

## **1. Introduction**

The following describes a previously unpublished pilot study investigating the effects of TGF- $\beta$ 3 treatment on survival after a single 10.5 Gy irradiation in DBA/2 mice.

## **2. Materials & Methods**

### *2.1. Animals and Treatment*

All procedures involving animals were conducted according to the Slovak Ethical Rules. Husbandry, care, and experimental use of the animals were in accordance with institutional guidelines under the approved protocols by the ethical committee and the Slovak Veterinary Office.

16 male and 16 female DBA/2 mice were bred locally in a nonbarrier unit and divided into two treatment groups: TGF- $\beta$ 3 treated ( $n = 8$  male +  $n = 8$  female) and controls ( $n = 8$  male +  $n = 8$  female). Mice were housed in cages of 8 according to experimental groups and fed mouse pellets and water ad libitum. Animals were collected for the experiment as they were bred and age therefore varied between 2 and 5 months old at the initiation of the experiment. Animals were age-matched between the groups.

For irradiation, animals were placed in a circular pen with wedge-shaped rooms and irradiated with a  $^{60}\text{Co}$ -source (Theratron Elite 100; Best Theratronics, Canada). Animals were given a 10.5 Gy dose at a dose rate of 55.8 Gy/h. Follow-up consisted of monitoring of general welfare and estimation of body weight by weighing the cage with the animals, subtracting the weight of the cage, and dividing by the number of animals in the cage. Moribund mice were euthanized by an overdose of anesthetics. The primary outcome of this experiment was animal survival. The experiment was terminated on day 161 after irradiation and surviving animals were euthanized via anesthetic overdose. Autopsy and histopathology were not performed.

TGF- $\beta$ 3 treatment consisted of one intraperitoneal injection of TGF- $\beta$ 3 at a dose of  $\sim 12.5$   $\mu\text{g/kg}$  body weight. The injection was administered 24 hours after irradiation.

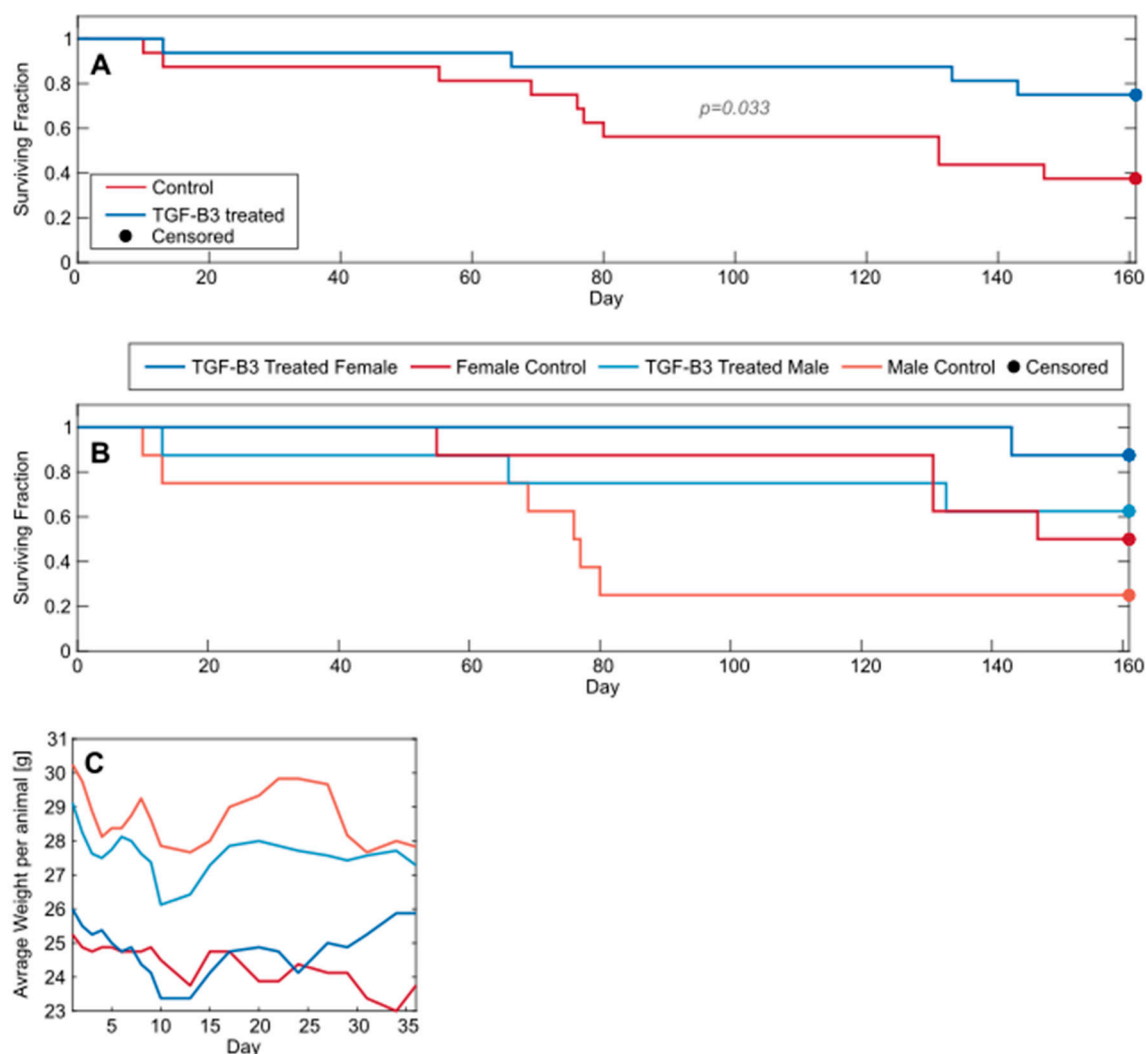
### *2.2. Statistical Analyses*

The Kaplan-Meier survival data in Figure S1. A and B were analyzed with the log-rank test using an online statistics tool [1].

## **3. Results**

161 days after a 10.5 Gy irradiation, TGF- $\beta$ 3 treated animals displayed a significant increase in survival at 75%, compared to controls at 37.5% ( $p = 0.033$ , log-rank test) (Figure S1A). When data from male and female mice were separated, treatment with TGF- $\beta$ 3 did not significantly increase survival for either group, although a trend in increased survival could be observed at 62.5% vs. 25% for males ( $p = 0.144$ ) and 87.5% vs 50% for females ( $p = 0.122$ ) (Figure S1B).

Estimated body weight followed similar patterns for TGF- $\beta$ 3 treated and control animals, with male animals weighing more than female animals throughout the measuring period (Figure S1C).



**Figure S1.** Survival and body weight of animals from Pilot Experiment. **A.** Pooled survival for male and female control (blue) and TGF- $\beta$ 3 treated (red) animals. The difference in total survival was significant ( $p = 0.033$ , Log-rank test). **B.** Group-wise survival for female TGF- $\beta$ 3 treated (dark blue) and control (dark red) animals and male TGF- $\beta$ 3 treated (light blue) and control (light red) animals. The difference between TGF- $\beta$ 3 treated and control animals was not significant within each sex ( $p = 0.144$  for males and  $p = 0.112$  for females, log-rank test). **C.** Average body weight for the first 35 days after irradiation for the same groups as **B**. Note that the body weight was measured by weighing the cage with the animals, subtracting the weight of the cage, and dividing by the number of animals contained in each cage. The weight of individual animals was thus not recorded.

## References

1. Statistics Kingdom. Kaplan Meier Survival Analysis. 2017. Available online: <https://www.statskingdom.com/kaplan-meier.html> (accessed on 24 October 2023).