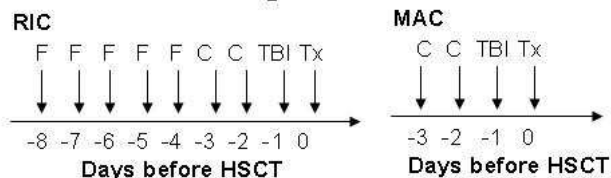


- SOP No:** AHP 71
- SUBJECT:** Haematopoietic stem cell transplantation (HSCT)
models:
a) Full MHC mismatched HSCT
b) Full MHC matched and minor histocompatibility antigen mismatched HSCT
- POLICY:** This technique must be performed by, or under the direct supervision of, an experienced operator.
- PRECAUTIONS:** Gloves, mask, long-sleeve gown, closed in shoes. Observe normal precautions for handling mice. 3M mask, safety glasses and nitril gloves are mandatory when handling animals during injection of cytotoxic drugs and for 5 days after the last injection of cytotoxic drugs.
- EQUIPMENT:** 27g needles & 1ml syringes.
Laminar flow hood for handling mice.
- PROCEDURE:**

1. Conditioning:
Reduced Intensity Conditioning (RIC)
Myeloablative (MAC)

A. RIC and MAC regimens



RIC: Mice receive fludarabine (F; 200 mg/kg) by *ip* injection daily from d-8 to d-4. Cyclophosphamide (C; 60 mg/kg) is given *ip* on d-3 & d-2 & 500cGy radiation (TBI) on d-1

MAC: Mice receive Cyclophosphamide (60 mg/kg) *ip* on d-3 & d-2 then on d-1, 2 doses of 500cGy separated by 3h to reduce gut toxicity.

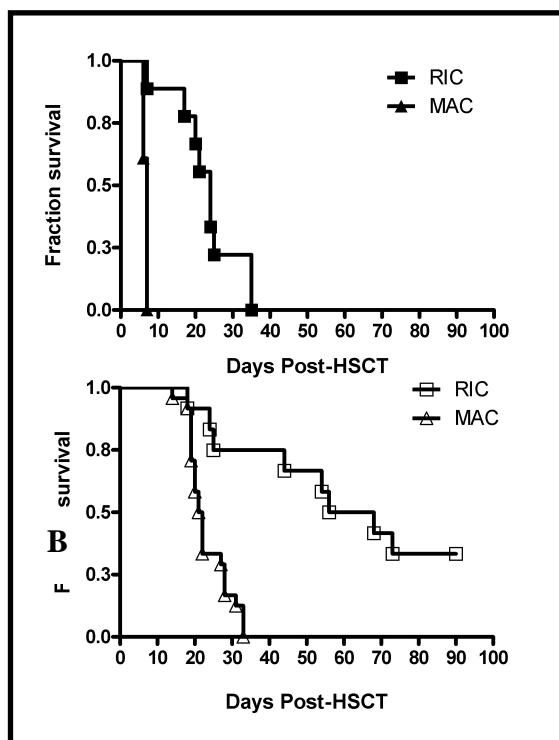
Mice from both cohorts are transplanted (Tx) on day 0

2. Stem cell source for HSCT:

**Bone marrow (10^7) + splenocytes (10^7) from donor mice
or
G-CSF mobilised blood (10^7) + non-mobilised
splenocytes (10^7) from donor mice.**

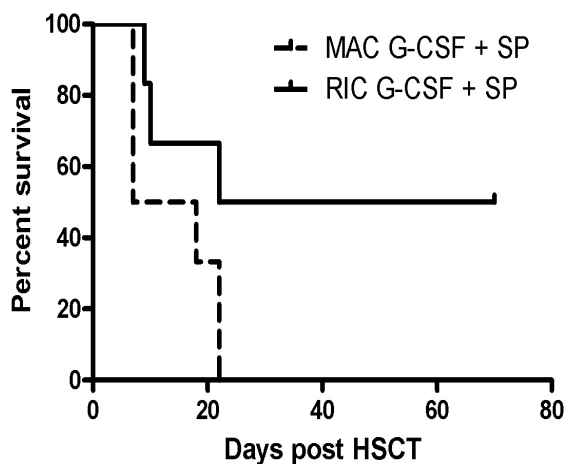
**Donor mice are given 10mg pegylated G-CSF/20gm
mouse or saline (subcutaneous injection) on d-4 and d-2
and blood collected by cardiac puncture on day 0.**

Expected timeframe for GVHD onset (Example)



**A: C57BL/6 [H-2^b] or UBI-GFP/BL6 [H-2^b] mice → BALB/c [H-2^d] mice
BM+Spleen⁷
MAC HSCT recipients (d+7±0)
RIC HSCT recipients (d+23±3)
G-CSF mobilised cells + non-mobilised spleen
MAC HSCT recipients (d+13.8±3)
RIC HSCT recipients (d+41±12)**

**B: C57BL/6 [H-2^b] or UBI-GFP/BL6 [H-2^b] mice → BALB.B [H-2^d] mice
BM+Spleen⁷
MAC HSCT recipients (d+23±1)
RIC HSCT recipients (d+60±8)**



GVHD onset after G-CSF mobilised HSCT. BALB/c mice receive RIC or MAC and G-CSF mobilised MNC + non-mobilized spleen cells from UBI-GFP/BL6 mice. HSCT recipients were monitored for GVHD onset. RIC HSCT recipients of G-CSF mobilised cells survive significantly longer than MAC HSCT recipients.

Anti-GVHD Treatment	Target	Dose	Volume*	Schedule	Route
KT3	All T cells	0.1 mg/mouse	500 µls maximum	d+1,d+3,d+5	IP
N418	All dendritic cells	1 mg/mouse	500 µls maximum	d-1, d+1, d+3	IP
RA83	Activated DC	200 µg/ml	500 µls maximum	To be determined	IP
PDCA-1	Plasmacytoid DC	1 mg/mouse	500 µls maximum	d-1, d+1, d+3	IP
MSC	Unknown	4 x 10 ⁵ -10 ⁶ /mouse	100 µls	d+1	IP
Etanercept ⁸	Anti-TNF-α	250 µg IV etanercept	200 µls	Weekly from d+6 post HSCT	IV

* The exact volume to be injected is dependent on the concentration of the antibody solution. Volumes will not exceed 500 µls.

GVHD monitoring (see attached score sheet)

Transplanted mice will be weighed daily and monitored for the onset and severity of GVHD using a score sheet modified from that of Hill et al⁸. Mice are given a score from 0-2 for posture, activity, skin & eye integrity, fur texture, weight and diarrhoea. Any animal reaching a cumulative grade of 8 will be sacrificed. BM, spleen and lymph nodes and GVHD target organs (large intestine, small intestine, liver, lung and skin) will be harvested and analysed.

Criteria	Grade 0	Grade 0.5	Grade 1	Grade 1.5	Grade 2
Weight loss	<10%	>10% to 15%	>15% to 20%	>20% to 25%	>25% to 35%
Posture	Normal	Slight hunching noted only at rest	Hunching noted only at rest	Constant hunching	Severe hunching impairs movement
Activity	Normal	Slightly decreased	Decreased	Significantly decreased	Stationary unless stimulated
Fur texture	Normal	Slight ruffling	Ruffled	Significant ruffling	Severe ruffling/poor grooming
Hair Loss	None	Slight loss	Severe loss	-	-
Eye Integrity	Normal	Obvious Pain	Eye(s) closed	-	-
Skin Integrity	Normal	Erythema	Erythema +	Erythema +	Erythema +

			scaling of paws/tail	significant scaling of paws/tail	obvious areas of denuded skin
Diarrhoea	None	None	Anal staining	Significant anal staining	Diarrhoea and blood

Cumulative weight loss is calculated daily using the starting weight (prior to any treatment) as the denominator and that day's weight as the numerator. Mice are monitored using a score sheet modified from that of Hill et al¹, and approved by the University of Queensland Animal Ethics Committee. Over the last three years working with these animals we have improved the score sheet to take into consideration other clinical symptoms that impact on the health and well being of the mice. Mice are monitored on a daily basis for weight, posture, fur texture, activity, eye integrity, skin integrity, hair loss and diarrhoea. Mice are given a score from 0-2 for each category. 0 indicates a healthy animal and a score of 2 indicates very poor condition. Mice are sacrificed if they have a total score of 8 (based on scores for weight, posture, fur texture, activity, eye integrity, skin integrity, hair loss and diarrhoea). The conditioning regime induces transient weight loss that may necessitate a score of 2, but the mice then regain weight and have reduced GVHD scores. If a mouse reaches 35% weight loss or a score of 2 in any other criteria they will be sacrificed.

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REVISED:

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