Developing *In Vivo* Prime Editing as a Potential Treatment Option for Heart Disease

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Introduction

- Heart disease comes in many forms, affects individuals at many ages, and can be a result of many factors, including cardiomyocyte malfunction caused by genetic mutations. Gene editing technologies have the potential to correct the underlying genetic causes of cardiac conditions, and prime editing (PE) approaches may be particularly attractive for their versatility and precision but have not previously been developed for treating cardiomyopathies *in vivo*.

Tenaya *In Vivo* Cardiac Prime Editor Prototype Installs Desired Edits in Cardiomyocytes in Mouse Heart

- To enable *in vivo* cardiomyocyte PE, we employed our proprietary, small, strong, cardiomyocyte-specific regulatory elements, optimized dual-AAV cassette arrangement, and novel cardiomyocyte-targeting AAV capsids (Abstract #1396).

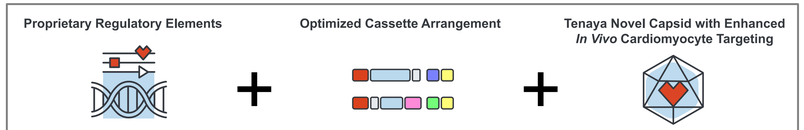

Figure 1. Designing an *in vivo* cardiac prime editor prototype. Through Tenaya's in-house regulatory engineering efforts, we identified small regulatory elements that mediate strong and highly cardiomyocyte-specific expression *in vivo* and are ideal for applications where size is limited and cardiac specificity is critical. To fit all essential elements in two AAV vectors, we identified an optimized split-PE cassette arrangement that expresses PE components at high levels and maintains the ability to be packaged efficiently in our novel cardiomyocyte-targeting AAV capsids.

- When tested in mice targeting a reference genome locus, *Dnmt1*, our prototype edited 22% of all genome copies in the heart at a high dose, providing proof of concept for dual AAV vector-based PE in the heart. The percentage editing of cardiomyocyte genome copies is likely higher than the measured value, as our PE machinery expresses specifically in cardiomyocytes, but genome copies from all cell types were collected and contributed to the denominator.

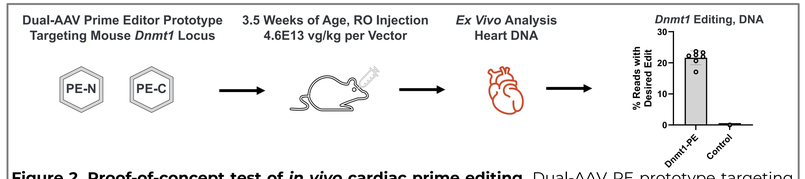

Figure 2. Proof-of-concept test of *in vivo* cardiac prime editing. Dual-AAV PE prototype targeting mouse *Dnmt1* locus was delivered by a proprietary novel cardiomyocyte-targeting AAV capsid, TNC734, systemically at 4.6E13 vg/kg per vector (n = 7). NGS analysis of DNA from whole heart lysate showed that 22% of reads that mapped to the editing site carried the desired edit.

- We then tested our prototype in a more challenging setting, covering a mutational hotspot (Abstract #523) in *Rbm20*, a gene primarily expressed in cardiomyocytes.

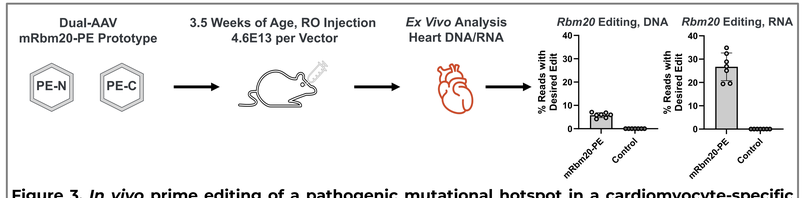

Figure 3. *In vivo* prime editing of a pathogenic mutational hotspot in a cardiomyocyte-specific gene. Dual-AAV PE prototype targeting mouse *Rbm20* was delivered by TNC734 capsid systemically at 4.6E13 vg/kg per vector (n = 7). NGS analysis of DNA and RNA from whole heart lysate showed 5.8% of *Rbm20* genome copies and 27% of *Rbm20* transcripts edited in the PE-treated group. While the DNA analysis captures all cell types in the heart, RNA analysis of *Rbm20* focuses only on cardiomyocytes due to *Rbm20*'s cardiomyocyte-specific transcription, and more accurately reflects the actual efficiency of our cardiomyocyte-specific prime editor. Based on the relationship between RNA and DNA editing levels observed for *Rbm20*, we can extrapolate that cardiomyocyte genome copies may account for ~22% of total genome copies in our whole heart DNA prep.

Developing Prime Editors with Enhanced Editing Efficiency

- To enhance *in vivo* editing efficiency, we designed additional versions of prime editors and first screened them *in vitro*. From this we identified multiple prime editor candidates that show potential to outperform the prototype.

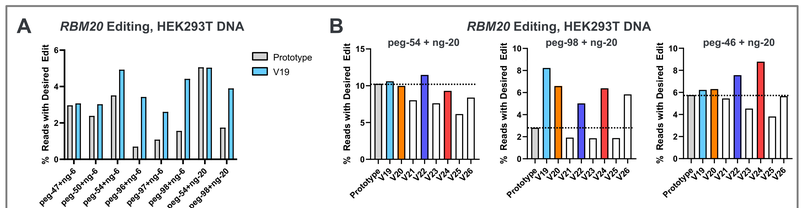

Figure 4. *In vitro* screening identified prime editor candidates with enhanced efficiency. HEK293T cells were transfected with DNA encoding PE components and harvested 72 hours post-transfection. Editing of the *RBM20* locus was assessed via NGS. (A) New design V19 showed improved or similar *RBM20 in vitro* editing efficiency compared to the prototype across multiple guide RNA pairs. (B) Besides V19, new designs V20, V22, V24 also showed improved or similar *RBM20 in vitro* editing efficiency compared to the prototype across multiple guide RNA pairs.

- To examine the *in vivo* prime editing efficiency of our new prime editor candidates, we tested the V19 design in mice targeting the *Dnmt1* locus. V19 administered at a low dose demonstrated nearly 50% higher editing efficiency compared to the prototype.

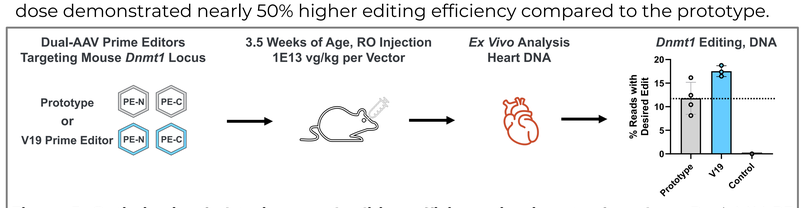

Figure 5. Optimization led to improved editing efficiency *in vivo* at a low dose. Dual-AAV PE prototype or V19 targeting *Dnmt1* were packaged in TNC734 capsid and administered systemically at 1E13 vg/kg per vector to mice at 3.5 weeks of age (n = 3 or 4). NGS of DNA from whole heart lysate showed the prototype and V19 prime editors installed the desired edit in 12% and 18% of genome copies, respectively, demonstrating that the optimized PE cassette V19 improves upon the prototype's *Dnmt1 in vivo* editing efficiency and, at a low dose, yields high editing efficiency, close to that of the prototype at a much higher dose (1E13 vg/kg vs 4.6E13 vg/kg), as shown in Fig. 2.

- We then evaluated new prime editor candidates V19, V20, V22, and V24 *in vivo* targeting the mouse *Rbm20* locus and observed improved editing efficiency.

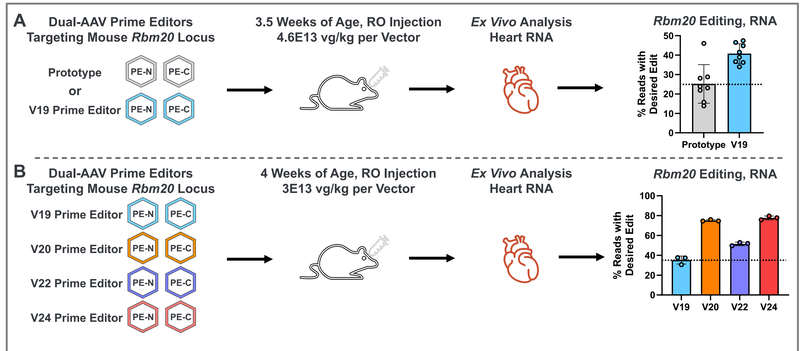

Figure 6. Optimized prime editors show improved editing efficiency *in vivo* on a hard-to-edit and cardiomyocyte-specific gene locus. Dual-AAV PE prototype, V19, V20, V22, or V24 targeting mouse *Rbm20* were packaged in a proprietary novel cardiomyocyte-targeting AAV capsid, TNC755 (Abstract #1396) and administered to mice systemically. (A) At 4.6E13 vg/kg per vector dosage, V19 mediated 40% editing efficiency at the *Rbm20* locus in cardiomyocytes assessed via RNA NGS analysis, outperforming the prototype (25% editing). (B) V20 and V24 further outperformed V19, showing 75% and 78% editing efficiency, respectively, at the *Rbm20* locus in cardiomyocytes at 3E13 vg/kg dosage.

In Vivo Cardiac Prime Editing Treatment Improves Heart Function in *Rbm20* Dilated Cardiomyopathy Mouse Model

- To demonstrate the therapeutic potential of *in vivo* cardiac PE, we performed a PE efficacy study in *Rbm20*$^{R636Q/+}$ mice, a model of human *RBM20* cardiomyopathy (Nishiyama et al. 2022). *RBM20* encodes a splicing regulator, and mutations in *RBM20* can disrupt splicing of downstream target genes and cause dilated cardiomyopathy.

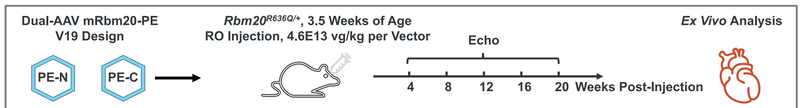

Figure 7. Treating *Rbm20*$^{R636Q/+}$ dilated cardiomyopathy disease mouse model with *in vivo* cardiac PE. *Rbm20*$^{R636Q/+}$ mice (n = 7 or 8) were injected systemically at 3.5 weeks of age with either vehicle or dual-AAV V19 PE targeting correction of *Rbm20*^{R636Q} mutation packaged in TNC755 capsid at 4.6E13 vg/kg per vector. Heart function was assessed via echocardiogram (echo) before injection and every 4 weeks post-injection. Mice were euthanized 20 weeks post-injection for *ex vivo* analysis.

- Our *Rbm20* PE treatment resulted in significant improvement of cardiac function, mitigation of ventricular dilation, and partial rescue of abnormal splicing of RBM20 target genes compared to vehicle control.

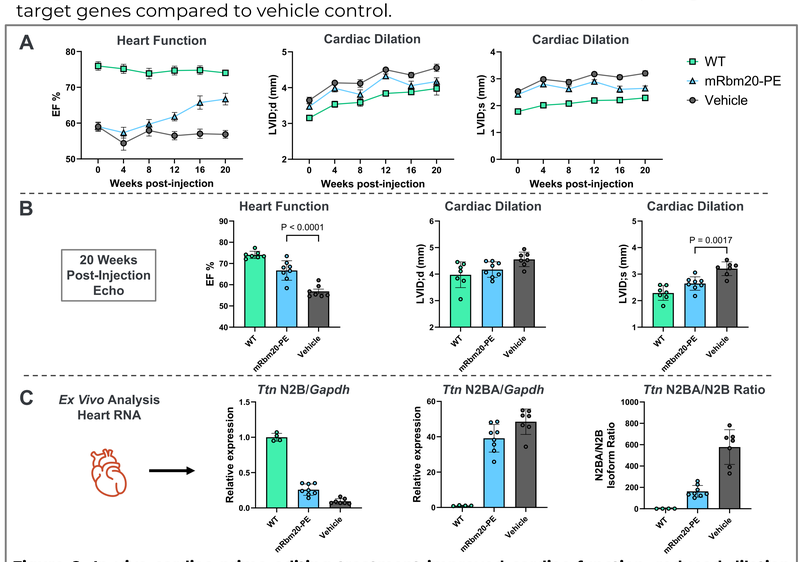

Figure 8. *In vivo* cardiac prime editing treatment improved cardiac function, reduced dilation, and partially reverted abnormal splicing in *Rbm20*$^{R636Q/+}$ mice. (A) Echo measurements from baseline to 20 weeks post-injection show that mRbm20-PE improves heart function and mitigates ventricular dilation over time compared to vehicle control. (B) Echo data at 20 weeks post-injection show that mRbm20-PE-treated mice had significantly better heart function and reduced cardiac dilation compared to vehicle control group. (C) The splicing pattern of *Ttn*, which is regulated by RBM20, was assessed via qPCR (expression normalized to *Gapdh*). In *Rbm20*$^{R636Q/+}$ mice, N2B isoform expression is downregulated and N2BA isoform expression is upregulated compared to WT mice. mRbm20-PE treatment partially rescues *Ttn* splicing pattern.

Conclusions

- For the first time, we established an *in vivo* cardiac prime editing platform and further engineered it to enhance cardiomyocyte genome editing efficiency.
- Our PE strategy successfully reverted the phenotype of a mouse disease model, demonstrating its potential for treating heart disease caused by genetic mutations.
- We plan to focus future efforts on improving PE efficiency and tailoring our PE strategy for specific human genetic mutations known to cause heart disease (Abstract #523).