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Haematopoietic cell transplantation for acute leukaemia and advanced myelodysplastic syndrome in Fanconi Anaemia

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Summary

Acute leukaemia or advanced myelodysplastic syndrome (MDS 5% blasts) in Fanconi Anaemia (FA) patients is associated with a poor prognosis. We report 21 FA patients with acute leukaemia or advanced MDS who underwent haematopoietic cell transplantation (HCT) at the University of Minnesota from 1988–2011. Six patients had biallelic *BRCA2* mutations. Eight patients received pre-transplant cytoreduction, with 3 achieving complete remission. HCT donor source included HLA-matched sibling (n=2), or alternative donors (n=19). Neutrophil engraftment was 95% for the entire cohort, and the incidence of acute GVHD was 19%. 5 year overall survival (OS) was 33%, with a relapse rate of 24%, with similar OS in patients with biallelic *BRCA2* mutations. Our study supports the use of HCT in the treatment of FA patients with acute leukaemia or advanced MDS, however, the role of chemotherapy prior to HCT remains unclear for this population. FA patients with biallelic *BRCA2* are unique and may benefit from higher dose chemotherapy relative to other complementation groups.

Keywords

Fanconi Anaemia; transplantation; leukaemia; MDS

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Author Contributions

RM analysed the data and wrote the manuscript, TD performed the statistical analysis, HZ collected and analysed the data, BH interpreted and reviewed the cytogenetic studies and critically reviewed the manuscript, JW and MM designed the research, contributed patients to the study, and critically reviewed the manuscript.

Disclaimer

The authors have no conflict of interest to declare.

Introduction

Fanconi Anaemia (FA) is a rare inherited disorder of chromosome fragility, associated with multiple congenital anomalies, bone marrow failure, and marked predisposition to malignancy (Wagner, *et al* 2008). Up to 98% of FA patients will develop bone marrow failure by the age of 40 years, with a median age of onset of 7 years (Kutler, *et al* 2003, Wagner, *et al* 2008). Whilst bone marrow failure remains a leading cause of death, the high incidence of both haematological and solid tumours in these patients is a significant cause of morbidity and mortality. FA patients have a 30% chance of developing haematological malignancy by the age of 40 years (Kutler, *et al* 2003), and rates of solid tumours, particularly squamous cell carcinoma, are projected to be even higher (Alter 2003). FA has significant genetic heterogeneity (Knies, *et al* 2012), which may have influence on rates of malignancy and treatment response. Notably, a subset of patients with biallelic *BRCA2* mutations have been shown to have a particularly high rate of malignancies at a very early age (Howlett, *et al* 2002, Offit, *et al* 2003, Wagner, *et al* 2004).

Haematological malignancy in FA patients most often manifests as myelodysplastic syndrome (MDS) or acute myeloid leukaemia (AML) (Alter 2003, Butturini, *et al* 1994). There are also isolated cases of acute lymphoblastic leukaemia (ALL) reported in the literature (Flatt, *et al* 2012, Sugita, *et al* 2000). The chemosensitivity associated with FA makes treatment challenging. Historically the presence of MDS has been recognized as a poor prognostic factor (Alter, *et al* 2000), and the development of leukaemia in FA patients has led to rapid almost uniform mortality (Auerbach and Allen 1991). The literature documenting the use of haematopoietic cell transplantation (HCT) in these patients is limited. A recent CIBMTR study reported outcomes for FA patients transplanted for acute leukaemia, MDS, or cytogenetic abnormalities, the majority of whom had no clinical disease, and survival for the leukaemia patients who received matched related donor transplants was 43% (Ayas, *et al* 2013). Prior to this the largest series published for FA patients with leukaemia or MDS contained only five patients (Ayas, *et al* 2004).

The role of chemotherapy is also not well understood. The Cincinnati group has demonstrated the safe use of a reduced intensity FLAG regimen in FA patients with AML (Mehta, *et al* 2007), however it is unclear whether patients attain complete remission. The recent CIBMTR study contains 6 patients who received chemotherapy prior to transplant, but the treatment regimens are not documented (Ayas, *et al* 2013). Therefore we still do not know if chemotherapy to reduce the burden of leukaemia prior to HCT leads to better transplant outcomes.

This study aimed to determine whether long term cure is possible in FA patients with haematological malignancy. This is the largest single institution cohort reported to date, with both HCT and pre-transplant chemotherapy documented, and is amongst the first to demonstrate that long term remissions are possible, even for patients with *BRCA2*.

Methods

Patients

A retrospective review was conducted of 157 FA patients that underwent HCT at the University of Minnesota between 1988 and 2011. Diagnosis of FA was made by demonstration of increased chromosomal breakage in patient lymphocytes by the addition of Diepoxybutane and/or Mitomycin C (Auerbach, *et al* 1989, Cervenka and Hirsch 1983). Inclusion criteria required all FA patients to have a confirmed diagnosis of acute leukaemia or advanced MDS on bone marrow biopsy. Advanced MDS was defined as having 5% blasts present in the bone marrow at diagnosis, as published by the WHO revised criteria of 2008, which defined refractory anaemia with excess blasts-1 (RAEB-1) as the presence of 5–9% blasts in the bone marrow with unilineage or multilineage dysplasia, and RAEB-2 as the presence of 10–19% blasts in the bone marrow (Vardiman, *et al* 2009). The use of these criteria in the diagnosis of MDS in FA patients has previously been validated by our institution (Cioc, *et al* 2010). Patients were assigned a diagnosis based on their highest recorded blast count.

Pre-transplant cytogenetic studies were conducted at the University of Minnesota for 18 patients, and at the referring institution for 2 of the patients. G-banding and FISH were used to characterise the clones.

All patients underwent first HCT at the University of Minnesota, and a proportion of patients received chemotherapy before progressing to HCT. All patients and/or guardians signed University of Minnesota Human Subjects Institutional Review Board approved informed consent in accordance with the Declaration of Helsinki. Studies were registered at <http://www.clinicaltrials.gov> under the identifiers NCT00005898, NCT 00167206, NCT 00258427 and NCT00352976.

Treatment

Chemotherapy was used in 8 patients prior to HCT, and has been documented in Table I. Two patients who received chemotherapy at outside institutions were unable to have specific doses recorded. The decision to give chemotherapy prior to transplant was at the discretion of the referring physician. Three patients received further chemotherapy to treat relapse following HCT, and are also documented in Table I.

Conditioning regimen was dependent on era of transplantation, with low dose cyclophosphamide used in combination with either busulfan or single dose total body irradiation (TBI), and a majority of patients received fludarabine to aid engraftment (Table I). In vivo T cell depletion was performed in 19 patients using anti-thymocyte globulin, and ex vivo T cell depletion was performed on all 15 bone marrow donor transplants using positive CD34⁺ selection. Two patients who relapsed after HCT underwent a second transplant, and conditioning regimens used are documented in Table I.

Patients were nursed in HEPA filtered rooms, and received standard antibacterial and antifungal therapy for infection prophylaxis. Cyclosporin A was given starting at day –3, and tapered at day +100 for matched sibling donors and day +180 for unrelated donors.

Patients received antibiotic prophylaxis until at least engraftment. Broad-spectrum intravenous antibacterial and as indicated antifungal and/or antiviral antimicrobials were used when patients developed fever. Patients received acyclovir prophylaxis if they were seropositive for herpes simplex virus and/or cytomegalovirus (CMV). Oral trimethoprim-sulfamethoxazole was given for *Pneumocystis jirovecii* pneumonia prophylaxis for 1 year after engraftment. CMV-seronegative recipients received CMV-safe (CMV-seronegative or filtered) blood products. All patients received granulocyte colony-stimulating factor 5µg/kg/day IV from the day after HCT until the absolute neutrophil count (ANC) $2.5 \times 10^9/L$ for two consecutive days. CMV reactivation was monitored weekly until at least day 100 post transplant.

Analysis of outcomes

Neutrophil engraftment was defined as having an ANC greater than $0.5 \times 10^9/L$ for 3 consecutive days. Platelet engraftment was defined as having a platelet count of at least $20 \times 10^9/L$ for 7 days unsupported by transfusion. Primary graft failure was defined as failure to achieve an ANC of $0.5 \times 10^9/L$ by day +42 post HCT. Acute and chronic graft versus host disease (GVHD) were defined using previously published criteria (Przepiorka, *et al* 1995, Shulman, *et al* 1980). Relapse was defined as the presence of the patient's original malignancy as diagnosed on bone marrow biopsy.

Kaplan-Meier curves were used to estimate the probability of survival through 5 years post-transplant. The log-rank test was used to complete the comparison (Kaplan and Meier 1958). Cumulative incidence treating non-event deaths as a competing risk was used to estimate the probability of relapse, neutrophil engraftment, and acute and chronic GVHD, treating non-event deaths as a competing risk (Lin 1997).

Results

Twenty-one FA patients were identified to have a diagnosis of acute leukaemia or advanced MDS, with an average age of 13.7 years (range 1.4–48.5). Patient and transplant characteristics are shown in Table I. There were 12 patients with AML, 8 with advanced MDS, and 1 patient treated for ALL. The median cell dose for donor bone marrow was $2.3 \times 10^8/kg$ total nucleated cells (range 0.1–11.1), and for cord blood was $3.0 \times 10^7/kg$ (range 1.5–31.1).

Cytogenetics

Of the 20 patients with cytogenetics available for review, 2 had normal cytogenetics and 18 had abnormal clones. The total number of cytogenetic abnormalities and major recurring abnormalities are documented in Table 1. The most frequent abnormalities observed included monosomy 7 (7L), loss of a portion of the long arm of chromosome 5 (5qL), gain of an extra copy of the distal long arm of chromosome 1 (1qG), gain of an extra copy of the distal long arm of chromosome 3 (3qG), and loss of the distal short arm of chromosome 17 to include the TP53 locus (17pL). In total, eight patients had high risk cytogenetics based on the presence of 5qL or 7L. Most of these clones were highly complex with numerous other structural and/or numerical abnormalities. Three of the 20 patients had 3qG without

concomitant 7L or 5qL but with concomitant other abnormalities. The cytogenetics for the BRCA2 patients did not differ in constellation from those of the other FA complementation groups.

Chemotherapy

Eight patients received chemotherapy for their primary malignancy prior to their HCT conditioning, three received chemotherapy to treat relapsed disease, and two patients went on to have a second transplant (Table I). Four patients also had more than one malignancy, with one case of T cell ALL and 5 cases of solid tumours occurring prior to the malignancy for which they received HCT. Table I documents the treatment each patient received for these other malignant conditions.

Of the eight patients that received chemotherapy prior to transplant, 3 were able to achieve remission, one of whom had the *BRCA2* mutation. Of the 5 patients that were not able to achieve remission, 3 were *BRCA2* patients.

Chemotherapy associated toxicity separate to HCT was seen in a total of 6 patients. Respiratory failure occurred in one patient, one patient developed persistent aplasia, and one patient developed both sepsis and persistent aplasia, all of which was associated with chemotherapy prior to undergoing HCT. One patient who received chemotherapy post HCT for relapsed disease developed cytarabine related neurotoxicity and retinal infarction. None of these four patients had the *BRCA2* mutation. The toxicities that occurred in *BRCA2* patients were related to treatment for separate malignancies, T cell ALL and medulloblastoma, and the patients developed a central nervous system bleed and persistent aplasia respectively.

Transplant outcomes

The cumulative incidence (CI) of neutrophil engraftment was 95% for the cohort (95% Confidence Limit (CL) 86–100%) at a median of 14 days (range 9–36). One patient did not engraft due to persistent malignant disease. For the entire cohort, the CI of grade II–IV GVHD and grade III–IV GVHD at day +100 was 19% (95% CL 3–35%) and 14% (95% CL 0–28%) respectively. The CI of chronic GVHD at 1 year was 5% for the entire cohort (95% CL 0–13%).

Survival

Overall survival (OS) at 5 years was 33% for the cohort (95% CL 15–53%) (Figure 1). The 5 year OS for patients that received fludarabine was 38% (95% CL 14–63), and 33% (95% CL 12–56) for the *BRCA2* patients. Median follow up was 9.6 years (range 3.4–18.8). Blast count at the time of HCT had no correlation with survival, with a relative risk of 1.0 (95% CL 0.98–1.03).

Looking at survival in relation to cytogenetics, patients with 5qL and/or 7L had a 5 year OS of 25% (95% CL 4–56%), those with 3qG had a 5 year OS of 100%, and the rest of the cohort had a 5 year OS of 11% (95% CL 1–39%) ($P = 0.02$). When survival for patients with

5qL and/or 7L were compared to the rest of the cohort, the result was not significant ($P = 0.81$).

Of the 8 patients that received chemotherapy prior to HCT, only two were alive at the time of reporting, and only 1 of the 3 patients who achieved remission with their pre-transplant chemotherapy (Patient 21 with T-ALL) was alive at the time of reporting.

The CI of relapse for the cohort was 24% (95% CL 6–42%), and 25% (95% CL 3–47%) and 50% (95% CL 13–87%) for the fludarabine and BRCA2 patients respectively. Causes of death included disease relapse ($n=7$), organ failure ($n=4$), interstitial pneumonitis ($n=2$) and fungal infection ($n=2$).

Discussion

While HCT for FA patients with marrow failure has been well documented, the role of HCT for FA patients with acute leukaemia or advanced MDS is less defined. This study demonstrates that long term remission can be achieved for these patients after HCT. While initial results are encouraging, survival for these patients remains significantly lower than that for FA patients with bone marrow failure (Farzin, *et al* 2007, Wagner, *et al* 2007). This demonstrates that a more effective HCT approach to these challenging patients is still required.

The role of pre-transplant cytoreduction in FA patients with advanced MDS or leukaemia remains unclear. Our study contained a number of patients that received chemotherapy prior to HCT, however the variety of different therapies precludes definitive conclusions regarding their effectiveness. Cytoreduction was not beneficial for these patients, most of whom had a diagnosis of AML, and is unlikely to be beneficial for patients with either AML or MDS, although this cannot be stated with certainty. The small size of the cohort also makes it hard to comment on the relationship between achieving remission prior to HCT and long term survival. What remains clear is that there continues to be significant toxicity associated with chemotherapy use in these patients. Aplasia was a significant toxicity associated with pre-transplant cytoreduction, stressing the importance of securing a donor before pursuing chemotherapy prior to HCT.

BRCA2 patients appear to be a unique population who require particular monitoring and tailored therapy to improve HCT outcomes and prevent relapse. Previous literature has documented the increased risk of haematological malignancy in patients with biallelic *BRCA2* mutations (Alter 2006, Barber, *et al* 2005, Wagner, *et al* 2004). Of the 21 patients in our cohort, 6 were found to have the *BRCA2* mutation present, and the relapse rate for these 6 patients was 50%. Three received pre-transplant cytoreduction for AML, none of whom achieved remission, but notably none of these BRCA2 patients experienced significant toxicities related to their chemotherapy. The only toxicities observed in the BRCA2 patients were related to treatment for prior malignancies (medulloblastoma and T-ALL), where higher dose chemotherapy was given. When we appreciate these observations in a broader context, it may imply that the BRCA2 patients require a more intensive therapy to prevent

relapse compared to other FA patients, but are still unable to tolerate doses used for the general population.

In this paper we chose to define advanced MDS as the presence of ≥ 5% blasts in the bone marrow, while previous literature has defined MDS as the presence of any myelodysplasia (Ayas, *et al* 2004, Guardiola, *et al* 2003). A recent CIBMTR report used the broader definition to examine HCT outcomes for MDS in FA, and reported a similar 5 year OS to that of patients with clonal abnormalities (57% v 58%) (Ayas, *et al* 2013). This demonstrates the importance of clearly defining MDS to be able to compare therapeutic options.

Cytogenetics have significant prognostic value in MDS and AML. Abnormal clones are generally characterised as high risk, intermediate risk or low risk dependent on the presence of specific chromosomal abnormalities and the overall complexity of the clone (Grimwade, *et al* 1998). None of the patients had any of the recurring rearrangements (e.g. t(8;21);inv(16); t(6;9); 11q23(MLL)) common in de-novo AML of non-FA patients (Grimwade, *et al* 1998, Martinez-Climent, *et al* 1995). Rather, the most frequent abnormalities observed in the cytogenetic clones (7L, 5qL, 3qG, 1qG, 17pL) have been previously documented as recurring abnormalities in FA, with the 1qG, 3qG and 7L accounting for the majority of clonal abnormalities observed in bone marrow of these patients (Cioc, *et al* 2010, Mehta, *et al* 2010, Meyer, *et al* 2012, Tonnies, *et al* 2003). The 3qG abnormality appears to be specific to FA, rarely occurring in non-FA patient with MDS or AML, and although not included in the WHO or other risk classifications for cytogenetics, has been proposed as a negative prognostic indicator with frequent evolution of the clone to include monosomy 7 (Cioc, *et al* 2010, Tonnies, *et al* 2003). Eight of the patients in this study had high risk cytogenetics based on the presence of 7L or 5qL, one of whom also had gain of 3q, and four patients had 3qG without concomitant 7L or 5qL. There was no significant survival difference related to the presence of high risk cytogenetics, and all patients with 3qG survived, however these results may be influenced by sample size.

One of the limitations in studying leukaemia and MDS in FA is its rarity. Whilst the cumulative risk for haematological malignancy in FA is high, the average incidence of acute leukaemia or advanced MDS in these patients is only approximately 9% (Auerbach and Allen 1991). We have reported the largest single institution cohort to date, but these cases have been collected over a period of 23 years, during which time therapy has changed significantly for HCT, treatment of leukaemia and supportive care issues. Future studies would ideally standardize therapy for these patients, however its rarity makes prospective trials challenging. The best way forward is to collect more data, as no registry currently exists for these patients. An international FA registry would be beneficial for collecting data on the use of chemotherapy prior to transplant, and to advance the collective knowledge of treating haematological malignancy in FA patients.

For FA patients who develop malignancies, it is important to minimize exposure to standard chemotherapy, to minimize DNA damage to fragile host cells. Cellular therapy utilizing a graft-versus-tumour effect may be an option, however, to date efforts have not been able to isolate this effect without inducing GVHD (Guardiola, *et al* 2003). As our understanding

and manipulation of the graft-versus-tumour process improves, specific cellular therapy combined with tailored immunosuppression may be used successfully in this patient population.

The treatment of FA patients who develop haematological malignancies remains challenging. It remains essential for FA patients to have complete blood counts performed at least every 3 months, and the authors recommend bone marrow examination at least annually for early detection of bone marrow failure, myelodysplasia, or cytogenetic clonal abnormalities. For those patients that develop acute leukaemia or advanced MDS, it is reasonable to pursue HCT, with an aim of remission and long term survival.

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Figure 1.
5 year overall survival and relapse for the cohort

Table 1

Patient and transplant characteristics. Matched sibling donors had antigen level typing at Human leukocyte antigen (HLA) -A, -B, and -DRB1. For unrelated donors, patients and donors had antigen level typing at HLA-A and -B and allele level typing at HLA-DRB1 until June 2005, when testing at the allele level for all loci was implemented. For UCB units, patients and donors were typed for HLA-A and -B at the antigen level and for -DRB1 at the allele level. Prospective allele level typing for HLA-C was incorporated for all donor sources beginning June 2004.

Pt	Sex	Complementation group	Malignancy	Number of cytogenetic abnormalities	Major recurring cytogenetic abnormalities	Blasts at Dx	Blasts at HCT	Treatment	Toxicity	Age at HCT	Year of HCT	Donor source	HLA match	Day of engraftment	Day of relapse	Alive post HCT	Cause of death
1	M		AML	Unknown		62	40	HCT <i>Cyclophosphamide</i> (40mg/kg) <i>TBI</i> (450cGy) <i>ATG</i> (195mg/kg)		28.0	1988	MSD	6/6	15		No	Fungal infection
2	M		AML	1	3qG	6	25	HCT <i>Cyclophosphamide</i> (40mg/kg) <i>TBI</i> (450 cGy)		28.5	1994	URD	6/6	23		18.8 yrs	
3	F	FANC-C	AML	1	1qG	14	40	HCT <i>Cyclophosphamide</i> (40mg/kg) <i>TBI</i> (450 cGy) <i>ATG</i> (150mg/kg) Post HCT <i>Cytarabine</i> 100mg/m ² /dose x 7 <i>Daunomycin</i> 20mg/m ² /dose x 3 <i>Cytarabine</i> 100mg/m ² /dose x 7 <i>Daunomycin</i> 20mg/ m ² /dose x 3 <i>Asparaginase</i> 6000IU/ m ² /dose x 1 <i>Cytarabine</i> 3g/m ² /day x 2 <i>Asparaginase</i> 6000IU/ m ² /dose x 1 <i>Cytarabine</i> 3g/m ² /day x 2 DLI	Neurotoxicity Retinal infarcts	21.9	1995	URD	5/6	13	175	No	Relapse

Pt	Sex	Complementation group	Malignancy	Number of cytogenetic abnormalities	Major recurring cytogenetic abnormalities	Blasts at Dx	Blasts at HCT	Treatment	Toxicity	Age at HCT	Year of HCT	Donor source	HLA match	Day of engraftment	Day of relapse	Alive post HCT	Cause of death
4	F		Vulval SCC Nasal SCC AML	0		25	35	Hemivulvectomy Resection HCT Cyclophosphamide (40mg/kg) TBI (450 cGy) ATG (150mg/kg)		27.7	1996	URD	6/6	Death		No	Organ failure
5	F	BRCA2	AML	>10	5qL, 7L+ other	29	30	Pre HCT Cytarabine 100mg/m ² /dose x 7 Daunomycin 20mg/m ² /dose x 3 HCT Cyclophosphamide (40mg/kg) TBI (450 cGy) ATG (150mg/kg)		3.7	1996	URD	5/6	Failure	32	No	Persistent disease
6	M		AML	0		72	0	Pre HCT Cytarabine 100mg/m ² /dose x 7 Daunomycin 20mg/m ² /dose x 3 Cytarabine 3g/m ² /day x 2 Fludarabine 40mg/m ² /dose x 1 HCT Cyclophosphamide (40mg/kg) Fludarabine (140mg/m ²) TBI (450 cGy) ATG (150mg/kg)	Respiratory failure	15.3	1999	UCB	5/6	36		No	Relapse
7	F		AML	6	5qL, 7L, 17pL + other	20	35	HCT Cyclophosphamide (40mg/kg) Fludarabine (140mg/m ²) TBI (450 cGy) ATG (150mg/kg)		33.3	2000	URD	6/6	11		No	Organ failure
8	M	FANC-A	AML	7	5qL, 17pL	25	77	HCT Busulfan (3.2mg/kg) Cyclophosphamide (40mg/kg) Fludarabine (140mg/m ²) ATG (150mg/kg)		21.7	2003	URD	5/6	19		No	Interstitial pneumonitis

Pt	Sex	Complementation group	Malignancy	Number of cytogenetic abnormalities	Major recurring cytogenetic abnormalities	Blasts at Dx	Blasts at HCT	Treatment	Toxicity	Age at HCT	Year of HCT	Donor source	HLA match	Day of engraftment	Day of relapse	Alive post HCT	Cause of death
9	M	FANC-A	AML	>10	Near triploid with relative loss of 5 and 17 + other	88	50	Pre HCT Cytarabine 200mg/m ² /dose x 3 Daunomycin 20mg/m ² /dose x 3 Etoposide 100mg/m ² /dose x 3 6-thioguanine 100mg/m ² /dose x 3 Dexamethasone 6mg/m ² /day x 3 IT cytarabine 70mg dose x 1 HCT Busulfan (3.2mg/kg) Cyclophosphamide (40mg/kg) Fludarabine (140mg/m ²) ATG (150mg/kg)		6.9	2004	DUCB	5/6	20	59	No	Relapse
10	M	FANC-A	AML	3	1qG, 3qG + other	61	46	AML 2002 induction * Cytarabine Daunomycin Etoposide IT methotrexate Low dose consolidation * Cytarabine Daunomycin Etoposide HCT Busulfan (3.2mg/kg) Cyclophosphamide (40mg/kg) Fludarabine (140mg/m ²) ATG (150mg/kg)	Sepsis Aplasia	16.5	2004	DUCB	5/6	16		8.6 yrs	
11	M	BRCA2	AML	2	7L + other	58	9	AAML0531 arm B induction * Cytarabine Doxorubicin Etoposide Gemtuzumab HCT Cyclophosphamide (40mg/kg)		1.6	2009	MSD	6/6	10	48	No	Relapse

Pt	Sex	Complementation group	Malignancy	Number of cytogenetic abnormalities	Major recurring cytogenetic abnormalities	Blasts at Dx	Blasts at HCT	Treatment	Toxicity	Age at HCT	Year of HCT	Donor source	HLA match	Day of engraftment	Day of relapse	Alive post HCT	Cause of death
								Fludarabine (140mg/m ²) Fludarabine (140mg/m ²) TBI (300 cGy) ATG (150mg/kg) 2 nd HCT Busulfan (3.2mg/kg) Cyclophosphamide (40mg/kg) Fludarabine (140mg/m ²) ATG (150mg/kg) Post HCT* Decitabine									Mitchell et al.
12	M	BRCA2	Medulloblastoma AML	3	other	93	21	Resection and chemotherapy <i>Methotrexate</i> <i>5g/m²/day x 1</i> <i>Vincristine</i> <i>1mg/m²/dose x2</i> <i>Cisplatin</i> <i>75mg/m²/dose x1</i> <i>Cyclophosphamide</i> <i>1.5g/m²/dose x1</i> Reduced intensity consolidation* <i>Low dose cyclophosphamide</i> <i>Low dose etoposide</i> <i>High dose methotrexate</i> <i>IT methotrexate</i> Pre HCT <i>Fludarabine</i> <i>30mg/m²/dose x3</i> <i>Cytarabine</i> <i>300mg/m²/dose x 3</i> <i>Mitoxantrone</i> <i>3mg/m²/dose x 5</i> <i>Cytarabine</i> <i>100mg/m²/dose x 7</i> HCT <i>Busulfan</i> <i>(3.2mg/kg)</i> <i>Cyclophosphamide</i> <i>(40mg/kg)</i> <i>Fludarabine</i> <i>(140mg/m²)</i> <i>ATG (150mg/kg)</i>	Aplasia	5.8	2011	UCB	5/6	9	21	No	Persistent disease

Pt	Sex	Complementation group	Malignancy	Number of cytogenetic abnormalities	Major recurring cytogenetic abnormalities	Blasts at Dx	Blasts at HCT	Treatment	Toxicity	Age at HCT	Year of HCT	Donor source	HLA match	Day of engraftment	Day of relapse	Alive post HCT	Cause of death	
								Post HCT <i>Rituximab</i> 375mg/m ² /dose x 2 <i>Mitoxantrone</i> 3mg/m ² /dose x 5 <i>Cytarabine</i> 100mg/m ² /dose x 7 <i>Decitabine</i> 15mg/m ² /dose x 10 2 nd HCT <i>Cyclophosphamide</i> (40mg/kg) <i>Fludarabine</i> (140mg/m ²) <i>TBI</i> (300 cGy) <i>ATG</i> (150mg/kg)									Mitchell et al.	
13	F		RAEB-2	2	7L + other	19	15	HCT <i>Cyclophosphamide</i> (40mg/kg) <i>TBI</i> (450 cGy)		11.7	1992	URD	5/6	22		No		Organ failure
14	M		RAEB-1	4	1qG + other	7	7	HCT <i>Cyclophosphamide</i> (40mg/kg) <i>TBI</i> (450 cGy) <i>ATG</i> (150mg/kg)		48.4	1995	URD	6/6	15		No		Interstitial pneumonitis
15	M	FANC-A	RAEB-2	3	other	12	12	HCT <i>Cyclophosphamide</i> (40mg/kg) <i>TBI</i> (450 cGy) <i>ATG</i> (150mg/kg)		12.7	1996	URD	6/6	13		No		Fungal infection
16	F	FANC-A	RAEB-2	1	7L	0.2	14	HCT <i>Cyclophosphamide</i> (40mg/kg) <i>Fludarabine</i> (140mg/m ²) <i>TBI</i> (450 cGy) <i>ATG</i> (150mg/kg)		6.7	2001	URD	6/6	15		11.9 yrs		
17	M	BRCA2	RAEB-1	8	5qL, 3qG, 17pL + other	6	2	HCT <i>Cyclophosphamide</i> (40mg/kg) <i>Fludarabine</i> (140mg/m ²) <i>TBI</i> (450 cGy) <i>ATG</i> (150mg/kg)		1.5	2001	URD	6/6	11	110	No		Relapse
18	F	BRCA2	Wilms tumor T-ALL RAEB-1	3	3qG + other	7	7	Resection CCG 1991 * standard risk	CNS bleed	4.9	2004	URD	6/6	13		8.9 yrs		
																	Page	

Pt	Sex	Complementation group	Malignancy	Number of cytogenetic abnormalities	Major recurring cytogenetic abnormalities	Blasts at Dx	Blasts at HCT	Treatment	Toxicity	Age at HCT	Year of HCT	Donor source	HLA match	Day of engraftment	Day of relapse	Alive post HCT	Cause of death	
								Cyclophosphamide and doxorubicin excluded Cyclophosphamide and doxorubicin excluded Cyclophosphamide and doxorubicin excluded HCT Busulfan (3.2mg/kg) Cyclophosphamide (40mg/kg) Fludarabine (140mg/m ²) ATG (150mg/kg)										Mitchell et al.
19	F		RAEB-2	8	other	12	0	Pre HCT Cytarabine 2g/m ² /day x 4 Fludarabine 25mg/m ² /dose x 4 HCT Busulfan (3.2mg/kg) Cyclophosphamide (40mg/kg) Fludarabine (140mg/m ²) ATG (150mg/kg)	Aplasia	1.3	2007	UCB	5/6	10		No	Organ failure	
20	F	FANC-A	Cervical dysplasia RAEB-2	9	3qG + other	2	15	LEEP HCT Cyclophosphamide (40mg/kg) Fludarabine (140mg/m ²) TBI (300 cGy) ATG (150mg/kg)		25.7	2009	UCB	5/6	12		3.4 yrs		
21	M	BRCA2	T-ALL	7	other	13	0	Pre HCT Vincristine 1.5mg/m ² /dose x 4 Daunomycin 25mg/m ² /dose x 4 Pegaspargase 2500 IU/m ² /dose x 1 Prednisone 50mg/m ² /day x 28 IT methotrexate 12mg dose x 1 IT cytarabine 70mg dose x 1 6 Mercaptopurine 420mg/m ² /week x 4		5.7	2002	MMRD	5/6	11		10.3 yrs		Page 17

Pt	Sex	Complementation group	Malignancy	Number of cytogenetic abnormalities	Major recurring cytogenetic abnormalities	Blasts at Dx	Blasts at HCT	Treatment	Toxicity	Age at HCT	Year of HCT	Donor source	HLA match	Day of engraftment	Day of relapse	Alive post HCT	Cause of death
								Cytarabine 75mg/m ² Cytarabine 100mg/m ² Vincristine 1.5mg/m ² /dose x 4 Pegaspargase 2500 IU/m ² /dose x 2 IT methotrexate 12mg dose x 3 HCT Busulfan (3.2mg/kg) Cyclophosphamide (40mg/kg) Fludarabine (140mg/m ²) ATG (150mg/kg)									Mitchell et al.

ALL - Acute lymphoblastic leukaemia; AML - Acute myeloid leukaemia; ATG - Anti-thymocyte globulin; CNS - Central nervous system; Dx - Diagnosis; DUCB - double umbilical cord blood; HCT - Haematopoietic cell transplantation; IT - intrathecal; LEEP - Loop electrode excision procedure; MMRD - Mismatched related donor; MSD - Matched sibling donor; RAEB - Refractory anaemia with excessive blasts; SCC - Squamous cell carcinoma; TBI - Total body irradiation; UCB - Umbilical cord blood; URD - Unrelated donor; yrs - Years; IqG - Gain of an extra copy of the distal long arm of chromosome 1; 3qG - Gain of an extra copy of the distal long arm of chromosome 3; 5qL - Loss of a portion of the long arm of chromosome 5; 7L - Monosomy 7; 17pL - Loss of the distal short arm of chromosome 17 including the TP53 locus

* Patients received chemotherapy at outside institutions where specific doses were not able to be retrieved