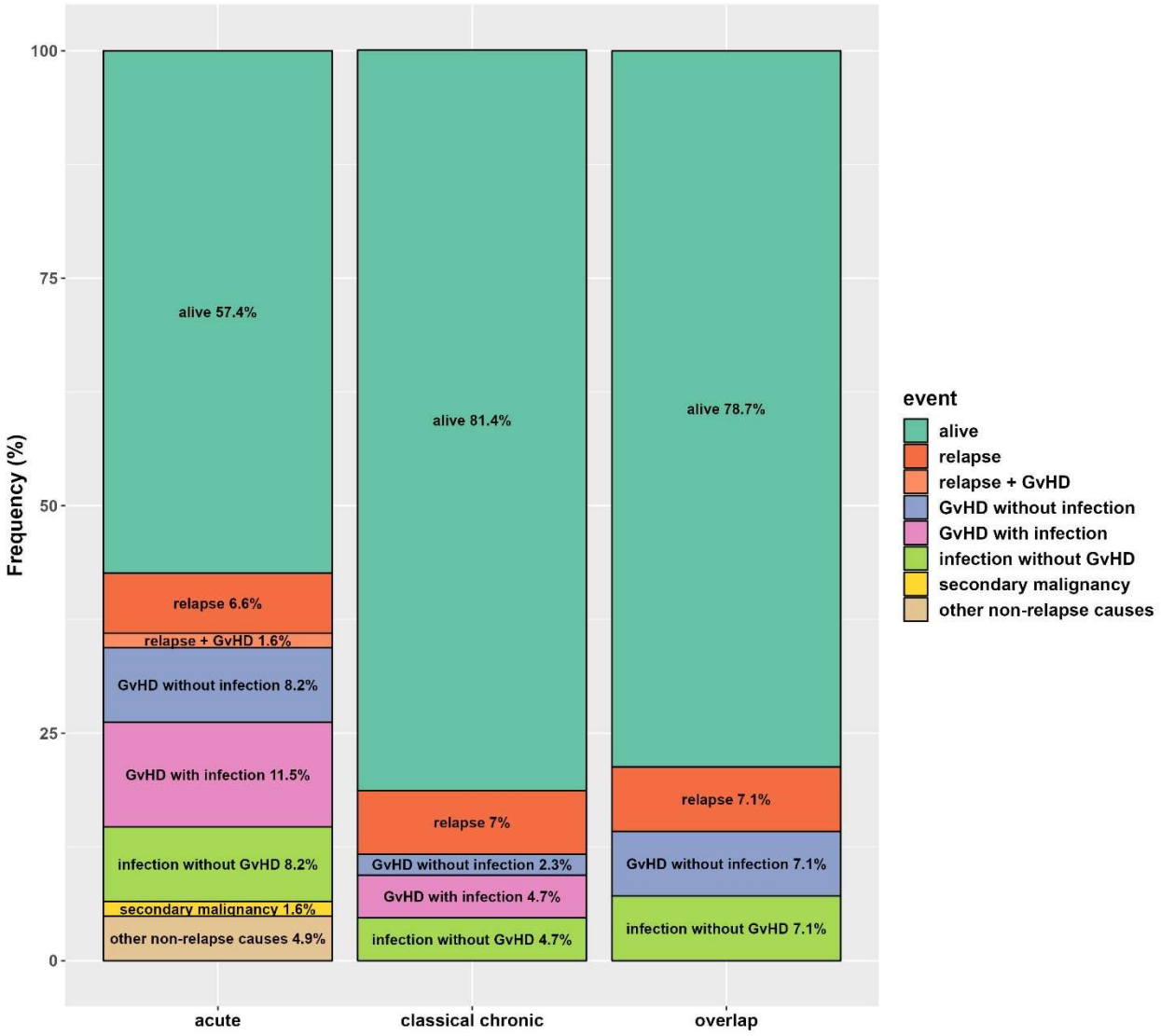

Figure 15: Causes of Death

21,3 % of patients who received RUX for aGvHD died with signs of aGvHD, so 50% of deaths in aGvHD were attributable to acute GvHD. In classical cGvHD …

11. Discussion

This study aimed to investigate the efficacy and safety of Ruxolitinib in the treatment of steroid-refractory acute and chronic GvHD. The findings of this study support existing data indicating that RUX is an effective and safe drug in second or further line treatment of acute GvHD and chronic GvHD.

The administration of RUX resulted in an improvement of GvHD grading and the ability to taper steroids in patients with all types of GvHD.

The overall response rate for RUX in the treatment of acute GvHD in our cohort was 68.9%. This could be further improved to 78.7% by the addition of further agents to ongoing RUX treatment. This was necessary in 41.5% of cases (**Table 27**). The most commonly added treatments were etanercept and ECP. Etanercept was predominantly added for aGvHD, while ECP was added for classical cGvHD (**Table 28**).

In chronic GvHD, best overall response rate was 62.8%. By addition of further therapy lines (**Table 28**), a best response rate of 74.4% could be achieved, with a CR-rate of 18.6%.

An overall response rate of 74.4% in cGvHD observed in this study is yet remarkable.

Table 18 suggests that especially cGvHD patients who had stable disease during RUX monotherapy did benefit from addition of another agent and improved to partial response, in both classical and overlap chronic GvHD.

The highly variable time to best response in cGvHD (**Table 14**) indicates the heterogenous pathophysiology of chronic GvHD in individual patients.

It is notable that in the NIH staging and grading system of chronic GvHD, persisting skin sclerosis is classified as stage 3, which is considered to be severe chronic GvHD, despite the localisation, affected BSA or absence of active inflammation. Consequently, the classification system lacks the capacity to convey the subjective clinical improvement reported by patients.

In the light of the limited informative value of the response of skin sclerosis in cGvHD in the NIH consensus staging and grading system, it is important to evaluate patient-reported outcomes such as quality of life and symptom scores in order to gain a more comprehensive understanding of improvements. As this study is retrospective in nature, it may be implemented in further studies.

These fibrotic changes in subcutaneous tissue caused by cGvHD are often permanent and can impair patients in their everyday lives. However, the extent of impairment depends on the localisation of skin sclerosis. In clinical practice, fibrotic changes frequently manifest on the forearm and shank, which is considerably less impairing than subcutaneous sclerotic changes that occur in the area of joints.

Ocular involvement is an organ manifestation that frequently persists at last follow up, as illustrated in **Table 29**. This indicates that, despite the absence of active inflammation, lacrimal gland damage frequently appears to be permanent.

The evidence for the efficacy of RUX in DLI-induced GvHD is currently limited, due to the exclusion of GvHD following unplanned DLI from the prospective trials. However, this represents an important real-world scenario. Our observation indicated that the response to RUX in patients with DLI-induced GvHD does not appear to differ from that observed in patients with GvHD occurring without DLI. Nevertheless, these novel findings necessitate further investigation due to the limited number of patients included in this analysis.

The study population comprised patients who had undergone extensive prior treatment, particularly in the context of cGvHD. Overall, 34.9% of cGvHD cases were refractory to three or more lines of treatment prior to the initiation of RUX. Adding to the results of REACH3, which investigated RUX in 2nd / 3rd line of treatment in cGvHD, our findings indicate that RUX is an effective treatment even in cases beyond the 3rd line of treatment for cGvHD.

Overlap cGvHD represents another type of cGvHD that was excluded from pivotal trials. Therefore, evidence for the efficacy of RUX in this GvHD type has been scarce thus far. In this retrospective analysis, patients with overlap cGvHD appear to achieve at least comparable CR-rates compared to classical cGvHD.

More than half of our patient cohort could discontinue systemic steroids in each GvHD type (**Table 23**), comparable to results of REACH3, where a steroid-sparing effect was observed. (222) All 81 survivors of our cohort (35 aGvHD, 35 classical cGvHD, 11 overlap cGvHD) had steroid dosage of ≤ 0,1 mgMPN/kg of body weight at last follow-up. Of these, 52 patients (20 acute, 24 classical chronic, 8 overlap chronic) were indeed steroid-free.

In patients with aGvHD, more than half of patients could discontinue RUX after a median of 7 months of treatment while the median treatment duration in cGvHD was 33.9 months (**Table 12**).

It is common for flare-ups of preexisting GvHD or new organ manifestations to occur during the tapering of immunosuppression. Given that aGvHD is a known risk factor for the later development of cGvHD, we observed that in patients who initiated RUX due to aGvHD, 57.4% (35/61) did not develop any further GvHD manifestations (either acute or chronic) following the initiation of RUX (**Table 24**). 34.4% (21/61) developed cGvHD prior to the discontinuation of RUX, with oral GvHD being the most common organ manifestation (23.0%, 14/61). This subsequently resulted in a longer duration of RUX treatment, which was not subjected to a detailed evaluation.

RUX was generally well tolerated, with the majority of adverse effects being haematological in nature. These were manageable through dose adjustments. It is not possible to make a direct comparison between the cytopenia data presented in **Table 30** and the incidence and severity of REACH trials, as the former includes preexisting cytopenia, which are certainly particularly prevalent in the context of aGvHD. Consequently, it would be reasonable to posit that the incidence of cytopenia would be greater at each point. However, it is noteworthy that the occurrence of grade 3/4 thrombocytopenia in classical cGvHD was less frequent than that observed in the REACH-3 study.

The two-year survival probability following the initiation of RUX was 57 % in the context of acute GvHD (range, 44 – 68 %), 86 % in classical cGvHD (range, 71 – 93 %) and 79 % in overlap cGvHD (range, 47 – 93 %).

The REACH-2 trial reported a median overall survival of 11.1 months in the RUX group. (138) The estimated probability of survival at 12 months in REACH3 was 81.4%. (139) In 2011, Xhaard et al. reported a 2-year survival of SR-aGvHD of less than 30%. (223)

The comparatively better survival may be associated with the earlier use of RUX in real-world, without awaiting the SR-criteria to be met.

In our study, 21,3 % of patients in the aGvHD group died from causes directly attributable to acute GvHD, corresponding to 50% of deaths in this group. This figure is consistent with the 22% reported from the REACH-2 trial.

One of the key advantages of retrospective real-world evidence is the long observation period. In our cohort, a large number of patients were treated with low-dose RUX for an extended time period with excellent tolerance and efficacy, without the use of steroids.

In contrast to the REACH-studies, our investigation included specific subgroups of GvHD, such as those induced by DLI and overlap cGvHD, which were therefore evaluated separately. Following DLI, there appears to be a strong correlation between GvHD and GvT effects, with GvHD being the most common and significant toxicity of DLI. (224) It has been demonstrated that overlap cGvHD is associated with a poorer prognosis, greater functional impairment and a higher symptom burden compared to classical cGvHD. (225)

It is important to note that the composition of the cohort differs significantly from that of the REACH-2 trial, as outlined in **Table 11.** 26.2 % (16/61 of the cohort) had previously failed multiple treatment lines, including steroids, prior to RUX initiation. Furthermore, a greater proportion of patients exhibited severe (grade 3-4) aGvHD (64% REACH-2 vs. 78.7%).

Notwithstanding the discrepancies, the response rate of 68.9% observed on day 28 in aGvHD (**Table 16**) was comparable to the 62% response rate on day 28 reported in the REACH-2 trial.

In patients with chronic GvHD, the observed best overall response rate in our cohort is inferior to the best overall response at any time observed in the REACH-3 study. Nevertheless, at 6 months, the overall response rate was 58.1% with 9.3% achieving CR in our cohort, while the ORR in REACH-3 was 49.7% with a 6.7% CR rate.

A comparison of the characteristics of patient and GvHD between a real-world context and the pivotal phase 3 studies demonstrates notable differences.

The majority of our patient cohort exhibited exclusion criteria for the respective REACH trial. On the one hand, this comprised heavily pretreated patients, while on the other, there was a more liberal and earlier use of RUX, with initiation before fulfilling SR-criteria. As mentioned above, DLI-induced and overlap chronic GvHD were also exclusion criteria.

A Chinese study from Dong Wang et al.in 2021 reported remarkable outcomes, with an ORR of 74.3% (52/70), including 34 patients in CR (48.5%). (226) The discrepancies to our cohort were readily apparent when the baseline characteristics of the SR-cGvHD were considered. In the Chinese study, 32,9% of patients exhibited mild cGvHD, 54,3% exhibited moderate cGvHD and only 12,8% exhibited severe cGvHD. (226) In comparison to our cohort, where only 7% exhibited mild cGvHD, 44,2 % had moderate cGvHD and 48,8% suffered from severe cGvHD.

Abedin et al. reported a response rate of 84% (16/19) at day 28 in aGvHD and 63% (15/24) on day 28 for cGvHD despite a median of 2 failed prior treatment lines. However, they also mentioned infectious events in 42% (18/43 patients). (227)

In a study of 46 patients with a mean of 3.7 prior therapies, Modi et al. evaluated the efficacy of RUX in the treatment of cGvHD. After six months of RUX, the observed response rates were 10% CR, 37% PR, and 15% SD. (228)

As the co-inhibition of JAK1/2 is associated with cytopenia, a common side effect of Ruxolitinib, there are specific JAK1 inhibitors, such as Itacitinib, under investigation with the aim to to reducing cytokine signaling without inducing cytopenia. However, single inhibition of JAK1 or JAK 2 has not been as effective as double blockade with Ruxolitinib. (3)

Limitations include the retrospective nature of this study, which introduces a risk of bias and incomplete data, and the less stringent assessment time points compared to prospective studies.

However, there are advantages to retrospective data analysis, including long follow-up and long-term survival results to support and complement data from pivotal phase 3 trials. The maximum treatment duration of RUX was 6,2 years.

It should be noted that the male/female ratio in our cohort may limit the generalisability of these results to a female cohort. The REACH-2 and REACH-3 cohorts also exhibited a preponderance of male patients.

An obvious disadvantage of Ruxolitinib is that it is only available as an oral tablet, which may not be fully absorbed in the presence of severe emesis or diarrhea, which are possible symptoms of acute GvHD.

Subtherapeutic serum concentrations of Ruxolitinib may contribute to the low response rates in patients with gastrointestinal involvement. (142)

Further evaluation of these data may include a more detailed subgroup analysis of organ-specific response rates, (lower GI aGvHD), response assessment in heavily pretreated patients and those on low steroid doses.

To prevent the occurrence of GvHD-related complications including infection, it is essential to achieve a rapid and durable response, with the aim of inducing tolerance.
Although RUX was a milestone in the treatment of GvHD, there are still patients who do not respond sufficiently to this treatment. Future studies should evaluate the treatment of GvHD beyond steroids and RUX, as well as treatment in addition to ongoing RUX, which is more straightforward in real-world evidence. In our cohort, by the addition of another agent to ongoing RUX in 41.5% of patients response could be further improved.

To control relapsed malignancy without exacerbating GvHD, promising approaches are under investigation, such as vaccine-based approaches or donor T cells genetically modified to express TCRs specific for leukaemia-associated antigens. (80)

Future aspects of GvHD treatment may include strategies that interrupt local inflammatory pathways by promoting tissue tolerance through regeneration and repair. (93)

Biomarkers to predict a patient’s response to a specific group of agents are currently under investigation.

The findings of this retrospective analysis of real-world evidence represent a significant complementation to the data derived from prospective phase 3 trials. This type of reporting allows for the identification of differences between subgroups, the effective combination of agents and long-term follow-up.

12. List of figures

13. List of tables

14. Literature Cited

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