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Complete donor T-cell engraftment 30 days after allogeneic transplantation predicts molecular remission in high-risk chronic lymphocytic leukaemia

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Chronic lymphocytic leukaemia (CLL) is often indolent, however some patients will demonstrate an aggressive disease course. Over the last decade, it has become possible to define high-risk cohorts, using such prognostic indicators as *IGHV* mutation status and cytogenetics (Miklos, 2009; Hamblin et al, 1999). This advance has led to clinical trials exploring the utility of early aggressive intervention in high-risk patients, including allogeneic haematopoietic cell transplant (allo-HCT).

Recent work has demonstrated that minimal residual disease (MRD) negativity at one year post-transplant strongly predicts better disease-free survival at two years (Farina et al, 2009) and better overall survival at three years (Ritgen et al, 2008), and may therefore be a surrogate marker for longer term remission and an important therapeutic goal following allo-HCT for CLL. However, disease progression may occur before the one-year landmark, necessitating therapeutic intervention. Therefore, to facilitate clinical management of these patients during the first year post-transplant, we evaluated allo-HCT and CLL-associated risk factors for predictive associations to molecular remission.

Twenty-one consecutive high-risk CLL patients from two clinical trials were analysed. Patients received a reduced intensity conditioning preparative regimen consisting of total lymphoid irradiation (TLI; 80 cGy in 10 fractions) and antithymocyte globulin (ATG; total 7.5 mg/kg) (Lowsky et al, 2005; Kohrt et al, 2009). Donor peripheral blood progenitor cells were infused on d+0. Ciclosporin prophylaxis was tapered by 6 months, and mycophenolate mofetil until d+28 for related donors, d+100 for unrelated donors. Nineteen patients from one trial received rituximab (375 mg/m²); infused on d+ 56, 63, 70, and 77. Two patients

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from a second trial had the same preparative regimen but did not receive rituximab. Median follow-up was 19 months, with a range of 12-46 months.

The protocol and consent forms were approved by the Institutional Review Board of the Stanford University Medical Center: all patients provided signed, informed consent. The studies are registered at the U.S. National Institutes of Health, ClinicalTrials.gov (Identifiers NCT00234013 and NCT00185640).

Assessment of MRD in the peripheral blood (PB) was performed by quantitative polymerase chain reaction using allele-specific oligonucleotides (ASO-Q-PCR) essentially as previously described (Ladetto et al, 2000), using *IGHV*-region consensus probes and rearranged-*IGH* allele-specific primers, including a complementarity-determining region 3 (CDR3) -primer. In some cases, it was necessary to design an ASO-probe spanning the CDR3 to achieve appropriate specificity and sensitivity. The method employed absolute quantitation, and used *GAPDH* as an endogenous control gene to measure the input quantity of amplifiable genomic DNA and ASO-*IGH* plasmid standards. The sensitivity for each assay was ≤ 10 copies/ug. Specificity was demonstrated using several negative controls including normal tonsillar DNA as a polyclonal control. TaqMan primers and probes were designed using PrimerExpress, version 3.0 (Applied Biosystems, Foster City, CA). MRD assays were performed on the ABI 7700 or 7900 (Applied Biosystems). Molecular remission was defined as ≤ 30 copies/ug white blood cell-DNA (approximately 1.8×10^{-4} white blood cells).

Molecular remission at d+365, as determined by Q-PCR MRD results, was compared with CLL and HCT risk factors for predictive associations. The CLL risk factors evaluated were *IGHV*-mutation status, unfavourable cytogenetics pre-HCT, prior alemtuzumab, and fludarabine refractoriness. The allo-HCT risk factors evaluated were lymph node > 5 cm at HCT, months from diagnosis to HCT, disease status at HCT, MRD level at HCT, number of prior treatment regimens, related vs. unrelated donor, female donor to male recipient, and time to establish complete donor engraftment of T-cells. There was no evaluation of the impact of rituximab administration because that was not randomized; 19 of 21 patients received it.

Complete donor T-cell engraftment was defined as $\geq 95\%$ donor for CD3 in the PB, assayed by routine DNA fragment analysis of serial tandem repeat polymorphisms in bead-separated lymphocytes.

Statistical calculations were done using GraphPad Prism, version 5.0a (GraphPad, San Diego, CA). Categorical variables were compared by the Fisher's exact test, while continuous variables were compared by unpaired, two-tailed t-tests and by log rank (Mantel-Cox) analyses. Significance levels were set at ≤ 0.05 .

Table I shows MRD and chimerism result data for the first year for all 21 patients; 13 patients had achieved molecular remission. Disease progression had occurred in six patients, one patient showed stable disease, and 14 patients were in clinical remission.

Analyses of the disease parameters described above showed no predictive value, with the exception of complete donor engraftment. Remarkably, as shown in Figure 1, complete donor T-cell (CD3) engraftment by d+30 was a strong predictor of molecular remission by one year ($p \leq 0.0008$ by log rank Mantel Cox), with a positive predictive value of 90.9%

The utility of parallel assessments of chimerism and MRD in predicting relapse in allo-HCT with reduced-intensity conditioning has been shown but remains controversial due to contradictory reports (Pérez-Simón et al, 2002; Pulsipher et al, 2008). However, in our

cohort of high-risk CLL patients treated on this protocol, complete donor T-cell engraftment at d+30 was an early predictor of molecular remission at d+365, a surrogate marker for longer-term remission as described above.

This predictive association improves disease stratification such that patients who show mixed T-cell chimerism at d+30 can be more closely monitored and perhaps treated earlier. While the mechanism of the association of complete donor T cell engraftment and molecular remission is not definitively known, T-cells are important contributors in the graft-versus-leukaemia (GvL) effect (Levenga et al, 2007). While patients with other diseases, such as acute myeloid leukaemia may already be cured by chemotherapy before allo-HCT, CLL patients are not cured by chemotherapy, and in the absence of effective GvL activity by the graft, CLL will recur. Accordingly, patients who do not achieve complete donor chimerism may have an inadequate GvL response, resulting in a higher risk of disease recurrence.

In conclusion, our analysis has identified that complete donor T-cell engraftment at d+30 following TLI-ATG allogeneic conditioning is a strong predictor for achieving molecular remission by one-year post-transplant. While confirmatory studies with other treatment regimens are required, monitoring donor T-cell engraftment status at d+30 may be an important treatment parameter indicating the likelihood of molecular remission by one-year post allo-HCT in CLL.

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Figure 1.

Log-rank analysis comparing patients with and without complete donor T-cell engraftment (T chim) at d+30 post-HCT by percent of each group in molecular remission. Full donor CD3 engraftment at day 30 predicts molecular remission by one year post-transplantation, $p=0.0008$.

Table 1

MRD and chimerism test results data. Grey shaded boxes show complete T-cell donor engraftment (all timepoints). Molecular remission at d+365 shown in bold. CR = clinical remission, PD = progressive disease, RT = Richter's transformation, SD = stable disease, ND = not done, Donor T-chim = donor T cell chimerism.

Case no.	Donor T-chim (%) d30	Residual Disease in PB (copies/ug DNA) d90	Donor T-chim (%) d90	Residual Disease in PB (copies/ug DNA) d180	Donor T-chim (%) d180	Residual Disease in PB (copies/ug DNA) d365	Donor T-chim (%) d365	Clinical status d365
3431	100	0	100	18	100	0	100	CR
3870	97	0	99	0	100	0	100	CR
3703	99	3	100	28	100	0	100	CR
4077	97	3	99	3	99	0	99	CR
3399	96	166	100	0	100	0	100	CR
3732	95	935	99	67	99	10	99	CR
3903	100	1,197	97	25	99	25	99	CR
3934	96	5,269	100	4	100	0	100	CR
3751	92	835	99	181	99	30	99	CR
3723	97	2,735	96	24	99	19	100	CR
3835	95	0	93	ND	95	10	99	RT
3879	87	122	97	14	100	0	100	CR
3409	50	5,327	30	37	70	0	100	CR
3455	90	3,201	99	18,339	98	18,128	99	PD
3860	32	91,500	64	18,089	88	6,368	97	SD
3873	86	20,465	88	1,440	96	2,280	95	CR
3632	36	236	4	3,238	2	846	98	PD
3975	91	273	60	899	85	12,813	85	PD
3740	93	66	84	15,042	47	129,021	19	PD
3926	96	31	55	45	39	127,580	51	PD
3697	62	0	61	59	47	282	49	CR