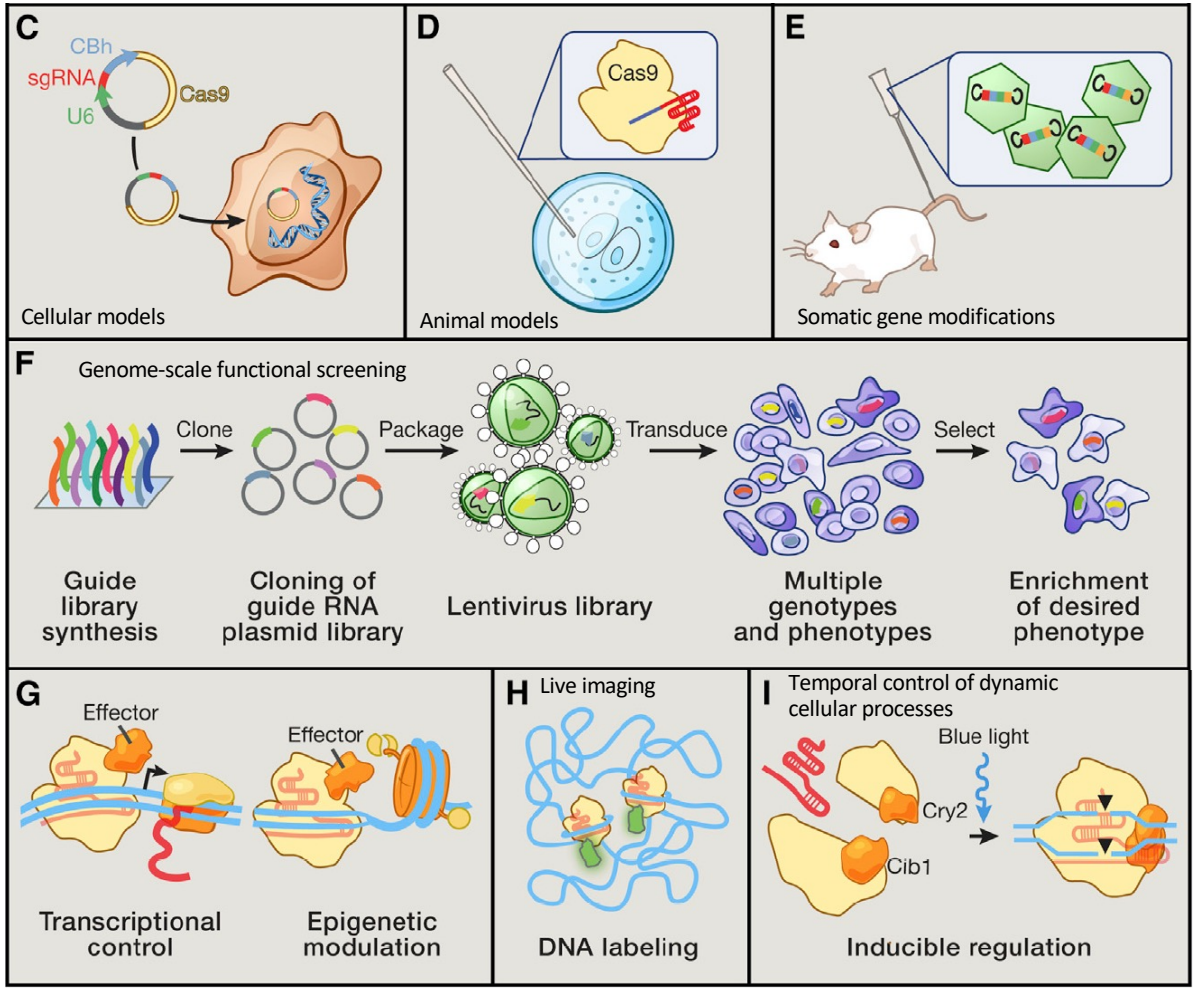
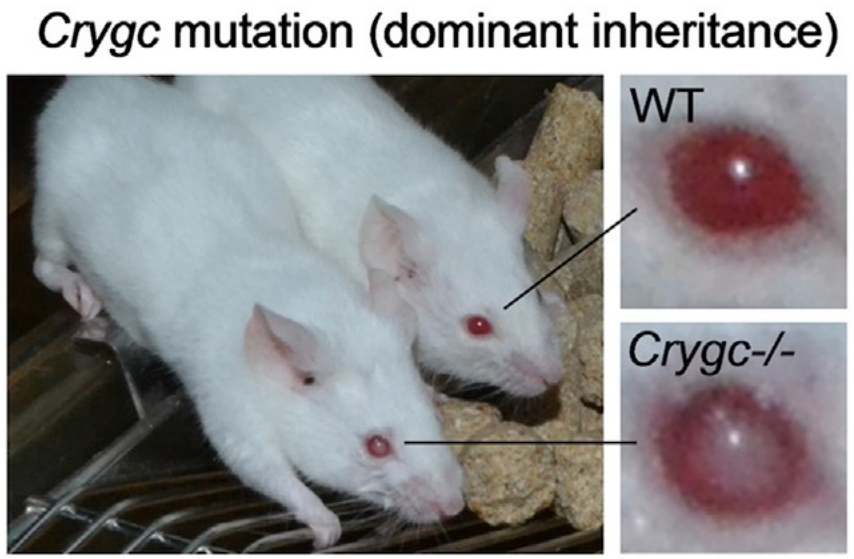
CRISPR/Cas9
APPLICATIONS

CRISPR/Cas engineering is enabling a broad range of applications



(Hsu et al., Cell, 2014)

Can CRISPR/Cas9 be used for correct genetic disorders?

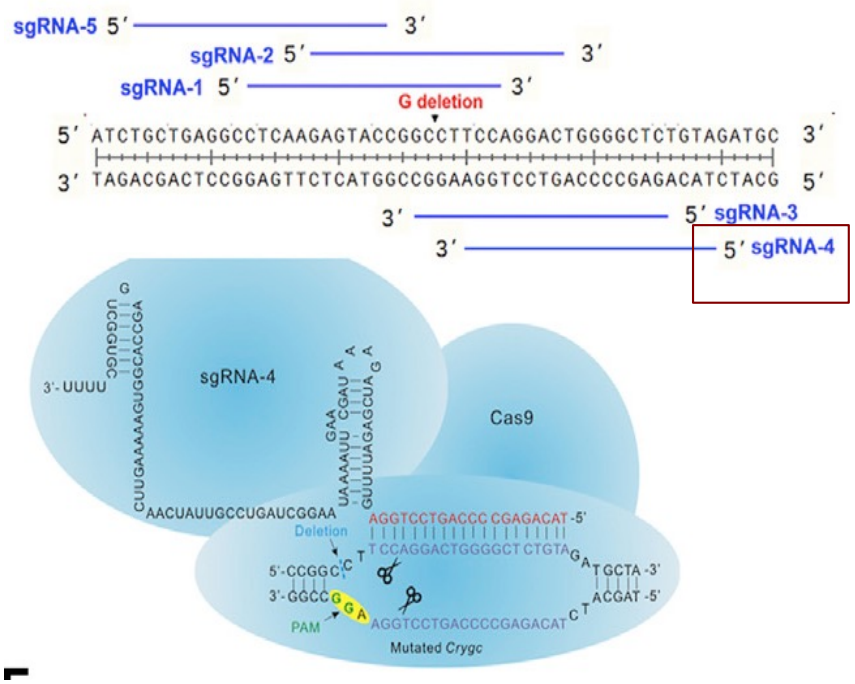


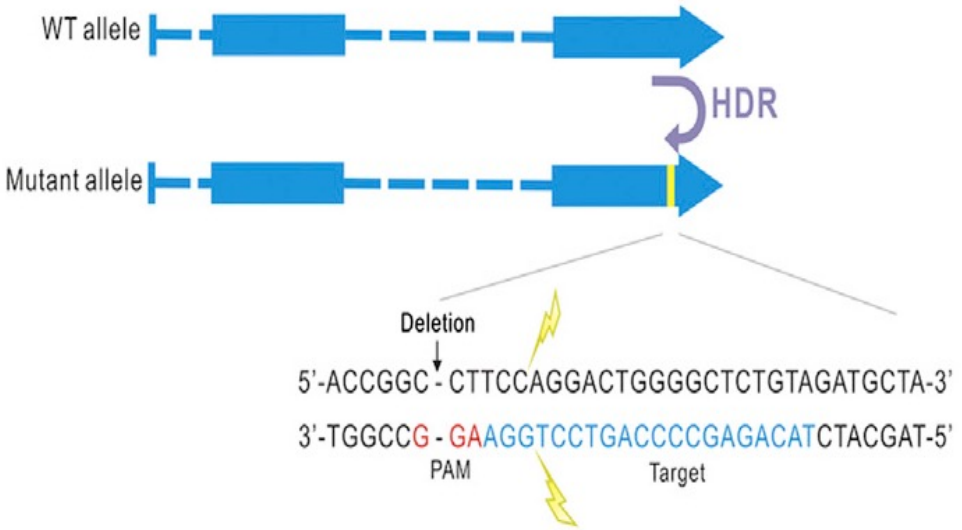
(Wu et al., Cell 2013)

1 bp deletion in exon 3 of Crygc gene leads to cataract

Can CRISPR/Cas9 be used for correct genetic disorders?

In vitro





(Wu et al., Cell 2013)

Can CRISPR/Cas9 be used for correct genetic disorders?

In vitro

sgRNA leads to HDR mediated repair

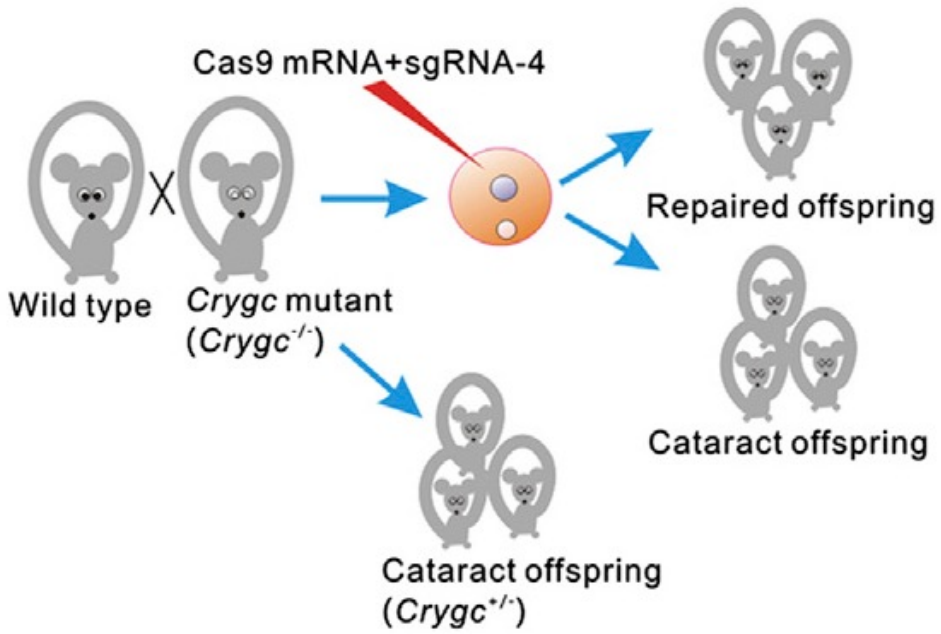
sgRNA	E14 ESC clones		m*Crygc* (*Crygc*$^{+/-}$) ESC clones		
	Cleavage at 1 Allele/Total	Cleavage at 2 Alleles/Total	Cleavage at WT Allele/Total	Cleavage at Mutant Allele/Total	HDR-mediated Repair/Total
sgRNA-1	4/36	0/36	0/36	10/36	7/36
sgRNA-2	23/36	7/36	17/36	25/36	2/36
sgRNA-3	3/36	0/36	0/36	7/36	5/36
sgRNA-4	0/36	0/36	0/36	11/36	16/36
sgRNA-5	4/36	26/36	27/36	26/36	0/36

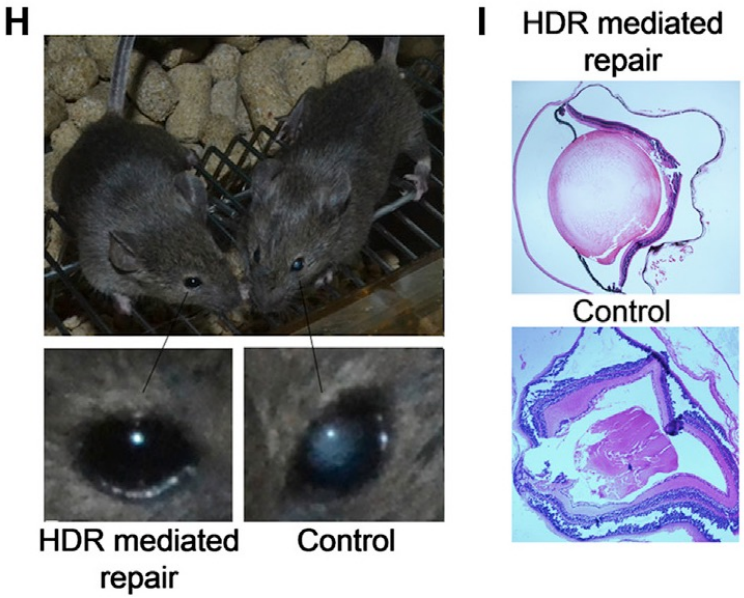
(Wu et al., Cell 2013)

sgRNA4 show high specificity for mCrygc allele and mediates HDR

Can CRISPR/Cas9 be used for correct genetic disorders?

In vivo





(Wu et al., Cell 2013)

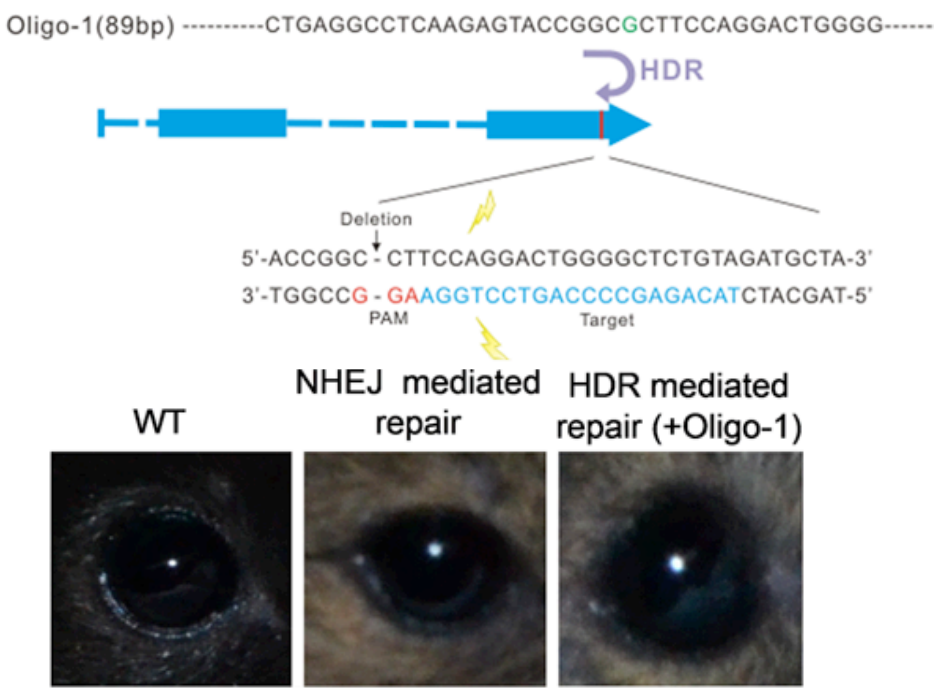
CRISPR/Cas9 system leads to gene correction via HDR using wt allele on the homologous chromosome

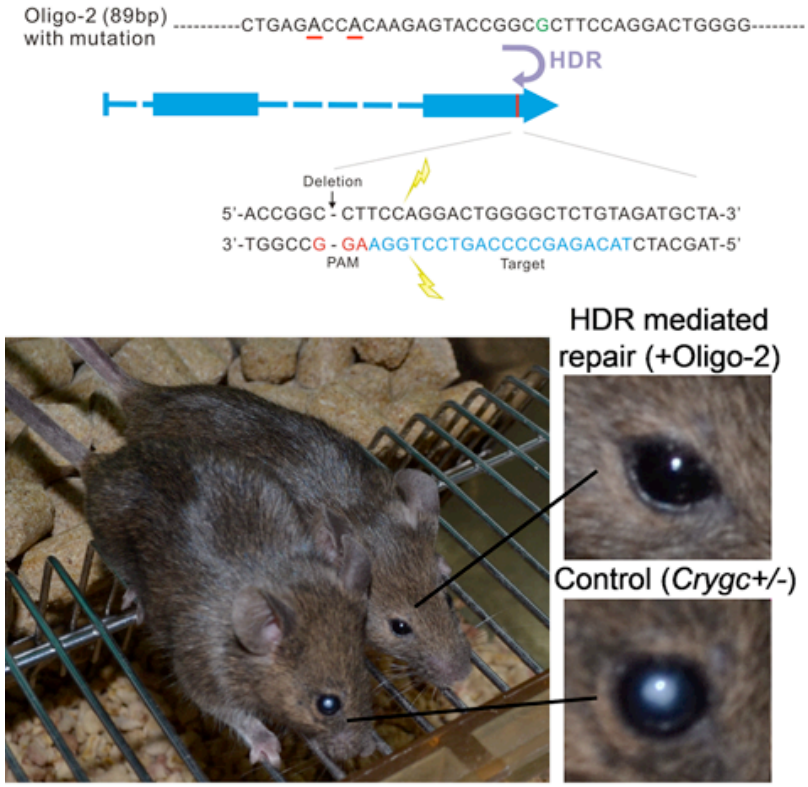
Can CRISPR/Cas9 be used for correct genetic disorders?

(Wu et al., Cell 2013)

NHEJ events can lead to correct reading frame

Is it possible to improve CRISPR/Cas9 sgRNA4 gene correction?

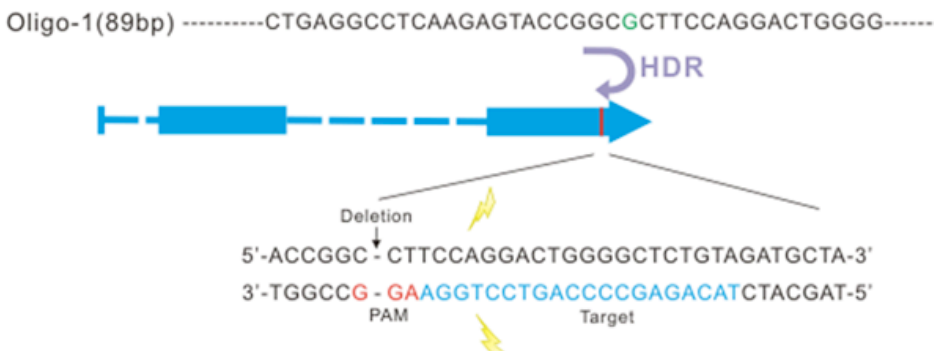




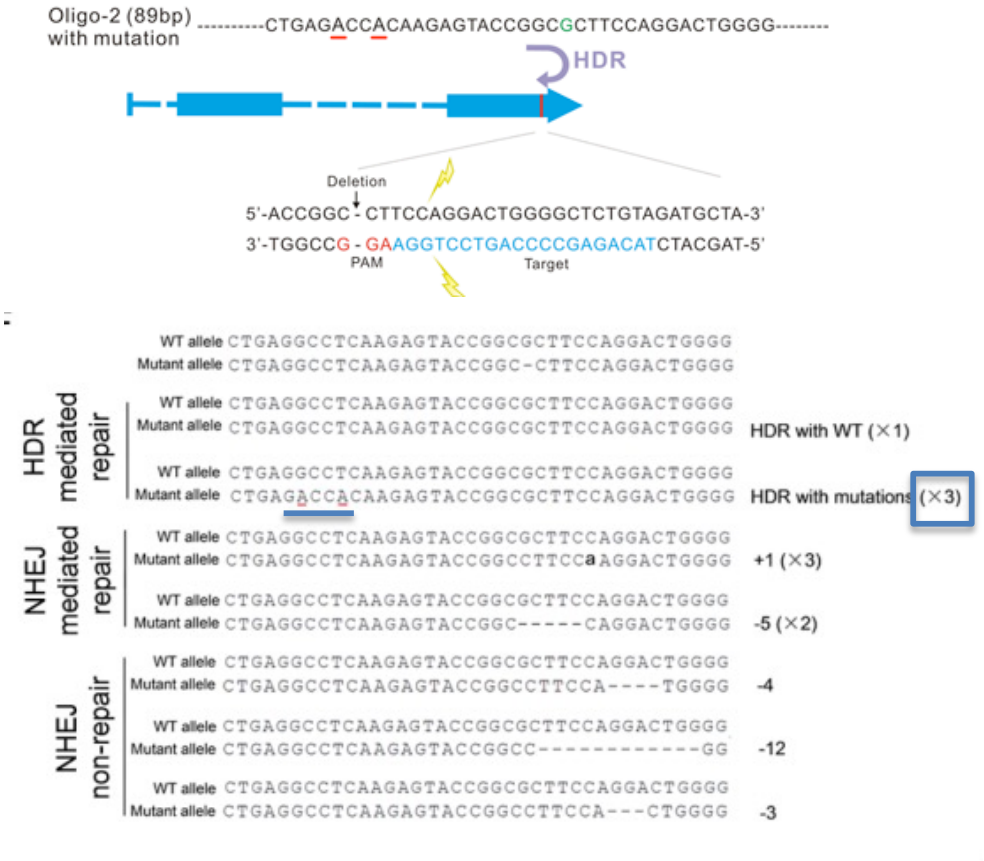
(Wu et al., Cell 2013)

Insertion of Oligo-1 that mimic wt allele and Oligo-2 that contains specific in frame mutation

Is it possible to improve CRISPR/Cas9 sgRNA4 gene correction?



Oligo	Injected Embryos	Blastocysts (Percentage of Injected Embryos)	Transferred Blastocysts	Live-Born Pups	Genetic Modification		NHEJ-Mediated Repair/Nonrepair	HDR-Mediated Repair
					WT allele	mutant allele		
-	172	157 (91%)	135	22	0	10	2/4	4
Oligo-1	245	213 (87%)	178	29	0	14	4/5	5
Oligo-2	221	190 (86%)	159	27	0	12	5/3	4



(Wu et al., Cell 2013)

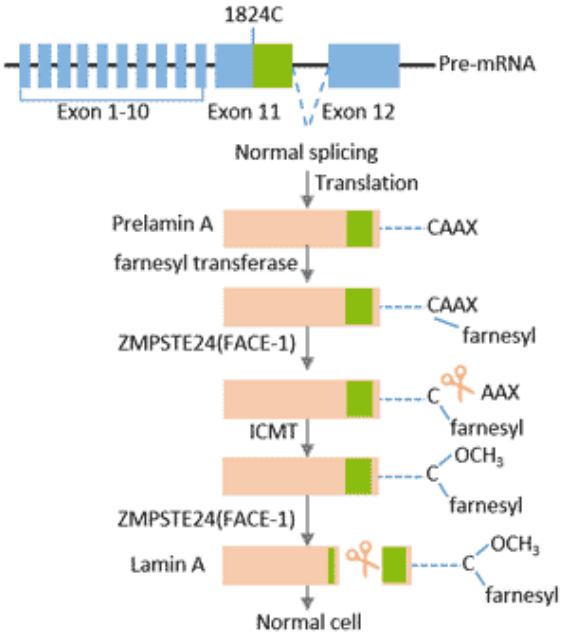
Insertion of Oligo-1 that mimic wt allele and Oligo-2 that contains specific synonymous mutations

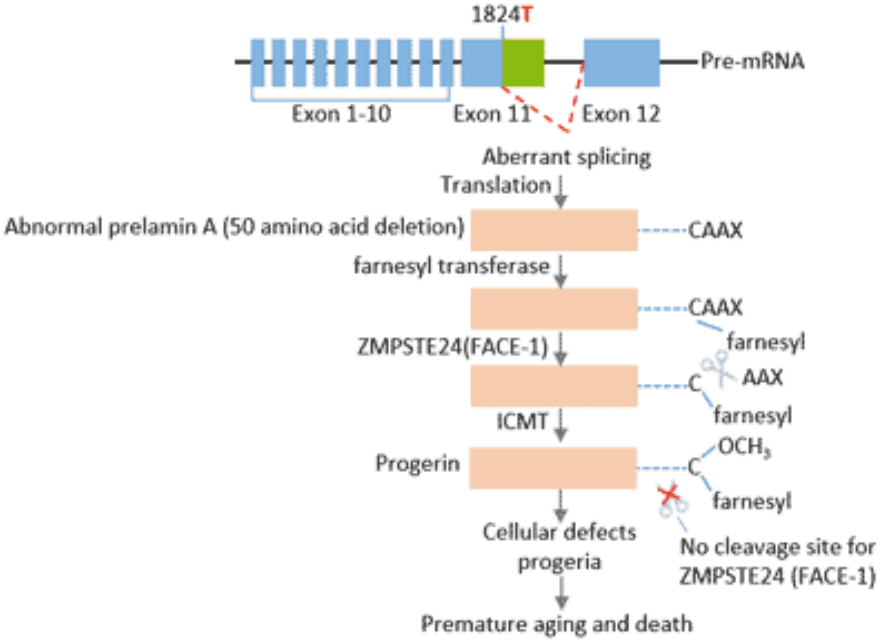
Article

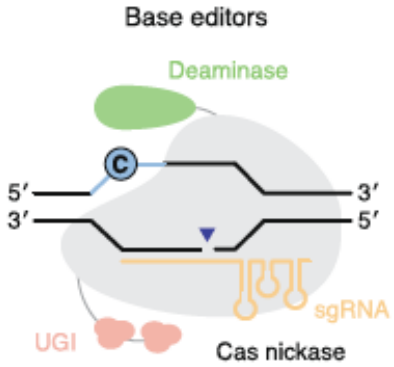
In vivo base editing rescues Hutchinson–Gilford progeria syndrome in mice

Luke W. Koblan[1,2,3,13], Michael R. Erdos[4,13], Christopher Wilson[1,2,3], Wayne A. Cabral[4], Jonathan M. Levy[1,2,3], Zheng-Mei Xiong[4], Urraca L. Tavarez[4], Lindsay M. Davison[5], Yantenew G. Gete[6], Xiaojing Mao[6], Gregory A. Newby[1,2,3], Sean P. Doherty[5], Narisu Narisu[4], Quanhu Sheng[7], Chad Krilow[4], Charles Y. Lin[8,9,12], Leslie B. Gordon[10,11], Kan Cao[6], Francis S. Collins[4]✉, Jonathan D. Brown[5]✉ & David R. Liu[1,2,3]✉

Nature | Vol 589 | 28 January 2021 |



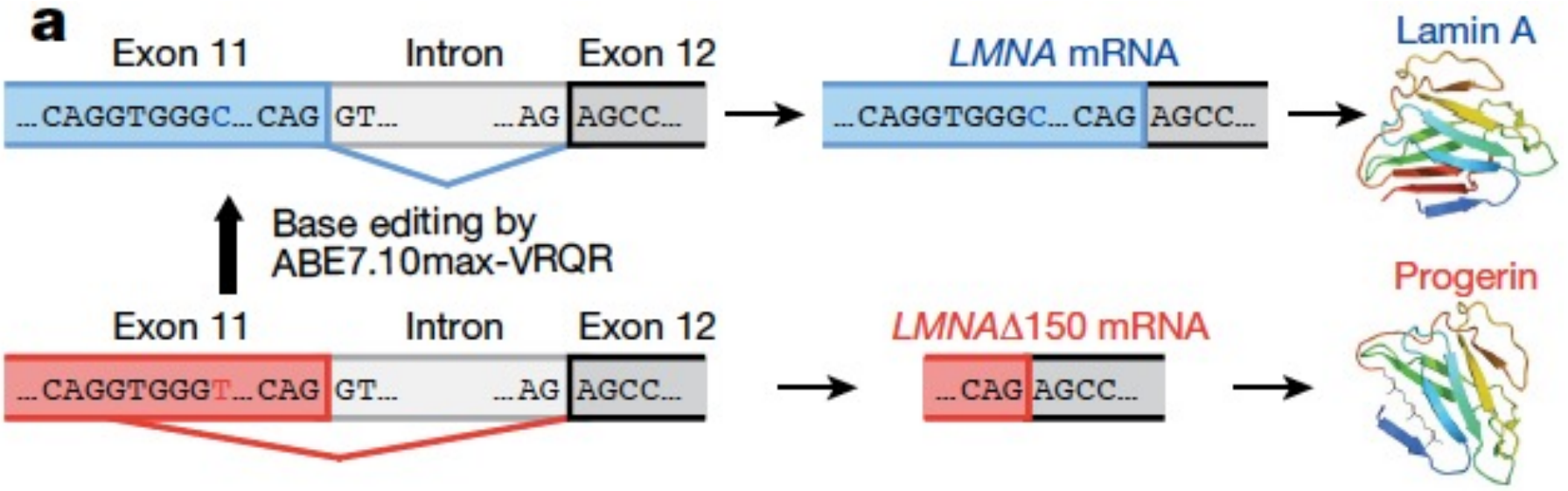


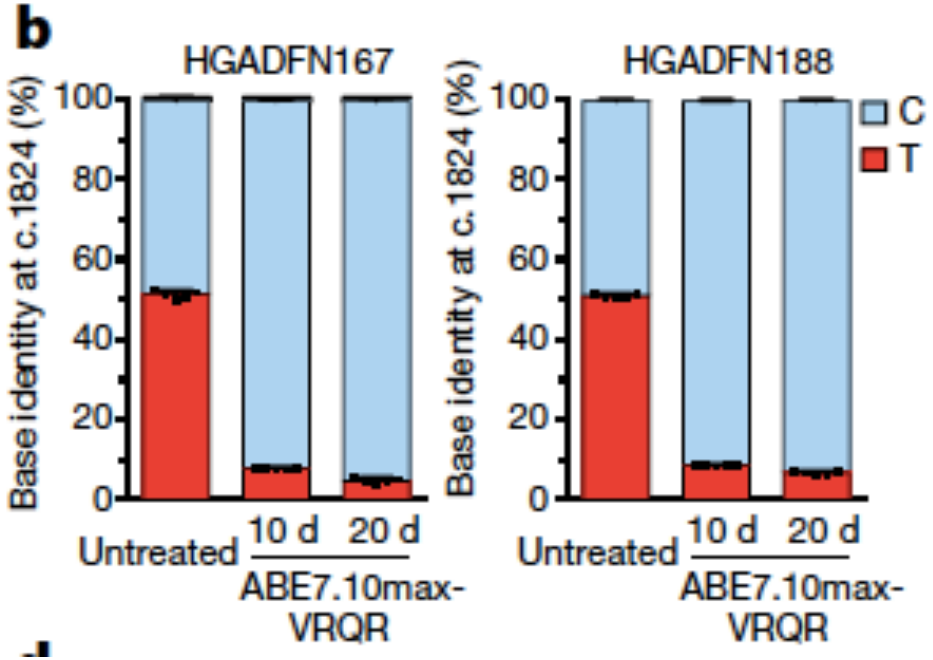


ABE: adenine base editor and converts A-T to G-C.

Adapted from Baek, Erikson, 2013

Does ABE approach correct HGPS mutation in patient cells?

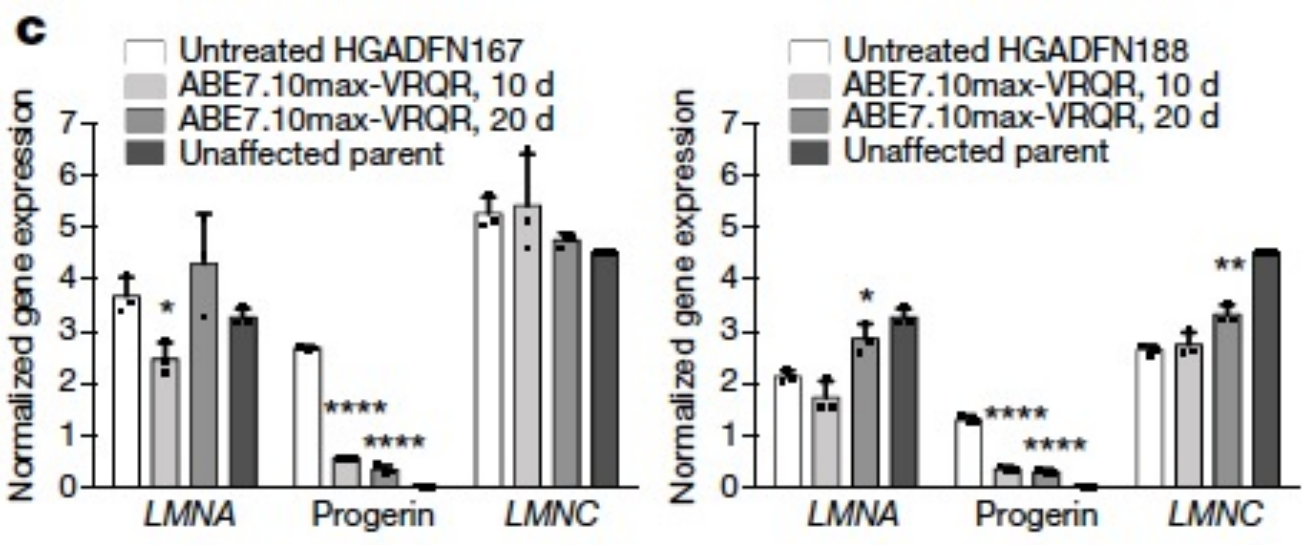




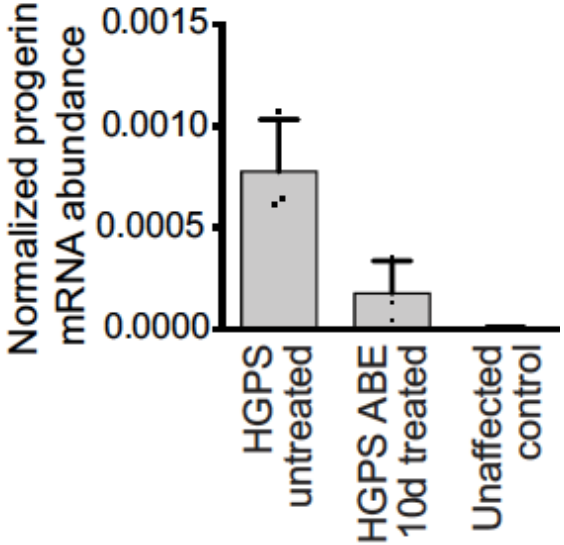
Lentiviral mediated delivery
ABE7-10fused with optimized SpCas9-VRQR+
SgRNA targeting HGP mutation

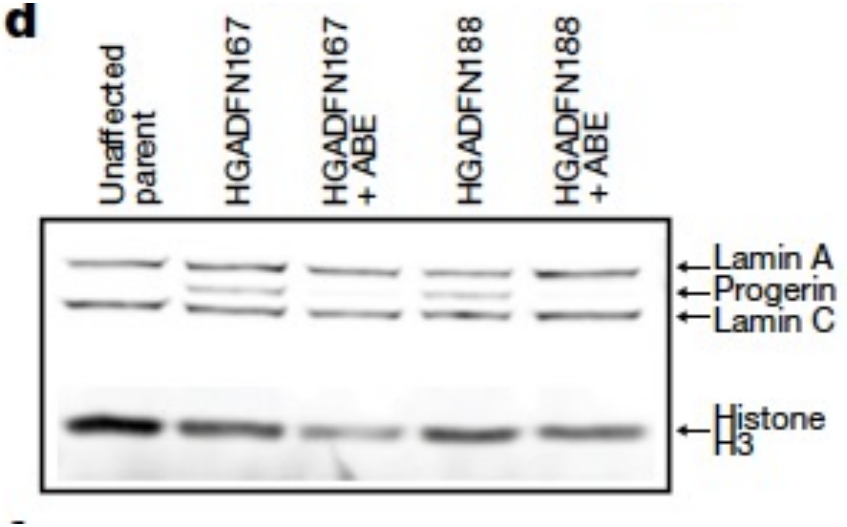
84-85% of correction 10 days
87-91% of correction 20 days

Does ABE approach correct HGPS mutation in patient cells?



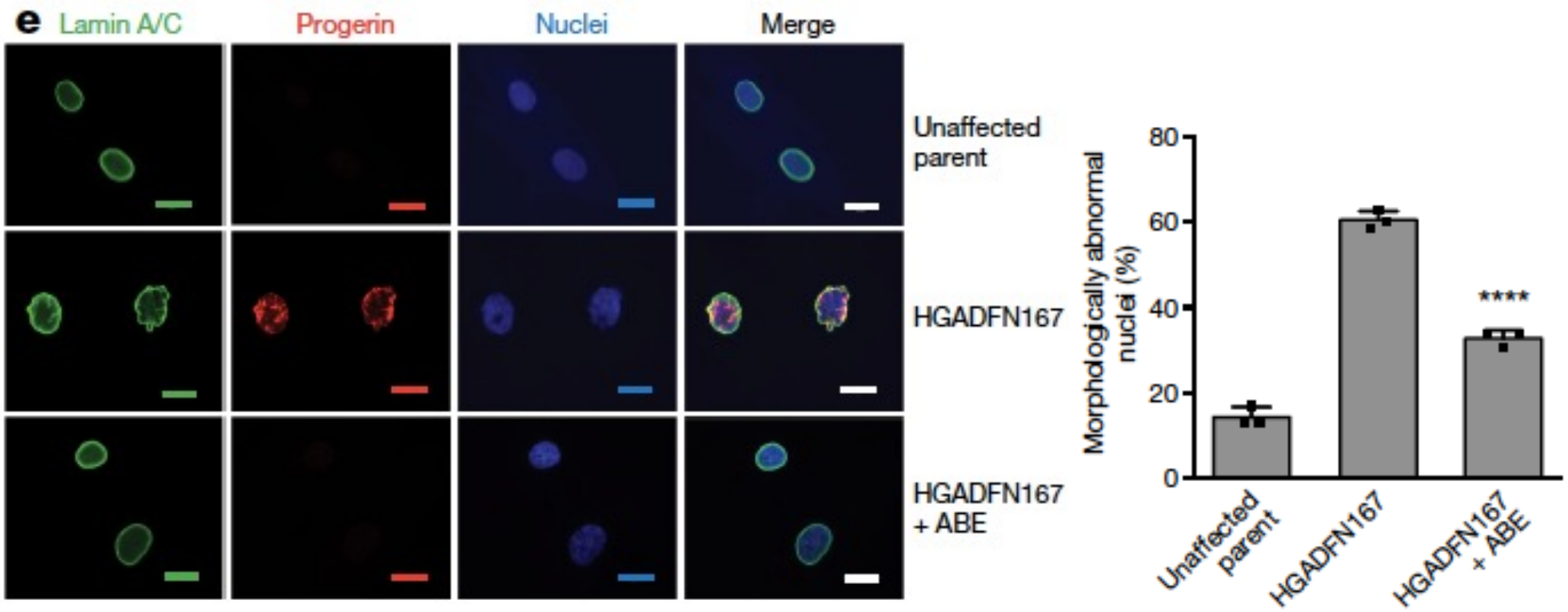
8.8-4.4fold reduction of progerin





6.1-15 fold reduction of progerin protein

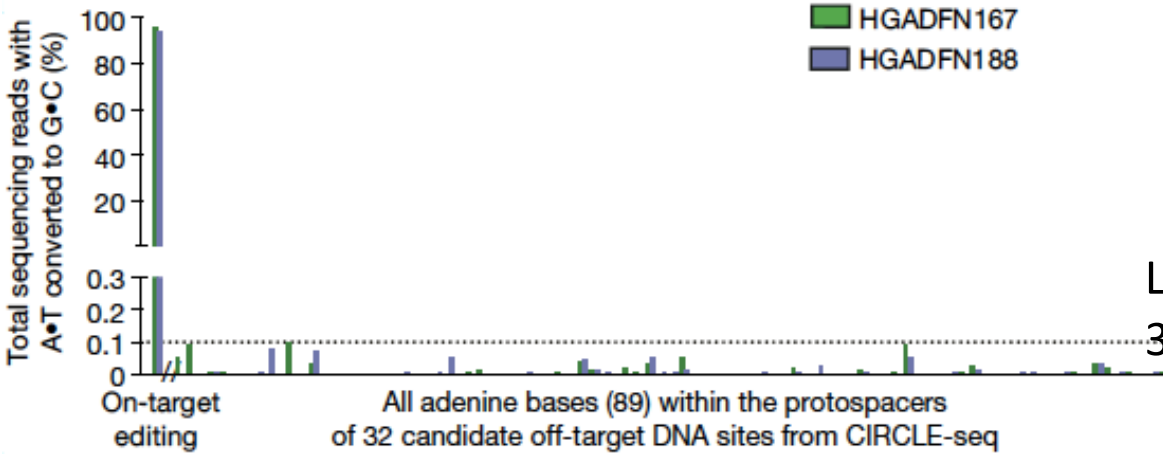
Does ABE approach correct HGPS mutation in patient cells?



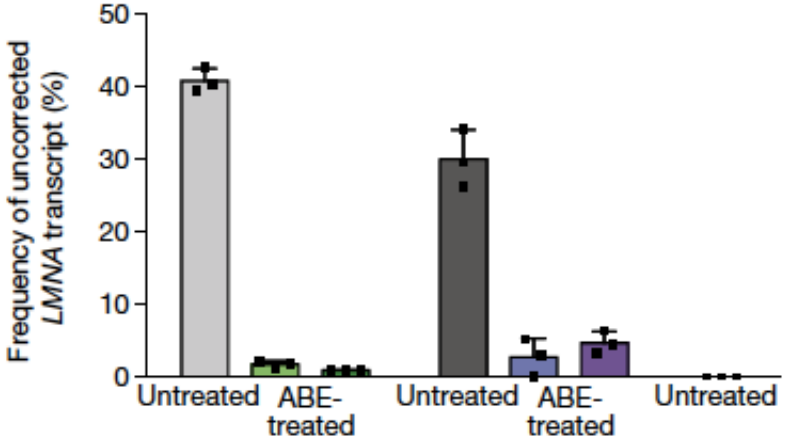
1.8 fold reduction of nuclear abnormalities

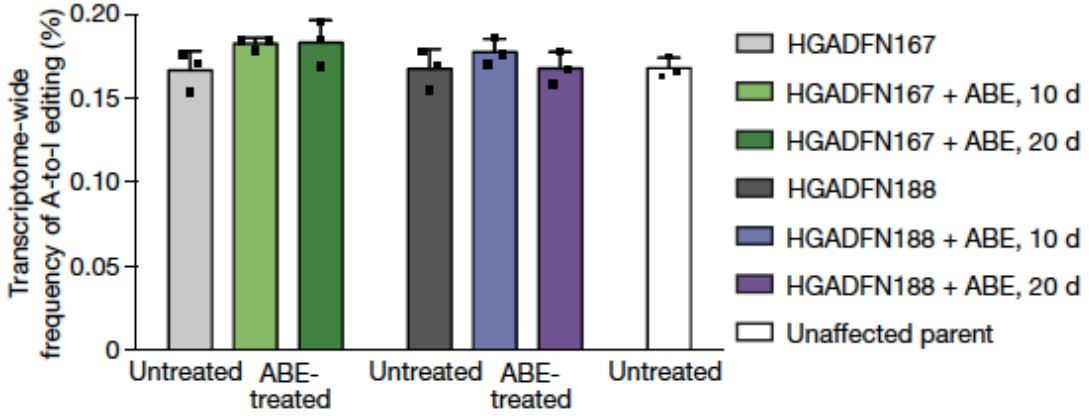
Base editing to correct LMNA c.1824 C→T mutation in cells from HGPS patients rescues the molecular and phenotypic consequences of the mutation

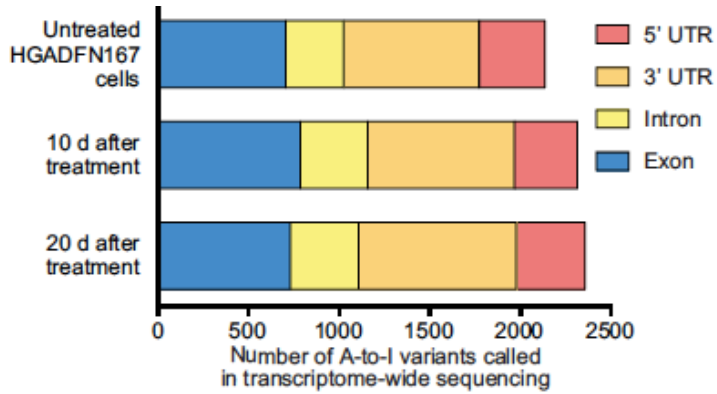
Does LMNA c.1824 C→ABE correction approach induce off targets?

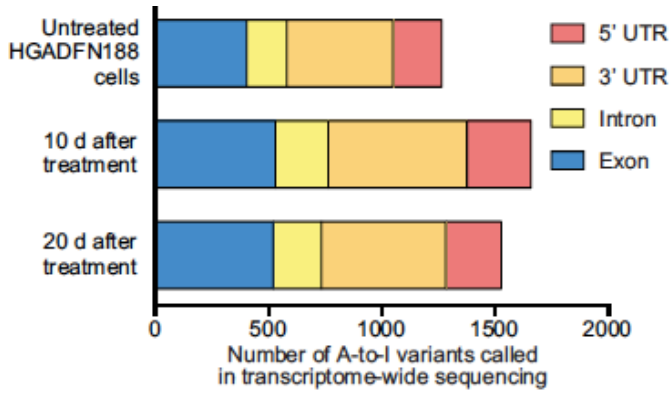


Less than 0.1% off-target DNA editing at 32 candidate off target loci

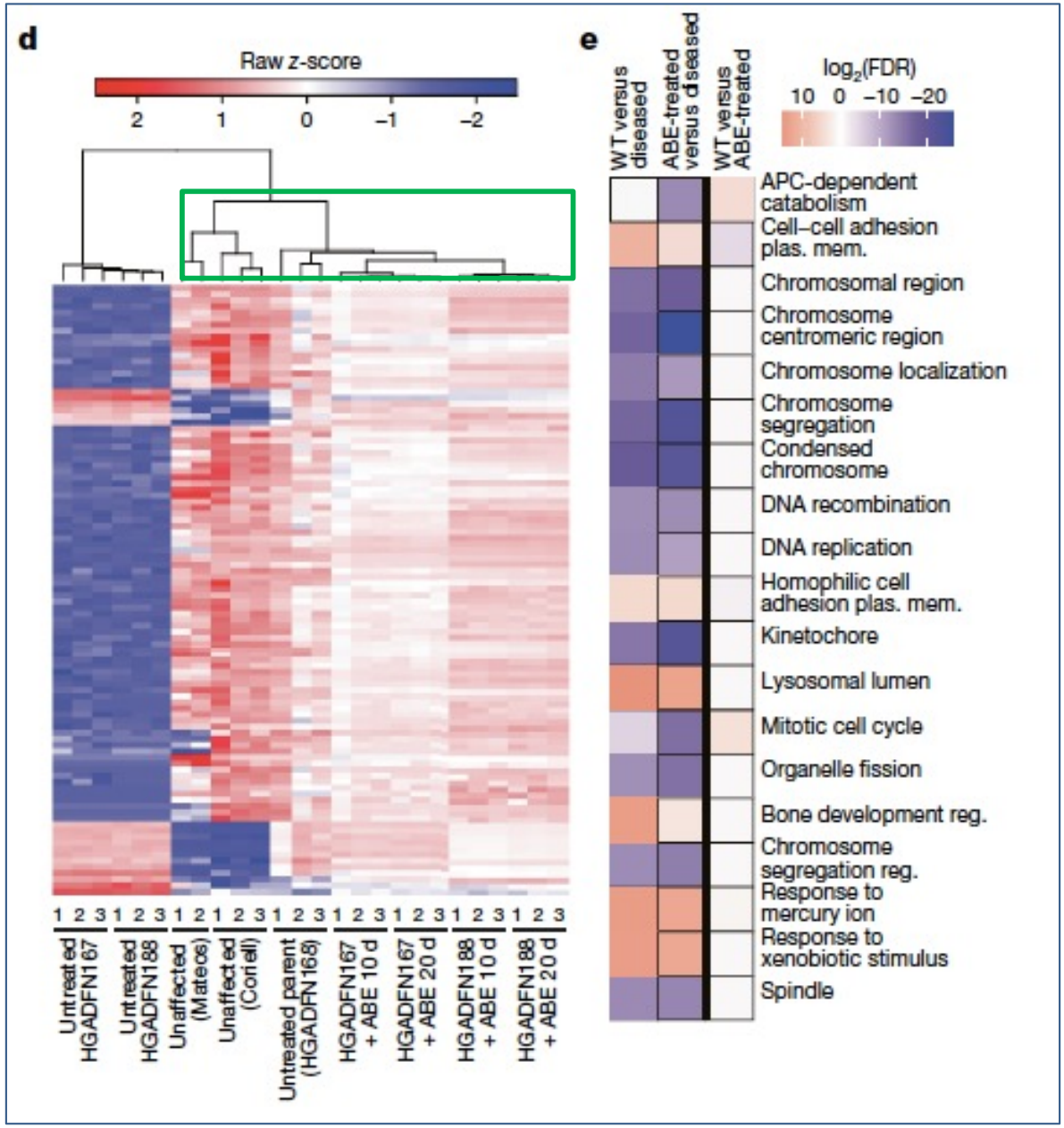








Does LMNA c.1824 C→ABE correction approach induce off targets?



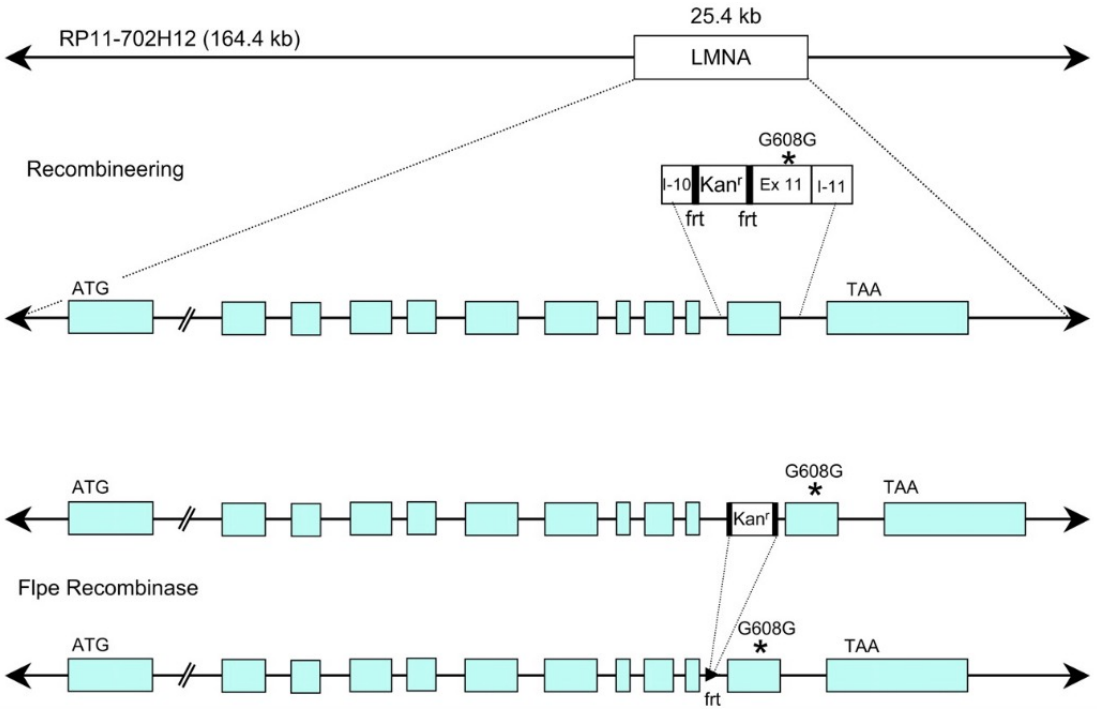
ABE treated transcriptomes cluster with unaffected Fb

Treating cells with LMNA-targeting sgRNA and ABEmax-VRQR didn't results in off–target DNA or RNA editing despite high level of on-target editing

In vivo ABE delivery in mice with progeria

Progeria mouse model:

C57BL/6-tg(LMNA*G608G)HCIns/J: includes complete human LMN c.1824C>T



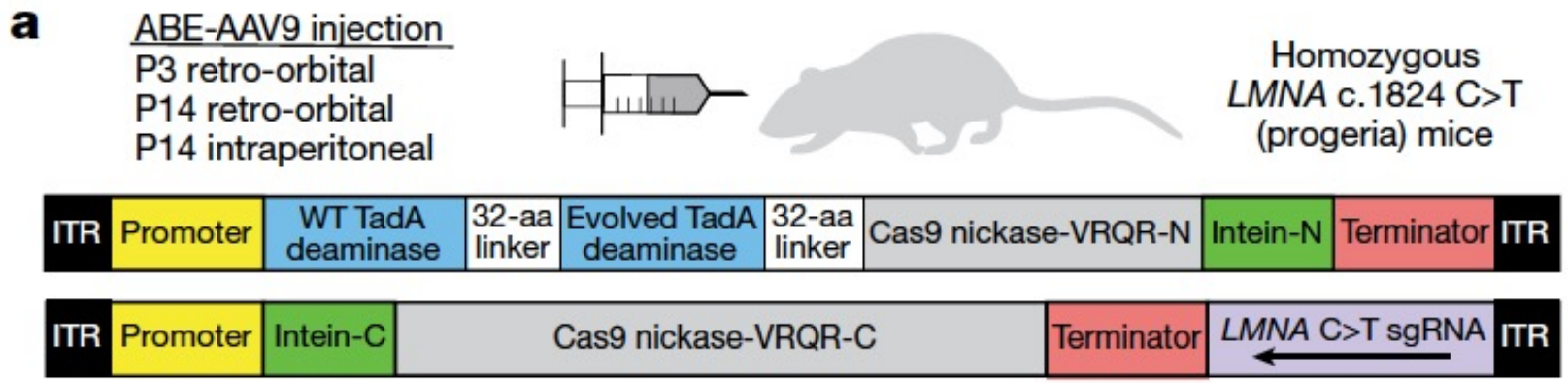
Systemic in vivo delivery of ABE and sgRNA characterized in cells

Varga et al, PNAS 2006

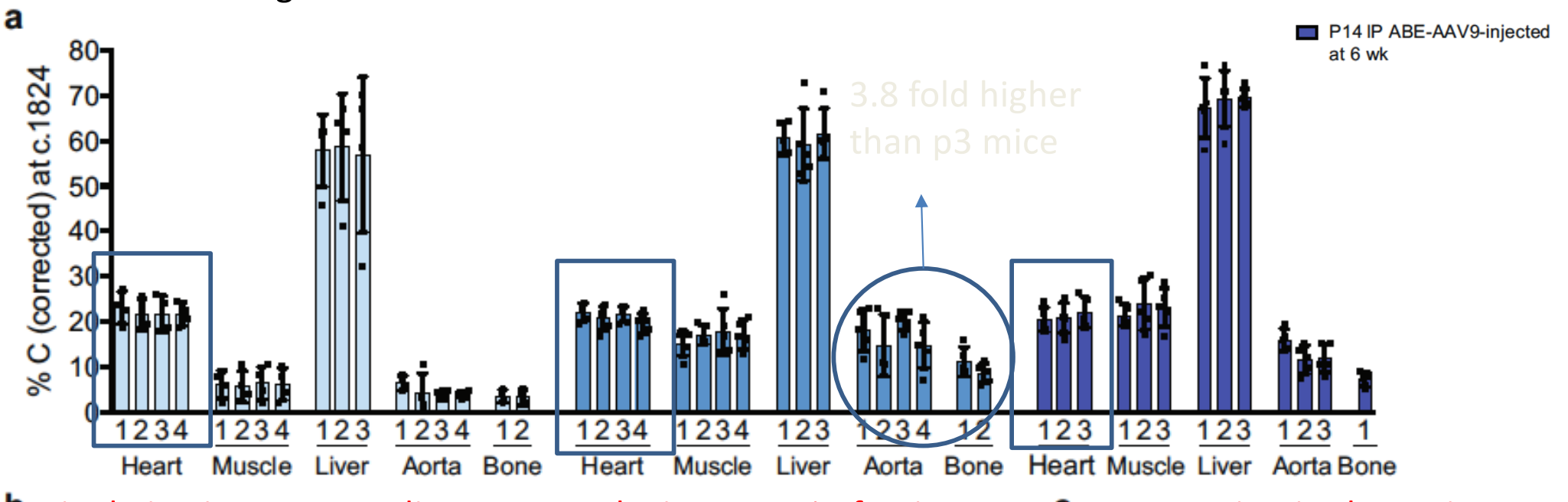
For the first 4 months after birth, homozygotes exhibit a slower rate of weight gain when compared to hemizygotes. In addition to developing kyphosis, hair loss, tight skin, loss of subcutaneous fat and joint contracture, homozygotes develop a significantly more severe vascular damage with VSMC loss in aortic vessel walls, calcification, and periadventitial thickening. Homozygotes die at an average of 7-8 months of age as the result of aortic stiffening and impaired cardiovascular functioning. The Donating Investigator reports that homozygous females are infertile and that a lower than expected number of homozygotes are born.

From Jackson Lab

In vivo ABE delivery in mice with progeria



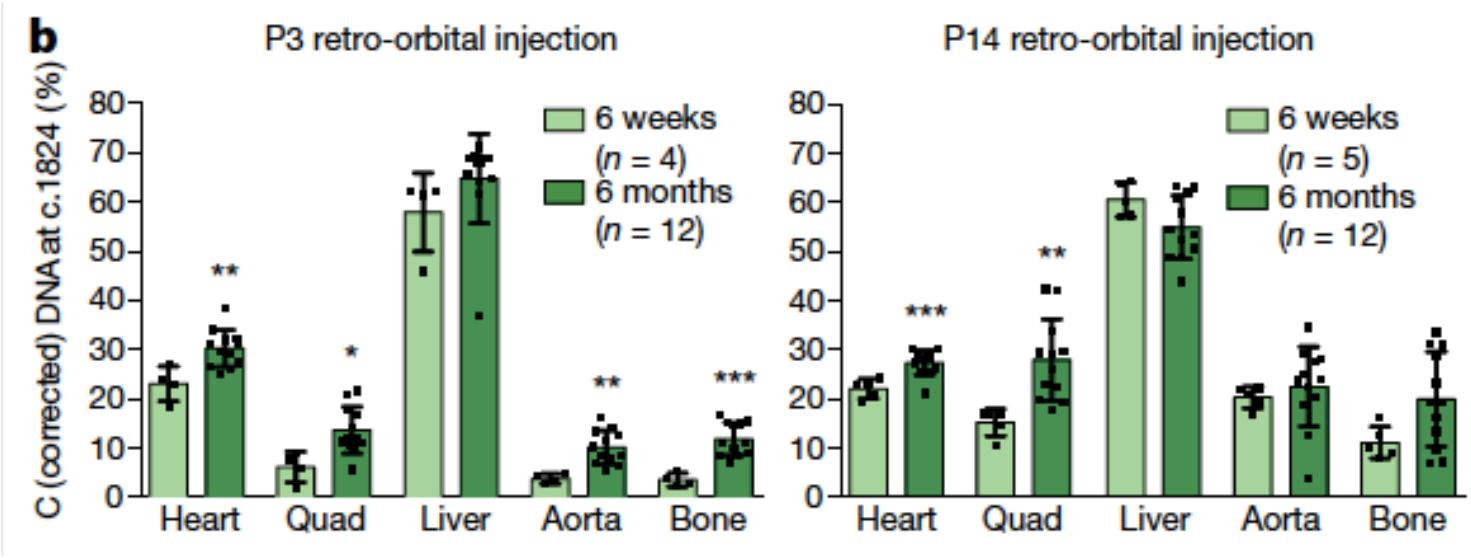
6 weeks of age



A single in vivo AB-encoding AAV results in 10-60% of point mutation correction in the various organs

In vivo ABE delivery in mice with progeria. Long term ABE treatment

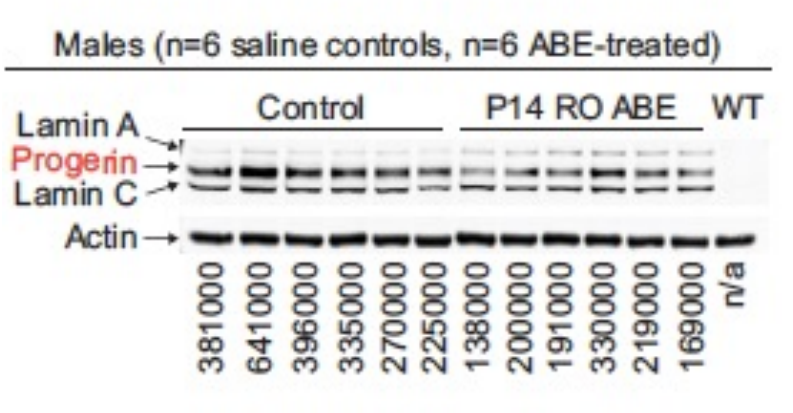
6 months after injection

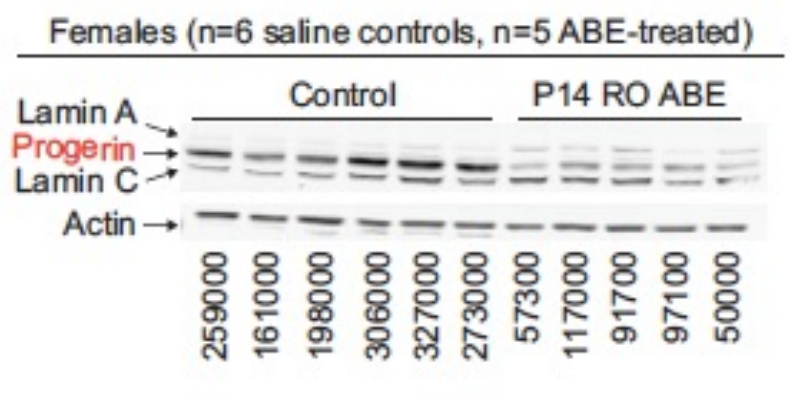


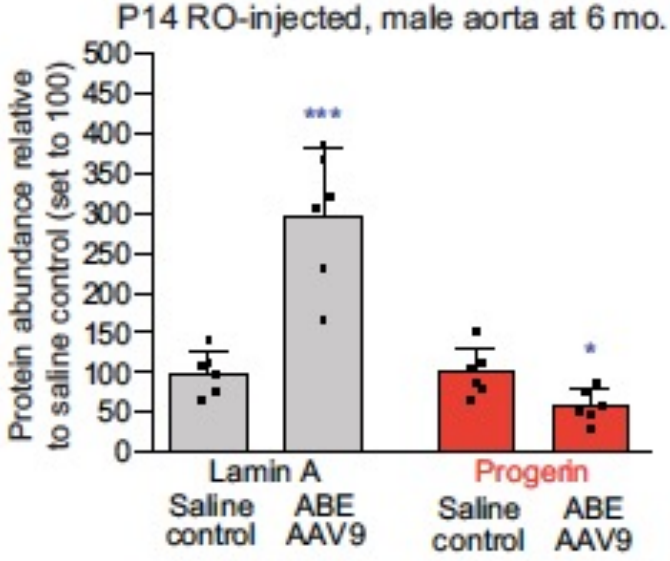
Increase in gene editing at 6 months, mainly for p3 mice but remains higher for p14 mice injected with higher doses off ABE encoding AAVs

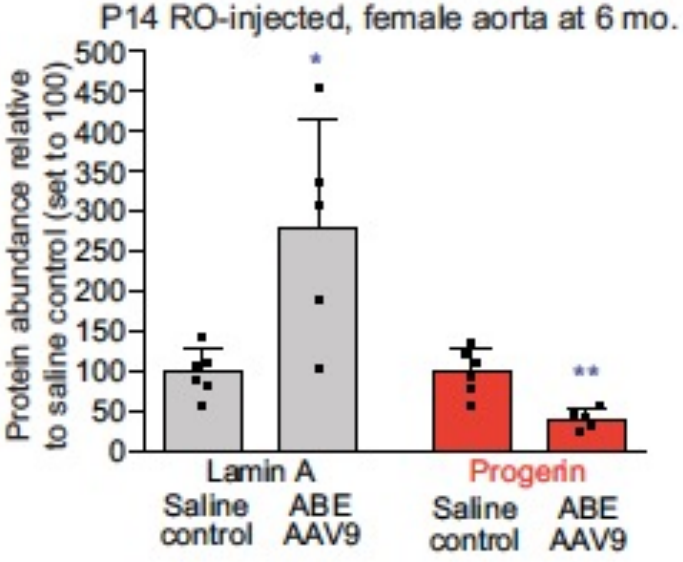
Base editing persists in vivo

In vivo ABE delivery in mice with progeria. Long term ABE treatment





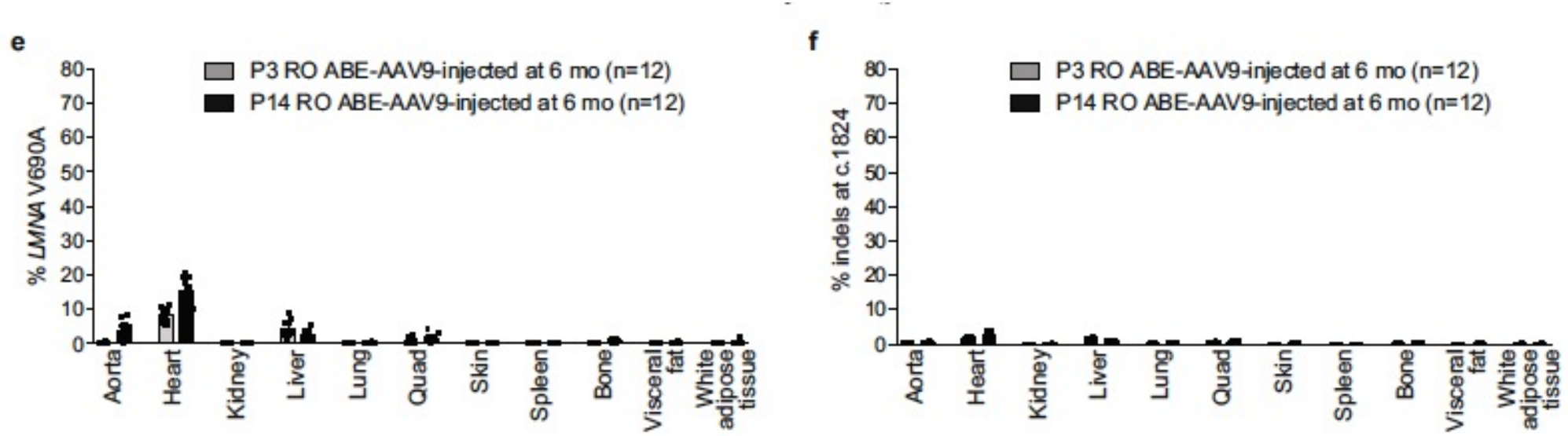




In vivo ABE treatment led to decrease in progerin transcript abundance and robust reduction of progerin protein

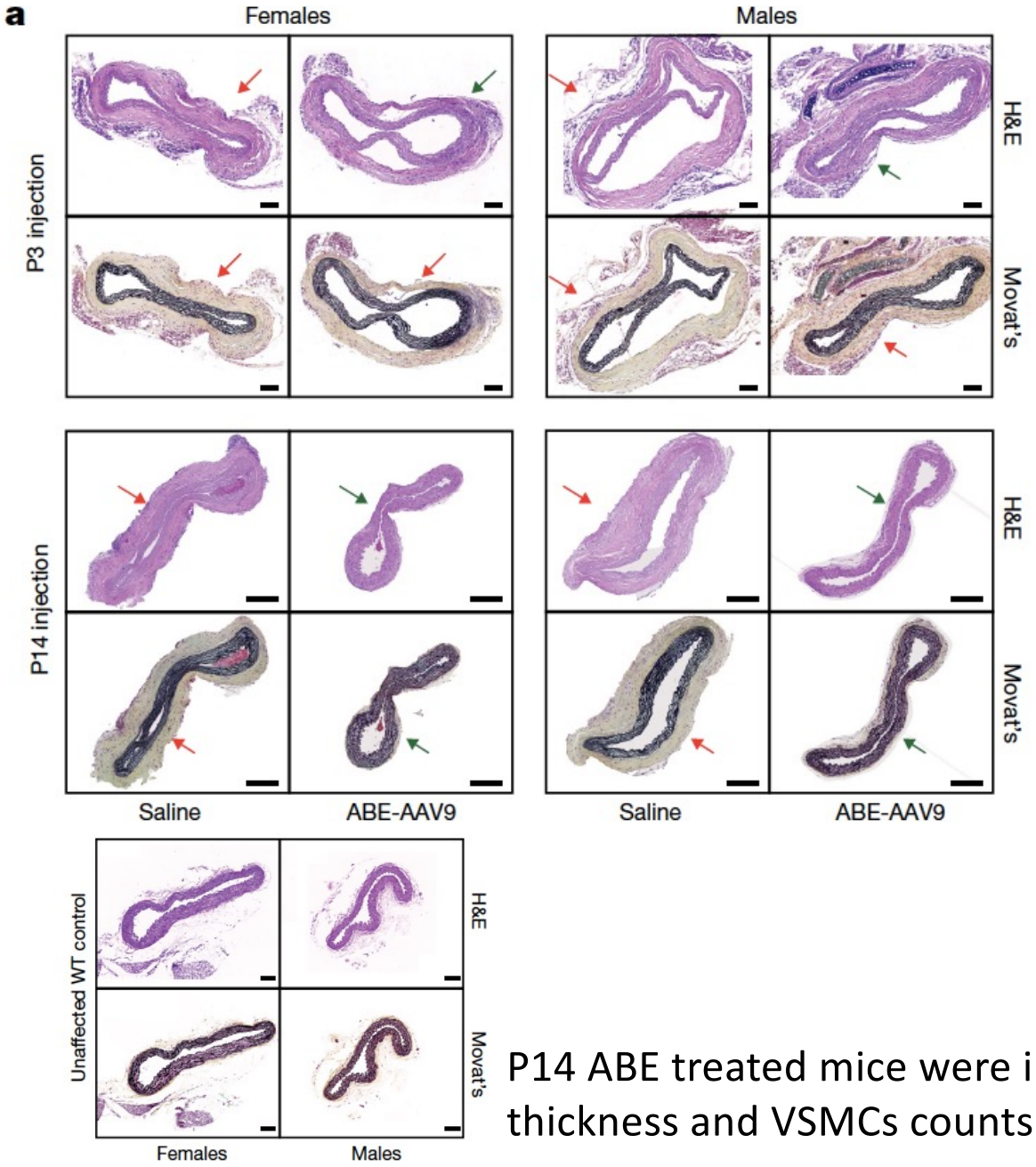
ABE correction of LMNA c.1824C>T alleli in mice can reduce progerin mRNA and protein levels in several clinically relevant tissues

In vivo ABE delivery in mice with progeria. Long term ABE treatment

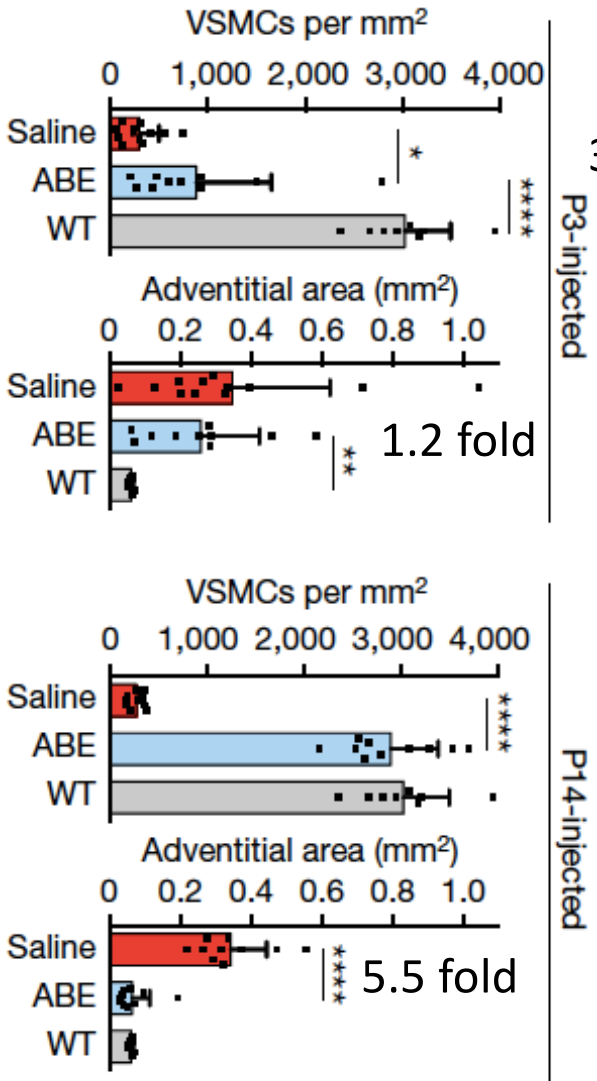


Bystander editing and indels observed at very low frequency compared to on target editing

ABE treatment improves vascular pathology



6 months mice



3.3 fold

1.2 fold

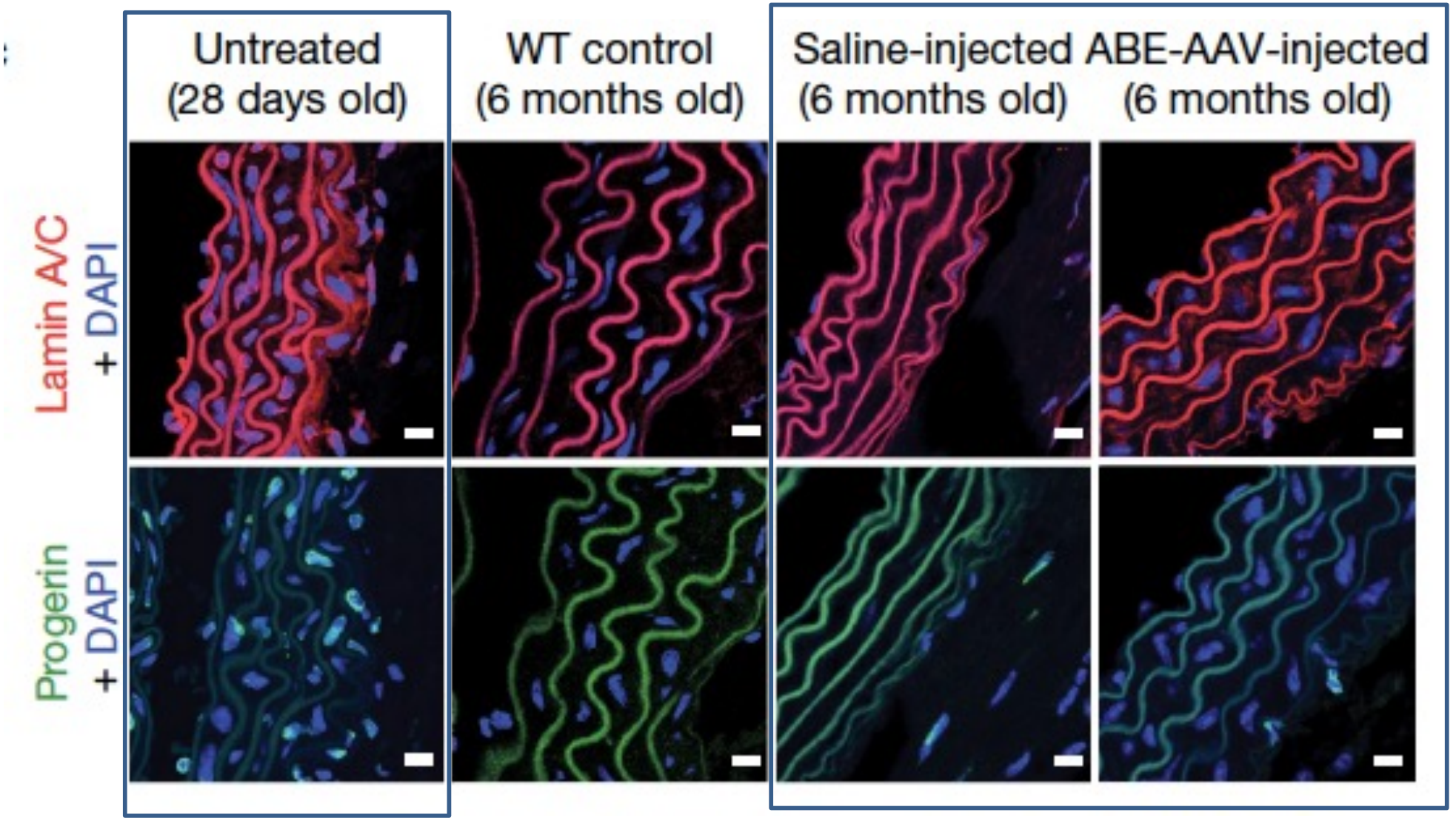
11 fold

5.5 fold

P14 ABE treated mice were indistinguishable for ctr for adventitial thickness and VSMCs counts

ABE treatment improves vascular pathology

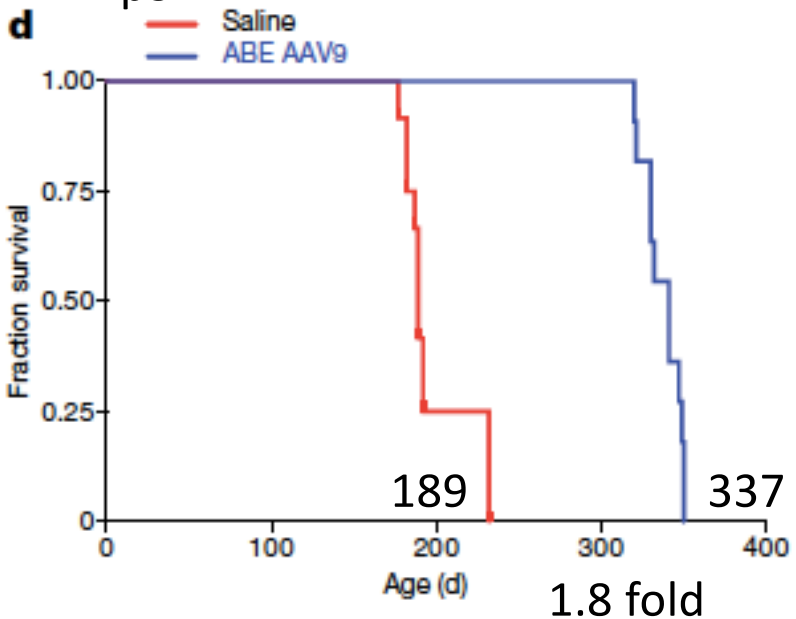
Increase in VSMCs count



Base editing of around 25% in aorta in enough to rescue two key vascular defects of progeria, preserving the expression of Lamin A/C and reducing progerin abundance

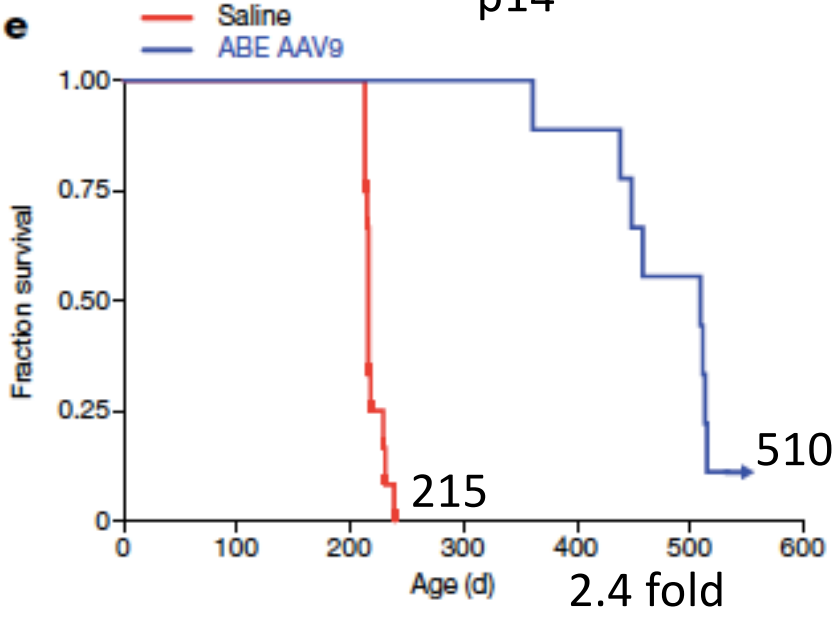
ABE treatment extends progeria mouse lifespan

p3

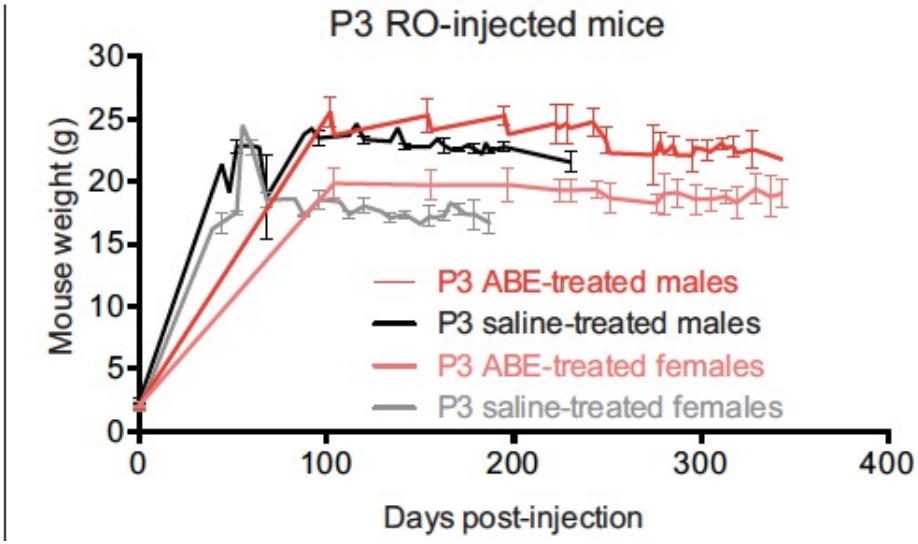


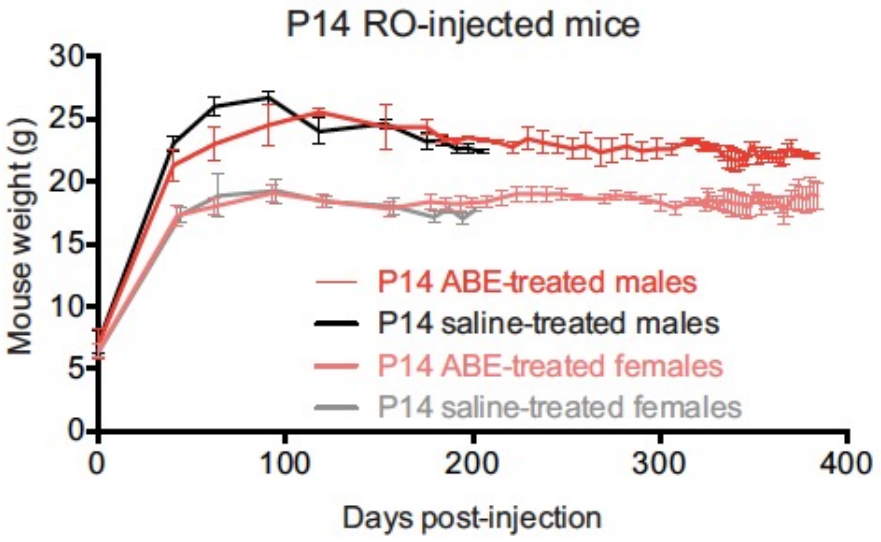
1.8 fold

p14



2.4 fold





Body weights were maintained

Conclusions

- Patients-derived cells ABE correct pathogenic allele (97-91% of on target editing) with minimal degree of off-target. Reduces mis-splicing and progerin protein resuces nuclear morphology defects
- A single dose of dual AAV9 encoding ABE and sgRNA in a progeria mouse model resulted in durable correction of pathogenic allele, reduction of progerin protein with different efficient in diverse tissue and organs. Treatment of p14 mice greatly improved cardiac phenotype and aortic health.
- A single dose of dual AAV9 encoding ABE and sgRNA in a progeria mouse model increases mice lifespan

Concerns:

- Immune response to treatment
- Timing of treatment to take account of the time of diagnosis
- Base editing amelioration to obtain results in other tissues
- No direct correlation between base editing, RNA levels and proteins levels