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CONTRACTING ORGANIZATION: North Carolina State University

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14. ABSTRACT

It is known that radiation therapy (RT) targeted to tumors especially melanomas can generate an in-situ tumor vaccine. In other words, inducing the release of antigens during cancer cell death, in association with proinflammatory signals, trigger the adaptive immune system to activate tumor-specific T cells and enhance tumor cell killing. The immune response of RT is especially pronounced in combination with immunotherapy. In our first-year funding cycle, with our vivo experiments, we have used genetically mutant circadian clock disrupted PER1/2 mutant mice and rotating shift mice showed a decreased adaptive immune response compared to their wild-type counterparts as an indication of 2-3-fold reduced CD4+ T lymphocytes in blood. In conclusion, these results suggest that circadian clock plays an important role in protecting host adaptive immune response which is important for efficient tumor cell killing against RT. In addition, we have established a circadian synchronization protocol in vitro using B16-F10 melanoma cell lines. During previous year funding cycle, our data suggest that a significantly reduced growth rate in untreated tumors in PER1/2 mutant mice vs. untreated wild-type mice, and a significantly reduced growth rate of wild-type AM immunotherapy treated mice vs. wild-type untreated mice. In addition, there were significantly fewer % gated CD4 (P=0.023872) and CD8 (P=0.004634) cells in the blood sample of AM immunotherapy treated wild-type mice relative to untreated mice. There were also significantly fewer % gated CD4 cells (P=0.034883) in the blood of PM immunotherapy treated wild-type mice. For this funding cycle, our results from figures 1-3 demonstrate that a healthy circadian clock protects mice from an aggressive tumor growth pattern, tumor mass, and further induces a more robust immune response against melanoma. In addition, our preliminary results suggest that anti CTLA4 therapy was more effective in clock disrupted females than wild type controls. Most importantly, Dual immuno therapy was effective against B16F10 melanoma mice model and most effective for genetically clock disrupted male mice. Overall, these results suggest that the time-of-day of dual immunotherapy treatment can have a significant effect on tumor growth in male mice with a normal circadian rhythm and in female mice with a disrupted circadian rhythm.

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1. INTRODUCTION:

Melanoma is the most lethal form of skin cancer. Despite the promise of immunotherapy, less than 50% of melanoma patients respond to monotherapy or combination therapy with targeted therapy. Although, radiation therapy (RT) plays a small role in the traditional management of metastatic melanoma for palliation, recent experimental and clinical evidence suggests a broader involvement of RT in enhancing tumor cell killing in immunotherapy by augmenting the patient's immune system. According to ClinicalTrials.gov, there are currently more than 100 active clinical trials underway for treatment of melanoma patients with RT, mostly in combination with targeted/immunotherapy. However, the majority of patients that receive RT suffer skin inflammation ranging from mild erythema to ulceration of the skin. Indeed, RT is often terminated early so as to limit further discomfort to patients; this cessation of treatment increases the risk of radio-resistance and tumor relapse. Minimizing toxicity is *critical* to improving the effectiveness of RT in combination with immunotherapy against melanoma. The *objective* of this proposal is to translate our findings on the circadian clock-controlled nature of DNA damage response and the immune system to minimize toxicities and improve RT and immunotherapy treatment efficacy in melanoma patients. Published data from our group and the preliminary data within this proposal show that DNA damage response (DDR) and pro-inflammatory signaling events are controlled by the circadian clock. We therefore *hypothesize* that skin toxicity and tumor shrinkage by the RT in combination with immunotherapy are regulated by the circadian clock. Completion of this project will *significantly contribute* to understanding the effectiveness of circadian clock-regulated therapies on healthy tissue toxicity, as well as tumor shrinkage, at the mechanistic level and determine whether active circadian rhythm has a protective role against the adverse effects of radiation treatment alone or in combination with immunotherapy. We also seek to test these therapies on both healthy and circadian-disrupted animals to determine treatment differences that may be necessary for individuals with abnormal sleep-wake cycles (e.g., shift workers). *Most importantly*, the incidence of melanoma in active duty *military personnel* has been increasing, and now exceeds that of the general population.

2. KEYWORDS:

Circadian rhythm, radiation therapy, time of day, sleep disruption, DNA damage response, skin toxicity, and melanoma

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Major Task 1: Test the circadian clock effect of IR treatment in vivo with wild type and PER mutant mouse models.

Subtask 1: Identify the circadian clock-related mechanisms underlying IR-induced DDR signaling, proinflammatory cytokine induction, and cell death in mouse skin using wild-type, and environmentally and genetically clock disrupted animals.

Subtask 2: Characterize the circadian clock impact underlying IR- induced skin erythema and fibrosis

Subtask 3: Characterize the role of the circadian clock in IR-induced adaptive immune response in spleen, lymph nodes, and blood samples

Milestone(s) Achieved: Both subtask 1 and 2 were completed and published in 2 major scientific journals i.e. PMIDs: 32422325 and 31919902. (for details please see under publications list and attached full text pdf. articles). Whereas, subtask 3 was completed in wild-type mice and found a significant sex difference in wild-type animals and this work was published in another peer-reviewed journal i.e. PMID: 32638080.

Major task 2 (under aim 2): Test the circadian function in RT and immunotherapy effectiveness on melanoma tumor growth and toxicity in healthy tissues using melanoma-prone genetic mouse models.

Subtask 1: Study skin toxicity and tumor shrinkage of B16-F10 tumor bearing mice or $BRAF^{CA}$ mice treated with IR alone, anti-CTLA4 injection alone, or both IR and anti-CTLA4 injection relative to mock/untreated control. Collect skin, blood, spleen, and tumor tissues 30 days post IR and/or immunotherapy treatments.

Subtask 2: Use skin, spleen, blood and tumor tissues to examine immunomodulation by the circadian clock, analyzing protein expression levels of DDR-signaling pathways including ATR/ATM-mediated checkpoint and apoptosis.

Subtask 3: Examine expression levels of cell proliferation/mitotic markers and DNA repair markers. Additionally use TUNEL assay and B- Gal staining kit to detect senescence levels.

Subtask 4: Score radiosensitivity of blood cells by chromosomal aberrations as means breaks per metaphase using a three-color FISH technique.

Milestone(s) Achieved: Approximately 50% of this goal was completed and rest of the project is in progress. The major findings of this task can be found under 'significant results' section and table 1, figures 1-3.

What was accomplished under these goals?

First 3 months of this study, we have successfully obtained IACUC and ACURO approval. The remaining last 9 months, we have been breeding the C57BL/6 genetic mice with established B16-F10 melanoma tumor models in the presence or absence of a molecular clock with Period 1 and Period 2 genes mutations (also called as PER mutant mice). The PER mutant animals were originally developed by Dr. David Weaver's group and are well-established and well-studied in circadian biology research. These animals already available in Gaddameedhi lab at WSU for this study. Both wild-type and PER mutant mice with melanoma tumors were irradiated at tumor sites using an X-Ray machine (X-RAD 320 model from precision X-Ray) during the morning (6-8 AM) or evening (4-6 PM). The following scientific activities were took place during the previous and current funding cycles:

Cell culture and collection

B16-F10-luc2 cells were cultured in RPMI+10% FBS, and collected for injection at between 70-85% confluency. Matrigel used for injections was thawed on ice prior to cell collection, and aliquoted into 25 ul volumes kept on ice. At collection, cells were trypsinized, counted using a hemocytometer, and washed 2x with 1x PBS. Calculations were performed such that 50,000 cells would be contained in every 25 ul, cells were resuspended in an according volume of serum-free RMPI, and placed on ice. The cell suspension was then added to each Matrigel aliquot, totaling 50 ul for each injection, and cells were transferred to the vivarium on ice for injection.

Cell Injection

All animal experiments were performed according to protocols approved by the Institute of Animal Care and Use Committee of Washington State University. Prior to injection male mice aged 10-15 weeks had their right flank and back shaved to allow for accurate injection and measurement via caliper. For injections, mice were weighed and anesthetized with 0.5-1.5% isoflurane in oxygen and cells were injected to the lower right back using insulin syringes.

Monitoring and intervention

Mice were weighed and tumor measurements were taken every other day. Tumor dimensions were measured using calipers and volume was determined by: $L \times W^2 \times 0.52$, with L corresponding to the longer measurement, and W the shorter perpendicular measurement. All interventions took place between 4-11 days post cell injection. Ionizing radiation was given using an X-RAD 320 equipped with an adjustable collimiter in daily fractions of 3 GY on days 4, 7, and 10. Anti-CTLA-4 (9H10, BioXcell) immunotherapy was given via intraperitoneal injection in 3 doses of 10 mg/kg on days 5, 8, and 12. All treatments were performed at ZT2 for AM groups, or ZT10 for PM groups.

Tissue collection

At sacrifice, blood was collected via cardiac puncture while animals were deeply isoflurane anesthetized. Blood was spun at 3 minutes at 5000 RCF, plasma was frozen, and the remaining fraction was used for flow cytometry. Fur was removed from the backs of mice via plucking and both unirradiated and irradiated (when applicable) sections of skin were collected, affixed to cardstock, covered with tin foil and snap frozen on dry ice. Lungs, liver, and a section of tumor were all also collected and snap frozen on dry ice for protein analysis. Spleen and the remaining tumor were collected, washed in 1x PBS, and placed in cold 1x PBS with 0.1% BSA on ice and cells were isolated for flow cytometry.

Significant results

Figure 1A showed significant decreases in both B16-F10 tumor growth rate and tumor weight **(figure 1B)** for wildtype female mice compared to clock-disrupted Per1/2$^{-/-}$ female mice. In addition, our flow cytometric immune cell profiling suggests that wildtype spleen and tumors have a 4-5-fold reduction in pro-tumorigenic M2 macrophages compared with Per1/2$^{-/-}$ counterparts **(figure 2)**. Warranting more precise examination, however, analysis of anti-tumorigenic CD8+ Effector T cells revealed inverted trends between spleen and tumor **(figure 3).** Female spleen samples had a significantly decreased population in Per1/2$^{-/-}$ mice compared to wildtype, whereas tumor cells from the same Per1/2$^{-/-}$ group showed an opposite elevation of the effector memory subset. As PD-1 is known to suppress expansion of CD8+ effector subsets (5), it will be interesting to compare PD-1+ lymphocyte counts between spleen and tumor in the future. *Taken together, these results demonstrate that a healthy circadian clock protects mice from an aggressive tumor growth pattern, tumor mass, and further induces a more robust immune response against melanoma.*

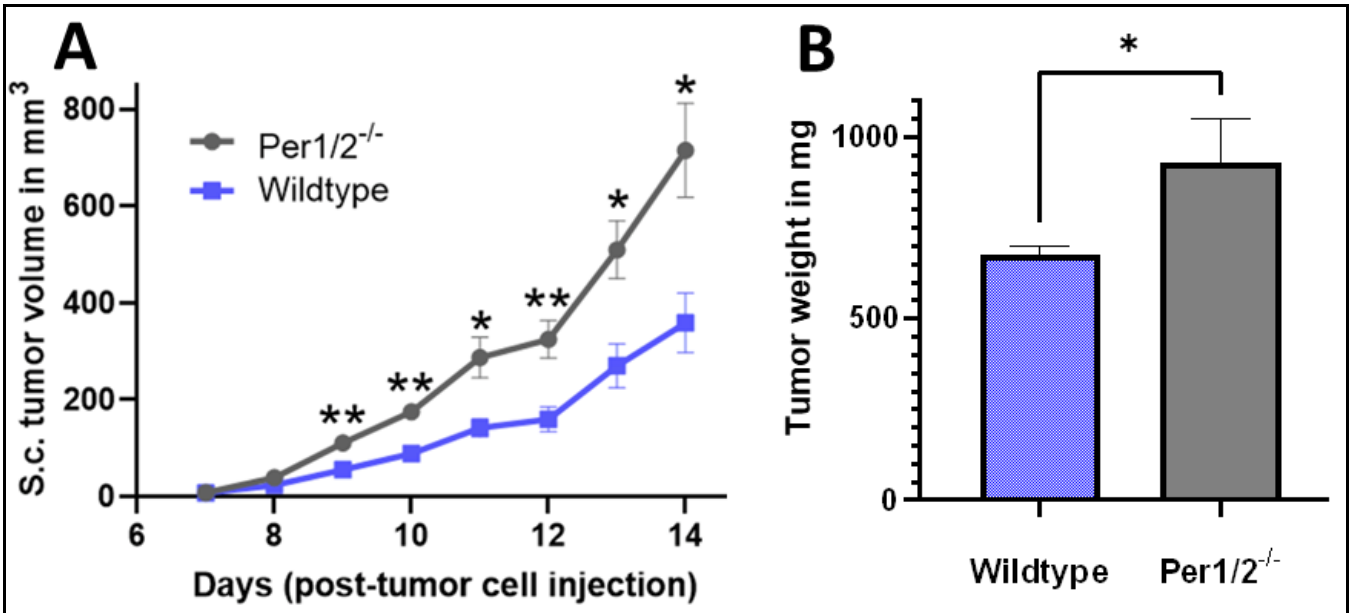


Figure 1: Circadian clock disruption by Per1/2 loss enhances melanoma tumor growth rate. (A) C57BL/6 wild-type (WT) and Per1/2$^{-/-}$ female mice (10-12 weeks old) were maintained under a LD12:12 cycle and subcutaneously injected (s.c.) with 0.2 million B16-F10 melanoma cells into a flank region. Tumor growth plot over 14 days after injection. (**B)** Mice from figure 1A were sacrificed 2 weeks after B16-F10 melanoma cells injection and tumor weight of all mice from each group were measured as shown in above bar graph. Two-tailed t-test performed for each timepoint; p-values *=p<0.05, **=p<0.01. Error bars = S.E.M. (n=6 mice each group).

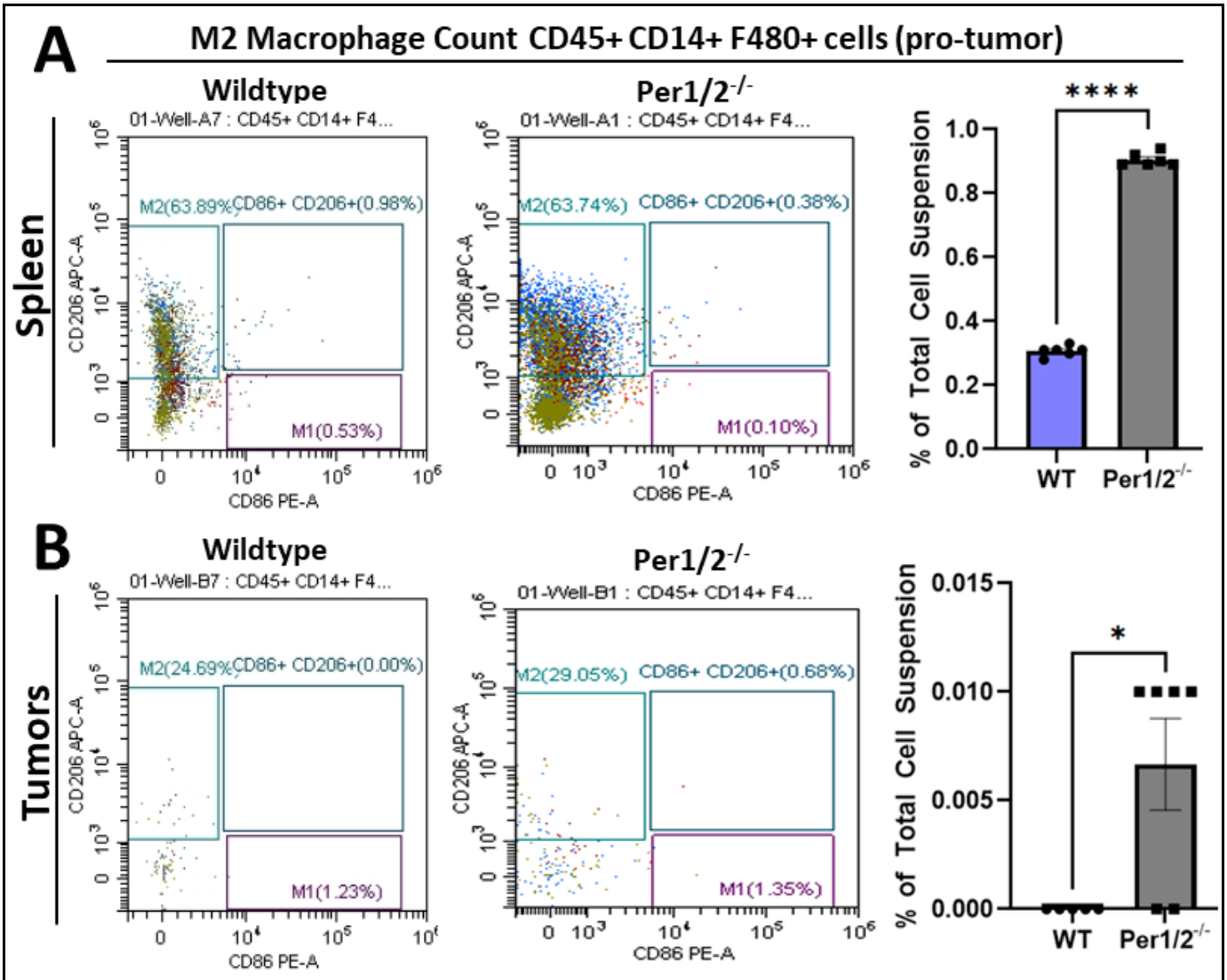


Figure 2: Circadian clock disruption by Per1/2 loss enhances pro-tumorigenic immune cell population with melanoma tumors. Representative flow cytometry images (left and middle) for Spleen **(A)** and B16-F10 Tumor cells **(B)** harvested from figure 1. Emphasis placed upon CD45+ CD14+ F480+ CD206+ CD86- population of pro-tumorigenic M2 macrophages (teal gate). Right side: quantification of M2 macrophage counts between Wildtype (WT) and Per1/2$^{-/-}$ C57BL/6 female mice spleen and tumors. One outlier removed from WT tumors group (ROUT Q < 1%). Two-tailed t-test performed for each timepoint; p-values *=p<0.05, ****=p<0.0001. Error bars = S.E.M. (n=6 mice each group).

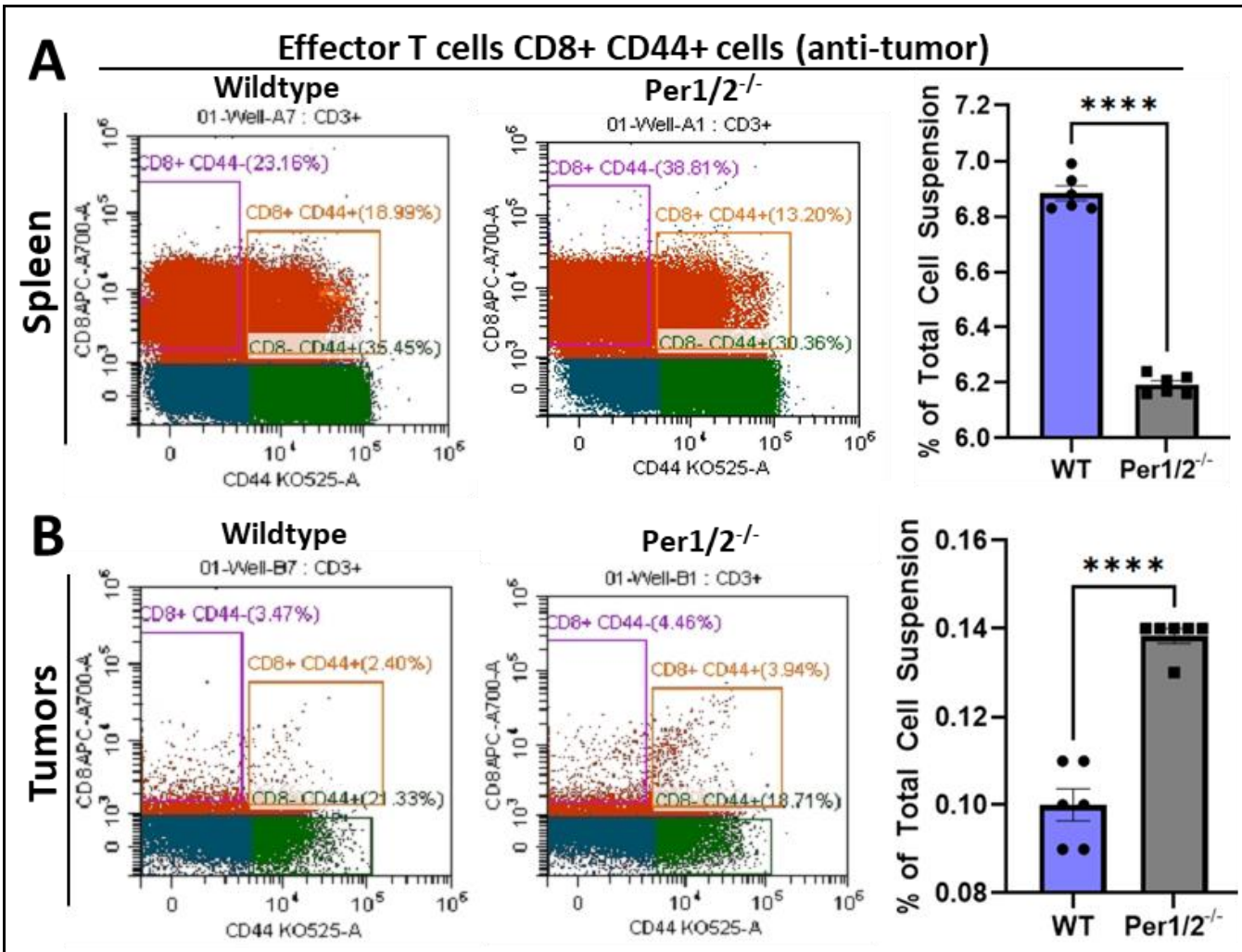


Figure 3: Circadian clock disruption by Per1/2 loss influences anti-tumorigenic immune cell population against melanoma tumors. Representative flow cytometry images (left and middle) for Spleen **(A)** and B16-F10 Tumor cells **(B)** harvested from figure 1. Emphasis placed upon CD3+ CD8+ CD44+ population of anti-tumorigenic Effector T cells (orange gate). Right side: quantification of Effector T cell counts between Wildtype (WT) and Per1/2$^{-/-}$ C57BL/6 female mice spleen and tumors. Two-tailed t-test performed for each timepoint; p-value ****=p<0.0001. Error bars = S.E.M. (n=6 mice each group).

In addition to the above report, the following technical progress has been made during the last year of the funding by understanding the Sex-Specific Time-of-Day and circadian clock effect on on Dual Immunotherapy Efficacy on Melanoma in Mouse model.

This study investigated the sex-specific time-of-day dual immunotherapy treatment effect on melanoma in mice. Mice were divided into four groups (Six mice used per group. Mice were housed up to a maximum of five mice per cage and at regular day shift i.e. Light: Dark::12:12):

- Wild-type (WT) male mice: These mice had a normal circadian rhythm and were treated with dual immunotherapy at ZT2 (2 hours after lights on) or ZT14 (14 hours after lights on).
- WT female mice: These mice had a normal circadian rhythm and were treated with dual immunotherapy at ZT2 or ZT14.
- Per1/Per2 mutant male mice: These genetically modified mice had a disrupted circadian rhythm at the molecular level. Mutations in the Per1 and Per2 genes prevented them from blocking the CLOCK-BMAL1 complex from binding to E-box elements in the promoter region of clock-control genes (CCGs). This led to the production of nonstop CCGs, including Bmal1, Clock, Rev-erbα, Rorα, NPAS2, and MTNR1A, which deviate from the normal rhythm. The circadian rhythm of these mice was entrained by the lights on at 7 AM (ZT0), so they were treated with dual immunotherapy at ZT2 (2 hours after lights on, which corresponds to inactive hours in mice) or ZT14 (14 hours after lights on).
- Per1/Per2 mutant female mice: These mice also had a disrupted circadian rhythm and were treated with dual immunotherapy at ZT2 or ZT14.

Tumor growth and treatment:

C57BL/6 WT and mPer1/mPer2 mutant male and female mice (8-12 weeks old) were separately caged with 1-5 mice per cage and maintained under a 12:12 light-dark cycle. They were subcutaneously injected with 0.05 million B16-F10 melanoma cells into a flank region. Dual immunotherapy was intraperitoneally administered as a combination of anti-CTLA-4 and anti-PD-1 antibodies on days 5, 8, and 11 after melanoma implantation. Tumor volume was measured every day from day 6 after implantation using a digital caliper and calculated using the formula:$V = (W^2 \times L)/2$. Mice were sacrificed when they were at risk of death due to tumor sizes approaching 1000 mm3 or after 4 weeks of transplantation.

Tumor growth was compared over 18 days after injection. Repeated measures of two-way ANOVA with Tukey's correction were used to compare tumor growth between the ZT2-treated and ZT4-treated groups using the statistical software package GraphPad Prism 10. $n = 6$ mice for each group. $p < 0.05$, $p < 0.001$, $*p < 0.0001$.

To understand the time-of-day immunotherapy effect of anti-CTLA4 antibody, flow cytometry analysis was performed to estimate the effector and regulatory T cell ratio changes in mice treated at ZT2 and ZT14, as well as their tumor infiltration. Spleen and tumor tissue were collected from mice at 28 days post-treatment, and the cells were stained and evaluated in flow cytometry. The flow cytometry data were analyzed to determine the percentage of effector and regulatory T cells, as well as the tumor infiltration of the cells. To evaluate the time-of-day immunotherapy effect of anti-PD1 antibody, the activity of effector T cells was studied.

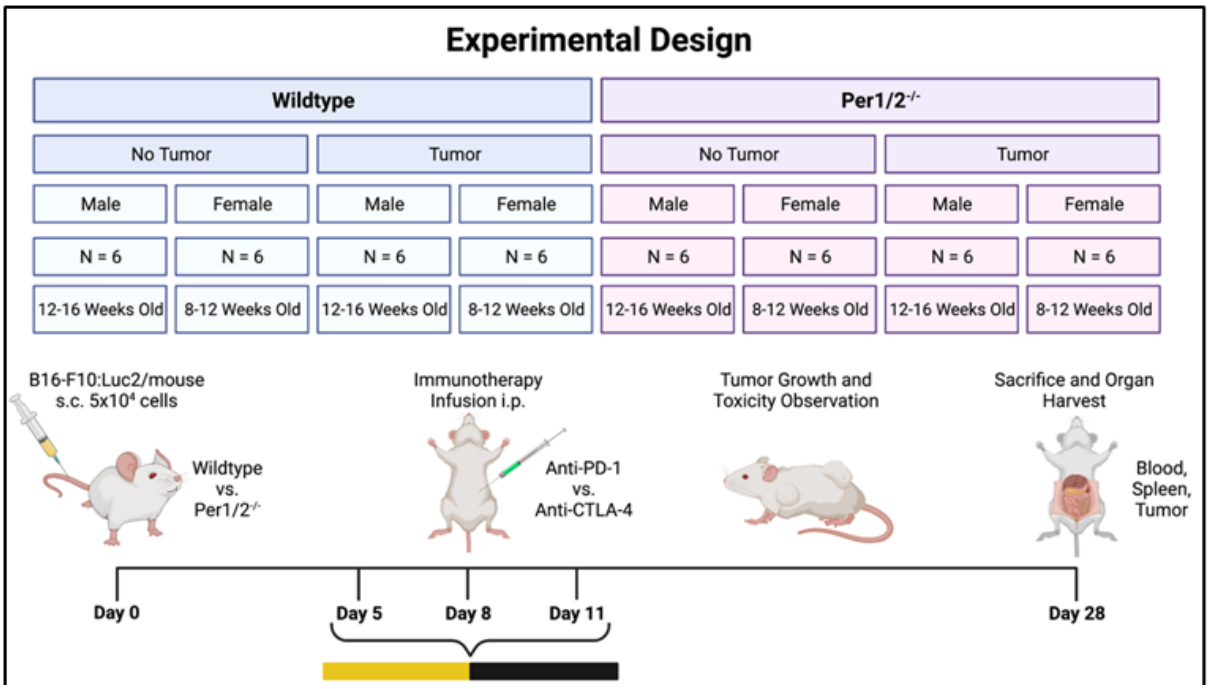


Figure-1. The diagram shows the experimental design of the proposed immunotherapy schedule among male and female melanoma mouse models from wild type B6 mice and mPer1/mPer2 mutant type B6 mice at different time points of the day with the number of experimental controls.

Additional results:

The results showed that the time of day of dual immunotherapy treatment was important for the tumor growth retardation of male mice with a healthy circadian rhythm and female mice with mutations on both Per1 and Per2 genes. Mice in these two groups had smaller tumors when they were treated at ZT14 (early active phase) than when they were treated at ZT2 (early inactive phase). This difference was not seen in male mice with a disrupted circadian rhythm (Per1/Per2 mutant male mice) or in wildtype female mice with a healthy circadian rhythm.

There was also a significant sex difference in the response to dual immunotherapy. Wildtype female mice had larger tumors than male mice when they were treated at ZT14. Interestingly, Per1/Per2 mutant male mice had smaller tumors than Per1/Per2 mutant female mice when they were treated at ZT2. However, the ZT14 treatment effect was similar in Per1/Per2 mutant male and female mice.

The time-of-day effect seen in wildtype male mice was not observed in Per1/Per2 mutant male mice. In fact, ZT2 treated Per1/Per2 mutant male mice also benefited equally as ZT14 treated ones. On the other hand, ZT2 treated Per1/Per2 mutant female mice were not as benefited as ZT14 treated Per1/Per2 mutant female mice and ZT2 or ZT14 treated wildtype female mice.

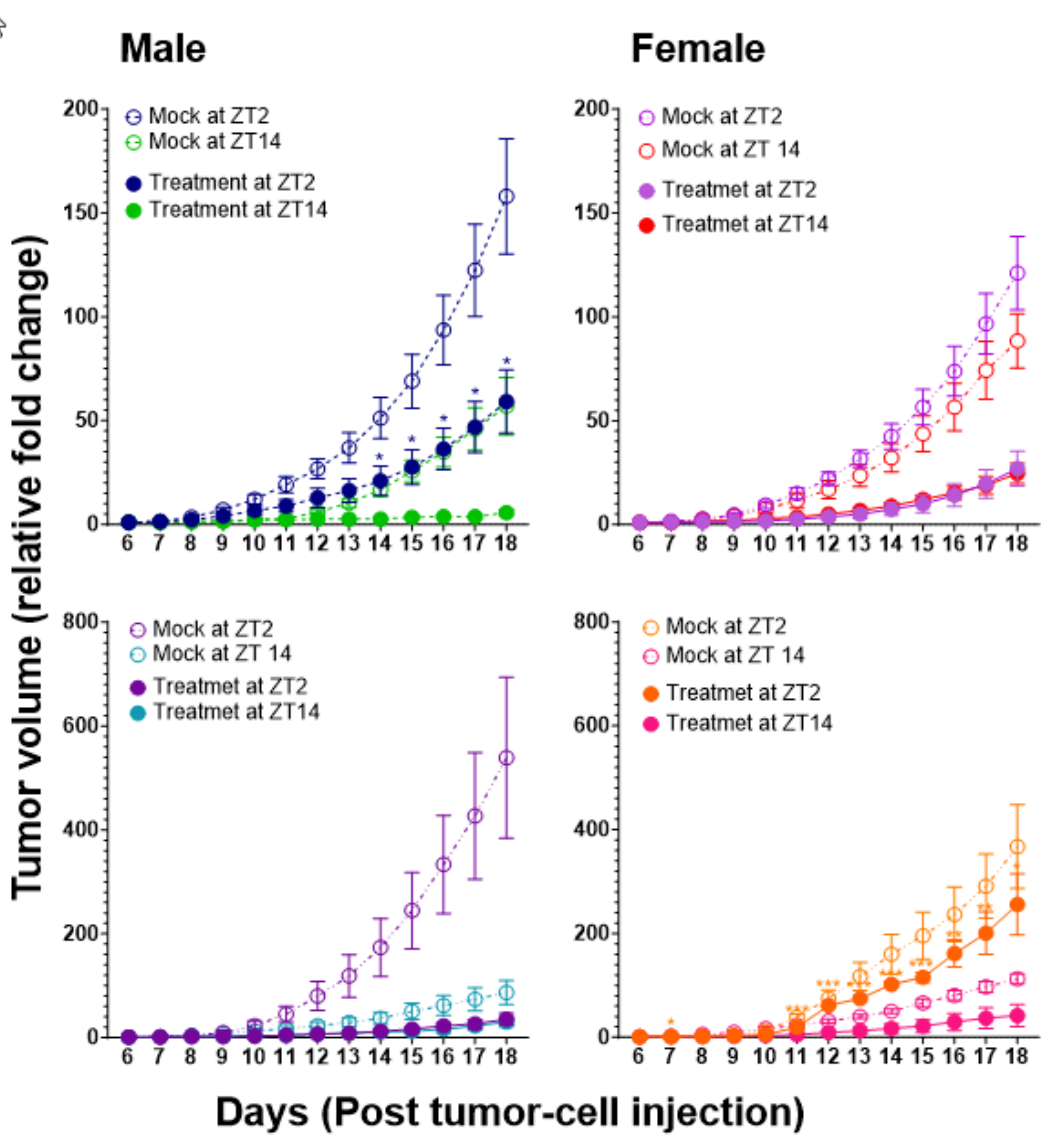
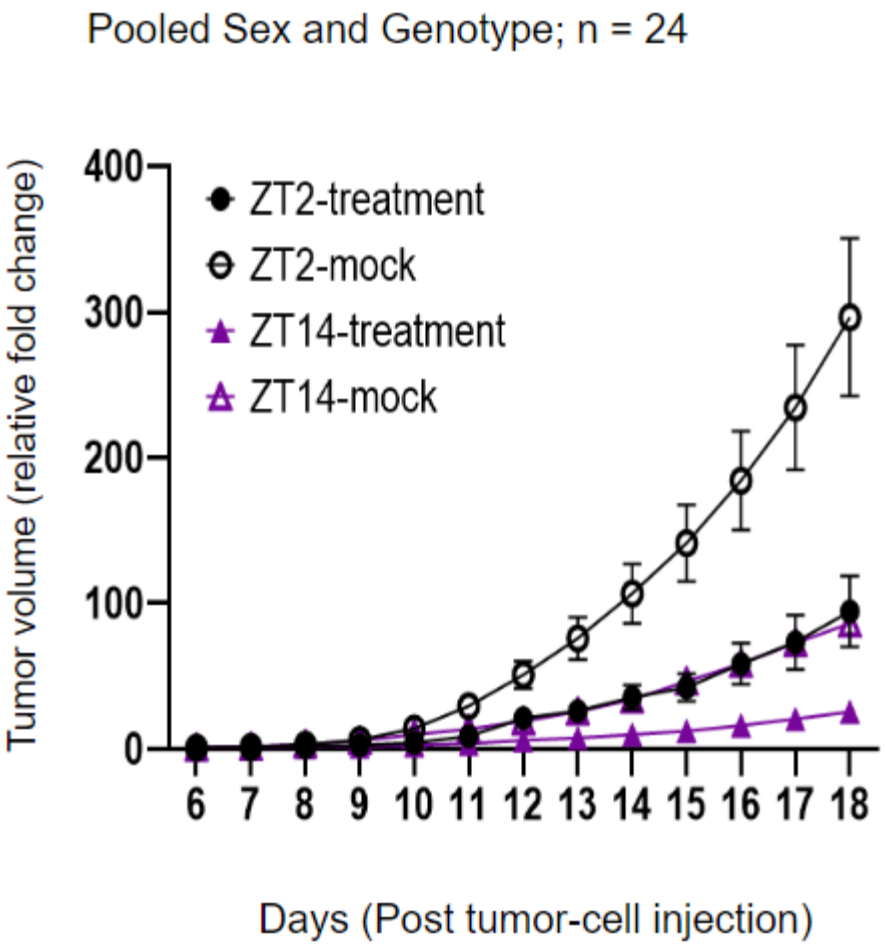
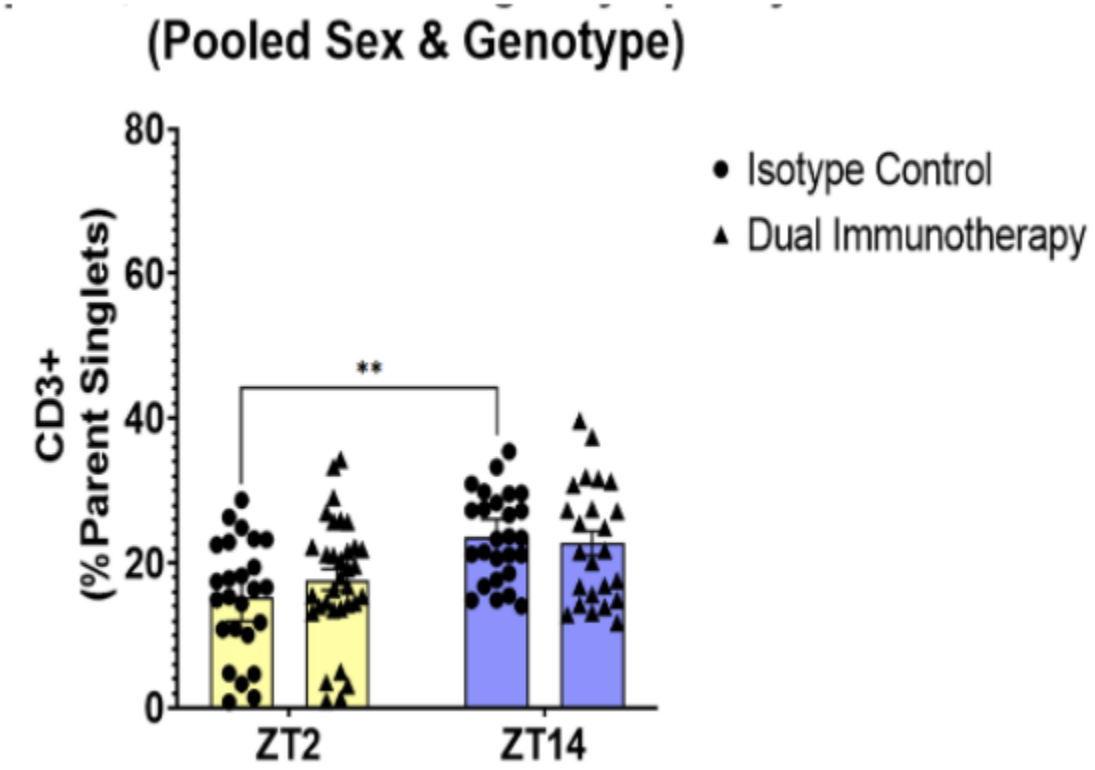


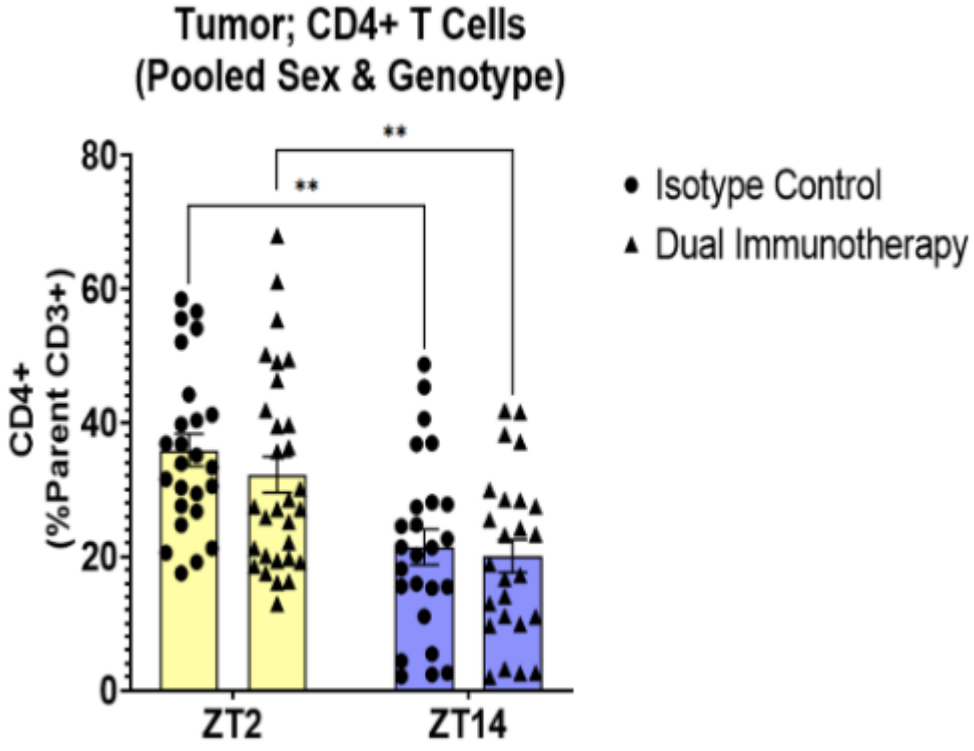
Figure-2. Tumor growth in C57BL/6 WT and Per1/2$^{-/-}$ mice after subcutaneous injection of B16-F10 melanoma cells. C57BL/6 WT and Per1/2-/- male and female mice (8-12 weeks old) were maintained under a LD12:12 cycle and subcutaneously injected (s.c.) with 0.05 million B16-F10 melanoma cells into a flank region. Tumor growth was measured over 18 days after injection. Repeated measures of two-way ANOVA with Tukey's correction were used to compare tumor growth between the ZT2-treated and ZT4-treated groups using the statistical software package GraphPad Prism 10. n = 6 mice for each group. $p < 0.05$, $p < 0.001$, *$p < 0.0001$.

Although dual immunotherapy outcomes differed between male and female mice, as well as between wildtype and Per1/Per2 mutant mice, when individual data from both sexes and genotypes were pooled (n=6 for each subgroup) and the time-of-day treatment effect was analyzed, ZT14 treatment showed significantly better treatment outcomes.

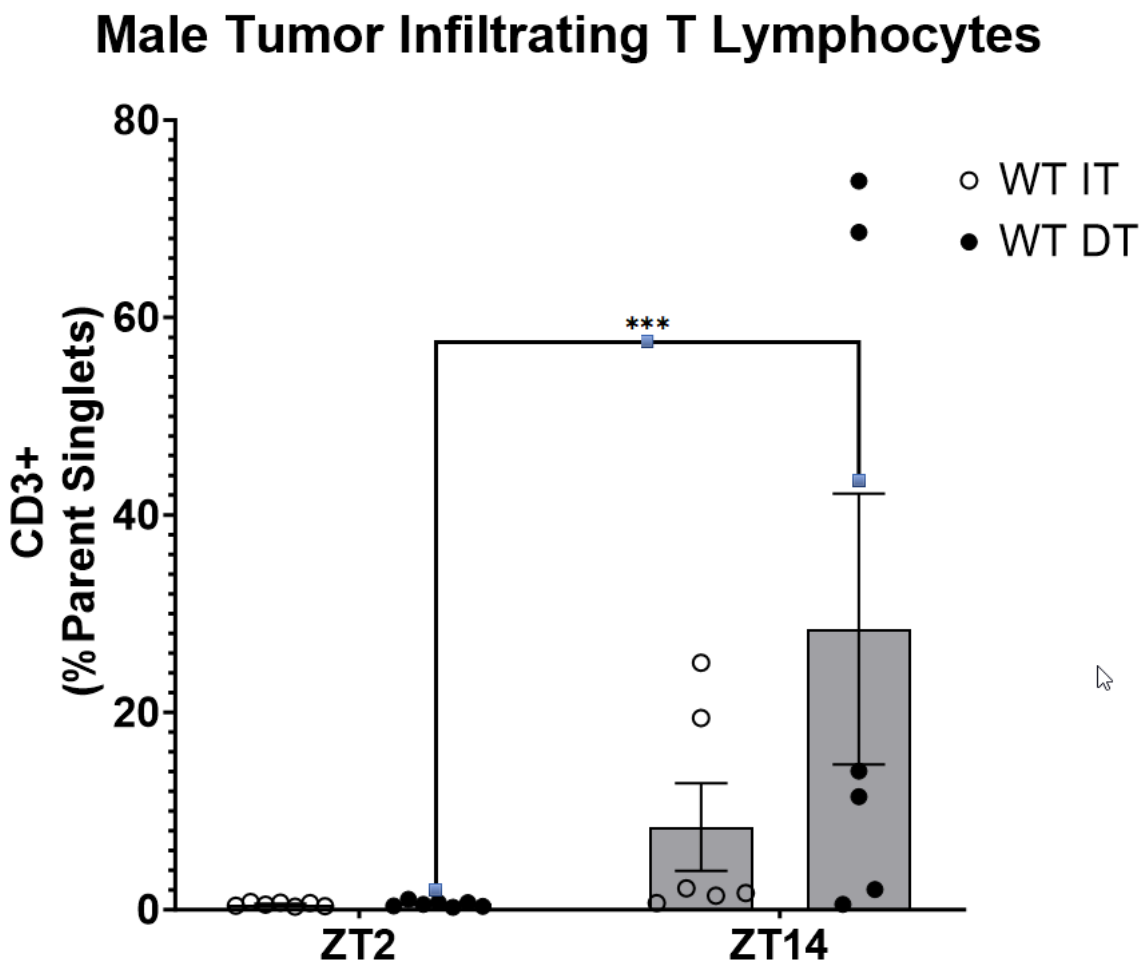


The flow cytometry results revealed a higher percentage of tumor infiltrating T cells and lower proportion of tumor-infiltrating CD4+ in the ZT14-treated groups, which supports the better treatment outcomes seen with ZT14 treatment.

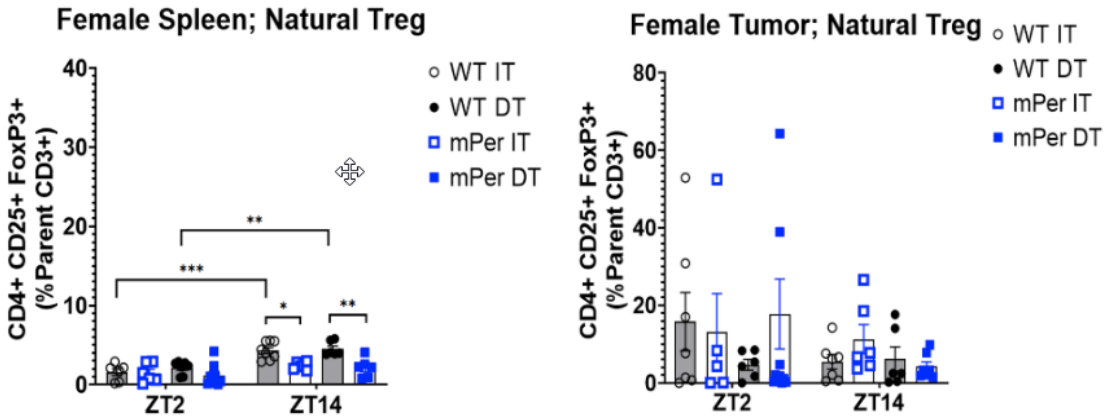




There was a notable ZT14 treatment effect on wildtype male mice in increasing T cell tumor infiltration in comparison to ZT2 treatment. This was evident in the flow cytometry data, which showed a higher percentage of T cells in the tumor tissue of ZT14-treated mice. This suggests that ZT14 treatment may be more effective at activating T cells and promoting their infiltration into tumors in wildtype male mice.



However, there was also a significant increase in protumorigenic regulatory T cells due to ZT14 dual immunotherapy in wildtype female mice, and in Per1/Per2 female mice there was an increase, but it was not statistically significant.



Consistent with a growing body of evidence, these findings suggest that administering immunotherapy during early active hours may provide superior outcomes compared to late active hour or inactive hour immunotherapy for the treatment of melanoma and other tumors.

Conclusion:

These results suggest that the time-of-day of dual immunotherapy treatment can have a significant effect on tumor growth in male mice with a normal circadian rhythm and in female mice with a disrupted circadian rhythm.

Future directions:

These findings warrant further investigation in larger clinical trials to determine if the time-of-day of dual immunotherapy treatment can improve outcomes in melanoma patients. It would also be interesting to investigate the mechanisms by which the circadian rhythm and sex affect the response to dual immunotherapy.

What opportunities for training and professional development has the project provided?

- 2022 The Society of Research on Biological Rhythms Biennial Conference, FL, **Invited Speaker**
- 2022 Center for Quantitative Life Sciences, Oregon State University, Corvallis, OR, **Invited Speaker**
- 2022 NCSU College of Veterinary Medicine, Cancer Research Seminar, Raleigh, NC, **Invited Speaker**
- 2021, University of Georgia, Athens, GA, **Invited Speaker**
- 2021, NCI Chronomedicine Speaker Series, Bethesda, MD, **Invited Speaker**
- 2021, Duke University, Durham, NC, **Invited Speaker**
- 2021, Oregon Institute of Occupational Health Sciences, Portland, OR, **Invited Speaker**
- 2021, Society for Investigative Dermatology (SID) Virtual Annual Meeting, **Invited Speaker**
- 2021, Wright State University, Dayton, OH, **Invited Speaker**

How were the results disseminated to communities of interest?

Nothing to Report.

What do you plan to do during the next reporting period to accomplish the goals?

.

Nothing to Report.

4. **IMPACT:**

What was the impact on the development of the principal discipline(s) of the project?

Nothing to Report.

What was the impact on other disciplines?

Nothing to Report.

What was the impact on technology transfer?

Nothing to Report.

What was the impact on society beyond science and technology?

Nothing to Report.

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Nothing to Report.

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

For objectives 1 and 2, although we proposed to complete major experiments in year 3 (this funding cycle), we could not be able to finish those experiments due to supply chain issues from COVID-19 as we had to wait approximately 5 months to get Matrigel which is needed for tumor cells injections. However, we are continuing these experiments now and planning to finish them during this coming no-cost extension cycle.

or example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

There are no significant changes on the impact of expenditures.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

N/A.

Significant changes in use or care of vertebrate animals

Significant changes in use of biohazards and/or select agents

None.

6. PRODUCTS:

- **Publications, conference papers, and presentations**

  Journal publications.

All these 10 peer-reviewed publications below were acknowledged with this federal grant support.

1. Goodenow D, Greer A, Cone S, and **Gaddameedhi S*** (**2022**): Circadian effects on UV-induced damage and mutations, *Mutation Research/Reviews in Mutation Research.* doi.org/10.1016/j.mrrev.2022.108413. *Corresponding author.
2. Dakup P, Greer A, **Gaddameedhi S*** (**2022**): Let's talk about sex: A biological variable in immune response against melanoma. *Pigment Cell Melanoma Res.* 35:268-27. doi: 10.1111/pcmr.13028. *Corresponding author.
3. Koritala BSC, Porter KI, Sarkar S, **Gaddameedhi S* (2022)**: Circadian disruption and cisplatin chronotherapy for mammary carcinoma. *Toxicol Appl Pharmacol.* 436:115863. doi: 10.1016/j.taap.2022.115863. *Corresponding author.
4. Sarkar S, Porter KI, Dakup PP, Gajula RP, Koritala BSC, Hylton R, Kemp MG, Wakamatsu K, **Gaddameedhi S* (2021)**: Circadian clock protein BMAL1 regulates melanogenesis through MITF in melanoma cells. *Pigment Cell Melanoma Res.* 34(5):955-965. doi: 10.1111/pcmr.12998. *Corresponding author.
5. Koritala BSC, Porter KI, Arshad OA, Gajula RP, Mitchell HD, Arman T, Manjanatha MG, Teeguarden J, Van Dongen HPA, McDermott JE*, **Gaddameedhi S* (2021)**: Night shift schedule causes circadian dysregulation of DNA repair genes and elevated DNA damage in humans, *J Pineal Res.* 70(3):e12726. doi: 10.1111/jpi.12726. *Corresponding author.
6. Dakup PP, Porter KI, Little AA, Zhang H, and **Gaddameedhi S*** (2020): Sex differences in the association between tumor growth and T cell response in a melanoma mouse model, Cancer Immunol Immunother. Jun 25. doi: 10.1007/s00262-020-02643-3. Online ahead of print. PMID: 32638080. *Corresponding author.
7. Dakup P, Porter K, and **Gaddameedhi S*** (2020): The circadian clock protects against acute radiation-induced dermatitis, Toxicol Appl Pharmacol. doi: 10.1016/j.taap.2020.115040. PMID: 32422325. *Corresponding author.
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10. Kumar PVA, Dakup P, Sarkar S, Modasia J, Madison SM, and **Gaddameedhi S*** (2019): It's About Time: Advances in Understanding the Circadian Regulation of DNA Damage and Repair in Carcinogenesis and Cancer Treatment Outcomes, Yale J. Biol. Med. 92 (2): 305-316. PMID: 31249491. *Corresponding author.

Books or other non-periodical, one-time publications.

Nothing to Report.

Other publications, conference papers and presentations.

Nothing to Report.

- **Website(s) or other Internet site(s)**

Nothing to Report.

- **Technologies or techniques**

Nothing to Report.

- **Inventions, patent applications, and/or licenses**

Nothing to Report.

- **Other Products**

Nothing to Report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

No change.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to Report.

What other organizations were involved as partners?

Nothing to Report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: N/A

QUAD CHARTS: N/A

9. APPENDICES: N/A