Genome Editing: Pathways to Translation

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Member, Organizing Committee
2nd International Summit on Human Genome Editing
November 28, 2018
Hong Kong

Statement of Organizing Committee 2015

- Basic and clinical **research**:
  - Endorsed basic and preclinical research on gene editing, including in human embryos and germ cells, subject to legal and ethical oversight:
  - "If, in the process of research, early human embryos or germline cells undergo gene editing, the modified cells should not be used to establish a pregnancy."
- Clinical **use**:
  - Somatic: Clinical translation can proceed under existing regulatory frameworks;
  - Germline: Editing poses risks, including inaccurate editing and difficulty predicting harmful effects; **"It would be irresponsible to proceed with any clinical use …"**

Genome Editing Clinical Trials (ZFNs)

Sponsor	NCT Number(s)	Year	Country	Disease	Gene Target	Nuclease	Ex vivo vs In vivo	Delivery	Pre-Clinical Publication	Clinical Publication
Penn/ Sangamo	00842634	2009	USA	HIV	CCR5	ZFN	Ex Vivo (T-Cell)	Electroporation (mRNA)	Perez et al NBT (2005)	Tebas et al NEJM (2014)
UCLA/UCSF Sangamo	01252641 01044654	2010	USA	HIV	CCR5	ZFN	Ex Vivo (T-cell)	Electroporation (mRNA)	Perez et al NBT (2005)	
City of Hope/ Sangamo	02500849	2015	USA	HIV	CCR5	ZFN	Ex Vivo (HSPC)	Electroporation (mRNA)	Digiusto et al MTMCD (2016) Holt et al NBT (2010)	
Sangamo	02695160 02702115 03041324	2016-2017	USA	Hem B MPS-I MPS-II	Albumin	ZFN	In vivo (liver)	AAV	Sharma et al Blood (2015)	
Bioverativ/ Sangamo	03653247	2018	USA	Sickle Cell Disease	BCL11A (erythroid enhancer)	ZFN	Ex Vivo (HSPC)	Electroporation (mRNA)	No	
Sangamo/ Bioverativ	03432364	2018	USA	β-Thal	BCL11A (erythroid enhancer)	ZFN	Ex Vivo (HSPC)	Electroporation (mRNA)	No	

Genome Editing Clinical Trials (TALENs)

Sponsor	NCT Number(s)	Year	Country	Disease	Gene Target	Nuclease	Ex vivo vs In vivo	Delivery	Pre-Clinical Publication	Clinical Publication
Servier/ Allogene	02808442 (peds) 02746952 (adult)	2016	USA Europe	ALL	TCR CD52	TALEN	Ex Vivo (CART-19)	mRNA	Poirot et al CCR (2015)	Qasim et al STM (2016)
Cellectis	03203369	2017	USA	BPDCN	TCR CD52	TALEN	Ex Vivo (CART-123)	mRNA		
Cellectis	03190278	2017	USA	AML	TCR CD52	TALEN	Ex Vivo (CART-123)	mRNA		
Sun Yat-sen	03057912	2017	China	HPV	HPV E6/E7	TALEN or Cas9/gRNA	In vivo	Plasmid/Gel		
Huazhong University of Science and Technology	03226470	2017	China	HPV	HPV E6/E7	TALEN	In vivo	Plasmid/ Polymer Gel	Hu et al JCI (2015)	

Genome Editing Clinical Trials (CRISPR/Cas9)

Sponsor	NCT Number(s)	Year	Country	Disease	Gene Target	Nuclease	Ex vivo vs In vivo	Delivery	Pre-Clinical Publication	Clinical Publication
Peking University (Cell Biotech)	02863913 02867345 02867332	2016	China	Bladder Prostate Renal Cell	PD-1	Cas9/gRNA	Ex Vivo (T-cells)			
Affiliated Hospital to Academy of Military Medical Sciences	03164135	2017	China	HIV	CCR5	Cas9/gRNA	Ex Vivo (HSPC)			
Chinese PLA General Hospital	03166878	2017	China	ALL/ Lymphoma	TCR B2M	Cas9/gRNA	Ex Vivo (CART-19)			
Shanghai Bioray Laboratory Inc.	03229876	2017	China	ALL	TCR HLA-1	Cas9/gRNA	Ex vivo (CART-19)			
UPenn/ Tmunity	03399448	2018	USA	Multiple Myeloma	TCRα TCRβ PD-1	Cas9/gRNA	Ex Vivo (CART-NY-ESO)	mRNA		
Chinese PLA General Hospital (Weidong Han)	03398967	2018	China	ALL/ Lymphoma	TCR	Cas9/gRNA	Ex Vivo (CART-19/22)			
CRISPR/Vertex	03655678	2018	Europe	β-Thal	BCL11A (erythroid enhancer)	Cas9/gRNA	Ex vivo (HSPC)	RNP		
CRISPR/Vertex		2018	USA	Sickle Cell Disease	BCL11A (erythroid enhancer)	Cas9/gRNA	Ex vivo (HSPC)	RNP		
Baylor	03690011	2018	USA	T-ALL	CD7	Cas9/gRNA	Ex Vivo (CART-CD7)			
Chinese PLA General Hospital	03545815	2018	China	Solid Tumors	TCR PD-1	Cas9/gRNA	Ex Vivo (CART-mesothelin)			

Lessons from trials of somatic genome editing

- Current mechanisms for trial oversight has ensured safety, rigor
  - Trade-offs: cumbersome, expensive, conservative, slow…

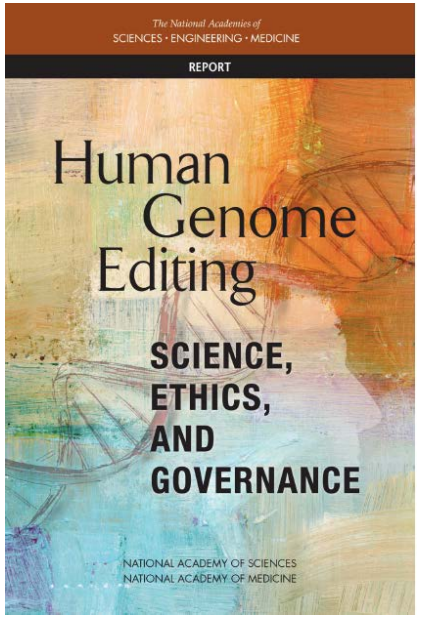
Statement of Organizing Committee 2015

- **“It would be irresponsible to proceed with any clinical use of germline editing unless and until…”**
  - Safety/ efficacy established after further pre-clinical research;
  - There is broad societal consensus about appropriateness of the application;
  - Only under appropriate regulatory oversight.
- “**At present, these criteria have not been met for any proposed clinical use…**
- **“As scientific knowledge advances and societal views evolve, the clinical use of germline editing should be revisited on a regular basis.”**
- Ongoing forum: “**The international community should strive to establish norms concerning acceptable uses of human germline editing** and to harmonize regulations in order to discourage unacceptable activities while advancing human health and welfare”

…Since 2015

- **May 2016 – International Society for Stem Cell Research**
- Guidelines Call for EMBRYO RESEARCH OVERSIGHT- EMRO
  - *Recommendation 2.1.1:* All research that (a) involves preimplantation stages of human development, human embryos, or embryo-derived cells or (b) entails the production of human gametes in vitro when such gametes are tested by fertilization or used for the creation of embryos shall be subject to review, approval, and ongoing monitoring by a specialized human embryo research oversight (EMRO) process capable of evaluating the unique aspects of the science.
- Rec 2.1.4: "Th**e ISSCR supports laboratory-based research** that entails modifying the nuclear genomes of gametes, zygotes and/or preimplantation human embryos, performed under a rigorous EMRO process…
- "Until further clarity emerges on both scientific and ethical fronts, the ISSCR holds that **any attempt to modify the nuclear genome of human embryos for the purpose of human reproduction is premature** and should be prohibited at this time."

…Since 2015



- **February 2017-- US NAS/NAM**

"Human Genome Editing: Science, Ethics, Governance"

  - "basic research involving both somatic and germline cells is essential to the advancement of science and should continue with existing regulatory structures."
  - "clinical trials of genome editing in somatic cells for the treatment or prevention of disease or disability should continue, subject to the ethical norms and regulatory frameworks that have been developed for existing somatic gene therapy research and clinical use"
  - "**Given both the technical and societal concerns, the committee concludes there is a need for caution in any move toward germline editing, but that caution does not mean prohibition. It recommends that germline editing research trials MIGHT be permitted, but only after much more research to meet appropriate risk/benefit standards for authorizing clinical trials."**

…Since 2015

- **July 2018- Nuffield Council on Bioethics**

—Genome Editing and Human Reproduction

- **”We can, indeed, envisage circumstances in which heritable genome editing interventions SHOULD be permitted.”**

>60+ Reports on Human Genome Editing (2015-2018)

Brokowski, C The CRISPR Journal 2018

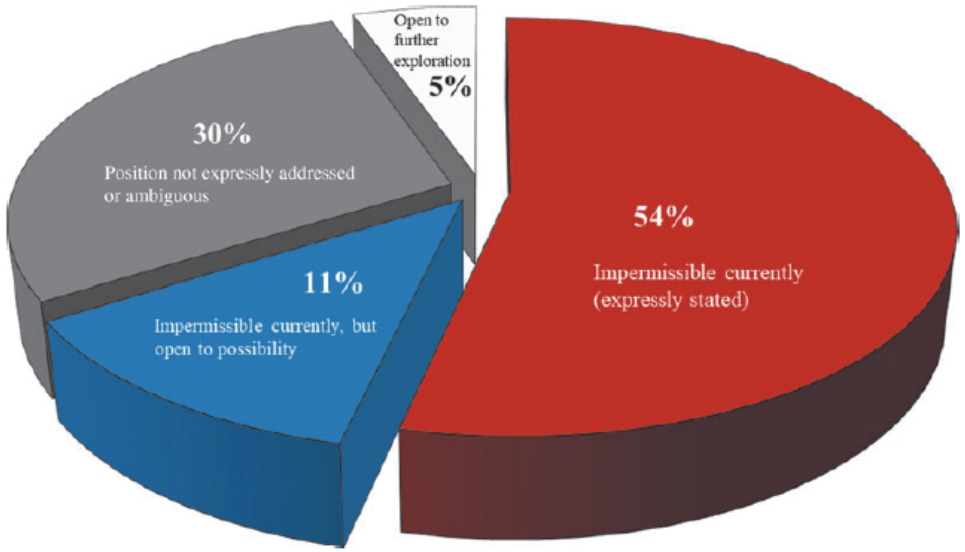


FIG. 3. Opinions on the moral permissibility of heritable genome editing. This pie chart displays the views of 61 ethics reports on germline editing. The views represented are not logically exhaustive. The majority (54%) expressly considered germline editing impermissible at the current time.[16–18,20–22,24,25,28,30,31,33,34,37,38,41,42,44,50,57,60–68,70–73] A further 11% also consider germline editing impermissible currently, but are expressly open to the possibility of allowing it under certain conditions.[1,15,23,26,39,45,47] In 30% of cases, the position is not expressly addressed or is ambiguous.[12–14,19,29,32,35,36,40,43,48,49,53–56,58,59,69] And 5% of the reports state an openness to further exploration.[46,51,52]

Genome editing in embryos (mice to monkeys)

Mice

One-Step Generation of Mice Carrying Mutations in Multiple Genes by CRISPR/Cas-Mediated Genome Engineering

Haoyi Wang,[1,6] Hui Yang,[1,6] Chikdu S. Shivalila,[1,2,6] Meelad M. Dawlaty,[1] Albert W. Cheng,[1,3] Feng Zhang,[4,5] and Rudolf Jaenisch[1,3,*]

Cell *153*, 910–918, May 9, 2013

One-Step Generation of Mice Carrying Reporter and Conditional Alleles by CRISPR/Cas-Mediated Genome Engineering

Hui Yang,[1,4] Haoyi Wang,[1,4] Chikdu S. Shivalila,[1,2,4] Albert W. Cheng,[1,3] Linyu Shi,[1] and Rudolf Jaenisch[1,3,*]

Cell *154*, 1370–1379, September 12, 2013

OXFORD

Human Molecular Genetics, 2018, Vol. 00, No. 00 1–11

doi: 10.1093/hmg/ddy367
Advance Access Publication Date: 16 October 2018
General Article

GENERAL ARTICLE

CRISPR/Cas9-mediated disruption of *SHANK3* in monkey leads to drug-treatable autism-like symptoms

Zhuchi Tu[1,†], Hui Zhao[2,3,†], Bang Li[1,†], Sen Yan[1], Lu Wang[4], Yongjin Tang[4], Zhujun Li[1], Dazhang Bai[1], Caijuan Li[1], Yingqi Lin[1], Yuefeng Li[5], Jianrong Liu[6], Hao Xu[4], Xiangyu Guo[1], Yong-hui Jiang[7,*], Yong Q. Zhang[2,*] and Xiao-Jiang Li[1,8,*]

JBC ACCELERATED COMMUNICATION

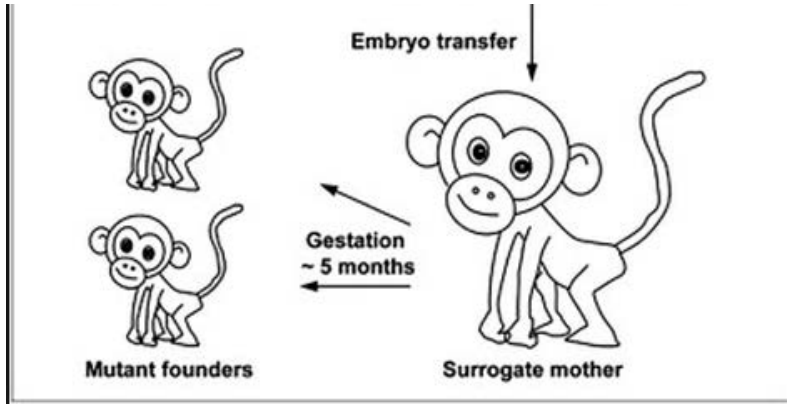
J. Biol. Chem. (2018) 293(30) 11654–11658

No off-target mutations in functional genome regions of a CRISPR/Cas9-generated monkey model of muscular dystrophy

Received for publication, June 10, 2018, and in revised form, June 21, 2018 Published, Papers in Press, June 25, 2018, DOI 10.1074/jbc.AC118.004404

Shuang Wang[‡§1], Shuaiwei Ren[‡§1], Raoxian Bai[‡§1], Puhao Xiao[‡§], Qin Zhou[‡§], Yin Zhou[‡§], Zhigang Zhou[‡§], Yuyu Niu[‡§], Weizhi Ji[‡§2], and Yongchang Chen[‡§3]

From the [‡]Yunnan Key Laboratory of Primate Biomedicine Research, Institute of Primate Translational Medicine, Kunming University of Science and Technology, Kunming 650500, China and the [§]Yunnan Provincial Academy of Science and Technology, Kunming 650051, China



Human embryos

Springer Link

CrossMark

Protein & Cell

10.1007/s13238-015-0153-5

Research article

CRISPR/Cas9-mediated gene editing in human tripronuclear zygotes

Puping Liang [1], Yanwen Xu [1], Xiya Zhang [1], Chenhui Ding [1], Rui Huang [1], Zhen Zhang [1], Jie Lv [1], Xiaowei Xie [1], Yuxi Chen [1], Yujing Li [1], Ying Sun [1], Yaofu Bai [1], Zhou Songyang [1], Wenbin Ma [1], Canquan Zhou [1] and Junjiu Huang [1]

(1) Guangdong Province Key Laboratory of Reproductive Medicine, the First Affiliated Hospital, and Key Laboratory of Gene Engineering of the Ministry of Education, School of Life Sciences, Sun Yat-sen University, Guangzhou, 510275, China

J Assist Reprod Genet
DOI 10.1007/s10815-016-0710-8

nature International weekly journal of science

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News & Comment > News > 2015 > April > Article

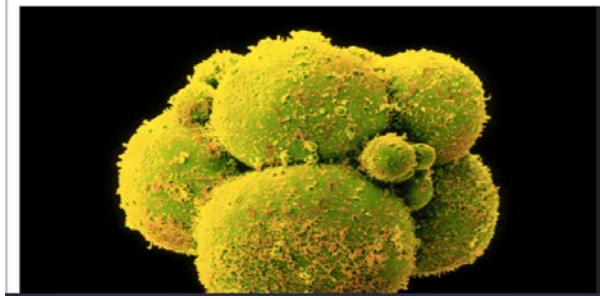
NATURE | NEWS

Chinese scientists genetically modify human embryos

Rumours of germline modification prove true — and look set to reignite an ethical debate.

David Cyranoski & Sara Reardon

22 April 2015



TECHNOLOGICAL INNOVATIONS

Online April 6, 2016

Introducing precise genetic modifications into human 3PN embryos by CRISPR/Cas-mediated genome editing

Xiangjin Kang [1] · Wenyin He [1] · Yuling Huang [1] · Qian Yu [1] · Yaoyong Chen [1] · Xingcheng Gao [1] · Xiaofang Sun [1] · Yong Fan [1]

5 OCTOBER 2017 | VOL 550 | NATURE

Genome editing reveals a role for OCT4 in human embryogenesis

Norah M. E. Fogarty[1], Afshan McCarthy[1], Kirsten E. Snijders[2], Benjamin E. Powell[3], Nada Kubikova[4], Paul Blakeley[1], Rebecca Lea[1], Kay Elder[5], Sissy E. Wamaitha[1], Daesik Kim[6], Valdone Maciulyte[3], Jens Kleinjung[7], Jin-Soo Kim[6,8], Dagan Wells[4], Ludovic Vallier[2,9,10], Alessandro Bertero[10]†, James M. A. Turner[3] & Kathy K. Niakan[1]

24 AUGUST 2017 | VOL 548 | NATURE

Correction of a pathogenic gene mutation in human embryos

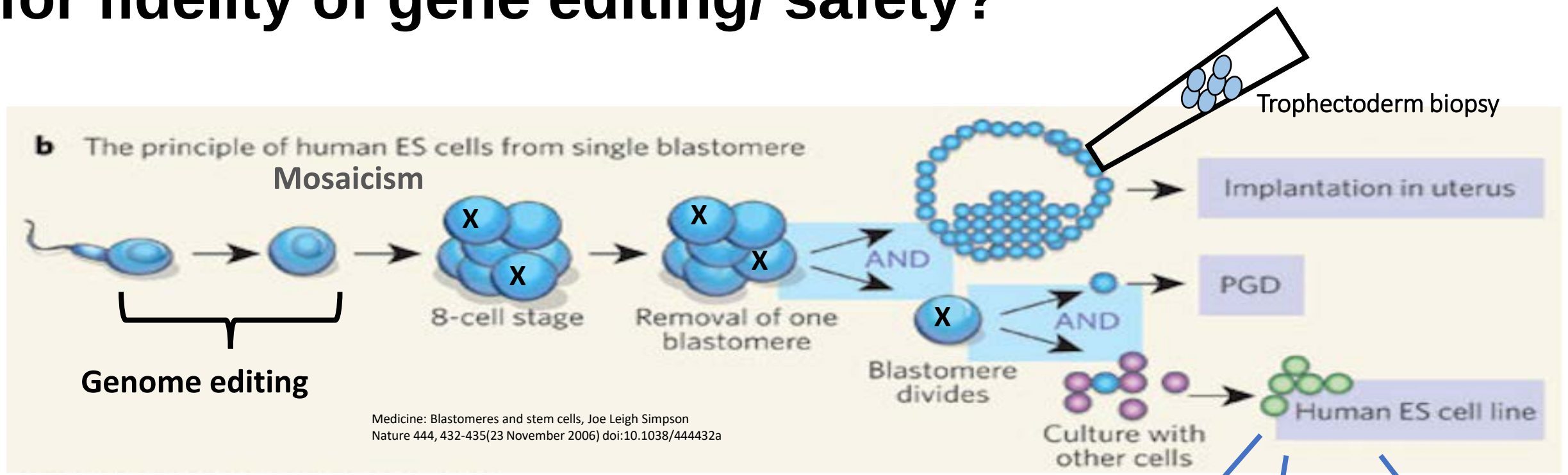
Hong Ma[1]*, Nuria Marti-Gutierrez[1]*, Sang-Wook Park[2]*, Jun Wu[3]*, Yeonmi Lee[1], Keiichiro Suzuki[3], Amy Koski[1], Dongmei Ji[1], Tomonari Hayama[1], Riffat Ahmed[1], Hayley Darby[1], Crystal Van Dyken[1], Ying Li[1], Eunju Kang[1], A.-Reum Park[2], Daesik Kim[4], Sang-Tae Kim[2], Jianhui Gong[5,6,7,8], Ying Gu[5,6,7], Xun Xu[5,6,7], David Battaglia[1,9], Sacha A. Krieg[9], David M. Lee[9], Diana H. Wu[9], Don P. Wolf[1], Stephen B. Heitner[10], Juan Carlos Izpisua Belmonte[3]§, Paula Amato[1,9]§, Jin-Soo Kim[2,4]§, Sanjiv Kaul[10]§ & Shoukhrat Mitalipov[1,10]§

Correction of β-thalassemia mutant by base editor in human embryos

Protein Cell 2017, 8(11):811–822
DOI 10.1007/s13238-017-0475-6

Puping Liang[1,2], Chenhui Ding[2], Hongwei Sun[1], Xiaowei Xie[1], Yanwen Xu[2], Xiya Zhang[1], Ying Sun[1], Yuanyan Xiong[1], Wenbin Ma[1], Yongxiang Liu[2], Yali Wang[2], Jianpei Fang[3], Dan Liu[4], Zhou Songyang[1,2,4]✉, Canquan Zhou[2]✉, Junjiu Huang[1,2]✉

Can embryos be effectively assessed for fidelity of gene editing/ safety?



Genetic QC

Epigenetic QC

Functional Assessments

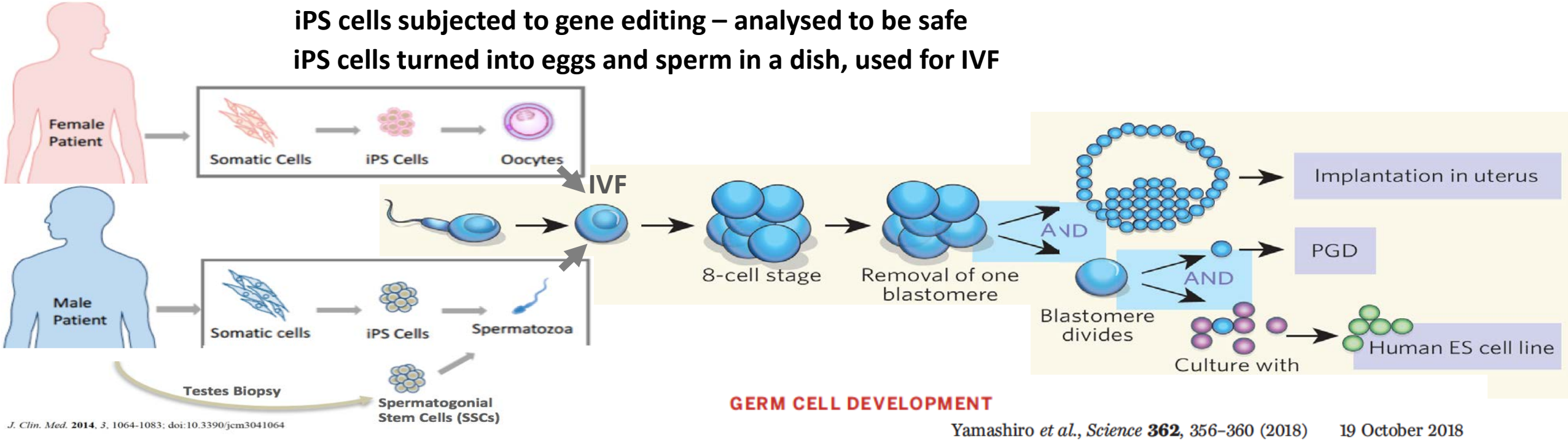
Safe Gene Editing in Embryos?

Editing in pluripotent stem cells coupled to in vitro gametogenesis

Skin or blood cells → reprogrammed to iPS cells

iPS cells subjected to gene editing – analysed to be safe

iPS cells turned into eggs and sperm in a dish, used for IVF



J. Clin. Med. 2014, 3, 1064-1083; doi:10.3390/jcm3041064

Sperm and eggs have been made from mouse iPS cells…
and mice have been born

GERM CELL DEVELOPMENT

Yamashiro *et al.*, *Science* **362**, 356–360 (2018) 19 October 2018

Generation of human oogonia from induced pluripotent stem cells in vitro

Chika Yamashiro[1,2], Kotaro Sasaki[1,2], Yukihiro Yabuta[1,2], Yoji Kojima[1,2,3,4], Tomonori Nakamura[1,2], Ikuhiro Okamoto[1,2], Shihori Yokobayashi[1,2,4], Yusuke Murase[1,2], Yukiko Ishikura[1,2], Kenjiro Shirane[5,6], Hiroyuki Sasaki[5,6], Takuya Yamamoto[3,4,7], Mitinori Saitou[1,2,3,4]*

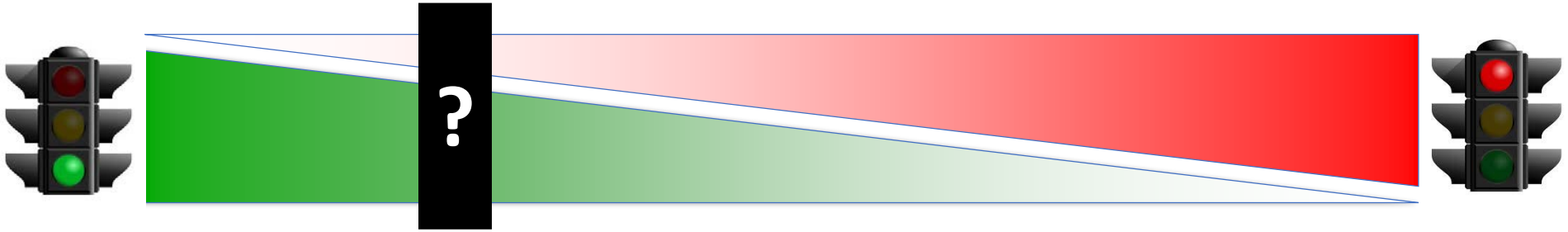
Figures adapted from Simpson JL Nature 2006 and Ishii T J Clin Med 2014

A responsible pathway for clinical translation

- Scientific consensus on optimal method for genome editing in embryos
  - Stage and methodology for maximizing on target editing, minimizing mosaicism
  - Methods to enable comprehensive assessment of genetic and functional outcome
  - Agreement on methods to evaluate efficacy, consensus on standards
- Definition of standards of technical competence for investigators
  - ISSCR Guidelines specify importance of relevant expertise of investigators…"Appropriate expertise and/or training of the investigators to perform the stated experiments must be ascertained in order to ensure the optimal use of research materials." (ISSCR Guidelines, p6 May 2016)

- Needed: A well defined translational pathway
  - Time for formal study group to define procedural standards for genome editing
  - Considerations of permissible "first in human" indications

Are there compelling medical indications?



Disease prevention

- Huntington's
- Tay Sach's
- Cystic Fibrosis
- Sickle cell anemia

Consider alternatives…

IVF, genetic diagnosis

Somatic therapy

When no alternative…

Couples, both affected

Infertility

Modifying Disease Risk

- HIV resistance (CCR5)
- Heart disease (PCSK9)
- Alzheimer's (APP A673T/+)
- Cancer (BRCA1/2)
- Resistance to global pandemics…

"Enhancements"

- Muscularity (MSTN)
- Height, skin color
- Learning and memory
  https://www.dnalc.org/view/1390-Genes-for-Learning-and-Memory.html

Permissible vs impermissible applications?

Encouraging active engagement of the research and clinical community

- Self-regulation by scientists is a long-standing professional norm
- Essential elements of self-regulation:
  - Transparency
  - Willing participation of scientists and practitioners

The criteria include:

- absence of reasonable alternatives;
- restriction to preventing a serious disease or condition;
- restriction to editing genes that have been convincingly demonstrated to cause or strongly predispose to that disease or condition;
- restriction to converting such genes to versions that are prevalent in the population and are known to be associated with ordinary health with little or no evidence of adverse effects;
- availability of credible pre-clinical and/or clinical data on risks and potential health benefits of the procedures;
- during the trial, ongoing, rigorous oversight of the effects of the procedure on the health and safety of the research participants;
- comprehensive plans for long-term multigenerational follow-up that still respect personal autonomy;
- maximum transparency consistent with patient privacy;
- continued reassessment of both health and societal benefits and risks, with broad, ongoing participation and input from the public; and
- reliable oversight mechanisms to prevent extension to uses other than preventing a serious disease or condition.
- “broad participation and input by the public, along with ongoing reassessment of both health and societal benefits and risks, should be a condition for moving clinical trials forward.”