Immune reconstitution following cellular therapy

IBTS Research Day 2023

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Irish Blood Transfusion Service
Seirbhís Fuilaistriúcháin na hÉireann

Trinity College Dublin
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The University of Dublin

Allogeneic Stem Cell Transplantation

The Stem Cell Transplant (SCT) Service in St James's Hospital was founded in 1984.

Currently the third-largest SCT unit in the UK and Ireland.

The Transplant programme:

- HPRA TE Licence (TE001)
- JACIE accreditation
- EBMT affiliation

HPRA An tÚdarás Rialála Táirgí Sláinte Health Products Regulatory Authority

EBMT European Society for Blood and Marrow Transplantation

JACIE Joint Accreditation Committee ISCT | EBMT

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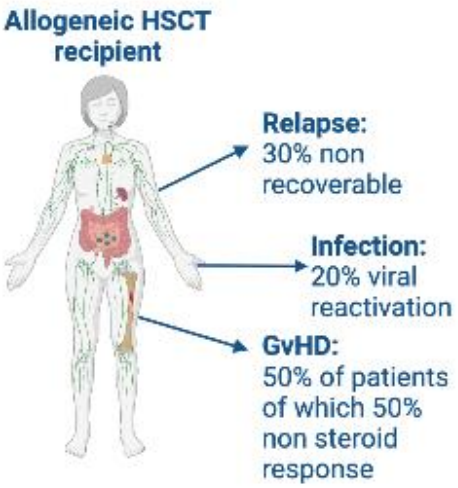
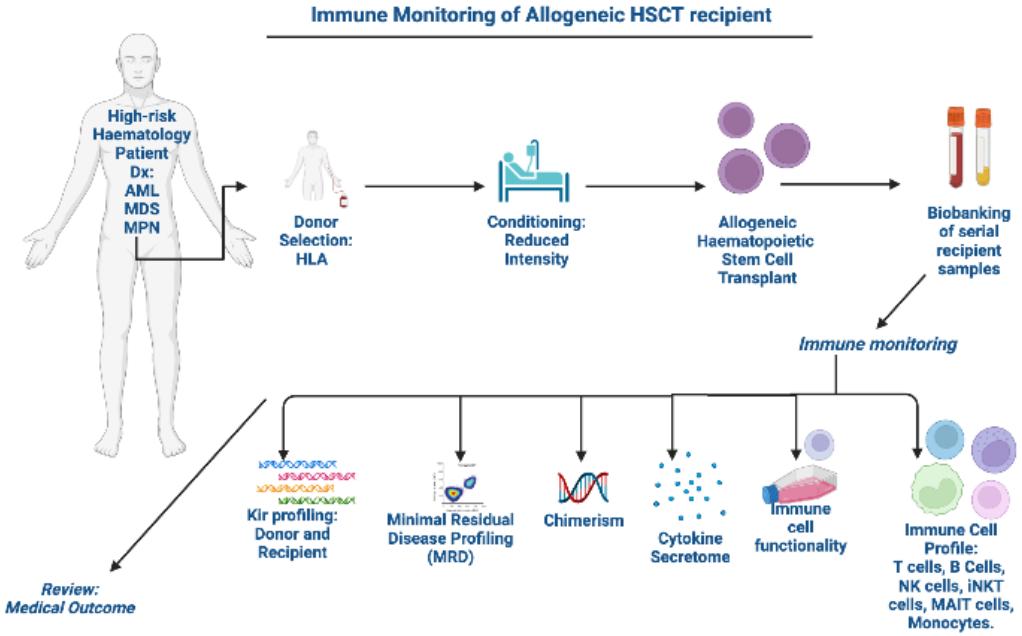
Study Design





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Study Group

Characteristic	Value
Number of patients	n=20
Sex	
Male (XY)	13 (65%)
Female (XX)	7 (35%)
Age at transplant	
median (range)	60 (47-71)
Diagnosis	
AML	13 (65%)
MDS	3 (15%)
Myelofibrosis	3 (15%)
CMML	1 (5%)

Study Group

Patients	n=20	
Clinical outcome at 12 months	**n**	**%**
Vital status: alive	19	95
Non-relapse mortality (NRM)	0	0
Relapse	3	15
aGvHD	12	60
cGvHD	4	20
Viral reactivation (CMV n=1, EBV n=5, ADV n=5)	11	55
Complications (including, relapse, non relapse mortality, aGvHD, cGvHD and viral reactivation)	18	90

Methodology: Flow panels

Fluorochrome	Panel 1	Panel 2	Panel 3	Panel 4
FITC	unstained	CD127	CD14	CD27
PE	unstained	Vα7.2TCR	CD56	CCR7
PerCP	unstained	CD3	CD3	CD3
APC	unstained	Vδ3 TCR	CD19	CD45RA
PE/Cy7	unstained	CD4	Vα24Jα18 TCR	CD4
APC/Cy7	unstained	CD25	Vδ1 TCR	CD8
Pacific Blue	unstained	CD161	CD16	Vδ2 TCR
eFluor506	unstained	Dead cell stain	Dead cell stain	Dead cell stain

Methodology: Flow panels

Cell subsets	Surface expression
Helper T cells	$CD3^+$ $CD4^+$
Cytotoxic T cells	$CD3^+$ $CD8^+$
Regulatory T cells	$CD3^+$ $CD4^+$ $CD25^+$ $CD127^{lo}$
Unconventional T cells	$CD3^+$ $CD4^-$ $CD8^-$ or $CD3^+$ $CD4^+$ $CD8^+$
Natural killer cells	$CD3^-$ $CD56^+$ $CD16^{+/-}$ (3 subsets depending on CD16 and CD56 expression)
B cells	$CD3^-$ $CD19^+$
Monocytes	$CD14^+$ $CD16^{+/-}$ (3 subsets depending on CD16 expression levels)
iNKT cells	$CD3^+$ Vα24Jα18 TCR^+
MAIT cells	$CD3^+$ $CD161^+$ Vα7.2$^+$
Vδ1 T cells	$CD3^+$ Vδ1 TCR^+
Vδ2 T cells	$CD3^+$ Vδ2 TCR^+
Vδ3 T cells	$CD3^+$ Vδ3TCR^+

Methodology: Flow panels

Differentiation status of T cell subsets	Surface expression
Naïve T cells	$CD3^+$ $CD45RA^+$ $CCR7^+$ CD4/CD8
Central memory T cells	$CD3^+$ $CD45RA^-$ $CCR7^+$ CD4/CD8
Effector memory T cells	$CD3^+$ $CD45RA^-$ $CCR7^-$ CD4/CD8
Terminally differentiated T cells	$CD3^+$ $CD45RA^+$ $CCR7^-$ CD4/CD8
Naïve Vδ2 T cells	$CD3^+$ $Vδ2^+$ $CD45RA^+$ $CCR7^+$
Central Vδ2 memory T cells	$CD3^+$ $Vδ2^+$ $CD45RA^-$ $CCR7^+$
Effector Vδ2 memory T cells	$CD3^+$ $Vδ2^+$ $CD45RA^-$ $CCR7^-$
Terminally differentiated Vδ2 T cells	$CD3^+$ $Vδ2^+$ $CD45RA^+$ $CCR7^-$

Preliminary Results

ASSOCIATION OF CD56⁺ T CELLS WITH THE ABSENCE OF CHRONIC GRAFT VERSUS HOST DISEASE IN PATIENTS UNDERGOING REDUCED-INTENSITY CONDITIONING ALLOGENEIC STEM CELL TRANSPLANTATION

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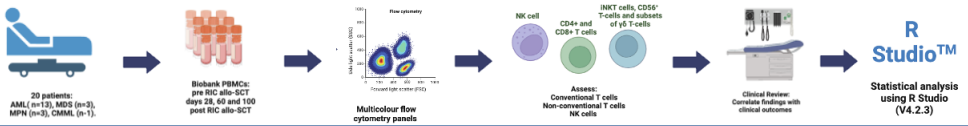
Presented at: HAEMATOLOGY ASSOCIATION OF IRELAND

Introduction

Despite the efficacy of reduced-intensity conditioning allogeneic stem cell transplantation (RIC allo-SCT) in treating blood cancer, a significant number of patients develop graft versus host disease (GvHD). GvHD occurs when donor T-cells recognise the recipient as foreign and mount an immune response that subsequently causes the destruction of host tissues. Chronic GvHD in particular, results in substantial morbidity and a reduction in the quality of life. We report preliminary results on post-transplant immune monitoring of innate lymphocytes, including CD56⁺ T-cells, gamma delta (γδ) T-cells and NK cells, in patients post-RIC allo-SCT for myeloid malignancy at St James's Hospital.

Methods



Results

GvHD manifested in 70% of cases (n=14). Within this group, 13 patients experienced acute GvHD (aGvHD) categorised as grade I (n=4), grade II (n=8) and grade III (n=1). Chronic GvHD (cGvHD) developed in 4 patients, and three of these patients had both aGvHD and cGvHD. The average time to onset of aGvHD was 61±34 days, whereas cGvHD was 299±141 days.

- At day 28 post-transplant, the mean absolute number of CD56⁺CD3⁺PBMC/μL was 3.96 ± 4.18. We observed significantly lower numbers of CD56⁺ T cells in patients who subsequently developed cGvHD compared to those who did not (mean 1.38 ±1.19 vs. 2.65 ±2.66, p=0.039, *figure 1a*).
- At day 100 post-transplant, the mean absolute number of CD56⁺CD3⁺PBMC/μL was 2.67 ±2.41. Again, a lower mean was observed in patients who subsequently developed cGvHD compared to those who did not (mean 0.87 ± 0.57 vs. 3.12 ± 2.49, p=0.039, *figure 1b*).
- At days 28, 60 and 100 post-transplant, we found no associations between CD56⁺ T-cells and steroid treatment, the incidence of relapse, recipient age, sex or donor age (p>0.05).
- In addition, at days 28, 60 and 100 we found no association between cGvHD and CD4⁺ T cells, CD8⁺ T cells, NK cells, invariant NKT cells and subsets of γδ T cells (p>0.05).

CD56⁺ T-cells in patients with cGvHD and no cGvHD post RIC allo-SCT

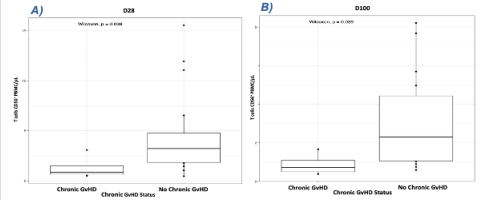


Figure 1: *Absolute numbers of CD3⁺CD56⁺ PBMC/μL in patients with Chronic GvHD and patients with no Chronic GvHD at day 28 post RIC allo-SCT (A) and day 100 post RIC allo-SCT (B).*

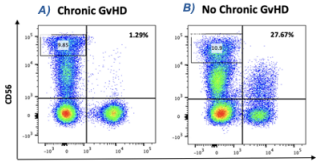


Figure 2: *Flow cytometry plots demonstrating CD3⁺CD56⁺ T cells in a patient with Chronic GvHD (A) versus a patient with no Chronic GvHD (B).*

Conclusion

Slower CD56⁺ T-cell recovery in the early post-transplant period associates with a higher incidence of subsequent cGvHD. CD56⁺ T cells are a subset of cytotoxic T cells, thought to develop from CD56 negative T cells upon activation, that display both natural and T cell receptor-mediated cytotoxic activity and enhance cytokine secretion. Additional patient testing and functionality studies are warranted to investigate the predictive value and the mechanism by which CD56⁺ T-cells may play a protective role against cGvHD post-RIC allo-SCT without compromising graft versus leukaemia effects.

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Preliminary Results

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ASSOCIATION OF CD56⁺ T CELLS WITH THE ABSENCE OF CHRONIC GRAFT VERSUS HOST DISEASE IN PATIENTS UNDERGOING REDUCED-INTENSITY CONDITIONING ALLOGENEIC STEM CELL TRANSPLANTATION

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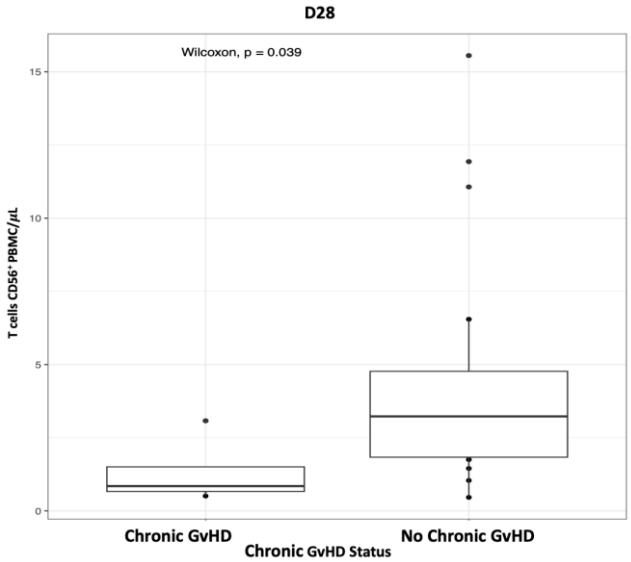

At day 28 post-transplant, the mean absolute number of $CD56^+CD3^+$PBMC/μL was 3.96 ± 4.18.

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Figure: *Absolute numbers of $CD3^+CD56^+$ PBMC/μL in patients with Chronic GvHD and patients with no Chronic GvHD at day 28 post RIC allo-SCT.*

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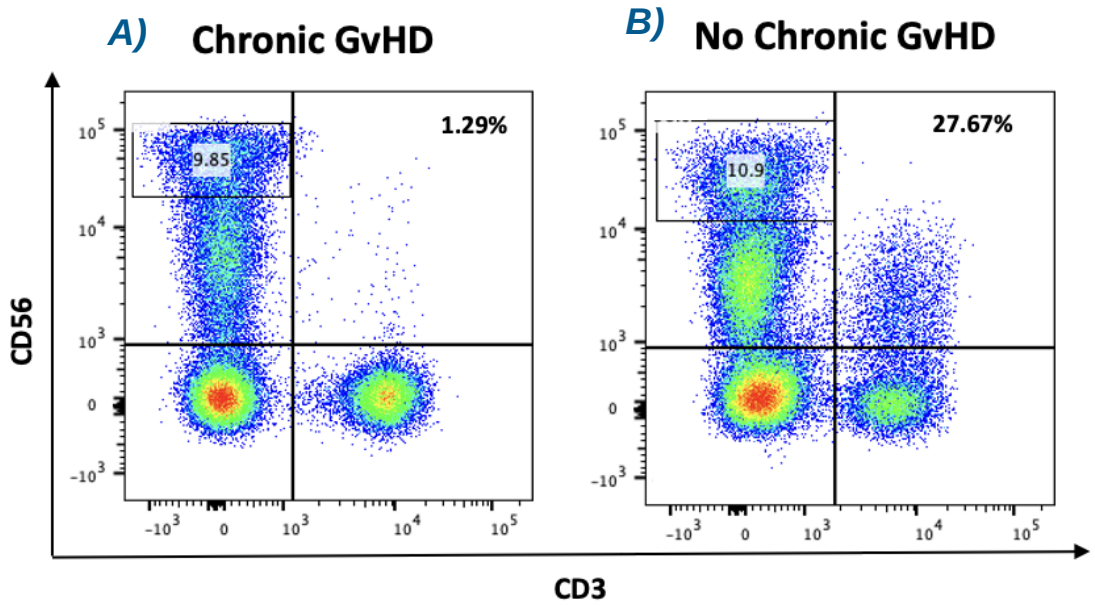

Figure: *Flow cytometry plots demonstrating CD3⁺CD56⁺ T cells in a patient with Chronic GvHD (A) versus a patient with no Chronic GvHD (B).*

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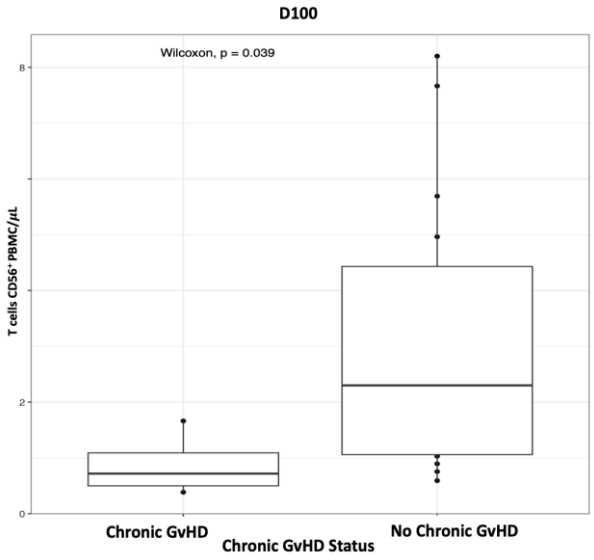

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Figure: *Absolute numbers of $CD3^{+}CD56^{+}$ PBMC/μL in patients with Chronic GvHD and patients with no Chronic GvHD at day 100 post RIC allo-SCT.*

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- Additional patient testing and functionality studies are warranted to investigate the predictive value and the mechanism by which CD56⁺ T-cells may play a protective role against cGvHD post-RIC allo-SCT without compromising graft versus leukaemia effects.

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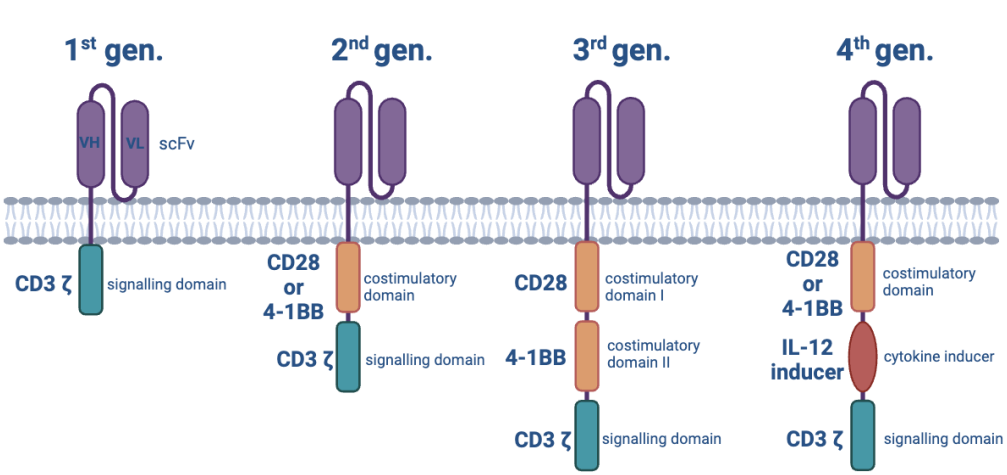
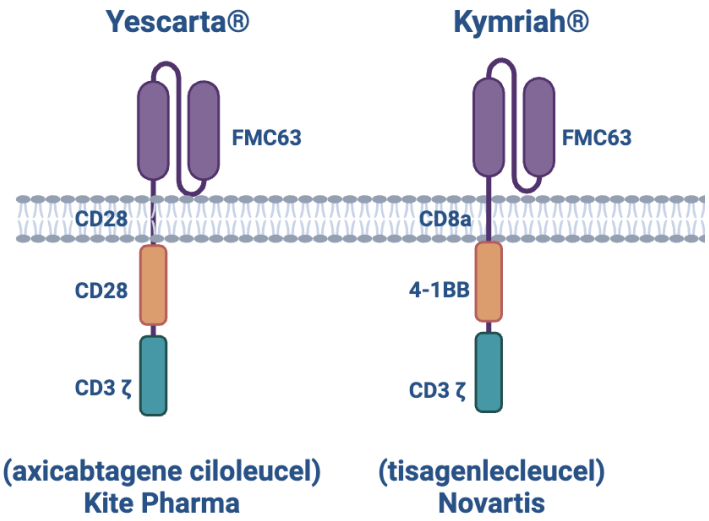
Chimeric Antigen Receptor T (CAR-T) Cells

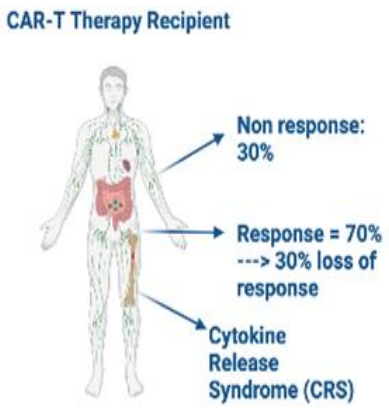


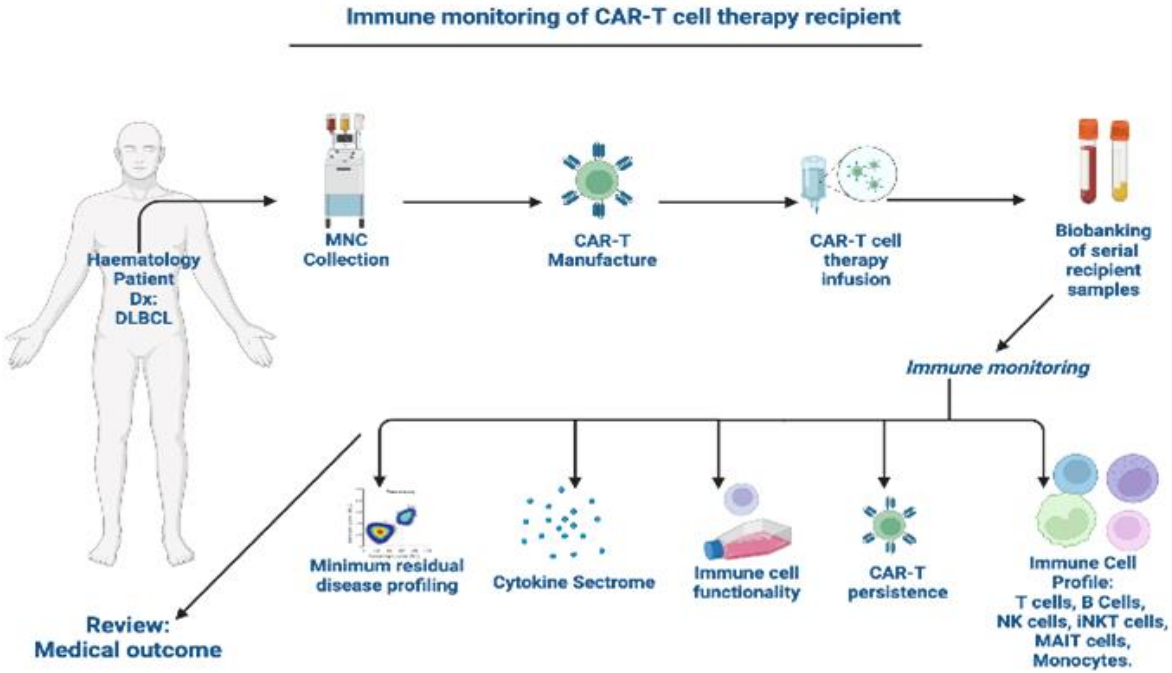


Created using BioRender.com

At present, two different second-generation CAR constructs have been licenced for the treatment of relapsed/refractory high-grade B-cell malignancies at St. James's Hospital, since 2021.

Study Design





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Study Group

Patients	n=10	
Sex	**n**	**%**
Male (XY)	7	70
Female (XX)	3	30
CAR-T product	**n**	**%**
Kymriah®	8	80
Yescarta®	2	20
Clinical outcome at 6 months	**n**	**%**
Complete remission (CR)	6	60
Partial response (PR)	1	10
Persistent disease (PD)	3	30

Methodology: Flow panels

Fluorochrome	Panel 5
FITC	PD-1
PE	CD19 CAR detection
PerCP	CD3
APC	CTLA-4
PE/Cy7	TIM-3
APC/Cy7	BTLA
Pacific Blue	LAG-3
eFluor506	Dead cell stain

AND Immune panels 1 – 4 as shown for allo-SCT

Preliminary Results

SERIAL MONITORING OF CIRCULATING CD19 CAR-T CELLS IN PATIENTS POST CAR-T CELL THERAPY

H Foy-Stones[1], CL Bacon[2], C Armstrong[2], A Kilgallon[3], DG Doherty[3], AM McElligott[3], T Hervig[4], N Casey[1], N Orfali [2], E Vandenberghe[2], R Henderson[2], E Higgins[2], N Gardiner[1].

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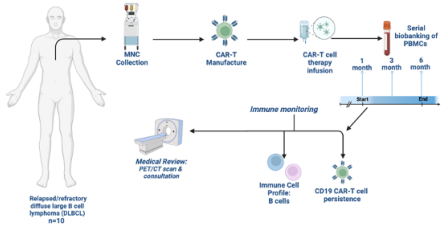
Presented at: Blood Cancer Network Ireland & Haematology Association of Ireland

INTRODUCTION

- Chimeric antigen receptor T cell (CAR-T) therapy has demonstrated success in the treatment of relapsed/refractory high-grade B-cell malignancies.
- Currently, the most utilised target for CAR-T cell therapy is the B-cell surface antigen CD19. However, with reported response rates of up to 50%, we are left with the question of what differentiates responders from non-responders, as various factors play a role in the success of CAR-T.
- Here we report preliminary results on serial monitoring of circulating CD19 CAR-T cells in patients' peripheral blood mononuclear cells (PBMCs) post-CAR-T cell therapy, to assess if CAR-T cell persistence is a useful indicator for patient outcome.

METHODS



RESULTS

Table 1: Characteristics of the study cohort.

Patients	n=10	
Sex	**n**	**%**
Male (XY)	7	70
Female (XX)	3	30
CAR-T product	**n**	**%**
Kymriah®	8	80
Yescarta®	2	20
Clinical outcome at 6 months	**n**	**%**
Complete remission (CR)	6	60
Partial response (PR)	1	10
Persistent disease (PD)	3	30

Objective 1: Monitoring CD19 CAR-T cell persistence.

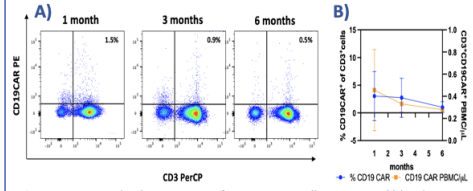


Figure 1: Longitudinal monitoring of CD19 CAR-T cells in patients' blood post-infusion by flow cytometry (A) and the mean percentages and numbers of T cells that express the CD19 CAR at 1, 3 and 6 months (B).

RESULTS

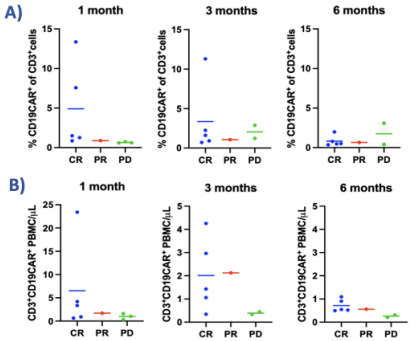


Figure 2: CD19 CAR-T cell percentages (A) and absolute numbers of $CD3^+CD19CAR^+$ PBMC/μL (B) at 1, 3 and 6 months post CAR-T in patients with a complete response (CR), partial response (PR) and persistent disease (PD).

Objective 2: Monitoring B cells as a surrogate marker of CD19 CAR-T cell function.

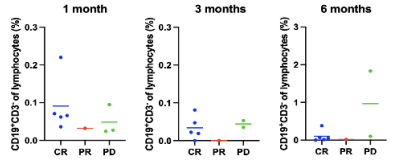


Figure 3: Monitoring of B cells [$CD3^-CD19^+$] at 1, 3 and 6 months post CAR-T cell therapy in patients with a complete response (CR), partial response (PR) and persistent disease (PD).

- CAR-T cell persistence decreases over time (*figure 1b*).
- A higher mean percentage of CD19 CAR-T cells was observed at 1 and 3 months post-infusion in the CR group compared to the PD group (*figure 2a*).
- In contrast, at 6 months, a higher mean percentage of CD19 CAR-T cells was observed in the PD group compared to the CR group (*figure 2a*).
- Higher absolute numbers of $CD3^+CD19CAR^+$ PBMC/μL were observed in the CR group compared to the PD group at 1, 3 and 6 months post CAR-T cell therapy *(figure 2b).*
- At 6 months post-CAR-T, we observed a small CD19 B-cell population in one patient with persistent disease (*figure 3*).

CONCLUSION

- We predict that longitudinal monitoring of CD19 CAR-T cells at further time points will shed light on whether the persistence of CD19 CAR is one of the contributing factors that can differentiate between responders and non-responders.

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Preliminary Results

Presented at:

Blood Cancer Network Ireland & Haematology Association of Ireland

SERIAL MONITORING OF CIRCULATING CD19 CAR-T CELLS IN PATIENTS POST CAR-T CELL THERAPY

H Foy-Stones[1], CL Bacon[2], C Armstrong[2], A Kilgallon[3], DG Doherty[3], AM McElligott[3], T Hervig[4], N Casey[1], N Orfali [2], E Vandenberghe[2], R Henderson[2], E Higgins[2], N Gardiner[1].

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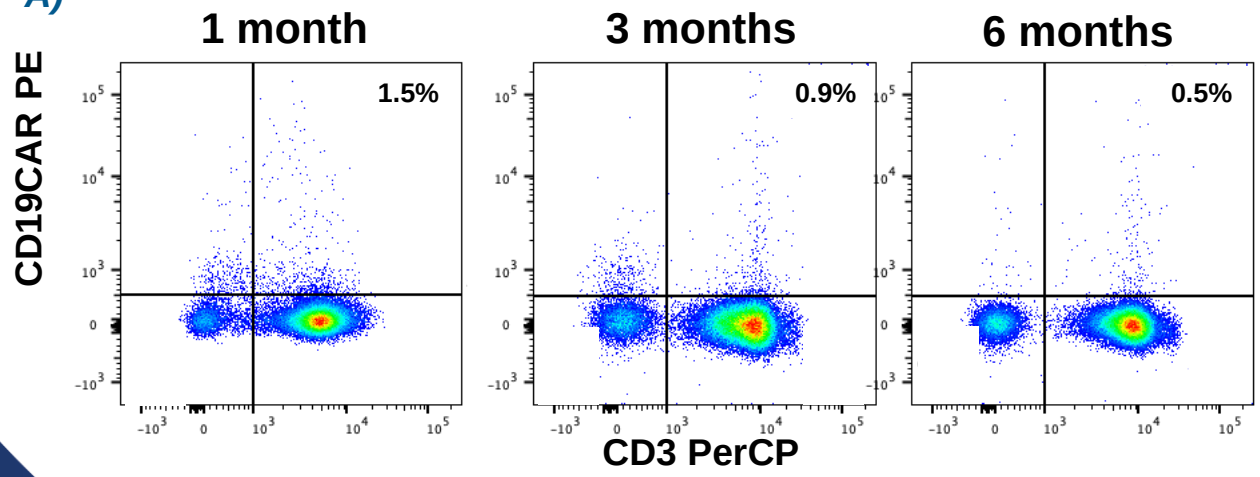
Results

A)



B)

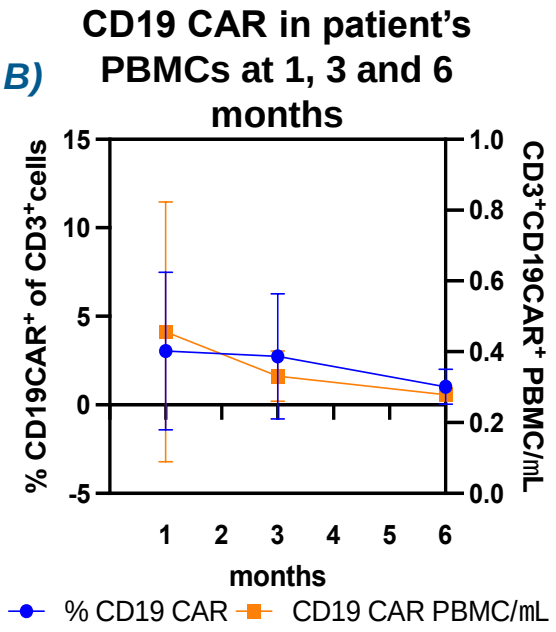


Figure: Longitudinal monitoring of CD19 CAR-T cells in patients' blood post-infusion by flow cytometry (A) and the mean percentages and numbers of T cells that express the CD19 CAR at 1, 3 and 6 months (B).

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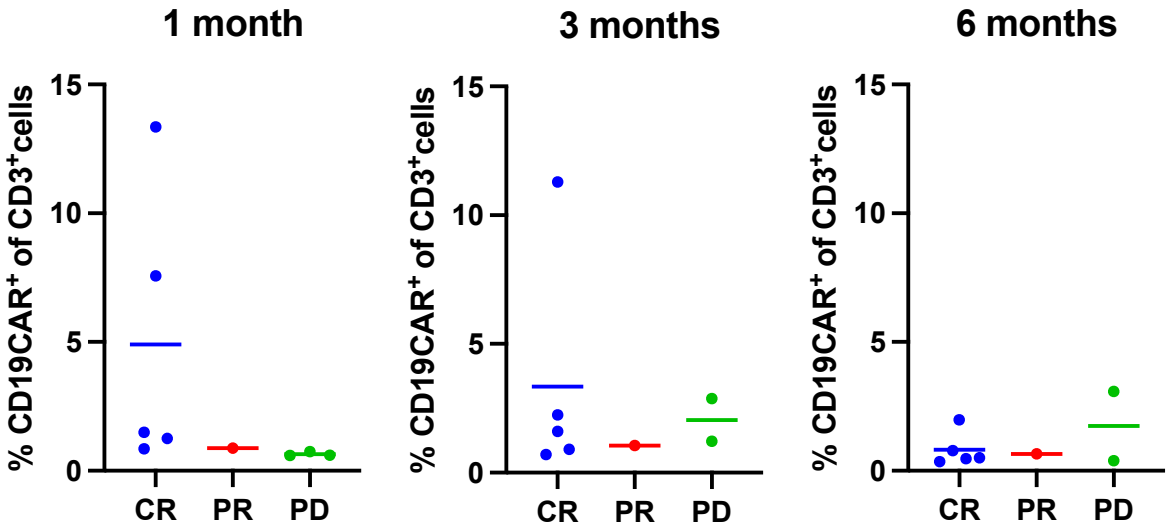

- ***Figure:*** CD19 CAR-T cell percentage at 1, 3 and 6 months post CAR-T cell therapy in patients with a complete response (CR), partial response (PR) and persistent disease (PD).

- At the 1 and 3 months post-CAR-T cell therapy, the mean CD19 CAR percentage was highest in the CR group.
- At 6 months we observed a higher CD19 CAR percentage in the PD group.

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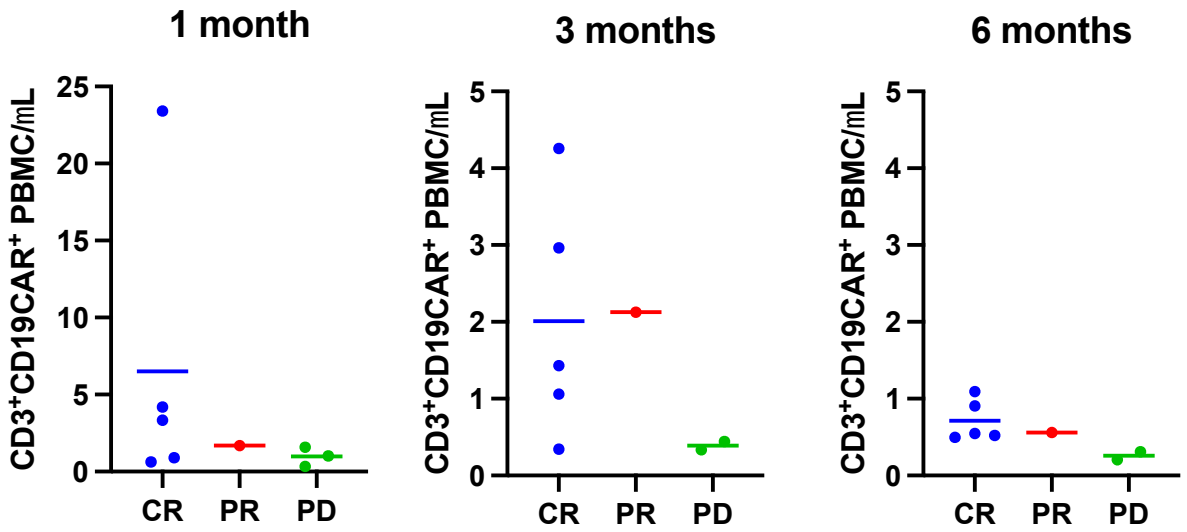
Results



- At the 1, 3 and 6 months post-CAR-T cell therapy, the mean $CD3^+CD19CAR^+$ PBMC/μL was highest in the CR group compared to the PD group.

Figure: $CD3^+CD19CAR^+$ PBMC/μL (B) at 1, 3 and 6 months post CAR-T cell therapy in patients with a complete response (CR), partial response (PR) and persistent disease (PD).

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SERIAL MONITORING OF CIRCULATING CD19 CAR-T CELLS IN PATIENTS POST CAR-T CELL THERAPY

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Conclusion

- We demonstrated that **CAR-T cell persistence decreases over time**.
- A **higher percentage of CD19 CAR-T cells** was observed at 1 and 3 months post-infusion in the **CR group** compared to the PD group.
- In contrast, at 6 months, a **higher percentage of CD19 CAR-T cells** was observed in the PD group compared to the CR group.
- **Higher absolute numbers of $CD3^{+}CD19CAR^{+}$ PBMC/μL** were observed in the **CR group** compared to the PD group at 1, 3 and 6 months post CAR-T cell therapy.
- The plan is to extend the numbers (n=30) and assess if the trend holds the predictive nature of the assay.

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Preliminary Results

EXPRESSION OF EXHAUSTION MARKERS ON THE SURFACE OF CAR-T CELLS AND T-CELLS IN THE RECIPIENTS OF CD19 CAR-T CELL THERAPY

H Foy-Stones[1], CL Bacon[2], C Armstrong[2], A Kilgallon[3], DG Doherty[3], AM McElligott[3], T Hervig[4], N Casey[1], N Orfali [2], E Vandenberghe[2], R Henderson[2], E Higgins[2], N Gardiner[1].

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Presented at: HAEMATOLOGY ASSOCIATION OF IRELAND

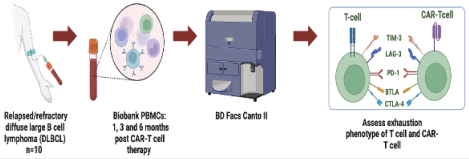
INTRODUCTION

- T-cell exhaustion is a state of functional impairment that occurs due to prolonged exposure to antigenic stimulation.
- In chronic infections and cancer, these exhausted T-cells can lose their ability to respond to antigens. The dynamics of T-cell exhaustion involves the expression of a number of receptors on T cells and their inhibitory ligands on other cells.
- We present our preliminary findings of exhaustion markers on circulating T-cells and CD19 CAR-T cells post CAR-T-cell infusion.

OBJECTIVES

- Correlate the exhaustion marker profile of T cells and CD19 CAR-T cells with the clinical outcomes post CAR-T cell therapy.

METHODS



RESULTS

TIM-3 expression on CD19 CAR-T cells

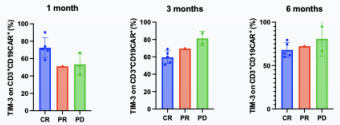


Figure 1: Percentage of TIM-3 expression on CD3⁺CD19CAR⁺ T-cells at 1, 3 and 6 months post-CAR-T in patients with a complete response (CR), partial response (PR) and persistent disease (PD).

- At 1, 3 and 6 months post-CAR-T cell therapy, the mean percentage of TIM-3⁺CD3⁺CD19CAR⁺ was 67%.
- On CD19 CAR T-cells, we observed a slightly higher mean percentage of TIM-3 in the CR group at 1 month post-infusion, and then in the PD and PR groups at 3 and 6 months post-infusion *(figure 1)*.

CTLA-4 expression on CD19 CAR-T cells

- The mean percentage of CTLA4⁺CD3⁺CD19CAR⁺ was 34%.
- CTLA-4 was seen at higher levels on CD19-CAR-T cells in the PD group at the 1, 3 and 6-month time points.

LAG-3 expression on CD19 CAR-T cells

- We observed, that LAG-3 expression levels were the lowest of all exhaustion markers that were monitored during our study. Across all time points, the mean percentage of LAG-3⁺CD3⁺CD19CAR⁺ was 6%.

RESULTS

PD-1 expression on CD19 CAR-T cells

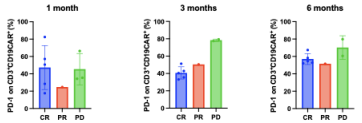


Figure 2: Percentage of PD-1 expression on CD3⁺CD19CAR⁺ T-cells at 1, 3 and 6 months post-CAR-T in patients with a complete response (CR), partial response (PR) and persistent disease (PD).

- The mean percentage of PD-1⁺CD3⁺CD19CAR⁺ was 51%.
- At 1-month post-infusion, the expression of PD-1 on CAR-T cells was similar between all groups, *(figure 2)*.
- At 3 and 6 months post-infusion, our PD group expressed slightly higher levels of PD-1 on CD19 CAR T-cells *(figure 2)*.

BTLA expression on CD19 CAR-T cells

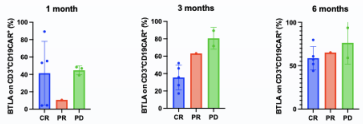


Figure 3: Percentage of BTLA expression on CD3⁺CD19CAR⁺ T-cells at 1, 3 and 6 months post-CAR-T in patients with a CR, PR and PD.

- The mean percentage of BLTA⁺CD3⁺CD19CAR⁺ was 50%.
- The expression of BTLA on CD19 CAR-T cells was slightly higher in the PD and PR groups at 3 and 6 months post-infusion *(figure 3)*.

Exhaustion marker expression on CD3⁺ T cells

- The mean percentage of exhaustion marker expression on CD3⁺ T cells post CAR-T cell therapy varied between each of the markers: TIM-3 (25.5%), PD-1 (18%), BTLA (12%) CTLA-4 (10%) and LAG-3 (0.11%).
- At 1 month, the CR group had higher percentages of TIM-3 and PD-1 on T cells compared to the PD group.
- At 3 and 6 months, the CR group had higher percentages of TIM-3, whereas the PD group had higher percentages of PD-1 and BTLA.

CONCLUSION

- We observed high levels of exhaustion markers on the surface of CAR-T cells and T-cells post-infusion. Despite the apparent negative aspects of T-cell exhaustion, our preliminary research has suggested that early expression at 1 month, might be associated with a positive overall outcome.
- Further research on additional patients at further time points is required to determine if these exhaustion markers are a negative or positive indicator of overall outcome post-CAR-T cell therapy.

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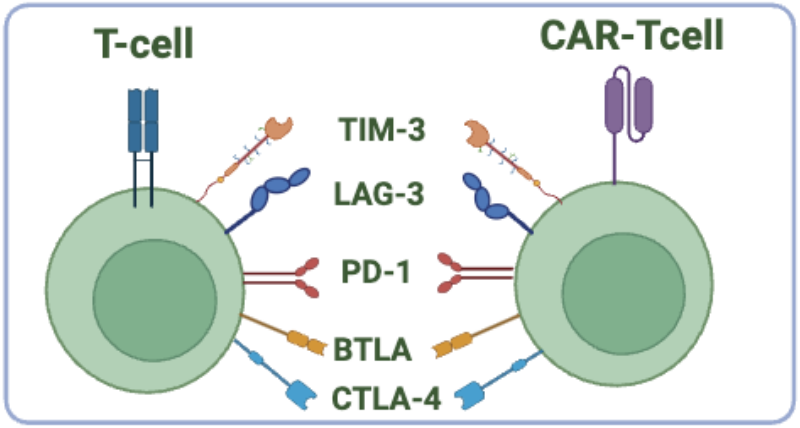
Exhaustion Markers

T cell exhaustion is a state of functional impairment, that occurs due to prolonged exposure to antigenic stimulation.



The dynamics of T cell exhaustion involve the expression of several receptors on T cells:

TIM-3 (T-cell immunoglobulin and mucin domain-3)
LAG-3 (Lymphocyte activation gene-3)
PD-1 (Programmed cell death protein 1)
BTLA (B and T lymphocyte attenuator)
CTLA-4 (Cytotoxic T-lymphocyte–associated antigen 4)

T-cell exhaustion also involves:
the expression of inhibitory ligands on other cells.

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Results

Expression of TIM-3 on CD19 CAR-T cells post CAR-T cell therapy

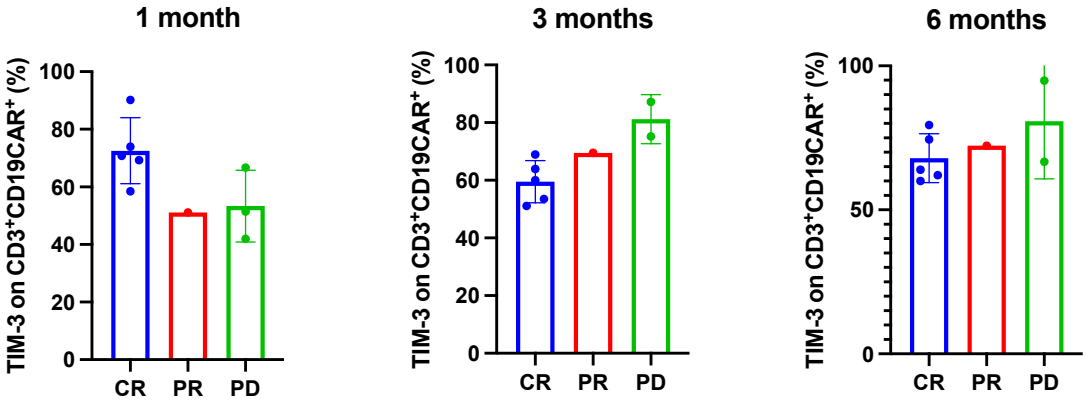

Figure: *Percentage of TIM-3 expression on $CD3^+CD19CAR^+$ T-cells at 1, 3 and 6 months post-CAR-T in patients with a complete response (CR), partial response (PR) and persistent disease (PD).*

- At 1, 3 and 6 months post-CAR-T cell therapy, the mean percentage of

  $TIM\text{-}3^+CD3^+CD19CAR^+$ was 67%.

- On CD19 CAR T-cells, we observed a slightly higher mean percentage of TIM-3 in the CR group at 1 month post-infusion, and then in the PD and PR groups at 3 and 6 months post-infusion.

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Results

Expression of PD-1 on CD19 CAR-T cells post CAR-T cell therapy

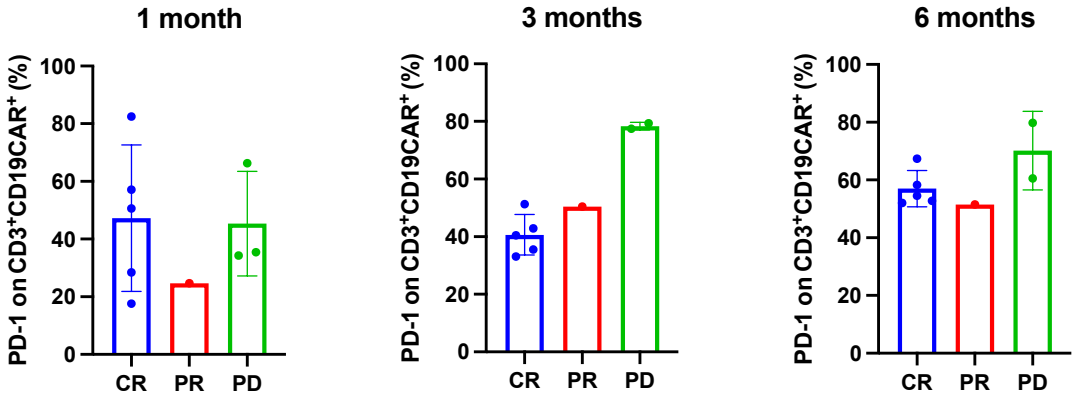

Figure: *Percentage of PD-1 expression on $CD3^+CD19CAR^+$ T-cells at 1, 3 and 6 months post-CAR-T in patients with a complete response (CR), partial response (PR) and persistent disease (PD).*

- The mean percentage of PD-1^+ $CD3^+CD19CAR^+$ was 51%.
- At 1-month post-infusion, the expression of PD-1 on CAR-T cells was similar between all groups.
- At 3 and 6 months post-infusion, our PD group expressed slightly higher levels of PD-1 on CD19 CAR T-cells.

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Results

Expression of BTLA on CD19 CAR-T cells post CAR-T cell therapy

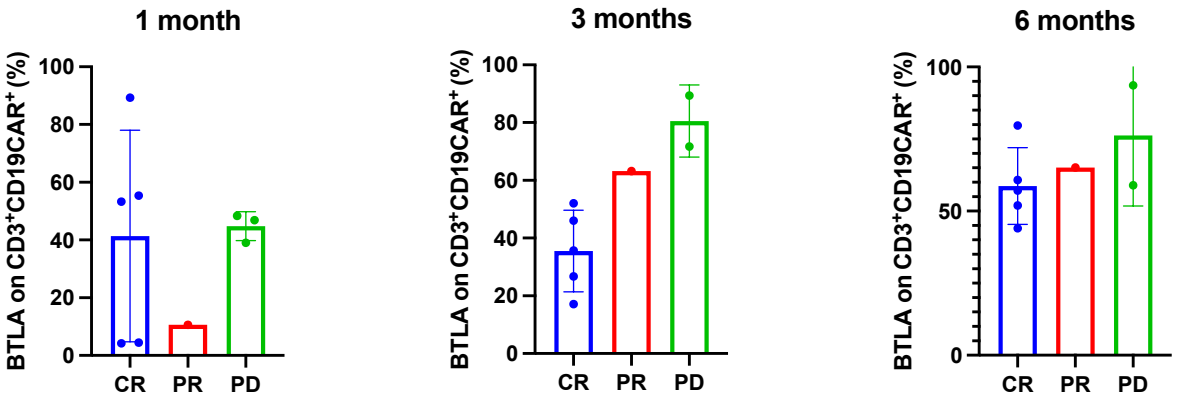

Figure: *Percentage of BTLA expression on $CD3^+CD19CAR^+$ T-cells at 1, 3 and 6 months post-CAR-T in patients with a CR, PR and PD.*

- The mean percentage of $BLTA^+CD3^+CD19CAR^+$ was 50%.
- The expression of BTLA on CD19 CAR-T cells was slightly higher in the PD and PR groups at 3 and 6 months post-infusion.

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- We observed **high levels of exhaustion markers** on the surface of CAR-T cells and T-cells post-infusion. Despite the apparent negative aspects of T-cell exhaustion, our preliminary research has suggested that early expression at 1 month, might be associated with a positive overall outcome.

- Further research on additional patients at further time points is required to determine if these exhaustion markers are a **negative or positive indicator** of overall outcome post-CAR-T cell therapy.

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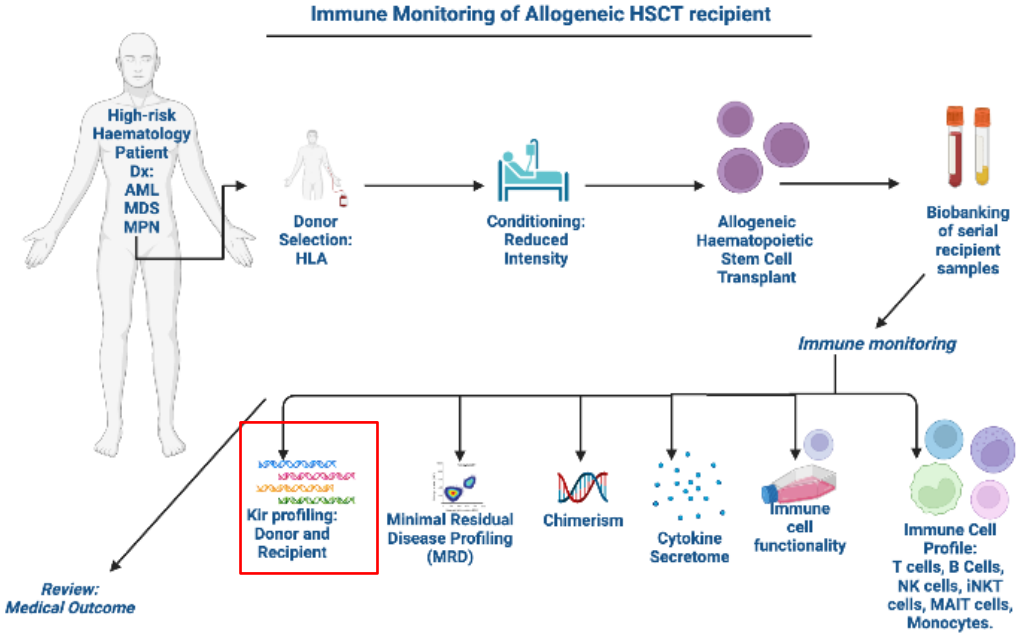
Planned future work: KIR profiling at IBTS



Killer cell immunoglobulin-like receptors (KIR):

Expressed on natural killer (NK) cells

Plan to analyse donor and recipient KIR profiles

Assess the correlation of the KIR profile with the clinical outcomes (GvHD, Relapse)

Acknowledgements – thank you!

PhD supervisors:

Dr Nicola Gardiner

Prof. Derek G. Doherty

Prof. Tor Hervig

Dr Anthony Mc Elligott

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SJH staff: Cryobiology laboratory, haematology consultants, CAR-T coordinators, nursing and phlebotomy.

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