

Consensus of German Transplant Centers on Hematopoietic Stem Cell Transplantation in Fanconi Anemia

Konsensus Empfehlungen Deutscher Transplantationszentren zur hämatopoetischen Stammzelltransplantation bei Fanconi Anämie

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Abstract

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Allogeneic hematopoietic stem cell transplantation (HSCT) is currently the only curative therapy for the severe hematopoietic complications associated with Fanconi anemia (FA). In Germany, it is estimated that 10–15 transplants are performed annually for FA. However, because FA is a DNA repair disorder, standard conditioning regimens confer a high risk of excessive regimen-related toxicities and mortality, and reduced intensity regimens are linked with graft failure in some FA patients. Moreover, development of graft-versus-host disease is a major contributing factor for secondary solid tumors. The relative rarity of the disorder limits HSCT experience at any single center. Consensus meetings were convened to develop a national approach for HSCT in FA. This manuscript outlines current experience and knowledge about HSCT in FA and, based on this analysis, general recommendations reached at these meetings.

Introduction

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Fanconi anemia (FA) is a rare inherited genomic instability disorder associated with congenital abnormalities, progressive bone marrow failure (BMF), and a predisposition to develop malignancies including myelodysplastic syndrome (MDS)/acute myeloid leukemia (AML) and squamous cell carcinoma (SCC) [23, 41]. Although there is phenotypic variability among FA individuals, hematologic abnormalities arise in the majority of patients and represent a significant clinical feature of the disorder. Some FA patients have mild hematologic changes and do not require thera-

Zusammenfassung

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Die allogene Stammzelltransplantation (HSCT) ist zurzeit die einzige kurative Therapie der schweren hämatopoetischen Komplikationen der Fanconi-Anämie (FA). In Deutschland werden etwa 10–15 Transplantationen pro Jahr bei Patienten mit FA durchgeführt. Da die FA auf einem Defekt der DNA-Reparatur beruht, können Standard-Konditionierungsprotokolle zu hoher Toxizität und Mortalität führen, während Regime mit reduzierter Intensität ein Risiko für Transplantatversagen in sich tragen. Hinzu kommt, dass die Entwicklung der Transplantat-gegen-Empfänger-Erkrankung ein wichtiger Risikofaktor für die Entwicklung von sekundären soliden Tumoren ist. Die relative Seltenheit der Erkrankung führt dazu, dass die Erfahrung jedes einzelnen Transplantationszentrums mit der Erkrankung begrenzt ist. Konsensus-Treffen mit dem Ziel der Entwicklung eines bundesweiten Vorgehens bei der Transplantation der FA wurden abgehalten. Dieses Manuskript beschreibt die langjährige Erfahrung und den aktuellen Kenntnisstand zur Transplantation bei FA sowie den darauf basierenden Konsens, der auf diesen Treffen erzielt wurde.

peutic intervention. Others have moderate to severe BMF that improves with androgen therapy [60]. In patients with BMF who fail to respond to androgens or who develop myeloid neoplasia, allogeneic hematopoietic stem cell transplantation (HSCT) can restore normal hematopoiesis. Unfortunately, early FA HSCT preparative regimens (cyclophosphamide ± radiation) based on HSCT for patients with acquired severe aplastic anemia (SAA) were fraught with severe complications [25, 27, 28, 31, 32, 45]. Excessive regimen-related toxicities (RRT), high rates of graft failure, graft-versus-host-disease (GVHD), secondary cancers, and poor survival outcomes were ob-

served. These were attributed to underlying congenital abnormalities, hypersensitivity to DNA damaging agents and putatively radiotherapy, delayed or absent tissue repair after GVHD induced injury, and/or presence of T cell mosaicism in some FA individuals. [25, 27, 28, 31, 32, 45].

Over the past 3 decades, modifications to FA HSCT conditioning regimens together with advances in transplant medicine (e.g. more accurate HLA typing, better supportive care, graft processing, etc.) have dramatically improved the prognosis of FA HSCT [20]. Reduction of chemotherapy and radiation doses (reduced intensity conditioning) lessened RRTs [27, 63]. Incorporation of fludarabine to preparative regimens enhanced immunosuppression and engraftment without adding toxicity [8, 29, 39, 68]. In vivo and/or ex vivo T cell depletion of allografts decreased the incidence and severity of GVHD [8, 17]. Radiation was eliminated in some conditioning regimens with excellent results [48, 54]. For some FA HSCTs, particularly in HLA matched sibling donor (MSD) transplants, survival now ranges from 70–100% with low graft failure and GVHD rates [48].

Challenges, however, remain. For FA patients who do not have a MSD and receive transplants from either mismatched related or unrelated donors, collectively termed alternative donor (AD), the outcomes of HSCT are less encouraging, albeit improving [68]. FA patients with advanced MDS or AML have a poorer prognosis [2]. Post-transplant infections represent a leading cause of transplant related morbidity and mortality [10] (Chao et al. in press). Long-term follow-up of FA patients indicate a 4.4 fold increased risk of developing head and neck SCC after HSCT [59], associated with GVHD development and debatably radiation exposure [15, 32, 59]. These challenges provide incentive for further improvements in FA HSCT.

Overview of FA HSCT nationally and internationally

The first HSCTs for FA in Germany were completed at the University of Ulm in the mid-1980s. Whereas the initial conditioning regimens included total body irradiation (TBI) or total lymphoid irradiation (TLI), since 1991, radiation has been eliminated. To date, 14 patients [6 matched unrelated (MUD), 5 MSD, 2 matched related transplants (MRD), and one haploidentical transplant] have received a cyclophosphamide fludarabine based regimen without radiation at the University of Ulm. Busulfan was also administered in patients with clonal disease and in the haploidentical transplant and anti-lymphocyte therapy with alemtuzumab (Campath-1H) or anti-thymocyte globulin (ATG) was applied in all patients except in those receiving MSD transplants. 10 of the 14 patients are alive, but unfortunately, 2 of the surviving patients are suffering from carcinomas.

Between October 1999 and April 2014, the Pediatric Blood and Marrow Transplantation Department at Charité University Medicine Berlin completed 39 consecutive MSD (n=4) and AD (n=35) HSCTs for FA. These transplants account for the majority of FA HSCTs performed in Germany. HSCT regimens titled German Fanconi Anemia 02 (GEFA02) (n=18) and German Fanconi Anemia 03 (GEFA03) (Chao et al. in press) (n=21), consisting of fludarabine based non-radiation conditioning, were utilized. The GEFA02 preparative regimen included fludarabine 30 mg/m²/day IV×6 days, busulfan 1 mg/kg/day in 2 divided doses PO×2 days, ATG-Fresenius 20 mg/kg/day IV×3 days, and Murromab-CD3 (OKT3, Orthoclone OKT3) 0.1 mg/kg/day IV×12 days and was employed from 1999 to 2005. Since 2005 to the

present time, the GEFA03 regimen which includes fludarabine 30 mg/m²/day IV×6 days, busulfan 1 mg/kg/day in 2 divided doses PO×2 days, cyclophosphamide 20 mg/kg/day IV×2 days, and alemtuzumab 5 mg/m²/day×1 day followed by 10 mg/m²/day IV×3 days has been used. Patients received Cyclosporine A (CSA) for GVHD prophylaxis. In this series, all but one patient experienced neutrophil engraftment. Mixed chimerism was observed in 4 patients who all converted to full donor chimerism after stem cell boost(s), and 4 second transplants were undertaken for graft failure. Approximately 38% of patients developed grade I acute GVHD (aGVHD), 10% grade II aGVHD, and no grade III-IV aGVHD was observed. Among the evaluable patients, 17% developed mild chronic GVHD (cGVHD) and no patient experienced moderate or severe cGVHD. 11 patients died: 8 from viral infection, 2 from AML disease progression, and one from SCC. The 2-year probability of survival was 76% (SE 7%) with a median follow-up of 5.7 years. A sub-analysis of GEFA03 patients showed a 2-year probability of survival of 90%, 100% neutrophil engraftment, 38% grade I aGVHD and no grade II-IV aGVHD, and no moderate or severe cGVHD.

At Eberhard Karl University in Tuebingen, 7 pediatric patients were transplanted for FA in more recent years. There were 4 MUD, 2 MSD, and one haploidentical donor transplants. The conditioning regimen included fludarabine 40 mg/m²/dose×4 doses, cyclophosphamide 300 mg/m²/dose×4 doses, and ATG Thymoglobulin 4.5 mg/kg total dose (0.5–2–2 mg/kg). For MUD transplants the dose of ATG was increased to 10 mg/kg (1–3–3–3 mg/kg). For the haploidentical transplant, 15 mg/kg ATG-Fresenius (1–4–5–5 mg/kg) was used and 4 Gy TBI, followed by a TCRαβ/CD19 negative depleted allograft of PBSCs. For MSD and MUD transplants, the GVHD prophylaxis included CSA and mycophenolate mofetil (MMF) and for the haploidentical transplant the patient received only MMF. All patients engrafted. 3 patients experienced aGVHD and among these patients, 2 developed grade I aGVHD and one grade III aGVHD. To date, only one patient has developed mild cGVHD. The overall survival was 100%.

The German experiences recapitulate worldwide FA HSCT experiences (e.g. a retreat from radiation based conditioning, utilization of fludarabine and anti-lymphocyte antibodies, and use of new stem cell sources such as haploidentical donors). Current conditioning regimens used in Germany are comparable to preparative regimens employed by others elsewhere and confer good outcomes. FA HSCT results published in the past 5 years are detailed in ► **Table 1** [1, 4, 12, 19, 34, 46–48, 62, 65, 67, 70, 71].

Radiation vs. no radiation

Historically, radiation was used in FA HSCT preparative regimens to facilitate engraftment. Yet, the early use of standard dose radiation was associated with severe adverse side effects and initial studies suggesting a marked hypersensitivity of FA patients towards radiation led to significant reduction of the radiation dose [25, 27]. Elimination of the modality proved successful in MSD transplants. Here, graft failure was less problematic and radiation was omitted without compromising engraftment. In mismatched and unrelated donor FA HSCTs, radiation was replaced by busulfan in some centers with good results [12]. Other centers have continued to employ radiation at lower doses for mismatched and unrelated donor transplants, arguing that single fraction low-dose radiation confers minimal toxicities not exceeding busulfan related toxicities [47, 48]. In Germany, radia-

Table 1 FA HSCT results published over last 5 years.

Reference	Conditioning Regimen	GVHD Prophylaxis	No. of Cases	Median Age	Engraftment *	aGVHD	cGVHD	Survival
Ayas et al. 2012 [1]	Cy, Flu, ATG	CSA	26 (19 MRD, 7 UCB)	7.8 yrs	19/19, 2/7	1/19, 1/7	1/19, 1/7	100%, 43% OS
Shimada et al. 2012 [62]	Cy, Flu, ATG	CSA, MTX; FK506, MTX	8 (3 MSD, 1 MRD, 2 MUD, 2 MMUD)	4.5 yrs	8/8	1/8	0/8	100% OS
Yabe et al. 2012 [70]	Cy, ATG, TAI/TBI; Cy, Flu, ATG	CSA, MTX	15 (MSD)	8 yrs	14/15	1/15	3/14	80% OS
Boulad et al. 2012 [12]	Cy, Bu, Flu, ATG		27 (AD)		27/27	3/27		83% OS
MacMillan et al. 2012 [46]	Cy, ATG, TBI, ± Flu		127 (AD)			18–50% grade II–IV	9–25%	61% 1 yr POS; 54% 5 yrs POS
Thaker et al. 2011 [67]	Flu, TBI	CSA, MMF	6 (AD)		5/6	4/6	3/5	50% OS
Stepensky et al. 2011 [65]	Cy, ± TAI/TLI, ± Bu, ± ATG; Flu ± Cy, ± Bu, ± ATG/Atgam, ± TBI	CSA; CSA, MMF; Tac; CSA, Tac; FK506, MMF, Tac	41 (MSD, AD; 17 w/o Flu, 24 w/ Flu)	10.3 yrs	17/17, 22/24	14/17, 11/24	4/17, 8/24	35%, 83% OS
Hamidieh et al. 2011 [34]	Cy, Bu, ± ATG; Cy, Flu, ATG	CSA ± MTX	53 (MSD, MRD, MMRD; 42 w/o Flu, 11 w/ Flu)	9 yrs	49/53	79%, 45%	17%, 36%	61% OS
MacMillan et al. 2010 [48]	Cy, Flu, ATG		22 (MSD)	8.5 yrs	22/22	0%	0%	82% OS
MacMillan et al. 2009 [47]	Cy, Flu, TBI, ATG	CSA	24 (AD; 22 w/ 3 Gy TBI, 2 w/ 1.5 Gy TBI)	8.8 yrs	22/22, 0/2	3/22, 0/2		86%, 100% OS
Ertem et al. 2009 [19]	Cy, Bu; Cy, Flu, ATG	CSA, MTX	8 (MSD)	12 yrs	8/8	0/8	1/8	87.5% OS
Baker et al. 2009 [4]	Cy, Flu, ATG, ± Bu	CSA, MP	5 (MRD, MUD)		4/5	2/5	0/5	80% OS
Yesilipek et al. 2009 [71]	Cy, TAI, ATG; Cy, Flu, ATG	CSA ± MMF	16 (MRD, MUD; 6 TBI, 10 Flu)					50%, 90% OS

* , primary stable engraftment; Cy, cyclophosphamide; Flu, fludarabine; ATG, anti-thymocyte globulin; CSA, cyclosporine A; MRD, matched related donor; UCB, unrelated cord blood; OS, overall survival; POS, probability of survival; MTX, methotrexate; MSD, matched sibling donor; MUD, matched unrelated donor; MMUD, mismatched unrelated donor; TAI, total abdominal irradiation; TBI, total body irradiation; MMF, mycophenolate mofetil; TLI, total lymphoid irradiation; Bu, busulfan; Tac, tacrolimus; MP, methylprednisolone

tion has been omitted for MSD, MUD, and mismatched unrelated donor FA HSCTs (excluding some haploidentical transplants). Interestingly, conflicting experimental and clinical data regarding the radiosensitivity of FA has emerged. While Marcou et al. demonstrated normal colony survival assays in irradiated fibroblasts from a FA patient, Djuzenova et al. using the same patient's fibroblasts showed increased DNA damage after radiation exposure [16, 49]. The patient experienced severe toxicities after radiotherapy for tonsillar carcinoma [49]. A newer study demonstrated that primary fibroblasts from patients with mutations in *FANCA*, *FANCD2*, and *FANCG* are not particularly sensitive for either ionizing or UV radiation [37]. Moreover, older studies clearly implicated radiation in the development of post-transplant head and neck SCC in FA patients [15], but more recent studies have not identified this link [55, 59]. The consensus group addressed the question whether radiation might be reintroduced in future German FA transplant protocols. The group agreed to continue with non-radiation protocols based on (a) established experience with non-radiation FA HSCT protocols, (b) good results achieved with these protocols, (c) potential to eliminate any possible short- and long-term toxicities associated with radiation, and (d) recent large FA HSCT reviews suggesting inferior survival for FA patients treated with radiation-containing preparative regimens [3, 55].

The backbone

Early FA HSCT conditioning regimens consisted of cyclophosphamide (200 mg/kg) and radiation. With increasing experience,

there have been refinements to the conditioning regimens. A 5–10 fold reduction in the cyclophosphamide dose was instituted. Likewise, radiation was decreased in dose or eliminated. Kapelushnik et al. were the first to report the use of fludarabine in FA HSCT [39]. Subsequently, numerous studies demonstrated that fludarabine accorded engraftment and survival benefits. Currently, fludarabine based regimens are regarded as standard in FA HSCTs [8, 14, 65]. Dose reduction of fludarabine, however, is warranted in FA patients with renal compromise (personal communication with W. Ebell). Might there be additional modification(s) to the FA HSCT preparative regimens to further reduce RRTs?

In 2007, Bonfim et al. reported on 43 FA patients who received transplants from HLA-matched related donors [11]. The conditioning regimen consisted of cyclophosphamide alone (total dose 60 mg/kg). 40 patients (93%) were alive with a median follow-up of 3.7 years. One patient experienced primary graft failure and 4 patients developed late graft failure. Grade II–III aGVHD and cGVHD were noted in 17 and 28.5% of patients, respectively [11]. A recently completed phase I/II study at Baylor College of Medicine in Houston, Texas, USA examined alemtuzumab and anti-CD45 antibodies (YTH-24 and YTH-54) (10/10 HLA matched) or alemtuzumab, anti-CD45 antibodies, and fludarabine (9/10 HLA matched) conditioning in 5 pediatric patients with FA or other DNA breakage/chromosomal instability syndromes [51]. For HLA mismatched donors, harvested peripheral blood stem cells (PBSC) were enriched for CD34+ cells [51]. 2 of the 5 participants showed donor engraftment at day +100. Median time to ANC >500/mm³ was 15 days. No aGVHD grade II–IV or cGVHD was observed and all patients are alive at one

year post-transplantation [51]. At Boston Children's Hospital, Boston, Massachusetts, USA 4 patients with dyskeratosis congenita were successfully transplanted with matched unrelated donor allografts ($\geq 9/10$ HLA matched) using fludarabine and alemtuzumab [43]. All patients engrafted neutrophils by day +30, and are alive and transfusion independent with a follow-up ranging from 6 months (1 patient) to 2 years (3 patients) [43]. All patients showed full donor myeloid chimerism by day +60. 3 of the 4 patients showed full donor lymphoid chimerism by year 2, and the fourth patient has high and increasing mixed donor lymphoid chimerism at day +180. There was no aGVHD and only one patient developed limited cGVHD of the skin [43]. Whether fludarabine and alemtuzumab only preparative regimen might be effective in FA HSCT is yet to be established.

Based on these and other published data and the German experience, particularly GEFA03, the consensus group agreed that fludarabine, cyclophosphamide, and busulfan will continue as the backbone agents; however, busulfan may be excluded in some transplants. Busulfan is unlikely to be necessary for "low risk" transplants (i.e. $\geq 9/10$ HLA matched related or unrelated HSCTs with BMF). Busulfan may be beneficial in FA patients with mismatched allografts to assist engraftment, and as an anti-leukemic agent in FA patients with bone marrow clonal aberration(s) and/or MDS/AML. Intravenous busulfan is preferred over oral busulfan due to its more consistent pharmacokinetics.

In vivo T cell depletion: Anti-thymocyte globulins vs. alemtuzumab

Both ATG(s) and alemtuzumab have been applied as in vivo T cell depleting agents in FA HSCTs to aid engraftment and to reduce the incidence and severity of GVHD. Whether one lymphocyte-depleting antibody may be advocated over the other is difficult to answer, as there are no prospective direct comparison studies in FA. Charité University Medicine Berlin used ATG-Fresenius with OKT3 in the GEFA02 protocol and alemtuzumab in the GEFA03 protocol. Comparison of GEFA02 and GEFA03 results, with the caveat that the backbone agents were not identical, demonstrated more stable engraftment (5/18 vs. 0/21 graft failure or mixed chimerism requiring stem cell boosts), less fatal viral infections (6/18 vs. 2/21), and a higher overall survival rate (10/18 vs. 18/21) with GEFA03. Moreover, published reports in other diseases and adult patients suggest superior outcomes with alemtuzumab. Marsh et al. retrospectively compared patients with acquired SAA who were conditioned for allogeneic HSCT with regimens including alemtuzumab ($n=100$) or ATG ($n=55$) [50]. Engraftment failure occurred in 9 and 11% of patients in the alemtuzumab and ATG groups, respectively. The 5-year survival rate was 90% for alemtuzumab and 79% for ATG. Overall survival was significantly better when using alemtuzumab (88%) compared with ATG (57%, $p=0.026$) for unrelated donor transplants. The risk for cGVHD was lower in the alemtuzumab group (11 vs. 26%, $p=0.031$) [50]. Similarly, Soiffer et al. examined the outcomes of reduced intensity conditioning transplants according to the use of in vivo T cell depletion with ATG ($n=584$), alemtuzumab ($n=213$), or no antibody therapy ($n=879$) in patients with hematopoietic malignancies [64]. Both grade II-IV aGVHD and cGVHD incidences were lower with alemtuzumab compared with ATG and unmanipulated grafts (19 vs. 38% for ATG and 40% for unmanipulated grafts, $p<0.0001$ and 24 vs. 40% for ATG and 52% for unmanipulated grafts,

$p<0.0001$, respectively) [64]. Relapse was more frequent with alemtuzumab and ATG (49 and 51 %, respectively, vs. 38 % unmanipulated, $p<0.001$). The 3-year probability of survival after adjusting for age, performance status, disease, disease status, GVHD prophylaxis, and donor source were 50% for alemtuzumab, 46% for T cell replete, and 38% for ATG containing regimens. The difference in survival outcomes between alemtuzumab and T cell replete groups was not statistically significant but the difference between ATG and unmanipulated T cell replete grafts was significant ($p=0.008$) [64]. Lower incidences of GVHD and RRTs and their sequelae related with alemtuzumab administration may explain the improved survival rates [47]. Of significance for FA patients is the lower rate of GVHD afforded by alemtuzumab, since its development is associated with a higher risk for secondary malignancy [59] and a negative impact on the quality of life.

Other GVHD prophylaxis

In a retrospective review of patients with FA ($n=37$) or acquired aplastic anemia ($n=73$) following MSD HSCT, FA patients had double the relative risk of grades II-IV aGVHD ($p=0.021$), and in younger patients, the relative risk was 7.93 ($p=0.014$) [32]. Moreover, the risk of requiring systemic corticosteroids and experiencing steroid-resistant aGVHD was higher in FA patients [32]. Increased cellular apoptosis in FA individuals has been linked to the higher severity of aGVHD [69]. Wang et al. assessed epithelial cell apoptosis and studied TP53 and miR-34a expression in the skin and gut biopsies from 5 non-transplanted FA patients, 20 FA patients with aGVHD, and 25 acquired aplastic anemia patients. Although FA and aplastic anemia patients received similar preparative regimens, epithelial apoptosis was higher in FA specimens than in samples from patients with acquired aplastic anemia patients [69]. Further studies of gut biopsies from FA patients showed that this deleterious effect was not related to TP53 gene overexpression but rather miR-34a which mimics p53 apoptotic effects in response to DNA damage [69]. In a large analysis of 795 FA HSCTs between 1972 and 2009, the incidence of cGVHD was 14% at 1 year and 19% at 5 years [55]. Older age at transplant and history of aGVHD were independent predictors of cGVHD [55]. The development of cGVHD correlated with an increased risk of developing head and neck SCC [59]. A principal goal in FA HSCT has been to reduce the incidence and severity of GVHD in order to abolish any of its potential short- and long-term complications. In addition to anti-lymphocyte antibody to reduce the incidence and severity of GVHD, the consensus group advises CSA as GVHD prophylaxis, with the addition of MMF for unrelated donors.

Immune reconstitution

Allogeneic HSCT is associated with profound immune deficiency owing to a multitude of factors including underlying disease, transplant conditioning regimen, stem cell source, degree of HLA matching, graft manipulation, development of GVHD, immunosuppressive medications for GVHD prophylaxis/therapy, and others. However, the speed of immune recovery has important repercussions regarding infectious complications and relapse following allogeneic HSCT [21,40]. The consensus group addressed post-transplant immune reconstitution as related to the

anti-lymphocyte antibody utilized in the preparative regimen. The focus was on alemtuzumab since the antibody is proposed for future studies.

There is evidence that alemtuzumab is more immunosuppressive than ATG because the former is directed against a variety of immune cells including T and B cells, macrophages, monocytes, and NK cells and the latter targets mainly T lymphocytes [57]. In a comparison of immune reconstitution following alemtuzumab (n=14) or ATG (n=13) in pediatric unrelated transplants, patients who received alemtuzumab demonstrated significantly slower immune recovery [61]. The presence of peripheral blood CD3+T cells (>30 cells/ μ L) (64.5 vs. 27 days), the median time to normal phytohemagglutinin response (283 vs. 88 days), and the median time to an antigen specific response (365 vs. 150 days) occurred later in those receiving alemtuzumab in comparison to patients given ATG [61]. Despite these delays in immune recovery, there was no increase in infectious complications in recipients of alemtuzumab [61].

Total lymphocyte and lymphocyte subset counts in peripheral blood were evaluated in the 39 patients who received GEFA02 (ATG/OKT3) and GEFA03 (alemtuzumab) preparative regimens. There was large patient-to-patient variability related to the clinical circumstance of the patient; however, as a group, total lymphocyte counts steadily rose and by day +100 the mean value was 1166/ μ L. NK cells were present at normal numbers immediately following transplantation and accounted for the majority of lymphoid cells during the early post-transplant period. B cells reached normal levels by day +60. CD8+ cells recovered by 3 months. CD4+T cells were considerably slower to recover and the CD4+to CD8+ratio remained <1 in the majority of patients during the first year following transplantation. CD4+reached normal levels by day +365. Memory (CD45RO+) helper (CD4+) and cytotoxic (CD8+) T cells predominated over naïve (CD45RA+) T cells during the one-year analysis period. Comparison of GEFA02 and GEFA03 immune reconstitution data showed modestly higher lymphocyte values for GEFA03 patients that were not statistically significant for most of the analyzed time points. This pattern of post-transplantation immune reconstitution is similar but not identical to reports by others [24,56]. In FA patients following HSCT, Perlingeiro et al. found a slightly different kinetic of recovery in the major lymphocyte subsets in 23 patients conditioned with one of 3 regimens (fludarabine, cyclophosphamide and ATG, cyclophosphamide alone, or fludarabine and irradiation) for related and unrelated donor transplants [56]. NK cells were the first to recover, followed by cytotoxic CD8+T cells and B cells, and finally CD4+T cells. Early lymphocyte recovery consisted of memory cells potentially derived from the graft. New thymic emigrant (CD31+CD45RA+) and naïve CD4+or CD8+T cells rose only at 6 months after HSCT. Only marginal differences were observed in the early recovery of CD8+T cells among those receiving a graft from a related donor versus an unrelated donor. Patients with GVHD displayed a markedly delayed recovery of NK cells and B cells as well as of regulatory T cells and both early thymic emigrant and total CD4+T cells [56]. Nonetheless, viral infection represented a major complication following FA allogeneic transplantation [10]. Indeed, in the Charité University Medicine Berlin cohort (n=39), viral reactivation/infection involving CMV, human herpes virus 6, Epstein-Barr virus (EBV), adenovirus, and combined viral infections, were the leading causes of death (8/11).

A logical response to improve post-transplant immune recovery may be to decrease the dose of alemtuzumab. A wide range of

doses (100–10 mg total dose) of alemtuzumab has been employed in allogeneic HSCT [38,57]. Chakraverty et al. examined de-escalating doses of alemtuzumab in reduced intensity conditioning regimen with fludarabine and melphalan in 106 adult MSD transplants [13]. The 20 mg total dose of alemtuzumab was associated with a greater risk of grade III-IV aGVHD and severe cGVHD in comparison with doses >20 mg. A total dose of 20 mg or lower was linked with incomplete saturation of CD52 binding sites, more rapid clearance, and greater risk of severe acute and chronic GVHD [13,57]. A total dose of 30 mg or lower was associated with a significant decrease in alemtuzumab levels by day 28 [13,57]. The 30 mg dose resulted in comparable GVHD outcomes but better lymphocyte recovery at one year post-transplantation than the 40 mg or 60 mg dose groups [13]. However, improved lymphocyte counts were not associated with lower rates of infections [13]. Bertz et al. reported no difference in grade III-IV aGVHD and extensive cGVHD or graft failure in 127 patients who received 10, 20, or 40 mg (total dose) alemtuzumab for unrelated transplants [7]. There was a significant difference in grade II-IV aGVHD incidence between the 20 and 40 mg dose cohorts [7]. In summary, the standard total dose of 100 mg appears more than is required in most transplant settings. Doses of less than 30 mg may be insufficient and predispose to increased risks of GVHD [57], however, comparative analyses of data from published literature are difficult due to differences in patient populations, diseases treated, and preparative regimens. Still more, the clinical benefit (i.e. decreased infections) is unclear. Finally, Bokhari et al. reported increased incidence of EBV induced post-transplant lymphoproliferative disease (PTLD) following a reduction in alemtuzumab dose from 50 to 30 mg [9]. For comparison, the alemtuzumab dose of 35 mg/ m^2 (1.16 mg/kg) used in GEFA03 resulted in a median total dose of 35 mg and mean total dose of 38 mg (range 11–75 mg). Other reduced intensity conditioning regimens for HSCT in patients with chronic granulomatous disease or DNA breakage repair disorder use alemtuzumab doses ranging from 0.5 mg/kg to 1 mg/kg [30,33]. The consensus group agreed to an alemtuzumab dose of 30 mg/ m^2 (1 mg/kg), since further dose reductions appear not to provide anti-infection benefits and may increase the risk of GVHD. A decreased alemtuzumab dose of 20 mg/ m^2 may be employed in patients with MSD allografts (10/10, genotypically identical), only of BM is used as stem cell graft. In patients with MSD allografts ($\geq 9/10$) and PBSC grafts, total alemtuzumab dose should remain 30 mg/ m^2 , due to increased risk of GVHD. The group also advocates prophylaxis and strict surveillance of infections, preemptive therapy and aggressive early treatment.

Selective allodepletion of the graft may be one method to improve immune reconstitution following allogeneic HSCT. CD34+positive selection of stem cell grafts enabled rapid and sustained engraftment, but immune reconstitution was delayed due to non-discriminate reduction of T cells in donor grafts [5]. Development of CD3, CD3/CD19 and more recently TCR $\alpha\beta$ /CD19 depletion strategies allow for T cell depletion of allografts with preservation of immune effector cells such as NK cells, monocytes, dendritic cells, and $\gamma\delta$ T cells to improve immune recovery and to better exploit graft versus malignancy effects [35]. In a randomized study of MSD and unrelated donor transplants, faster NK cell recovery was observed in patients with CD3/CD19 depleted PBSC grafts as compared with the CD34 positive selection patients [35]. Lang et al. recently reported on the results of 35 patients with high-risk malignant and nonmalignant disorders transplanted with TCR $\alpha\beta$ /CD19 depleted haploidentical

grafts. Within the first month post-transplantation, rapid immune reconstitution with good levels of CD3+T cells, CD3+CD4+T cells, and CD56+NK cells was observed [42]. In addition, expansion of TCR $\gamma\delta$ +T cells occurred faster than TCR $\alpha\beta$ +T cells in the early post-transplant period and may contribute to anti-infectious and anti-tumor immunity [42,44,53]. Graft engineering technologies may benefit FA patients, especially if they receive a mismatched PBSC allograft.

Haploidentical transplantation

In patients who lack an HLA matched related or unrelated donor, haploidentical stem cell transplantation is a feasible alternative. The immediate availability of a haploidentical donor in most families and the possibility for post-transplant cellular therapies either in the prevention of relapse or treatment of viral infections by adoptive T cell transfer are advantages of this type of transplantation [52]. Haploidentical HSCTs have been undertaken for both malignant and nonmalignant disorders since the late 1970s [6,22,58]. In a recent publication, Zecca et al. reported on 12 FA patients who were given T cell depleted, CD34+ positively selected cells from a haploidentical related donor after a reduced intensity conditioning regimen including fludarabine (120 mg/m²), cyclophosphamide (1200 mg/m²), ATG-Fresenius (40 mg/kg) with and without radiation (2 Gy TBI) [72]. Engraftment was achieved in 9 of 12 patients (75%) and the cumulative incidence of graft rejection was 17% (95% confidence interval [CI], 5–59%). The frequencies of grades II–IV aGVHD and cGVHD were 17% (95% CI, 5–59%) and 35% (95% CI, 14–89%), respectively. The cumulative incidence of transplant-related mortality was 17% (95% CI, 5–59%). The 5-year overall survival, event-free survival (probability of survival from time of transplant to occurrence of any event- graft failure, rejection or death from any cause- or the date of last follow up), and disease-free survival (probability of survival without evidence of disease at anytime after transplantation) were 83% (95% CI, 62–100%), 67% (95% CI, 40–93%), and 83% (95% CI, 62–100%), respectively [72]. Another group published their experience with 3 FA haploidentical HSCTs [66]. The patients received cyclophosphamide, fludarabine, and radiation. All 3 patients engrafted. One patient died from infection on day +37. Of the 2 remaining patients, both developed \geq grade II aGVHD and one patient developed mild cGVHD [66]. Dufort et al. also reported on 3 FA patients who were given fludarabine, cyclophosphamide, ATG, and TLI prior to haploidentical transplantation [18]. The patients engrafted, did not experience GVHD, and are alive at last follow-up (5, 6, and 16 months) [18].

Charité University Medicine Berlin performed one haploidentical transplant for FA using the GEFA03 conditioning regimen. The patient had MDS classified as refractory anemia with 7% blasts and complex karyotype including a chromosome 3 abnormality. He received CD34+ positively selected PBSC from a paternal uncle and engrafted neutrophils and platelets. The patient did not experience any aGVHD, but did develop mild cGVHD. Approximately 18 months post-transplantation, the marrow showed recipient marrow reconstitution and progression to AML. The patient subsequently received multiple donor lymphocyte infusions, reinduction chemotherapy, and a second haploidentical transplant from his father. He eventually died of AML 8 years after the first transplantation. At Hannover Medical School Children's Hospital, one FA patient with AML underwent

a paternal haploidentical HSCT. The patient received pre-transplantation therapy with low-dose cytarabine and developed prolonged aplasia and pulmonary aspergillosis. The patient went on to receive a megadose of CD34+ positively selected PBSC and granulocyte transfusions until engraftment after conditioning with cyclophosphamide, fludarabine, and ATG. She developed mild cGVHD involving her skin and is alive and well 15 years after transplantation. In Tuebingen, one FA patients received a haploidentical transplants from a paternal grandmother. Here, the conditioning regimen included cyclophosphamide, fludarabine, ATG, irradiation, and TCR $\alpha\beta$ /CD19 depletion of the graft. The patient engrafted neutrophils and platelets. The patient developed grade I aGVHD but no cGVHD and is alive. Haploidentical transplantation in FA is emerging as an alternative therapy for some FA patients. There appears to be more experience using ATG in this setting. Concepts using post stem cell infusion cyclophosphamide for T cell allodepletion are being developed for FA patients receiving mismatched allografts including haploidentical transplants [66].

Cord blood transplantation

Although the first successful related cord blood transplantation (CBT) occurred in a patient with FA [26], there is a paucity of information regarding FA patients and CBT. EBMT retrospectively analyzed the results of unrelated cord blood transplantation (UCBT) in 93 FA patients from 26 centers worldwide transplanted between 1994 and 2005 [29]. The median age at transplantation was 8.6 years. The majority of patients received an HLA mismatched cord blood allograft (1 HLA difference 35 cases and 2 or 3 HLA differences 45 cases). The median number of nucleated cells and CD34+ cells infused was 4.9×10^7 /kg and 1.9×10^5 /kg, respectively. The transplants were completed using varying preparative regimens; 61% of patients received fludarabine [29]. Cumulative incidence of neutrophil recovery was $60 \pm 5\%$ at day +60. The incidence of grade II–IV aGVHD and cGVHD was $32 \pm 5\%$ and $16 \pm 4\%$, respectively. Overall survival was $40 \pm 5\%$. In multivariate analysis, factors associated with favorable outcome were use of fludarabine in the conditioning regimen, $\geq 4.9 \times 10^7$ /kg nucleated cells infused, and negative recipient CMV serology [29]. More recent reports describe small institutional experiences and demonstrate conflicting results. Ayas et al. reported on 7 FA patients who received partially matched unrelated cord blood grafts. The patients were conditioned with cyclophosphamide (5 mg/kg/day for 4 days), fludarabine (30 mg/m²/dose for 5 days), and rabbit ATG (5 mg/kg/day for 4 days) [1]. The therapy was generally well tolerated with mucositis and CMV viremia representing major therapy-related complications [1]. 4 patients failed to engraft and another patient had secondary graft failure 7 months post-transplantation. One patient developed grade II aGVHD of the skin that evolved to cGVHD. At last follow-up ranging from 29 to 34 months, 3 patients are alive but only 2 are transfusion independent [1]. Jaing et al. administered fludarabine (30 mg/m²/day for 6 days), cyclophosphamide (60 mg/kg/day for 2 days), and rabbit ATG (2.5 mg/kg/day for 3 days) in 3 FA patients who received unmanipulated umbilical cord blood allografts [36]. None of the patients developed significant regimen related toxicity and all engrafted within 10–19 days [36]. All patients are well with stable or full donor chimerism after a median follow-up of 64 months [36]. In summary, CBT in FA is evolving.

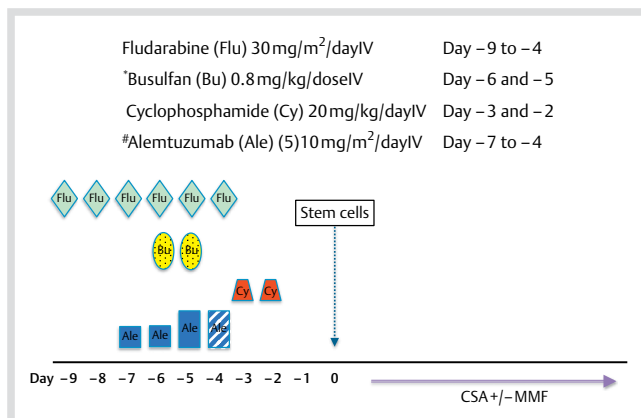


Fig. 1 Recommended allogeneic hematopoietic stem cell transplantation schema based on GEFA03. * Omit busulfan for low risk patients defined as cytopenia without evidence of leukemia or cytogenetic aberrations and with HLA identical donor (i.e. $\geq 9/10$ MSD, MRD, or MUD). Busulfan levels recommended. #Decrease alemtuzumab dose to 20 mg/m² in patients with MSD allografts (10/10) and only if BM is used as stem cell source. In patients with MSD ($\leq 9/10$), unrelated donor, or PBSC grafts, total alemtuzumab dose should remain 30 mg/m². The total dose of conditioning drugs is: fludarabine 180 mg/m², IV busulfan (Busilvex®) 1.6 mg/kg, cyclophosphamide 40 mg/kg, and alemtuzumab 30 mg/m² for MUD, MMRD, or PBSC (5 mg/m² days -7 and -6 followed by 10 mg/m² days -5 and -4) reduced to 20 mg/m² for MSD (5 mg/m² days -7 and -6 followed by 10 mg/m² day -5).

Conclusions and recommendations

HSCT represents a curative option for the severe hematological complications associated with FA. The therapeutic procedure is, however, characterized by unique challenges in this patient population. The consensus meetings on HSCT in FA aims to build upon previous German and international experiences and incorporate new data and technologies to improve outcomes. The current conclusions and recommendations are as follows:

1. Chemotherapy-only conditioning regimens resulted in equal or better outcomes than regimens that included radiotherapy, also in patients with MDS/AML. There is no reason to reintroduce radiotherapy into conditioning regimens for FA.
2. The chemotherapeutic backbone in the German GEFA03 study, the ESID-EBMT recommendations, and other modern conditioning therapies consist of fludarabine and cyclophosphamide [30]. This combination also forms the basis of the current recommendation. • **Fig. 1** illustrates the preparative regimen.
3. The addition of busulfan to the fludarabine cyclophosphamide backbone is probably not necessary in patients transplanted for BMF without blood cell clonal aberration(s) and/or MDS/AML, if transplanted from a $\geq 9/10$ HLA matched related or unrelated donor.
4. Busulfan is recommended to facilitate engraftment in patients with mismatched/haploidentical allografts ($< 9/10$) and as anti-leukemic agent in patients with bone marrow clonal aberrations and/or MDS/AML. The intravenous formulation is preferred due to more consistent blood levels. To gain more knowledge about the pharmacology of busulfan in FA, pharmacological monitoring of busulfan levels is recommended.
5. To prevent GVHD, in vivo T cell depletion is recommended in all FA patients, including patients transplanted from an MSD.

6. Alemtuzumab is considered superior to ATG in FA patients because of its better GVHD prophylactic effect.
7. In addition to anti-T cell antibody, CSA (day -1 until day +100, then taper) is employed for GVHD prophylaxis. MMF (day -1 to day +28, then taper) is added to CSA in unrelated donors only. Continuation or tapering of CSA and MMF is dependent on the presence or absence of GVHD.
8. Bone marrow is preferred as a stem cell source over PBSC because of its lower risk for GVHD.
9. For matched ($\geq 9/10$) PBSC grafts, limitation of the total T cell dose of the unmanipulated graft or ex vivo reduction of the T cell dose may be considered to avoid GVHD. For mismatched/haploidentical ($< 9/10$) transplants, for T cell depletion is mandatory.
10. Haploidentical transplantation concepts in FA are under development; no specific concept can be favored over another at this time.
11. Because CBT in FA is still evolving, unrelated cord blood as a stem cell source is not generally recommended for transplantation of FA patients at this time.
12. Although most transplant experience stems from HSCTs conducted in children with FA, these recommendations are intended for pediatric and adult patients with the condition.

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