

Evaluating the Application of Microchips to Monitor Temperature Change After Immunotherapy

Katie Potts, Alexandria Hernandez, Ben Matran, Guoxiang Yang, Michael Campagna, Jing Jiao, Mingshan Cheng
Innovation and Product Development, The Jackson Laboratory, Sacramento, CA 95838 USA

INTRODUCTION

Immunotherapy provides a powerful strategy to eradicate cancer, however it can trigger potentially severe side effects, including Cytokine Release Syndrome (CRS). The excessive release of cytokines can cause an increase in temperature and potentially severe organ failure. Therapeutic induced CRS can be monitored using humanized mouse models. Common methods to monitor therapeutic induced CRS include monitoring body conditioning scores of mice, collecting blood for serum cytokine analysis, and monitoring the change in mouse temperature. Rectal thermometers are commonly used to determine temperatures in mice. However, the acceptable frequency of use for this method is limited by its relative invasiveness. With this limited use it is difficult to capture the exact time point when mice experience the change in temperature after immunotherapy. As an alternative to taking rectal temperatures, mice can also be implanted with temperature monitoring microchips. These microchips paired with under-cage matrix scanners allow for continuous monitoring of temperatures in mice. This study seeks to determine if microchip temperatures and rectal temperatures are consistent, the exact time CRS occurs, and if microchips have an impact on cytokine levels and expected body weight loss.

METHODS

- On day 0, thirty-two, 6-week-old female NOD.Cg-Prkdc^{scid} H2-K1^{tm1Bpe} H2-Ab1^{em1Mvw} H2-D1^{tm1Bpe} Il2rg^{tm1Wjl}/SzJ (025216) mice were humanized by IV injection of human peripheral blood mononuclear cells (PBMC) at 15x10⁶ cells/mouse, 4 hours post irradiation at 100cGy.
- On day 1, 20 mice were injected with temperature microchips in the intrascapular area (Figure 2) while under anesthesia by isoflurane (Figure 1). Mice were scanned using a noninvasive handheld scanner to check implantation of the temperature microchips.
- On day 5, rectal temperatures (Figure 3) and handheld temperature scans were taken to establish baseline temperatures.
- Mice were grouped to target groups based on bodyweight and baseline temperature. Home cages of mice with microchips were placed on matrix scanners (Figure 4) overnight to continuously monitor temperatures.
- On day 6, microchipped and non-microchipped mice were IV injected with Phosphate Buffered Saline (PBS) or OKT3 (0.25 mg/kg) to induce toxicity. Cages of mice with microchips were placed on matrix scanners to start recording temperature. The remaining home cages of mice were housed on static racks without ventilation.
- Six hours post treatment, all mice were retro orbital (RO) bled for cytokine analysis, rectal temperatures and handheld temperature scans were taken.
- Cages of mice with microchips were placed back on matrix scanners for continuous temperature monitoring.
- Bodyweights, clinical observations and temperatures were taken daily after inducing toxicity.
- Mice were taken down on day 13.
- Serum cytokine levels were measured by the BD Cytokine Bead Array (CBA) Human Th1/Th2 Cytokine Kit II (Catalog No. 551809). The flow cytometry data was analyzed using FCAP Array software (BD).

FIGURE 1. Isoflurane Chamber setup



FIGURE 2. Intrascapular Area Microchip Implantation



FIGURE 3. Taking Rectal Temperatures

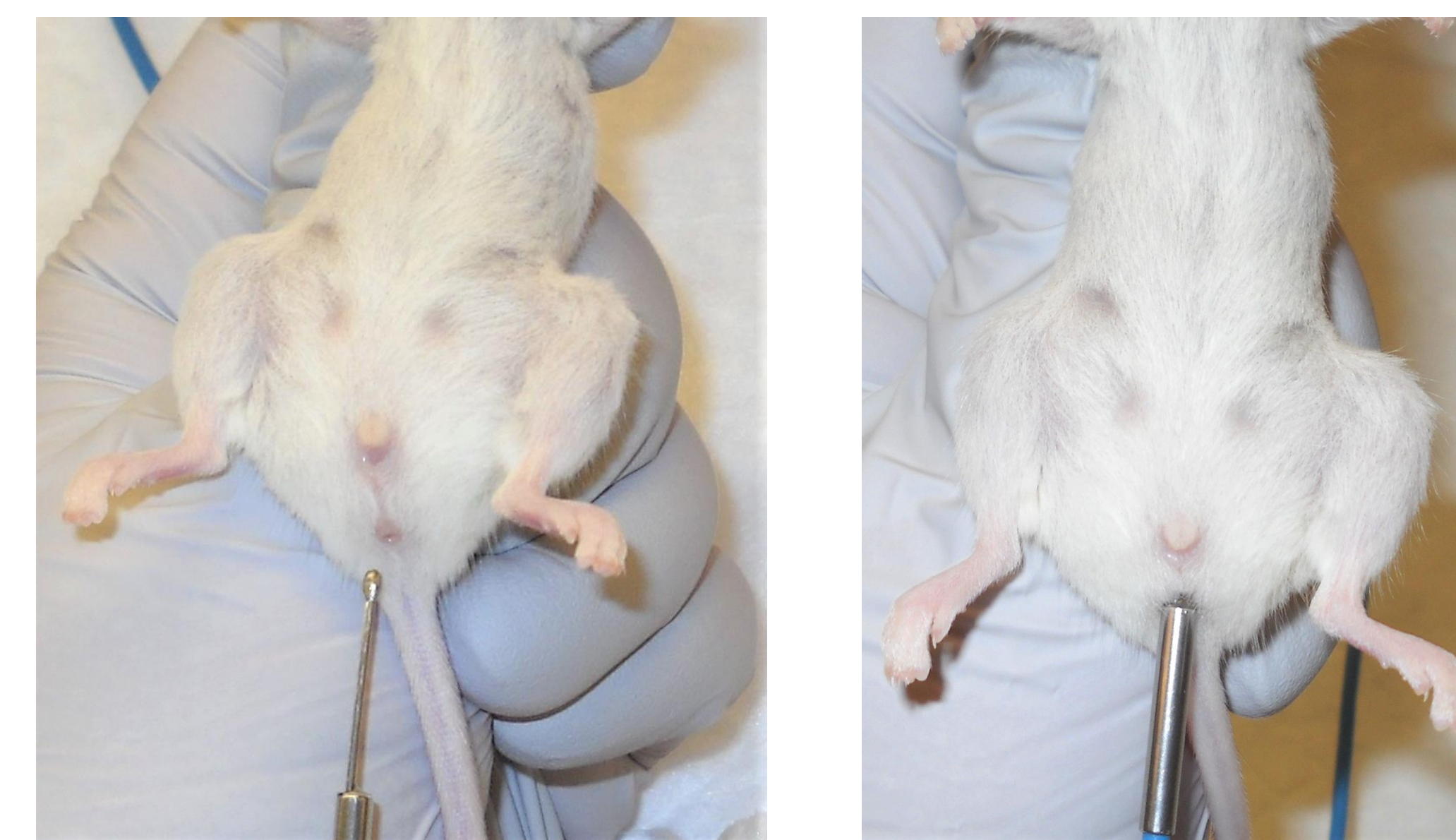


FIGURE 4. Cages on Matrix Scanners

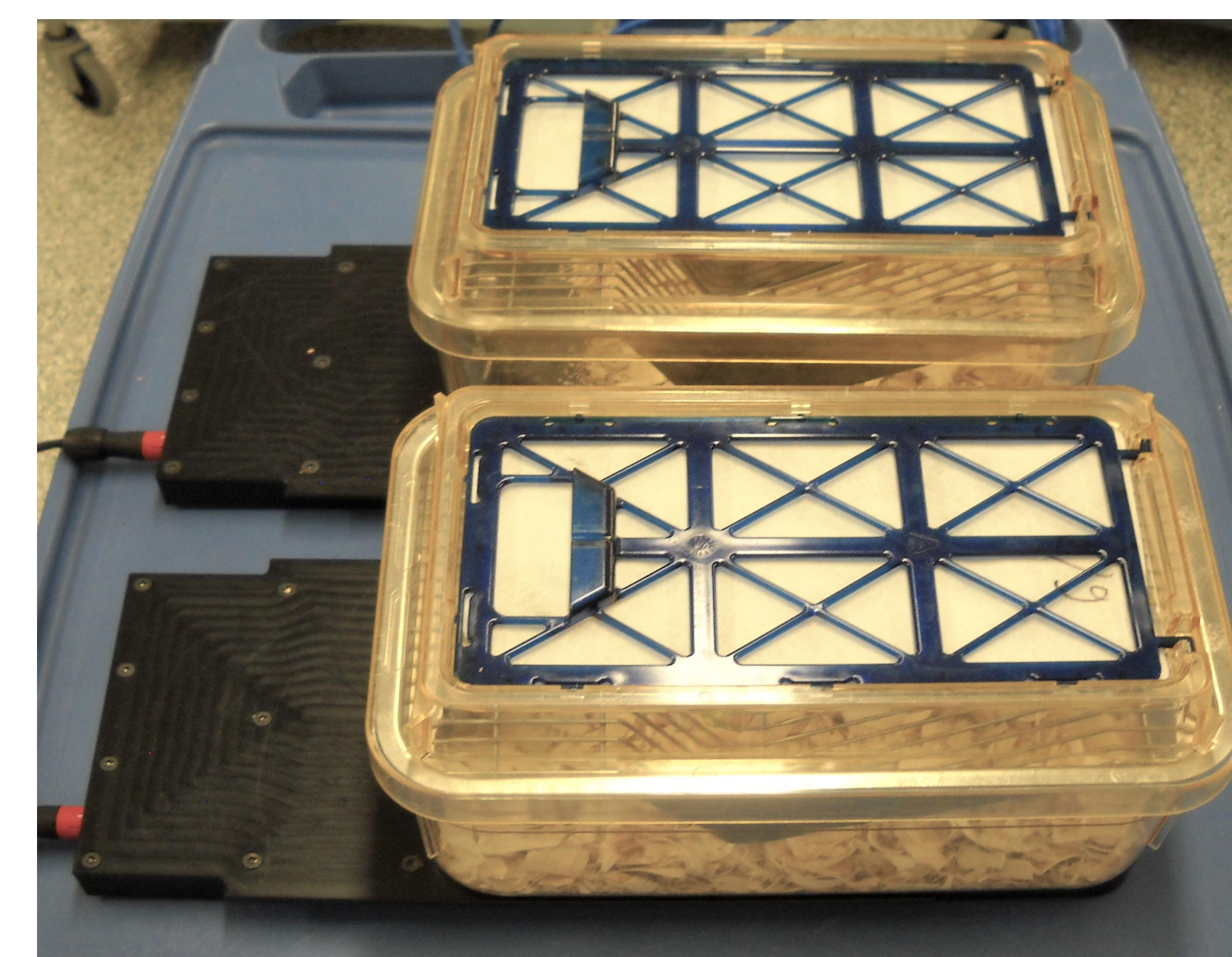
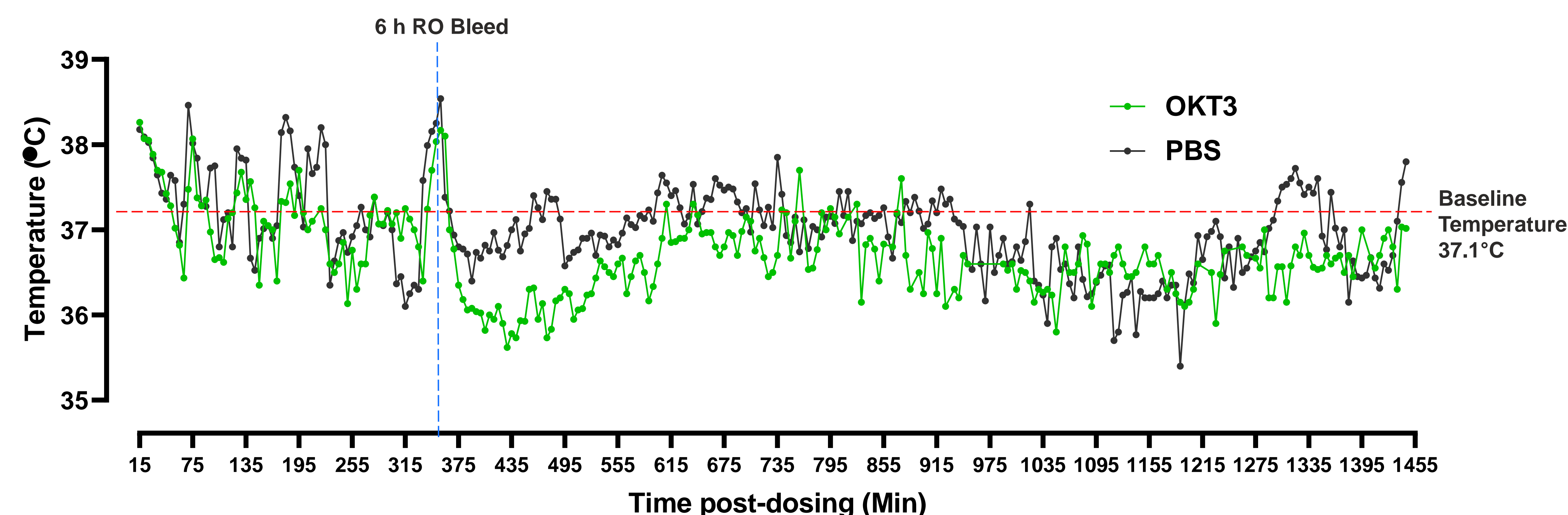


FIGURE 8. Recorded Microchip Matrix Temperatures for First 24 h after Immunotherapy



Graph showing continuous monitoring of mean temperatures of microchipped mice for the first 24 h after treatment with PBS or OKT3, baseline temperature established on day 5, and time of 6 h RO bleeds.

FIGURE 5. Microchip size

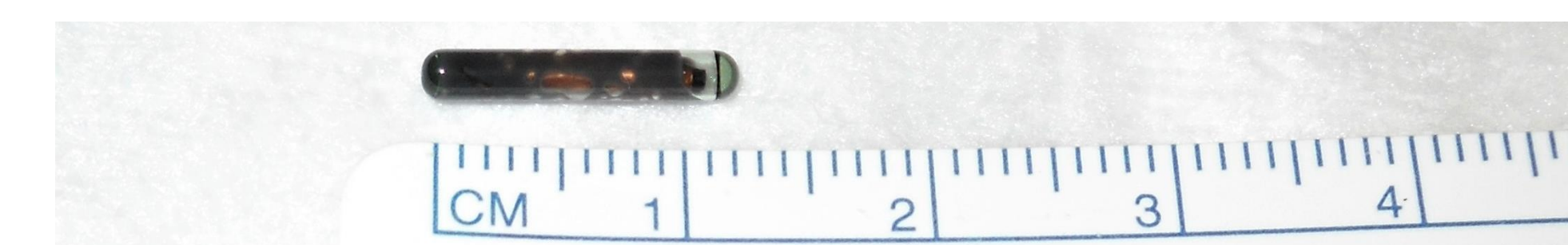
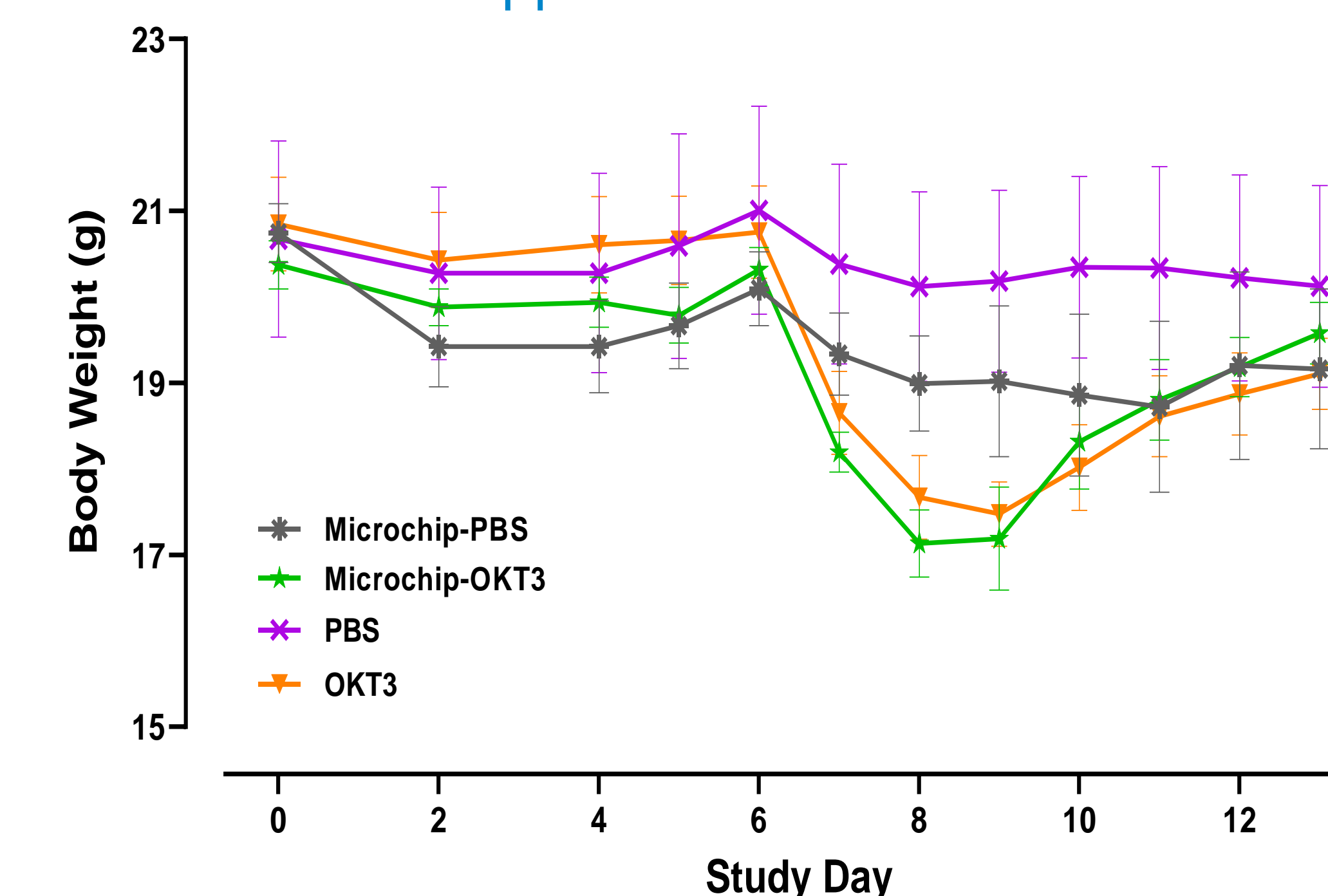
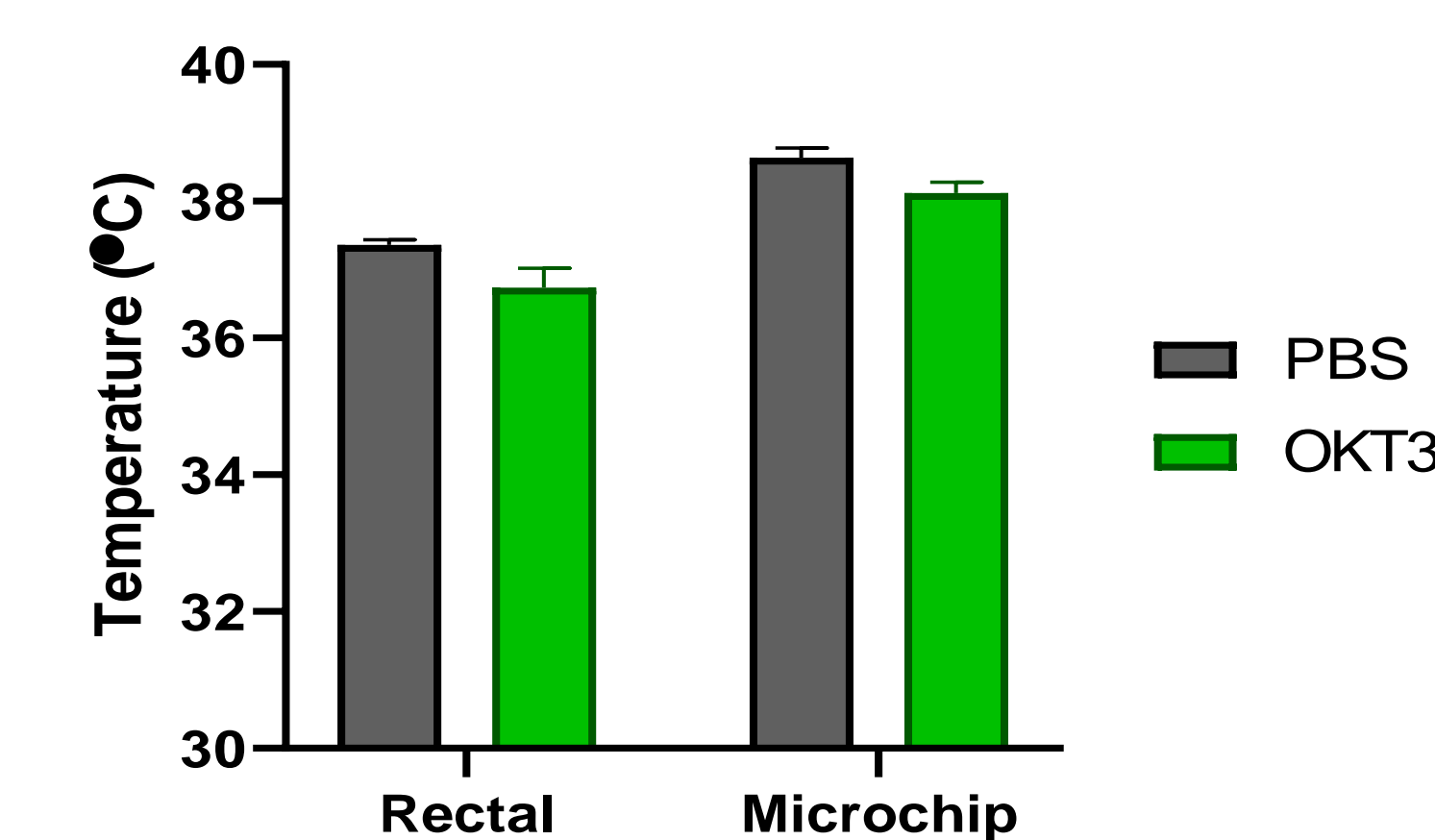


FIGURE 6. Bodyweight Comparison in Microchipped and Non-microchipped Mice



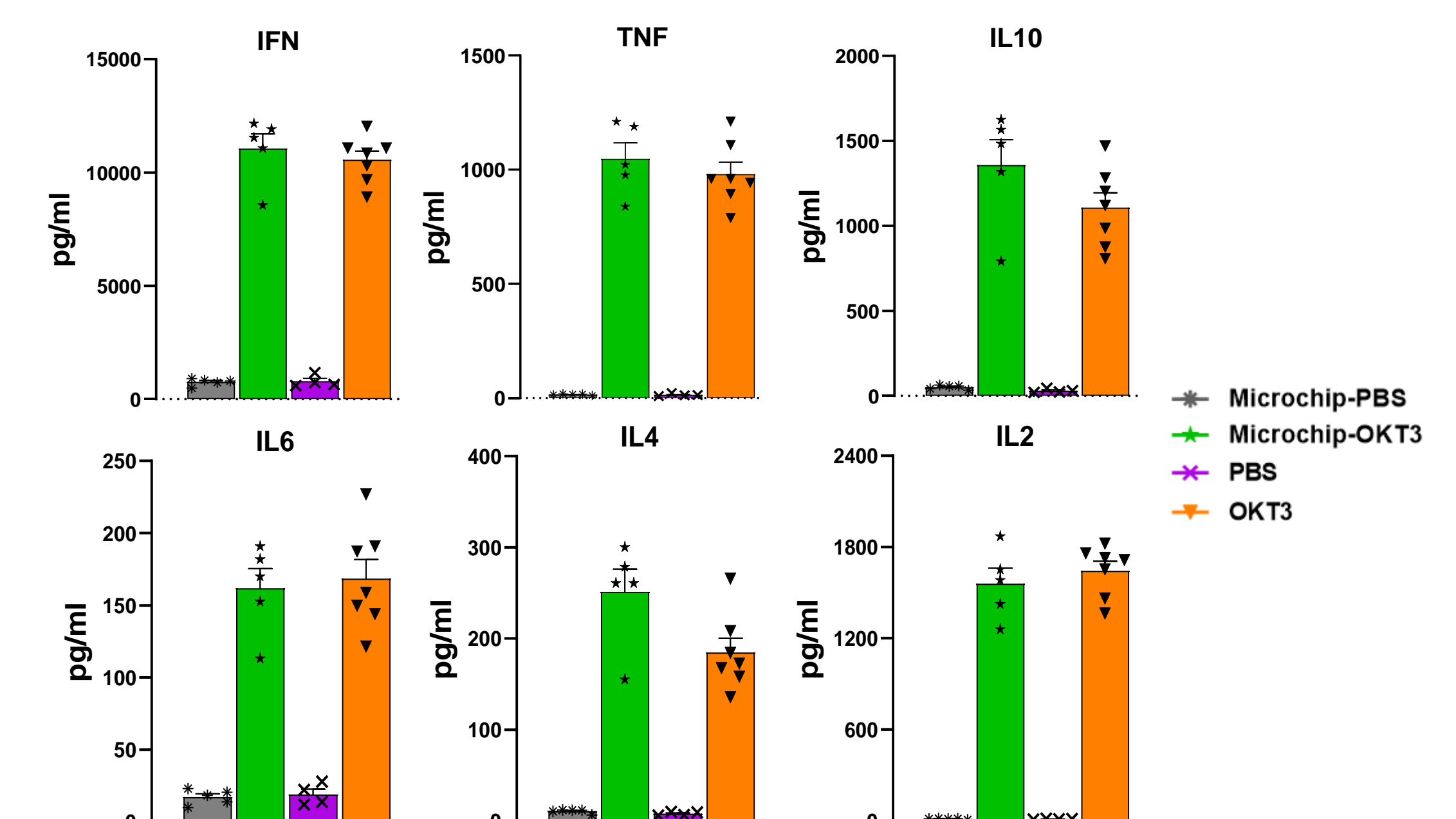
Average +/- SD bodyweight of microchipped mice and non-microchipped mice for PBS and OKT3 treated groups. Bodyweight loss was similar between microchipped and non-microchipped mice for both PBS and OKT3 treated groups.

FIGURE 7. Comparison of Rectal and Microchip Temperatures in Microchipped Mice at 6 h Post Treatment



Average +/- SD rectal temperatures and microchip temperatures in the same mice. Rectal temperatures are slightly lower compared to microchip temperatures. However, the temperature variation between OKT3 and PBS treated groups is consistent between temperature methods.

FIGURE 9. Serum Cytokine Levels in Microchipped and Non-microchipped Mice



Serum cytokine levels in microchipped and non-microchipped mice were similar. However, IL-10 and IL-4 showed a slight variation in levels in the OKT3 treated mice from both groups.

RESULTS

- The OKT3 treated groups had observable bodyweight loss 24 h to 96 h (Day 6 – Day 10) after treatment compared to the PBS treated groups, which is consistent between microchipped and non-microchipped mice.
- Rectal and microchip temperatures followed similar relative trends within the same animal, but rectal temperatures were consistently lower in absolute value.
- Initial increase in temperature immediately after dosing is reflective of using a heat lamp to aid in IV dose. Mice returned to baseline temperature until there was an increase in temperature at 5.5 h (330 min) after dosing. Maximum temperature was reached at 6 h (360 min).
- Cytokine analysis confirmed mice had significant cytokine release at 6 h post OKT3 treatment which is consistent with the temperature increase seen while mice were being continuously monitored for temperature. There was an increase in temperature at 6 h post PBS treated groups as well, but this is inconsistent with cytokine levels.
- At 6 h (360 min) when the mice were RO bled for cytokine analysis, there was a temporary decrease in temperature. Both PBS and OKT3 treated groups decreased in temperature following the bleed. However, OKT3 groups had a greater decrease in temperature suggesting the mice may have been less physiologically stable.
- Two hours after blood collection (480 min) there was an increase in temperature suggesting a recovery from blood loss. However, the OKT3 treated groups remained lower in temperature than the PBS group, suggesting the OKT3 treated groups remained less stable from the influx of cytokines. Both groups returned to baseline temperature 37.1°C at 12 h (720 min) post dose suggesting a complete recovery from blood loss and possibly a reduction in cytokine levels for the OKT3 treated groups.
- There were many fluctuations in temperatures for the remaining 24-72 h, most likely due to the diurnal variation in activity level of mice. There were no visible surges in temperatures other than 6 h (360 min) post dose. However, the OKT3 treated groups stayed consistently lower in temperature than the PBS groups.

CONCLUSION

- Microchips are an effective approach to determine the changes in temperature due to immunotherapy with no impact on bodyweight loss or cytokine release.
- Microchip temperatures are slightly higher than rectal temperatures.
- The matrix scanner successfully allowed for non-invasive, continuous monitoring of temperature in a way that allowed for easier identification of transient changes within certain groups.

FUTURE DIRECTIONS

- Determine the exact temperature curve of CRS by not collecting blood from the mice at 6 h.
- Test out different immunotherapy treatments to determine if the increase in temperature occurs at the same time across treatments.