Humane end point refinement for total body irradiation and humanization of NCG mice

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charles river

1 Abstract

Humanization of mice has proved to be a promising avenue for translational research of human hematopoiesis and immunity. The utility of immunodeficient strains which lack adaptive and/or innate immune function such as the NOD-*Prkdc*^{em26Cd52}*Il2rg*^{em26Cd22}/NjuCrl (NCG) mice have also allowed for better engraftment rates of humanized cells. Developed using CRISPR/Cas9, the NCG mouse carries a human-like variant of the *Sirpa (SIPR a)* gene which, in part, permits xenotransplantation of immature hematopoietic stem cells. The process of humanization however can carry with it side effects of irradiation dose and the eventual onset and development of xenogenic graft-versus-host-disease (GvHD). GvHD in mice typically presents as unthriftiness, hunched posture and weight loss, fur loss, reduced mobility or tachypnea. Often, total weight loss alone is used as a parameter for end point criteria without consideration of other parameters and despite widespread use of humanized mice, humane endpoint criteria for humanization have not been clearly outlined in the veterinary science and humanized mouse literature. To address this issue, we developed a cage side scoring system specifically for NCG mice following the process of humanization with human peripheral blood mononuclear cells (hPBMC) or human CD34$^+$ cells which can include total body irradiation (TBI). This scoring system included criteria surrounding overall appearance, physical exam parameters, behavioural scoring, and body condition scoring. Utilizing this method at our institution, we were able to calculate a cage side cumulative score, in addition to total body weight loss criteria to provide a quantitative measure and refinement to humane end points. This, in comparison to a subjective assessment of morbidity or moribundity, helped to determine preemptive euthanasia prior to animals becoming moribund, provide better communication between husbandry technicians and veterinary staff and ultimately improve animal welfare by minimizing pain and distress associated with GvHD, the effects of TBI and the humanization process.

2 Materials and Methods

40, 6-8 week old female NCG mice were IV injected with human PBMCs via the tail vein.
389, 4-6 week old female NCG mice were IV injected with cord blood derived CD34$^+$ cells injected via the tail vein following TBI. All animals were weighed and health checked three times weekly until study end.

For the initial PBMC pilot studies, no humane end point guidelines were utilized outside of internal clinical health monitoring standard operating procedures. Following experimental manipulation, some animal loss was attributed to GvHD per pathologist consultation. Animals that received human PBMCs were euthanized due to low body condition, generalized alopecia and scaly skin which was negative for *Corynebacterium bovis*. Other animals in the initial pilot study were euthanized due to >20% body weight loss as a sole humane endpoint.

The humanized mouse end point guidelines were then developed and initiated following the initial CD34$^+$ humanization studies and applied to all other studies to improve technician confidence and communication between stakeholders regarding the clinical health of the animals. These guidelines accounted for appearance, physical exam parameters, behavioral scoring, body condition score (BCS) in addition to weight loss parameters and were designed to be done cage side. This also allowed for euthanasia determination prior to animals becoming moribund or found dead.

3 Results

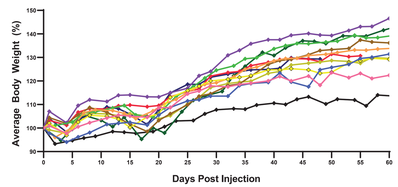

Figure 1: Body weights (g) are shown as average over time (percentage body weight change compared to initial weight at Day 0). Data compiled from twelve studies from animals injected with cord blood derived CD34$^+$ cells; each line represents an individual study.

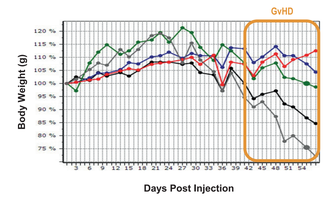

Figure 2: Representative study displaying individual animal body weights (g) over time from animals injected with donor-qualified PBMCs. GvHD onset was tracked by body weight changes.

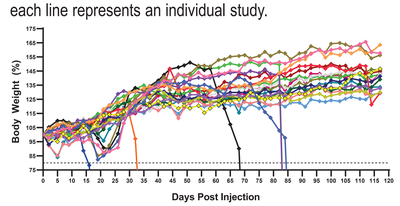

Figure 3: Representative CD34$^+$ study displaying individual animal body weights (g) over time. Animals with decreases in body weight are visible as well as animals that recover after being placed on watch. Dashed line indicates 20% overall body weight loss.

Figures 1-2 indicate body weight over time for both CD34$^+$ and PBMC studies. Guidelines were created and put into place in mid-April 2019. Figure 3 shows one study with individual animal body weight over time highlighting that weight loss typically occurred within the first 30 days of the study. The weight loss also coincided with symptoms of TBI and higher guideline scoring. Data complied from eleven studies, evaluating n=374 animals. Five studies were initiated before the guidelines were implemented and contain data from both before and after the guidelines were implemented.

Table 1: Percentage of animals found dead or euthanized before and after the guidelines were implemented as well as percentage of animals that recovered after being placed on watch.

	Animals Found Dead	Animals Euthanized	Animals that Recovered (Removed from Watch)	n
Before Guidelines	**16.0%**	5.6%	0.0%	144
After Guidelines	**6.1%**	26.7%	10.8%	343

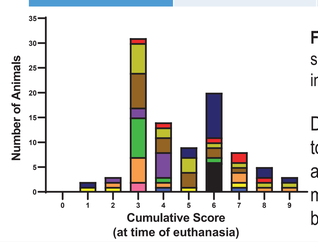

Figure 4: Cumulative score at time of euthanasia. Two distinct scores were noted during the course of the study denoting instances where animals were likely to be euthanized.

During weeks 2-4 post TBI or injection, animals were likely to receive a score of "3" but met the weight loss criteria and were euthanised. Following week 4, animals were more likely to score between "4-6" and were euthanized based on a combination of weight loss and clinical signs.

4 CR Humanized Mouse End-point Guidelines

The Charles River Humanized Mouse End Stage Illness Guidelines consisted of weight guidelines as well as an assessment scoring table. Animals were weighed every other weekday (three times weekly).

Weight Guidelines

- Any overall or single weight loss of 20-25.0% initiates daily assessment scoring and weights.
  - If weight loss continues or scores >8 on the assessment scoring table, euthanasia is required.
- Any animal with an overall or single weight loss of >25% requires immediate euthanasia.

Assessment Scoring Table

- The assessment scoring table consisted of four portions as seen in the table with an example score.
  - A more detailed scoring list can be found as part of the handout.

Appearance (0-3)	1
Physical exam parameters (0-2)	2
Behavioral scoring system (0-3)	3
Body condition score (0-3)	2
Cumulative score	8

- A given cumulative score the appropriate interpretation and action would be taken based on the following table.

Cumulative Score	Interpretation	Criteria/Action
0	Normal	▪ Continue regular monitoring, no action required
1-5	Mild to moderate signs of morbidity	▪ Monitor daily and notify supervisor and principle scientist
6-8	Significant signs of morbidity	▪ Euthanasia is recommended ▪ Contact veterinary personnel, supervisor and principle scientist to discuss changes in monitoring frequency and end point of study/animal
9-11	Moribund	▪ Immediate euthanasia

5 Conclusion

As humanized mice models are becoming more widely utilized in research, veterinary staff and animal care technicians require refinement of end point criteria to better assess the condition of the animal. Based on the PBMC and CD34$^+$ humanized mice in these pilot studies, following TBI, sole weight loss of >20% did not automatically correlate with higher severity assessment scores for euthanasia determination. Once animals are placed "on watch" after losing >20% weight loss, daily weights and observations are required to accurately assess the animals and avoid animals being found dead. Based on results from Table 1, the percentage of animals found dead decreased after the implementation of the guidelines. Technicians were also empowered to confidently euthanize an animal based on these parameters and the guidelines have also been helpful in training and acclimating new technicians to study goals and expectations relating to humanized mice.

6 Acknowledgements

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