CRISPR: The Next Health Care Revolution

Natalia Rivera-Torres, Ph.D.

April 6th, 2023

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Gene Editing Institute

About Me

Education

- B.S. in Molecular and Cellular Biology-UPRRP
- M.S. in Biology-DSU
- Ph.D. in Medical and Molecular Sciences- UD

Principal Investigator CC-GEI

- Identifying new clinically-relevant genomic targets for which CRISPR-directed gene editing can be utilized as a therapeutic modality.

- Pre-clinical development for CCGEI-101 FDA package

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The Gene Editing Revolution

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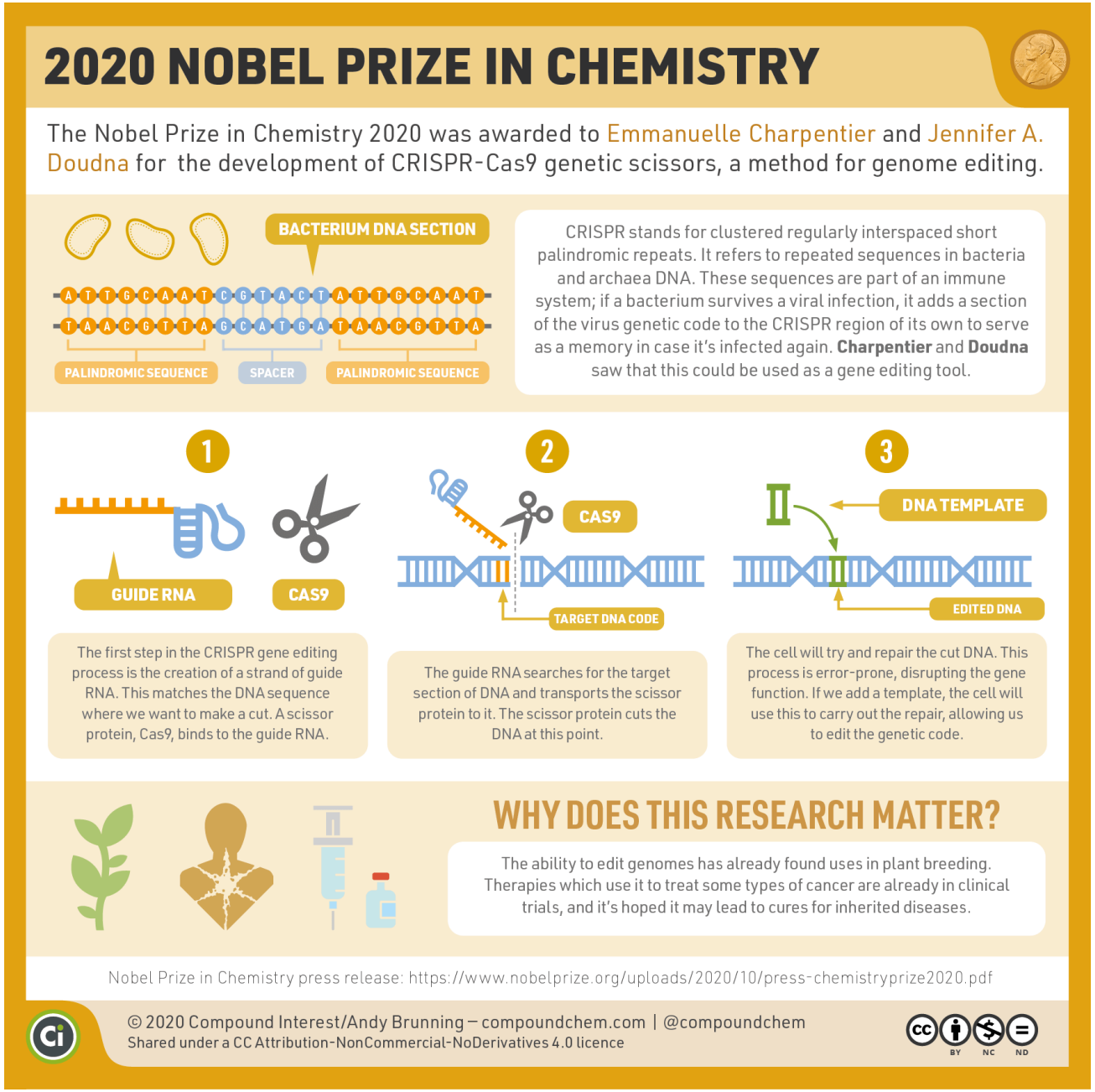
The Nobel Prize in Chemistry 2020 was awarded jointly to Emmanuelle Charpentier and Jennifer A. Doudna "for the development of a method for genome editing."

Jennifer A. Doudna

Prize share: 1/2

Emmanuelle Charpentier

Prize share: 1/2



The Nobel Prize in Chemistry 2020. NobelPrize.org. Nobel Media AB 2020. Thu. 12 Nov 2020. https://www.nobelprize.org/prizes/chemistry/2020/summary/

Forefront of Medical Science

GEI delivers scientific excellence in a patient-care setting, which represents the true potential of theoretical processes to become meaningful diagnostic tools and precision medicine that improves health and, in the long term, offers healing to the hopeless.

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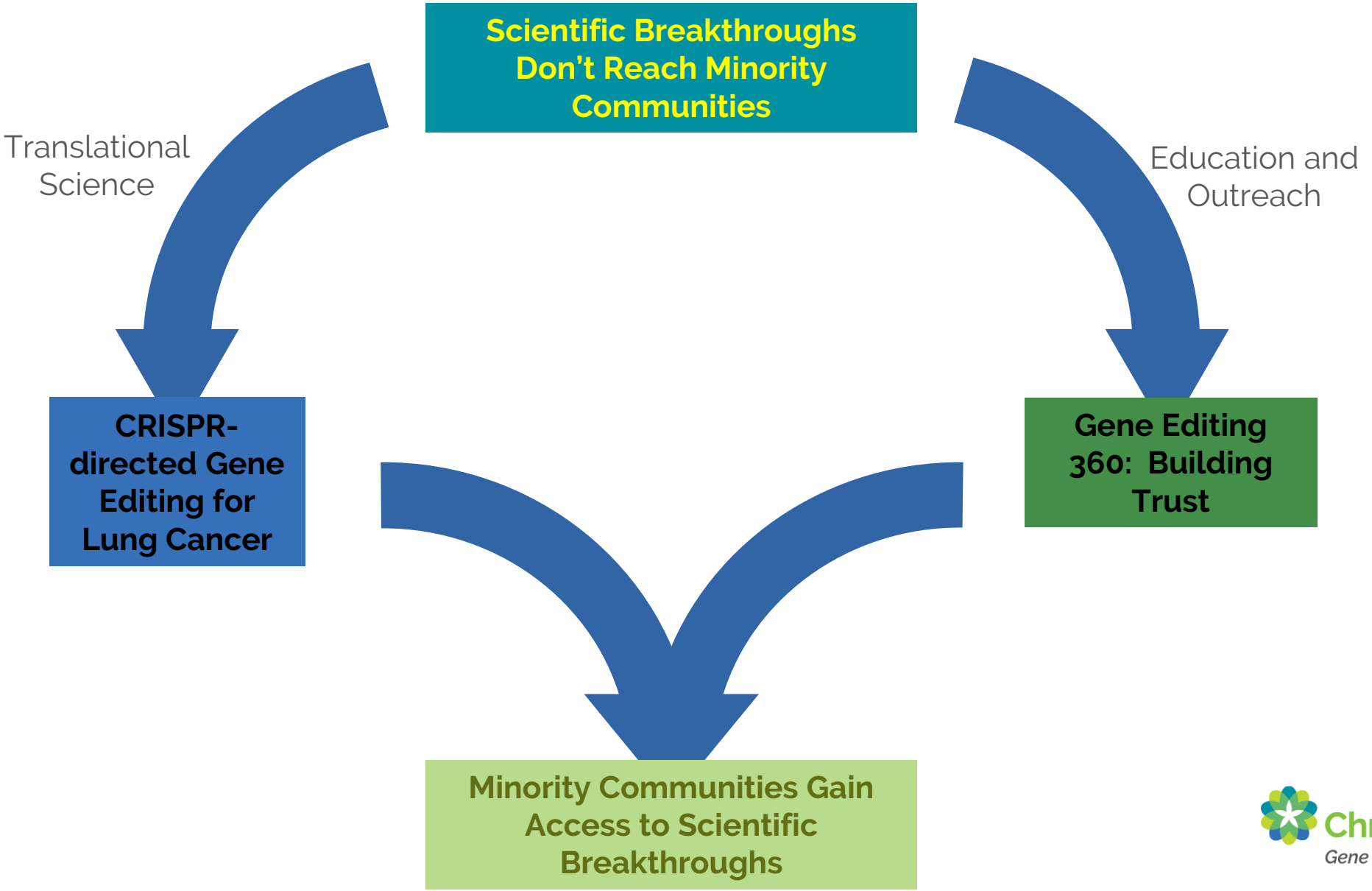
Our **Commitment**



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Molecular Therapy
Nucleic Acids
Original Article

AMERICAN SOCIETY of GENE & CELL THERAPY

gRNA Sequence Heterology Tolerance Catalyzed by CRISPR/Cas in an *In Vitro* Homology-Directed Repair Reaction

Amanda M. Hewes,[1] Brett M. Sansbury,[1,2] Shaul Barth,[3] Gabi Tarcic,[3] and Eric B. Kmiec[1,2]

[1]Gene Editing Institute, Helen F. Graham Cancer Center & Research Institute, Christiana Care Health System, Newark, DE, USA; [2]Department of Medical and Molecular Sciences, University of Delaware, Newark, DE, USA; [3]Novellus, Jerusalem Bio-Park, 1st Kiryat Hadassah, Hadassah Ein-Kerem Medical Center Campus, Jerusalem, Israel, 9112001

CRISPR and associated Cas nucleases are genetic engineering tools revolutionizing innovative approaches to cancer and inherited diseases. CRISPR-directed gene editing relies heavily on proper DNA sequence alignment between the guide RNA (gRNA)/CRISPR complex and its genomic target. Accurate hy-

template to mend the break site.[1] In the normal lifespan of an organism, HDR occurs during and as a result of meiosis with sister chromatids crossing over or providing genetic information to repair a damaged site;[2] it is presumably error free. In human gene editing, it is anticipated that these HDR pathways will direct gene repair at a

International Journal of
Molecular Sciences

MDPI

Editorial

On the Origins of Homology Directed Repair in Mammalian Cells

Brett M. Sansbury and Eric B. Kmiec *

Gene Editing Institute, Helen F. Graham Cancer Center & Research Institute, ChristianaCare, Newark, DE 19713, USA; Brett.Sansbury@ChristianaCare.org
* Correspondence: Eric.B.Kmiec@christianacare.org

Abstract: Over the course of the last five years, expectations surrounding our capacity to selectively modify the human genome have never been higher. The reduction to practice site-specific nucleases designed to cleave at a unique site within the DNA is now centerstage in the development of effective molecular therapies. Once viewed as being impossible, this technology now has great potential and, while cellular and molecular barriers persist to clinical implementations, there is little doubt that these barriers will be crossed, and human beings will soon be treated with gene editing tools. The

International Journal of
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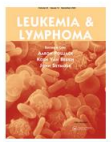
Review

A Consensus Model of Homology-Directed Repair Initiated by CRISPR/Cas Activity

Kevin Bloh [1,2] and Natalia Rivera-Torres [1,*]

[1] Gene Editing Institute, Helen F. Graham Cancer Center & Research Institute, ChristianaCare, 4701 Ogletown-Stanton Road, Newark, DE 19710, USA; kbloh@udel.edu
[2] Department of Medical and Molecular Sciences, University of Delaware, Newark, DE 19710, USA
* Correspondence: Natalia.riveratorres@christianacare.org; Tel.: +1-302-623-4754

Abstract: The mechanism of action of ssODN-directed gene editing has been a topic of discussion within the field of CRISPR gene editing since its inception. Multiple comparable, but distinct, pathways have been discovered for DNA repair both with and without a repair template oligonucleotide. We have previously described the ExACT pathway for oligo-driven DNA repair, which consisted of a two-step DNA synthesis-driven repair catalyzed by the simultaneous binding of the repair oligonucleotide (ssODN) upstream and downstream of the double-strand break. In order to better



Taylor & Francis

Leukemia & Lymphoma

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/ilal20

Modeling pediatric AML FLT3 mutations using CRISPR/Cas12a- mediated gene editing

Natalia Rivera-Torres, Kelly Banas & Eric B. Kmiec

To cite this article: Natalia Rivera-Torres, Kelly Banas & Eric B. Kmiec (2020) Modeling pediatric AML FLT3 mutations using CRISPR/Cas12a- mediated gene editing, Leukemia & Lymphoma, 61:13, 3078-3088, DOI: 10.1080/10428194.2020.1805740

To link to this article: https://doi.org/10.1080/10428194.2020.1805740

MOLECULAR CANCER RESEARCH | NEW HORIZONS IN CANCER BIOLOGY

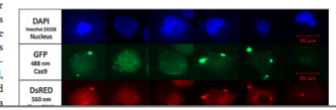
Kinetics of Nuclear Uptake and Site-Specific DNA Cleavage during CRISPR-Directed Gene Editing in Solid Tumor Cells

Kelly Banas[1,2], Natalia Rivera-Torres[1], Pawel Bialk[1], Byung-Chun Yoo[1], and Eric B. Kmiec[1,2]

ABSTRACT

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)–directed gene editing is approaching clinical implementation in cancer. Thus, it is imperative to define the molecular framework upon which safe and efficacious therapeutic strategies can be built. Two important reaction parameters include the biological time frame within which the CRISPR/Cas complex enters the nucleus and executes gene editing, and the method of discrimination that the CRISPR/Cas complex utilizes to target tumor cell, but not normal cell, genomes. We are developing CRISPR-directed gene editing for the treatment of non–small cell lung carcinoma

Visual Overview: http://mcr.aacrjournals.org/content/molcanres/18/6/891/F1.large.jpg.



genes

MDPI

Article

The Diversity of Genetic Outcomes from CRISPR/Cas Gene Editing is Regulated by the Length of the Symmetrical Donor DNA Template

Amanda M. Hewes [1], Brett M. Sansbury [1,2] and Eric B. Kmiec [1,2,*]

[1] Gene Editing Institute, Helen F. Graham Cancer Center & Research Institute, Christiana Care Health System, Newark, DE 19713, USA; Amanda.M.Hewes@ChristianaCare.org (A.M.H.); sansbury@udel.edu (B.M.S.)
[2] Department of Medical and Molecular Sciences, University of Delaware, Newark, DE 19716, USA
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Keywords: symmetrical homology arms; single-stranded donor template; homology directed repair; CRISPR/Cas12a; CRISPR/Cas9; gene editing

The CRISPR Journal
Volume 4, Number 1, 2021
Mary Ann Liebert, Inc.
DOI: 10.1089/crispr.2020.0022

The CRISPR Journal

RESEARCH ARTICLE

Deconvolution of Complex DNA Repair (DECODR): Establishing a Novel Deconvolution Algorithm for Comprehensive Analysis of CRISPR-Edited Sanger Sequencing Data

Kevin Bloh,[1,2,†] Rohan Kanchana,[1,†] Pawel Bialk,[1] Kelly Banas,[1,2] Zugui Zhang,[3] Byung-Chun Yoo,[1] and Eric B. Kmiec[1,2,*]

Abstract

During CRISPR-directed gene editing, multiple gene repair mechanisms interact to produce a wide and largely unpredictable variety of sequence changes across an edited population of cells. Shortcomings inherent to previously available proposal-based insertion and deletion (indel) analysis software necessitated the development of a more comprehensive tool that could detect a larger range and variety of indels while maintaining the ease of use of tools currently available. To that end, we developed *Deconvolution of Complex DNA Repair* (DECODR). DECODR can detect indels formed from single or multi-guide CRISPR experiments without a limit on indel

COMMUNICATIONS BIOLOGY

ARTICLE

https://doi.org/10.1038/s42003-019-0705-y OPEN

Understanding the diversity of genetic outcomes from CRISPR-Cas generated homology-directed repair

Brett M. Sansbury[1,2], Amanda M. Hewes[2] & Eric B. Kmiec[1,2*]

Gene Therapy (2021) 28:105–113
https://doi.org/10.1038/s41434-020-00192-z

BRIEF COMMUNICATION

Precise and error-prone CRISPR-directed gene editing activity in human CD34+ cells varies widely among patient samples

Shirin R. Modarai[1] · Sambee Kanda[1] · Kevin Bloh[1] · Lynn M. Opdenaker[2] · Eric B. Kmiec[1]

Received: 22 March 2020 / Revised: 5 August 2020 / Accepted: 19 August 2020 / Published online: 1 September 2020

Abstract

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and their associated CRISPR-associated nucleases (Cas) are among the most promising technologies for the treatment of hemoglobinopathies including Sickle Cell Disease (SCD). We are only beginning to identify the molecular variables that influence the specificity and the efficiency of CRISPR-directed gene editing, including the position of the cleavage site and the inherent variability among patient samples selected for CRISPR-directed gene editing. Here, we target the beta globin gene in human CD34+ cells to assess the impact of these two variables and find that both contribute to the global diversity of genetic outcomes. Our study demonstrates a unique genetic profile of indels that is generated based on where along the beta globin gene attempts are made to correct the SCD

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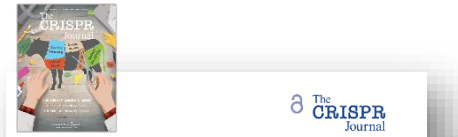
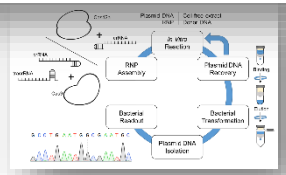
From Discovery to Product…





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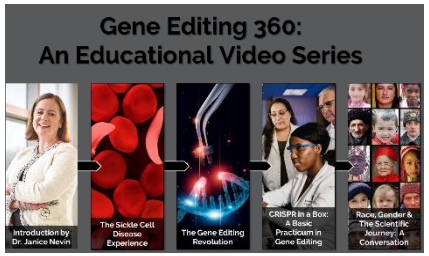

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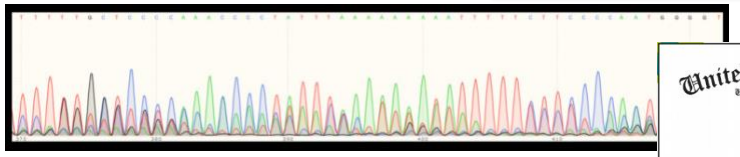
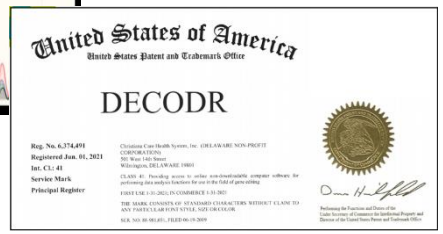
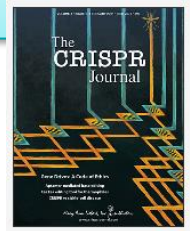
In vitro system and protocol	CIAB developed to support grant	Educational platform supports product & cause with DETV	CIAB Complete Education Kit in action in classroom labs	National distribution

2018 2019 2020 2021

1	2	3	4
Search for better Sanger deconvolution approach	DECODR patented, published & well received	Development team improves product for commercial users	DECODR adopted on leading platform







58

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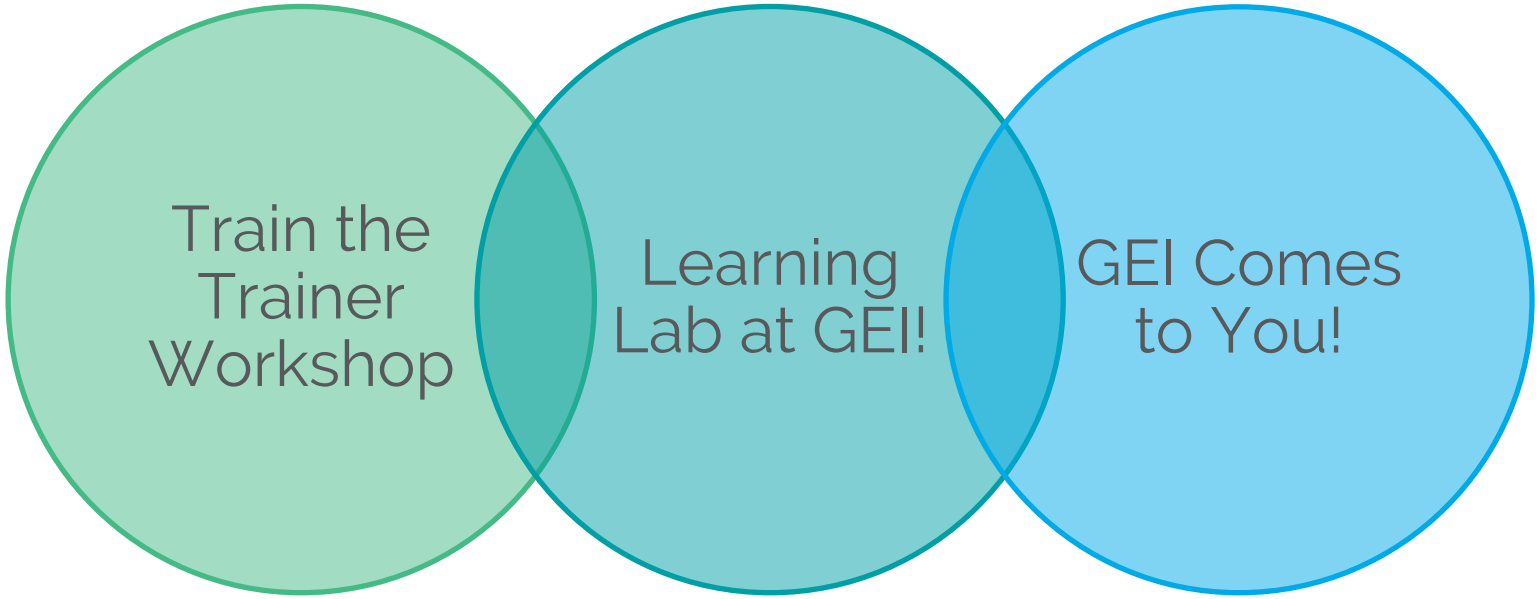
What is Gene Editing 360™?

Gene Editing 360™ is GEI's signature education platform designed to deliver educational programs and resources while also providing foundational information about CRISPR gene editing for high school and college students.

Core Programs



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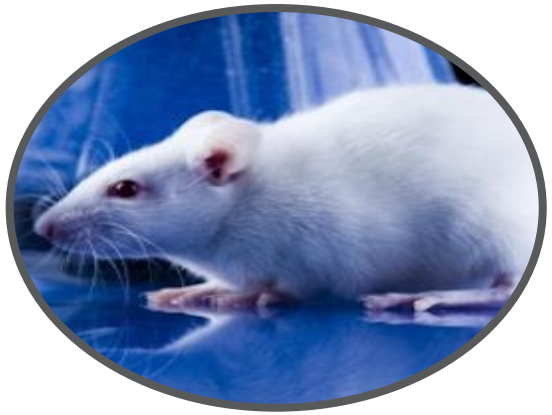
Gene Editing:
Applications & Current Research

Drug Development

- Eliminate HIV
- Cancer immunotherapy
- Repair genetic blindness



Animal Models

- Model human disease
- Universal transplant organs
- Huntington's disease

Agriculture

- Control pesticide resistance
- Sustainable, storable foods
- Accelerated growth crops

Gene Drives

- Disease prevention
- Eliminate malaria
- Control invasive species

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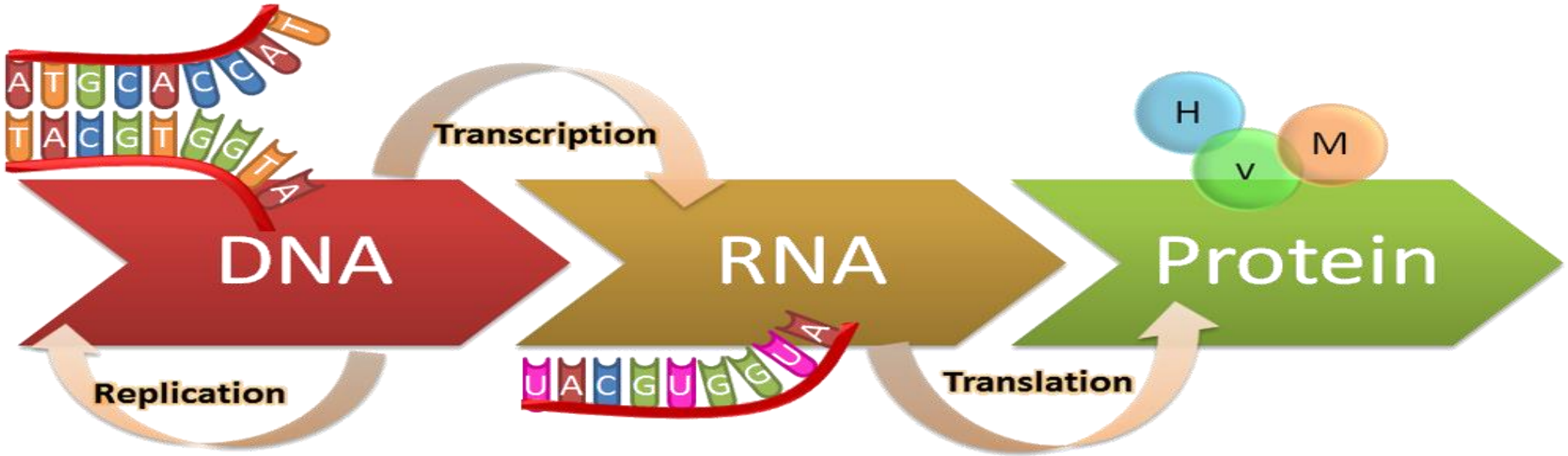
The Central Dogma of Biology



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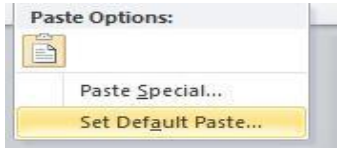
Editing?

Cutting

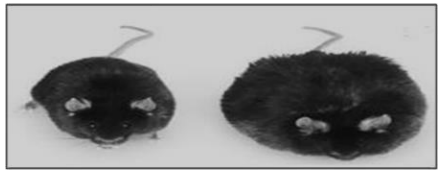
Pasting



Changing

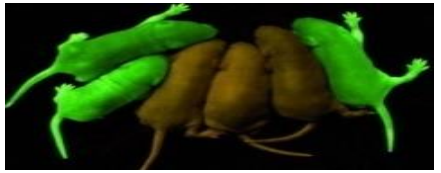
Gene Editing?

Deleting
Knockout



Leptin

Inserting
Transgenic



GFP

Replacing
Knock-in

Rhodopsin GFP

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Gene Edited DNA Repair

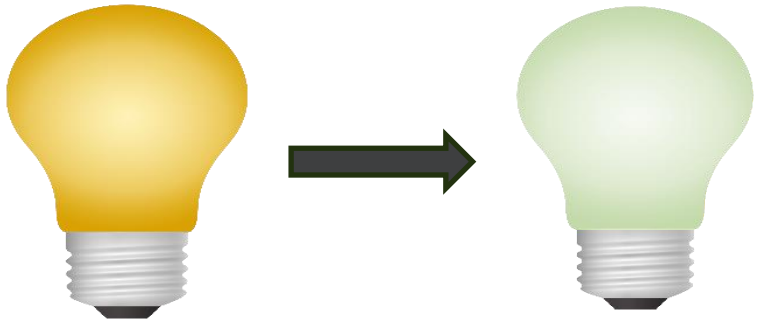
5’-TGACCACCCTGACCTACGGCGT GCAGTGCTTCAGCCGCTATCGA-3’
3’-ACTGGTGGGACTGGATGCCGCA CGTCACGAAGTCGGCGATAGCT-5’

Non-Homologous End Joining

5’-CCCTGACCTACGGCG GTGCTTCAGCCGC-3’
3’-GGGACTGGATGCC CGAAGTCGGCG-5’

5’-CCCTGACCTACGG------GCTTCAGCCGC-3’
3’-GGGACTGGATGCC------CGAAGTCGGCG-5’

Knock-Out



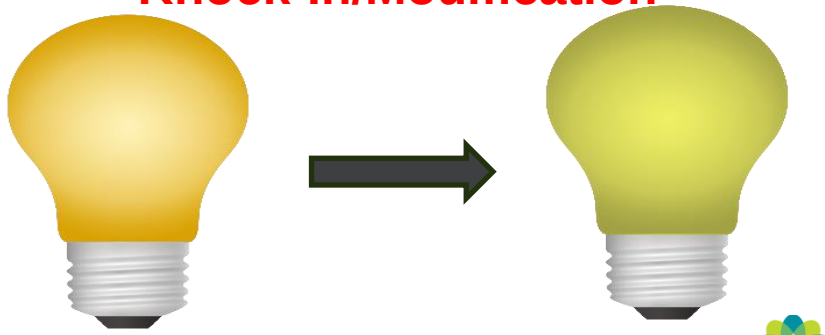
Homology-Directed Repair

5’-CCCTGACCTACGGCGT GCAGTGCTTCAGCCGC-3’
3’-GGGACTGGATGCCGCA CGTCACGAAGTCGGCG-5’

ssDNA

5’-CCCTGACCTACGGCGT TCAGTGCTTCAGCCGC-3’
3’-GGGACTGGATGCCGCA AGTCACGAAGTCGGCG-5’

Knock-In/Modification



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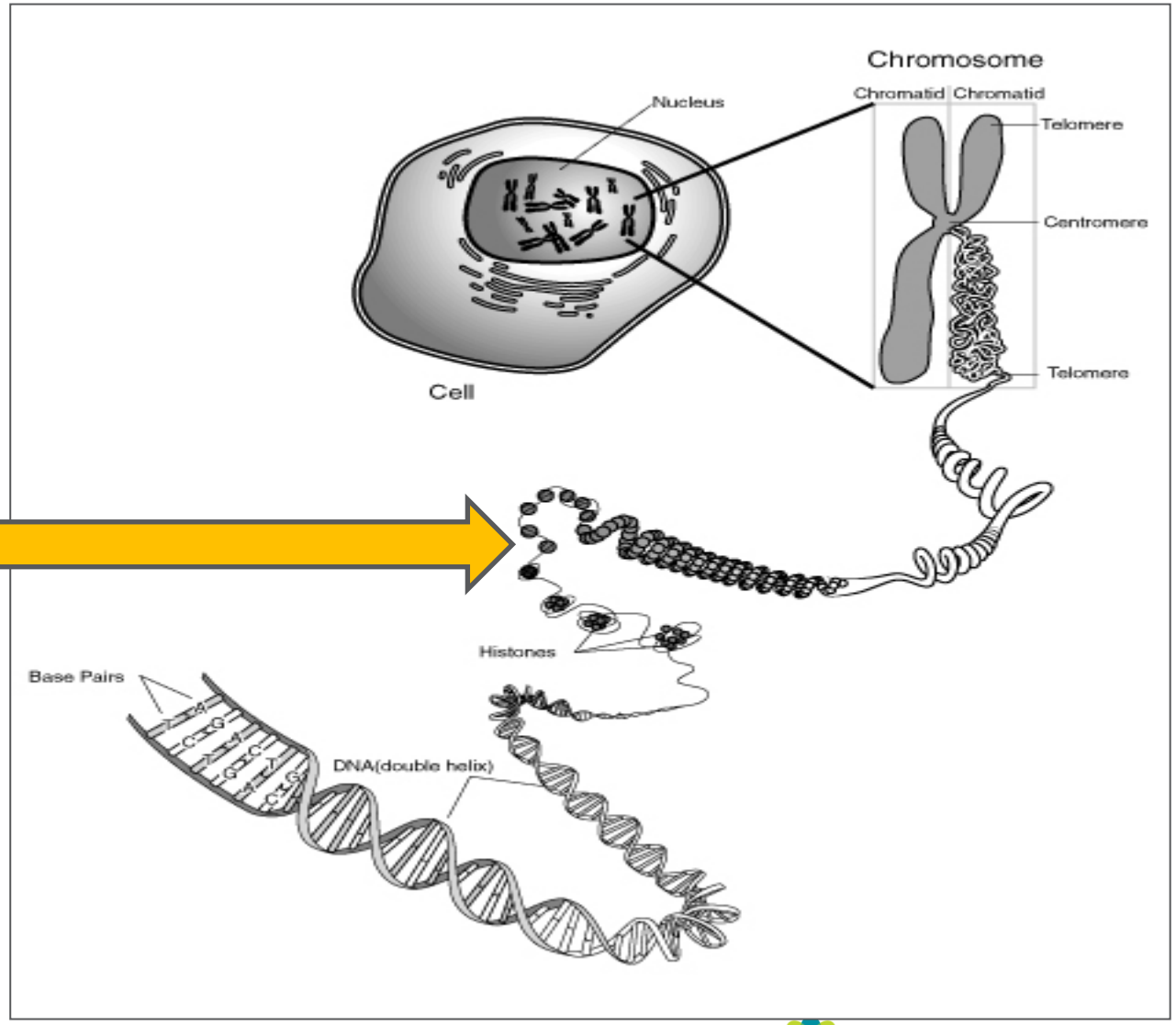
Cas9 Nuclease

CRISPR-guide RNA



CRISPR/Cas9 System

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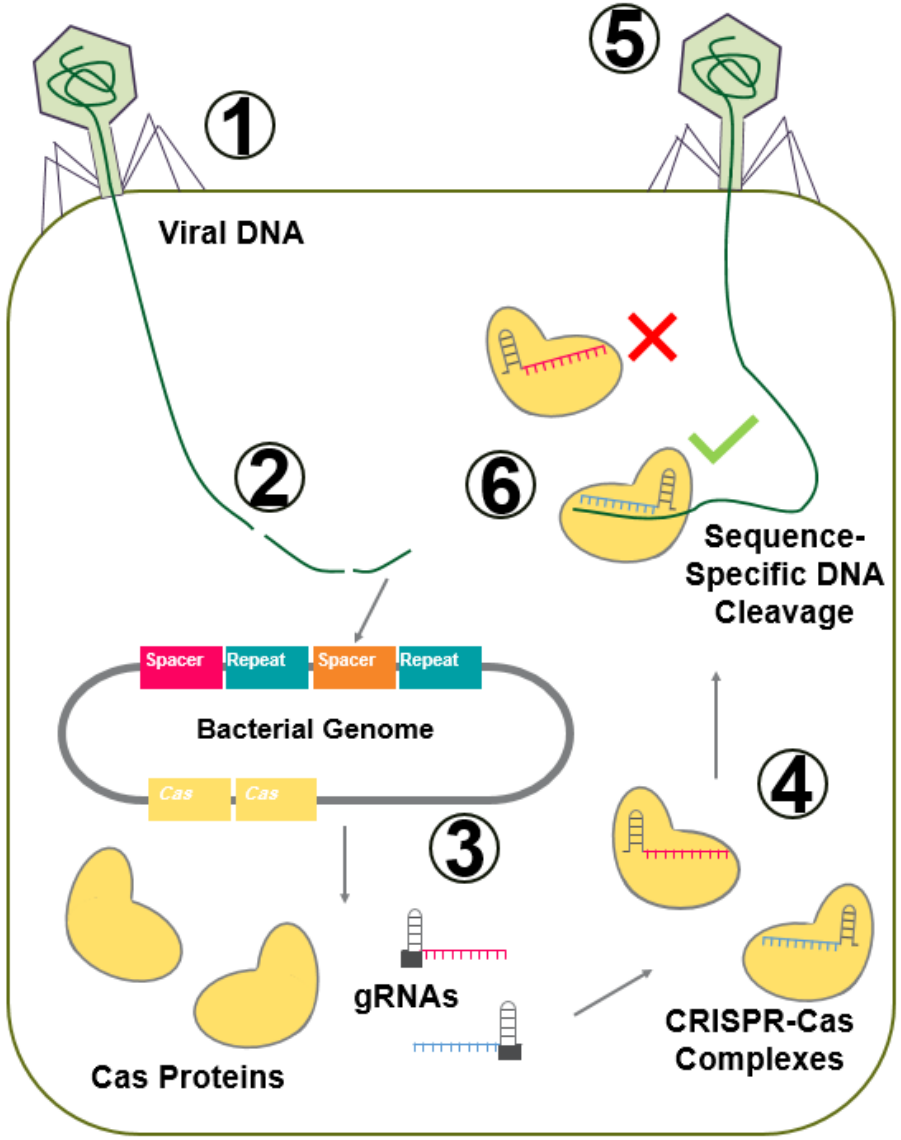
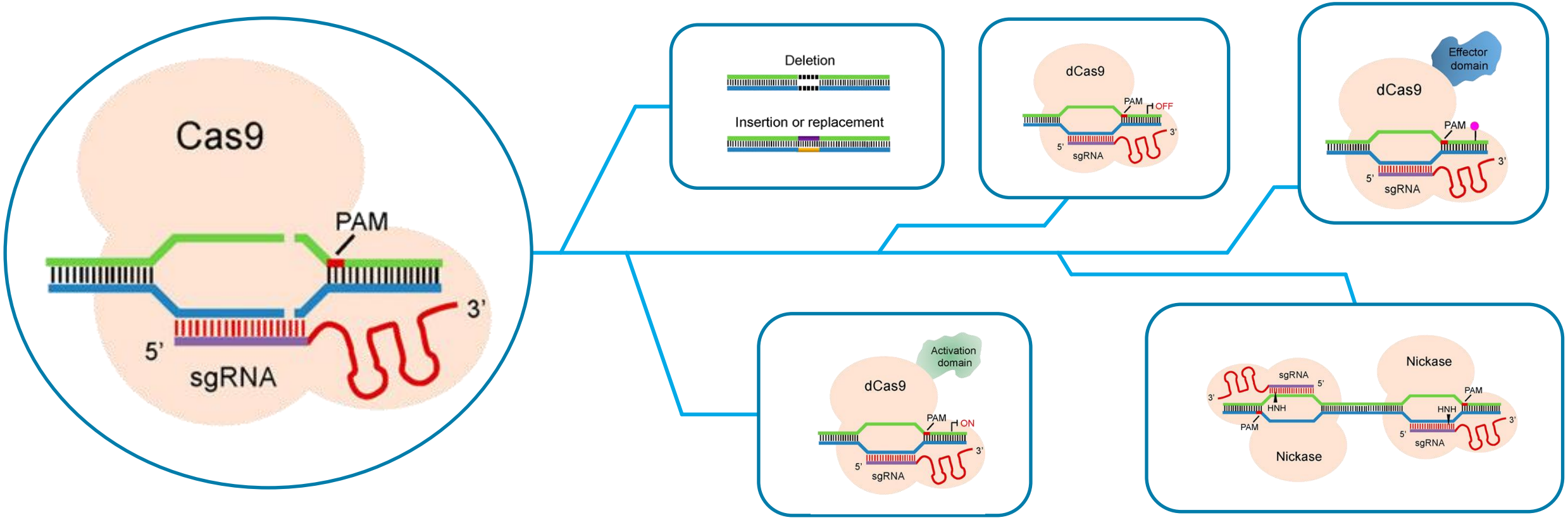
CRISPR in Nature: A Bacterial Defense

1. Viral infection
2. Viral DNA integrates into host genome
3. CRISPR components are produced
4. Formation of tracrRNA:crRNA complexes
5. Viral reinfection
6. CRISPR-directed DNA Cleavage



The Gene Editing Revolution



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Human Genome Editing, Ethics & Policy

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A Powerful Tool for Medical Research

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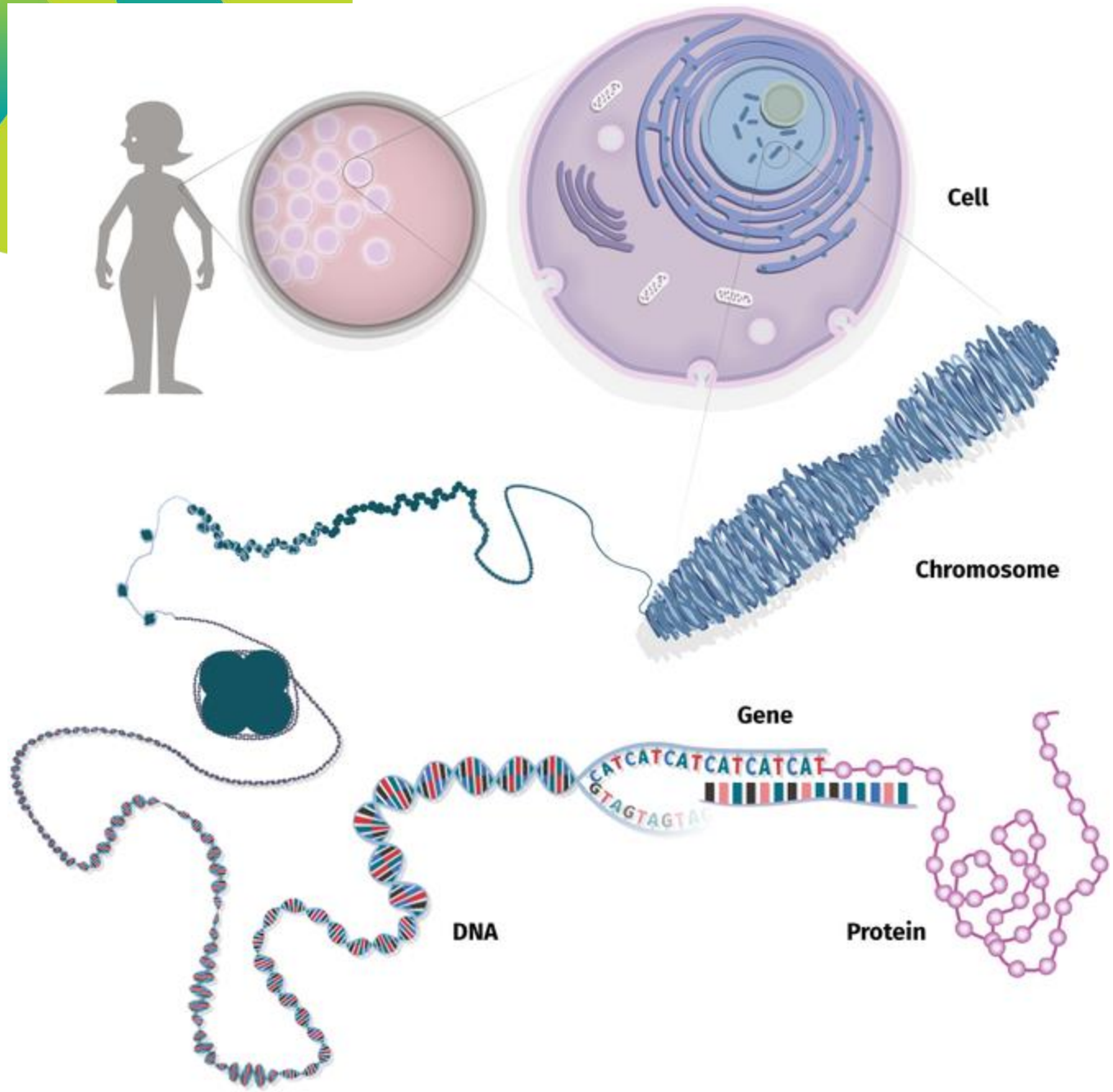

We use CRISPR to tell the body where to heal itself.

Sometimes this involves correcting a genetic mutation such as in Sickle Cell Disease.

Other times, we can mark a gene for destruction because that gene is blocking a therapy that treats a disease, as in the case of lung cancer.

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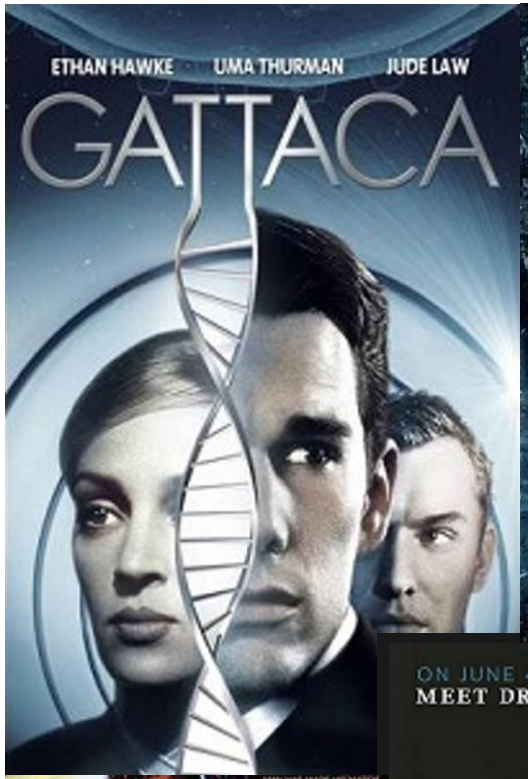
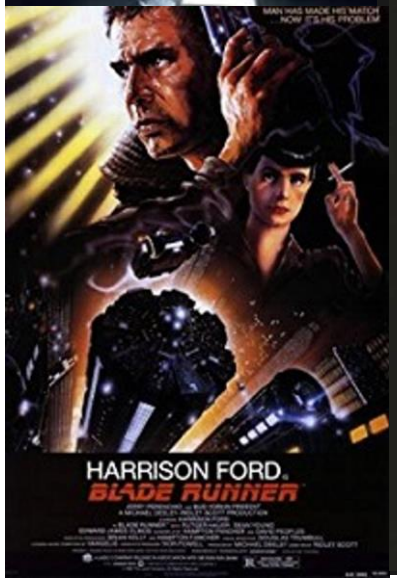

ETHAN HAWKE
UMA THURMAN
JUDE LAW
GATTACA


THE AMAZING
SPIDER-MAN
COMING SOON
IN 3D


MATT DAMON
JODIE FOSTER
ELYSIUM
FROM THE DIRECTOR OF 'DISTRICT 9'
AUGUST


BIG MEETS BIGGER
DWAYNE JOHNSON
RAMPAGE
SEE IT IN 3D
COMING SOON


HARRISON FORD
BLADE RUNNER


ON JUNE 4TH...
MEET DREN.
SPLICE
THE FUTURE IS BORN 06.04.10


JURASSIC WORLD

How should we regulate genome editing?

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A Report on the Social and Ethical Issues of Genetic Engineering with Human Beings

President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research

November 1982

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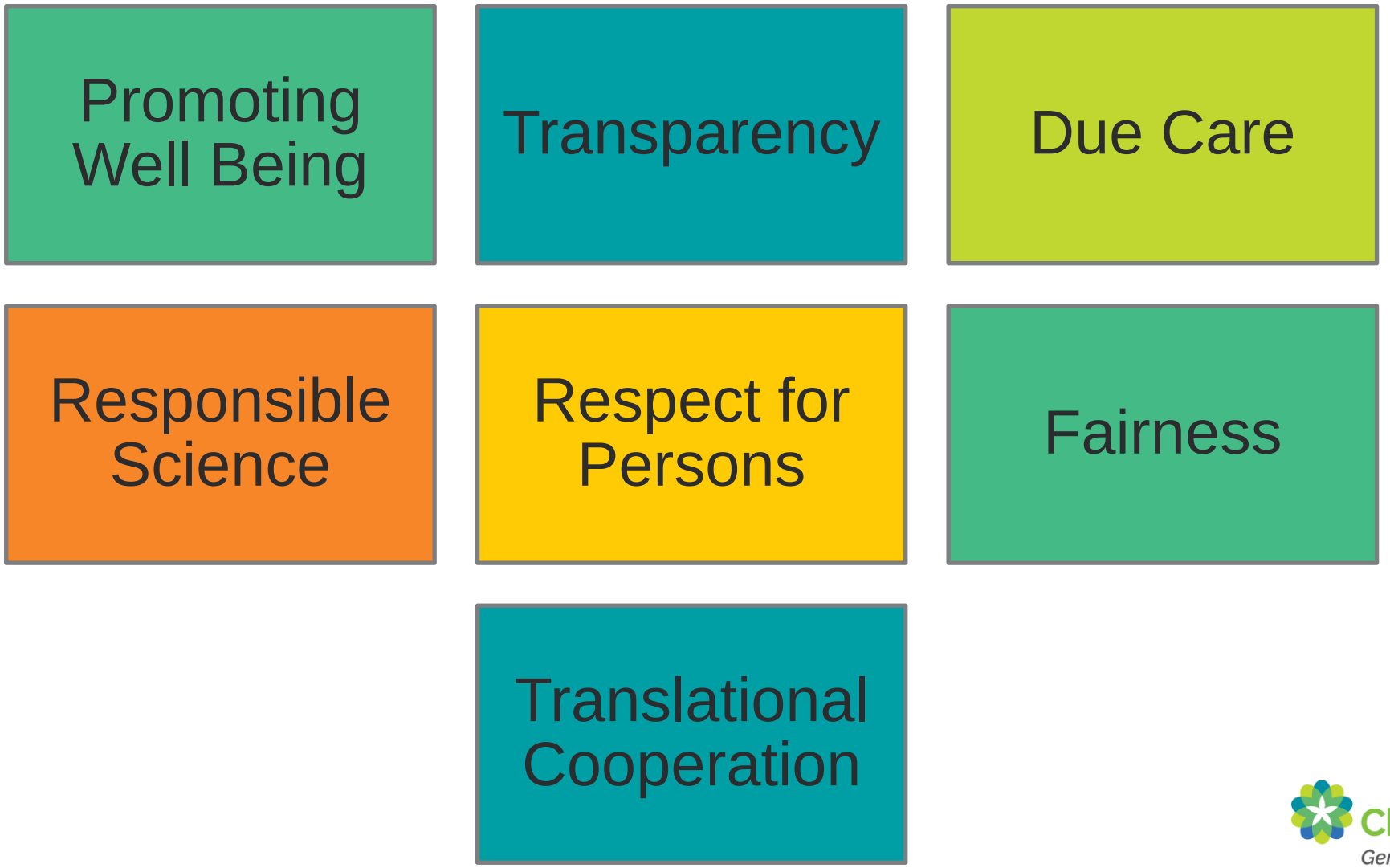
Governance Principles for Human Genome Editing



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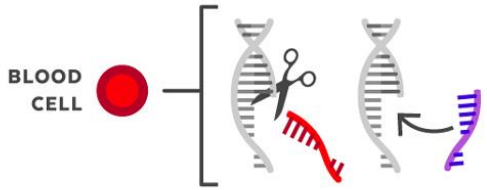
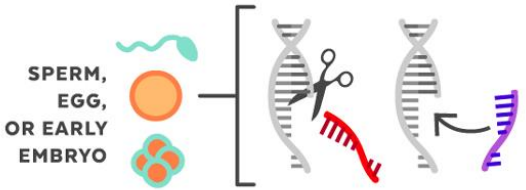
SOMATIC GENE EDITING VS. GERMLINE GENE EDITING

EDIT



Somatic therapies target genes in specific types of cells (blood cells, for example).



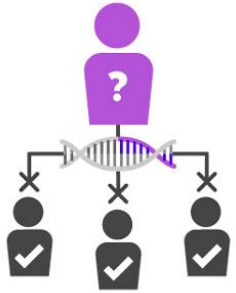
Germline modifications are made so early in development that any change is copied into all of the new cells.

COPY

The edited gene is contained only in the target cell type. No other types of cells are affected.

The edited gene is copied in every cell, including sperm or eggs.

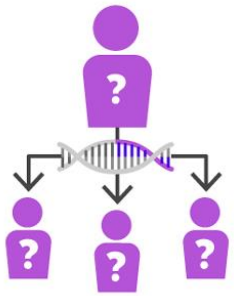
RISKS



Any changes, including potential off-target effects, are limited to the treated individual.

NEXT GENERATION

The edited gene is not passed down to future generations.



If the person has children, the edited gene is passed on to future generations.

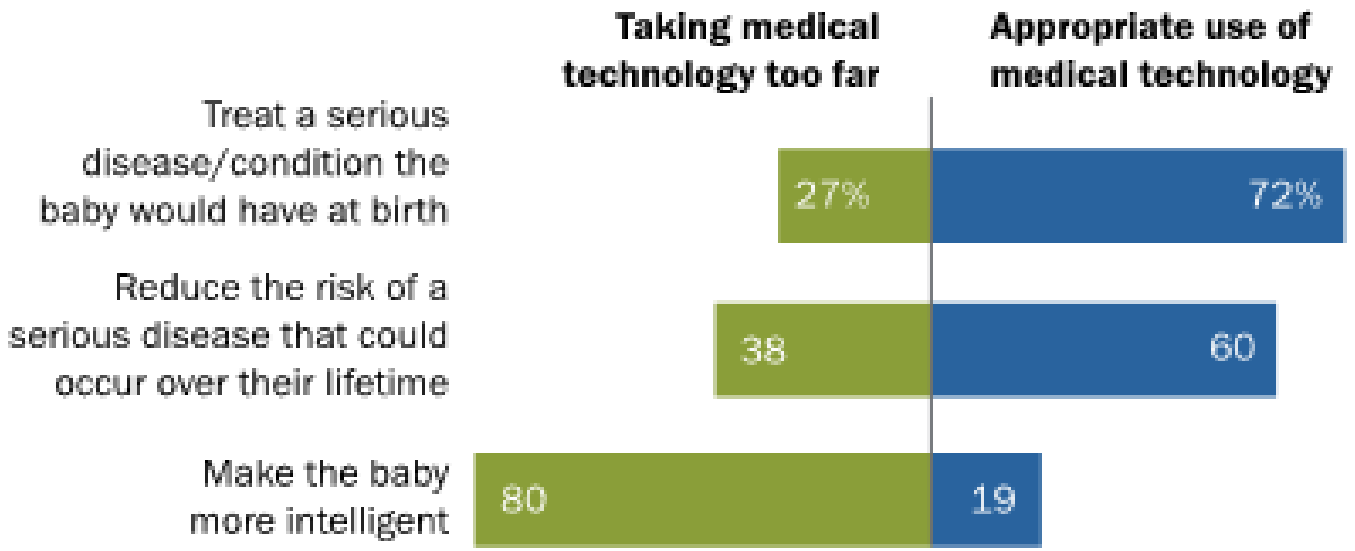
CONSENSUS

Somatic cell therapies have been researched and tested for more than 20 years and are highly regulated.

Human germline editing is new. Heritability of germline changes presents new legal and societal considerations.

A majority of U.S. adults say changing a baby's genes to treat a serious congenital disease is appropriate

% of U.S. adults who say changing a baby's genetic characteristics for each of the following reasons is ...



Note: Respondents who did not give an answer are not shown.
Source: Survey conducted April 23-May 6, 2018.
"Public Views of Gene Editing for Babies Depend on How It Would Be Used"

PEW RESEARCH CENTER

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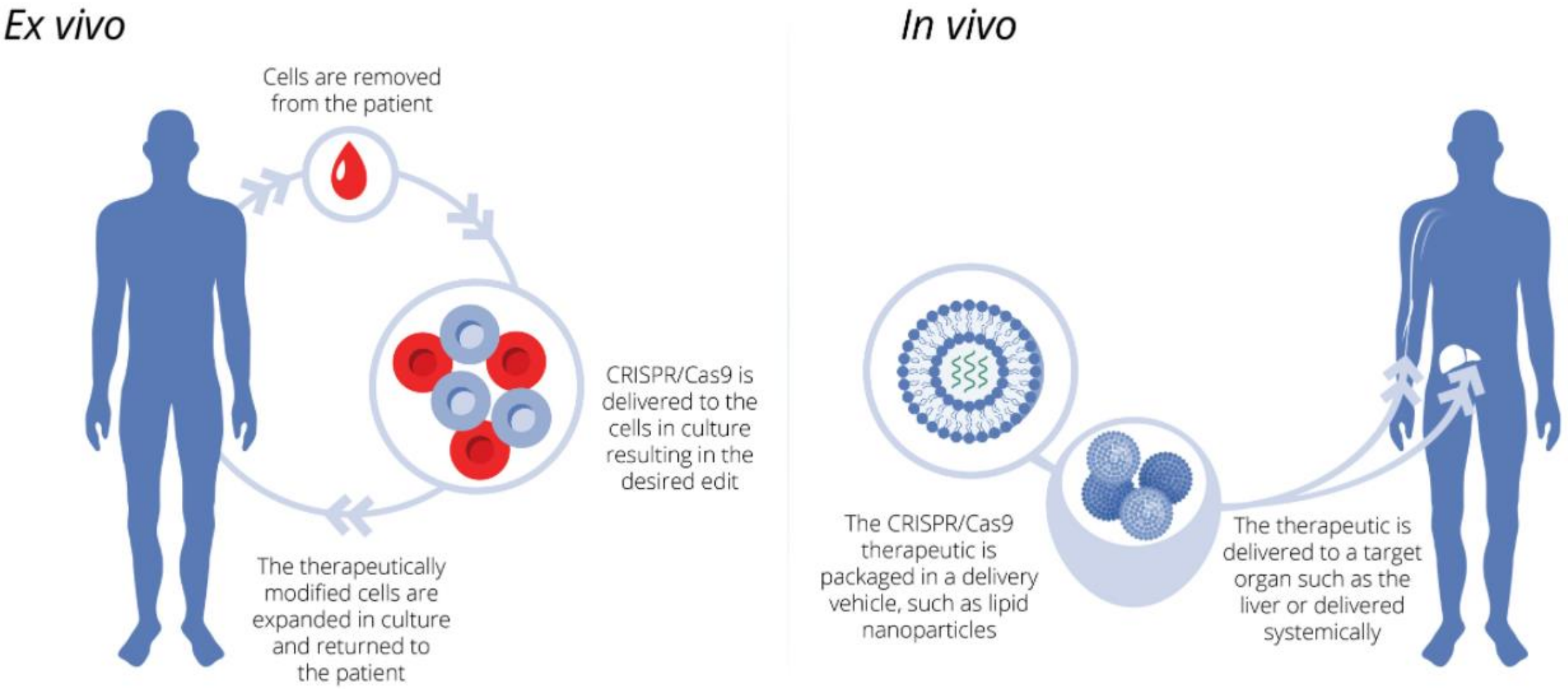
CRISPR in the clinic?



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* Lack of knowledge of genetic diversity makes things more complex…

Ongoing clinical trials for *in vivo* genome editing therapy.

Description	Clinical Trials ID, Phase, Start Year	Delivery Method	Affiliation	Ref.
Inserting a normal copy of *IDUA* gene in hepatocytes to target MPS I patients using zinc-finger nuclease (SB-318)	NCT02702115 Phase 1 / 2 2016	AAV2/6 *via* intravenous (IV) infusion	Sangamo Therapeutics, USA	[36]
Inserting a normal copy of *F9* gene in hepatocytes to target hemophilia-B using zinc-finger nuclease (SB-FIX)	NCT02695160 Phase 1 2016	AAV2/6 *via* intravenous (IV) infusion	Sangamo Therapeutics, USA	[37]
Disrupting *E7* oncogene from HPV to target cervical cancer using zinc-finger nuclease	NCT02800369 Phase 1 2016	Suppository containing ZFN-603 or ZFN-758 *via* intratumoral injection	Huazhong University of Science and Technology, China	[38]
Disrupting *E6/E7* oncogene from HPV to target cervical cancer using TALEN	NCT03226470 Phase 1 2017	Suppository containing T27 and Suppocire *via* intravaginal injection	Huazhong University of Science and Technology, China	[39]
Disrupting *E6/E7* oncogene from HPV to target cervical cancer using TALEN and CRISPR-Cas9	NCT03057912 Phase 1 2017	A gel containing TALEN or CRISPR-Cas9 plasmid, C32–447, Poloxmer 407 *via* intravaginal injection	Sun Yat-Sen University, China	[40] [39]
Inserting correct *IDS* gene in hepatocytes to target MPS II patients using zinc-finger nuclease (SB-913)	NCT03041324 Phase 1 / 2 2017	AAV2/6 *via* intravenous (IV) infusion	Sangamo Therapeutics, USA	[41]
Correcting *CEP290* gene in retinal to target LCA10-IVS26 patients using CRISPR-SaCas9 (EDIT-101)	NCT03872479 Phase 1 / 2 2019	AAV5 *via* subretinal injection	Editas Medicine, Inc., USA	[27]
Clearing HSV infection targeting herpetic stromal keratitis using CRISPR-SpCas9 (BD111)	NCT04560790 Phase 1 / 2 2020	VLPs *via* corneal injection	Shanghai BDgene, China	[42]
Knockout *TTR* gene in hepatocytes to target ATTR amyloidosis patients using CRISPR-spCas9 (NTLA-2001)	NCT04601051 Phase 1 2020	LNPs *via* intravenous administration	Intellia Therapeutics, UK	[43]

CRISPR In The Clinic: Lung Cancer Case Study

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Lung Cancer Case Study

- Cells in the lung mutate growing uncontrollably and clustering together to form a tumor.

- Causes:
  - Smoking
  - Radon
  - Hazardous Chemicals
  - Particle pollution
  - Genes

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Lung cancer symptoms usually do not appear until the cancer has spread to other parts of the body. At this point, it is harder to treat lung cancer.

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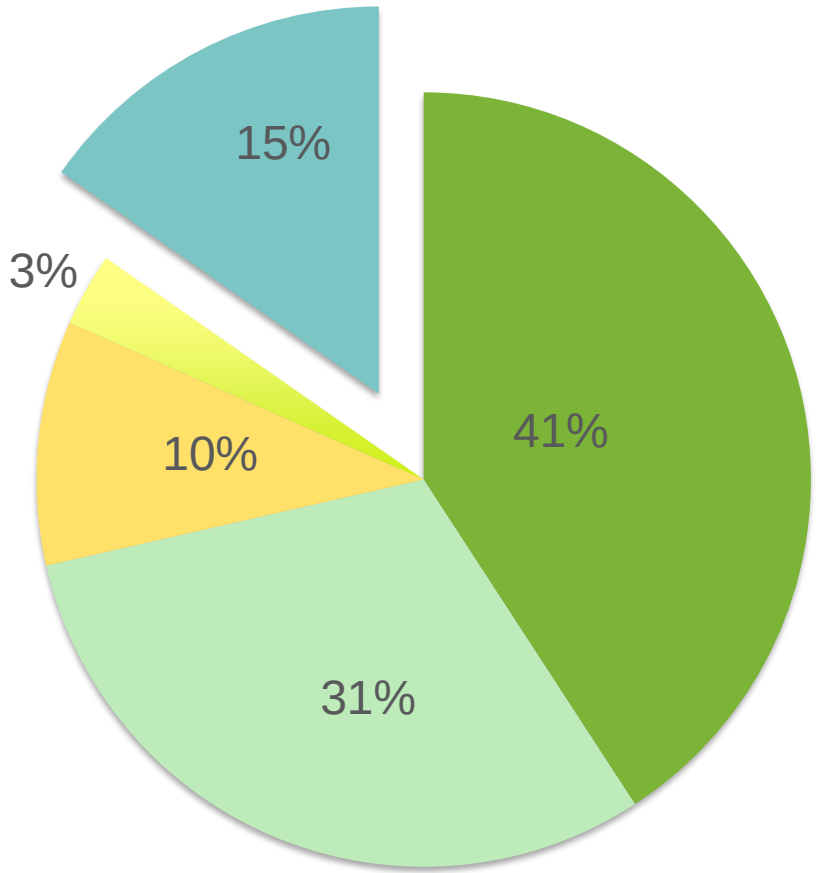
Lung Cancer Classification

Small cell lung carcinoma (SCLC)



Non-small cell lung carcinoma (NSCLC)

- Adenocarcinoma
- Squamous cell carcinoma
- Large cell carcinoma
- Other

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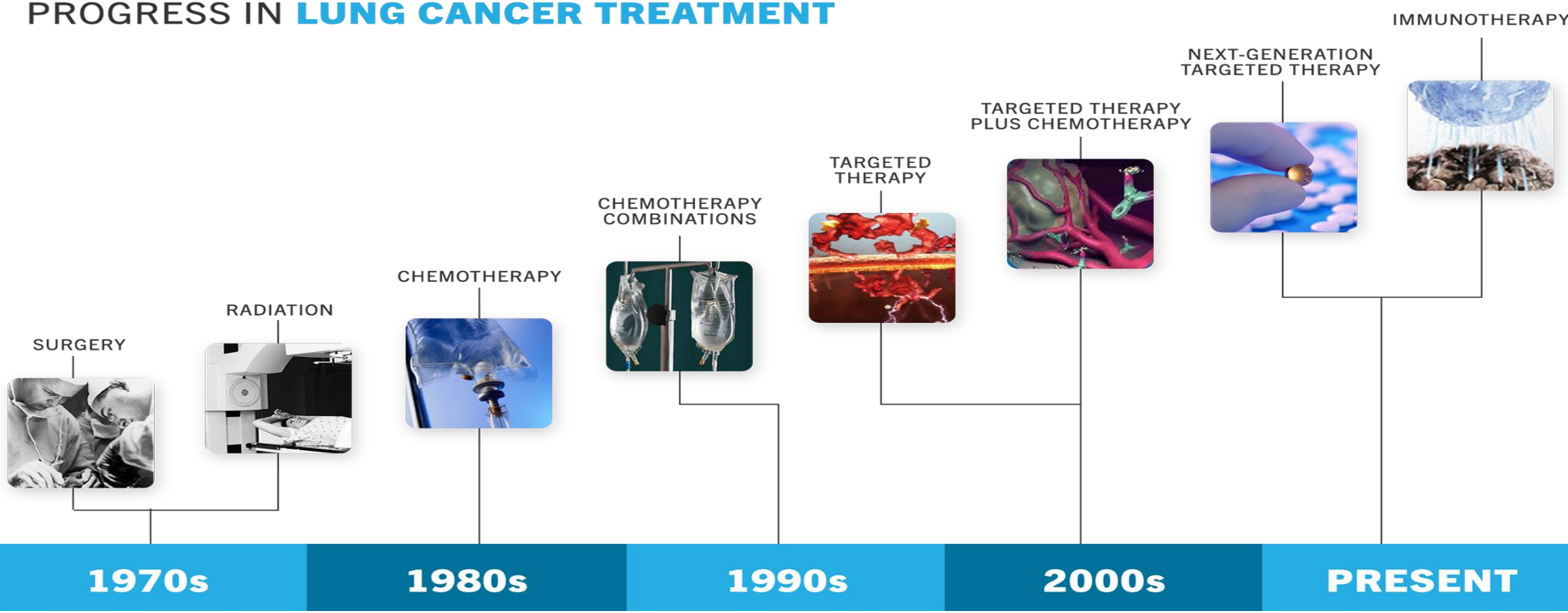
Modified from the LUNGevity Foundation

PROGRESS IN **LUNG CANCER TREATMENT**



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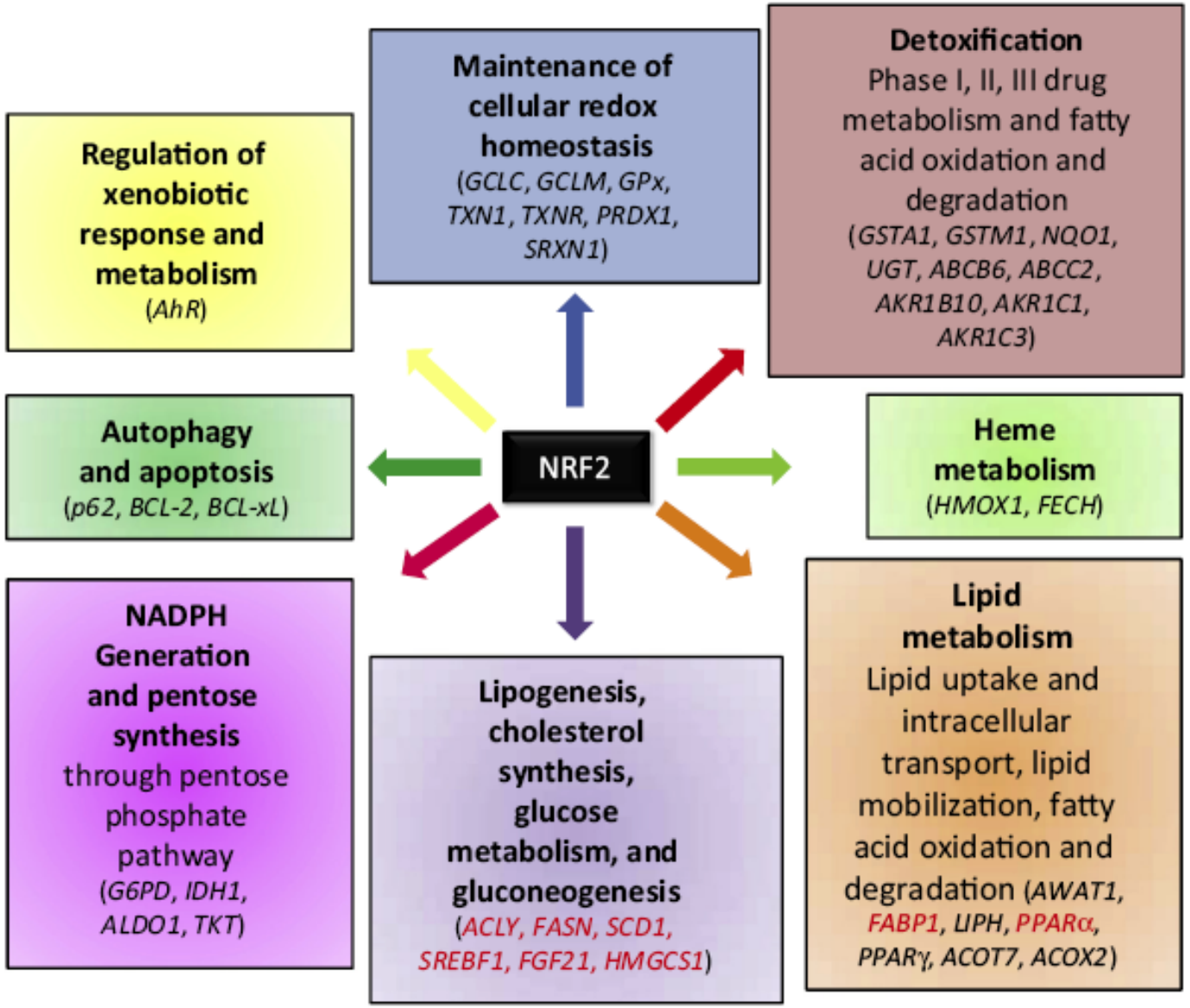
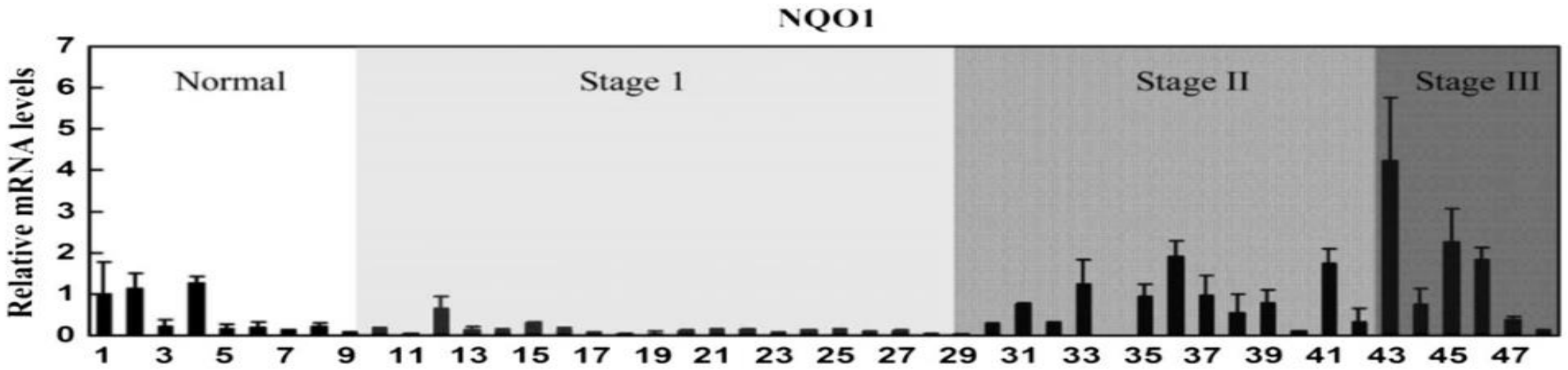
Chemoresistance of Non-small Cell Lung Carcinoma (NSCLC)



- **NRF2 is a multifunctional transcription factor**
- **Chemotherapy has been shown to activate the transcriptional activity of the NRF2 target genes**
- **Other mechanisms that lead to the upregulation of NRF2**
  - mutations in KEAP1 or NRF2, epigenetic modifications
- **Upregulation of NRF2**
  - enhanced resistance of cancer cells to chemotherapy *(Yang et al, 2011; Hayden et al, 2014)*

Menegon S, Columbano A, Giordano S. The Dual Roles of NRF2 in Cancer. Trends Mol Med. 2016. doi:10.1016/j.molmed.2016.05.002.

Nrf2 expression is associated w/ worse overall survival and recurrence-free survival in NSCLC patients



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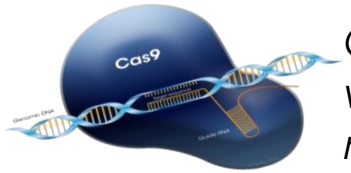
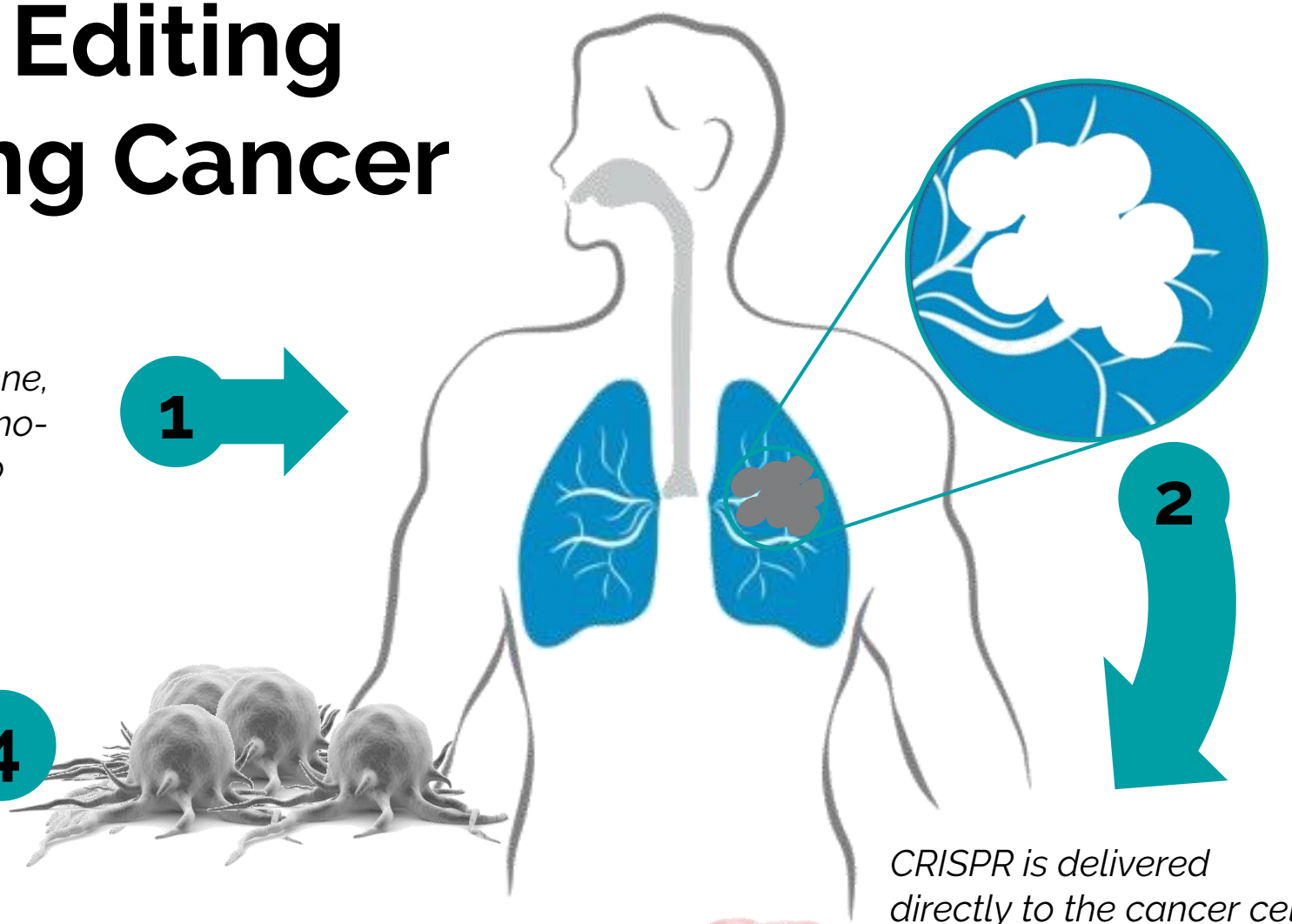
A Novel Gene Editing Approach to Lung Cancer



CRISPR targeting the Nrf2 gene, which is responsible for chemo-resistance, is administered to patient

1



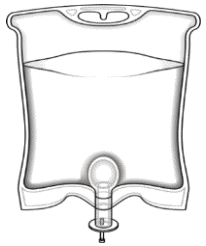
2

CRISPR is delivered directly to the cancer cells

3

CRISPR eliminates Nrf2 gene, creating edited cancer cells

4



Edited cancer cells, lacking Nrf2, are now more susceptible to standard chemotherapy strategies

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Molecular Therapy
Oncolytics
Original Article

AMERICAN SOCIETY of GENE & CELL THERAPY

Functional Gene Knockout of *NRF2* Increases Chemosensitivity of Human Lung Cancer A549 Cells *In Vitro* and in a Xenograft Mouse Model

Pawel Bialk,[1,3] Yichen Wang,[1,3] Kelly Banas,[1,2,3] and Eric B. Kmiec[1,2]

[1]Gene Editing Institute, Helen F. Graham Cancer Center & Research Institute, Christiana Care Health System, 4701 Ogletown-Stanton Road, Suite 4300, Newark, DE 19713, USA; [2]Department of Medical and Molecular Sciences, University of Delaware, Willard E. Hall Education Building, Newark, DE 19716, USA

Recent studies point to the evolution of drug resistance in lung cancer as being centered, at least in part, on the upregulation of various genes involved in controlling efflux or drug inactivation. Among the most important of these genes is Nuclear Factor Erythroid 2-Related Factor (*NRF2*), considered the master regulator of 100–200 target genes involved in cellular responses to oxidative and/or electrophilic stress. With increased focus on the development of combinatorial approaches for cancer treatment, we utilized CRISPR/Cas9 to disable the *NRF2* gene in lung cancer cells by disrupting the NRF2 nuclear export signal (NES) domain; phenotypically, the protein is largely blocked from transiting into the nucleus after translation. In tissue culture, cells with this gene knockout were found to have a reduced proliferation phenotype and are more sensitive to chemotherapeutic agents, such as cisplatin and carboplatin. These observations were confirmed in xenograft mouse models wherein the homozygous knockout cells proliferate at a slower rate than the wild-type cells, even in the absence of drug treatment. Tumor growth was arrested for a period of 16 days, with a dramatic decrease in tumor volume being observed in samples receiving the combined action of CRISPR-directed gene editing and chemotherapy.

INTRODUCTION

Lung cancer is the leading cause of cancer mortality in the United States, accounting for more than 1 in 4 cancer deaths. It kills more people than breast, prostate, and colon cancer combined;[1] yet, despite these grim statistics, there are reasons to be optimistic about the potential to reduce mortality. Advances in treatment have shown promise, and emerging targeted treatments (see Hirsch et al.[2]) for various forms of lung cancer will soon be made more widely available. Some of these therapies include the use of endothelial growth factor receptor (EGFR) monoclonal antibodies and vascular EGFR inhibitors.[3–4] EGFR tyrosine kinase inhibitors continue to be a superior choice as first-line treatment in patients with EGFR mutation-positive non-small-cell lung cancer (NSCLC).[5–8] Despite these positive results, however, EGFR mutations account for approximately 17% of the driver mutations in lung adenocarcinoma. The other 85% of the mutations reside in genes such as K-RAS, ALK, HER2, or in unknown genes, demonstrating the need to design combinatorial strategies even when some specific mutations in target genes are known.

To this end, immunotherapy is also becoming part of cancer treatment plans, but transformative clinical benefit is often limited to the patients containing infiltrated T cells or biomarkers of a specific type.[9,10] As such, combinatorial strategies for immunotherapy are now being clinically evaluated in a similar fashion to other strategies for tyrosine kinase inhibitors. Chemotherapy remains an important option in the treatment of lung cancer, but issues involving efficacy and toxicity can become problematic with extended care. In most cases, resistance to a variety of chemotherapy drugs can develop with extended treatment.[11] Pharmacogenomic studies point to the evolution of drug resistance being centered on the upregulation of the variety of genes involved in controlling the efflux of anticancer drugs or directing transcriptional activation among others.

Nuclear Factor Erythroid 2-Related Factor (*NRF2*) is considered the master regulator of 100–200 target genes involved in cellular responses to oxidative and/or electrophilic stress. Targets include GSH mediators, antioxidants, and genes controlling efflux pumps.[12] NRF2 is also known to regulate the expression of genes involved in protein degradation and detoxification, and it is negatively regulated by Kelch-like ECH-associated protein 1 (KEAP1), a substrate adaptor for the Cul3-dependent E3 ubiquitin ligase complex. Under normal conditions, Keap1 constantly targets NRF2 for ubiquitin-dependent degradation, maintaining a low expression of NRF2 on downstream target genes. However, chemotherapy has been shown to activate transcriptional activity of the NRF2 target genes, often triggering a cytoprotective response; enhanced expression of NRF2 occurs in response to environmental stress or detrimental growth conditions. Other mechanisms that lead to NRF2 upregulation include mutations

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https://doi.org/10.1016/j.omto.2018.10.002.

[3]These authors contributed equally to this work.

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E-mail: eric.b.kmiec@christianacare.org

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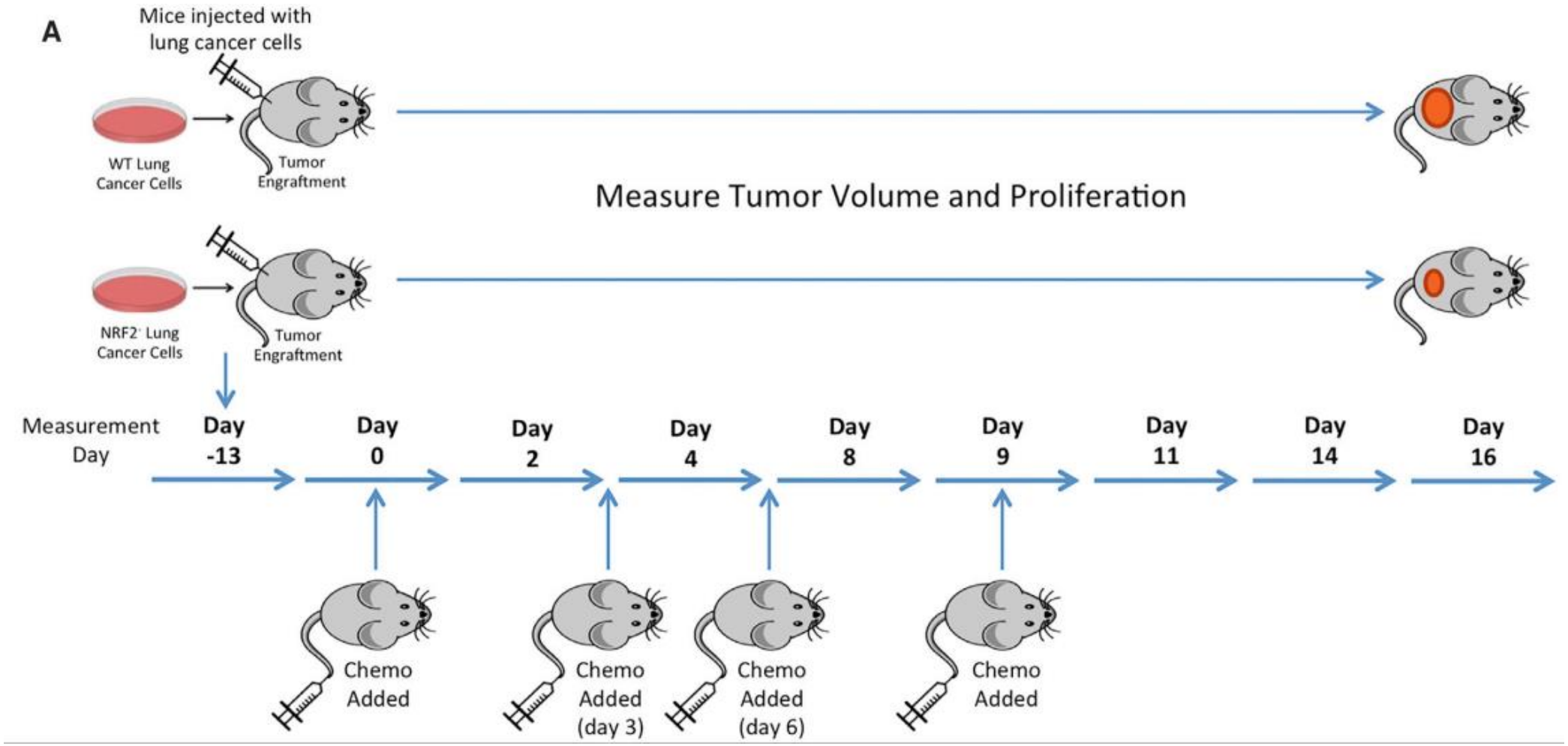
Restored Chemosensitivity in Mice with NRF2 Knockout in Tumors



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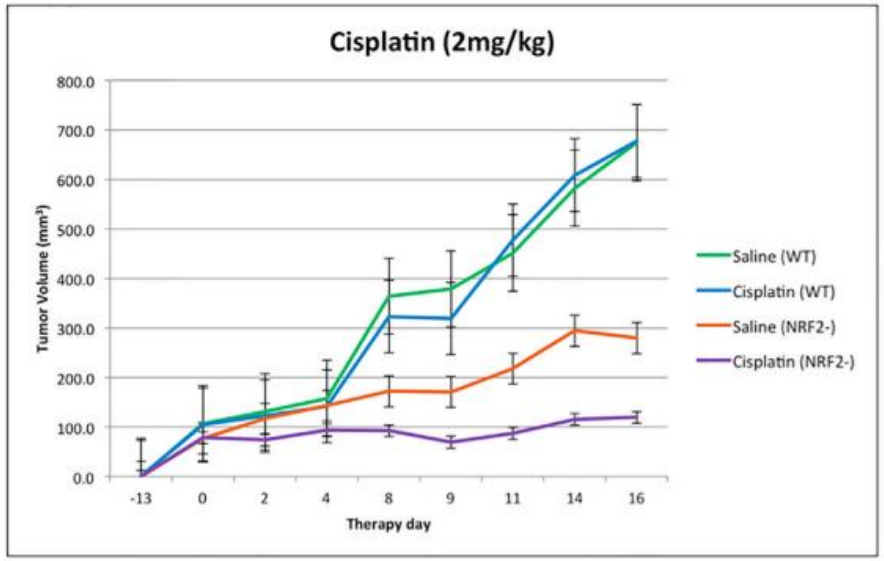
Restored Chemosensitivity in Mice with NRF2 Knockout in Tumors

B



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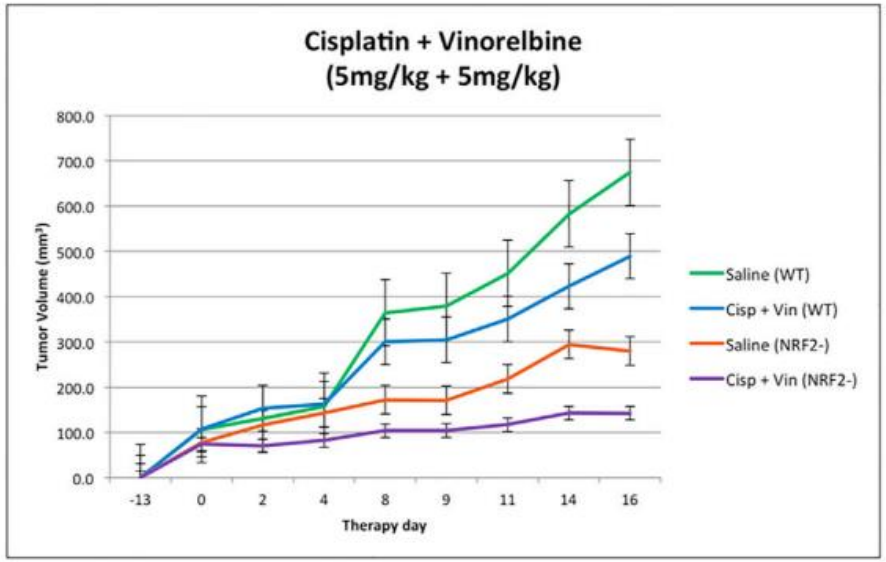

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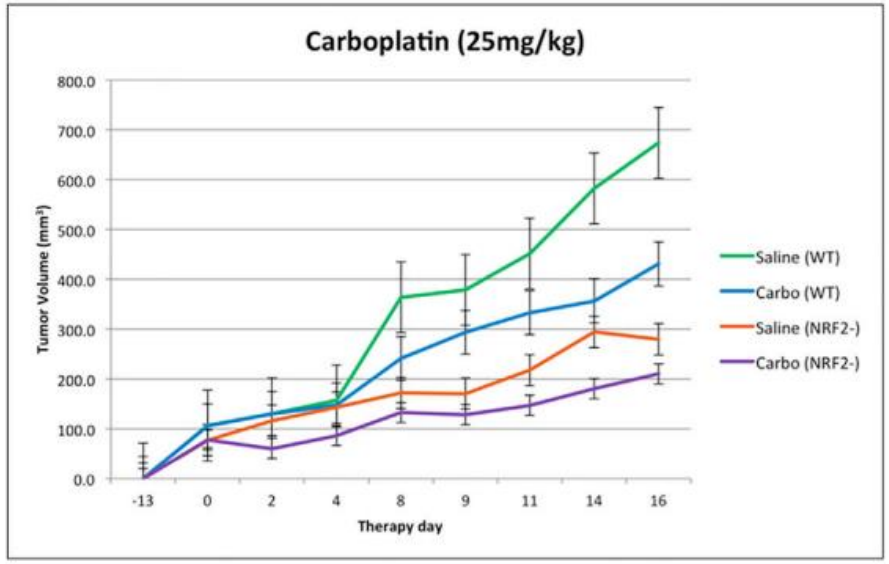

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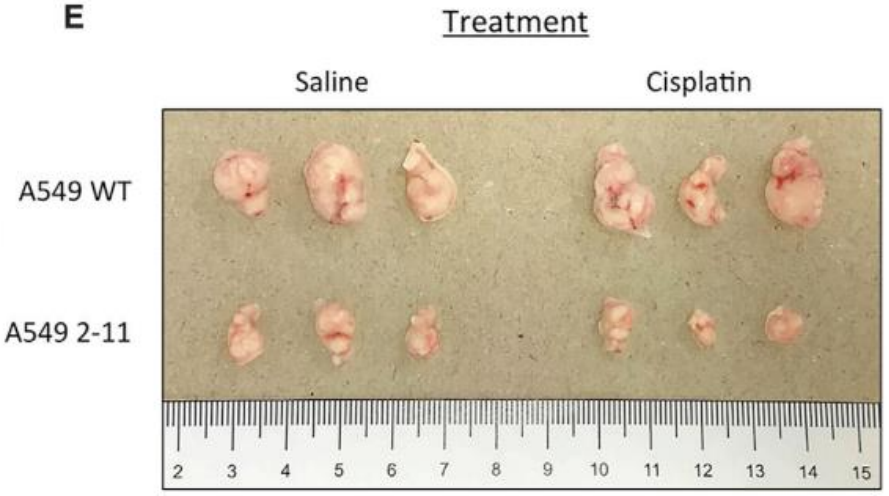
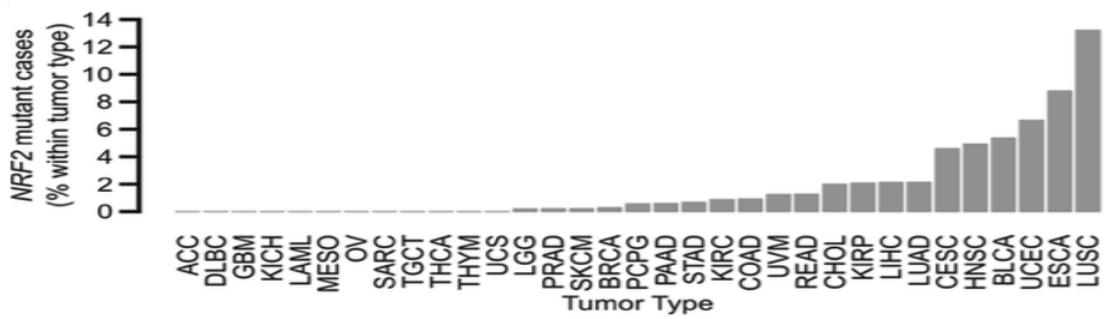
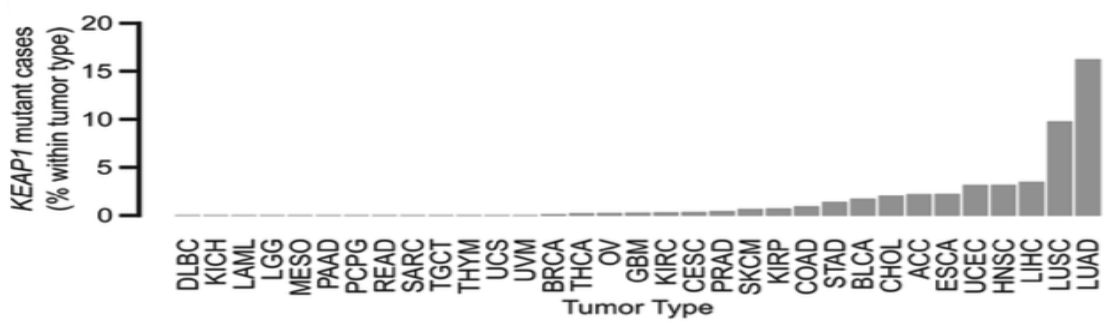
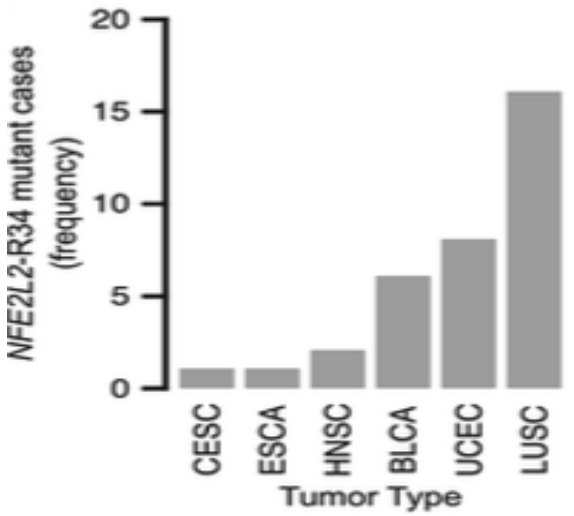


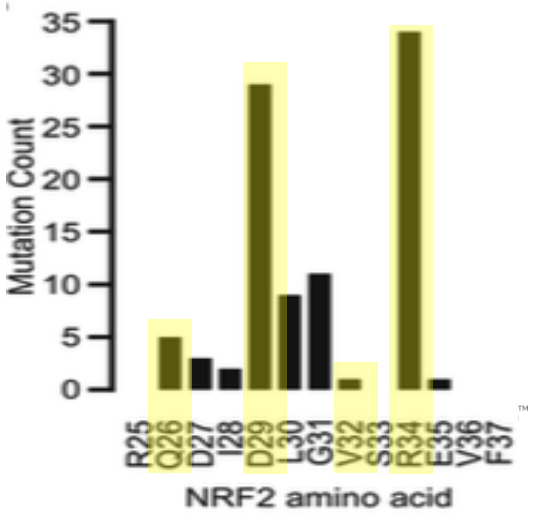
A catalog of somatic NRF2 gain-of-function mutations in cancer

- Mutant forms of NRF2 are unique to cancer cells
  - Treatment strategy
- 14% of NRF2 mutations occur in LUSC
  - Followed by esophageal, uterine-endometrial, bladder-urothelial, head and neck, cervical cancers
- R34 mutation found in 5 tumor types
  - Creates new recognition site for Cas9







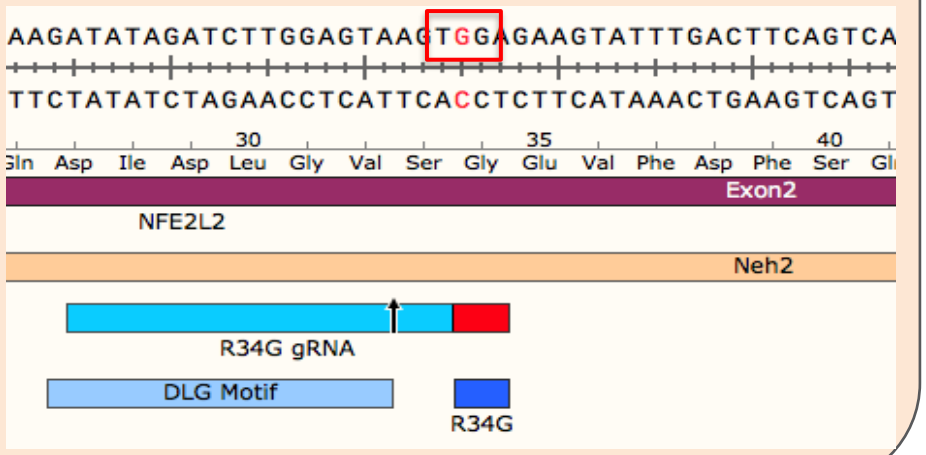


(Kerins, M.J. & Ooi, A., 2018)

Cancer-Specific Gene Editing

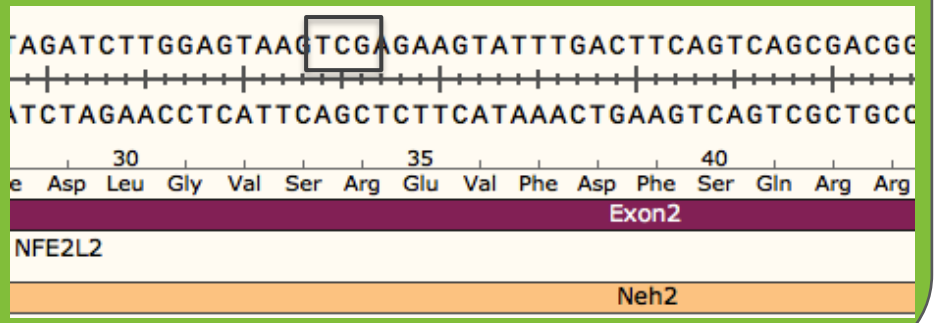
Mutant NRF2 (R34G)

NEW CRISPR recognition site



Wildtype NRF2

NO CRISPR recognition site



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Lung Adenocarcinoma (LUAD)	Lung Squamous Cell Carcinoma (LUSC)
Well studied	Less studied
Known oncogenic drivers (KRAS, EGFR)	Lack of treatment options
Targeted therapy	Failed targeted therapy
Good treatment response & prognosis	Poor response & prognosis
Median OS = >30 months	Median OS = <15 months

CRISPR News MEDICINE

CRISPR Makes Resistant Lung Cancer Cells Vulnerable to Chemotherapy

Resistance to chemotherapy is a major challenge in the treatment of non-small cell lung cancer. By profiling the spectrum of outcomes arising from CRISPR-based knockout of the NRF2 protein, which contributes to chemoresistance, Eric Kmiec Ph.D. and Kelly Banas Ph.D. of the ChristianaCare Gene Editing Institute have developed a new therapeutic strategy that increases the sensitivity of cancer cells to traditional chemotherapy.

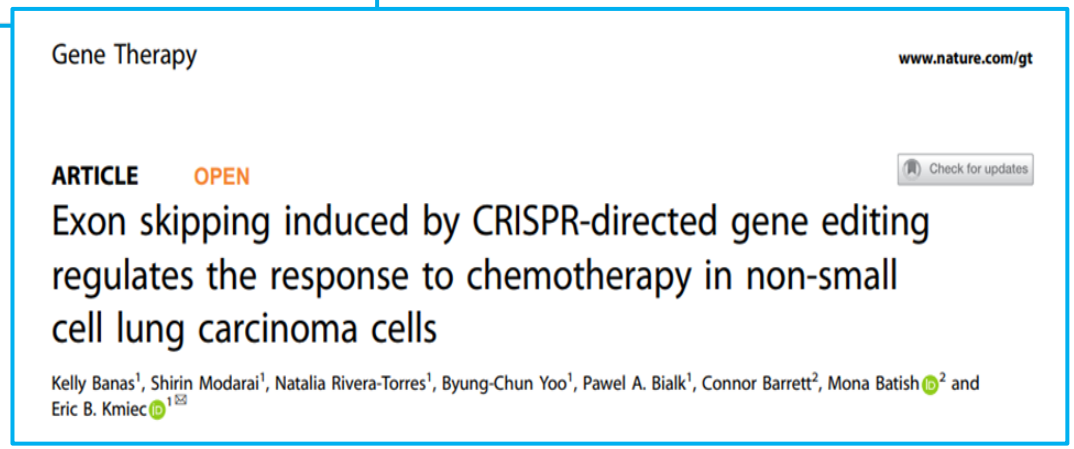
By: Rebecca Roberts - Apr. 5, 2022

Many types of cancer can be treated with relative ease, but this is not the case for late-stage non-small cell lung cancer (NSCLC). There is a pervasive sense of hopelessness among these patients owing to the current lack of treatment options, therapeutic failure due to drug resistance, and low survival rates. It was this sense of despair that drove researchers from ChristianaCare's Gene Editing Institute to develop an innovative CRISPR therapy that could make chemotherapy-resistant NSCLC cells vulnerable to standard chemotherapy once again.


MOLECULAR CANCER RESEARCH | NEW HORIZONS IN CANCER BIOLOGY

Kinetics of Nuclear Uptake and Site-Specific DNA Cleavage during CRISPR-Directed Gene Editing in Solid Tumor Cells

Kelly Banas[1,2], Natalia Rivera-Torres[1], Pawel Bialk[1], Byung-Chun Yoo[1], and Eric B. Kmiec[1,2]


Gene Therapy www.nature.com/gt

ARTICLE OPEN

Check for updates

Exon skipping induced by CRISPR-directed gene editing regulates the response to chemotherapy in non-small cell lung carcinoma cells

Kelly Banas[1], Shirin Modarai[1], Natalia Rivera-Torres[1], Byung-Chun Yoo[1], Pawel A. Bialk[1], Connor Barrett[2], Mona Batish[2] and Eric B. Kmiec[1]

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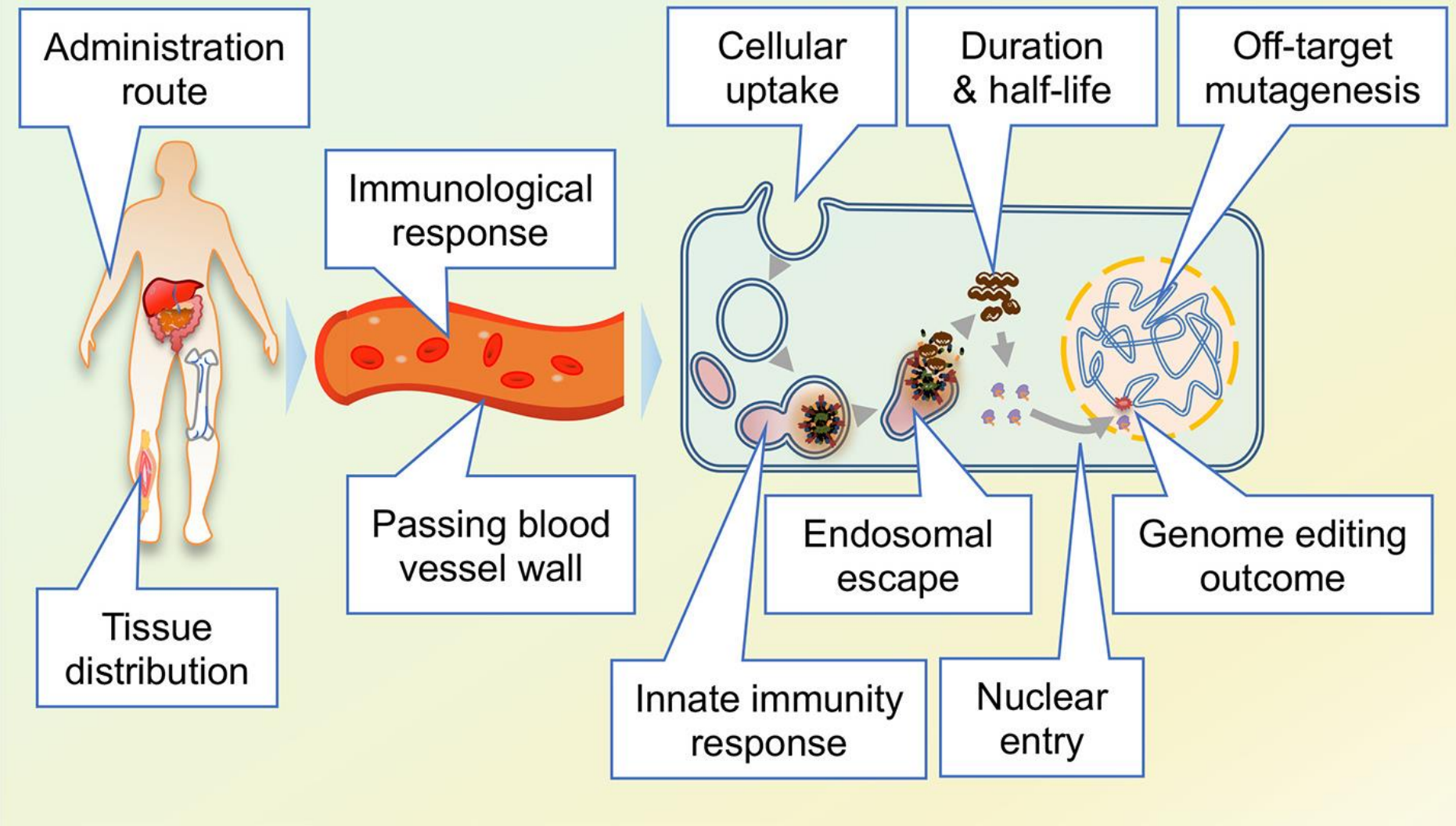
Challenges need to be overcome for genome editing delivery



E.A.Taha et al. 2022

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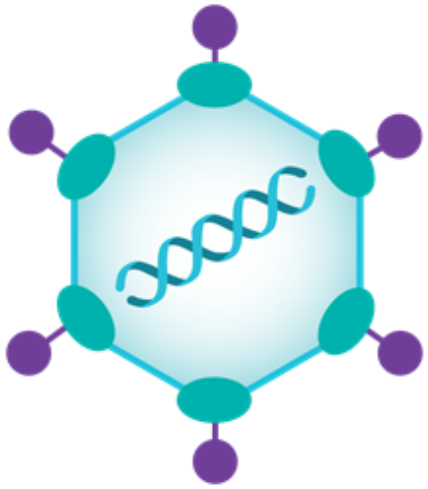
Genetic Medicine Challenge

Genetic medicine requires efficient delivery of the nucleic acid-based drug to the target cell to affect gene expression. Two clinically-validated genetic medicine technologies are:

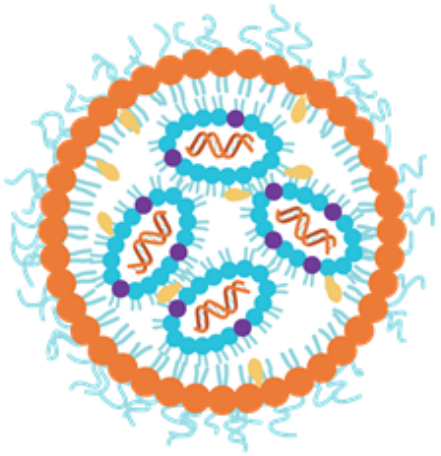
Viral Vectors



Delivers gene using a modified virus

High immune response
Potential genomic insertion
Limited gene size
Potential anti-vector immunity
Cell culture Required

Lipid Nanoparticles



Delivers gene using an LNP

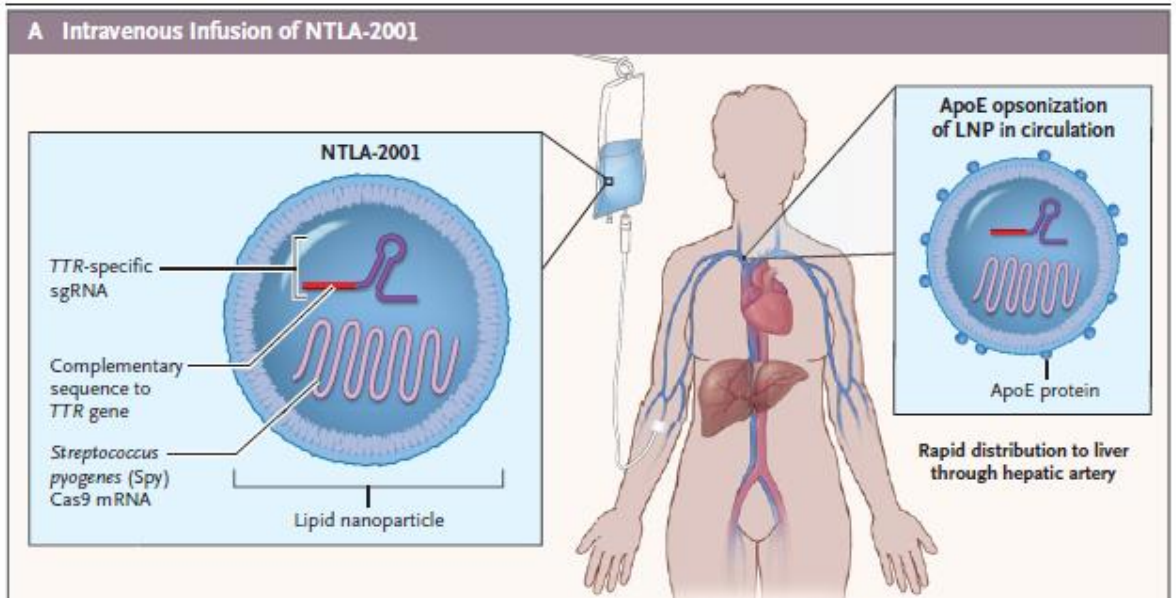
Low immune response
No genomic insertion
Increased gene size
Lower potential anti-vector immunity
Cell-free manufacturing

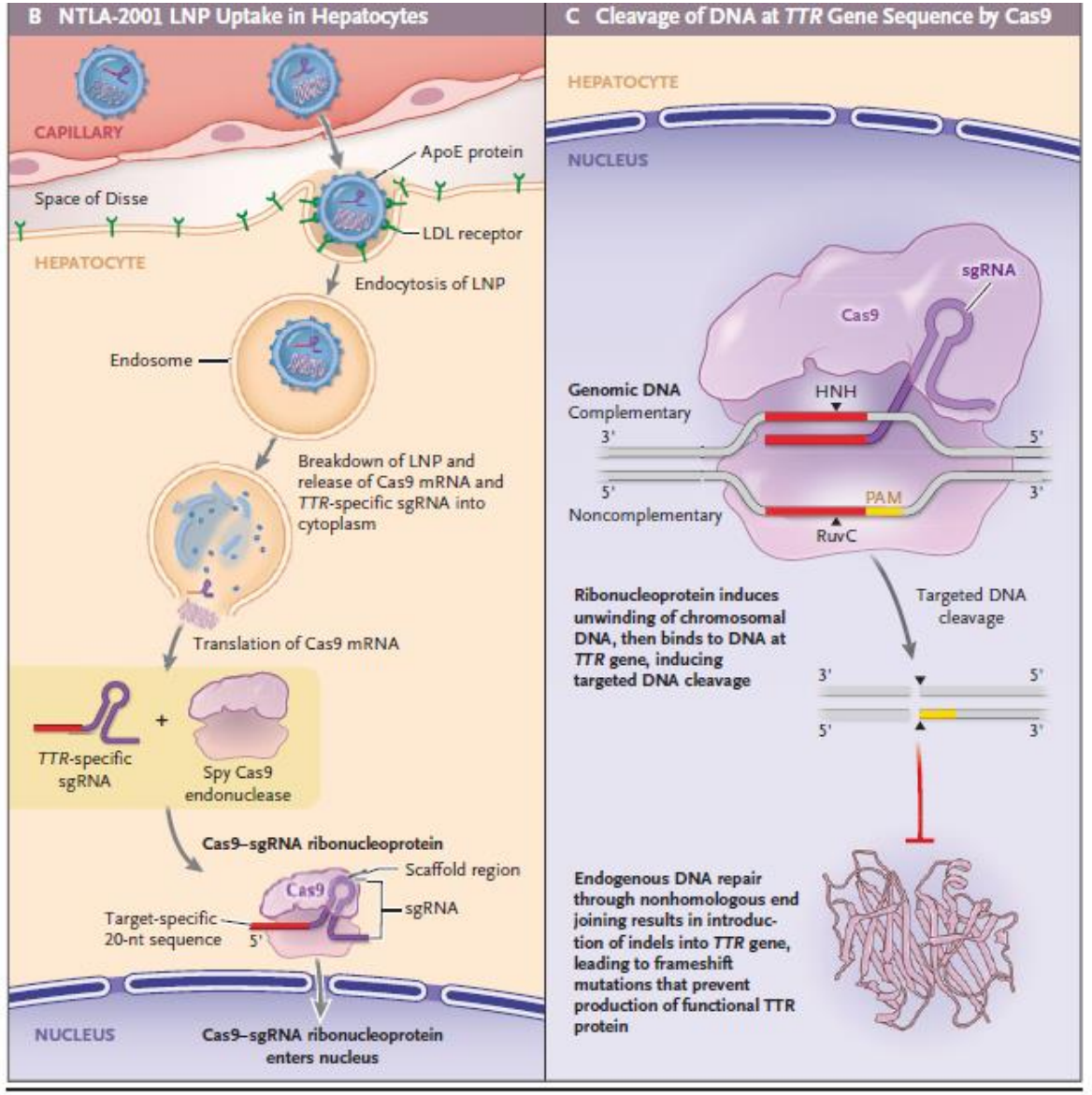
The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 AUGUST 5, 2021 VOL. 385 NO. 6

CRISPR-Cas9 In Vivo Gene Editing for Transthyretin Amyloidosis

Julian D. Gillmore, M.D., Ph.D., Ed Gane, M.B., Ch.B., Jorg Taubel, M.D., Justin Kao, M.B., Ch.B., Marianna Fontana, M.D., Ph.D., Michael L. Maitland, M.D., Ph.D., Jessica Seitzer, B.S., Daniel O'Connell, Ph.D., Kathryn R. Walsh, Ph.D., Kristy Wood, Ph.D., Jonathan Phillips, Ph.D., Yuanxin Xu, M.D., Ph.D., Adam Amaral, B.A., Adam P. Boyd, Ph.D., Jeffrey E. Cehelsky, M.B.A., Mark D. McKee, M.D., Andrew Schiermeier, Ph.D., Olivier Harari, M.B., B.Chir., Ph.D., Andrew Murphy, Ph.D., Christos A. Kyratsous, Ph.D., Brian Zambrowicz, Ph.D., Randy Soltys, Ph.D., David E. Gutstein, M.D., John Leonard, M.D., Laura Sepp-Lorenzino, Ph.D., and David Lebwohl, M.D.





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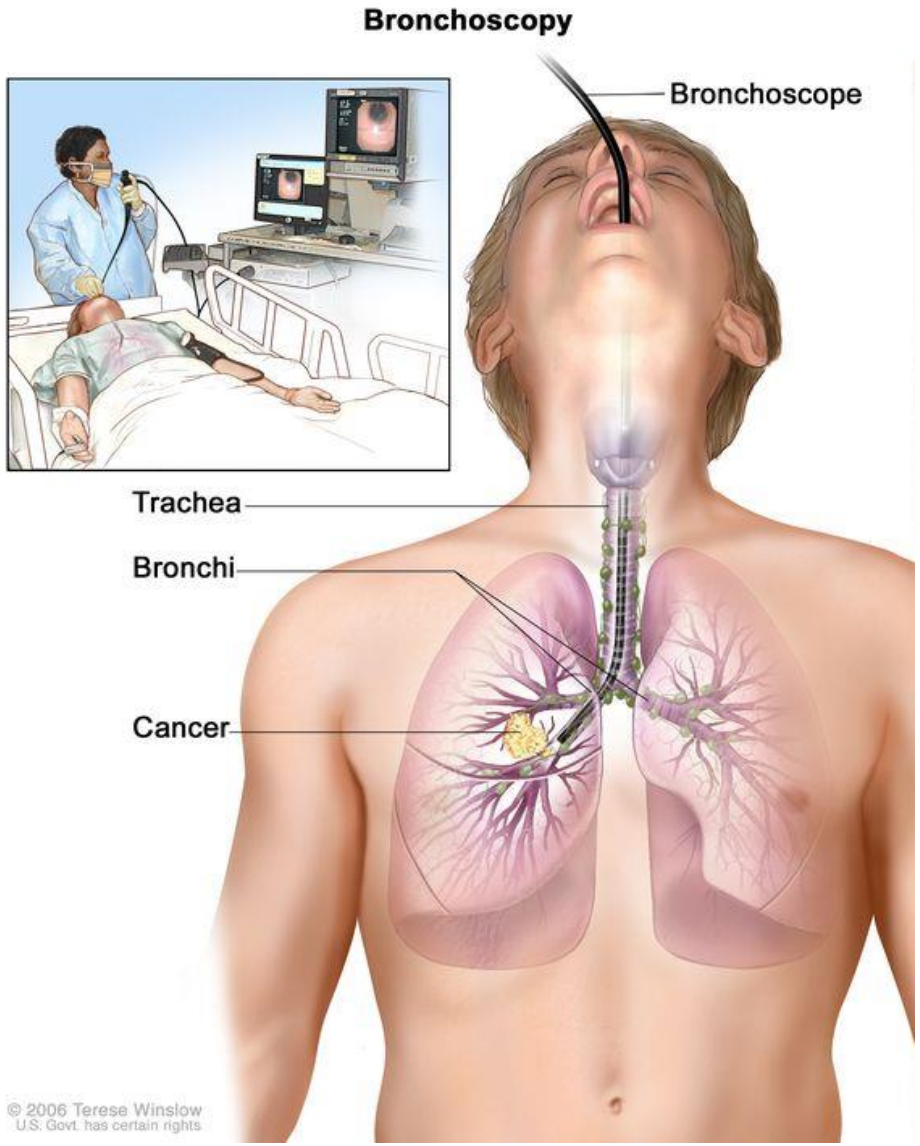
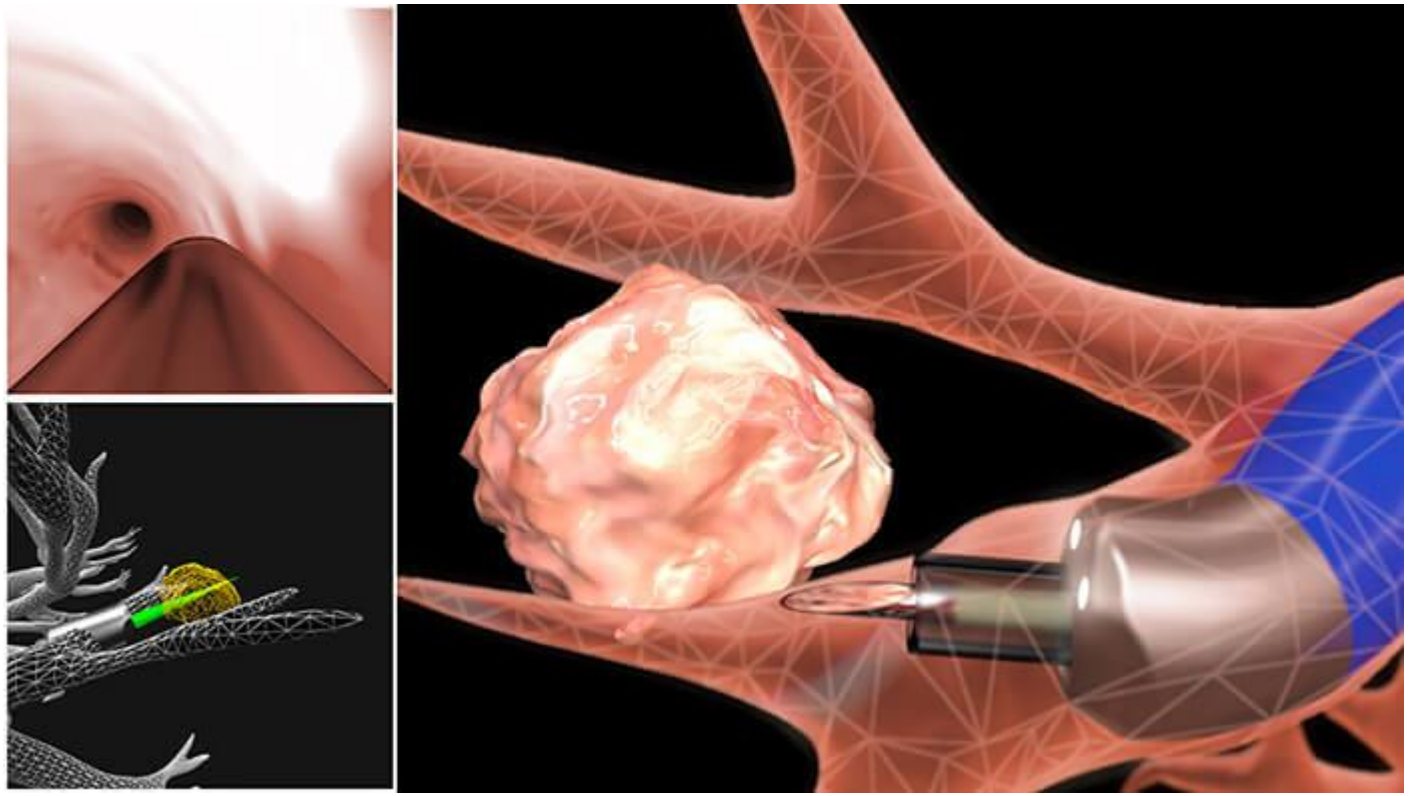
Intra-tumoral Injection





Advancing Care with Interventional Bronchoscopy | University Hospitals (uhhospitals.org)

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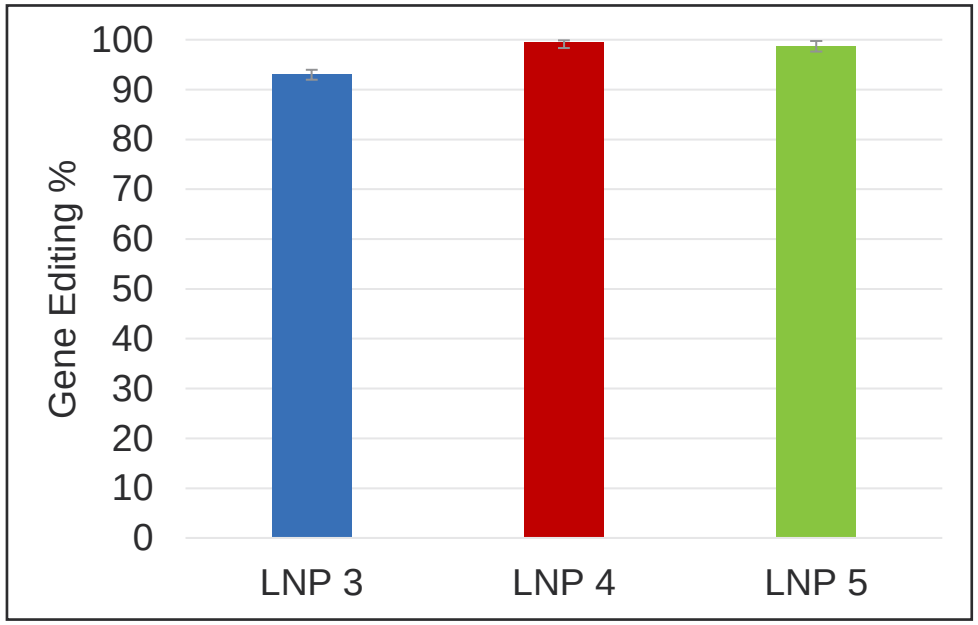
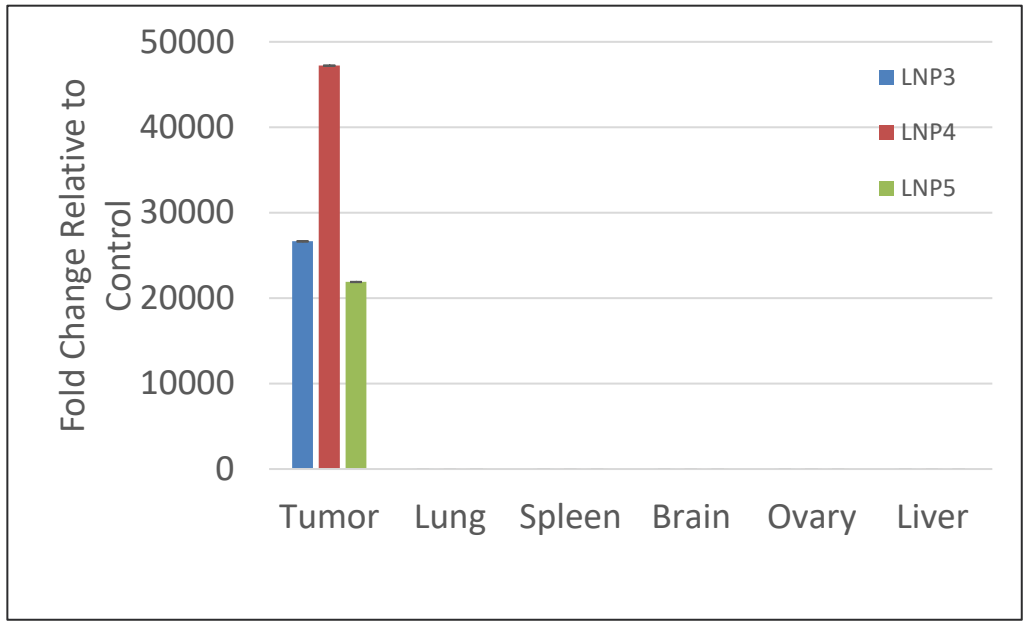
Biodistribution of LNP in NCG Mice





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THANK YOU

www.christianacare.org/geneeditinginstitute

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