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Analysis of sequential treatments for hematological diseases by advanced statistical methods

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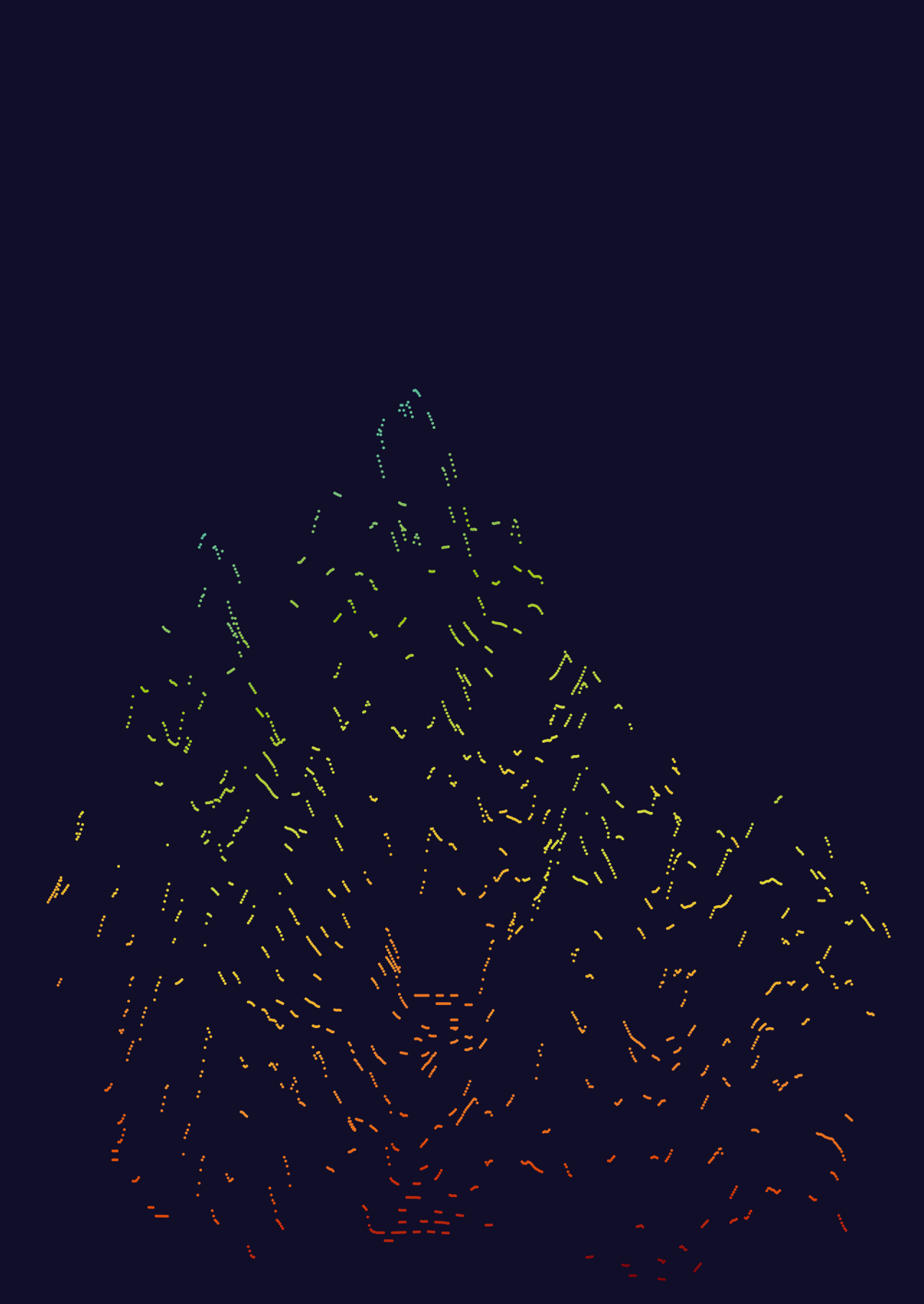

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General introduction

With observational studies researchers can gain valuable knowledge about diseases, treatments and associations between them from real-world data, but they have no control on the interventions or when which measurements are done. This calls for careful assessment of potential biases and optimal use of the available data. Investigating treatment outcomes becomes more challenging when the treatment consists of sequential interventions, more complex outcomes than for instance overall survival are considered, or when risk factors are analyzed that have different effects on different outcomes or are affected by events during follow-up. In these cases, more advanced statistical methods are often required. In this thesis, two complex clinical settings are investigated: allogeneic hematopoietic stem cell transplantation (alloSCT) for patients with acute leukemia and immunosuppressive therapy (IST) for patients with acquired aplastic anemia (AA). Both cases will first be introduced, followed by a brief overview of commonly used methodological approaches, promising advanced approaches and the aims of this thesis.

Allogeneic hematopoietic stem cell transplantation for patients with acute leukemia

The aim of alloSCT for patients with acute leukemia is to eradicate the disease by replacing patient hematopoiesis by donor-derived hematopoiesis and by introducing donor-derived alloreactive T cells that can eliminate the malignant hematopoietic cells of the patient. The latter process is called the Graft-versus-Leukemia (GvL) effect and may result in lifelong immunity against the malignancy.[1] However, if these alloreactive T cells target non-hematopoietic tissues of the patient, Graft-versus-Host-Disease (GvHD) may develop.[2] The success of alloSCT as treatment against acute leukemia depends on establishing sufficient GvL without inducing severe GvHD.

Both the GvL effect and GvHD result from an alloimmune response of donor-derived T cells recognizing a 'nonself' peptide/HLA complex on patient cells: the peptide, HLA molecule or both are not present in the donor due to genetic differences.[2] Vice versa, patient-derived T cells recognizing nonself peptide/HLA complexes on donor-derived hematopoietic cells can cause graft rejection. Since all nucleated cells present HLA and any peptide presented in nonself HLA may provoke an alloimmune response, patients with an HLA-mismatched donor have high risks of developing severe GvHD and graft rejection.[3] Therefore, HLA-matched donors are generally preferred. In the setting of fully HLA-matched alloSCT, alloimmune T-cell responses are directed against immunogenic nonself peptides presented in self HLA. These peptides are called minor histocompatibility antigens (MiHAs). Due to the high genetic diversity in peptides and T-cell receptors, the patient and donor may have hundreds of different MiHA-specific T cells, which are, in theory, capable of graft rejection, GvL and/or GvHD, depending on the origin of these T cells and the tissue expression of the MiHAs.[2] Patients with an HLA-matched unrelated donor (UD) have about twice as many disparities as patients with an HLA-matched sibling donor (called related donor, RD).[4] They have generally higher risks of GvHD and graft rejection, and a stronger GvL effect leading to a lower relapse risk.[5]

The alloSCT strategy consists of several steps: the conditioning of the patient, infusion of the graft, interventions to prevent severe GvHD and, in some strategies, interventions to improve the GvL effect. All steps influence the lymphohematopoietic status and

recovery of the patient and the risks of graft rejection, infections, GvHD, relapse and mortality.

Conditioning, graft infusion and lymphohematopoietic recovery

In the case of alloSCT for acute leukemia, the conditioning regimen has three aims: to make space for the hematopoietic stem cells (HSCs) of the donor, to reduce the tumor burden, and to prevent graft rejection. The first two aims are achieved by eliminating most HSCs of the patient, both healthy and malignant, and the third aim by suppressing the patient's immune system, including the MiHA-specific T cells. This is done by administering chemotherapy with or without irradiation and/or antibody therapy against immune cells during the days before graft infusion. The myelosuppressive potency of the conditioning regimen determines how many HSCs of the patient survive the conditioning regimen to compete with the donor HSCs and how many leukemia cells survive this stage.[6]

After the conditioning, the stem cell graft from the donor is infused. Unmanipulated grafts consist of HSCs and immune cells. The donor HSCs home to the patient's bone marrow (BM), where they compete with the surviving patient-derived HSCs to repopulate the BM.[7] This competitive repopulation often leads to a state of mixed chimerism (MC): both patient- and donor-derived HSCs are present. Both populations produce immune cells. The recovery of innate immune cells closely follows the BM repopulation, as they have a relatively high turnover. In contrast, *de novo* generation of T cells requires a functioning thymus, and it takes months before *de novo* T cells appear after alloSCT. The early T-cell recovery depends on homeostatic proliferation (i.e., expansion of mature memory/effector T cells in a lymphopenic environment) of both the patient T cells that survived the conditioning regimen and the donor T cells that were present in the graft, and the expansion of T cells in response to antigens they encounter.[8]

T-cell alloreactivity and the need for GvHD prophylaxis

From the moment the graft is infused, alloreactive T cells encounter nonself antigens: any surviving patient-derived alloreactive T cells may encounter nonself antigens on hematopoietic cells of the donor while infused donor alloreactive T cells may encounter nonself antigens on hematopoietic and non-hematopoietic cells of the patient. In HLA-matched alloSCT, alloreactive T cells are usually naïve T cells: they have never encountered the antigen before and need costimulatory signals to become appropriately activated.[2] These can be given by professional antigen presenting cells (APCs) such as dendritic cells. Around the time of alloSCT, these APCs become activated in several ways: the conditioning regimen causes tissue damage leading to release of danger signals, the destruction of epithelial cells allows translocation of microbial products over the intestinal barrier, and infections and viral reactivations occur often due to the low immunity of the patient.[9,10] Without intervention, the resulting activation of APCs would lead to massive activation of alloreactive T cells. Therefore, to prevent lethal GvHD (and graft rejection) patients usually receive systemic immunosuppression for several months after alloSCT. During this period, the tissue damage and the epithelial barrier are repaired and initial immunological recovery takes place, providing some protection against infections. Moreover, the patient-derived professional APCs are gradually replaced by donor-derived APCs, which are less likely to induce a strong alloimmune response by donor-derived T cells.[11]

T-cell depletion and posttransplant cyclophosphamide to reduce the risk of GvHD

Even if GvHD prophylaxis is used, the allo-immunological pressure after HLA-matched alloSCT is considerable: about a third of the patients develop GvHD and GvHD is the main cause of non-relapse mortality (NRM).[5] To reduce the risk of severe GvHD, T-cell depletion (TCD) can be applied. With *ex vivo* TCD, the graft is manipulated by selecting certain cell subsets (e.g., CD34+ selection by immunomagnetic procedures) or by removing certain cell subsets (e.g., CD52+ immune cells by alemtuzumab, depletion of (subsets of) T cells by immunomagnetic procedures).[12,13] With *in vivo* TCD, patients receive alemtuzumab and/or anti-thymocyte globulin (ATG) intravenously. While TCD can effectively reduce the risk of GvHD[14], some studies have shown an increase in the risks of relapse and infections.[15-17] These studies demonstrate the downside of TCD: also the alloreactive T cells responsible for the GvL effect and the non-alloreactive T cells responsible for the protection against viruses are affected.

Another method to reduce the GvHD risk is posttransplant cyclophosphamide (PTCY): patients receive an unmanipulated graft, followed by cyclophosphamide and start of GvHD prophylaxis a few days later, when the alloreactive T cells have been activated but before they start eliminating their target cells. Cyclophosphamide affects mostly activated T cells, leading to preferential recovery of regulatory T cells and non-alloreactive T cells.[18,19] While this leads to a better protection against infections compared to TCD[20], the GvL effect is still suppressed and the risk of relapse remains higher compared to non-TCD alloSCT[21].

Donor lymphocyte infusions to boost the GvL effect

To boost the GvL effect after alloSCT, additional alloreactive donor T cells may be given to the patient. This can be done by the administration of unmodified donor lymphocyte infusions (DLI), which contain alloreactive and non-alloreactive T cells and other immune cells. The higher the T-cell dose the more effective and potentially toxic the DLI, i.e., the stronger the GvL effect and the higher the risk of severe GvHD. Therefore, the dose depends partly on the indication of the DLI.[22] Firstly, DLI can be given therapeutically to patients with a relapse after alloSCT, often in combination with chemotherapy to reduce the tumor burden. Establishment of a strong alloimmune response is vital for these patients. Therefore, the DLI dose is relatively high, and an increased risk of inducing GvHD is accepted. While some patients with overt relapse can be rescued with this treatment, the majority dies of relapse (insufficient GvL effect) or GvHD (too strong alloimmune response).[23-25] Secondly, DLI can be administered preemptively to patients with MC or minimal residual disease (MRD), which may be signs of an impending relapse. In this case, there is more time for awaiting the effect of DLI, and the starting dose is lower. Based on the persistence of MC and/or MRD, subsequent DLIs are given over time with increasing dose.[22] The goal of this gradual dose escalation is to achieve a sufficient GvL effect with the lowest dose possible, thereby minimizing the risk of inducing severe GvHD. Lastly, DLI can be administered prophylactically to all patients without GvHD, i.e., to all patients without a sign of alloreactivity. As relapses may occur without any foreboding signs, one may choose to administer prophylactic DLI to boost the GvL effect even in the absence of MC and/or MRD to minimize the risk of relapse. The dose is usually comparable to that of

preemptive DLI, as these patients do not have a relapse yet and the risk of inducing severe GvHD should be minimized.

The alloreactive potential of DLI also depends on the genetic disparity and the presence of pro-inflammatory conditions that gradually diminish after alloSCT. Therefore, the DLI dose is also determined by donor type and timing after alloSCT: patients with an UD often receive a lower dose than patients with a RD, and earlier DLI are given at a lower dose than later DLI.[22] However, despite adjusting the dose to donor type and timing, alloreactivity by DLI is highly variable: some patients succumb to severe GvHD, while others do not show any sign of GvHD and GvL and may relapse.

Combining interventions to optimize the balance between GvHD and GvL

Some alloSCT strategies combine TCD or PTCY with prophylactic DLI to improve the balance between GvHD and GvL. The idea is to perform the alloSCT in two steps: to first introduce donor-derived hematopoiesis with minimal risk of severe GvHD, and then introduce donor-derived immunity to establish a sufficient GvL effect. Because the second step occurs after the initial recovery has taken place, the alloreactive T cells arrive in a less pro-inflammatory environment, leading to a lower risk of GvHD compared to if they had been infused directly after the conditioning. The strategy relies on the antitumor effect of the conditioning itself to control the leukemia until the DLI can be given. After the prophylactic DLI, preemptive DLI can be given if the patient still has MC or MRD.

The complex dynamics of lymphohematopoietic recovery and clinical events after alloSCT and DLI

Disentangling the effects of the different factors, mechanisms and interventions on the recovery after alloSCT is challenging. Patient factors, donor factors, conditioning intensity, the use and type of GvHD prophylaxis, TCD and/or PTCY all influence the competitive repopulation, homeostatic proliferation and/or allo-immunological pressure after alloSCT, determining the sizes of the emerging patient- and donor-derived lymphohematopoietic cell populations. The patient- and donor-derived populations can coexist or one population can eliminate the other via an alloimmune response, leading to graft rejection or a GvL effect. The latter may be accompanied by GvHD. While immunity is low, patients have a high risk of infections, which lead to a proinflammatory environment stimulating alloimmune responses. Posttransplant interventions such as DLI further complicate the dynamics. The potency of each DLI depends on many factors, i.e., patient- and donor-related factors, the DLI product and the conditions at the time of DLI. The DLI itself may affect the lymphohematopoietic recovery, cause GvHD and temporarily increase the mortality risk. The GvL effect of the DLI is often hard to quantify: therapeutic DLI are usually combined with other treatments, conversion from MC to FDC may also have occurred without preemptive DLI and for patients with FDC and no MRD receiving prophylactic DLI, it's impossible to say what would have happened if no DLI had been administered. Capturing these dynamics and estimating the effects of risk factors is complex. Some of the commonly used methodological approaches and more advanced approaches will be explained in the methodological section of the introduction.

Immunosuppressive treatment for patients with acquired aplastic anemia

AA is a hematological disease characterized by a hypocellular BM and hematopoietic failure leading to pancytopenia. Without treatment, patients may succumb to anemia, bleeding or infections. In the majority of the cases, acquired AA seems to be caused by an autoimmune reaction against hematopoietic cells.[26,27] There are two main treatment options: replacing the patient hematopoiesis and immunity by alloSCT or suppressing the autoimmune reaction by IST. AlloSCT leads to rapid and enduring hematopoietic recovery at the risk of transplant-related morbidity and mortality, mostly because of GvHD.[28] Therefore, currently alloSCT is only recommended for patients of 40 years or younger who have a suitable HLA-matched RD. The majority of adult patients with AA are treated with an IST regimen based on ATG and ciclosporin.[29] This treatment has moderate side effects compared to alloSCT but is less effective: only two-third of the patients respond, often only partially: these patients become transfusion-independent but their blood counts remain low.[30] Improvement of hematopoiesis after IST can take six months or even longer, as the autoimmune response first needs to be sufficiently suppressed after which the few surviving HSCs need time to repopulate the BM. During this period, patients remain at risk for bleeding and life-threatening infections due to their pancytopenia. After achievement of a response, the IST is tapered with the aim to stop. However, 30% of the responders develop relapse of the disease, requiring to restart or increase the dose of the IST, or even proceed to alloSCT.[31] Additionally, patients with AA receiving IST often have clonal evolution of hematopoietic cells, which may eventually lead to other BM diseases, most importantly acute myeloid leukemia (AML) and paroxysmal nocturnal hemoglobinuria (PNH).[31]

As older patients may have a higher risk of treatment-related toxicity and mortality and a lower likelihood of achieving a response, there is still debate whether ATG-based IST should be the first-line treatment of choice for patients aged 60 or older, instead of a less intensive treatment.[29,32-34] The arguments in this discussion are usually based on short-term response descriptions, overall survival and cumulative incidences of different types of failures. However, these estimates do not give a good overview of the likelihood of treatment success over time for several reasons. IST patients often need to be treated for months before a response becomes visible, while those who respond remain at high risk for several failure types: recurrence of the disease, development of another BM disease and death due to the complications of cytopenia or due to treatment toxicity. Some failures can be reversed by change of treatment (e.g., recurrence of disease can be treated by increasing the IST dose, restarting the IST, starting other IST or alloSCT). Patients can also experience different types of failure over time. For instance, a patient may first show a response, then relapse, develop AML and die. Moreover, some failure types are less severe than others: recurrence of the disease is less severe than development of AML. A single 'treatment success' measure capturing these highly dynamic outcome possibilities would be valuable for the evaluation of the treatment toxicity and efficacy over time. However, the estimation of such an endpoint is a challenge, as will be explained in the methodological section.

Methodological challenges and common methodological approaches

Measuring treatment outcomes

To assess treatment success after alloSCT for acute leukemia GvHD-relapse-free survival (GRFS) is often used: the probability of surviving without experiencing any clinically relevant GvHD or relapse.[35] A limitation of composite endpoints like GRFS is that they do not give any information on the reason of failure, in this case relapse (not enough GvL), GvHD (too strong alloimmune response) or death without relapse and GvHD, often related to treatment toxicity. Not all failures are equally severe and risk factors often have different effects on different components of the composite endpoint (for instance, having an unrelated donor decreases the risk of relapse but increases the risk of GvHD). Another limitation of composite endpoints is that subsequent events are ignored. For instance, GRFS does not consider that GvHD can resolve, and that patients who developed GvHD also likely established a GvL effect protecting them from relapse. Their prognosis may even be better than that of patients who never developed GvHD. Therefore, considering GvHD as definitive treatment failure seems too strict. To overcome these limitations, GRFS is often reported together with relapse-free survival, overall survival and cumulative incidences of GvHD, relapse and non-relapse mortality separately. The reader has to combine the results of all these analyses to obtain a full picture of the clinical recovery.

Analyzing the outcome of IST for aplastic anemia faces similar problems. In this setting, assessment of treatment success is usually based on overall survival and the recovery of the blood cell counts: (partial) recovery indicates (partial) disease response. As mentioned before, the timing of the disease response is variable, with most responses occurring within 3 months but some also beyond 6 months[30], and the response can be lost. The probability of reaching a response over time can be shown by cumulative incidence curves, but these give no information on what happened after achievement of a response. This is a major limitation in a setting where even after a response, failure often occurs. Therefore, usually the current response at certain times (most often 6 months) is reported. Aside from only giving information at one time point, these descriptive analyses give no information on temporary responses before this time point. Peffault de Latour et al. provided information on loss of response between 3 and 6 months in the table legends[30], but most often the temporary responses are not described at all. Prabahran et al.[33] reported outcomes of subsequent events by showing overall survival curves and risks of relapse and clonal evolution from different stages: from start of IST, from the 6-month response, after relapse and after second-line alloSCT. While this approach allows to zoom in on certain phases of the treatment, it requires a multitude of analyses (for each phase and outcome measure) and figures with different timescales. For each analysis, the reader needs to consider who is at risk (e.g., only those who have a response at 6 months) and the time between the start of the study and the start of the analysis. Combining all information to obtain a full picture of the recovery is challenging and becomes even impossible if some analyses start at the time of an intermediate event instead of a fixed time since start of the main analysis.

Investigating the effects of events and biomarkers during follow-up

Risk factors can be categorized into baseline risk factors, known at or before the start of the treatment, and time-dependent risk factors which can change after treatment, such

as infection. Especially the effect of the latter can be complex to estimate correctly. The most important rule is to only use information known at the present or past to predict the future. Otherwise, immortal time bias will occur. Immortal time refers to a period in which the event of interest (e.g., death) cannot occur due to the design of the analysis. Bias occurs for example when responders are defined by having a response during follow-up and are compared with non-responders from the start of treatment. The patients in the first group cannot die until they have achieved a response (otherwise, they would not have been selected for this group), while those in the latter group can. Thus, considering the response during follow-up as known at baseline favors the first group in this case. Even though this problem was already recognized in 1983[36], this is still a commonly made mistake. For instance, Zhou et al. found chronic GvHD to be the strongest predictor in their prognostic model for longer survival in patients with chronic myelomonocytic leukemia receiving alloSCT, but did not take into account that patients needed to survive for at least a few months before they could develop chronic GvHD.[37] The non-GvHD group could die from day 1, leading to an overestimation of the effect of chronic GvHD on survival. In the setting of IST for AA, two studies aimed to show the impact of experiencing relapse, PNH and AML on survival, but did not consider this bias. In their figures, at the beginning of follow-up the 'no event' group has temporarily lower survival compared to the groups with an adverse event.[38,39]

There are several relatively commonly used approaches to prevent this bias. In intention-to-treat analyses, groups are defined at baseline based on the treatment they are *intended* to receive instead of who actually received it. However, this method can only be used in settings where treatment allocation is known at time of start, it cannot be used for clinical developments like GvHD, and it usually attenuates outcome differences between groups. This attenuation occurs because often the treatment group contains some patients who actually did not receive the treatment and vice versa: the groups become more similar and the differences in outcome often smaller than if all patients could have been allocated to the correct group. Landmark analyses at certain time points after start only include the patients who are still at risk for the event of interest and split the group based on events that occurred until the landmark time. Immortal time bias is prevented while at least some of the information during follow-up can be used to define the groups, but information of patients who already had the event of interest before the landmark time is lost. Moreover, there is often no clear optimal landmark time: earlier landmark times include more patients but the groups may still contain a considerable number of patients that have the group-defining event after the landmark time, while later landmark times throw away more information. Often, multiple landmark times are chosen, but this may require correction for multiple testing. A method that can include all patients and event data is the time-dependent Cox proportional hazards model. In this model, covariates can change their value over time, assuming that the values of the covariates are constant until the next observation. For clinical events this is often acceptable – for treatments the exact starting time is known and the time of events like relapse are defined as the day of observing the relapse – but this may be a problem when analyzing biomarkers such as MRD markers and lymphocyte counts. Their values can change significantly in between two measurements, which may relate to the development of events such as relapse and death. Other limitations of the time-dependent Cox model are that it does not consider measurement error and that no absolute risks can be calculated since the probability of the intermediate event is not modelled explicitly.

Promising advanced methodological approaches

Multi-state model to capture complex sequences of events

The main limitation of GRFS as an endpoint can be overcome by including recovery after GvHD and calculating current GvHD-relapse-free survival (cGRFS): the probability of being alive without relapse and currently not having GvHD.[40,41] This can be done by multi-state models, which capture sequences of events, allowing to keep track of the clinical trajectories of patients in detail. In a multi-state model patients move between states at the occurrence of clinical events or treatments. Transitions define which routes between states are allowed. Figure 1 shows the structure of a multi-state model incorporating GvHD, relapse and death. The most common multi-state model is the time-inhomogeneous Markov model, which assumes that the hazard of making a certain transition only depends on the current state and the time since the start of the analysis.

Another advantage of the multi-state model is that the effects of risk factors can be modelled on each of the transitions separately, usually by means of transition-specific Cox proportional hazards models. For example, in the model of Figure 1, donor type and conditioning intensity are likely relevant for all transitions, while disease risk only needs to be modelled for the transitions to Relapse. Each transition hazard zooms in on a specific part of the process and all this information needs to be combined to get a full picture of the recovery. The model does this by using all transition hazards to calculate the probability of being in a certain state or set of states. This can be done non-parametrically (without taking any risk factors into account) or semi-parametrically by considering transition-specific Cox models for one or more transitions. The latter allows to show the clinical impact of the risk factors on different outcome measures, such as cGRFS (probability of being in 'Alive without relapse/GvHD') and relapse-free survival (probability of being in 'Alive without relapse/GvHD' or GvHD) in Figure 1.

In conclusion, the multi-state framework can overcome all described limitations of the composite endpoint: it keeps track of which failures and recoveries occur, captures sequences of events, enables to investigate the effects of risk factors on different

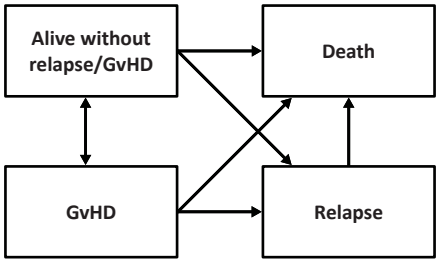

Figure 1. Example of a multi-state model. The boxes represent states, the arrows transitions. All patients start in the starting state 'Alive without relapse/GvHD'. From here, they move through the model at the occurrence of events. The other states can be absorbing (impossible to leave, e.g. Death) or intermediate (possible to leave, e.g. GvHD and Relapse). From Relapse, patients can only move to Death: achievement of remission after relapse is not considered. In contrast, patients in the state GvHD can return to the state 'Alive without relapse/GvHD' if their symptoms disappear. If they relapse during GvHD, they move to the state Relapse: this model considers relapse a more important failure than GvHD.

components of the recovery process and can translate them to the total impact on clinically relevant outcome measures. Despite appearing to be the ideal framework for analyzing complex recovery patterns of patients with hematological diseases, a recent systematic review by Bonneville et al. on studies reporting multivariable Cox proportional hazards models in the setting of malignant hematological diseases showed that only 2 of the 299 included papers involved a multi-state model.[42] This is likely due to the requirement of high-quality clinical data and sufficient clinical, biological and statistical knowledge to translate a clinical research question into a multi-state model. Multi-state modelling demands careful choices in which clinical events are relevant, which transitions are allowed and which risk factors should be modelled in which way for which transitions.

Joint model to investigate effects of biomarkers

The main limitations of the time-dependent Cox model for analyzing biomarkers such as MRD markers and lymphocyte counts are the assumptions that the measurement values are constant between visits, that there is no measurement error, and that the availability of the measurements is not related to the failure status.[43] The latter indicates that the biomarker needs to be exogeneous, which is by definition untrue. The joint model does not depend on these assumptions.[44] It captures biomarkers and clinical events simultaneously by linking two submodels, one for the longitudinal measurements and one for the risks of the clinical events, via an association structure (Figure 2). This allows to model the measurement trajectories over time (which are not yielded by the time-dependent Cox model) while appropriately accounting for both the heterogeneity in subject-specific trajectories and measurement error, and enables the estimation of an association between the longitudinal measurements and the risks of clinical events.

While joint models seem to be the method of choice for analyzing the impact of biomarkers on survival outcomes, they have been applied rarely in the field of hematology.[45,46] As for multi-state models, their disuse is likely due to the required clinical, biological and statistical knowledge to correctly specify the model.

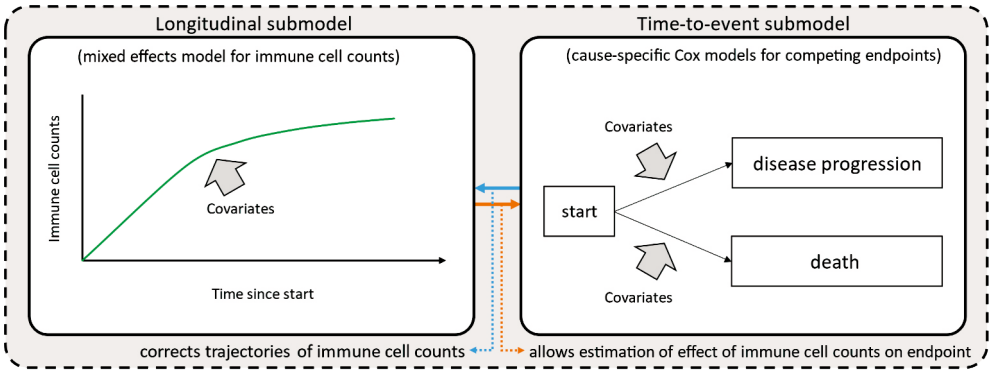

Figure 2. Example of a joint model. The model consists of two components: a longitudinal submodel for the immune cell counts and a time-to-event submodel for the clinical events, which are linked together via an association structure.

Aim of the thesis

The aim of this thesis is to investigate how careful selection of a specific setting and application of advanced statistical methodology such as multi-state and joint models can be used to investigate complex mechanisms or research questions in observational studies in the field of hematology. **Chapters 2-5** investigate the lymphohematopoietic and clinical recovery after alloSCT for acute leukemia and **Chapter 6** investigates the multi-step treatment of and recovery after IST for AA.

In **Chapter 2**, we aim to investigate how selection of a specific alloSCT strategy, TCD alloSCT followed by prophylactic or preemptive DLI, can be used to disentangle the effects of competitive repopulation and allo-immunological pressure on the patient- and donor-derived lymphohematopoietic recovery. The reduction of allo-immunological pressure early after alloSCT by the TCD will provide us an opportunity to investigate the impact of the conditioning intensity on the competitive repopulation in the absence of strong allo-immunological pressure. By selecting a cohort with different DLI strategies, prophylactic versus preemptive DLI and with different starting times of first DLI based on the anticipated relapse risk, we will be able to study the impact of introducing allo-immunological pressure after the competitive repopulation has taken place.

In **Chapter 3**, we will dive deeper into the immune cell kinetics after alloSCT and aim to investigate the complex associations between immune cell kinetics and alloreactivity by using joint modeling. Also in this case, we will use a setting of TCD alloSCT followed by DLI to study the impact of DLI on the immune cell kinetics. The joint model framework will also enable us to estimate the impact of the number of circulating immune cells on the risks of GvHD and relapse. However, we will need to take into account that the actual administration of DLI not only depends on the treatment plan, but also on the clinical circumstances, which may influence the immune cell counts. To take this properly into account, we will perform an intention-to-treat analysis.

In **Chapter 4**, we will focus on the clinical outcomes after DLI and aim to identify factors that influence the alloreactivity of DLI, taking into account the dynamic nature of GvHD, which can lead to death, resolve, and decrease the risk of relapse, by using a multi-state model. We will investigate the effects of conditioning intensity, donor type, presence of patient-derived APCs in the BM, lymphopenia and viral infections in relation to the timing and dose of the DLI. Using the multi-state framework the clinical relevance of any found associations will be demonstrated by assessing the impact of these risk factors on different outcomes after DLI, such as cGRFS.

In **Chapter 5**, we aim to investigate how the transplantation strategy affects the alloreactivity of DLI by considering a different clinical setting, PTCY alloSCT followed by DLI, than in the previous chapters. By keeping the patient selection and interventions after alloSCT similar, the impact of the transplantation strategies on the conditions at the time of DLI and the alloreactivity of DLI can be investigated. We will assess chimerism conversion after DLI and compare this with the results of **Chapter 2**, and compare the DLI conditions and risk of DLI-induced GvHD with the results of **Chapter 4**.

In **Chapter 6**, we move to the AA setting and aim to investigate whether and how the multi-state framework can be used to develop a dynamic measure of "treatment success" that can better capture the complex clinical recovery and failure patterns of patients with

AA receiving IST compared to conventional analysis approaches. We will use the model to evaluate treatment outcome in different age groups. The multi-state framework will also allow us to investigate the effects of risk factors such as age and the presence of a GPI-deficient cell clone on different phases of the recovery and assess their impact on overall treatment outcomes.

In **Chapter 7**, the results of this thesis will be summarized and discussed in the light of the current literature and other methodological approaches.

REFERENCES

1. Horowitz, M., Gale, R., Sondel, P. et al. Graft-versus-leukemia reactions after bone marrow transplantation. Blood 75, 555-562 (1990). doi: 10.1182/blood.V75.3.555.555
2. Falkenburg, J. H. F. & Jedema, I. Graft versus tumor effects and why people relapse. Hematology. American Society of Hematology. Education Program 2017, 693-698 (2017). doi: 10.1182/asheducation-2017.1.693
3. Beatty, P. G., Clift, R. A., Mickelson, E. M. et al. Marrow transplantation from related donors other than HLA-identical siblings. N Engl J Med 313, 765-771 (1985). doi: 10.1056/nejm198509263131301
4. Martin, P. J., Levine, D. M., Storer, B. E. et al. Genome-wide minor histocompatibility matching as related to the risk of graft-versus-host disease. Blood 129, 791-798 (2017). doi: 10.1182/blood-2016-09-737700
5. Shouval, R., Fein, J. A., Labopin, M. et al. Outcomes of allogeneic haematopoietic stem cell transplantation from HLA-matched and alternative donors: a European Society for Blood and Marrow Transplantation registry retrospective analysis. Lancet Haematol 6, e573-e584 (2019). doi: 10.1016/s2352-3026(19)30158-9
6. Storb, R. & Sandmaier, B. M. Nonmyeloablative allogeneic hematopoietic cell transplantation. Haematologica 101, 521-530 (2016). doi: 10.3324/haematol.2015.132860
7. Quesenberry, P. J., Colvin, G. & Abedi, M. Perspective: fundamental and clinical concepts on stem cell homing and engraftment: a journey to niches and beyond. Exp Hematol 33, 9-19 (2005). doi: 10.1016/j.exphem.2004.10.012
8. Elfeky, R., Lazareva, A., Qasim, W. & Veys, P. Immune reconstitution following hematopoietic stem cell transplantation using different stem cell sources. Expert review of clinical immunology 15, 735-751 (2019). doi: 10.1080/1744666x.2019.1612746
9. Ghimire, S., Weber, D., Mavin, E. et al. Pathophysiology of GvHD and Other HSCT-Related Major Complications. Front Immunol 8, 79 (2017). doi: 10.3389/fimmu.2017.00079
10. Ferrara, J. L., Levine, J. E., Reddy, P. & Holler, E. Graft-versus-host disease. Lancet 373, 1550-1561 (2009). doi: 10.1016/s0140-6736(09)60237-3
11. Chakraverty, R. & Sykes, M. The role of antigen-presenting cells in triggering graft-versus-host disease and graft-versus-leukemia. Blood 110, 9-17 (2007). doi: 10.1182/blood-2006-12-022038
12. Saad, A. & Lamb, L. S. Ex vivo T-cell depletion in allogeneic hematopoietic stem cell transplant: past, present and future. Bone Marrow Transplant 52, 1241-1248 (2017). doi: 10.1038/bmt.2017.22
13. Barge, R. M., Starrenburg, C. W., Falkenburg, J. H. et al. Long-term follow-up of myeloablative allogeneic stem cell transplantation using Campath "in the bag" as T-cell depletion: the Leiden experience. Bone Marrow Transplant 37, 1129-1134 (2006). doi: 10.1038/sj.bmt.1705385
14. Busca, A. & Aversa, F. In-vivo or ex-vivo T cell depletion or both to prevent graft-versus-host disease after hematopoietic stem cell transplantation. Expert Opin Biol Ther 17, 1401-1415 (2017). doi: 10.1080/14712598.2017.1369949
15. Soiffer, R. J., Lerademacher, J., Ho, V. et al. Impact of immune modulation with anti-T-cell antibodies on the outcome of reduced-intensity allogeneic hematopoietic stem cell transplantation for hematologic malignancies. Blood 117, 6963-6970 (2011). doi: 10.1182/blood-2011-01-332007
16. Bacigalupo, A. Antilymphocyte/thymocyte globulin for graft versus host disease prophylaxis:

efficacy and side effects. Bone Marrow Transplant 35, 225-231 (2005). doi: 10.1038/sj.bmt.1704758
17. Soiffer, R. J., Kim, H. T., McGuirk, J. et al. Prospective, Randomized, Double-Blind, Phase III Clinical Trial of Anti-T-Lymphocyte Globulin to Assess Impact on Chronic Graft-Versus-Host Disease-Free Survival in Patients Undergoing HLA-Matched Unrelated Myeloablative Hematopoietic Cell Transplantation. J Clin Oncol 35, 4003-4011 (2017). doi: 10.1200/jco.2017.75.8177
18. Nunes, N. S. & Kanakry, C. G. Mechanisms of Graft-versus-Host Disease Prevention by Post-transplantation Cyclophosphamide: An Evolving Understanding. Front Immunol 10, 2668 (2019). doi: 10.3389/fimmu.2019.02668
19. Zhao, C., Bartock, M., Jia, B. et al. Post-transplant cyclophosphamide alters immune signatures and leads to impaired T cell reconstitution in allogeneic hematopoietic stem cell transplant. Journal of Hematology & Oncology 15, 64 (2022). doi: 10.1186/s13045-022-01287-3
20. Montoro, J., Roldán, E., Piñana, J. L. et al. Ex vivo T-cell depletion vs post-transplant cyclophosphamide, sirolimus, and mycophenolate mofetil as graft-vs-host disease prophylaxis for allogeneic hematopoietic stem cell transplantation. European journal of haematology 106, 114-125 (2021). doi: 10.1111/ejh.13529
21. Nagler, A., Labopin, M., Dholaria, B. et al. Graft-versus-Host Disease Prophylaxis with Post-Transplantation Cyclophosphamide versus Cyclosporine A and Methotrexate in Matched Sibling Donor Transplantation. Transplant Cell Ther 28, 86.e81-86.e88 (2022). doi: 10.1016/j.jtct.2021.11.013
22. Falkenburg, J. H. F., Schmid, C., Kolb, H. J. & Kuball, J. in The EBMT Handbook: Hematopoietic Cell Transplantation and Cellular Therapies (eds Anna Sureda et al.) 531-539 (Springer International Publishing, 2024).
23. Schmid, C., Labopin, M., Nagler, A. et al. Donor lymphocyte infusion in the treatment of first hematological relapse after allogeneic stem-cell transplantation in adults with acute myeloid leukemia: a retrospective risk factors analysis and comparison with other strategies by the EBMT Acute Leukemia Working Party. J Clin Oncol 25, 4938-4945 (2007). doi: 10.1200/jco.2007.11.6053
24. Schmid, C., Labopin, M., Nagler, A. et al. Treatment, risk factors, and outcome of adults with relapsed AML after reduced intensity conditioning for allogeneic stem cell transplantation. Blood 119, 1599-1606 (2012). doi: 10.1182/blood-2011-08-375840
25. Eefting, M., von dem Borne, P. A., de Wreede, L. C. et al. Intentional donor lymphocyte-induced limited acute graft-versus-host disease is essential for long-term survival of relapsed acute myeloid leukemia after allogeneic stem cell transplantation. Haematologica 99, 751-758 (2014). doi: 10.3324/haematol.2013.089565
26. Young, N. S., Calado, R. T. & Scheinberg, P. Current concepts in the pathophysiology and treatment of aplastic anemia. Blood 108, 2509-2519 (2006). doi: 10.1182/blood-2006-03-010777
27. Pool, E. S., Luk, S. J., Ijsselsteijn, M. E. et al. Imaging mass cytometry reveals the order of events in the pathogenesis of immune-mediated aplastic anemia. Blood 146, 951-963 (2025). doi: 10.1182/blood.2025028723
28. Zhang, Y., Huo, J., Liu, L. et al. Comparison of Hematopoietic Stem Cell Transplantation Outcomes Using Matched Sibling Donors, Haploidentical Donors, and Immunosuppressive Therapy for Patients With Acquired Aplastic Anemia. Front Immunol 13, 837335 (2022). doi: 10.3389/fimmu.2022.837335
29. Kulasekararaj, A., Cavenagh, J., Dokal, I. et al. Guidelines for the diagnosis and management of adult aplastic anaemia: A British Society for Haematology Guideline. Br J Haematol 204, 784-804 (2024). doi: 10.1111/bjh.19236
30. Peffault de Latour, R., Kulasekararaj, A., Iacobelli, S. et al. Eltrombopag Added to Immunosuppression in Severe Aplastic Anemia. N Engl J Med 386, 11-23 (2022). doi: 10.1056/NEJMoa2109965
31. Young, N. S. Aplastic Anemia. N Engl J Med 379, 1643-1656 (2018). doi: 10.1056/NEJMra1413485
32. Tjon, J. M., de Groot, M. R., Sypkens Smit, S. M. A. et al. Short-term efficacy and safety of antithymocyte globulin treatment in elderly patients with acquired aplastic anaemia. Br J Haematol 180, 459-462 (2018). doi: 10.1111/bjh.14372

33. Prabahran, A., Durrani, J., Coelho-Da Silva, J. et al. Safety and efficacy of immunosuppressive therapy for elderly patients with severe aplastic anaemia. Br J Haematol 205, 1170-1179 (2024). doi: 10.1111/bjh.19648
34. Fattizzo, B., Gurnari, C., Giammarco, S. et al. Elderly Patients With Aplastic Anemia: Treatment Patterns and Outcomes in the Real World. Am J Hematol 100, 584-591 (2025). doi: 10.1002/ajh.27611
35. Holtan, S. G., DeFor, T. E., Lazaryan, A. et al. Composite end point of graft-versus-host disease-free, relapse-free survival after allogeneic hematopoietic cell transplantation. Blood 125, 1333-1338 (2015). doi: 10.1182/blood-2014-10-609032
36. Anderson, J. R., Cain, K. C. & Gelber, R. D. Analysis of survival by tumor response. Journal of Clinical Oncology 1, 710-719 (1983). doi: 10.1200/jco.1983.1.11.710
37. Zhou, J. Y., Wang, S., Yuan, H. L. et al. Impact of a novel prognostic model on allogeneic hematopoietic stem cell transplantation outcomes in patients with CMML. Am J Hematol 98, 1394-1406 (2023). doi: 10.1002/ajh.26999
38. Patel, B. A., Groarke, E. M., Lotter, J. et al. Long-term outcomes in patients with severe aplastic anemia treated with immunosuppression and eltrombopag: a phase 2 study. Blood 139, 34-43 (2022). doi: 10.1182/blood.2021012130
39. Chattopadhyay, S., Lionel, S., Selvarajan, S. et al. Relapse and transformation to myelodysplastic syndrome and acute myeloid leukemia following immunosuppressive therapy for aplastic anemia is more common as compared to allogeneic stem cell transplantation with a negative impact on survival. Annals of Hematology 103, 749-758 (2024). doi: 10.1007/s00277-024-05621-2
40. Solomon, S. R., Sizemore, C., Zhang, X. et al. Current Graft-versus-Host Disease-Free, Relapse-Free Survival: A Dynamic Endpoint to Better Define Efficacy after Allogenic Transplant. Biol Blood Marrow Transplant 23, 1208-1214 (2017). doi: 10.1016/j.bbmt.2017.02.022
41. Eefting, M., de Wreede, L. C., Halkes, C. J. et al. Multi-state analysis illustrates treatment success after stem cell transplantation for acute myeloid leukemia followed by donor lymphocyte infusion. Haematologica 101, 506-514 (2016). doi: 10.3324/haematol.2015.136846
42. Bonneville, E. F., Schetelig, J., Putter, H. & de Wreede, L. C. Handling missing covariate data in clinical studies in haematology. Best Practice & Research Clinical Haematology 36, 101477 (2023). doi: 10.1016/j.beha.2023.101477
43. Baart, S. J., van der Palen, R. L. F., Putter, H. et al. Joint Modeling of Longitudinal Markers and Time-to-Event Outcomes: An Application and Tutorial in Patients After Surgical Repair of Transposition of the Great Arteries. Circ Cardiovasc Qual Outcomes 14, e007593 (2021). doi: 10.1161/circoutcomes.120.007593
44. Rizopoulos, D. (Boca Raton: Chapman and Hall/CRC, 2012).
45. Huang, A., Chen, Q., Fei, Y. et al. Dynamic prediction of relapse in patients with acute leukemias after allogeneic transplantation: Joint model for minimal residual disease. International Journal of Laboratory Hematology 43, 84-92 (2021). doi: 10.1111/ijlh.13328
46. Tang, X., Alatrash, G., Ning, J. et al. Increasing chimerism after allogeneic stem cell transplantation is associated with longer survival time. Biol Blood Marrow Transplant 20, 1139-1144 (2014). doi: 10.1016/j.bbmt.2014.04.003

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