185. Gene Correction via CRISPR and Others

(Wisconsin Alumni Research Foundation)

Asset Overview

Product Type	Gene Therapy
Disease Area	Others
Indication	Pompe Disease and Other Autosomal Recessive Disorders
Current Stage	Lead Optimization
Target	Pompe disease cells
MoA	Cas9-ribonucleoprotein-based genome editors can correct two distinct mutant alleles within a single human cell precisely
Brief Description	• Pompe disease is a rare autosomal recessive genetic disease (mutations from both parent carriers have to be inherited by the offspring to see symptoms) that typically affects children during development but can also present as an adult. Pompe disease is characterized by a lack of functional acid alpha-glucosidase (GAA) enzyme. The lack of GAA leads to a glycogen buildup in cells throughout the body, which results in muscular weakness and wasting. Newborns in which the disease is misdiagnosed rarely survive past one year. • UW–Madison researchers have developed a complexed CRISPR-Cas system (S1mplex; P170309US01) for treating patients with inherited autosomal recessive conditions. The work focuses on Pompe disease. The inventors identified new guide RNA target sites and repair templates that could be used for gene therapy strategies and cell therapeutic strategies. • The inventors demonstrated successful editing of fibroblast and induced pluripotent stem cells from three Pompe patients at UW Hospital having heterozygous mutations. Using their complexed CRISPR-Cas technology markedly increased editing precision (18.4-fold) in two different cell lines (HEK and hPSC), easing concerns about off-target effects.
Intellectual Property	US20200140858A1
Publication	Design of efficacious somatic cell genome editing strategies for recessive and polygenic diseases. Nat Commun. (2020)
Inventors	Krishanu Saha, Jared Matthew Carlson-Stevermer, Lucille Katherine Kohlenberg

Highlights

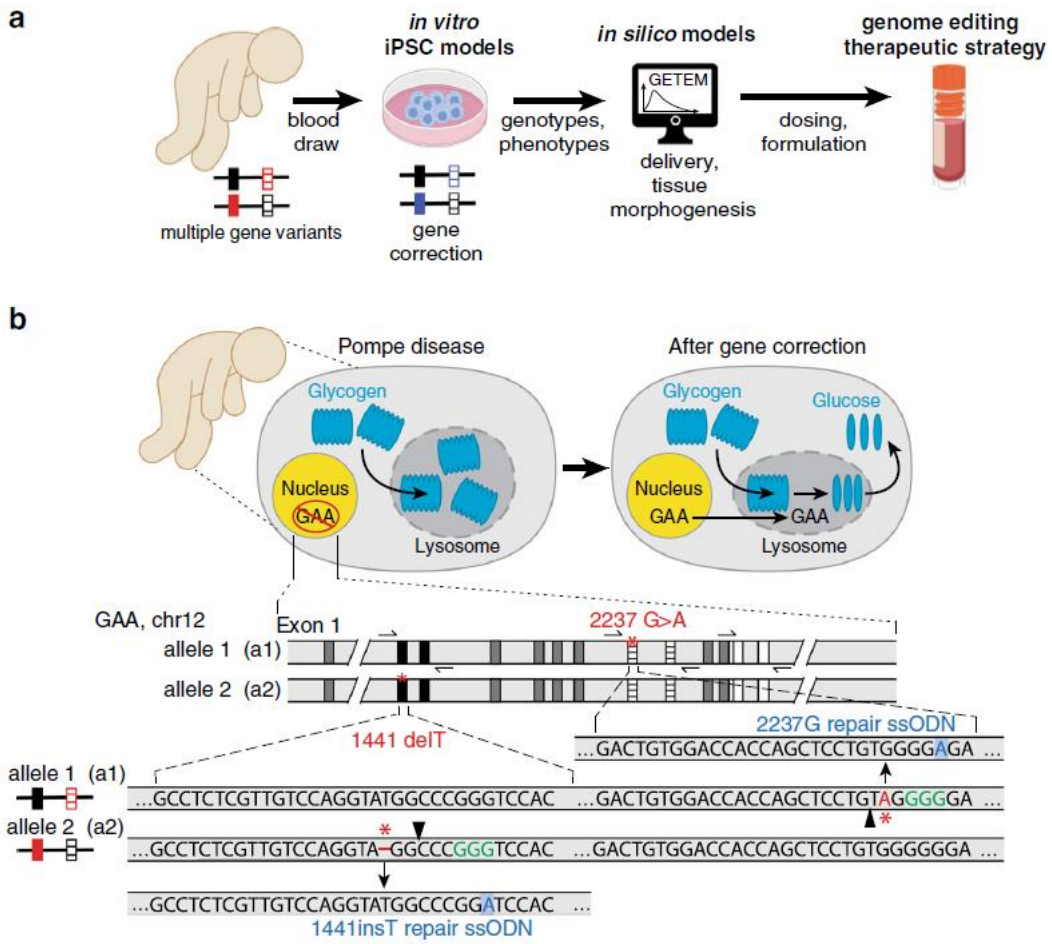
- Paired with complexed CRISPR-Cas (S1mplex), precise-to-imprecise editing ratios were improved 18.4-fold.
- Intervention utilizing multiple gene correction within the same human cell is possible.
- Rapid improvements in glycogen processing is observed (as short as 24 hours).

185. Gene Correction via CRISPR and Others

(Wisconsin Alumni Research Foundation)

Key Data

A combined in vitro and in silico strategy to evaluate the efficacy of different gene correction therapeutic strategies



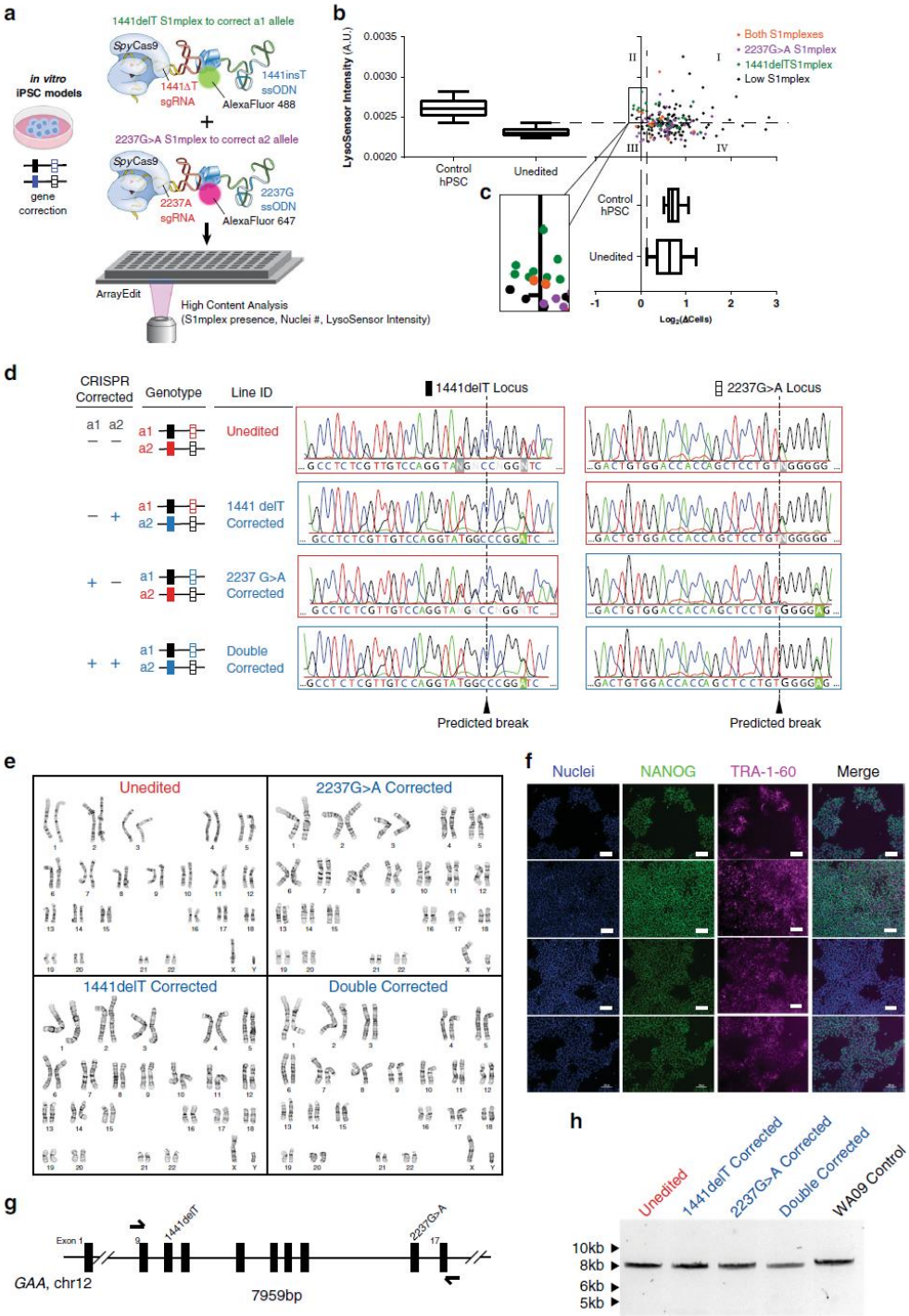
a Schematic indicating the modeling approach in which samples from patients are collected ex vivo are then genome-edited in vitro. Genotypes and phenotype outcomes from the in vitro studies are inputs for an in silico model that simulates the delivery of the therapeutic in vivo as well as tissue morphogenesis. The results of the in silico model can ultimately guide dosing and formulation decisions. **b** Editing strategy for gene correction of Pompe-diseased induced pluripotent stem cells (iPSCs). Pompe disease is caused by two defective copies of the acid-α-glucosidase (GAA) gene. This enzyme is responsible for breakdown of glycogen within lysosomes inside cells. Without GAA, glycogen build up can cause downstream health issues. After correction, GAA expresses a functional protein leading to a reduction in glycogen. The schematic indicates the editing locations within GAA locus and CRISPR gene correction strategy. In the Pompe patient-derived line, cells harbor compound heterozygous mutations in GAA. Allele one, a1, contains a point mutation that causes a premature stop codon (GAA:c.[1441=2237G>A]) while allele two, a2, carries a one base pair deletion (GAA:c.[1441delT;2237=]). For the CRISPR gene correction strategy, single guide RNAs (sgRNAs; the predicted DNA double-strand break by SpyCas9 is denoted by the arrowhead) were designed to be specific to only the diseased allele by containing the mutant bases (red) within the seed region. Single-stranded oligonucleotides (ssODNs) used for genomic repair contained the wildtype sequence at the mutation site (blue) as well as a silent mutation "wobble" to remove the PAM site (green) to prevent re-cutting of the corrected allele while preserving the amino acid sequence of GAA.

185. Gene Correction via CRISPR and Others

(Wisconsin Alumni Research Foundation)

Key Data

Gene correction of two distinct mutant alleles within a single human cell



To be continued

Key Data

Gene correction of two distinct mutant alleles within a single human cell

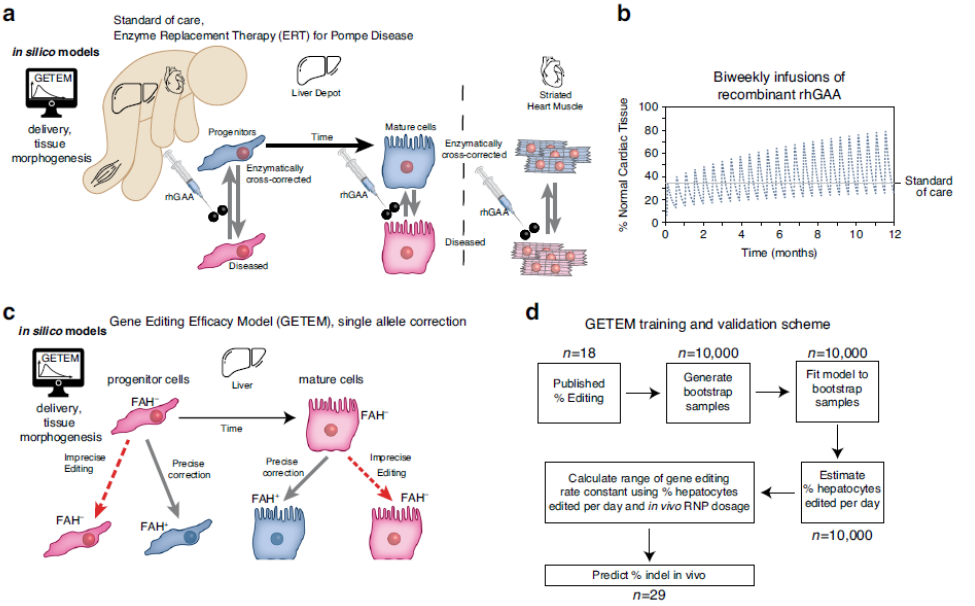
a ArrayEdit-based isolation of iPSC clones corrected at either or both loci. CRISPR S1mplex design for the gene correction of compound heterozygous mutations. S1mplexes targeting 1441delT mutant were labeled with an AlexaFluor488 compound while S1mplexes targeting the 2237G>A mutation were labeled with an AlexaFluor647. These ribonucleoprotein genome editors were mixed prior to transfecting into cells and subsequently plated on the ArrayEdit platform to conduct high-content analysis (see Supplementary Fig. 1). b Left: LysoSensor quantification per µFeature of two mock transfections after 7 days of growth. Normal control hPSCs were significantly more intense than unedited, Pompe diseased iPSCs on ArrayEdit. Bottom right: the growth rate of unedited and control hPSCs following a mock transfection to establish a baseline for growth. Growth rates were calculated by measuring the per-day change in the number of cells of the µ Feature. Top right: LysoSensor intensity was plotted against growth rate per µ Feature to identify edited colonies. Dashed lines indicate regions of interest. (n = 145 independent cell lines). c Magnification of quadrant II from (b). M Features in this region were selected for genomic analysis to isolate edited clones. (n = 17 independent cell lines). d Sanger sequencing traces of corrected cell lines. The unedited line contains mutations at both alleles: 1441delT mutation causes a breakdown of sequence trace, whereas a single point mutation demonstrates a heterozygosity 2237G>A locus. SpyCas9 cut site is denoted by a dotted line. e Karyotypes of all isolated gene-corrected lines as well as unedited cells. No abnormalities were detected at a band resolution of 500. f Immunocytochemistry of pluripotency markers in gene-corrected lines. All lines were positive for pluripotency markers NANOG and TRA-1-60 (scale bar: 100 µm). g Schematic of long PCR covering both SpyCas9 cut sites. Arrows denote primers. The expected PCR amplicon is 7959 bp in length. h Gel analysis of long-range PCR described in (g) in each isolated cell line. No significant deviances from the expected length were detected, and no other notable bands were observed. WA09 control cells are hPSCs.

185. Gene Correction via CRISPR and Others

(Wisconsin Alumni Research Foundation)

Key Data

Derivation, empirical training, and validation of in silico gene therapy efficacy model for Pompe disease correction in a developing infant



To be continued

Key Data

Derivation, empirical training, and validation of in silico gene therapy efficacy model for Pompe disease correction in a developing infant

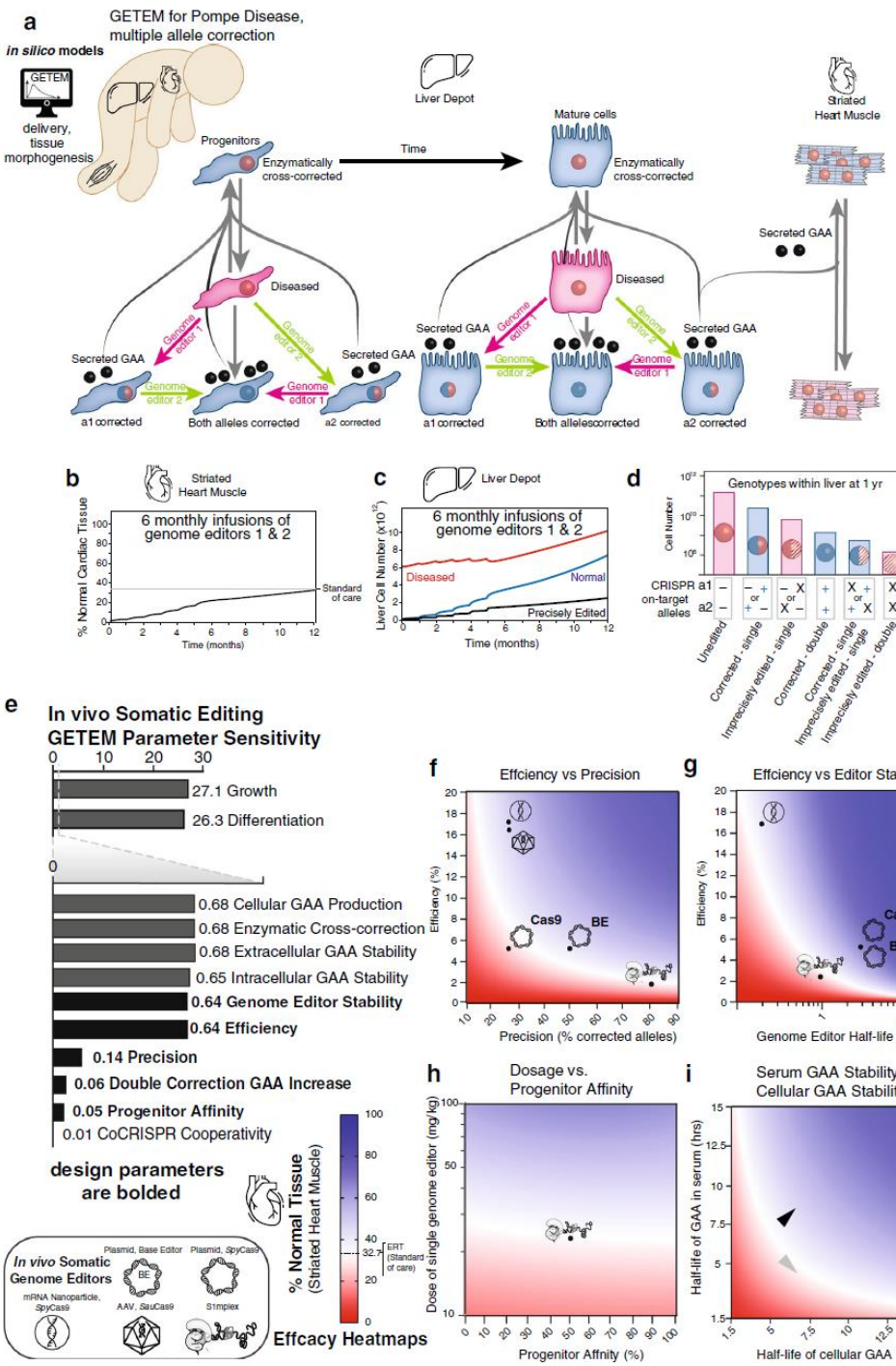
a Schematic showing enzymatic cross-correction of somatic tissues by injected rhGAA (enzyme replacement therapy, ERT). For a given developing liver of a patient, progenitor cells proliferate and can asymmetrically divide into mature cells. Injected rhGAA is absorbed by the liver as well as striated muscle tissue (both heart and skeletal muscle). b Percentage of normal cardiac tissue within a heart of a Pompe diseased infant after one year of biweekly ERT at 20 mg/kg. Percentages of normal skeletal muscle tissue and hepatocytes are in Supplementary Fig. 9. c Schematic for in silico gene editing model for previously published precise correction of a single base pair in mouse disease models, d Flowchart for training and validating the GETEM model using previously published studies of in vivo somatic cell genome editing of a mouse liver by intravenous injection. e (top) Absolute change of edited cells over time predicted by the GETEM for the studies utilized in (d). For studies that employed selection in the liver, arrows indicate the time at which the selective pressure favoring edited cells was applied (for Fah−/− models, this indicates the removal of NTBC supplementation in the diet; and, for Otc editing, this indicates the induction of a high protein diet.). f Validation of the model using additional published editing strategies. Arrows on days 21 and 24 indicate removal of selection pressure on the treated mice as per published experimental protocol. Arrow on day 2 in the mRNA LNP indicates redosing of the LNP. (n represents biological replicates as previously reported in the literature, mean ± s.d). g Percentage of total sequencing reads from primary fibroblasts of Pompe diseased patients that were treated with S1mplexes targeting W746X D645N or R660H GAA mutations. Results indicate gene correction and imprecise editing for three different mutations. h Percentage of edited alleles that are precisely edited in (g).

185. Gene Correction via CRISPR and Others

(Wisconsin Alumni Research Foundation)

Key Data

In vivo somatic cell gene correction strategies involve tradeoffs between efficiency, precision, progenitor affinity, and editor stability



To be continued

185. Gene Correction via CRISPR and Others

(Wisconsin Alumni Research Foundation)

Key Data

In vivo somatic cell gene correction strategies involve tradeoffs between efficiency, precision, progenitor affinity, and editor stability

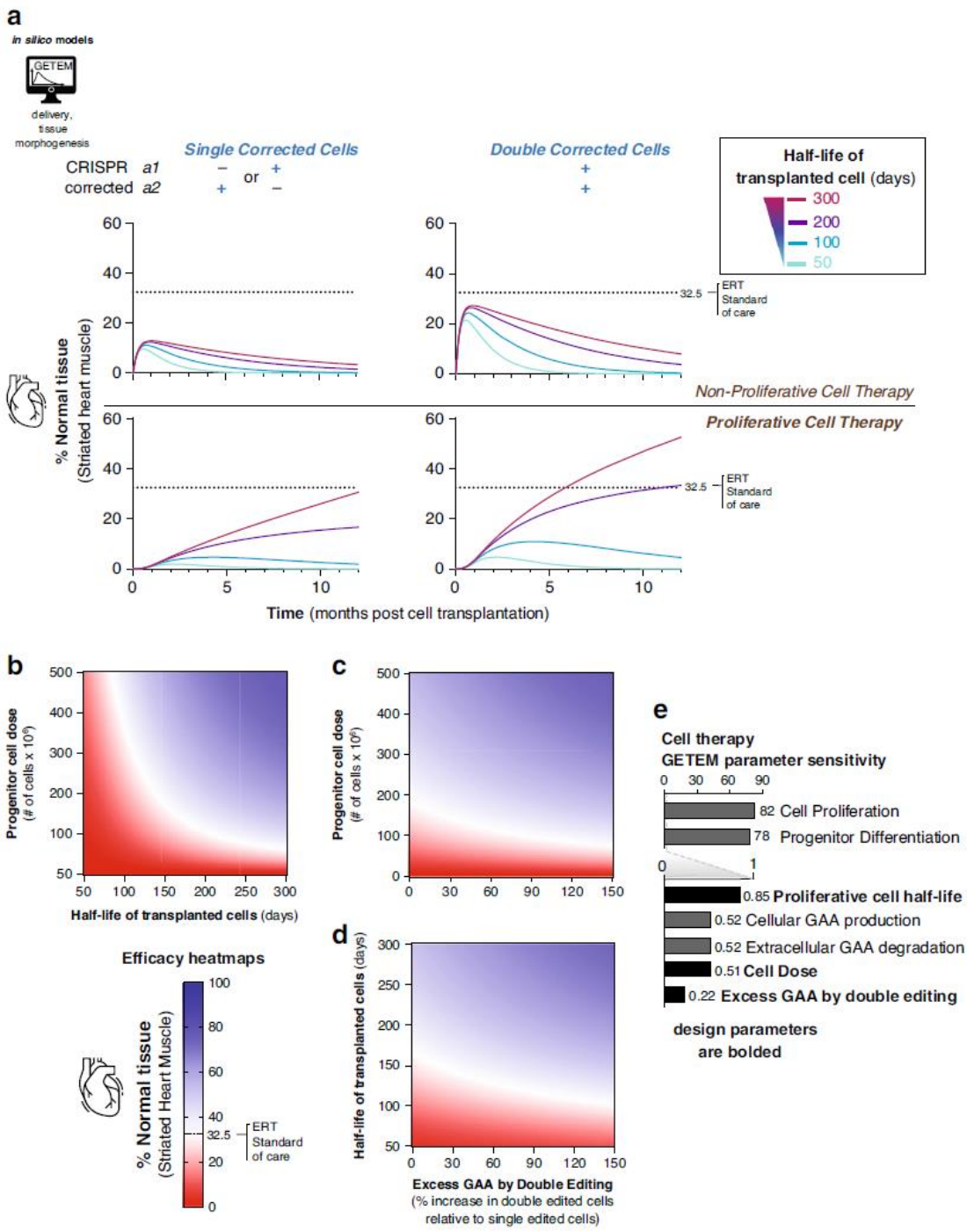
a In silico Gene Therapy Efficacy Model (GETEM) for Pompe disease correction in a developing infant. Schematic showing gene correction for two diseased alleles in a liver indicating correction of alleles, a1 and a2, by genome editors 1 and 2 to form gene-corrected cells capable of secreting GAA to enzymatically correct other unedited cells. Secreted GAA is also absorbed by striated muscle tissue (both heart and skeletal muscle). b Percentage of normal cardiac tissue within a developing heart of a Pompe diseased infant after the administration of six doses of genome editors at 23.9 mg/kg. c Cell numbers indicating growth of diseased, normal, and precisely-edited cells in the gene-edited liver depot after the administration of 6 doses of genome editors at 23.9 mg/kg. d Distribution of genotypes in the gene-edited liver depot after the administration of 6 doses of genome editors at 23.9 mg/kg. e Sensitivity analysis of the model indicating the absolute values of parameter sensitivity of tissue morphogenesis factors, genome editor factors, and cell/tissue biology intrinsic factors. f Tradeoff between genome editor efficiency and genome editor stability, focusing on the percentage of enzymatically cross-corrected heart tissue. Heatmap indicates that lower efficiency editors could be efficacious if the extracellular editor stability increases. g Tradeoff between genome editor efficiency and precision, focusing on the percentage of enzymatically cross-corrected heart tissue. Heatmap indicates that lower efficiency editors can be efficacious if higher precision editors are used. h Tradeoff between increasing genome editor dose and progenitor affinity, focusing on the percentage of enzymatically cross-corrected heart tissue. i Using GETEM, heatmap indicating tradeoff in heart muscle correction in the developing infant between the degradation rates of GAA in the serum and cellular GAA, indicating that stabilization of GAA in the serum can improve clinical outcome (gray arrowhead indicates pre-stabilization, black arrowhead indicates post-stabilization).

185. Gene Correction via CRISPR and Others

(Wisconsin Alumni Research Foundation)

Key Data

Durable gene-corrected cell therapy for Pompe disease requires persistent proliferative cells



To be continued

Key Data

Durable gene-corrected cell therapy for Pompe disease requires persistent proliferative cells

a Plots showing the degree of phenotypically normal cardiac tissue when either 10 billion non-proliferative single- or double-corrected cells are dosed or 0.1 billion proliferative single- or double-corrected cells are dosed. For these plots, the GAA production for double-corrected cells is 150% excess that of single corrected cells for four different half-lives of dosed cells. For long-lasting correction, proliferative progenitor cells need to have a half-life exceeding 100 days. b Heatmap showing the proliferative cell dose (assuming double-corrected cells with 75% excess GAA production compared to single-corrected cells) against the half-life of dosed cells. If the dosed cells have a half-life similar to endogenous hepatocytes, a 75 million cell dose is sufficient for matching ERT. c Heatmap showing the proliferative cell dose (assuming that the dosed cells have 250-day half-life) against the GAA production rate of dosed cells, demonstrating that dose has a higher effect on therapeutic efficacy than GAA production gained from double correction. d Heatmap showing that the GAA production against the half-life of the dosed cells (assuming that 250 million cells were dosed), indicating that the half-life of the dosed cells has a higher effect on therapeutic efficacy than the GAA production gained from double correction. e Sensitivity analysis for model parameters relevant to cell therapy. In addition to growth and differentiation rates of the transplanted cells, outcomes are highly sensitive to the proliferative cell half-life, cell dose and excess GAA produced by double gene correction. These later parameters, relevant to the design of therapy with autologous gene-corrected cells, are bolded.