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Review Article

CRISPR-Based Correction of Monogenic Disease in Patient-Derived Induced Pluripotent Stem Cells

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Abstract

Background: Isogenic pairs anchor monogenic disease modelling in induced pluripotent stem cells, and CRISPR correction has made them routine across the monogenic disease spectrum.

Objective: To reassess where the residual risk in generating corrected isogenic pairs now lies, given that correction efficiency is no longer the limiting obstacle.

Methods: Narrative review of gene editing studies, clone screening reports and early clinical applications.

Findings: Templated correction in human induced pluripotent stem cells ran at 1.0%, whereas prime editing reached 4.3-24.8% in human embryonic stem cells. Large on-target lesions appeared in 33% of 27 edited clones and in up to 40% of edited clones, all passing standard PCR and Sanger checks; copy-neutral loss of heterozygosity occurred in 7 of 648 clones.

Conclusions: The evidence supports treating on-target structural damage, not efficiency, as the main threat to isogenic validity; it does not show that amplicon sequencing alone validates a corrected pair.

Keywords: Induced pluripotent stem cells; Genome editing; Isogenic controls; Loss of heterozygosity; Monogenic disease

Introduction

The isogenic pair is the central experimental object of monogenic disease modelling in induced pluripotent stem cells. A patient line carries the causal allele on a genetic background that also carries several million other variants, and the only way to attribute a cellular phenotype to the allele rather than to the background is to correct the allele and compare the corrected clone with its parent. The logic was established before CRISPR, with zinc-finger nucleases used to generate pluripotent lines differing exclusively at two early-onset Parkinson point mutations [1], and with targeted correction of alpha-1 antitrypsin deficiency in patient iPSCs [2]. It is quantitatively justified: across 711 iPSC lines derived from 301 healthy donors in the HipSci collection, donor identity accounted for 5-46% of the variance in phenotypes including differentiation capacity and cellular morphology [3]. Between-donor comparisons of unrelated patient and control lines are therefore uninterpretable at the effect sizes most disease models report.

CRISPR-Cas9 made the correction step tractable, and correction has now been demonstrated across the monogenic disease spectrum, including Huntington disease [4], Duchenne muscular dystrophy [5], and long-QT syndrome [6]. The literature that followed concentrated its methodological anxiety on two questions: how to raise homology-directed repair efficiency in a cell type that repairs badly, and how to bound off-target cleavage elsewhere in the genome. Both concerns were reasonable and both have been substantially addressed.

This review argues that the residual risk has moved. Correction efficiency was the early obstacle and is now the lesser one; the harder problem is unintended damage at the on-target site itself — large deletions, loss of heterozygosity, and terminal chromosomal truncation — which the PCR-and-Sanger workflow used to validate almost every published isogenic pair cannot detect, and

which appears in a third or more of edited clones in the studies designed to look for it. A second, related pressure comes from p53: the same double-strand break that drives correction selects against cells with an intact damage response. The isogenic pair is only as good as the screening that produced it.

Efficiency: the obstacle that receded

Homology-directed repair in pluripotent cells is poor, and the numbers make clear how much of the reported variation is cell-type rather than reagent. Using identical Cas9 ribonucleoprotein reagents, allelic repair from a donor template reached approximately 9% in unsynchronised HEK293T at 30 pmol, rose to about 20% with nocodazole arrest, and reached 38% in HEK293T after sequential cell-cycle synchronisation at 100 pmol; the same optimised protocol delivered approximately 2% in H9 human embryonic stem cells and 0.6-1.3% in primary neonatal fibroblasts [7]. Even under optimal timing, the maximum ratio of HDR to total editing was around 33%, so two-thirds of breaks still resolved through end joining. In human iPSCs, a direct comparison at matched loci gave 1.0% HDR against 3% NHEJ with Cas9 and an ssODN donor, against 0.6% HDR and 0.4% NHEJ for a comparator reTALEN [8]. Larger interventions are not uniformly worse: FACS-scored multi-kilobase replacement at THY1 in human iPSCs reached 11.6% homozygous and 14.6% heterozygous, with homozygous deletion efficiency of 24% for a 2.7 kb deletion and 8% for an 86 kb deletion [9].

Against these population rates sits the clone-level reality. Correction of a SAMHD1 nonsense mutation in patient iPSCs by Cas9 nuclease plus donor returned zero correctly edited clones from 72 single clones screened [10]. That outcome is not an efficiency percentage; it is the distribution's left tail, and it is what a laboratory actually experiences.

Knockout, by contrast, was never the bottleneck. Average indel efficiency exceeded 80% in human pluripotent stem

cell lines using stably integrated Cas9 or transient Cas9 RNP [11], and RNP delivery achieves site-specific mutation frequencies up to 79% in human cells including pluripotent stem cells while yielding at least twofold more hESC colonies than plasmid transfection [12]. The gap between >80% indel formation and ~1% templated correction in the same cell class defines the problem that a decade of protocol work — donor design, synchronisation, transient selection schemes that recover edited clones within about two weeks across five hPSC lines without leaving a cassette behind [13], and allele-specific design strategies for homozygous versus heterozygous outcomes [14] — was built to close.

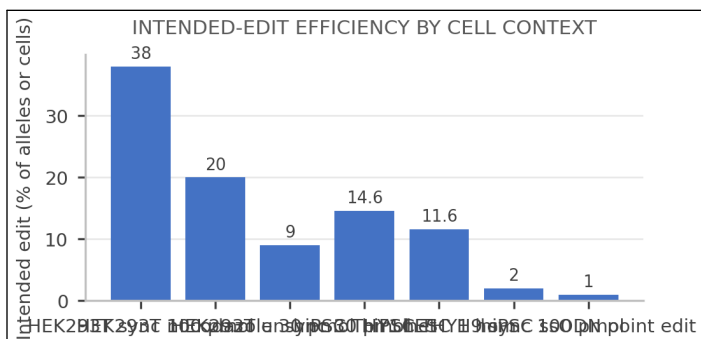


Figure 1: Intended-edit rates fall as cells become less transformed and more pluripotent, from 38% in synchronised HEK293T to 2% in H9 hESCs with identical Cas9 RNP reagents and 0.6-1.3% in primary neonatal fibroblasts [7], with hiPSC values of 14.6% heterozygous and 11.6% homozygous multi-kilobase THY1 replacement scored by FACS [9] and 1.0% for an ssODN point edit [8]; the replacement and point-edit values are not directly comparable and are shown together only to bracket the achievable range, and a patient hiPSC SAMHD1 correction returned 0 of 72 clones screened [10].

Editing chemistries that avoid the double-strand break

Base and prime editing changed the arithmetic by removing the requirement for a double-strand break and a donor. Cytosine base editing with BE3 converts 15-75% of total cellular DNA at the target base with typically at most 1% indel formation, measured in four transformed human and murine cell lines [15]. Adenine base editing with ABE7.10 converts A-T to G-C at approximately 50% efficiency in human cells with product purity typically at least 99.9% and indels typically no more than 0.1% [16].

Prime editing extended the range further, with more than 175 distinct edits spanning all twelve transition and transversion classes plus insertions and deletions in four human cell lines and post-mitotic mouse neurons [17].

Table 1: Intended-edit efficiency, indel byproduct and large-deletion burden by editing chemistry, with the cell context of each measurement stated because no single study compares all three chemistries in one patient iPSC line at one locus [8,7,10,15,16,18,19,26].

Chemistry	Intended edit (cell context)	Indel byproduct	Large deletions
Cas9 + ssODN	1.0% (hiPSC)	3% NHEJ same locus	reference
Cas9 RNP, timed	38% (HEK293T); 2% (H9 hESC)	~67% of breaks	reference
Cas9 + donor, patient	0 of 72 clones (hiPSC)	--	reference
Cytosine base editor	15-75% (4 transformed lines)	typically <=1%	~20-fold below Cas9
Adenine base editor	~50%, purity >=99.9% (human cells)	typically <=0.1%	~20-fold below Cas9
Prime editing PE3	4.3-24.8% (hESC); 6.5% reporter (hiPSC)	2.7-8.1%	~20-fold below Cas9

Those figures come from transformed lines and must not be carried into a pluripotent context unmodified. In human embryonic stem cells, PE3 installed intended edits at 4.3-24.8% with unwanted indels at 2.7-8.1%; across PE3 conditions indel frequencies ran 2-13%, against 1-5% for base editors in the same study, and deletions up to 80 bp and insertions up to 42 bp were installed precisely [18]. In human iPSCs, adenine base editor mRNA converted up to 80% of a GFP reporter to BFP, base-editing rates across TP53 sgRNAs ranged from 1% to 90%, and a matched target gave 52.4% for ABE against 8.8% for CBE, while prime editing of the same reporter reached only about 6.5% [10]. The clone-level accounting in that study is the more instructive figure: of 21 subclones from an ABE correction of a patient iPSC line, 5 (24%) died during expansion, 6 (29%) were unmodified, and 4 (19%) were fully corrected as intended. Architecture improvements continue to move the population rates — transient MLH1dn co-expression in PE4/PE5 raised prime editing

efficiency an average 7.7-fold over PE2 and 2.0-fold over PE3 and improved the edit-to-indel ratio 3.4-fold in mismatch-repair-proficient cells, with PEmax adding 2.8-fold on average in HeLa, across 191 edits in 7 mammalian cell types [19].

Nicking chemistries also improve precision at the cut. In HEK293T and fibroblasts, Cas9 nuclease gave HDR of $21 \pm 2.4\%$ against 60.7% indels, an HDR-to-indel ratio of 0.53, whereas the paired nickase gave $2.55 \pm 0.73\%$ initially and 18% after optimisation with only 0.2-0.7% indels, a ratio of 19 [20]. Efficiency, in short, is now a design variable rather than a barrier.

Unintended damage at the on-target site

The events that matter are the ones the standard workflow does not see. In human iPSCs, 33% of 27 clones edited across 9 targeted loci had acquired large on-target genomic defects, including insertions and loss of heterozygosity, and all of them had passed standard PCR and Sanger sequencing [21]. A second study reported large mono-allelic deletions and loss of heterozygosity escaping standard PCR and Sanger quality control in up to 40% of edited human stem cell clones, after both HDR-based insertion and NHEJ editing, detectable only by quantitative genotyping PCR and SNP genotyping [22]. The apparently clean homozygous corrected clone is, in a substantial fraction of cases, hemizygous.

The lesions extend well beyond the amplicon. Of 10 single HEK293T clones transfected with Cas9 nuclease, 3 had lost a distal FISH probe, and array-CGH mapped a 7.5 Mb terminal loss downstream of the UROS cut site; cells bearing a truncation signal rose to 22.6% with nuclease alone and 20.1% with nuclease plus ssODN, and TP53-null fibroblasts showed up to a 10-fold higher truncation risk than wild-type fibroblasts [20]. In primary human haematopoietic stem and progenitor cells edited at the globin locus with indel rates of 86% and 88%, copy-neutral loss of heterozygosity appeared in 7 of 648 clones (1.1%), across an interval running 5.2 Mb from the cut site to the

telomere and containing 167 genes; in HEK293T, 2 of 27 LOH clones (7.4%) retained a copy-neutral signal [23]. Copy-neutral LOH is invisible to copy-number assays as well as to sequencing of the target amplicon.

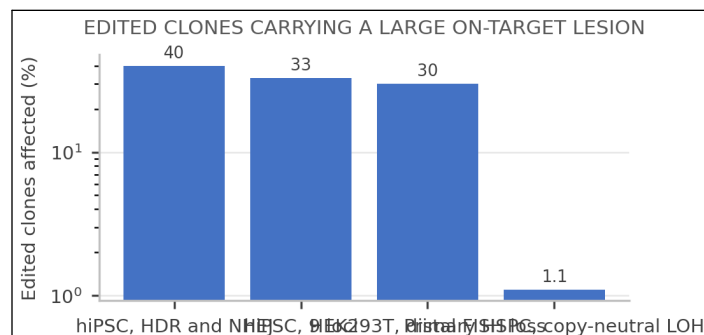


Figure 2: Large on-target lesions in edited clones, reported as up to 40% of human stem cell clones after HDR and NHEJ editing [22], 33% of 27 hiPSC clones across 9 loci [21], 3 of 10 HEK293T clones losing a distal FISH probe [20], and 7 of 648 primary HSPC clones with copy-neutral LOH [23]; the assays differ in sensitivity as well as in biology, so the ordering is not a ranking of risk.

The asymmetry between the two failure modes deserves emphasis. Off-target cleavage is dispersed across the genome, generally sub-genic in extent, and can be enumerated by unbiased genome-wide assays before a clone is ever picked. On-target structural damage is concentrated at precisely the locus under study, can span megabases, and is systematically hidden by the assay used to declare the edit successful. For a disease model, a hemizygous deletion of the gene being interrogated is not a background nuisance; it is a confound aligned with the hypothesis.

Kilobase-scale deletions, lesions distal to the cut, and crossover events after Cas9 repair have been described in mouse and human cells using long-range PCR and long-read approaches [24], and single-cell whole-genome sequencing has established chromothripsis as an on-target consequence of Cas9-induced breaks arising through micronuclei and chromosome bridges [25]. Nickase-based chemistries reduce this burden without abolishing it: base editors and prime editors still generate deletions greater than 100 bp, at approximately 20-fold lower frequency than Cas9 nuclease, measured in cancer

cell lines, human embryonic stem cells and human primary T cells [26].

p53 as a selection filter on the surviving clones

The double-strand break also decides which cells survive to be screened. The toxic response to Cas9-induced breaks in human pluripotent stem cells is P53-dependent, and precise engineering efficiency is severely reduced in P53-wild-type hPSCs [11]; Cas9 editing induces a p53-mediated DNA damage response whose corollary is that the cells tolerating editing best are those with a compromised pathway [27]. That corollary has been observed directly: expression profiling of 165 paired human cancer cell lines and their Cas9-expressing derivatives showed p53 pathway upregulation specifically in TP53-wild-type lines, and genetic characterisation of 42 line pairs showed the emergence and expansion of p53-inactivating mutations, with Cas9 less active in TP53-wild-type than TP53-mutant lines [28].

Pluripotent lines bring standing variation into this selection. Exome sequencing of 140 independent hESC lines, 26 of them prepared for potential clinical use, found 5 unrelated lines carrying 6 dominant-negative TP53 mutations; RNA-seq from a further 117 hPSC lines yielded 9 more TP53 coding mutations, all in the DNA-binding domain, with mutant allelic fraction above 50% in 3 lines and rising with passage number [29]. Editing imposes a selective pressure on top of that pre-existing pool, and the p53 status of a corrected clone is a property of the correction, not of the patient.

Screening that would actually detect the damage

None of these events is invisible; they are simply invisible to the assay in routine use. Amplicon PCR and Sanger sequencing confirm the base at the target position and report a heterozygous deletion of one whole allele as a homozygous edit. Detecting the difference requires assays addressed to allele dosage and haplotype: quantitative genotyping PCR and SNP genotyping across the locus [22], copy-number and structural analysis in the megabase

neighbourhood [21], FISH with distal probes and array-CGH for terminal loss [20], and SNP-array-based detection of copy-neutral LOH extending to the telomere [23]. Long-range amplification is what exposed the kilobase-scale rearrangements in the first place [24]. Off-target profiling by GUIDE-seq, validated across 13 RNA-guided nucleases in 2 human cell lines [30], answers a different and largely orthogonal question and does not substitute for on-target characterisation. Published guidance on reproducibility in iPSC-derived disease models argues for exactly this kind of explicit quality control, alongside multiple independent clones per genotype [31]. A frank gap remains: no primary study reports a clean karyotype-abnormality rate among CRISPR-edited iPSC clones against unedited controls from the same parental line.

Table 2: Reported on-target damage by study, the assay that revealed it, and the detection rate among edited clones [21,22,20,23,26].			
Study	Cells	Assay	Rate
[21]	hiPSC, 9 loci	copy number, LOH genotyping	33% of 27 clones
[22]	human stem cells	quantitative genotyping PCR, SNP array	up to 40%
[20]	HEK293T, fibroblast	distal FISH, array-CGH	3 of 10 clones
[23]	primary HSPC	SNP array, copy-neutral LOH	7 of 648 clones
[26]	hESC, T cells, lines	long deletion sequencing	~20-fold below Cas9

Distance from a corrected dish to a therapy

Corrected iPSCs are a model system, and the therapeutic milestones so far have come from other cell types. Ex vivo editing of the BCL11A enhancer modified approximately 80% of alleles in the CD34+ product, and two patients treated after myeloablation showed high-level allelic editing, pancellular fetal haemoglobin induction, transfusion independence and elimination of vaso-occlusive episodes beyond one year [32] — a somatic knockdown strategy, not a corrected pluripotent graft. Ex vivo correction of CFTR F508del in upper airway basal

stem cells from 10 cystic fibrosis patients reached 30-50% allelic correction and restored 20-50% of non-CF CFTR function in differentiated epithelia [33]. Those are the benchmarks a corrected iPSC product would have to meet, with clonal genomic integrity added to the list.

Conclusion

The efficiency literature has done its work: templated correction in human pluripotent cells that once ran at 1.0% [8] is now addressable by chemistries reaching 4.3-24.8% in hESCs [18] with roughly 20-fold fewer large deletions [26]. What has not kept pace is validation. Two independent studies found large on-target lesions in 33% of 27 iPSC clones [21] and up to 40% of edited clones [22], every one of which had passed the standard checks. An isogenic pair validated only by amplicon sequencing carries an untested assumption of one-third failure, and any phenotypic difference it reports may reflect a megabase deletion, a copy-neutral LOH tract, or a p53-compromised clone rather than the corrected allele. The remedy is procedural rather than technical: allele-dosage and structural screening at the locus, TP53 status reported for both members of the pair, and more than one independent corrected clone before a phenotype is attributed to a gene.

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