SUPPLEMENTARY APPENDIX

Multi-state analysis illustrates treatment success after stem cell transplantation for acute myeloid leukemia followed by donor lymphocyte infusion

Matthias Eefting,[1*] Liesbeth C. de Wreede,[2*] Constantijn J.M. Halkes,[1] Peter A. von dem Borne,[1] Sabina Kersting,[1] Erik W.A. Marijt,[1] Hendrik Veelken,[1] Hein Putter,[2] Johannes Schetelig,[3] and J.H. Frederik Falkenburg[1]

[1]Department of Hematology, Leiden University Medical Center, the Netherlands; [2]Department of Medical Statistics and Bioinformatics, Leiden University Medical Center, the Netherlands; and [3]Medical Department I, University Hospital Carl Gustav Carus, Technische Universität Dresden & DKMS, German Bone Marrow Donor Center, Germany

**ME and LCdW contributed equally*

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Correspondence: l.c.de_wreede@lumc.nl

Appendix

Transplantation protocol, engraftment, and assessment of GvHD

Patients were transplanted with a myeloablative conditioning regimen, consisting of cyclophosphamide 60 mg/kg i.v. for 2 days, and TBI 9 Gy (n=77) or busulphan (Busilvex©) 3.2 mg/kg i.v. for 4 days (n=2). In addition to this standard conditioning, all patients transplanted with a donor other than a fully matched sibling donor received pre-transplant alemtuzumab 15 mg i.v. for 2 days, and cyclosporine 3 mg/kg as GvHD-prophylaxis from day -1 until day 60 in the absence of GvHD. In all patients *in vitro* T-cell depletion (TCD) of the stem cell product was performed by incubation of the graft with alemtuzumab (20 mg) for 30 min at room temperature under continuous agitation, also known as 'Campath in the bag' (n=73) or by $CD34^+$-cell MACS-sorting (n=6).[1] The day of granulocyte engraftment was defined as the first of 3 consecutive days of absolute granulocyte counts > 0.5 x 10^9/L. Assessment and grading of GvHD was performed using modified Glucksberg and Shulman criteria.[2, 3]

Prophylactic DLI

Until June 2007 only patients with mixed-chimerism but without early relapse of AML or MDS were eligible for prophylactic DLI at 6 months after SCT. Mixed-chimerism was defined as ≥1% patient hematopoiesis in any cell fraction (leukocytes, mononuclear cells and/or granulocytes) measured by PCR or FISH. If severe GvHD (overall grade II or higher) was present, DLI infusion was postponed. DLI-dosing depended on the time-point after SCT and on donor type. Median first DLI-dose of patients transplanted with a sibling donor was 3.0x10^6 $CD3^+$-cells/kg (range 1.0-3.0x10^6 CD3+cells/kg) and 1.5x10^6 $CD3^+$-cells/kg (range 1.5-2.4x10^6 CD3+cells/kg) for patients transplanted with an unrelated donor, respectively. From June 2007 onwards, all patients (n=40) except intermediate risk AML (n=8) were eligible for low dose prophylactic DLI at 3 months after SCT. The starting dose of DLI consisted of a predefined dose of 0.3x10^6 $CD3^+$-cells/kg or 0.15x10^6 $CD3^+$-cells/kg for patients with a sibling donor or unrelated donor, respectively. Escalating doses of DLI were performed in case of persisting mixed-chimerism at 3 months after DLI (see Table 1 for details).

Multi-state models

A multi-state model consists of different states (indicated by boxes) and transitions (indicated by arrows) (see Figures 1 and A1 for an illustration of the model used in this study). Patients experience a transition (i.e., event) when they pass from one clinical situation (state) to the next, and they remain in this state until the next transition takes place or until they are censored at the end of their follow-up. The model is used to estimate transition probabilities between states, which indicate the time-dependent probabilities of being in a certain state at a certain moment, based on the state (same or other) where the patient stayed before. It can be perceived as an extension of standard survival analysis, which can be modeled with a two-state model: state 1 representing SCT, state 2 death, and the transition probability in the time interval 0 to t between these 2 states being equal to 1-survival (t). Transition probabilities are either represented by means of graphs (analogous to survival curves) or as numbers at a fixed point in time. As in standard survival analysis, the building blocks of the transition probabilities are the hazards, indicating the instantaneous probability of experiencing an event of a certain type conditional on being at risk for this event.

In the model presented here, it is assumed that Markovianicity holds, which implies that the future only depends on the past through the present, in other words, if the state of a patient at a specific point in time is known, it no longer matters through which route and when she/he arrived there. Transition hazards were estimated by means of Nelson-Aalen estimators, and transition probabilities by the Aalen-Johansen estimator. Standard errors of the transition probabilities were calculated by means of the Greenwood estimator.[4] They were used to estimate 95% point-wise linear confidence intervals. Only results up to 60 months after SCT are shown because of the increased imprecision afterwards due to the smaller number of patients still at risk.

All patients started at 'SCT' (state 1). We considered Relapse and NRM (states 7 and 8) as primary failures, which are the absorbing states in the model, meaning no further transition is possible once either of them is reached. The following intermediate states were defined: 'Start IS', 'Stop IS', 'DLI', 'Start IS after DLI' and 'Stop IS after DLI' (states 2 to 6, respectively). All patients experiencing (severe) GvHD requiring IS entered

the state ‘Start IS’ at the day IS for GvHD was started. After cessation of IS, these patients entered the state ‘Stop IS’. All patients receiving prophylactic DLI entered the state ‘DLI’. Patients who experienced GvHD requiring IS after receiving prophylactic DLI entered the state ‘Start IS after DLI’. After cessation of IS after prophylactic DLI, patients entered the state ‘Stop IS after DLI’.

References

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Figure A1. Multi-state model with transition counts. In contrast to Figure 1, transitions to the absorbing states 'NRM' and 'Relapse' are also depicted by arrows. The number between brackets within each state indicates the observed number of patients in that state at the end of their follow-up. The number on each arrow indicates the observed number of transitions in our dataset (consisting of 78 patients).

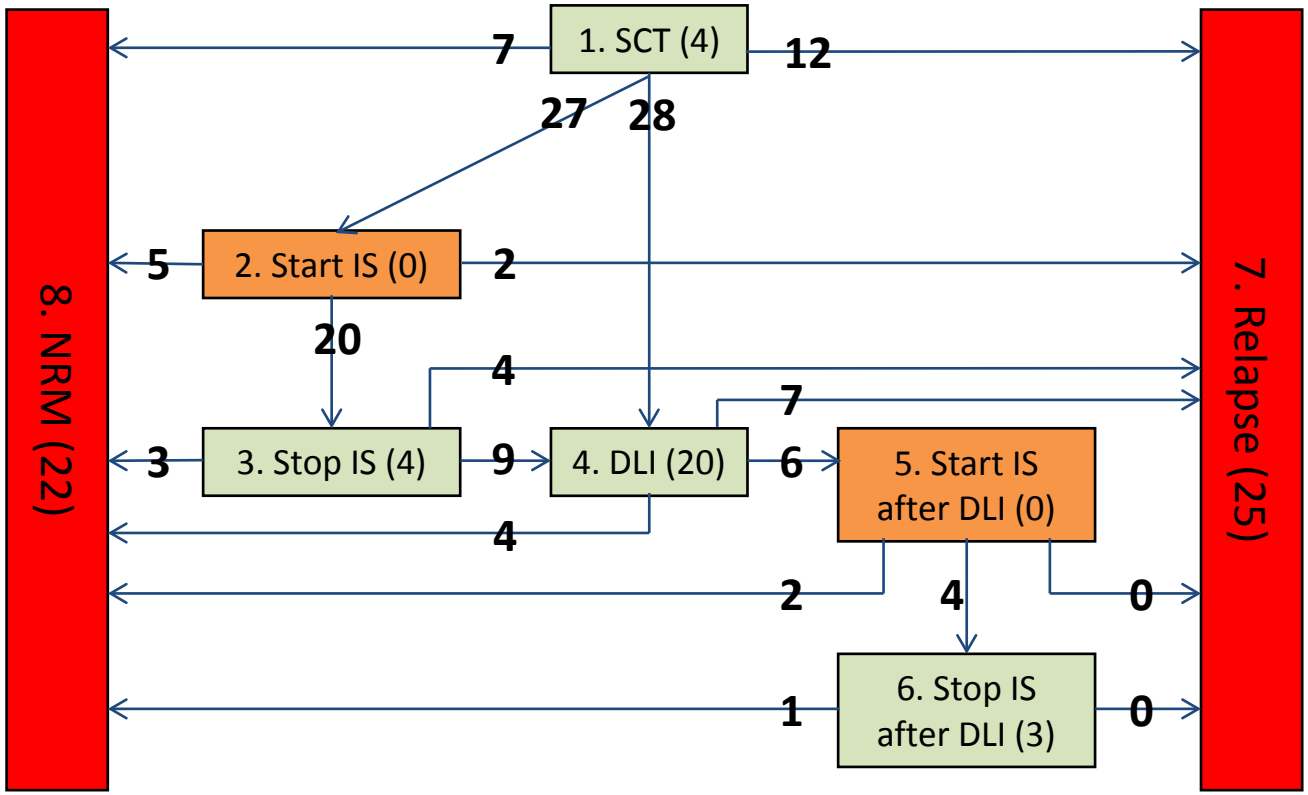

Figure A2. Cumulative incidence curves of prophylactic DLI and its competing risks relapse before DLI and NRM before DLI.

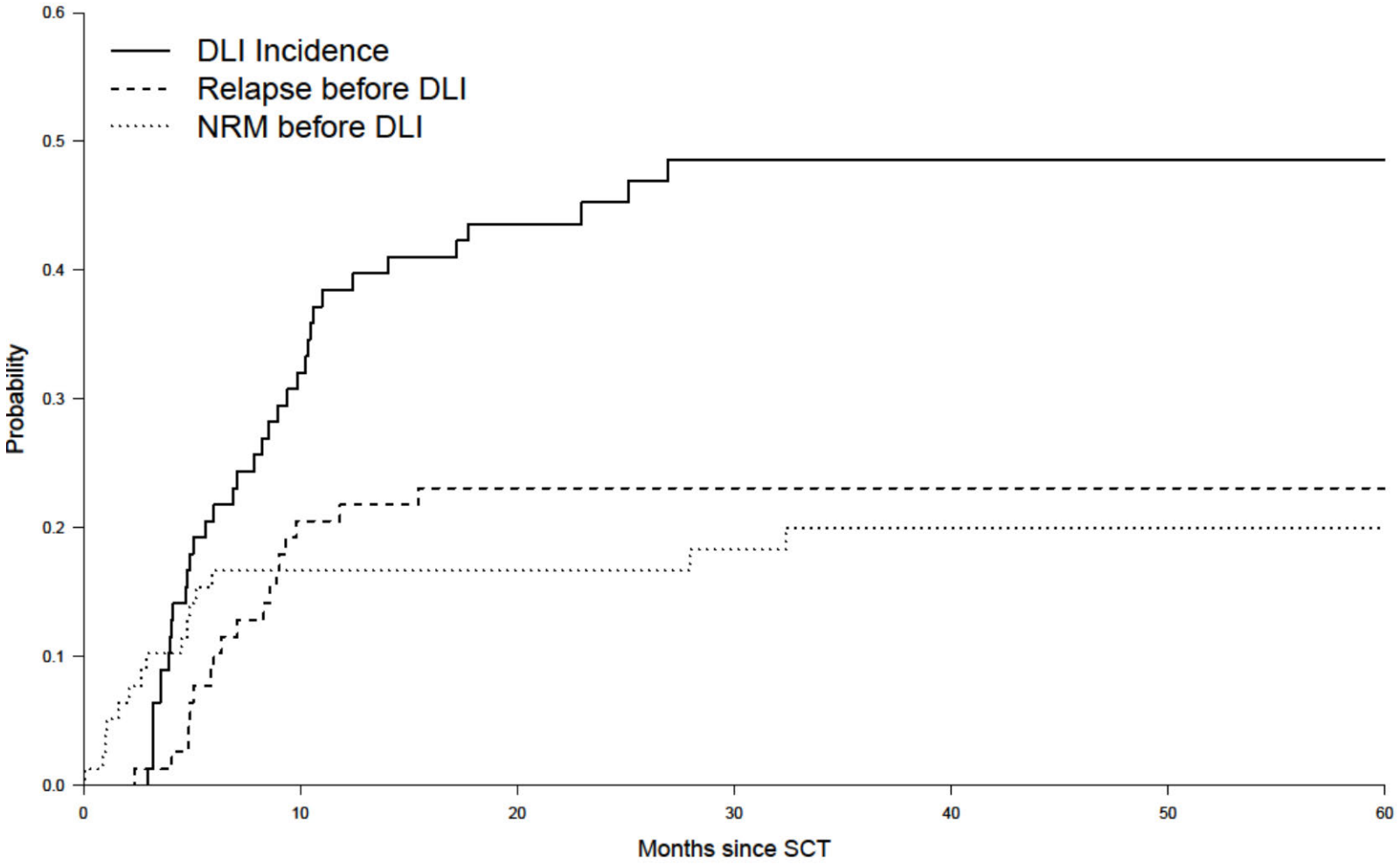

Figure A3. Transition probabilities to all states from SCT. Transition probabilities (black) and associated 95% point-wise linear Confidence Intervals (red) derived from the multi-state model of Figure 1. At each point in time, the black curve indicates the probability of a patient being in the respective state, given that he/she was in the SCT state at time 0. Since all patients started in the 'SCT' state, the probability of being in that state was 1 at time 0 and decreased afterward, because patients could only leave this state. For all other states, the probability to have entered this state at time 0 was 0. For the 'Relapse' and 'NRM' states, which are absorbing states, the probabilities can only increase over time since patients cannot leave these states anymore. For the 5 intermediate states, probabilities increase and decrease over time. Note that the scale of the y-axis of the first figure (SCT) differs from that of the other figures.