The Lumbering Crawl Toward Human Germline Editing

Eli Y. Adashi and I. Glenn Cohen

It is in the nature of novelty that consensus is hard to come by. Such is clearly the lot of groundbreaking biomedical advances. History is no stranger to this phenomenon. The prospect of human germline editing is no exception. A fine illustration as any of this state of affairs is offered by Professor Drabiak in this issue of the *Journal of Law, Medicine, & Ethics*. Squarely on the side of the skeptics, Professor Drabiak carefully lays out the relative shortcomings of the technology in question. Our main focus is to complement Professor Drabiak’s extensive account by highlighting the present state of the science of human germline editing. No judgement is being passed on the notion of human germline editing nor on the potential application thereof — we have discussed our own views in depth elsewhere.

- The mixing of Mitochondrial Replacement Therapy (MRT) and germline gene editing. Throughout the article, Professor Drabiak moves back and forth between policy treatment, concerns, and news events regarding MRT and those pertaining to gene editing. While the two technologies have much in common and some would label “MRT” as “gene editing,” the concerns pertaining to the latter often do not affect the former. This is particularly true if, as recommended by the National Academies, but not acknowledged by Professor Drabiak in her account, one were to limit MRT to male-only embryos thus avoiding transmission of heritable changes.
- The effectiveness of the Congressional Rider. Professor Drabiak briefly mentions the appropriations rider, initially put in place in 2015 and renewed annually since, that prohibits FDA from considering applications “for an exemption for investigational use … in research in which a human embryo is intentionally created or modified to include a heritable genetic modification.” The FDA has interpreted the rider to bar the approval MRT, even for male-only embryos. Because of the rider, any attempt to use gene editing or MRT in the U.S. would violate the Food, Drug, and Cosmetics Act and subject the party to legal liability. It is unclear what additional measures Professor Drabiak, who is clearly against these technologies, could seek, or why this means that the U.S., in her view, compares unfavorably to peer nations?
- The legality of medical tourism from the U.S. for MRT or gene editing. In her discussion of the case of Dr. Zhang, Professor Drabiak writes that “despite the FDA asserting jurisdiction over MRT and genome editing, physicians may circumvent FDA regulations and unilaterally integrate experimental techniques modifying the human germline into clinical practice.” The answer, though, depends on the legality of the technique in the destination country, as well as on what elements of the process occur intra-

---

*Eli Y. Adashi, M.D., M.S., is Professor of Medical Science at Warren Alpert Medical School, Brown University in Providence. R. I. Glenn Cohen J.D., is James A. Altwood and Leslie Williams Professor of Law at Harvard Law School and Faculty Director of the Petrie-Flom Center for Health Law Policy, Biotechnology, and Bioethics at Harvard University in Cambridge, MA.*
versus extra territorially and the physician’s home country’s own assertions of jurisdiction.\textsuperscript{9} While Professor Drabiak acknowledges the FDA cease and desist letter issued to Dr. Zhang, it is not clear why she concludes it is not effective, as it appears to us to have been.\textsuperscript{10}

On April 18, 2015, on the pages of \textit{Protein & Cell}, Liang et al. reported on the first ever attempt to edit the genome of the human embryo.\textsuperscript{11} Seeking to inactivate the wild-type \textsuperscript{-}Globin gene by applying the CRISPR/Cas9 endonuclease, the authors strove to generate a targeted Double-Strand Break (DSB) in the genome of triploid nuclear (inviable) zygotes.\textsuperscript{12} Amongst the outcomes noted, a low (14.5\%) measure of editing efficiency stood out.\textsuperscript{13} Modest editing specificity was similarly apparent due to on- and off-site insertions/deletions (indels).\textsuperscript{14} Limited editing uniformity was also in evidence due out.\textsuperscript{18} In the first set of experiments, intact MII oocytes were fertilized by co-incubation with mutant sperm, edited 18h later, and the results analyzed in day-3 cleavage-stage embryos.\textsuperscript{19} In the second set of experiments, intact MII oocytes were ICSI (Intracytoplasmic Sperm Injection)-fertilized as well as edited and the results analyzed in day-3 cleavage-stage embryos.\textsuperscript{20} Editing of zygotes 18h post-fertilization increased the representation of non-mosaic wild-type homozygosity by 41\%.\textsuperscript{21} The mosaicism rate, however, stood at 24\%.\textsuperscript{22} Editing of zygotes at the time of fertilization increased the prevalence of non-mosaic wild-type homozygosity by 53\% while reducing the mosaicism rate to 2.4\%.\textsuperscript{23} A modicum of on-target indels was noted for both experimental protocols.\textsuperscript{24} Taken together, these observations suggest that zygotic editing at the time of fertilization enhances editing efficiency and uniformity if not specificity and that further optimization will be

\textbf{In 1963, Joshua Lederberg, PhD, one of the finest scientific minds of his time and a Nobel awardee for the discovery of bacterial conjugation, suggested that “we might anticipate the in vitro culture of germ cells and the direct control of nucleotide sequences in human chromosomes, coupled with recognition, selection and integration of the desired genes...” In hindsight, we can only assume that Professor Lederberg conjectured a functionality that we might refer to as editing the human germline. Fifty-five years later, that functionality is no longer in the realm of postulation. Actualization however, is a whole other matter.}

to a significant measure of mosaicism.\textsuperscript{15} The modest nature of the results notwithstanding, this proof-of-principle undertaking was nevertheless highly cited by dint of sheer novelty. Inclusion in “Nature’s 10” listing for the year followed suit. In the aftermath, four additional communications explored the feasibility of DSB-dependent editing of the human embryo. The utility of DSB-independent editing was explored as well before too long.

It was not until August 24, 2017, however, on the pages of \textit{Nature}, that a more substantive effort to assess the feasibility of human germline editing was undertaken by Ma et al. of the Oregon Health & Science University.\textsuperscript{16} The disease target selected, Familial Hypertrophic Cardiomyopathy (FHC), an autosomal dominant malady, was attributable to a mutant Sarcomere Gene.\textsuperscript{7} Incurable if amenable to symptomatic care, FHC all too often culminates in sudden cardiac death.\textsuperscript{17} Two distinct sets of experiments were carried required if clinical application is to be entertained. These and previous observations bring one to an important fork in the proverbial road leading to mutation-free embryo transfers. One road will likely comprise selection via Preimplantation Genetic Diagnosis (PGD) in day-3 cleavage-stage embryos. The other road, however, might entail universal zygotic editing at the time of fertilization. These two technologies could not be more different. Selection via PGD is, of course, purely diagnostic in nature in that editing the multicellular embryo is infeasible at this time. Zygotic editing, on the other hand, is entirely restorative in nature. Neither approach is free of relative shortcomings. Selection via PGD has been found to display a significant falloff in the number of embryos available for transfer.\textsuperscript{25} When screening for dominant disorders by PGD, 30\% of egg retrievals failed to reach the embryo transfer stage.\textsuperscript{26} The corresponding figure for recessive disorders was 20\%.\textsuperscript{27} This reality is further driven
home in the PGD-dependent selection of savoir-sib-
lings wherein advanced maternal age may be associ-
ated with a retrieval to transfer falloff exceeding 90%.
These observations suggest that PGD, while broadly
applicable to the selection of mutation-free embryos,
might be constrained by circumstances characterized
by rate-limited pools of selectable embryos. PGD is
also of limited utility in the context of rare parental
cell lineages which give rise to universal embryonic
affliction. Zygotic editing, on the other hand, may
be uniquely suited for rate-limiting complements of
embryo populations. Herein however, both mutant
and intact zygotes are exposed to the editing mix, a
reality the impact of which remains to be further
evaluated. Technologies to validate editing rigor prior
to embryo transfer have yet to materialize as well.
Looking ahead, consideration might also be given to
a third road to mutation-free embryo transfers, that
is, the editing of gametes or else of stem cells destined
to become gametes. Progress along these lines would
facilitate the validation of editing rigor as well as elimi-
nate the prospect of embryonic mosaicism.

In 1963, Joshua Lederberg, PhD, one of the finest
scientific minds of his time and a Nobel awardee
for the discovery of bacterial conjugation, suggested
that “we might anticipate the in vitro culture of germ
cells and the direct control of nucleotide sequences in
human chromosomes, coupled with recognition, selection
and integration of the desired genes...”28 In
hindsight, we can only assume that Professor Leder-
berg conjectured a functionality that we might refer to
as editing the human germline. Fifty-five years later,
that functionality is no longer in the realm of postula-
tion. Actualization however, is a whole other matter.

Reference
1. K. Drabiak, “Untangling the Promise of Human Genome
Editing,” Journal of Law, Medicine and Ethics 46, no. 4
Replacement as a Guide to Genome Editing,” Cell 164, no.5
3. Drabiak, supra note 1.
Path Forward for Mitochondrial Replacement,” Science 351,
5. I.G. Cohen and E.Y. Adashi, “The FDA is Prohibited from
6. Id.
7. Drabiak, supra note 1.
8. Id.
Edge Medicine: The Case of Mitochondrial Replacement
Therapy,” Indiana Journal of Global Legal Studies 25, no. 439
10. Drabiak, supra note 1.
11. P. Liang et al., “CRISPR-Cas9-Mediated Gene Editing in
Human Tripronuclear Zygotes,” Protein & Cell 6, no.5 (2015):
363-372.
12. Id.
13. Id.
14. Id.
15. Id.
16. H. Ma et al., “Correction of a Pathogenic Gene Mutation in
17. Id.
18. Id.
19. Id.
20. Id.
21. Id.
22. Id.
23. Id.
24. Id.
25. J. Steffann et al., “Could Failure in Preimplantation Genetic
Diagnosis Justify Editing the Human Embryo Genome?” Cell
26. Id.
27. Id.