Tracking Functional Immune Reconstitution Following Allogeneic Haemopoietic Stem Cell Transplantation

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Background

- Allogeneic haemopoietic stem cell transplantation (allo-HSCT) is used to cure haematological malignancies; however, it causes significant immunocompromise
- The post-transplant course is often complicated by opportunistic and vaccine-preventable infections
- Patients remain at risk of such infections until the immune system recovers; a process which can take many months to years[1,2]
- Antimicrobial prophylaxis and vaccination are used to prevent post-transplant infection; however, the optimal timing of these interventions is poorly-defined, limiting their effectiveness

Aims

- To characterise the kinetics of immune recovery following allo-HSCT in the modern era
- To correlate the laboratory findings with the clinical data to identify a panel of biomarkers of immune recovery with potential to guide the timing of antimicrobial prophylaxis and vaccination

Methods

Table 1. Overview of Methods

	Patients (n=20)	Healthy Donors (n=10)
Samples Collected	PBMCs and serum	PBMCs and serum
Collection Timepoints	Baseline* 3-, 6-, 9- and 12-months post-HSCT	Time of consent
Clinical data	Yes (Detailed) 12-month follow-up	Yes (to confirm eligibility)

*Within 96 hours of commencing conditioning therapy.
Abbreviations: PBMCs – peripheral blood mononuclear cells.

Table 2. Immunological Assays Performed at Each Timepoint

Assay	To Determine
Immunophenotyping	Immune cell counts
Immunoglobulin Isotyping	Concentrations of serum isotypes (e.g. IgM, IgG subtypes, IgA)
T-Cell Proliferation Assay	T-Cell proliferation in response to *in-vitro* PBMC stimulation with various pathogens and vaccines
Cytokine Profiling	Cytokine concentrations in response to i*n-vitro* PBMC stimulation with various pathogens and vaccines

Results

Clinical Outcomes

- Eighteen patients (90%) experienced at least one microbiologically-diagnosed infection.
- Six (30%) patients developed invasive fungal disease
- Five patients (25%) died, with 3 of the 5 (60%) deaths attributable to invasive fungal disease.

Immune Cell Counts

- Median B-, T- and CD8 T-cell counts returned to the normal range at 6 months post-transplant
- Median CD4 T-cells remained below the lower limit of normal (LLN) over the entire 12 months of follow-up

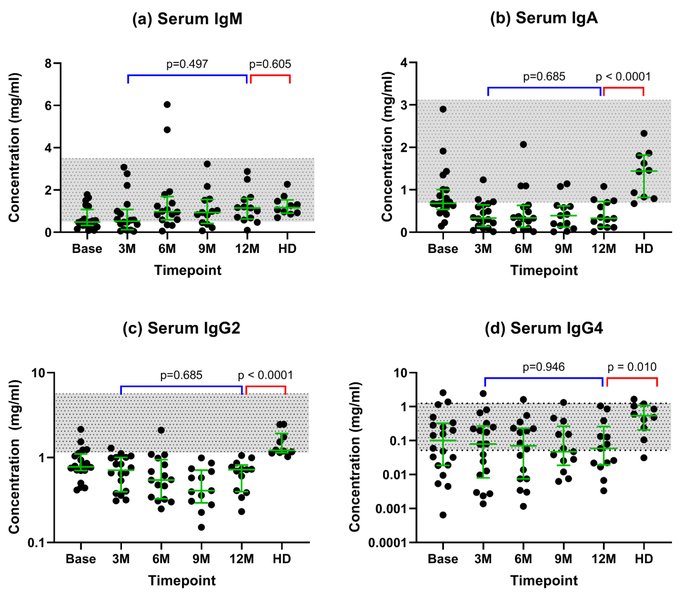

Figure 1 (a-d). Recovery of Immunoglobulin Isotypes Following Allo-HSCT
Wilcoxon signed-rank test was used to compare 3M with 12M data and Mann-Whitney U test was used to compare the 12M data with the healthy donors.
Grey band = normal range; Green bars = median and interquartile range; Black dots = different participants; Blue/red bars represent comparison groups for p-values

Base = baseline (n=20); 3M = 3 months (n=18); 6M = 6 months (n=16); 9M = 9 months (n=13); 12M = 12 months (n=13) ; HD = healthy donors (n=10); Ig = immunoglobulin.

Immunoglobulin Isotyping

- Median serum IgA and IgG_2 levels remained below the LLN for the entire 12 months of follow-up (Figure 1[b, c])
- Serum IgA, IgG_2 and IgG_4 were significantly lower at 12 months post-transplant compared with healthy donors (Figure 1[b-d])

T-Cell Proliferation Assay

- T-cell proliferation was significantly lower in allo-HSCT recipients compared with healthy donors in response to all pathogens and vaccines tested until at least 6 months post-transplant (Fig. 2a-f)
- Only two (10%) patients mounted an immune response to *Aspergillus* (Stimulation Index > 1.5) and not until the 12-month timepoint post-transplant (Fig. 2b)

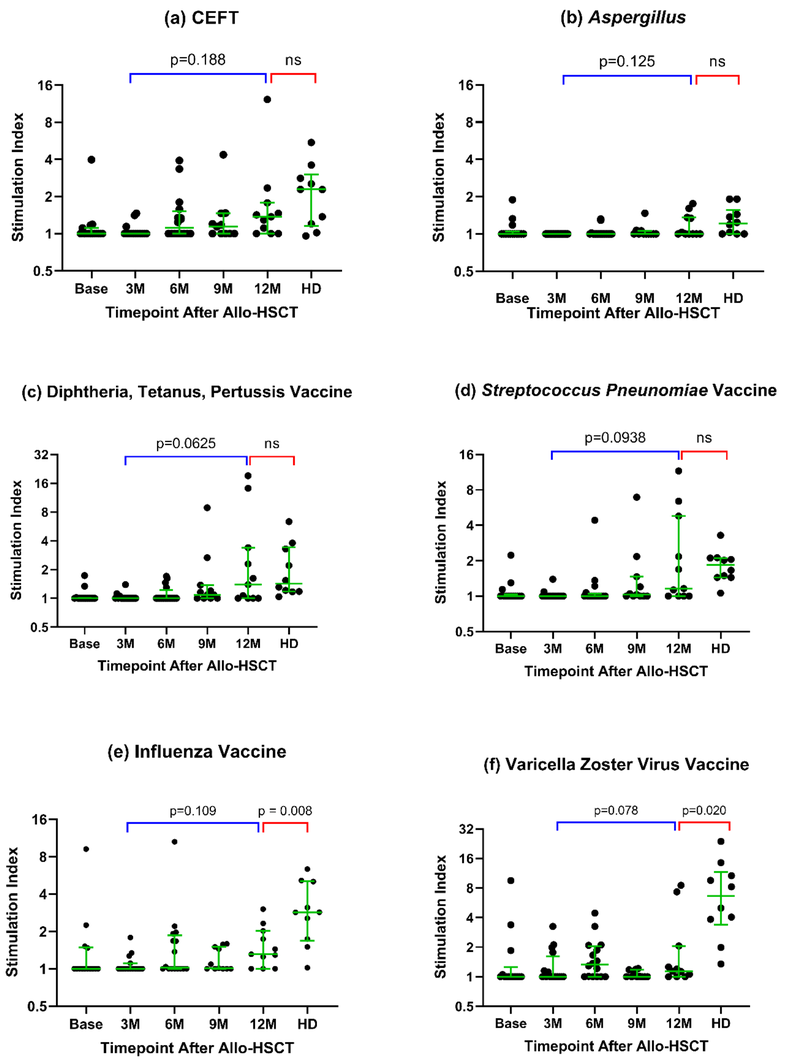

Figure 2(a-f). Lymphocyte Proliferation Assay
Wilcoxon signed-rank test was used to compare 3M with 12M data and Mann-Whitney U test was used to compare the 12M data with the healthy donors.
Green bars = median and interquartile range; Black dots = different participants; Horizontal bars = p-value for differences; Stimulation index = ratio of proliferation in the stimulated sample compared with corresponding unstimulated sample.

CEFT – Cytomegalovirus-Epstein-Barr virus-Tetanus-Influenza Peptide Pool; ns = not significant; Base = baseline (n=14); M = months; HD = healthy donors (n=10)

Cytokine Profiling

- Several cytokines were secreted in significantly lower amounts by allo-HSCT recipients compared with healthy donors in response to the various pathogens and vaccines (Figure 3[a,b)]
- T-cell proliferation correlated with the secretion of particular cytokines, depending on the pathogen or vaccine tested (e.g. *Aspergillus*: IFN-γ, IL-17F, IL-22, IL-23; p<0.05) (Figure 4)

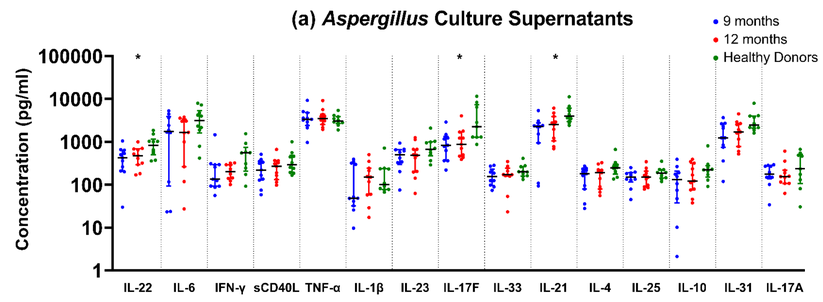

Figure 3(a,b). Culture Supernatant Cytokine Concentrations in Response to *in-vitro* PMBC Stimulation with *Aspergillus and CMV-EBV-Flu-Tetanus Peptide Pool*
* Indicates a statistically significant difference ($p < 0.05$) as compared with healthy donors. Compared using the Mann-Whitney U Test.

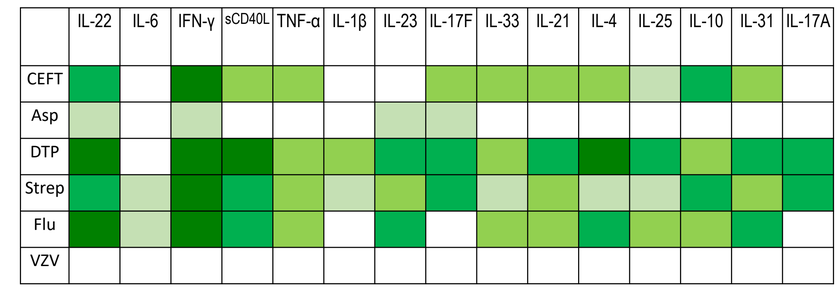

Figure 4. Correlation Between Cytokine Secretion and T-Cell Proliferation
Shaded squares indicate a statistically significant positive correlation, using Spearman's rank co-efficient
Abbreviations: CEFT = Cytomegalovirus-Epstein-Barr virus-Tetanus-Influenza Peptide Pool; Asp = Aspergillus; DTP = Diphtheria, Tetanus, Pertussis; Strep – Streptococcus pneumoniae; Flu – Influenza; VZV – Varicella Zoster Virus.

Clinical Correlation

- Those receiving myeloablative conditioning had higher CD4 T-cell counts and IgA levels at 12 months post-HSCT than those receiving non-myeloablative conditioning (p=0.002 and p=0.045, respectively).

Conclusions

- Immune recovery can be measured; but, remains a slow and highly variable process with significant immune deficits present 12 months post-transplant
- Several immune parameters, including CD4 T-cell counts, selected cytokines (IFN-γ, IL-1β, IL-4, IL-6, IL-17, IL-21, IL-31, TNF-α, IL-22, IL-23 and sCD40L) and immunoglobulin isotypes could serve as biomarkers of immune function following allo-HSCT
- Further studies are needed to validate this panel of biomarkers and to evaluate its clinical utility in guiding the timing of antimicrobial prophylaxis and vaccination

Acknowledgements

Funding: Unrestricted investigator-initiated grant from Merck, Sharp and Dohme

References

1. Gea-Banacloche J, Komanduri KV, et al. National Institutes of Health Hematopoietic Cell Transplantation Late Effects Initiative: The Immune Dysregulation and Pathobiology Working Group Report. Biol Blood Marrow Transplant. 2017;23(6):870-81.
2. de Silva HD, Ffrench RA, et al. Contemporary analysis of functional immune recovery to opportunistic and vaccine-preventable infections after allogeneic haemopoietic stem cell transplantation. Clinical & translational immunology. 2018;7(10):e1040.

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