SUPPLEMENTARY APPENDIX

Predictors of recovery following allogeneic CD34+-selected cell infusion without conditioning to correct poor graft function

Maria M. Cuadrado,[1] Richard M. Szydlo,[1,2] Mike Watts,[3] Nishil Patel,[4] Hanna Renshaw,[4] Jude Dorman,[5] Mark Lowdell,[6] Stuart Ings,[3] Chloe Anthias,[1] Alejandro Madrigal,[1] Stephen Mackinnon,[5] Panagiotis Kottaridis,[5] Ben Carpenter,[5] Rachael Hough,[5] Emma Morris,[5] Kirsty Thomson,[5] Karl S. Peggs[5,7] and Ronjon Chakraverty[5,7]

[1]Anthony Nolan Research Institute; [2]Department of Haematology, Imperial College London; [3]Wolfson Cellular Therapy Unit, University College Hospital London NHS Trust; [4]Department of Haematology, Royal Free London NHS Trust; [5]Department of Haematology, University College Hospital NHS Trust; [6]Centre for Cell, Gene & Tissue Therapeutics, Royal Free London NHS Trust and [7]Department of Hematology, Cancer Institute, University College London, London, UK

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Correspondence: *RONJON CHAKRAVERTY* - r.chakraverty@ucl.ac.uk

Supplementary table 1. Recent series of CD34-Selected Stem Cell Infusions.

Report	n/ median follow up (years)	Time from allo-SCT (days)	CD34 dose x10^6/kg (median)	CD3 dose x 10^3/kg (median)	% Recovery	Acute GVHD III-IV (n)	Chronic GVHD (n)	Overall survival	Comments
Askaa et al. 2014	18/ 4.1	113	4.9	11	72	2	9	2y 48%	6/18 patients with MF
Klyuchnicov et al. 2014	32/ 2.5	150	3.4	9	81	4	6	2y 45%	14/32 patients with MF
Stasia et al. 2014	41/ 3.5	140	3.5	6	75	0	0	3y 63%	
Ghobachi et al. 2017	9 G-CSF 9 G-CSF + Pl 8 Cryo Total 26/ NA	138	3.1 G-CSF 10.9 G-CSF + Pl 1 Cryo	<10^5/kg	81	0	8	1yr 65%	No clear difference in response rates according to product

Abbreviations: Pl-Plerixafor; Cryo- CD34 selection on cryopreserved products; MF –myelofibrosis; NA- not available

Supplementary comment 1.

$CD34^+$ stem cell selection and infusion

A CD34+ cell selection from G-CSF-mobilized PBSC was performed using the CliniMACS CD34 enrichment system (Miltenyi Biotec GmbH, Germany); this system uses a monoclonal anti-CD34 antibody (QBEnd10), directly conjugated to an iron oxide/dextran particle approximately 50 nm in size ('CD34 reagent'), to label target cells. PBS buffer was added to the PBSC harvest to a volume of 500ml before washing using a COBE 2991 Cell Processor (Terumo, UK) to reduce the incubation volume to 100ml. CD34 reagent was added followed by a 30-minute agitation cycle. After washing the product twice to remove non-reacting reagent, the harvest was loaded on to the CliniMACS device processing kit and the labelled $CD34^+$ cells captured by passing the cells through the magnet allowing the unlabelled $CD34^-$ cells to pass through to waste. To enhance the purity of the $CD34^+$ cells, three rounds of magnetic selection were applied before the magnet was withdrawn and the purified $CD34^+$ cells flushed into a collection bag for infusion. Cell counts were performed using a KX-21automated cell counter (Sysmex Ltd., UK). Final $CD3^+$ or $CD34^+$ cell frequencies were determined by staining separately with anti-CD3-FITC alone, or anti-CD34 HPCA-2-PE in conjunction with anti-CD45-FITC monoclonal antibodies (Becton Dickenson, UK) before flow cytometry (Coulter FC500, Beckman Coulter, UK) and data analysis using the Nordic gating protocol as described previously

Supplementary Table 2. Univariate analysis for Primary and Secondary Poor GF.

	N	Primary Poor GF N (%)	Secondary Poor GF, N (%)	P-Value
HCT-CI				
- Low risk	45	18 (40%)	27 (60%)	
- Intermediate risk	15	2 (13%)	13 (87%)	0.1
- High risk	2	1 (50%)	1 (50%)	
R/D sex				
- Unmatched	31	8 (26%)	23 (74%)	
- Matched	31	13 (42%)	18 (58%)	0.2
Donor type				
- Related Donor	28	5 (18%)	23 (82%)	
- Matched Unrelated Donor	18	8 (44%)	10 (56%)	0.05
- Mismatched Unrelated Donor	16	8 (50%)	8 (50%)	
ABO status				
- No incompatibility	35	14 (40%)	21 (60%)	
- Major incompatibility	14	3 (21%)	11 (79%)	0.4
- Minor incompatibility	13	4 (31%)	9 (69%)	
CMV status				
- R- D-	23	13 (57%)	10 (44%)	
- Other	39	8 (21%)	31 (80%)	0.004
EBMT Risk Score*				
- Early stage	19	5 (26%)	14 (74%)	
- Intermediate	28	10 (36%)	18 (64%)	
- Advanced	9	3 (33%)	6 (67%)	0.7
- None malignant disease	6	3 (50%)	3 (50%)	
Recipient age (median)				
- Age < 49 years	31	12 (39%)	19 (61%)	
- Age ≥ 49 years	31	9 (29%)	22 (71%)	0.4
Donor age (median)				
- Age < 38 years	27	11(41%)	16 (59%)	
- Age ≥ 38 years	35	10 (29%)	25 (71%)	0.3
Acute GvHD				
- Grades 0-I	42	12 (29%)	30 (72%)	
- Grades II-IV	20	9 (45%)	11 (55%)	0.2
Chronic GvHD				
- None	37	13 (35%)	24 (65%)	
- Mild	11	4 (36%)	7(64%)	
- Moderate	9	2 (22%)	7 (78%)	0.2
- Severe	3	0	3 (100%)	
- Non Evaluable**	2	0	2 (100%)	

Abbreviations: HCT-CI- Hematopoietic cell transplantation-specific comorbidity index; R/D – Recipient / Donor; GvHD – Graft versus host disease.
*EBMT Risk Score: Early disease stage includes Acute Leukemia (AL) transplanted in first complete remission (CR), MDS transplanted untreated or in first CR. Intermediate disease stage includes AL in second CR, CML in all other stages than first chronic phase or blast crisis, MDS in second CR or in PR, lymphoma and multiple myeloma in second CR, in PR or stable disease. Late disease stage includes AL in all other disease stages, CML in blast crisis, MDS in all other disease stages and lymphoma and multiple myeloma in all disease stages other than those defined as early or intermediate. Stage is not applicable for aplastic anemia, primary immunodeficiencies and sickle cell disease; **Patient died before 100 days post Allo-SCT.

Supplementary Table 3. Multivariate analysis for Secondary poor GF.

	N	OR (CI 95%)	P VALUE
CMV status			
- R-D-	39	1.0	
- Others	23	5.4 (1.6-18.8)	0.008
Donor type			
- Related Donor	28	1.0	
- Matched unrelated donor	18	0.4 (0.4-0.8)	0.2
- Mismatched unrelated donor	16	0.18 (0.4-0.8)	0.03

Abbreviations: R-D-: CMV Serostatus negative for recipient and donor

Supplementary table 4. Univariate analysis for variables related to CMV and recovery after CD34^{+}- selected infusion.

	N	Recovery, N (%)	P-Value
CMV Reactivation post allo-SCT			
- **No**	**27**	**24 (89%)**	
- **Yes**	**35**	**23 (66%)**	**0.04**
Day of CMV Reactivation post Allo-SCT (median)			
- < Day 30	16	10 (63%)	
- ≥ Day 30	16	11 (68%)	0.7
- Missing Values	3		
CMV PCR (median)			
- < 16,500 copies	16	10 (63%)	
- ≥ 16,500 copies	14	9 (64%)	0.9
- Missing Values	5		
Duration of CMV Treatment (median)			
- < 50 days	15	10 (67%)	
- ≥ 50 days	18	12 (67%)	1.0
- Missing Values	2		
Number of CMV Reactivations (median)			
- ≤ 1	16	9 (56%)	
- > 1	17	13 (77%)	0.2
- Missing Values	2		
CMV at the time of CD34^{+}- selected Infusion			
- No	25	17 (68%)	
- Yes	10	6 (60%)	0.7
CMV disease			
- No	28	20 (71%)	
- Yes	7	3 (43%)	0.2

Abbreviations: Allo-SCT – Allogeneic Stem Cell Transplantation.