Gene editing in medicine

08.04. 2021

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Research Programs Unit, University of Helsinki

and

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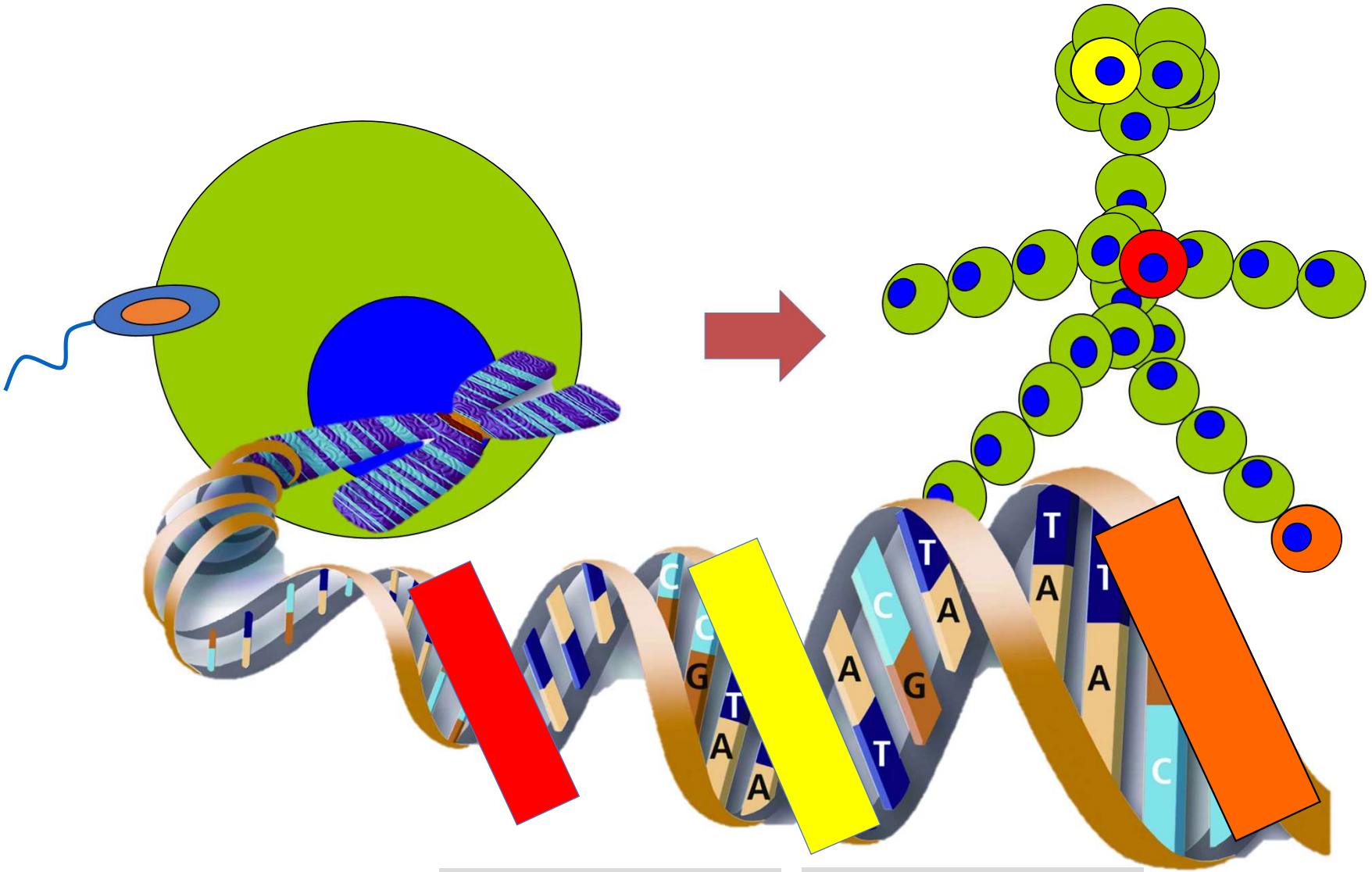
By guiding the DNA-protein interactions you can control the (biological) world. Gene editing does it for you.

GIF

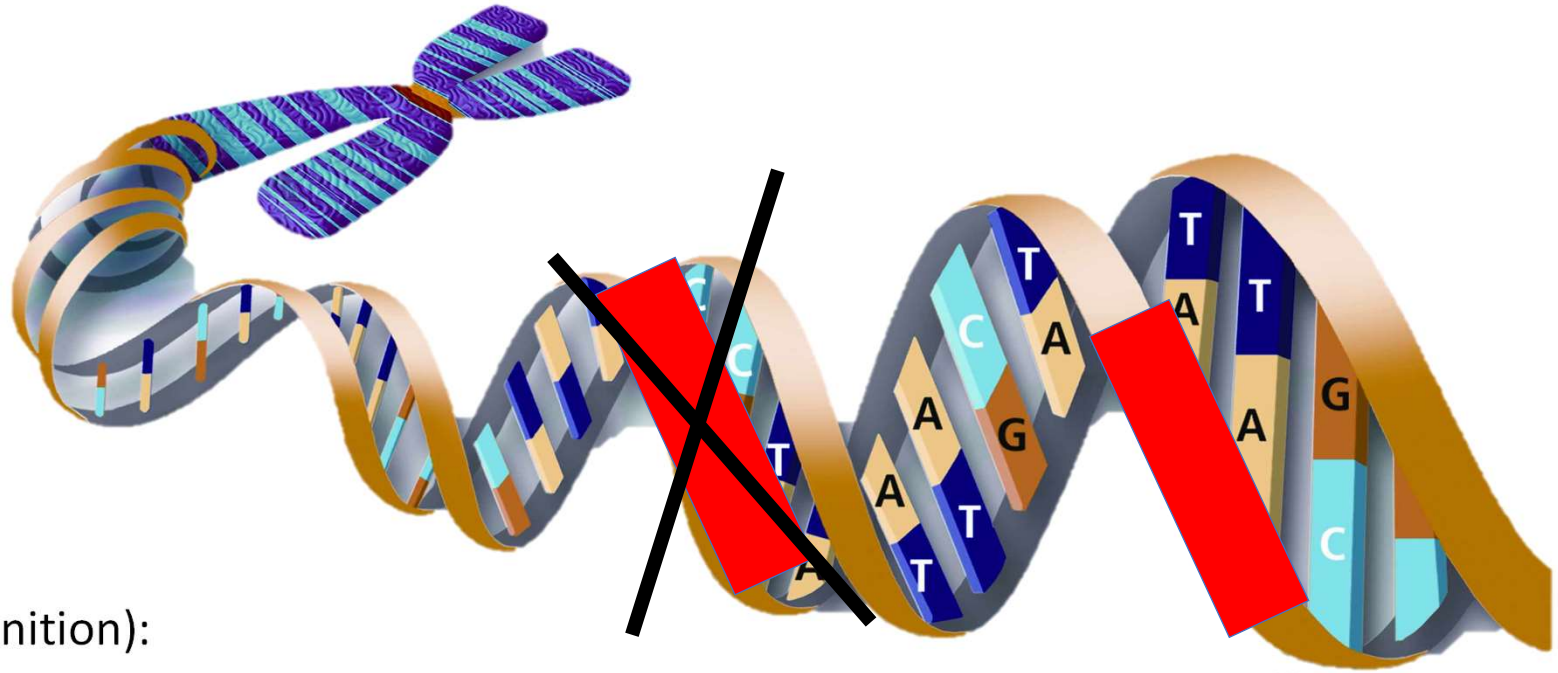
54 Tweet

3 ”How”s for getting genetic therapy to clinic

1. Biological/medical: how to identify the disease cause?
2. Technical: how to fix the problem?
3. Societal: how to implement the solution?


GENE= RECIPE
DNA= LETTERS

Gene therapy medicines



(EMA definition):

- contain genes that lead to a therapeutic, prophylactic or diagnostic effect.
- work by inserting 'recombinant' genes into the body, usually to treat a variety of diseases, including genetic disorders, cancer or long-term diseases. A recombinant gene is a stretch of DNA that is created in the laboratory, bringing together DNA from different sources.
- Synthetic oligonucleotides are not gene therapy medicines

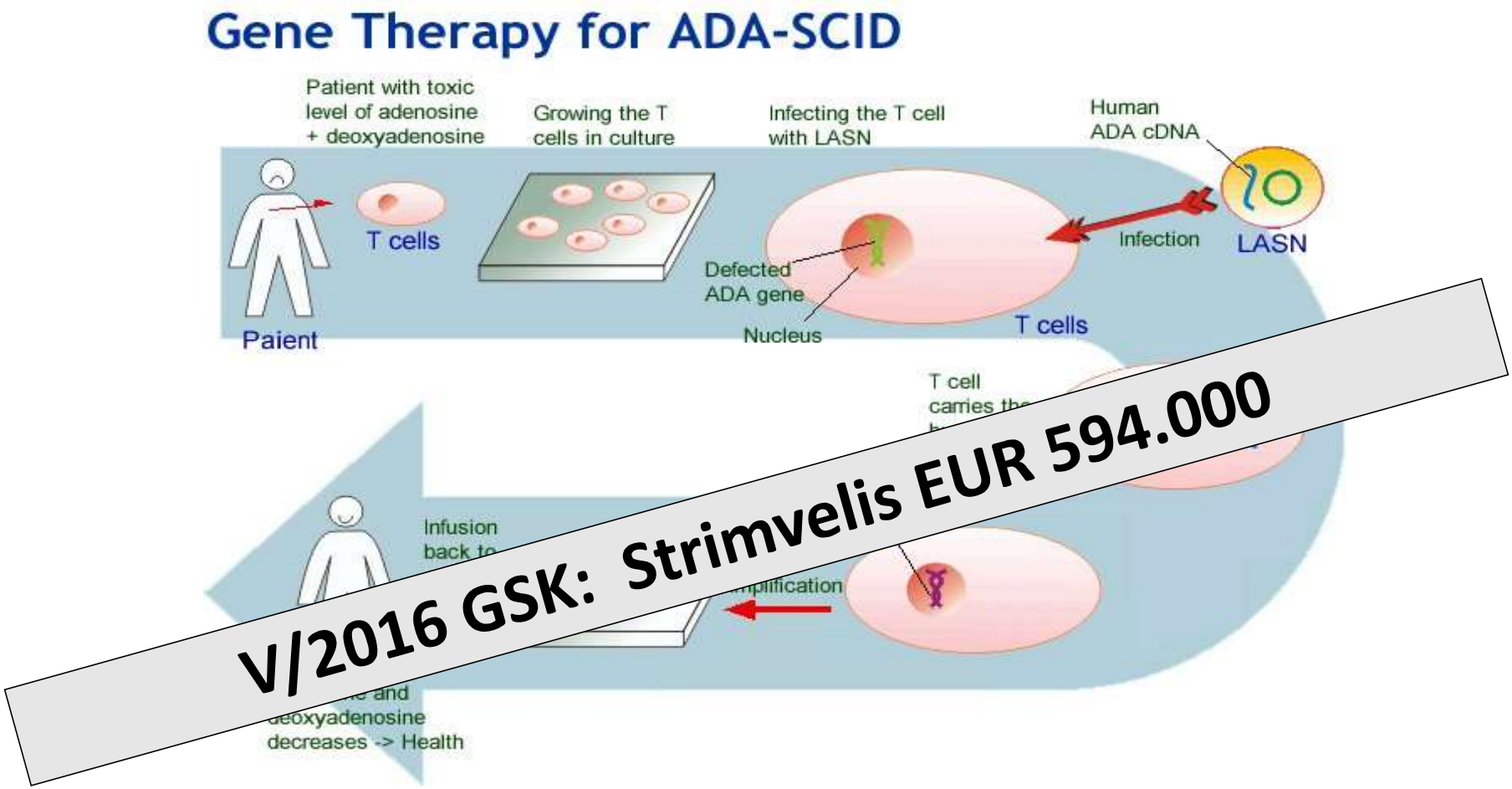
Gene therapy products in EU 2021

Table 3 Gene therapy products approved by the EMA[a]				
Trade name	**Product**	**Condition**	**Vector**	**EMA Approval**
Glybera®	Alipogene tiparvovec	Lipoprotein lipase deficiency		10/2012†2017
Imlygic®	Talimogene laherparepvec	Regionally or distantly metastatic unresectable melanoma	HSV-1/GM-CSF	12/2015 Cancer medicine
Strimvelis®[b]	Autologous CD34+ cells transduced to express ADA	Adenosine deaminase deficiency (ADA)	Y-retrovirus/ADA	05/2016
Kymriah®[c]	Tisagenlecleucel	• Relapsed or refractory B-cell accute lymphoblastic leukemia • Relapsed or refractory diffuse large B-cell lymphoma	LV-CAR (CD19R)	09/2018 Cancer medicine
Yescarta®[c]	Axicabtagene ciloleucel (CAR-T)	• Relapsed or refractory DLBCL and primary mediastinal large B-cell lymphoma • Some types of non-Hodgkin lymphoma	Y-retrovirus	08/2018 Cancer medicine
LUXTURNA®[d]	Voretigene neparvovec	Inherited retinal dystrophy caused by biallelic RPE65 mutations	AAV2-RPE65	11/2018
Zynteglo®[e]	Autologous CD34+ cells encoding βA-T87Q-globin gene	β-thalassemia with regular blood transfusions	LV-ß-globin	05/2019
Zolgensma®	Onasemnogene abeparvovec	Spinal muscular atrophy 1	AAV9	03/2020

†: Taken out of the market.

[a] (Gene Therapy Net, 2020); [b] (Novartis, 2020); [c] (Dolgin, 2019); [d] (Master, 2019); [e] (DBGen, 2019); [f] (Deena Beasley, 2019)

Maldonado R et al. Journal of Community Genetics 2020



Maria Pia Cicalese et al (2016)
Blood-2016-01-688226

- 18 patients, donor not available, not responding to ERT.
- autologous $CD34^{+}$ retrovirally transduced
- survival 100%
- 2.3 to 13.4 (median: 6.9) years. Severe infections from 1.17 to 0.17 events/py

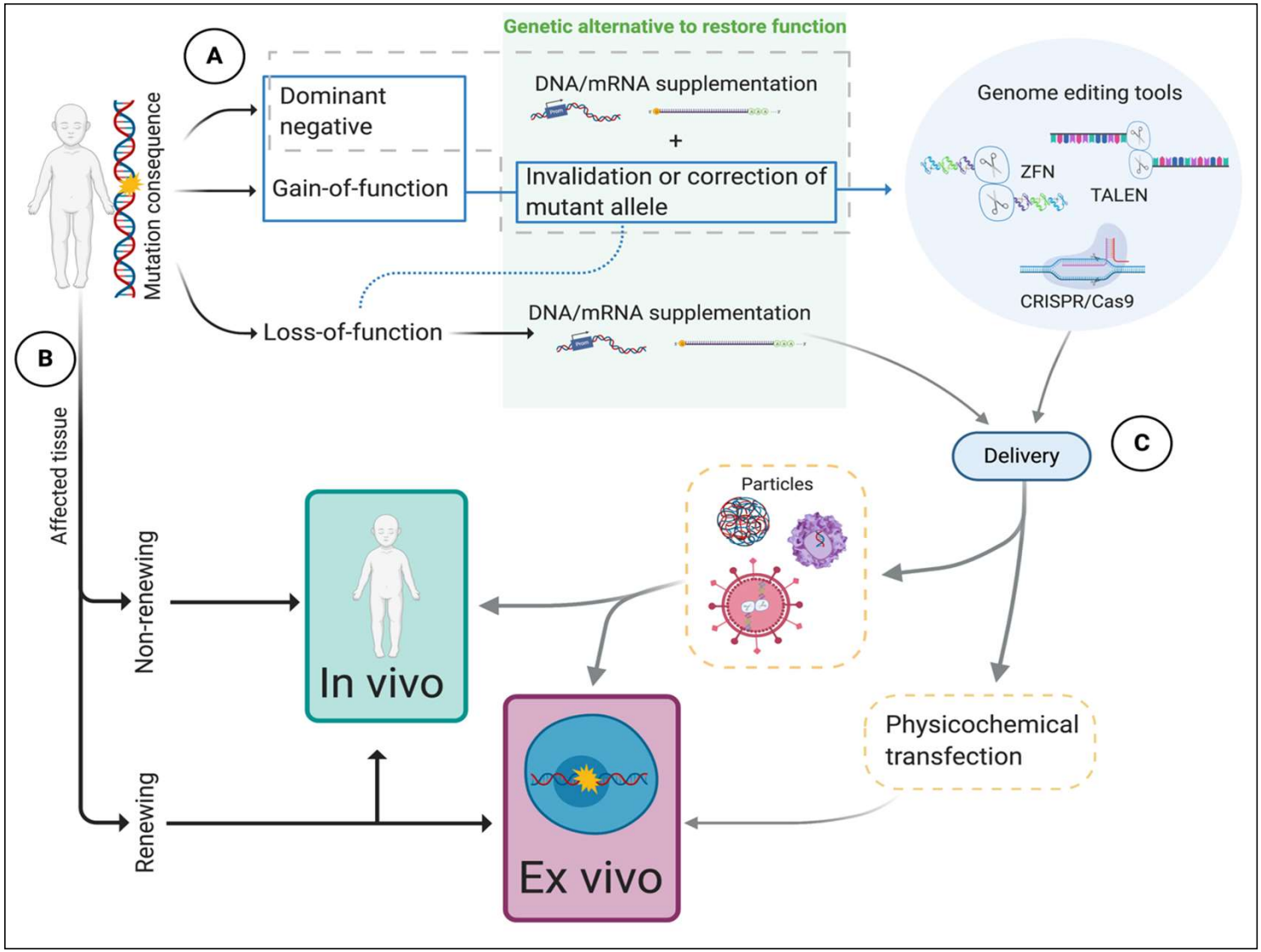
The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 APRIL 19, 2018 VOL. 378 NO. 16

Gene Therapy in Patients with Transfusion-Dependent β-Thalassemia

A.A. Thompson, M.C. Walters, J. Kwiatkowski, J.E.J. Rasko, J.-A. [illegible], G.J. Schiller, E. Payen, M. Semeraro, D. Moshous, F. Lefrere, H. [illegible], L. Caccavelli, J.-S. Diana, F. Suarez, F. Monpoux, V. Brousse, C. Poirot, C. Pondarré, Y. Beuzard, S. Chrétien, T. Lefebvre, D.T. Teachey, U. An[illegible]aile, M. Kletzel, E. Vichinsky, S. Soni, G. Veres, O. Negre, R.W. Ross, [illegible], L. Sandler, M. Asmal, O. Hermine, M. De Montalembert, [illegible]ma, S. Blanche, P. Leboulch, and M. Cavazzana

V/2019 Bluebird Bio: Zyntenglo 1.575 M€

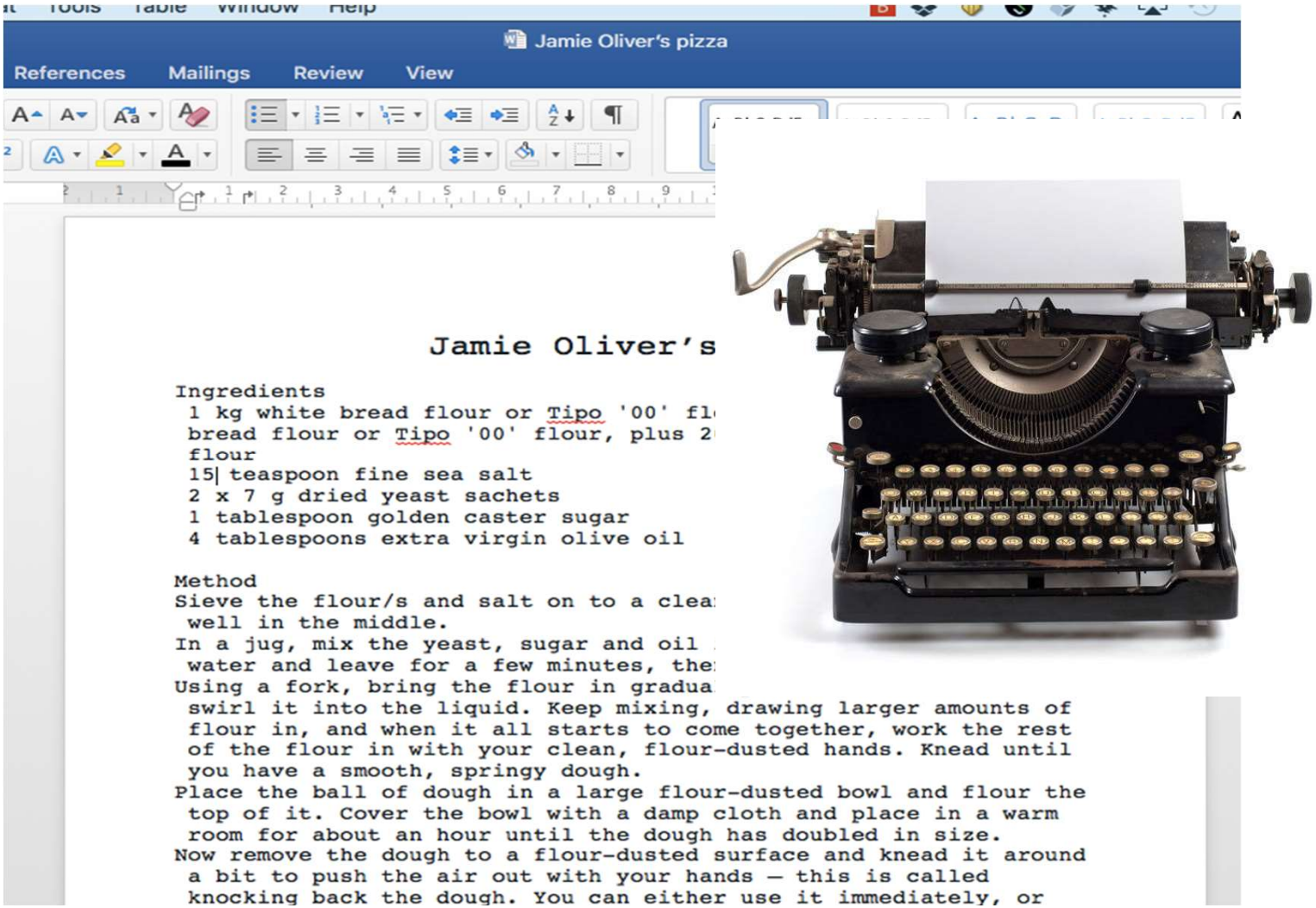


Important variables in gene therapy:

A) mutation type
B) affected tissue
C) delivery method

Maldonado R et al. *Journal of Community Genetics* 2020

“old” gene therapy vs. editing



Genome editing technologies

1. Hybrid meganuclease

2. Zinc-Finger Nuclease

ZFN

Zinc finger domains

3. Transcription Activator-like Effector Nuclease

TALEN

TALE subunits

active FokI catalytic subunit heterodimer

The Nobel Prize in Chemistry 2020

© Nobel Media. Ill. Niklas Elmehed.

Emmanuelle Charpentier

Prize share: 1/2

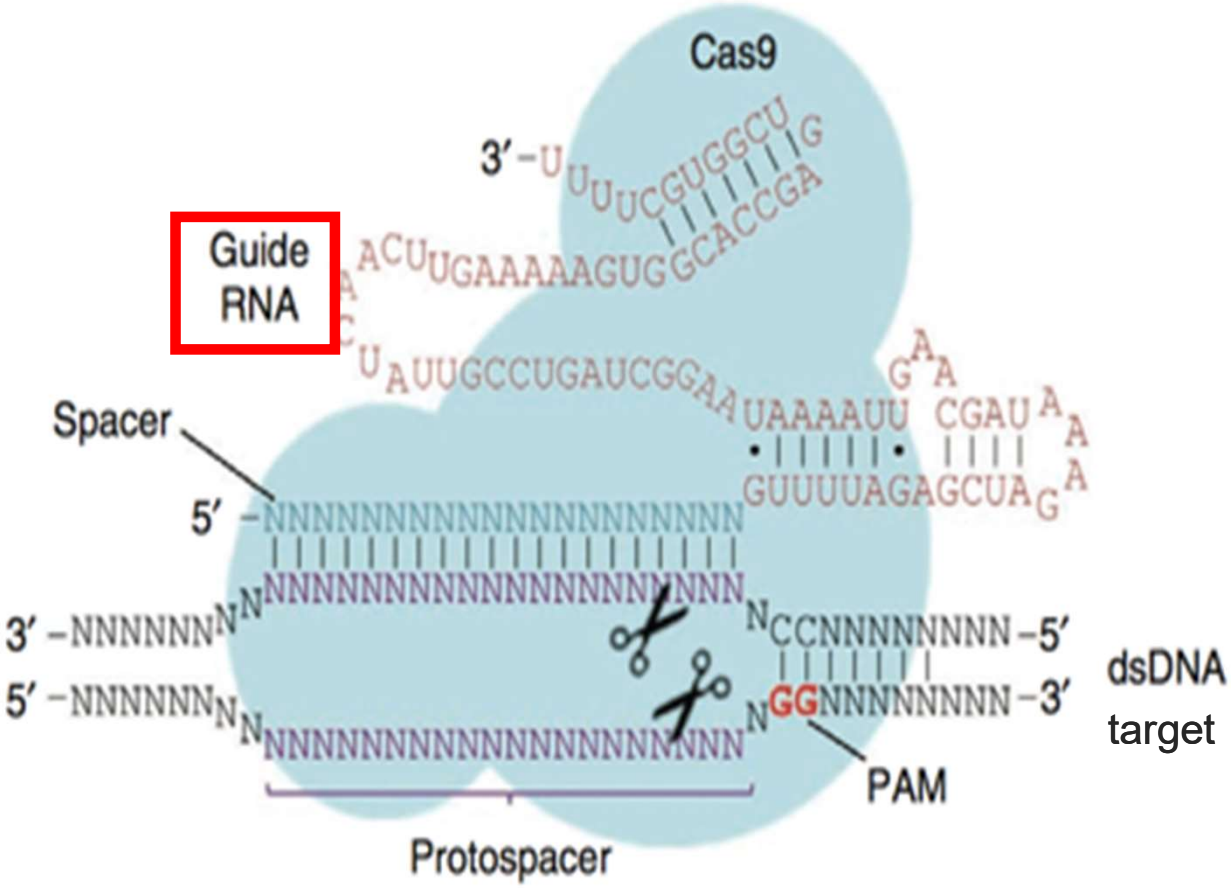
© Nobel Media. Ill. Niklas Elmehed.

Jennifer A. Doudna

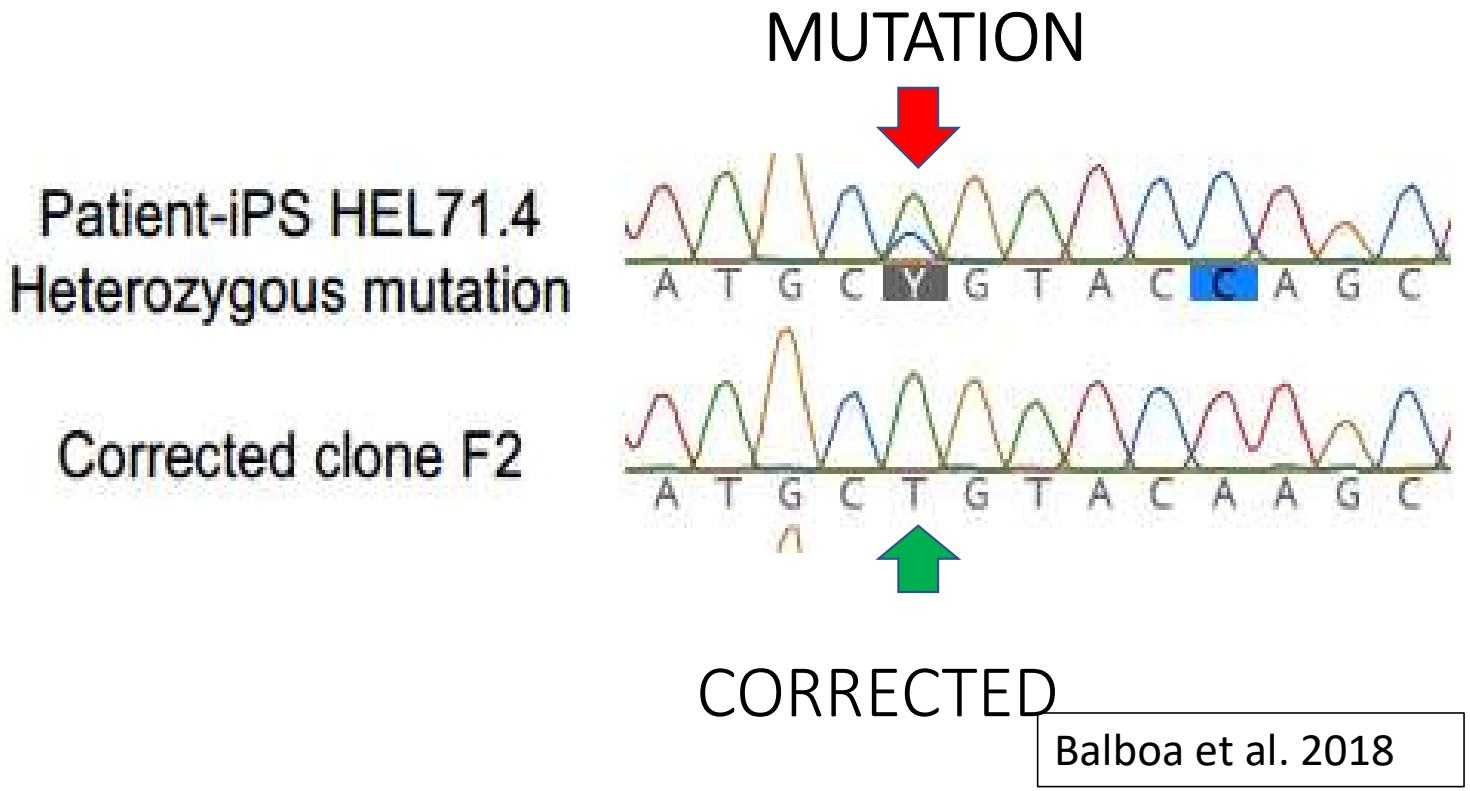
Prize share: 1/2

The Nobel Prize in Chemistry 2020 was awarded jointly to Emmanuelle Charpentier and Jennifer A. Doudna "for the development of a method for genome editing."

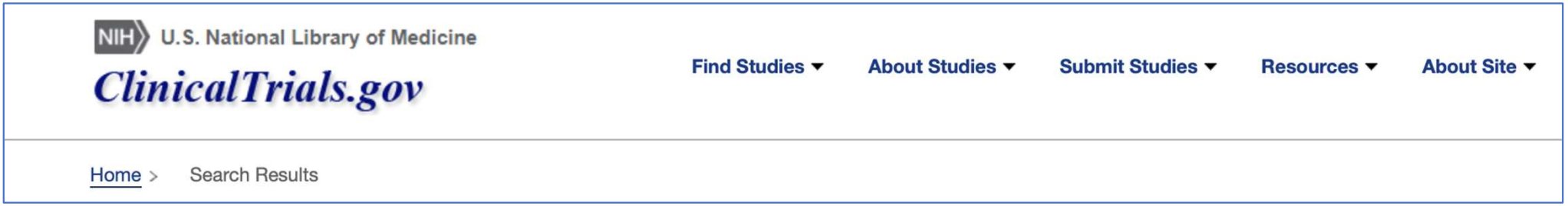
CRISPR-Cas9



Genetic correction of a patient mutation



Balboa et al. 2018



Genome editing clinical trials 4/2021

ZFNs	15
TALENs	7
CRISPRs	46 (of which 7 in diagnostics)

No market approvals for gene editing medicinal products

REPORT

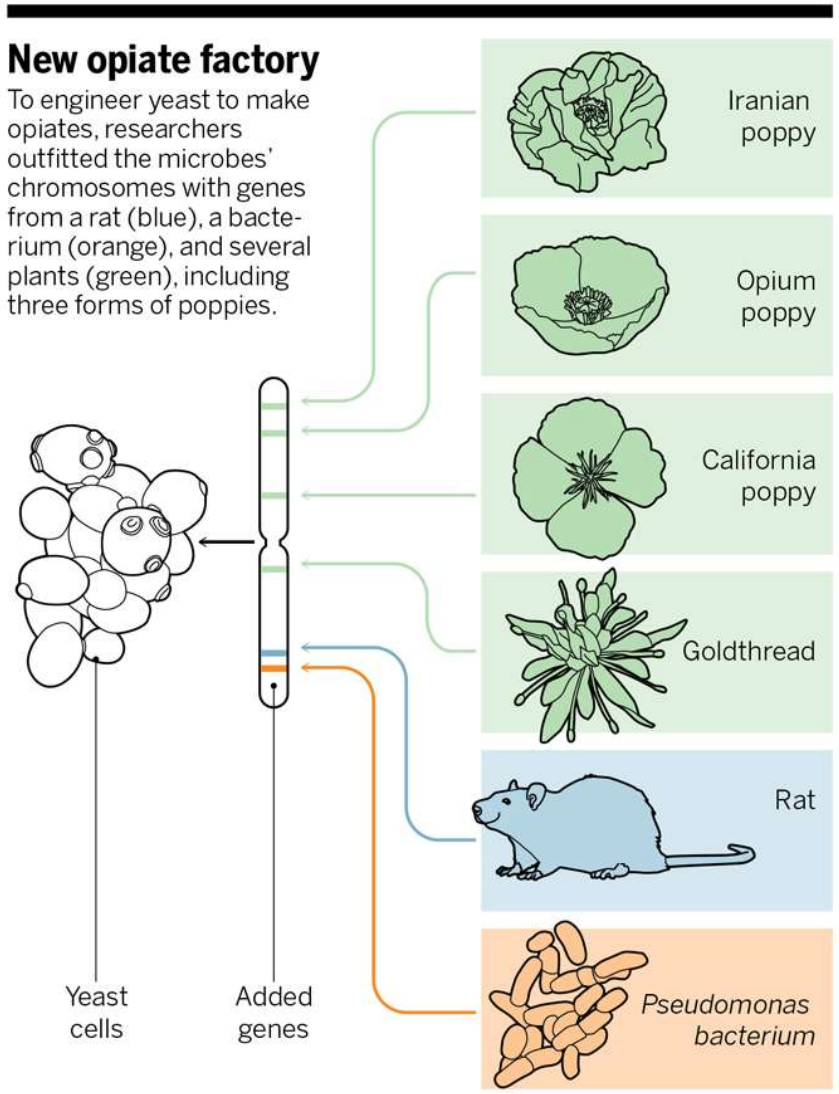
Complete biosynthesis of opioids in yeast

Stephanie Galanie[1], Kate Thodey[2], Isis J. Trenchard[2], Maria Filsinger Interrante[2], Christina D. Smolke[2,*]

+ See all authors and affiliations

Science 04 Sep 2015:
Vol. 349, Issue 6252, pp. 1095-1100
DOI: 10.1126/science.aac9373

- Synthetic biologists engineered 21 genes in total, including many added from a diverse set of species (see graphic); making hydrocodone took 23 genes.



The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Gene Editing of *CCR5* in Autologous CD4 T Cells of Persons Infected with HIV

Pablo Tebas, M.D., David Stein, M.D., Winson W. Tang, M.D., Ian Frank, M.D., Shelley Q. Wang, M.D., Gary Lee, Ph.D., S. Kaye Spratt, Ph.D., Richard T. Surosky, Ph.D., Martin A. Giedlin, Ph.D., Geoff Nichol, M.D., Michael C. Holmes, Ph.D., Philip D. Gregory, Ph.D., Dale G. Ando, M.D., Michael Kalos, Ph.D., Ronald G. Collman, M.D., Gwendolyn Binder-Scholl, Ph.D., Gabriela Plesa, M.D., Ph.D., Wei-Ting Hwang, Ph.D., Bruce L. Levine, Ph.D., and Carl H. June, M.D.

N Engl J Med 2014; 370:901-910 | March 6, 2014 | DOI: 10.1056/NEJMoa1300662

First CRISPR clinical trial 2016-2019

ARTICLES
https://doi.org/10.1038/s41591-020-0840-5

nature medicine

Check for updates

Safety and feasibility of CRISPR-edited T cells in patients with refractory non-small-cell lung cancer

You Lu[1,14] ✉, Jianxin Xue[1,14], Tao Deng[2,14], Xiaojuan Zhou[1,14], Kun Yu[2,14], Lei Deng[3], Meijuan Huang[1], Xin Yi[4], Maozhi Liang[5], Yu Wang[6], Haige Shen[6], Ruizhan Tong[1], Wenbo Wang[7], Li Li[1], Jin Song[4], Jing Li[4], Xiaoxing Su[8], Zhenyu Ding[1], Youling Gong[1], Jiang Zhu[1], Yongsheng Wang[1,5], Bingwen Zou[1], Yan Zhang[1], Yanying Li[1], Lin Zhou[1], Yongmei Liu[1], Min Yu[1], Yuqi Wang[4], Xuanwei Zhang[1], Limei Yin[1], Xuefeng Xia[4], Yong Zeng[2], Qiao Zhou[9], Binwu Ying[10], Chong Chen[11], Yuquan Wei[11], Weimin Li[12] and Tony Mok[13]

Clustered regularly interspaced short palindromic repeats (CRISPR)–Cas9 editing of immune checkpoint genes could improve the efficacy of T cell therapy, but the first necessary undertaking is to understand the safety and feasibility. Here, we report results from a first-in-human phase I clinical trial of CRISPR–Cas9 *PD-1*-edited T cells in patients with advanced non-small-cell lung cancer (ClinicalTrials.gov NCT02793856). Primary endpoints were safety and feasibility, and the secondary endpoint was efficacy. The exploratory objectives included tracking of edited T cells. All prespecified endpoints were met. *PD-1*-edited T cells were manufactured ex vivo by cotransfection using electroporation of Cas9 and single guide RNA plasmids. A total of 22 patients were enrolled; 17 had sufficient edited T cells for infusion, and 12 were able to receive treatment. All treatment-related adverse events were grade 1/2. Edited T cells were detectable in peripheral blood after infusion. The median progression-free survival was 7.7 weeks (95% confidence interval, 6.9 to 8.5 weeks) and median overall survival was 42.6 weeks (95% confidence interval, 10.3–74.9 weeks). The median mutation frequency of off-target events was 0.05% (range, 0–0.25%) at 18 candidate sites by next generation sequencing. We conclude that clinical application of CRISPR–Cas9 gene-edited T cells is generally safe and feasible. Future trials should use superior gene editing approaches to improve therapeutic efficacy.

NATURE MEDICINE | MAY 2020 | 732–740

Single Ascending Dose Study in Participants With LCA10

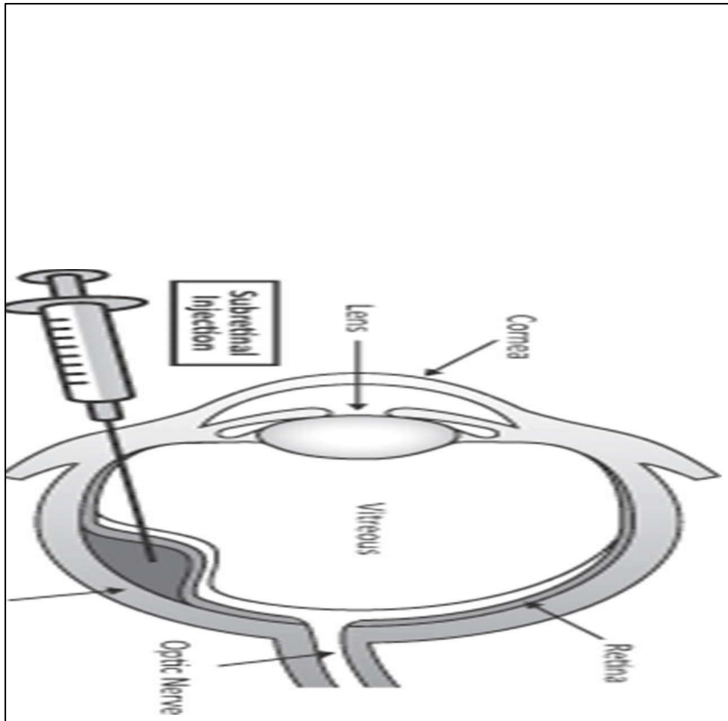
The safety and scientific validity of this study is the responsibility of the study sponsor and investigators. Listing a study does not mean it has been evaluated by the U.S. Federal Government. Know the risks and potential benefits of clinical studies and talk to your health care provider before participating. Read our disclaimer for details.

ClinicalTrials.gov Identifier: NCT03872479

Recruitment Status : Recruiting
First Posted : March 13, 2019
Last Update Posted : November 22, 2019

"….safety, tolerability and efficacy ….in participants with **LCA10** caused by a ….mutation involving c.2991+1655A>G in intron 26 of the **CEP290 gene"**

1st *in vivo* CRISPR therapy



CRISPR blood disease clinical trials 2021

1	Recruiting	CRISPR-Edited Allogeneic Anti-CD19 CAR-T Cell Therapy for Relapsed/Refractory B Cell Non-Hodgkin Lymphoma	• Lymphoma, Non-Hodgkin • Relapsed Non Hodgkin Lymphoma • Refractory B-Cell Non-Hodgkin Lymphoma • (and 4 more...)	• Genetic: CB-010 • Drug: Cyclophosphamide • Drug: Fludarabine	• Oncology Hematology Care Cincinnati, Ohio, United States
2	Recruiting	A Safety and Efficacy Study Evaluating CTX130 in Subjects With Relapsed or Refractory T or B Cell Malignancies	• T Cell Lymphoma	• Biological: CTX130	• Research Site 2 Duarte, California, United States • Research Site 5 Stanford, California, United States • Research Site 4 Miami, Florida, United States • (and 3 more...)
3	Recruiting	A Safety and Efficacy Study Evaluating CTX120 in Subjects With Relapsed or Refractory Multiple Myeloma	• Multiple Myeloma	• Biological: CTX120	• Research Site 4 Chicago, Illinois, United States • Research Site 3 Portland, Oregon, United States • Research Site 1 Nashville, Tennessee, United States • (and 4 more...)
4	Recruiting	CRISPR (HPK1) Edited CD19-specific CAR-T Cells (XYF19 CAR-T Cells) for CD19+ Leukemia or Lymphoma.	• Leukemia Lymphocytic Acute (ALL) in Relapse • Leukemia Lymphocytic Acute (All) Refractory • Lymphoma, B-Cell • CD19 Positive	• Genetic: XYF19 CAR-T cell • Drug: Cyclophosphamide • Drug: Fludarabine	• Xijing Hospital Xi'an, Shannxi, China
5	Recruiting	A Safety and Efficacy Study Evaluating CTX110 in Subjects With Relapsed or Refractory B-Cell Malignancies (CARBON)	• B-cell Malignancy • Non-Hodgkin Lymphoma • B-cell Lymphoma	• Biological: CTX110	• UCSF Medical Center San Francisco, California, United States • Mayo Clinic Jacksonville, Florida, United States • Emory University Winship Cancer Institute Atlanta, Georgia, United States • (and 8 more...)
6	Recruiting	A Safety and Efficacy Study Evaluating CTX001 in Subjects With Transfusion-Dependent β-Thalassemia	• Beta-Thalassemia • Thalassemia • Genetic Diseases, Inborn • (and 2 more...)	• Biological: CTX001	• Stanford University Stanford, California, United States • Columbia University Manhattan, New York, United States • The Children's Hospital at TriStar Centenni Nashville, Tennessee, United States • (and 7 more...)

Cancer

Cancer

Cancer

Cancer

The NEW ENGLAND JOURNAL of MEDICINE

BRIEF REPORT

CRISPR-Cas9 Gene Editing for Sickle Cell Disease and β-Thalassemia

H. Frangoul, D. Altshuler, M.D. Cappellini, Y.-S. Chen, J. Domm, B.K. Eustace, J. Foell, J. de la Fuente, S. Grupp, R. Handgretinger, T.W. Ho, A. Kattamis, A. Kernytsky, J. Lekstrom-Himes, A.M. Li, F. Locatelli, M.Y. Mapara, M. de Montalembert, D. Rondelli, A. Sharma, S. Sheth, S. Soni, M.H. Steinberg, D. Wall, A. Yen, and S. Corbacioglu

3 ”How”s for getting genetic therapy to clinic

1. Biological/medical: how to identify the disease cause?
2. Technical: how to fix the problem?
3. Societal: how to implement the solution?

Societal questions

- Regulatory requirements -> price
- Safety: how many patients need to be tested?
- Other variables in ethical questions:

risk/benefit; cost/alternative treatments; private/public health systems; possibilities for re-treatments

- Germline treatments
- Environmental engineering, e.g. malaria mosquitos

Science History Institute

Chemistry · Engineering · Life Sciences

https://www.sciencehistory.org

How safe is safe enough?

DISTILLATIONS

USING STORIES FROM SCIENCE'S PAST TO UNDERSTAND OUR WORLD

HEALTH

The Death of Jesse Gelsinger, 20 Years Later

Gene editing promises to revolutionize medicine. But how safe is safe enough for the patients testing these therapies?

By Meir Rinde | June 4, 2019

Photo illustration by Clay Cansler

JCI The Journal of Clinical Investigation
Published by The American Society for Clinical Investigation | Founded 1908

J Clin Invest. 2008 Sep 2; 118(9): 3132–3142.
Published online 2008 Aug 7. doi: 10.1172/JCI35700
PMCID: PMC2496963
PMID: 18688285

Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1

Salima Hacein-Bey-Abina,[1,2] Alexandrine Garrigue,[2] Gary P. Wang,[3] Jean Soulier,[4] Annick Lim,[5] Estelle Morillon,[2] Emmanuelle Clappier,[5] Laure Caccavelli,[1] Eric Delabesse,[6] Kheira Beldjord,[7,8] Vahid Asnafi,[7,8] Elizabeth MacIntyre,[7,8] Liliane Dal Cortivo,[1] Isabelle Radford,[8] Nicole Brousse,[9] François Sigaux,[4] Despina Moshous,[10] Julia Hauer,[2] Arndt Borkhardt,[11] Bernd H. Belohradsky,[12] Uwe Wintergerst,[12] Maria C. Velez,[13] Lily Leiva,[13] Ricardo Sorensen,[13] Nicolas Wulffraat,[14] Stéphane Blanche,[10] Frederic D. Bushman,[3] Alain Fischer,[2,10] and Marina Cavazzana-Calvo[1,2]

▸ Author information ▸ Article notes ▸ Copyright and License information Disclaimer

Associated Data

▸ Supplementary Materials

Abstract

Go to:

Previously, several individuals with X-linked SCID (SCID-X1) were treated by gene therapy to restore the missing IL-2 receptor γ (*IL2RG*) gene to $CD34^+$ BM precursor cells using gammaretroviral vectors. While 9 of 10 patients were successfully treated, 4 of the 9 developed T cell leukemia 31–68 months after gene therapy. In 2 of these cases, blast cells contained activating vector insertions near the LIM domain–only 2 (*LMO2*) proto-oncogene. Here, we report data on the 2 most recent adverse events, which occurred in

J Clin Invest

CRISPRed cells seem safe

Stadtemauer EA, et al. *Science367: (2020)*

RESEARCH ARTICLE SUMMARY

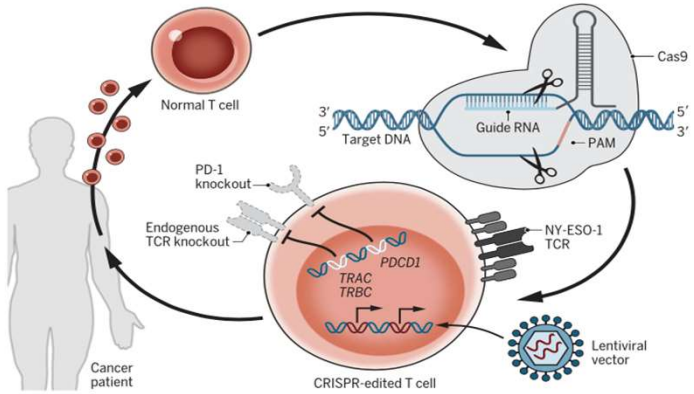
CLINICAL TRIALS

CRISPR-engineered T cells in patients with refractory cancer

Edward A. Stadtmauer*†, Joseph A. Fraietta*, Megan M. Davis, Adam D. Cohen, Kristy L. Weber, Eric Lancaster, Patricia A. Mangan, Irina Kulikovskaya, Minnal Gupta, Fang Chen, Lifeng Tian, Vanessa E. Gonzalez, Jun Xu, In-young Jung, J. Joseph Melenhorst, Gabriela Plesa, Joanne Shea, Tina Matlawski, Amanda Cervini, Avery L. Gaymon, Stephanie Desjardins, Anne Lamontagne, January Salas-Mckee, Andrew Fesnak, Donald L. Siegel, Bruce L. Levine, Julie K. Jadlowsky, Regina M. Young, Anne Chew, Wei-Ting Hwang, Elizabeth O. Hexner, Beatriz M. Carreno, Christopher L. Nobles, Frederic D. Bushman, Kevin R. Parker, Yanyan Qi, Ansuman T. Satpathy, Howard Y. Chang, Yangbing Zhao, Simon F. Lacey*, Carl H. June*†

INTRODUCTION: Most cancers are recognized and attacked by the immune system but can progress owing to tumor-mediated immunosuppression and immune evasion mechanisms. The infusion of ex vivo engineered T cells, termed adoptive T cell therapy, can increase the natural antitumor immune response of the patient. Gene therapy to redirect immune specificity combined with genome editing has the potential to improve the efficacy and increase the safety of engineered T cells. CRISPR coupled with CRISPR-associated protein 9 (Cas9) endonuclease is a powerful gene-editing technology that potentially allows the ability to target multiple genes in T cells to improve cancer immunotherapy.

RATIONALE: Our first-in-human, phase 1 clinical trial (clinicaltrials.gov; trial NCT03399448) was designed to test the safety and feasibility of multiplex CRISPR-Cas9 gene editing of T cells from patients with advanced, refractory cancer. A limitation of adoptively transferred T cell efficacy has been the induction of T cell dysfunction or exhaustion. We hypothesized that removing the endogenous T cell receptor (TCR) and the immune checkpoint molecule programmed cell death protein 1 (PD-1) would improve the function and persistence of engineered T cells. In addition, the removal of PD-1 has the potential to improve safety and reduce toxicity that can be caused by autoimmunity.



CRISPR-Cas9 engineering of T cells in cancer patients. T cells (center) were isolated from the blood of a patient with cancer. CRISPR-Cas9 ribonuclear protein complexes loaded with three sgRNAs were electroporated into the normal T cells, resulting in gene editing of the *TRAC*, *TRBC1*, *TRBC2*, and *PDCD1* (encoding PD-1) loci. The cells were then transduced with a lentiviral vector to express a TCR specific for the cancer-testis antigens NY-ESO-1 and LAGE-1 (right). The engineered T cells were then returned to the patient by intravenous infusion, and patients were monitored to determine safety and feasibility. PAM, protospacer adjacent motif.

A synthetic, cancer-specific TCR transgene (NY-ESO-1) was also introduced to recognize tumor cells. In vivo tracking and persistence of the engineered T cells were monitored to determine if the cells could persist after CRISPR-Cas9 modifications.

RESULTS: Four cell products were manufactured at clinical scale, and three patients (two with advanced refractory myeloma and one with metastatic sarcoma) were infused. The editing efficiency was consistent in all four products and varied as a function of the single guide RNA (sgRNA), with highest efficiency observed for the TCR α chain gene (*TRAC*) and lowest efficiency for the TCR β chain gene (*TRBC*). The mutations induced by CRISPR-Cas9 were highly specific for the targeted loci; however, rare off-target edits were observed. Single-cell RNA sequencing of the infused CRISPR-engineered T cells revealed that ~30% of cells had no detectable mutations, whereas ~40% had a single mutation and ~20 and ~10% of the engineered T cells were double mutated and triple mutated, respectively, at the target sequences. The edited T cells engrafted in all three patients at stable levels for at least 9 months. The persistence of the T cells expressing the engineered TCR was much more durable than in three previous clinical trials during which T cells were infused that retained expression of the endogenous TCR and endogenous PD-1. There were no clinical toxicities associated with the engineered T cells. Chromosomal translocations were observed in vitro during cell manufacturing, and these decreased over time after infusion into patients. Biopsies of bone marrow and tumor showed trafficking of T cells to the sites of tumor in all three patients. Although tumor biopsies revealed residual tumor, in both patients with myeloma, there was a reduction in the target antigens NY-ESO-1 and/or LAGE-1. This result is consistent with an on-target effect of the engineered T cells, resulting in tumor evasion.

CONCLUSION: Preliminary results from this pilot trial demonstrate that multiplex human genome engineering is safe and feasible using CRISPR-Cas9. The extended persistence of the engineered T cells indicates that preexisting immune responses to Cas9 do not appear to present a barrier to the implementation of this promising technology. ■

ON OUR WEBSITE

Read the full article at http://dx.doi.org/10.1126/science.aba7365

The list of author affiliations is available in the full article online.
*These authors contributed equally to this work.
†Corresponding author. Email: edward.stadtmauer@pennmedicine.upenn.edu (E.A.S.); cjune@upenn.edu (C.H.J.)
Cite this article as E. A. Stadtmauer *et al.*, *Science* **367**, eaba7365 (2020). DOI: 10.1126/science.aba7365

Unanswered questions in genomic editing: best techniques

- Off- targets (biological safety)
- Targeting methods (safety, efficacy)
- Tissue specificity and accessibility (safety, efficacy)
- Immunity, long-term effects

By guiding the DNA-protein interactions you can control the (biological) world. Gene editing does it for you.

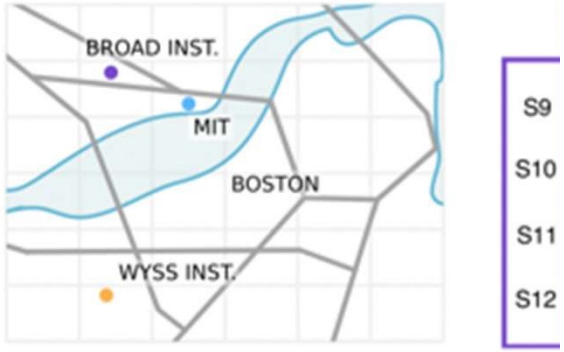
GIF

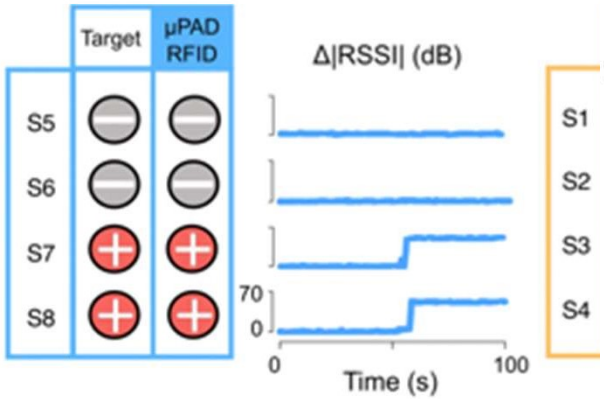
54 Tweet

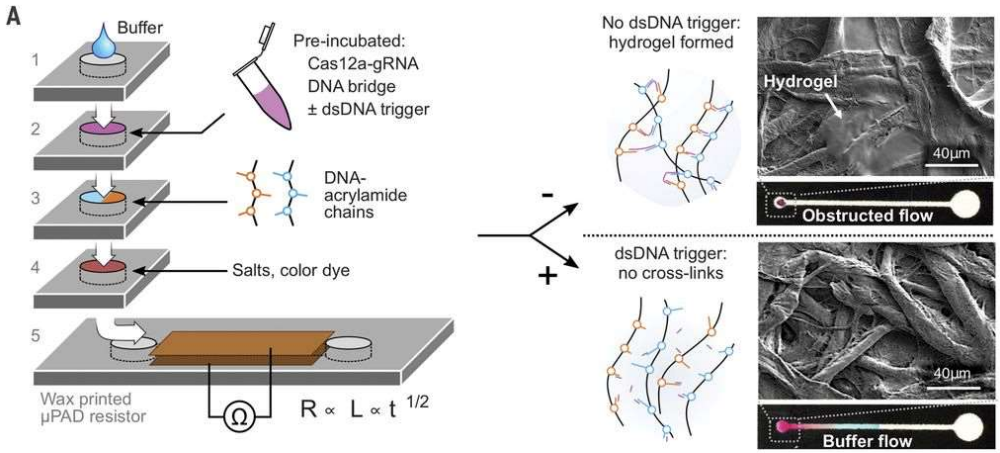
THANK YOU!

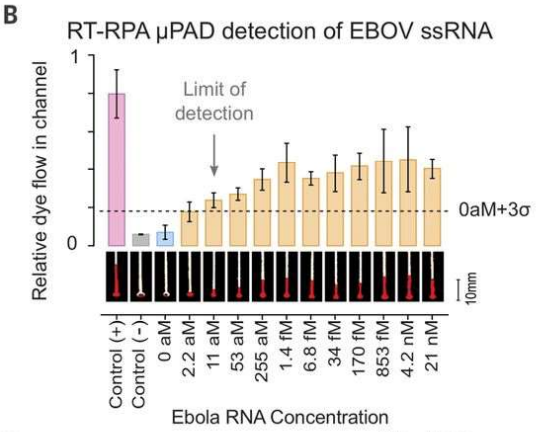
Financial support:

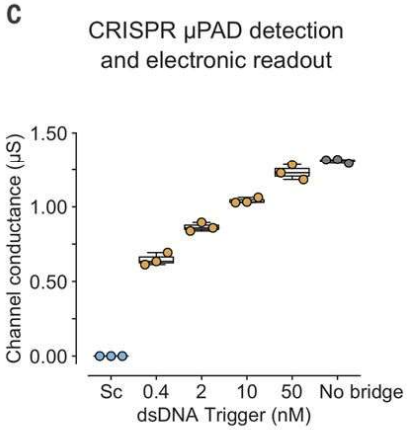
- **Suomen Akatemia**
- **Helsingin yliopisto**
- **HUS tutkimusvarat**
- **Valtion erikoissairaanhoidon tutkimusmääräraha**
- **Lastentautien tutkimussäätiö**
- **Paulon säätiö**
- **Georg och Mary Ehrnroth stiftelse**
- **Silmä- ja kudospankkisäätiö**

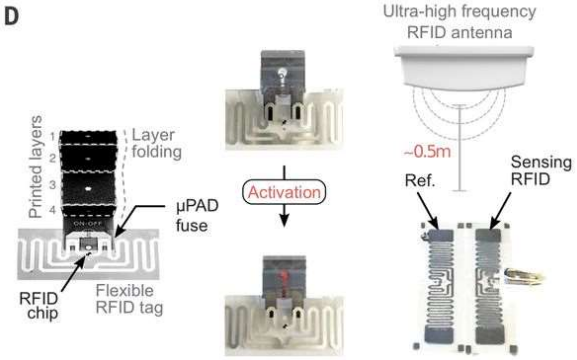


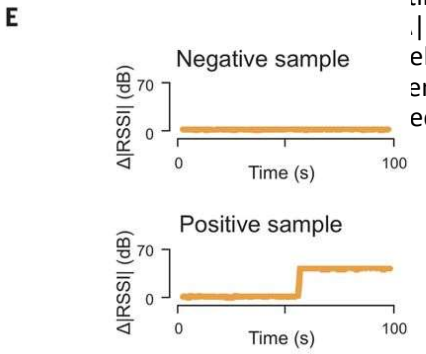












= 607,558

- Samples (S1-12) containing either 0 aM
incubated with the ssDNA gel bridging a
are shown adjacent to the samples. We
a positive result (the presence of EBOV
reference RFID tag, where a complete lo
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experimenter.

ere amplified by RT-RPA,
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ement of the twelve samples
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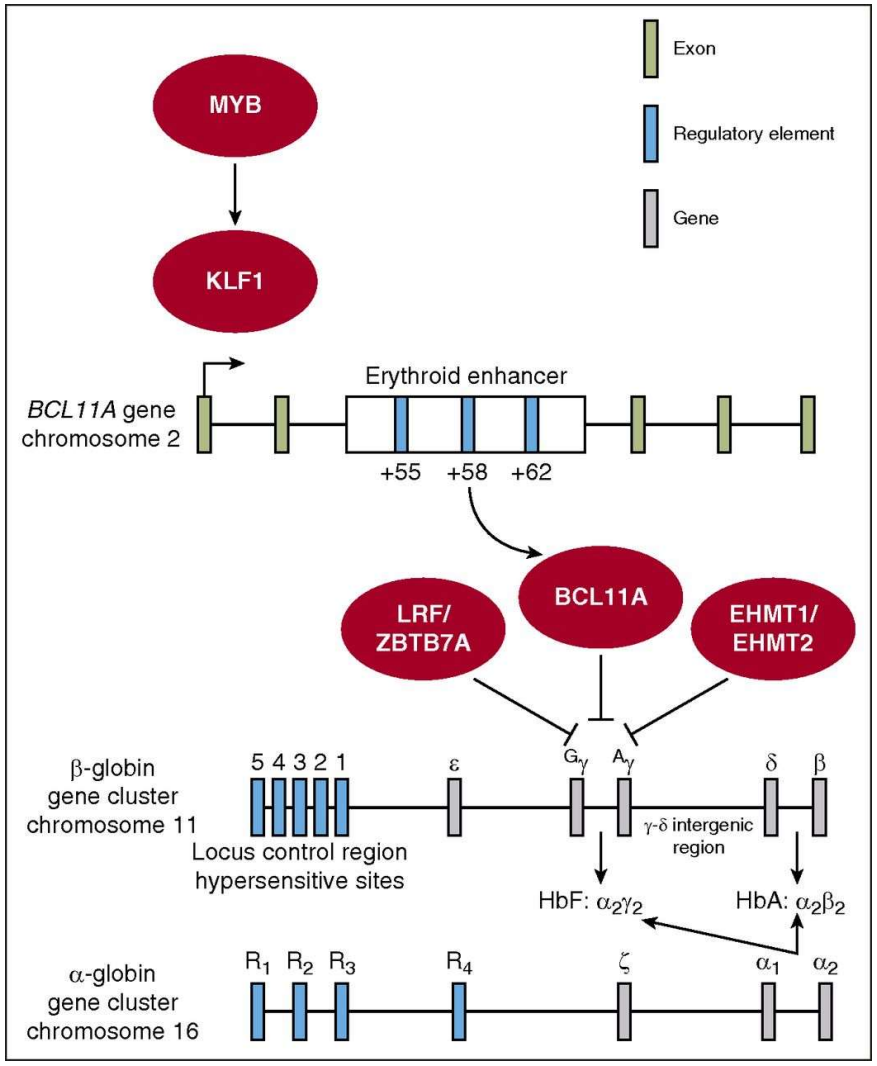

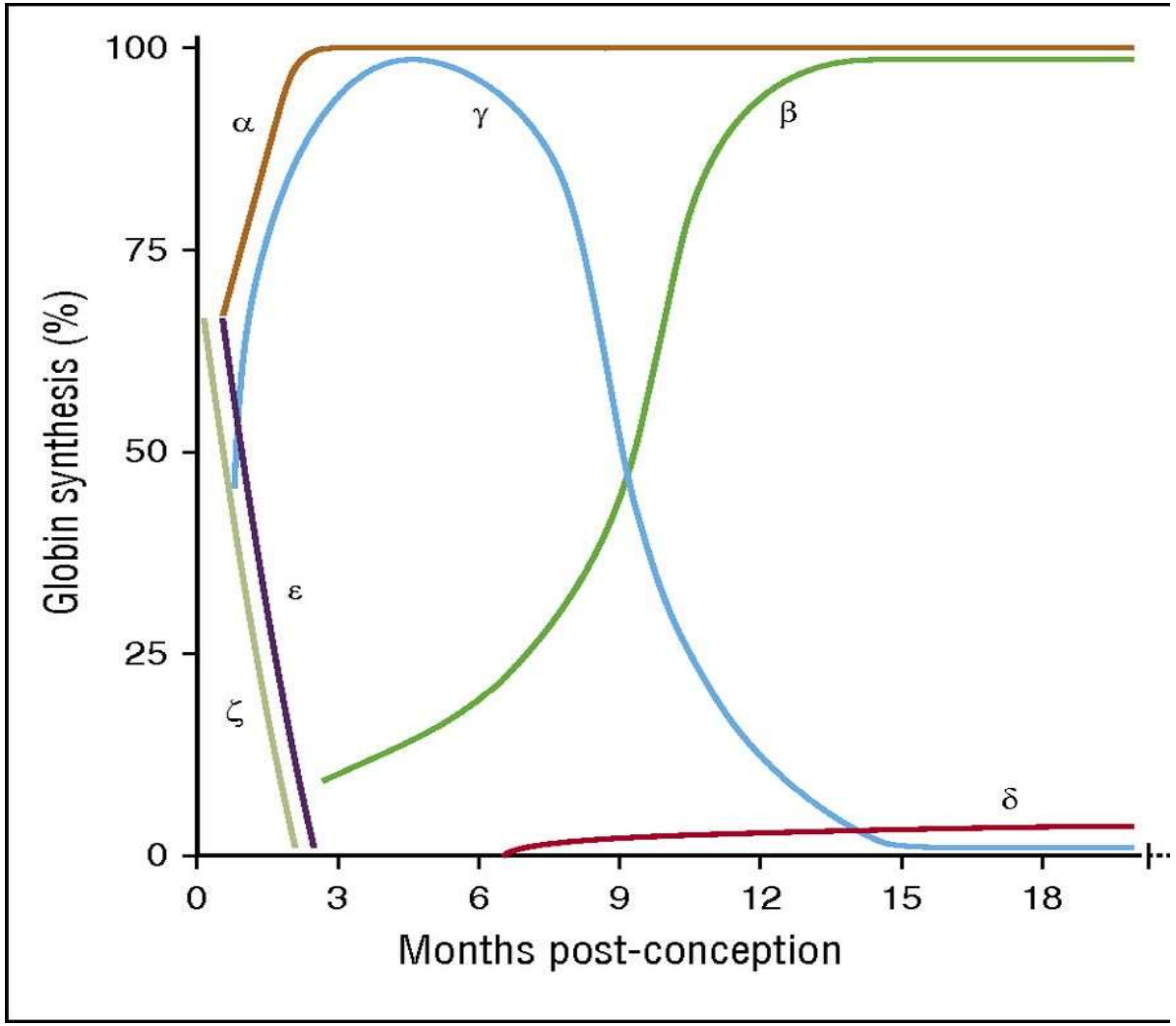

Matthew C. Canver et al. Blood 2016

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Geenitekniikka: Crispr-muokkausta kokeillaan muun muassa syöpään

Geenimuokkaus nujersi vaikean veritaudin

Yhdysvaltalaisessa kokeessa kolmen potilaan luuytimien solut saatiin korjattua terveiksi geenisaksiksi sanotulla menetelmällä. Siinä on yhä puutteensa.

Niko Kettunen

YHDYSVALLOISSA on onnistuttu parantamaan kolmelta ihmiseltä hengenvaarallinen verisairaus geenimuokkauksen avulla.

Potilaat kärsivät aiemmin jatkuvista verisuonten tukkeumista ja tarvitsivat kuukausittaisia verensiirtoja pysyäkseen hengissä.

Kokeellisessa hoidossa heidän luuytimiensä kantasoluja muokattiin niin, että ne tuottavat nyt terveitä punasoluja.

Ensin hoidettu koehenkilö on selvinnyt jo 15 kuukautta ilman verensiirtoja. Jos potilaiden tila pysyy vakaana, he ovat käytännössä parantuneet sairaudestaan.

KOKEEN takana on kaksi lääkeyhtiötä, Crispr Therapeutics ja Vertex. Tutkijat julkistivat alustavia tuloksia veritautitutkijoiden verkkokokouksessa kesäkuussa.

Kaksi hoidetuista sairasti beetatalassemiaa ja yksi sirppisoluanemiaa. Ne ovat samankaltaisia sairauksia. Niissä veren hemoglobiiniproteiinit eivät muodostu oikein. Sairauksia on pääosin väestöllä, jonka juuret ovat Afrikassa.

Sirppisoluanemiassa punasolut kaareutuvat sirppimäisiksi ja ne takkuuntuvat verisuoniin, jolloin happi ei kulje kunnolla. Taudin vakavinta muotoa sairastavat tarvitsevat kuukausittaisia verensiirtoja. Niiden avulla he voivat elää 40–50-vuotiaiksi.

Yksi potilaista on 34-vuotias kolmen lapsen äiti **Victoria Gray**. Ennen hoitoa hän joutui käymään jatkuvasti sairaalassa kipujen takia, minkä lisäksi hän sai säännöllisesti uutta verta.

"Kipu oli niin pahaa, että se lamautti", Gray kertoo uutiskanava NPR:n haastattelussa.

Nyt Gray on selvinnyt yhdeksän kuukautta ilman verensiirtoja ja elää täysin normaalia elämää. Hänen luuytimensä muokatut kantasolut näyttävät toimivan oikein ja tuottavat niin sanottua sikiöhemoglobiinia.

Verisolukoe oli tauolla pandemian takia, mutta jatkuu. Mukana on 90 potilasta. Heistä puolet sairastaa betatalassemiaa ja puolet sirppisoluanemia.

KANTASOLUBIOLOGIAN dosentti, perinnöllisyyslääkäri **Kirmo Wartiovaara** pitää hoitoa loistavana saavutuksena.

Hän kuitenkin huomauttaa, ettei tästä ole hoidoksi kaikille sirppisoluanemiasta kärsiville vielä pitkään aikaan. Hoito maksaa miljoonia ja siinä on riskejä.

"Nämä ensimmäiset kokeilut ovat niin kalliita, että vain rikkaimmat maat tai rikkaimmat vakuutusyhtiöt pystyisivät ne kustantamaan. Se on todellakin eliittilääketiedettä", Wartiovaara sanoo. Nyt hoitoa kehittävä lääkeyritys maksaa viulut.

Wartiovaaran ryhmä tutkii Helsingin yliopistossa vastaavanlaista verisolujen muokkausta.

"Teemme täysin samaa, mutta hieman eri kohtaan geeniperimässä. Yritämme samalla tavalla saada sikiöhemoglobiinia nousemaan verisoluissa, ja se näyttää onnistuvan."

MITÄ kokeessa tehtiin? Hoito perustuu crispr-cas9 -geenitekniikkaan.

Sillä voidaan tarkasti muokata yksittäisten geenien toimintaa. Viallinen geeni voidaan kopauttaa pois toiminnasta tai leikata ja liimata sen tilalle pätkä haluttua dna:ta.

> "Pitää punnita, otatko 0,1 prosentin riskin."
>
> **Dosentti Kirmo Wartiovaara**

Geenejä muokkaava aine ohjelmoidaan laboratoriossa ja siirretään soluun. Muokkaus perustuu pätkään ohjaavaa rna-molekyyliä, jonka avulla leikkuri löytää oikean kohdan solun dna:sta.

Sitten bakteerilta peräisin oleva entsyymi saksii dna:n kaksoiskierteen auki. Leikkurin rna toimii mallina, kuin reikäkorttina, jonka avulla solu korjaa leikatun dna:n halutuksi.

Ihmisen elimistössä verta tuottavat luuytimen kantasolut. Potilailta otettiin näitä kantasoluja, muokattiin niitä, ja ruiskutettiin takaisin luuytimeen.

TUTKIJAT eivät suoraan korjanneet sitä geeniä, jonka takia hemoglobiini ei muodostu oikein.

Siinä oli se riski, että solut ryhtyvät tuottamaan normaalia aikuisten hemoglobiinia, jollaista näiden potilaiden elimistössä ei ole koskaan muodostunut. Se olisi näin ollen ollut vieras proteiini, jota elimistö olisi voinut hyökkäyksellä.

Sen sijaan tutkijat muokkasivat toista kohtaa kantasolujen dna:sta niin, että solut tuottavatkin nyt sikiöiden hemoglobiinia, jonka tuotanto normaalisti lopputuu ihmisen syntymän aikoihin.

"He poistivat pienen palan dna:sta, minkä seurauksena solut ikään kuin kuvittelevat olevansa sikiössä. Geenivirhe saa olla siellä, sillä ei ole enää välliä", Wartiovaara sanoo.

Elimistö ei hylji näitä muokattuja soluja, sillä ne ovat samanlaisia kuin potilaan oma elimistö on aikoinaan äidin kohdussa tuottanut. Kun muutos on nyt tehty, kantasolujen pitäisi toimia normaalisti läpi elämän.

LUUYTIMEN kantasolusiirtoja on toki tehty jo kymmeniä vuosia esimerkiksi sirppisoluanemian hoitamiseksi.

Niissä kantasolujen luovuttajan täytyy kuitenkin olla lähisukulainen, eikä kaikille potilaille löydy luovuttajaa. Crispr-tekniikalla voidaan nyt korjata suoraan henkilön omaa elimistöä.

Hoidossa on riskinsä. Crispr-leikkurin tiedetään joskus tekevän vahingollisia muutoksia dna:han. Ne voivat johtaa syövän kehittymiseen. Riski on pieni, mutta pitää ottaa huomioon.

"Pitää punnita, otatko 0,1 prosentin riskin sairastua syöpään, mutta vältät sadan prosentin riskin, että kuolet tautiin 45-vuotiaana. Aika moni varmasti hyväksyisi riskin. Mutta esimerkiksi sairaan viisivuotiaan lapsen äidille kysymys voi olla mutkikkaampi", Wartiovaara pohtii.

Ihmisalkioilla tehdyissä kokeissa havaittiin aivan äskettäin, että muokatuista soluista saattoi kadota kokonainen kromosomi, kun tarkoitus oli korjata vain yhtä geeniä. Testimielessä muokatusta 18 alkiosta viidesosaan tuli hallitsemattomia muutoksia. Crispr saattoi leikata pois tuhansia emäspareja.

CRISPR-MUOKKAUSTA käytetään nyt jo useissa ihmiskokeissa ympäri maailmaa. Kiinassa on yritetty hoitaa syöpää sen avulla.

Kiinalaistutkijat muokkasivat potilaiden immuunisoluja niin, että ne hyökkäisivät ärhäkämmin kasvainten kimppuun. Ne eivät saa olla liian ärhäköitä, ettevät ne vahingolta terveyttä kuluta.

Koe ei parantanut potilaiden syöpää lopullisesti. Se osoitti ainakin, että tekniikka vaikuttaa turvalliselta ja sitä voidaan kehittää eteenpäin.

Yhdysvalloissa puolestaan yritetään parantaa sokeuteen johtavaa perinnöllistä silmäsairautta. Tässä kokeessa tutkijat ohjelmoivat viruksia kantamaan geenivirheen korjaavaa crispr-mekanismia ja ruiskuttavat näitä viruksia silmään.

Virukset hakeutuvat soluihin ja muokkaavat vialliset geenit kuntoon. Näkökyvyn rappeutuminen pysähtyy tai ainakin hidastuu merkittävästi.

AIKUISEN elimistön muokkauksessa on rajansa.

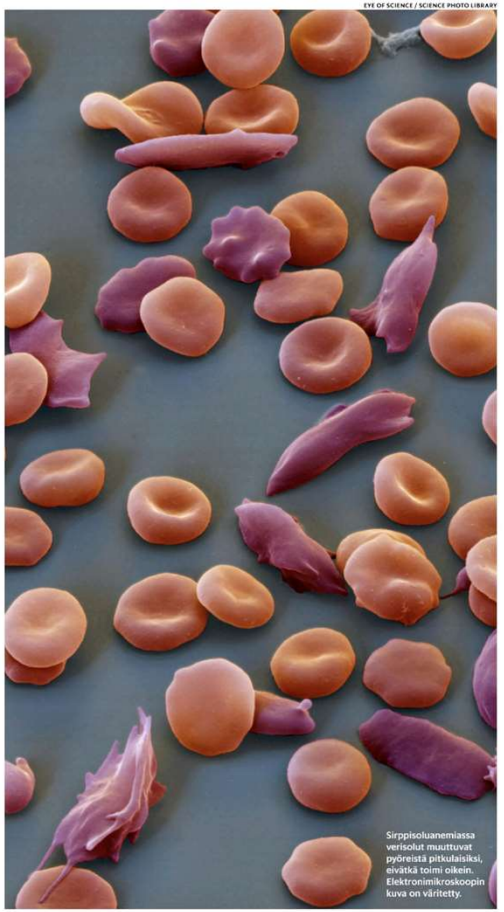
Luuytimen solujen kaltaisia kantasoluja on sinänsä helppo muokata, kun ne elimistöön siirrettynä tuottavat jatkossa lisää terveitä soluja.

Suuri osa elintemme ja kehomme soluista eivät kuitenkaan enää aikuisena uusiudu. Ne täytyy korjata yksitellen, ja se on tehtävä virusten avulla.

Koska vaikkapa sydämessä on soluja valtavasti, täytyy geenivirheiden korjaukseen ruiskuttaa miljoonia viruksia, jotta saataisiin edes osa soluista korjattua. Miljoonat virukset täytyy valmistaa laboratoriossa, eikä ihmisen elimistö välttämättä kestä tällaista viruslastia, vaikkei kyse olekaan tartuttavista viruksista.

"Hoitavan geenin saaminen joka ikiseen soluun on hankala pulonkauta", Wartiovaara sanoo.

"Veri on poikkeus, koska sitä on niin helppo kasvattaa. Ei tarvitse olla kovinkaan monta kantasolua, muutamalla kymmenellä tuhannella päästään jo aika pitkälle. Näin on varsinkin, jos riittää, että osa henkilön soluista on terveitä. Isossa ruiskussa luuydinnäytettä niitä on jo viisi miljoonaa."



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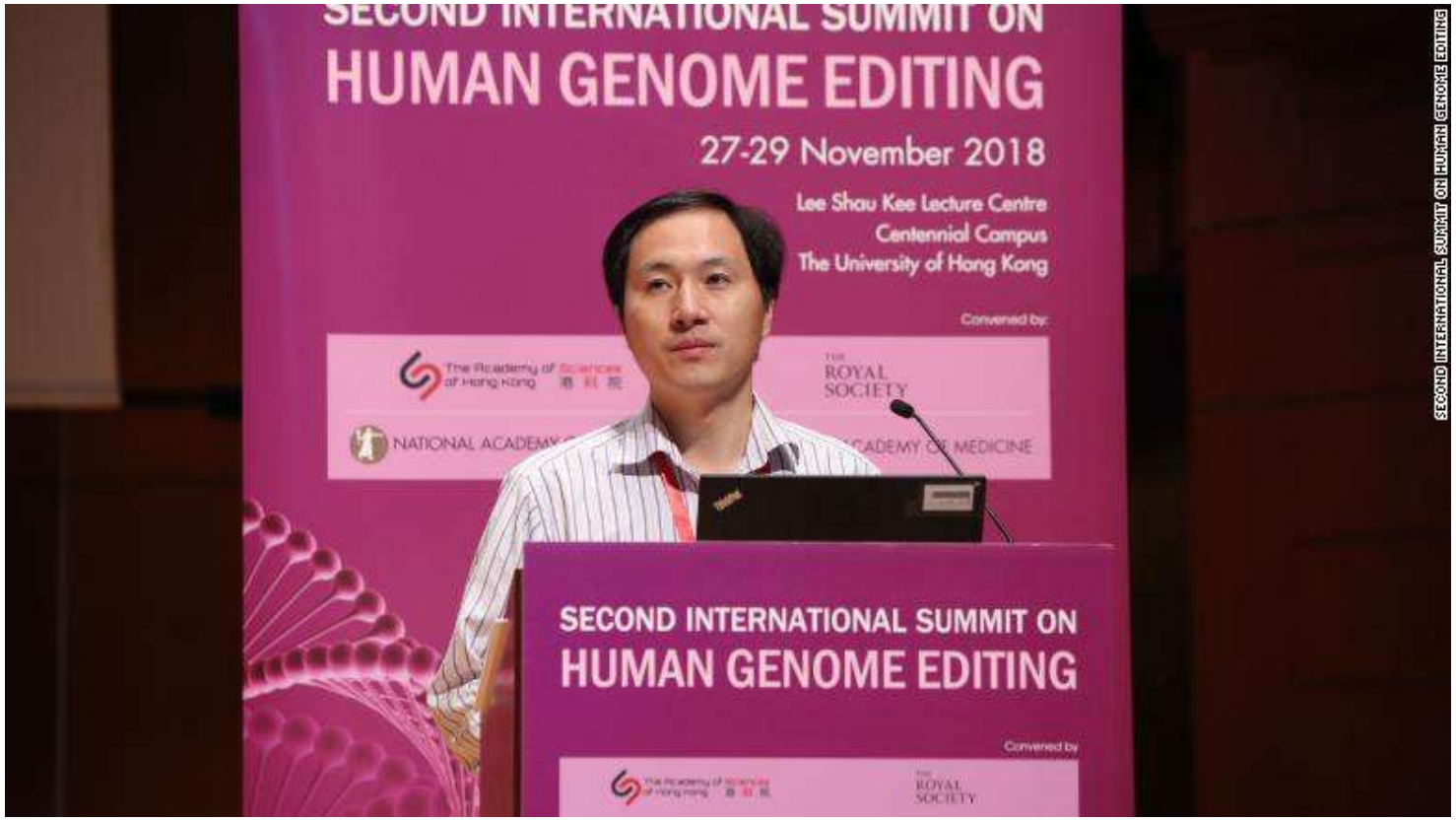
Sirppisoluanemiassa verisolut muuttuvat pyöreistä pitkulaisiksi, eivätkä toimi oikein. Elektronimikroskoopin kuva on väritetty.

He Jiankui 2018: ”First genetically edited babies are born”



nature International weekly journal of science

NATURE | NEWS FEATURE

Welcome to the CRISPR zoo

Birds and bees are just the beginning for a burgeoning technology.

Sara Reardon

09 March 2016