

MIH-0005 hVEGF Minicircle Pilot

Kylee Harrington, BS

Aleksander D. Szymaniak, PhD

Last Updated: 16Jul2024

Study Design

C57BL/6, Male, 8-Weeks Old

Group ID	Model	Inducer	Quantity
1	Pilot Injection VEGF Minicircle Safety	Pilot VEGF DNA Injection	3
2	VEGF Minicircle Safety	VEGF DNA Injections	18
Total			21

All animals injected with 50uL of VEGF DNA into the right quadricep muscle using an 29G insulin syringe while under isoflurane anesthesia. Pilot animals (n=3) injected prior to other animals and euthanized last (28 Days-Post Injection) for tissue and blood collections.

Timeline

- Pilot IM Injection (n=3) - 11JUN2024
- Blood Collection for VEGF Quantification ELISA (n=6) - 18JUN2024
- VEGF ELISA Analysis – 20JUN2024
- Remaining IM Injections (n=18) - 24JUN2024



Timeline

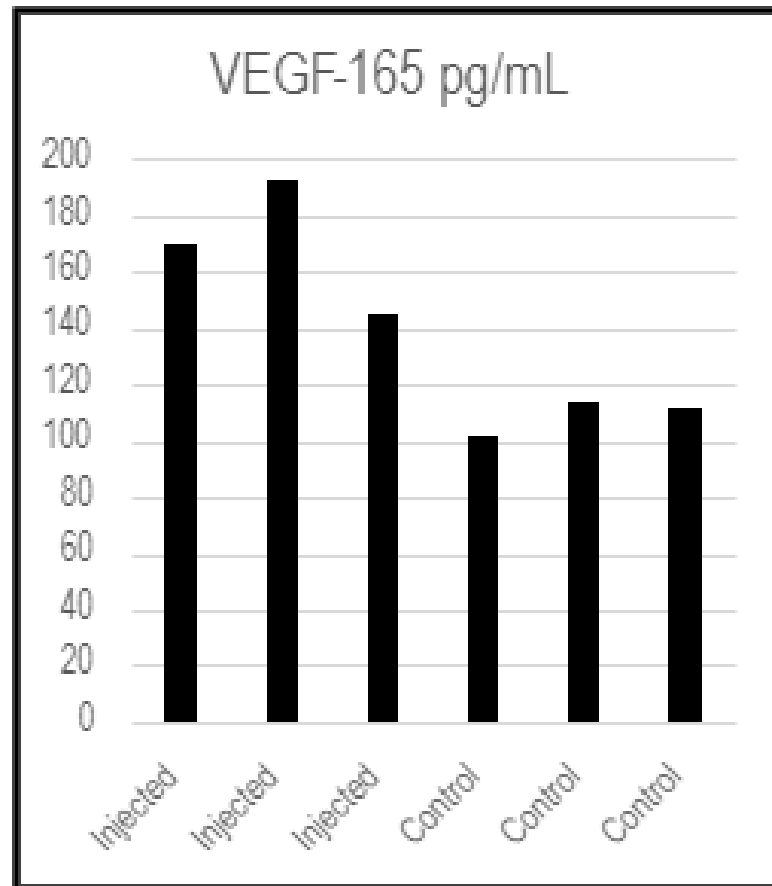
- Blood and Tissue Collections
 - 4 Hours-Post (n=3) – 24JUN2024
 - 24 Hours-Post (n=3) - 25JUN2024
 - 2 Days-Post (n=3) - 26JUN2024
 - 4 Days-Post (n=3) - 28JUN2024
 - 8 Days-Post (n=3) - 02JUL2024
 - 14 Days-Post (n=3) - 08JUL2024
 - 28 Days-Post (n=3; pilot injection animals) - 09JUL2024



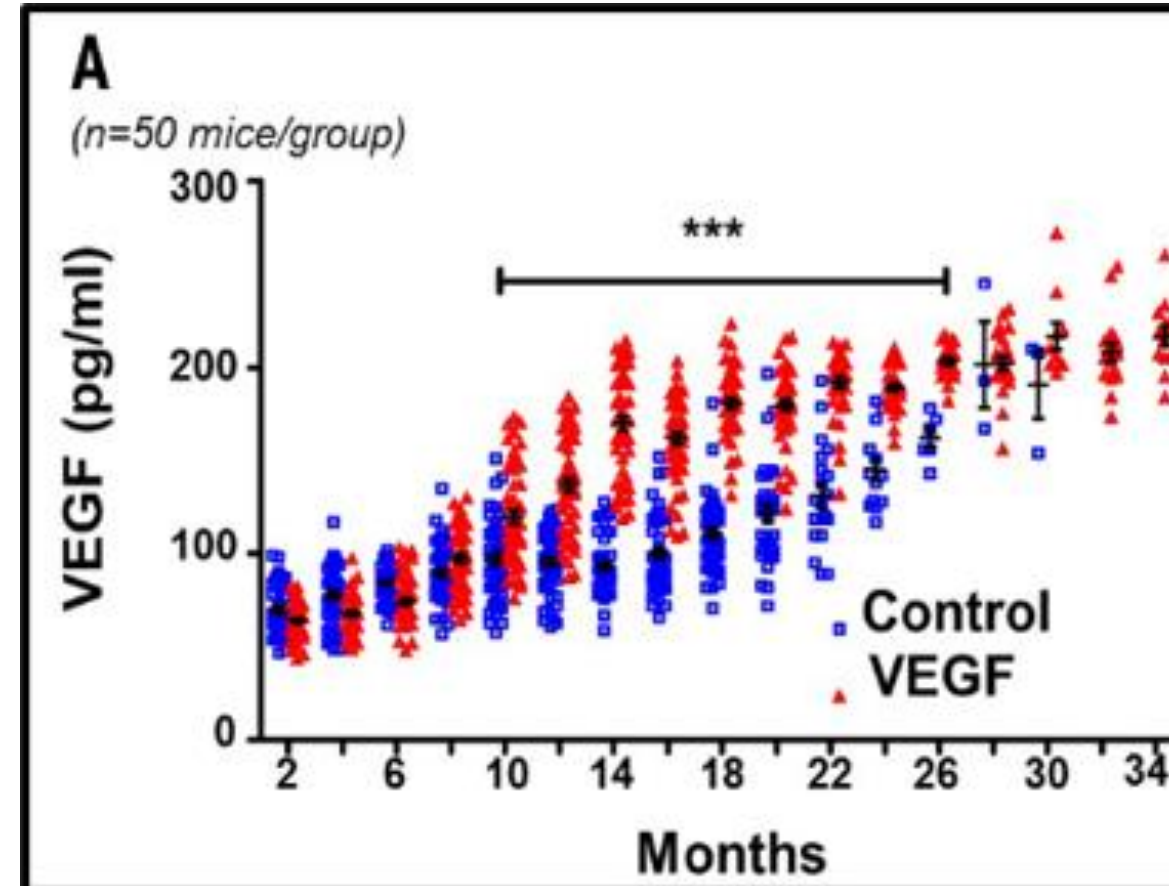
Executive Summary

- **MINICIRCLES SUCCESSFULLY EXPRESS ENCODED hVEGF!**
- Study ran smoothly without any deviations or complications.
- Pilot VEGF ELISA was very promising. Trend was in the right direction in the injected samples.
- Body weights are all normal, indicating no adverse health effects.
- LFTs were normal, indicating no liver toxicity.
 - Interestingly, ALT was slightly elevated relative to other samples in the Day 14 samples.
 - **These samples had consistently higher VEGF expression.**
- Terminal ELISA data was very promising as well.
 - Need to nail down a PK profile after injection
- Recommend a follow-up dose finding study and multidose study.
 - How much Minicircle DNA needs to be dosed to arrive at 100-200pg/mL?

Pilot VEGF ELISA Data Indicates VEGF Expression in Serum 7 days post-injection



MIH-0005 Data



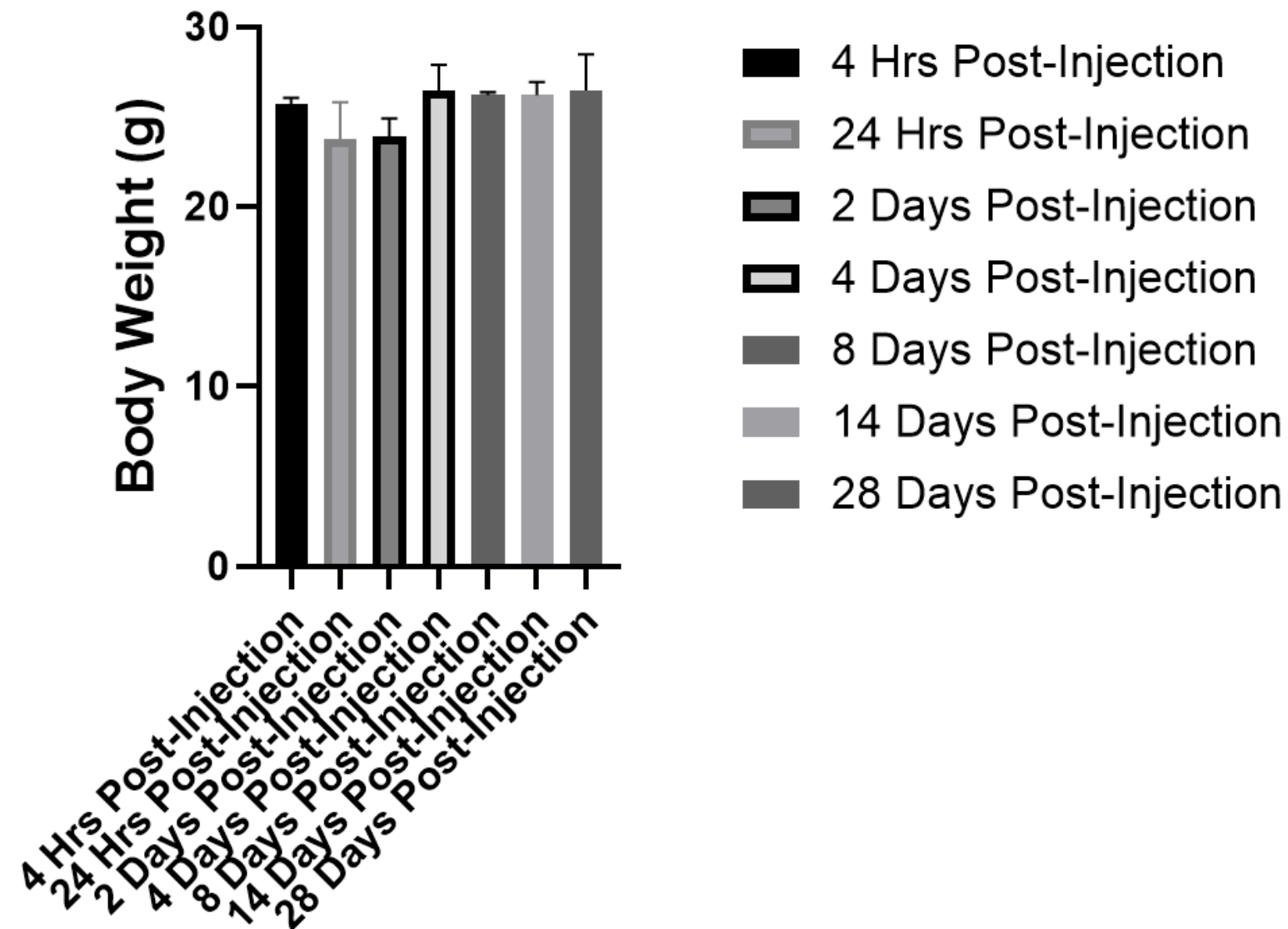
Target Data from Science Paper

Grunewald, M et al. "Counteracting age-related VEGF signaling insufficiency promotes healthy aging and extends life span." *Science (New York, N.Y.)* vol. 373,6554 (2021): eabc8479. doi:10.1126/science.abc8479

Endpoints

- Terminal Blood and Tissues
- VEGF ELISA (terminal blood)
- DNA Extraction (terminal tissues)
- HESKA Dri-Chem Chemistries (terminal blood)
 - ALT
 - AST
 - ALP
 - GGT **Values below the limit of detection for machine

Terminal Body Weights – No Adverse Health Concerns

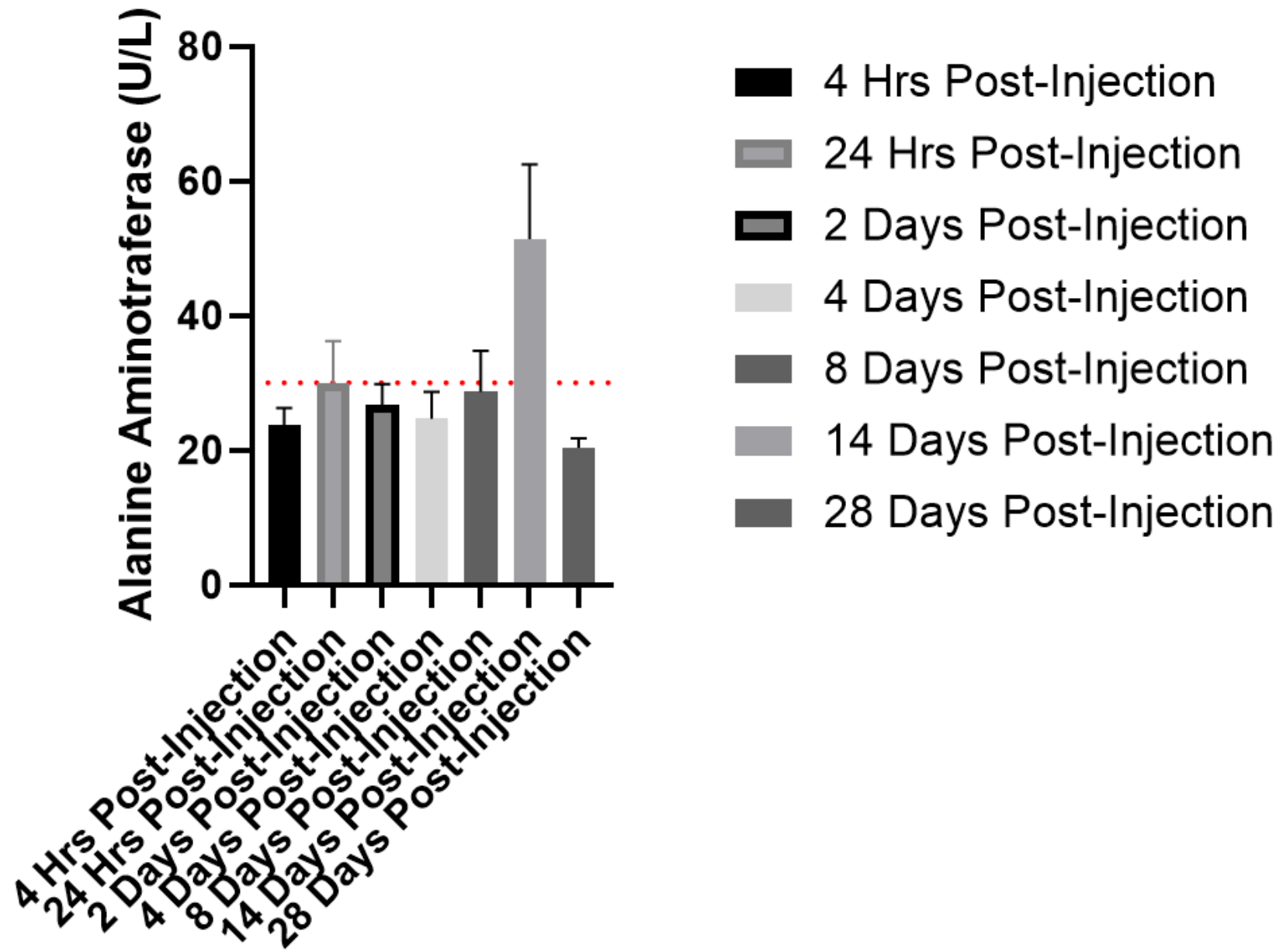


All mice received VEGF DNA via intra-muscular injection into the right quadricep muscle. A terminal body weight was taken prior to the mice (n=3) being euthanized and having blood and tissues collected 4 hours, 24 hours, 2 days, 4 days, 8 days, 14 days, and 28 days post-injection. Error bars are \pm SEM.

ALT Values Are Within Expected Range

Day 14 Samples with High VEGF Expression Are Slightly Elevated, But Not Concerning

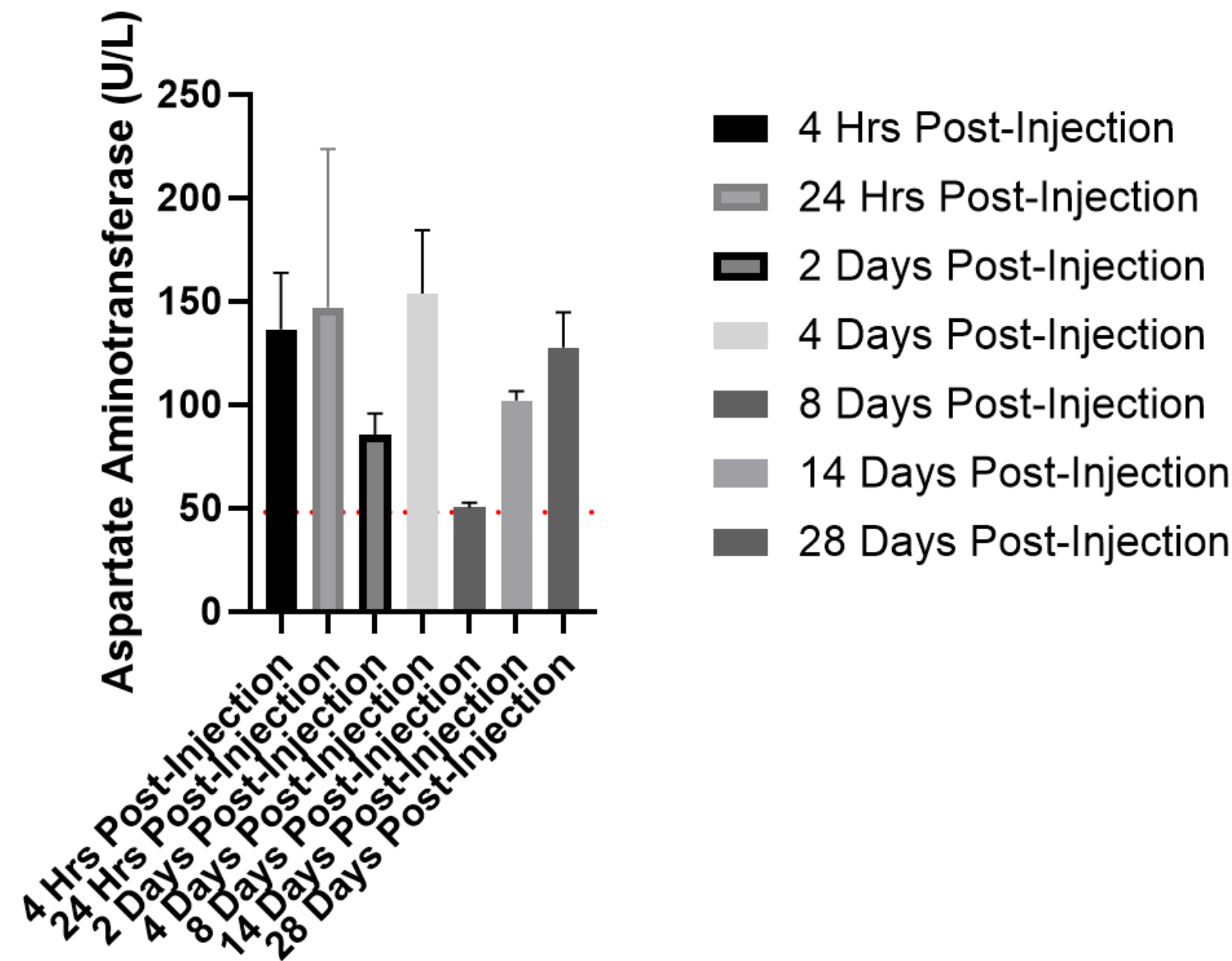
190 U/L is still healthy



All mice received VEGF DNA via intra-muscular injection into the right quadricep muscle. The mice (n=3) were then euthanized and had blood and tissues collected 4 hours, 24 hours, 2 days, 4 days, 8 days, 14 days, and 28 days post-injection. Following blood collection, ALT values were taken with a HESKA Dri-Chem machine. The red dotted line within the graph represents the median value from young, C57BL/6 males (*Otto, Gordon P et al. "Clinical Chemistry Reference Intervals for C57BL/6J, C57BL/6N, and C3HeB/FeJ Mice (Mus musculus)." Journal of the American Association for Laboratory Animal Science : JAALAS vol. 55,4 (2016): 375-86*). Error bars are \pm SEM.

AST Values Are Within Expected Range

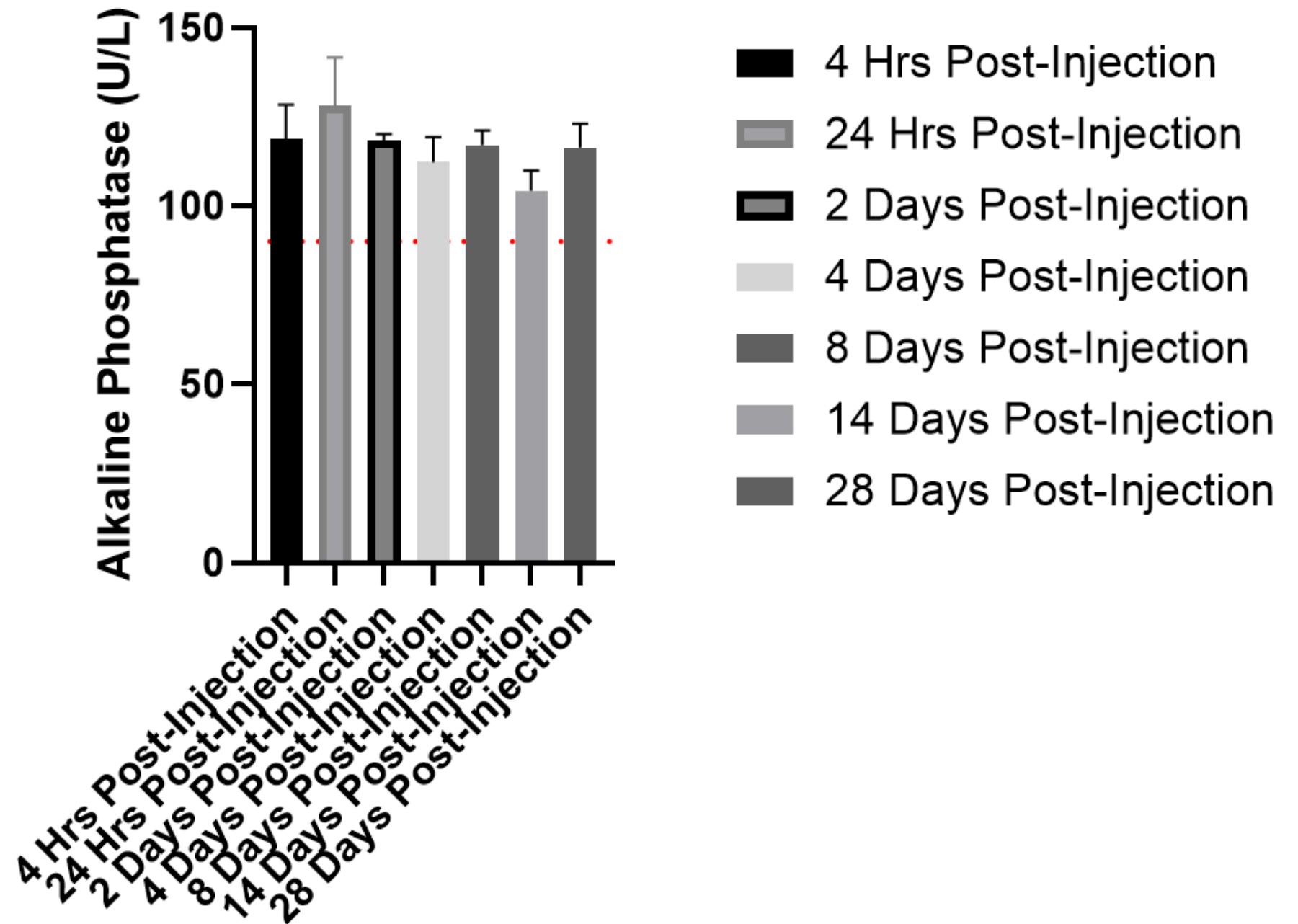
380 U/L is still healthy



All mice received VEGF DNA via intra-muscular injection into the right quadricep muscle. The mice (n=3) were then euthanized and had blood and tissues collected 4 hours, 24 hours, 2 days, 4 days, 8 days, 14 days, and 28 days post-injection. Following blood collection, AST values were taken with a HESKA Dri-Chem machine. The red dotted line within the graph represents the median value from young, C57BL/6 males (Otto, Gordon P et al. "Clinical Chemistry Reference Intervals for C57BL/6J, C57BL/6N, and C3HeB/FeJ Mice (*Mus musculus*).*" Journal of the American Association for Laboratory Animal Science : JAALAS vol. 55,4 (2016): 375-86*). Error bars are \pm SEM.

ALT Values Are Within Expected Range

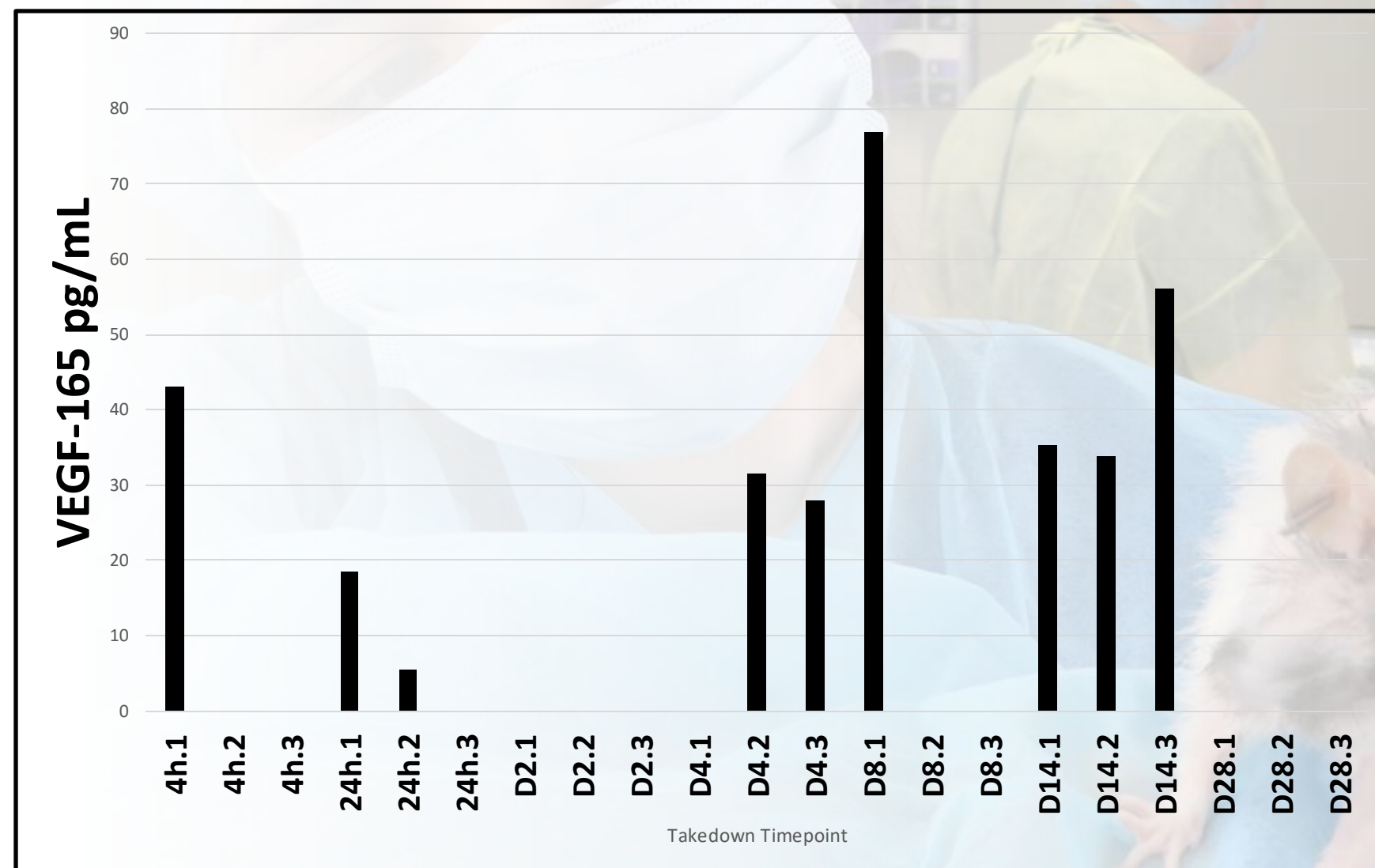
190 U/L is still healthy



All mice received VEGF DNA via intra-muscular injection into the right quadricep muscle. The mice (n=3) were then euthanized and had blood and tissues collected 4 hours, 24 hours, 2 days, 4 days, 8 days, 14 days, and 28 days post-injection. Following blood collection, ALP values were taken with a HESKA Dri-Chem machine. The red dotted line within the graph represents the median value from young, C57BL/6 males (*Otto, Gordon P et al. "Clinical Chemistry Reference Intervals for C57BL/6J, C57BL/6N, and C3HeB/FeJ Mice (Mus musculus)." Journal of the American Association for Laboratory Animal Science : JAALAS vol. 55,4 (2016): 375-86*). Error bars are \pm SEM.

Terminal ELISA Data Indicates Successful Expression of hVEGF by Minicircles

- ~45% of injected animals express hVEGF in serum after Minicircle injection!
- hVEGF expression levels are similar over time, suggesting prolonged expression after a single injection.
- Findings will be verified with dose-finding and multidose studies prior to lifespan work.
- Target expression of hVEGF at 100-200 pg/mL (Science Paper) will be addressed in dose finding studies.



Executive Summary

- **MINICIRCLES SUCCESSFULLY EXPRESS ENCODED hVEGF!**
- Study ran smoothly without any deviations or complications.
- Pilot VEGF ELISA was very promising. Trend was in the right direction in the injected samples.
- Body weights are all normal, indicating no adverse health effects.
- LFTs were normal, indicating no liver toxicity.
 - Interestingly, ALT was slightly elevated relative to other samples in the Day 14 samples.
 - **These samples had consistently higher VEGF expression.**
- Terminal ELISA data was very promising as well.
 - Need to nail down a PK profile after injection
- Recommend a follow-up dose finding study and multidose study.
 - How much Minicircle DNA needs to be dosed to arrive at 100-200pg/mL?