In-Vivo Toxicity of miR-302 Precursors in Mice

Outsourced Institution: WJWU & LYNN Institute for Stem Cell Research,

12145 Mora Drive, Santa Fe Springs, CA 90670, USA

Lead Scientist: Shi-Lung Lin, PhD

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Sponsor Institution: Mello Biotechnology, Inc.,

12145 Mora Drive, Ste 6, Santa Fe Springs, CA 90670, USA

Project Scientists: Melody Lin, M.S.

Lead Scientist: Jack Chen, Ph.D.

Email: jack.chen@mellobiotech.com

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ABSTRACT

MiR-302 is a standard marker for human pluripotent stem cells. Mello Biotech's purified *Natural microRNA Precursor*™ F6 contains purified miR-302 precursors dissolved in Mello's proprietary F5 formulation. Extracted from Dicer-negative microRNA expressing cells, miR-302 precursors are hairpin-like small RNA molecules that can be processed to yield mature and functional miR-302 microRNAs following delivery into target cells. The mature miR-302 microrRNAs have been reported to play an important role in a wide range of stem cell-related activities, such as wound healing, tissue repairing, stem cell generation and maintenance that eventually lead to anti-aging and rejuvenation. The aim of this study is to determine the *in vivo* toxicity of miR-302 precursors in mice.

SUMMARY

In this study, four separate experiments were carried out to determine the in vivo toxicity of miR-302 precursors in mice. Collectively, our data showed that miR-302 precursors caused no detectable *in vivo* toxicity in mice.

1) A single subcutaneous injection (SC) of miR-302 precursors into mouse dermis showed no detectable toxicity at the maximal injectable level (=200 µg pre-miR-302).

2) 4 weekly SC injections of miR-302 precursors into mouse dermis did not cause any acute (<1 month) or chronic (>1 year) toxicity *in vivo*.

3) A single intravenous injection (IV) of miR-302 precursors into mouse tail veins caused no detectable toxicity from up to of 400 µg miR-302 precursors per mL of blood volume in mice *in vivo.*

4) 4 weekly IV injections of miR-302 precursors into mouse tail veins did not cause any acute (<1 month) or chronic (>1 year) toxicity *in vivo*.

5) LD50 of miR-302 precursors (dissolved in F5) in wild-type mice is approximately 750 µg/mL of blood volume (total = 3 mg of F1 in 300 µL F5).

MATERIALS AND METHODS

To achieve the goal of this study, 4 separate experiments were carried out. Unless otherwise stated, all mice were purchased from Harlan Laboratories. The following table describes the materials and methods employed in each experiment:

Experiment #	Mouse Strain	miR-302 Precursor (pre-miR-302) Preparation	Treatment Protocol
1	8-week old male SCID-beige mice	1 mg cold-dried pre-miR-302 dissolved in 1 mL F2	4 weekly subcutaneous (SC) injections (200 μL each time) in each of 5 mice; left and right hind legs rec'd pre-miR-302 and control (saline), respectively; another 5 mice rec'd no injections
2	21-week old male SCID-beige mice	1 mg lyophilized pre-miR-302 dissolved in 1 mL F3	4 weekly intravenous (IV) injections (200 μL each time) into the tail vein of each mouse; 5 mice each for receiving pre-miR-302 and saline injections
3	8-week old female SCID-beige mice	1 mg lyophilized pre-miR-302 dissolved in 1 mL F3	4 weekly intravenous (IV) injections (200 μL each time) into the tail vein of each mouse; 4 mice rec'd pre-miR-302 while 2 mice rec'd saline injections
4	8-week old female wild-type pet mice (from Petco)	5 mg lyophilized pre-miR-302 dissolved in 1 mL F5	4 weekly intravenous (IV) injections (200 μL each time) into the tail vein of each mouse; 4 mice rec'd pre-miR-302 while 2 mice rec'd saline injections

RESULTS & OBSERVATIONS

In this study, various strains of mice received either SC or IV injections of miR-302 precursors dissolved in different formulations. In experiment 1, all 10 mice were observed for up to 3 months following the 4 weekly SC injections. All mice presented the same normal and health condition during and after the period of this experiment. Injection wounds were quickly healed within 2~3 days in all mice. No difference could be found between the treatment and control sides. No difference could be found between treated and blank control mice. No skin rash, no non-stop bleeding, no ataxia, no cachexia, no tumor or any kind of sickness could be found. Overall, no physical or behavioral change or any abnormality was detected in all mice of this experiment.

In experiment 2, 2 treated and control mice were sacrificed immediately following the 12-week (3-month) experiment. The remaining 3 treated and control mice were kept for long-term observation over 12 months. All 10 mice showed the same normal and health conditions during and after this experiment. Injection wounds quickly healed within 3~5 days in all mice; wounds in pre-miR-302-injected mice healed 1 day quicker than the wounds in saline-injected mice. There was no bleeding, ataxia, cachexia, tumor or any kind of abnormality to be observed. Overall, no physical or behavioral changes or any abnormality was detected in all mice in this experiment.

Major organs from the 2 sacrificed control and treated mice were collected and subjected to histological analysis. As shown in **Fig. 1**, histological examination further revealed that no damage or difference could be found in major organs, such as heart, liver, spleen, kidney, brain, spinal cord and bone marrow.

Experiment 3 was essentially a repeat of experiment 2, except female mice were used instead of male. All 6 mice exhibited the same normal and health conditions during and after this experiment. Once again, there was no skin rash, bleeding, ataxia, cachexia,

tumor or any overt sickness to be observed. It is fairly simple to detect any potential abnormality in hairless nude mice if there is any. Overall, no physical or behavioral changes were detected in all mice during this experiment.

These mice were further observed for 2 months after the last injection and then sacrificed. Histological examination revealed no detectable damage in major organs, including heart, liver, spleen, kidney, brain, spinal cord and bone marrow.

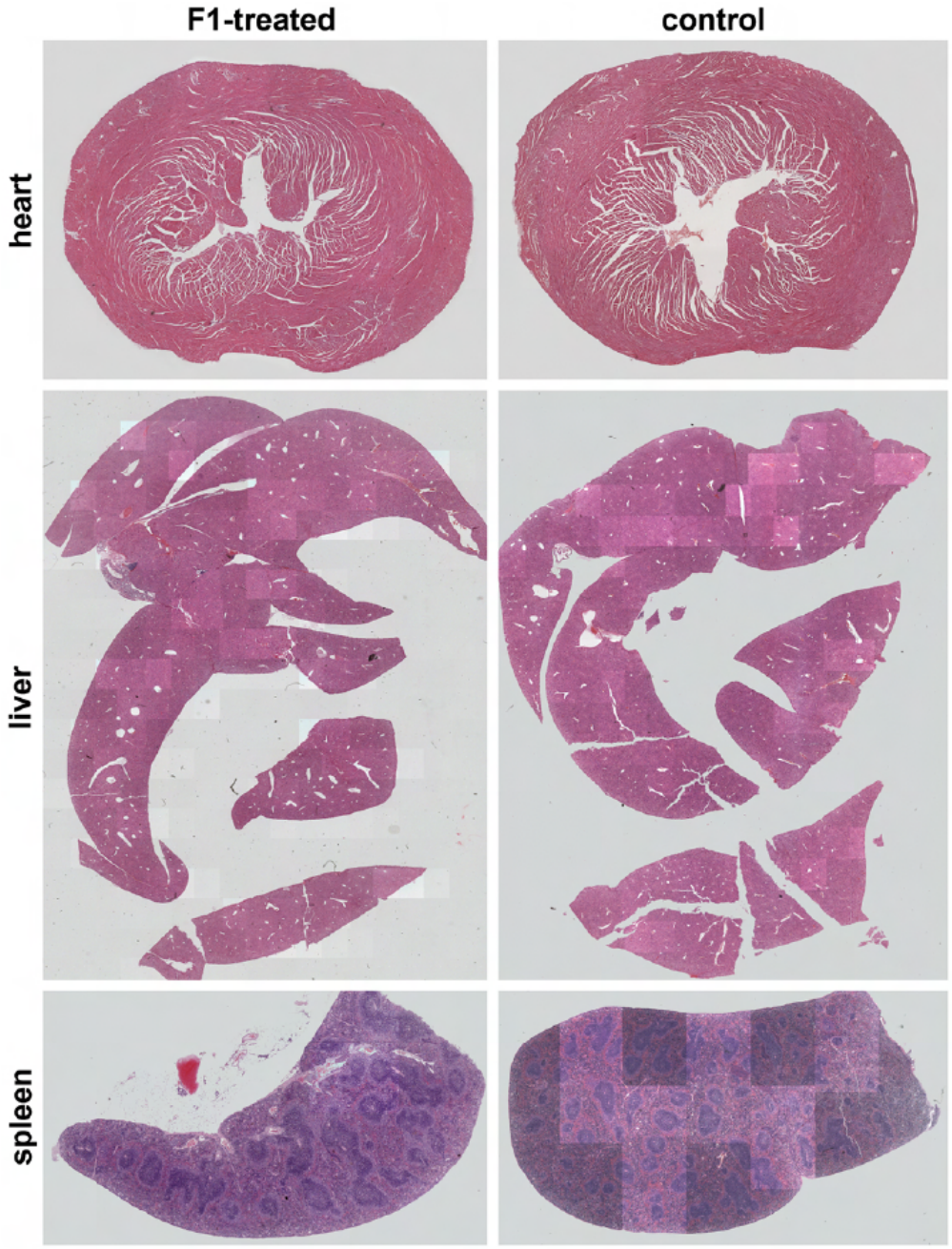

Fig. 1 Representative histological examination of pre-miR-302 (F1)- and saline-treated mice.

In experiment 4, 6 female pet mice received IV injections containing higher doses of miR-302 precursors dissolved in Mello's proprietary F5 formulation and are currently still under observation. This experiment therefore is still ongoing. Thus far, all treated mice look normal and healthy. Injection wounds once again healed quickly in all mice, within 3~5 days. Interestingly again, injection wounds in mice receiving F5-formulated miR-302 precursors healed 1~2 days quicker than the wounds in mice receiving saline injections. There was again no skin rash, bleeding, ataxia, cachexia, tumor or any overt sickness to be observed thus far. Overall, no physical or behavioral changes or any abnormality were observed in any of the mice at this moment.

