# Using Precision Therapies to Protect the Developing Brain from Viral Infection and Injury

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## Background

Prenatal viral infections can cause severe damage to the developing brain. Among the most threatening prenatal pathogens, Zika virus can cause microcephaly, ocular abnormalities, congenital contractures, seizures, and fetal demise, termed congenital Zika syndrome.[1] Currently, there is no prenatal standard of care or treatment for Zika infection.[2]

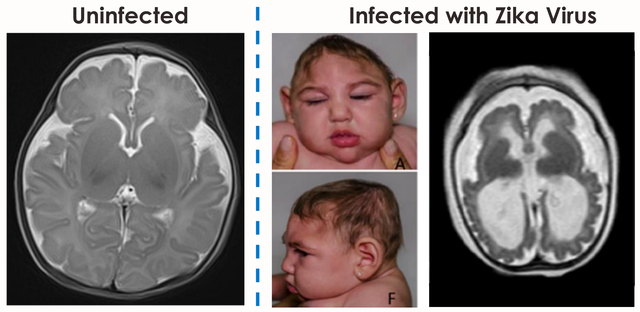


**Figure 1. Virally induced brain injury.** Prenatal viral infection can cause severe brain injury, as seen by visual examination (collapsed skull) and MRI (tissue loss, images not to scale).[3]

## Objective

Our goal is to identify effective and safe treatments for prenatal Zika infection by repurposing targeted medicines.

## Therapeutic Approach

Whole exome genetic sequencing (WES) of severely affected patients identified mTOR (regulator of autophagy) and HEXB (a lysosomal enzyme) as modifiers of injury.[4] Here, we repurpose and test four drugs that manipulate the autophagy-lysosomal pathway in unique ways; chloroquine, metformin, trehalose, and PD146176. Chloroquine serves as our positive control.[5]

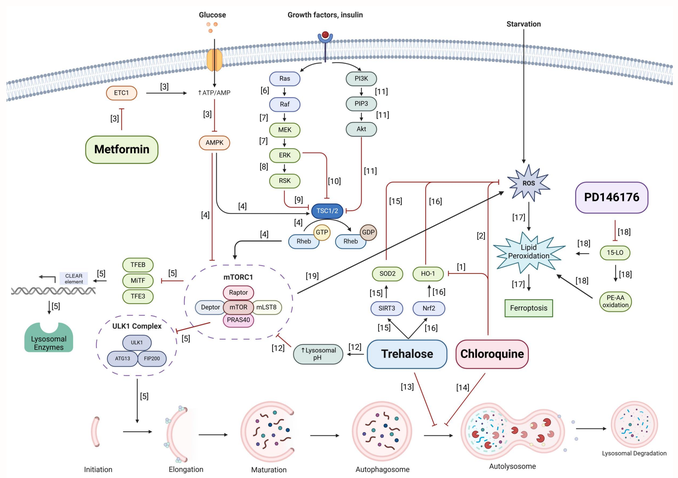


**Figure 2. Therapeutic approach.** Chloroquine, metformin, trehalose, and PD146176 manipulate autophagy and/or the lysosome by distinct and relevant mechanisms.

## Experimental Approach

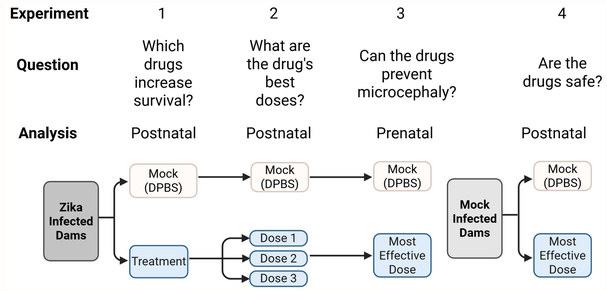


**Figure 3. Experimental approach.** We outlined four experiments that use mock- and Zika-infected mice to determine the optimal doses of treatments that are effective and safe, as evaluated both prenatally and postnatally.

## Methods

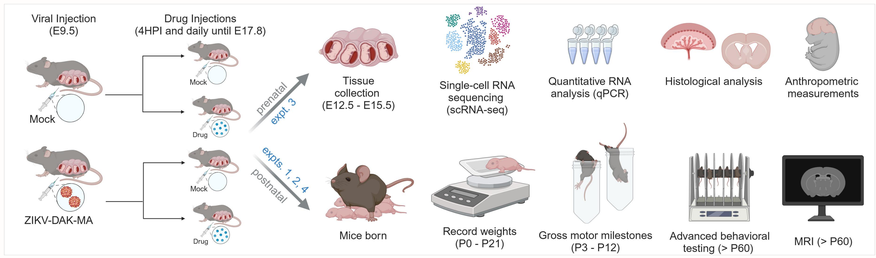


**Figure 4. Experimental system.** Experimental design of prenatal Zika infection in a humanized immunocompetent (hSTAT2$^{KI/KI}$) mouse model with pipelines for both prenatal and postnatal analyses.

## Preliminary Data: Prenatal Viral Infection

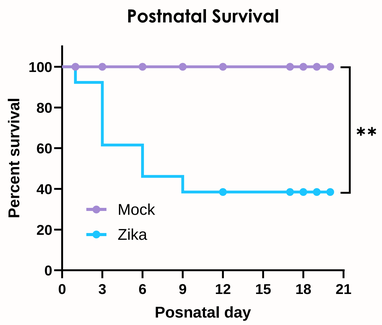


**Figure 5. Postnatal survival.** Prenatal Zika infection caused 62% death for mice by P21.[6]

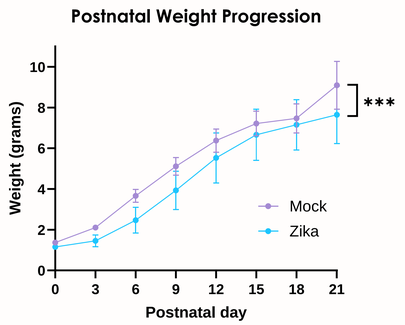


**Figure 6. Postnatal weight progression.** Prenatal Zika infection caused delays in weight gain in mice.[6]

## Preliminary Results: Treatments

| | | Treatment | | | | |
|---|---|---|---|---|---|---|
| | | Mock | Chloroquine 50 mg/kg/day | Metformin 100 mg/kg/day | Trehalose 3,333 mg/kg/day | PD146176 80 mg/kg/day |
| Infection | Mock | n = 1 | 100, n = 2 | - | - | - |
| | Zika | 67, n = 3 | 0, n = 2 | 100, n =1 | 0, n =1 | - |

**Table 1. Percentage of successful pregnancies.** Prenatal Zika infection can cause spontaneous abortions. This is exacerbated with chloroquine (50 mg/kg/day) and potentially trehalose (3,333 mg/kg/day).

## Metformin Increases Survival

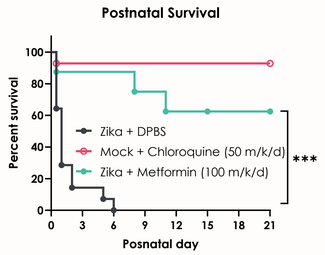


**Figure 7. Postnatal survival after treatment.** Metformin rescues survival after infection from 0% to 62.5% by P21. Chloroquine causes 93% survival (active data collection).

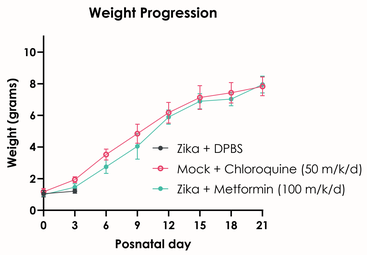


**Figure 8. Postnatal weight progression after treatment.** Metformin may rescue weight progression after viral infection. Mock + Chloroquine in active data collection.

## Conclusions

Metformin may improve postnatal outcomes for prenatal viral infection. The interaction between chloroquine at 50 mg/kg/day and this viral strain (DAK-MA) may be toxic. Trehalose at 3,333 mg/kg/day may also be toxic. Daily IP injections during pregnancy might cause adverse postnatal outcomes for mice.

## Future Directions & Research Timeline

Immediate next steps include growing our sample sizes for each experimental condition, determining the effects of daily IP injections, if any, on outcome, and establishing a dose-dependent curve for each drug. Long-term research plan outlined below.

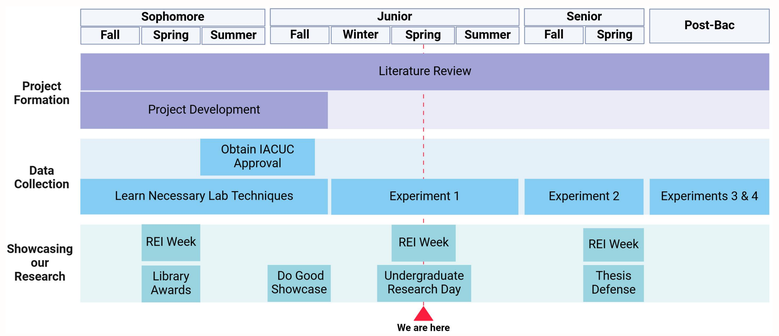


**Figure 9. Timeline.** We will continue to work on experiments while sharing our work.

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**References**

**Figures:** Created with BioRender & GraphPad Prism