Supplementary material

Title: Conjunctival T cell profile in Allogeneic Haematopoietic Stem Cell Transplant patients after instilling Topical Cyclosporine-A 0.1% cationic emulsion

Short title: Conjunctival T cell changes after topical cyclosporine in HSCT patients

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Supplementary Table 1: Indication for allo-HSCT in each patient

Subject ID	Diagnosis	GVHD Prophylaxis Regimen	Conjunctival T cell profile *
IKERVIS001	Myelodysplastic syndrome with excess blasts	Cyclosporin, Methotrexate	Profile 2
IKERVIS002	Acute Myeloid Leukemia in second complete remission 2	Cell selection FK506	-
IKERVIS003	Acute Myeloid Leukaemia	Cyclosporin, Methotrexate	-
IKERVIS004	Acute Myeloid Leukemia	Cyclosporin, Methotrexate	-
IKERVIS005	Acute myeloid leukaemia	Cyclosporin, Methotrexate	-
IKERVIS006	Acute myeloid leukemia	Antithymocyte Globulin, Cyclosporin, Methotrexate	Profile 2
IKERVIS007	Mycosis Fungoides	Tacrolimus, Mycophenolate Mofetil	-
IKERVIS008	Myelodysplastic syndromes	PTCY, Cyclosporin, Mycophenolate sodium	-
IKERVIS009	Chronic myeloid leukemia in blast crisis	Tacrolimus	Profile 2
IKERVIS010	Anaplastic large cell lymphoma, block negative	Cyclosporin, Mycophenolate sodium	Profile 1
IKERVIS011	Acute myeloid leukaemia	Tacrolimus	-
IKERVIS012	Chronic myeloid leukaemia in second chronic phase	Cyclosporin, Methotrexate	Profile 1
IKERVIS013	Relapsed Acute Myeloid Leukemia in complete remission 2	Cyclosporin, Mycophenolate sodium	Profile 1
IKERVIS014	Acute Myeloid Leukemia	Cyclosporin, Mycophenolate sodium	Profile 1
IKERVIS015	PRV Transformed	Cyclosporin, Mycophenolate sodium	-
IKERVIS016	Severe aplastic anemia	Post transplant cyclophosphamide FK506 orally	Profile 1
IKERVIS019	Acute Myeloid Leukemia	Tacrolimus	-
IKERVIS020	Relapse Acute Myeloid Leukemia in complete remission 2	Tacrolimus	-

IKERVIS021	Chronic Myeloid Leukaemia	Methotrexate, cyclosporine, mycophenolate mofetil	Profile 1

HSCT: hematopoietic stem cell transplantation

*Refer to Supplementary Table 5

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Supplementary Table 2: demographics of patients and donors

	Patient			Donor			
Subject	**Age**	**Gender**	**Ethnicity**	**Age**	**Gender**	**Donor relation**	**HLA matching**
IKERVIS001	47	M	Others	43	M	Brother	Full matched Sibling
IKERVIS002	57	F	Chinese	26	M	Son	Haploidentical
IKERVIS003	64	M	Chinese	59	M	Brother	Full matched Sibling
IKERVIS004	30	M	Malay	30	M	Unrelated	Full matched Unrelated
IKERVIS005	60	F	Chinese	57	M	Brother	Full matched Sibling
IKERVIS006	37	M	Chinese	32	M	Unrelated	Full matched Unrelated
IKERVIS007	27	M	Chinese	48	M	Unrelated	Full matched Unrelated
IKERVIS008	57	M	Chinese	28	M	Nephew	Haploidentical
IKERVIS009	54	M	Chinese	56	M	Brother	Full matched Sibling
IKERVIS010	35	M	Chinese	42	F	Sister	Full matched Sibling
IKERVIS011	59	M	Malay	23	M	Son	Haploidentical
IKERVIS012	59	F	Chinese	64	F	Sister	Full matched Sibling
IKERVIS013	30	F	Chinese	34	M	Brother	Full matched Sibling
IKERVIS014	61	F	Chinese	25	M	Unrelated	Full matched Unrelated
IKERVIS015	62	F	Chinese	23	F	Unrelated	Mismatched Unrelated
IKERVIS016	50	M	Chinese	16	M	Son	Haploidentical
IKERVIS019	63	M	Chinese	31	M	Son	Haploidentical
IKERVIS020	50	F	Malay	42	M	Brother	Haploidentical
IKERVIS021	26	M	Chinese	24	M	Brother	Full matched Sibling

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Supplementary Table 3: Subjects not included in conjunctional T cell analysis

Subject ID	Conjunctival T cell data	Reason
IKERVIS002	Not available	Machine was under maintenance
IKERVIS003	Only data from one visit	Due to MRSA infection
IKERVIS004	Only data from one visit	Due to CP-CRE infection
IKERVIS005	Only data from one visit	Due to MRSA
IKERVIS007	Only data from one visit	Lab technician not available
IKERVIS008	Only data from one visit	Withdrawn
IKERVIS015	Only data from two visits	Too few cells
IKERVIS019	Only data from one visit	Withdrawn
IKERVIS020	Only data from one visit	Lab technician not available at visit 4, will collect impression at visit 5 (last visit).

HSCT: hematopoietic stem cell transplantation; MRSA: methicillin-resistant staphylococcus aureus; CP-CRE: carbapenemase producing carbapenem-resistant Enterobacteriaceae

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Supplementary Table 4: Data from study visit number included in the analysis in Table 2

	Number of months post-HSCT that data was collected for use in analysis when laboratory and Schirmer's test were last performed*
IKERVIS 001	12
IKERVIS 002	12
IKERVIS 003	12
IKERVIS 004	6
IKERVIS 005	12
IKERVIS 006	12
IKERVIS 007	12
IKERVIS 008	6
IKERVIS 009	12
IKERVIS 010	12
IKERVIS 011	12
IKERVIS 012	12
IKERVIS 013	12
IKERVIS 014	12
IKERVIS 015	6
IKERVIS 016	12
IKERVIS 019	3
IKERVIS 020	6
IKERVIS 021	6

*for patients with post-HSCT data < 12 months, they were still followed up by the haematologists and did not have any ocular complaints (were not referred back to eye)
HSCT: hematopoietic stem cell transplantation

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Annex A: Detailed description of the methodology used in the collection of data

Non-invasive keratography tear break up time

The Oculus Keratograph 5M (Oculus, Wetzlar, Germany) was used to perform the non-invasive keratograph tear break up time (NIKBUT). Participants were first seated and allowed to blink freely while fixing on a target ahead. Once ready, participants were asked to blink twice and then refrain from further blinking. The fully automated instrument captured breaks or distortions in the image of the projected Placido rings on the cornea and the timings were automatically recorded. One reading was taken per eye and averaged, higher readings indicating more tear stability.[23]

Conjunctival redness

Conjunctival redness was measured by the Oculus Keratograph 5M (Oculus, Wetzlar, Germany). After scanning the ocular surface and images captured, grading of the conjunctival hyperemia (0-4) was automatically performed with subtraction of major conjunctival blood vessels, and temporal bulbar, nasal bulbar and average readings will be obtained. Higher redness scores indicate more hyperemia.[24]

Tear osmolarity

Tear osmolarity was measured with the TearLab system (OcuSense, San Diego, CA), a simple and highly specific point-of-care method of measuring tear osmolarity in mOsM.[29] Briefly, a non-traumatic touch pen was applied to the lower tear meniscus of each eye, and as little as 50 nL of tear collected instantly. After docking the pen into the reader, the result was obtained within seconds. One osmolarity reading on each eye will be analysed.

Lipid layer thickness (LipiView)

The lipid layer thickness of the tear film was assessed using the interferometer (LipiView® ocular surface interferometer, TearScience Inc, Morrisville, NC). The participant was seated in front of the instrument with his head positioned on the chin and forehead rest. The instrument captured the interferometer pattern of the tear film, which was subsequently used to calculate the lipid layer of the tear film, while the participant was blinking normally. This procedure took around 30 seconds for each eye.

Infra-red Meibography

This was performed as described by *Arita et al*.[27] with the exception that the Oculus Keratograph 5M (Oculus, Wetzlar, Germany) was used. The main advantage of this type of Meibography was that eversion of the tarsal plates and transillumination with a separate light source were not required. This procedure was previously approved by the IRB (CIRB Ref 2008/611/A) and for another clinical protocol (CIRB 2010/316/A).

Schirmer test

Schirmer test was done with the standard 5 mm wide Test Strips (Clement Clark) with a notch for folding, without prior anaesthesia. The strips were positioned over the inferior temporal half of the lower lid margin in both eyes, and participants' eyes were closed. The extent of the wetting was recorded after 5 minutes, and strips stored at -80˚C until further analysis.[35]

Tear cytokines

Proteins were eluted using our previous protocol.[35] The schirmer strips were longitudinally halved, and from the half strip, we eluted proteins into 110 microliters of assay buffer. Tear cytokines were evaluated using the multiplex bead-based indirect immunofluorescent assay (Milliplex, Merck-Millipore, Billerica, MA). Cytokine concentrations were normalized to the length of wetting of the Schirmer strips, adding 3 mm for the rounded portion of the strip proximal to the notch. The advantage of this technique was that 15 cytokines could be assayed using a minimum amount of tear.

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Corneal fluorescein staining score (CCLRU)

Fluorescein staining was performed as previously described.[35] A drop of saline was instilled on the fluorescein strip (Fluorets) then shaken off so that no visible drop remained. The cornea staining was subsequently imaged by the Oculus Keratograph 5M (Oculus, Wetzlar, Germany) and scored in 5 corneal zones as in the Center for Contact Lens Research Unit (CCLRU) scale. In each zone, the grade was 0-4, with 0.5 unit intervening steps, a greater number indicating more intense or greater area of staining.[28]