

SOP No: AHP 70

SUBJECT: Hu-(NOD-) SCID Mouse Model of GVHD.
Induction of xenogeneic graft vs host disease in SCID or NOD-SCID mice by IP transplantation of human peripheral blood mononuclear cells (PBMC).

POLICY: This procedure must be performed by, or under the direct supervision of, an experienced operator.

PRECAUTIONS: Gloves, mask, long-sleeve gown, closed in shoes, tetanus immunization. Observe normal precautions for handling mice and human products.

EQUIPMENT: 22g needles & 1ml syringes.
Sterile human PBMC from normal donors negative for HIV, hepatitis B & C, HTLV, syphilis. Frozen PBMC work well.
Laminar flow hood for handling mice.
Animal irradiator.

PROCEDURE:

- 1. Conditioning:** On the day before transplant, record body weight and inject each individually ear-tagged 5-7 week old female SCID or NOD-SCID mouse (≥ 16 g body weight) with anti-asialo GM1 antibody (Waco; 20ul, IP) or TM β -1 antibody (BioXCell; 1mg in ≤ 500 ul, IP) and irradiate at 325 cGy (SCID) or 350 cGy (NOD-SCID).
- 2. Transplant:** On the following day, inject IP 50×10^6 freshly prepared PBMC in ≤ 500 ul PBS or RPMI 1640).
- 3. Experimental:** Blood can be sampled or treatments (antibodies, other drugs, cells) can be given at any time, as required, by IP, SC or IV injection.
- 4. Monitoring:** Clinical scoring should begin for each mouse on the day after transplant and should be repeated daily at approximately the same time (see attached Clinical Score sheet).
- 5. Termination:** Sacrifice mice when the Clinical Score ≥ 5 or at end of experiment (maximum 60 days).

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REVISED:

REFERENCES

1. Sandhu, J.S., R. Gorczynski, B. Shpitz, S. Gallinger, H.P. Nguyen, and N. Hozumi. 1995. A human model of xenogeneic graft-versus-host disease in SCID mice engrafted with human peripheral blood lymphocytes. *Transplantation* 60:179-184.

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Clinical Score Sheet To Assess Each SCID or NOD-SCID Mouse Transplanted with Human Cells

| Mouse ID / strain | | Experiment # | | | Clinical Scores* (see table below for scoring criteria) | | | | | | | Comment |
|-------------------|------|---|------------|---------------|--|---------------|----------------|-------------------|----------------------|-----------------|------------------------|---------|
| Exp. / Cohort | | Cage _____ Mouse _____ | | | Weight loss score | Posture score | Activity score | Fur texture score | Skin integrity score | Diarrhoea score | Overall Clinical Score | |
| Day | Date | Treatment (eg, irradiated, or blood sampling) | Weight (g) | % Weight loss | | | | | | | | |
| -1 | | 325cGy + 20ul ASGM-1 <i>ip</i> | | | | | | | | | | |
| 0 | | | | | | | | | | | | |
| 1 | | | | | | | | | | | | |
| 2 | | | | | | | | | | | | |
| 3 | | | | | | | | | | | | |
| 4 | | | | | | | | | | | | |
| 5 | | | | | | | | | | | | |
| 6 | | | | | | | | | | | | |
| 7 | | | | | | | | | | | | |
| 8 | | | | | | | | | | | | |
| 9 | | | | | | | | | | | | |

* Animals receive a score between 0 and 2 for each of the criteria below. Animals must be sacrificed when the Overall Clinical Score ≥ 5 or a single score of 2, otherwise at the end of the experimental period (normally 30 days, maximum of 60 days post-transplant).

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| Criterion | Score = 0 | Score = 1 | Score = 2 |
|---|------------------|-------------------------------------|---|
| Weight loss (<i>cf.</i> Day - 1) | <10% | >10% to 25% | >25% |
| Posture | normal | Hunching noted only at rest | Severe hunching impairs movement |
| Activity | normal | Mild to moderately decreased | Stationary unless stimulated |
| Fur texture | normal | Mild to moderate ruffling | Severe ruffling/poor grooming |
| Skin integrity | normal | Scaling of paws/tail | Obvious areas of denuded skin |
| Diarrhoea | normal | Anal staining | Anal stain with blood |