Novel uses of CRISPR/cas9 (Clustered Regularly Interspaced short palindromic repeats/CRISPR-associated protein-9) in oncology

Aaron Swomley, PharmD, PhD
Pharmacy Resident, PGY1
University of Utah Health
Aaron.Swomley@utah.edu
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Disclosure

- Relevant Financial Conflicts of Interest
  - **CE Presenter, Aaron Swomley, PharmD, PhD:**
    - No relevant conflicts of interest exist
  - **CE mentor, Rebecca Martin, PharmD, BCOP:**
    - No relevant conflicts of interest exist
- Off-Label Uses of Medications
  - This presentation will not discuss off-label uses of medications

Pharmacist learning objectives:

Explain the process of CRISPR/Cas9

Diagram the steps required of CRISPR/Cas9 for eukaryotic gene editing

Define CRISPR/Cas9 in terms of oncology treatment

Compare uses of CRISPR/Cas9 in different oncologic disease states

Technician learning objectives:

Review the steps of CRISPR/Cas9 production

Name a disease state that may benefit from gene editing

Differentiate between different types of gene editing

Abbreviations

- BSA – Body surface area
- CAS – Crisper-associated protein
- CRISPR – Clustered regularly interspaced repeats
- CRS – Cytokine release syndrome
- DNA – Deoxyribonucleic acid
- EGFR – Epidermal growth factor receptor
- ERK – Extracellular signal-regulated kinase
- GBM – Glioblastoma
- G-CSF – Granulocyte colony stimulating factor
- GVHD – Graft versus host disease
- HDR – Homology directed repair
- ICANS – Immune effector cell-associated neurotoxicity syndrome
- IM – Intramuscular
- LNP – Lipid nanoparticle
- MAP3K – Mitogen associated protein-3 kinase
- ZFN – Zinc finger nuclease
- MLK3 - Mixed-lineage kinase-3
- mTOR - Mammalian target of rapamycin
- NSCLC – Non-small cell lung cancer
- NHEJ – Non-homogenous end joining
- NPM1 – Nucleophosmin
- OS – Overall survival
- PAM – Protospacer adjacent motif
- PBMC – Peripheral blood mononuclear cells
- PFS – Progression free survival
- PTPN23 – Protein tyrosine phosphatase N23
- RNA – Ribonucleic acid
- TALEN - Transcription activator-like effector nucleases
- TTR – Transthyretin
- UBXN1 - UBX domain containing protein-1
- VEGF – Vascular endothelial growth factor

Pre-Question: Which of the following may be treated with CRISPR/Cas9 in the future?

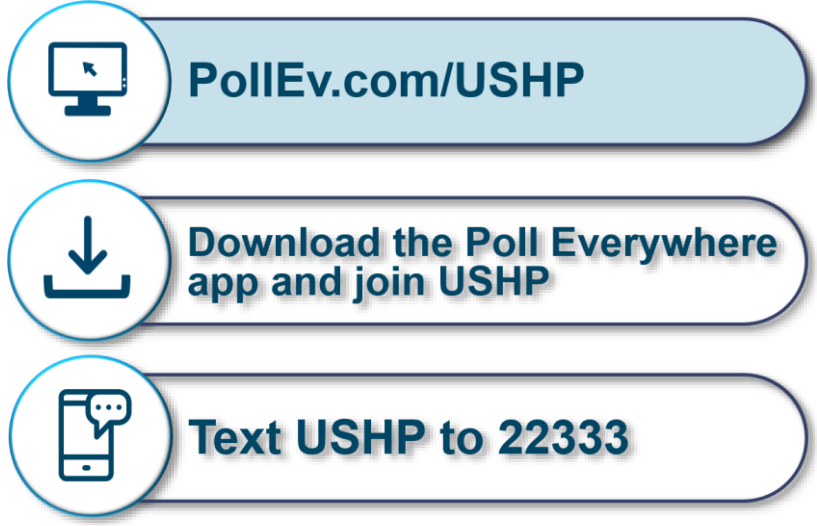
A. Alzheimer disease

B. Thyroid cancer

C. Cystic fibrosis

D. Sickle cell anemia

E. All of the above



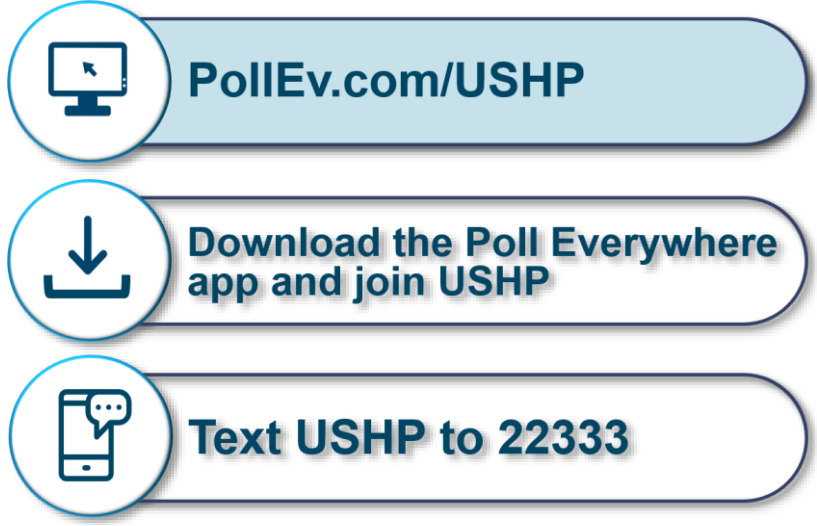
Pre-Question: The original mechanism of CRISPR-Cas9 can be best defined as:

A. A method for industrial quantification of alternative splicing

B. Viral mechanism for immune evasion

C. Biomimetic recombinant antibody manufacturing

D. A complex bacterial immune system against bacteriophage DNA



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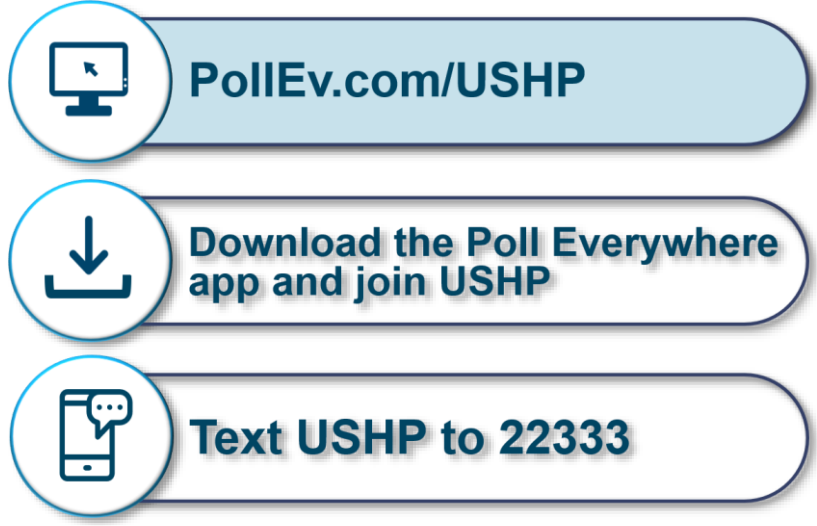
Pre-Question: CRISPR/Cas9 may potentially be used in what ways to treat different malignancies?

A. Correction of a defective tumor suppressor gene

B. Knockout of an oncogene

C. Enhanced sensitivity to chemotherapy

D. All of the above



CRISPR/Cas9

The CRISPR/Cas9 System

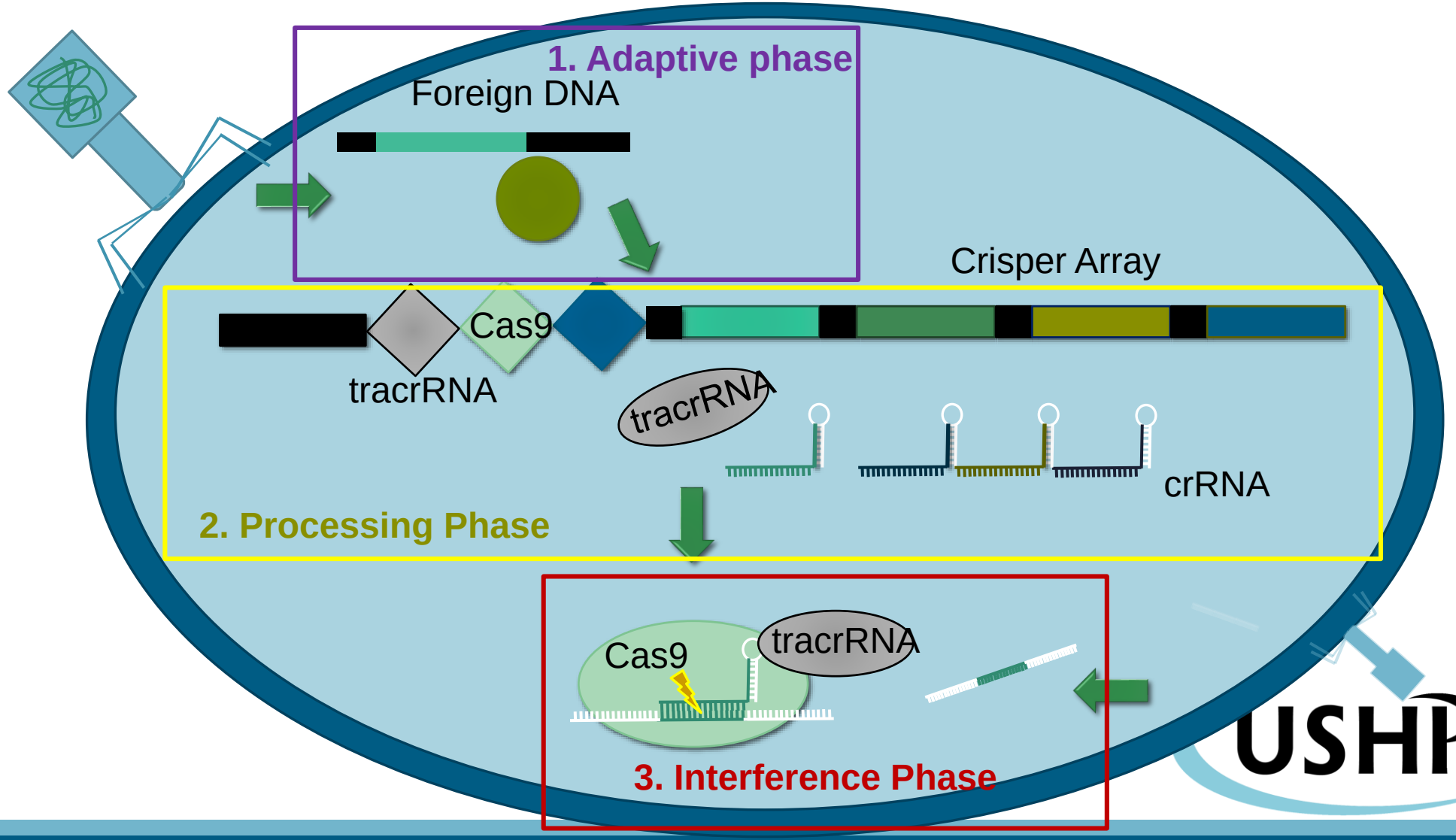
The CRISPR system found in prokaryotes and archaea

Bacterial immune system against bacteriophage

Crisper-associated protein (Cas)

Best characterized and most widely studied Cas9 from streptococcus *pyogenes*

CRISPR/Cas9 Mechanism



Current gene editing options

Zinc-finger nucleases (ZFN)

- Fok1 nuclease
- 2 proteins required
- Specificity is lacking
- Target motif (18-36 bp)
- Lacks multitargeted KO potential
- Very expensive
- Perhaps more off-target effects
- >10 weeks production time

Transcription activator-like effector nucleases (TALENS)

- Fok1 nuclease
- 2 proteins required
- Design is more streamlined than ZFN
- Target motif (24-59 bp)
- Highly specific
- 2 proteins required
- Expensive (cheaper than ZFN)
- >4 weeks production time
- Multitargeted KO possible

CRISPR/Cas9

- Cas9 nuclease
- 1 protein required
- Requires protospacer adjacent motif (PAM)
- Target motif (20-24 bp)
- Multiple target KO possible
- Much cheaper
- Much faster (~ 1 week)

Double Stranded DNA repair (NHEJ or HDR)

Non-homogenous end-joining (NHEJ)

- Does not use a donor template
- Gain or loss of size of DNA
- May result in indels (insertions or deletions)

Homology directed repair (HDR)

- Uses a donor template (endogenous or exogenous)
- Maintains size of DNA
- Results in correction of DNA or insertion of a gene

Who may benefit from CRISPR in the near-term?

Patients with relapsed/refractory disease that have exhausted all other options

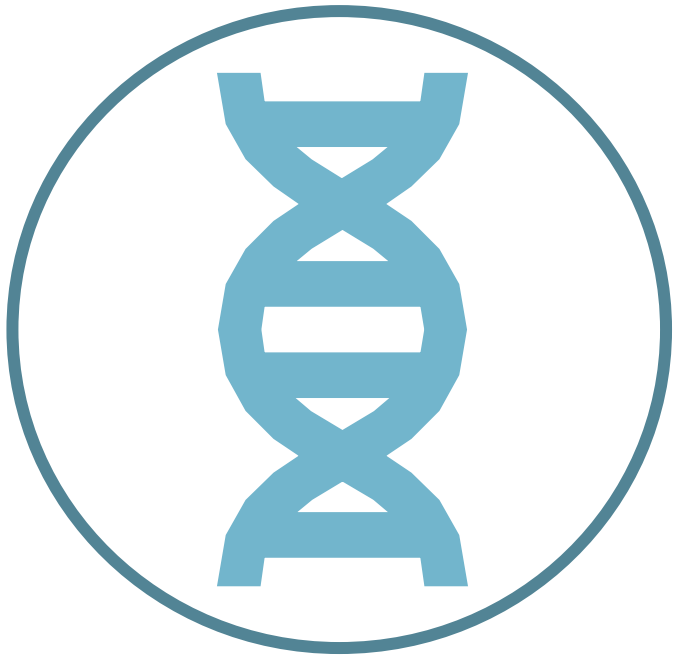
- Targeted molecular therapies
- Immunotherapy
- Effective chemotherapeutic options

Malignancies with a genetic target

- Tumor suppressor correction
- Oncogene knockout

Material delivery

- **Lipid nanoparticle**
  - Large range of potential cargo (e.g., DNA encoding Cas9, sgRA, Cas9 mRNA, etc.)
  - Ease of large-scale production
  - Low-toxicity
  - Lower efficiency of delivery
  - Timed release of CRISPR-Cas9 may reduce off-target effects



Viral delivery mechanism

Adeno-associated virus

Single-stranded DNA
4.7 kb capacity
High transduction efficiency
Low immunogenicity
Long-term Cas9 expression

Lentivirus

Single-stranded RNA
10 kb capacity
High transduction efficiency
Complicated packaging

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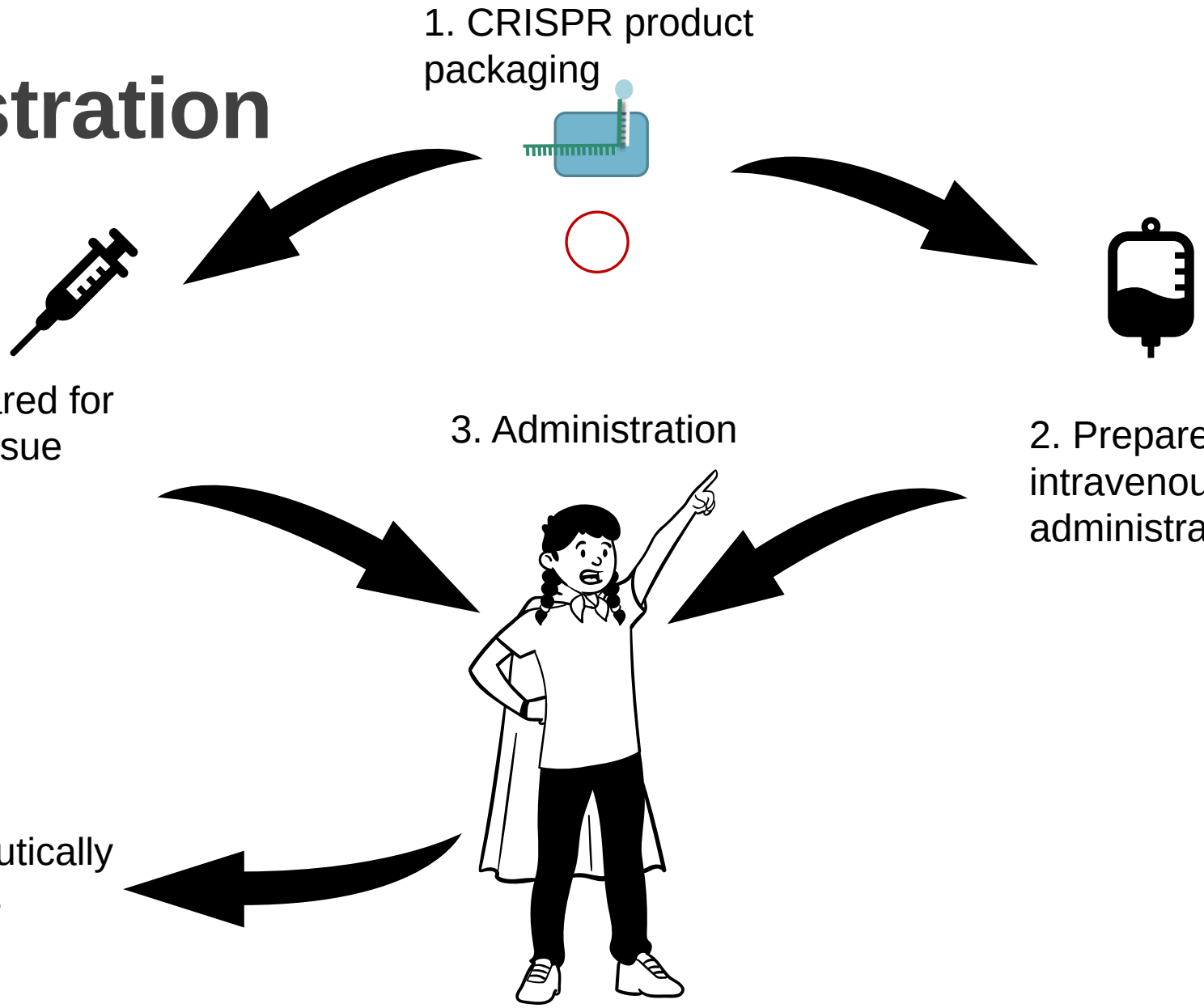
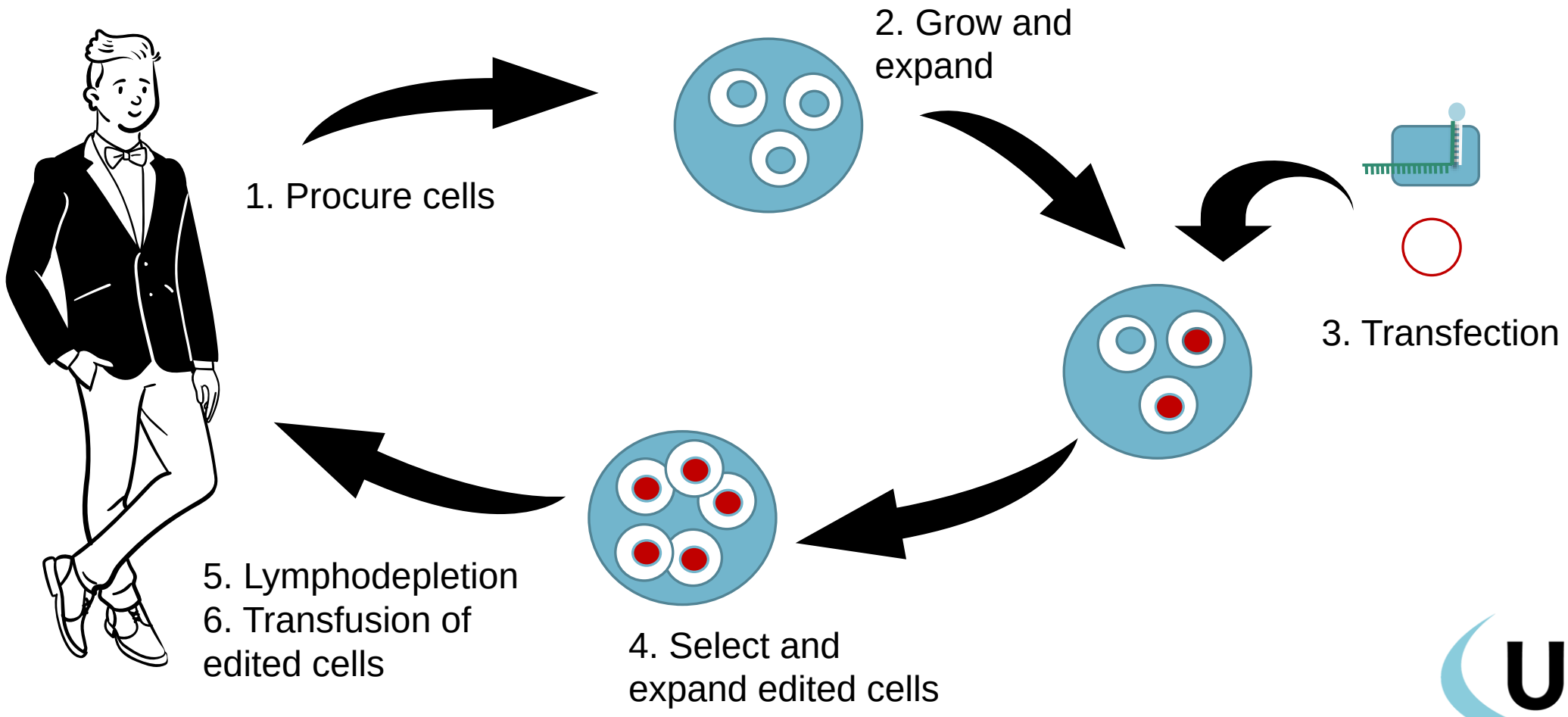
Behr M et al., *Acta Pharm Sin B*. 2021

In-Vivo administration



Ex-vivo administration



Uddin F et al., *Front Oncol*. 2020

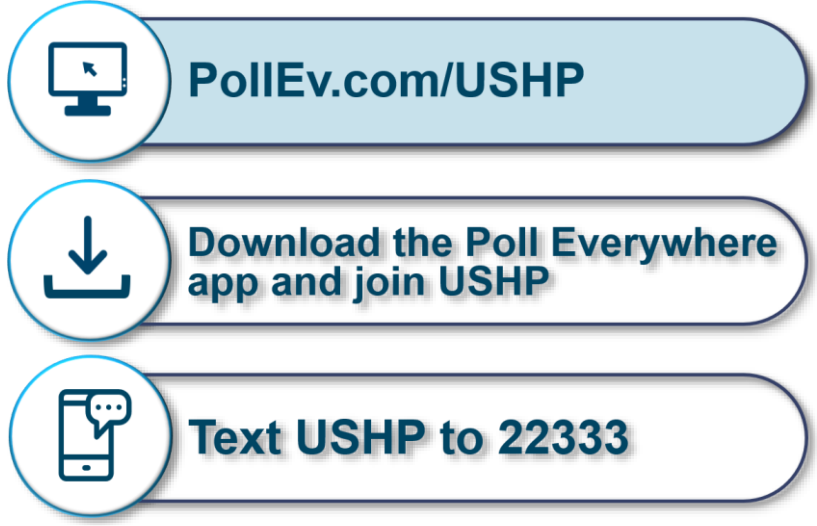
Question: Which step of the CRISPR/Cas9 mechanism includes the transcription and translation of CRISPR-associated proteins and crRNA?

A. Adaptive phase

B. Processing phase

C. Transcriptive phase

D. Interference phase

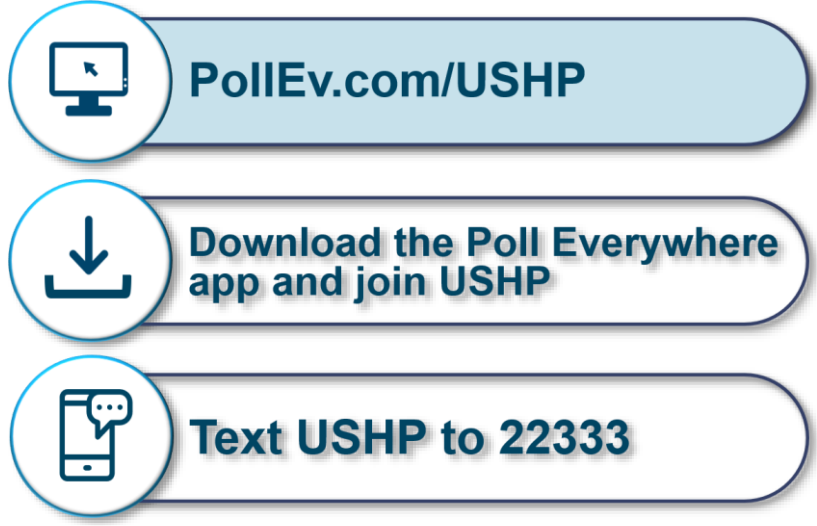


Question: CRISPR/Cas9 differs from traditional methods of gene editing using:

A. CRISPR/Cas9 uses FOK1 endonuclease that recognizes 18-36 bp sequence

B. CRISPR/Cas9 requires a 2-protein construct that is highly specific and very small in size allowing it to be more easily packaged

C. CRISPR/Cas9 uses a PAM for highly-specific sequence recognition and is less expensive and more easily produced



CRISPR/Cas9 in human disease:

Non-small cell lung cancer (*ex vivo* administration)

Transthyretin amyloidosis (*in vivo* administration)

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

Lu Y, Xue J, Deng T, Zhou X, Yu K, Deng L, Huang M, Yi X, Liang M, Wang Y, Shen H, Tong R, Want W, Li L, Song J, Li J, Su X, Ding Z, Gong Y, Zhu J, Wang Y, Zou B, Zhang Y, Li Y, Zhou L, Liu Y, Yu M, Wang Y, Zhang X, Yin L, Xia X, Zeng Y, Zhou Q, Ying B, Chen C, Wei Y, Li W, and Mok T.

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

Design	Phase I dose-escalating clinical trial; 4 cohorts
Population	18-70yr with histologically or cytologically confirmed stage IIIB or IV NSCLC • Disease progression after three or more systemic therapies including molecular targeted therapy
Intervention/Comparison	Reception of CRISPR/Cas9-modified PD-1 edited T-cell infusions
Outcomes	**Primary Outcome:** Sufficient and viable edited T cells manufacturing by patients (defined by >90% edited for >50% of patients) **Secondary Outcomes:** Objective response rate, editing efficiency, 8-week disease control rate, progression free survival, and overall survival **Safety Outcomes:** Adverse events, off-target effects **Additional Measures:** *in vivo* tracking of T-cells, diversity and dynamics of TCR clones
Enrollment Time	August 26, 2016 – March 21, 2018
Statistical Analysis	Kaplan-Meier analysis for time-event variables (PFS and OS), two-tailed T-test for IFN-γ production pre-/post edited cells, Wilcoxon rank-sum test for TCR diversity, one-way ANOVA for immunohistochemistry analysis $P \leq 0.05$

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Lu Y, et al., *Nat Med*. 2020

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

- Anti-PD-1 checkpoint inhibitors in NSCLC
  - 5-yr survival rates of 15.5-23%
- Immune escape through PD-L1
- Could disruption of T-cell PD-1 independently decrease immune escape?

T-cell product preparation

PBMC's isolated from 60-80 mL from each NSCLC patient

Approximately 5-10x10^6 cells transfected with CRISPR plasmid using electroporation

Cells were cultured and expanded to a final yield of 0.5-1.5x10^9 cells per bag with >95% cell viability at 20-28d

Product stored at 2 °C – 8 °C during transportation and awaiting use

Product infused via a central or peripheral line to the patient

T-cell lymphodepletion

Cyclophosphamide (20 mg/kg) prior to the first infusion

Rationale:

Allows for expansion of T-cells following reinfusion

Elimination of regulatory T-cells and production of a “cytokine sink”

Potential proliferation and differentiation into memory-like T-cells

T-Cell product administration

30-minutes prior to administration, IM diphenhydramine

0.9% normal saline used to establish an IV line

Gently shake the bag 3-4 times to suspend the cells

Start the infusion and shake the bag gently every 20 minutes, gently pinch the bottom of the bag to prevent aggregation

Infuse at 15-20 drops/min and monitor the patient every 10-15 minutes

May increase the rate to a maximum of 60 drops/min per patient tolerability

T cell product Post-Infusion Monitoring

Patients hospitalized for ≥ 7 days post-infusion

Immediately after infusion:

- Monitored every 30-minutes for 2-hours or until resolution of potential symptoms
- Cytokine release syndrome (CRS)
- Tumor lysis syndrome (TLS)
- Neurotoxicity (ICE/ICANS)
- GvHD

Medications classes to avoid*

- High-dose systemic steroids
- Immunomodulators
- G-CSF
- Human growth factors
- Antitumor therapies

* Unless compelling indication

T-cell product adverse event monitoring and management

CYTOKINE RELEASE SYNDROME (CRS)

IMMUNE EFFECTOR CELL-ASSOCIATED NEUROTOXICITY SYNDROME(ICANS)

GRAFT VS. HOST DISEASE (GVHD)

Cytokine Release Syndrome Grading

CRS Parameter	Grade 1	Grade 2	Grade 3	Grade 4
Fever	Temperature ≥38°C	Temperature ≥38°C	Temperature ≥38°C	Temperature ≥38°C
With hypotension	None	Not requiring vasopressors	Requiring a vasopressor with or without vasopressin	Requiring multiple vasopressors (excluding vasopressin)
And/or hypoxia	None	Requiring low-flow nasal cannula	Requiring high-flow nasal cannula, facemask, or nonrebreather	Requiring positive pressure (e.g., BiPAP, CPAP, intubation or mechanical ventilation)

Lee DW, *Biol Blood Marrow Transplant*. 2019

Cytokine Release Syndrome Management

CRS Severity	Tocilizumab	Corticosteroids	Hypotension management
Grade 1	Tocilizumab may be considered	N/A	N/A
Grade 2	Administer tocilizumab 8 mg/kg IV over 1 hour (max 800 mg) May repeat every 8 hours as needed if not responsive to IV fluids or oxygen Limit to ≤3 doses in 24-hours, maximum of 4 total doses	Manage per institutional guidelines if no improvement after initial tocilizumab therapy. Continue corticosteroids until event is grade ≤1, then taper	Mange per institutional guidelines
Grade 3	Per Grade 2	Per Grade 2	Manage per institutional guidelines
Grade 4	Per Grade 2 (if unresponsive to tocilizumab and steroids, consider other anticytokine agents)	Per Grade 2	Manage per institutional guidelines

Immune effector cell encephalopathy (ICE) score

Domain	Assessment	Maximum Score
Orientation	Year, month, city, hospital	4 points
Naming	Name 3 objects (button, pencil, cup)	3 points
Following command	Ability to follow commands (e.g., “Show me 3 fingers” or “Close one eye, and open your mouth”	1 point
Writing	Ability to write a standard sentence (Noun and a verb)	1 point
Attention	Ability to count backward from 100 by 10’s	1 point

ICANS Grading

Neurotoxicity	Grade 1	Grade 2	Grade 3	Grade 4
ICE Score	7-9	3-6	0-2	0 (unarousable)
Depressed LoC	Awakens spontaneously	Awakens to voice	Awakens to tactile stimuli	Unarousable or requires vigorous tactile stimuli
Seizure	N/A	N/A	Any clinical seizure (focal or general) that resolves rapidly or nonconvulsive	Life-threatening or prolonged (>5 min) or repetitive
Motor findings	N/A	N/A	N/A	Deep focal motor weakness
Elevated ICP/Cerebral Edema	N/A	N/A	Focal/local edema on imagine	Diffuse cerebral edema

Lee DW, *Biol Blood Marrow Transplant*. 2019

ICANS Management

Severity	Management
Grade 1	Supportive care
Grade 2	Consider administration of dexamethasone 10mg* IV q6 hours unless already on equivalent dose for CRS Continue dexamethasone until event is ≤ Grade 1, then taper
Grade 3	Administer dexamethasone 10mg* IV q6 hours unless subject is already on the equivalent dose of steroids for CRS. Continue dexamethasone until event is ≤ Grade 1, then taper
Grade 4	Administer methylprednisolone 1000 mg IV per day for 3 days If symptoms improve, manage as above

* Or equivalent

Acute GvHD Grading

Stage	Skin	Liver (bilirubin)	Lower GI (stool output/day)	Upper GI
0	No active GvHD rash	<2 mg/dL	<500 mL/d or <3 episodes/d	None or intermittent N/V or anorexia
1	Maculopapular rash (<25% BSA)	2-3 mg/dL	500-999 mL/d or 3-4 episodes/d	Persistent N/V or anorexia
2	Maculopapular rash (25-50% BSA)	3.1-6 mg/dL	1-1.5 L/d or 5-7 episodes/d	-
3	Maculopapular rash (>50% BSA)	6.1-15 mg/dL	>1.5 L/d or >7 dpisodes/d	-
4	Generalized erythroderma (>50% BSA) in addition to bullous formation/desquamation >5% BSA	>15 mg/dL	Severe abdominal pain w/ or w/o ileus or grossly bloody stool	-

Harris AC, et al., *Biol Blood Marrow Transplant.* 2016

Acute GvHD Management

Grade	Management
1	Skin: Topical steroids or immunosuppressants
2-4	Initiate IV methylprednisolone 2mg/kg/d* Taper steroid after ≥3 days of steroids; to decrease by 50% of total dose every 5 days GI: In addition to steroids, start anti-diarrheal agents

*or equivalent. Consider IV over oral dosage form if concern for malabsorption

Patient Characteristics, inclusion

Stage IIIB or IV NSCLC	Age 18-70	PD-L1 positive (≥ 1%)
POD through third-line treatment	ECOG PS: 0-2	Life expectancy ≥ 3 months

Patient characteristics

Characteristic	Pre-A (n=2)	A (n=4)	B (n=3)	C (n=3)	Total (n=12)
Age, median	55.5	56	55	53	54.5
Male, (%)	100%	50%	33%	100%	67%
Smoker, (%)	100%	50%	0%	100%	58%
Squamous, (%)	50%	0%	33%	0%	17%
EGFR+, (%)	0%	25%	33%	33%	25%
ALK+, (%)	0%	0%	0%	0%	0%
PD-L1+ ≥50%, (%)	100%	50%	0%	67%	50%

Dose escalation phase I: 4 cohorts

Pre-A (Safety Cohort) N=2	Cohort A N=3	Cohort B N=3	Cohort C N=3
1x $2x10^7$ cells/kg	2x $1x10^7$ cells/kg	2x $2x10^7$ cells/kg	2x $3x10^7$ cells/kg

- Each receiving 20%, 30%, and 50% on days 1, 3, and 5
- Day -3, lymphodepletion with cyclophosphamide 20 mg/kg
- Day 28, cycle-2 of T-cell infusion without lymphodepletion

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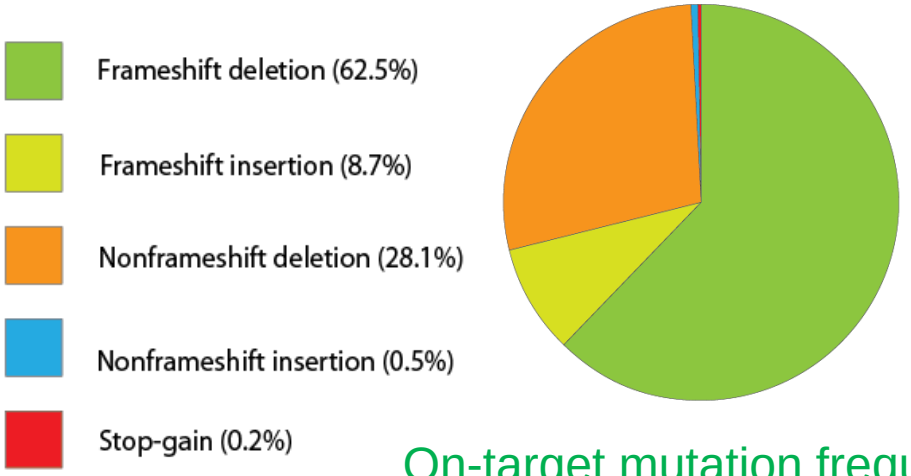
Lu Y, et al., *Nat Med*. 2020

Safety: Treatment-Related adverse events

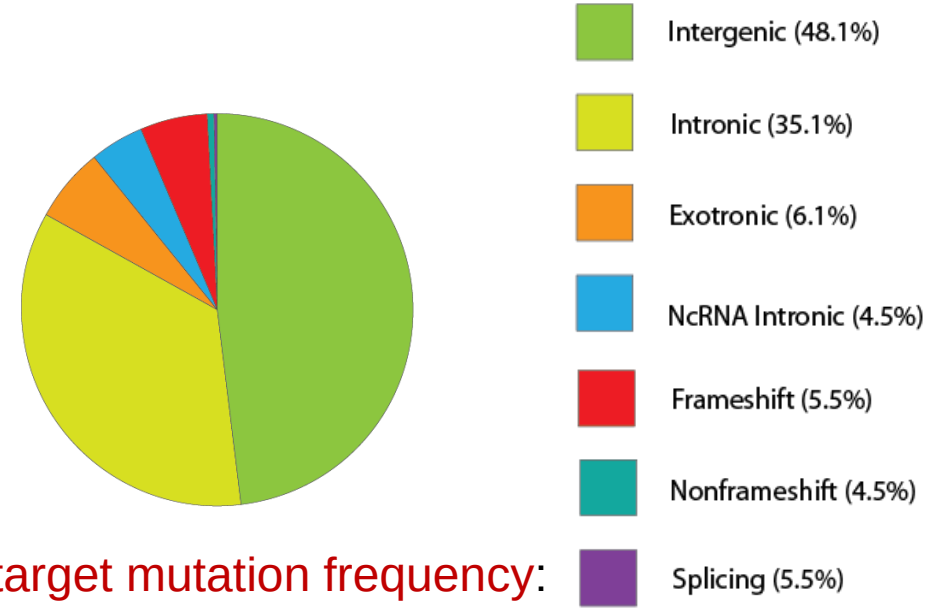
	Total	1	2	≥ 3
Any Event	11 (92)	8 (67)	3 (25)	0
Lymphopenia	3 (25)	2 (17)	1 (8)	0
Fatigue	3 (25)	3 (25)	0	0
Leukopenia	2 (17)	1 (8)	1 (8)	0
Fever	2 (17)	2 (17)	0	0
Arthralgia	2 (17)	2 (17)	0	0
Rash	2 (17)	2 (17)	0	0
Neutropenia	1 (8)	0	1 (8)	0
Infusion-related	1 (8)	0	1 (8)	0
Hyperhidrosis	1 (8)	1 (8)	0	0
Arrythmia	1 (8)	1 (8)	0	0
Hypertension	1 (8)	1 (8)	0	0
AST elevation	1 (8)	1 (8)	0	0
ALT elevation	1 (8)	1 (8)	0	0
Thrombocytopenia	1 (8)	1 (8)	0	0
Anemia	1 (8)	1 (8)	0	0

Safety: On vs off-target mutation characterization



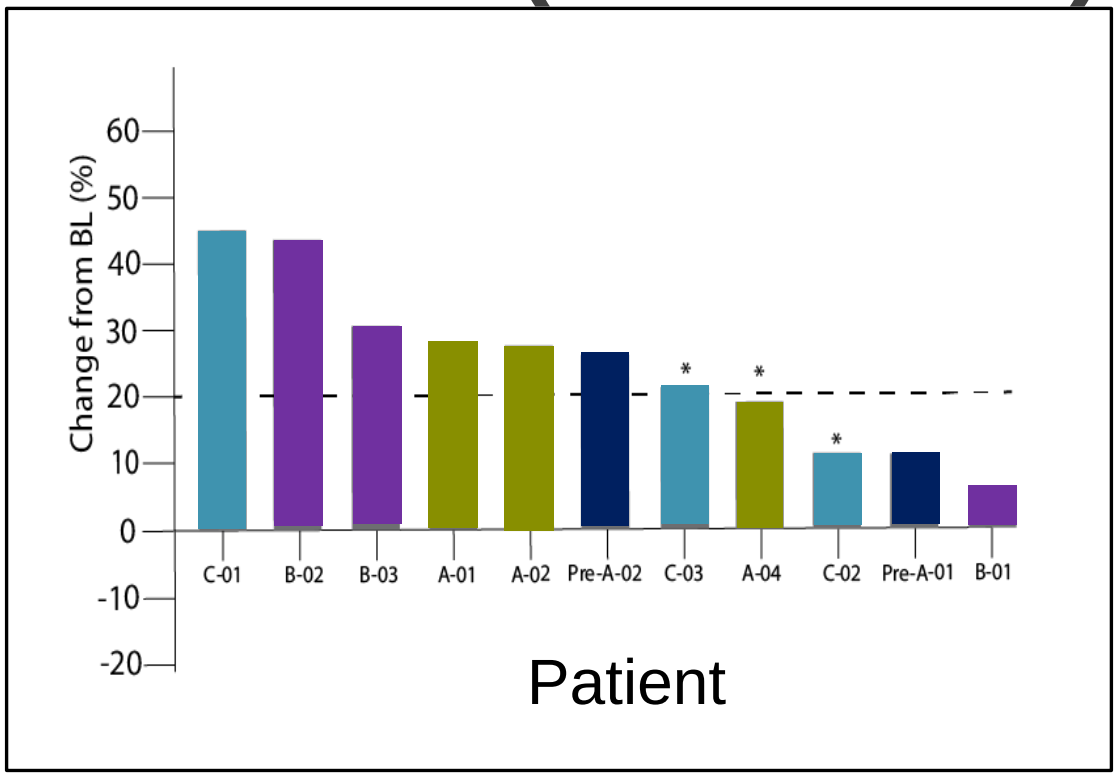
On-target mutation frequency:
1.69% (range 0.03-11.08%)

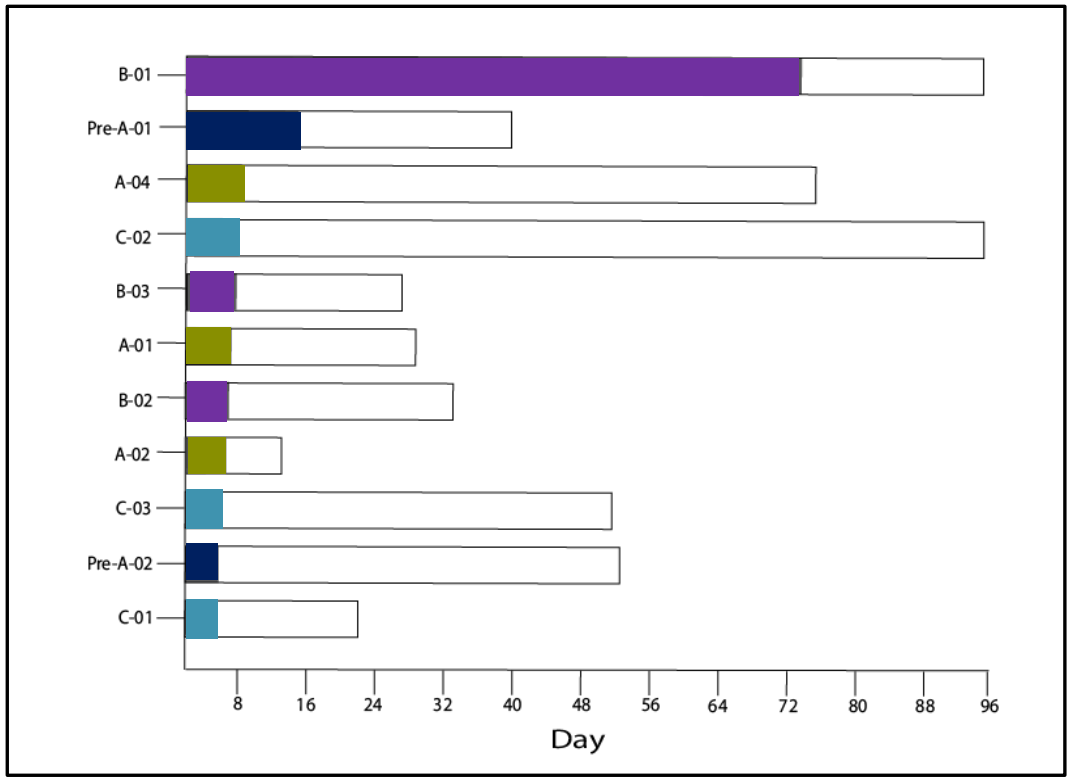
On target : Off target modification ratio
On : Off = 48.7



Off-target mutation frequency:
0.05% (range, 0-0.25%; all sites)

Efficacy: Best percentage change from baseline (tumor size)





Efficacy End-points

Median PFS
7.7 weeks (95% CI 6.9 – 8.5 weeks)

Median OS
42.6 weeks (95% CI 10.3 – 74.9 weeks)

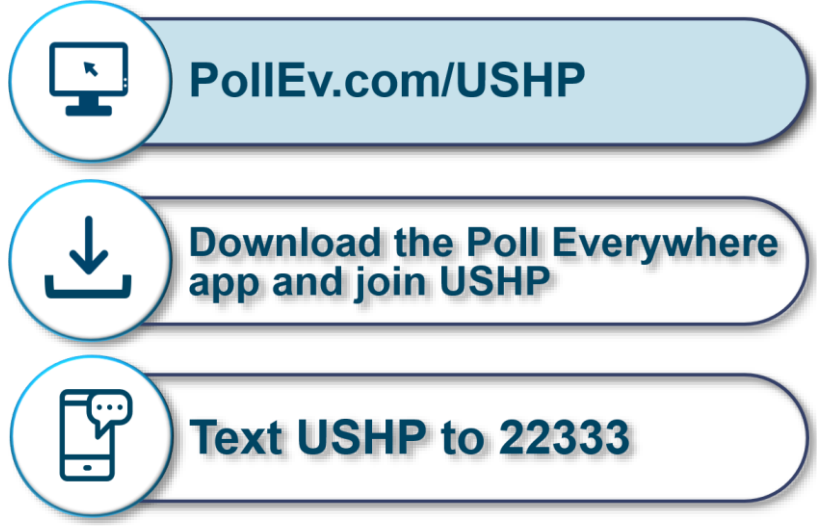
No partial response

Two stable disease

91.7% had died from tumor progression

No treatment-related death

Question: Diagram the steps of CRISPR/Cas9 in the ex-vivo administration of modified T-cells:



A. Procurement of T-cells, transfection of cells with CRISPR plasmid, lymphodepletion, infusion of cells, monitoring for toxicity

B. Procurement of T-cells, lymphodepletion, transfection of cells with CRISPR plasmid, monitoring for toxicity, infusion of cells

C. Lymphodepletion, transfection of cells with CRISPR plasmid, procurement of T-cells, infusion of cells, monitoring for toxicity

D. Procurement of T-cells, transfection of cells with CRISPR plasmid, infusion of cells, lymphodepletion, monitoring for toxicity

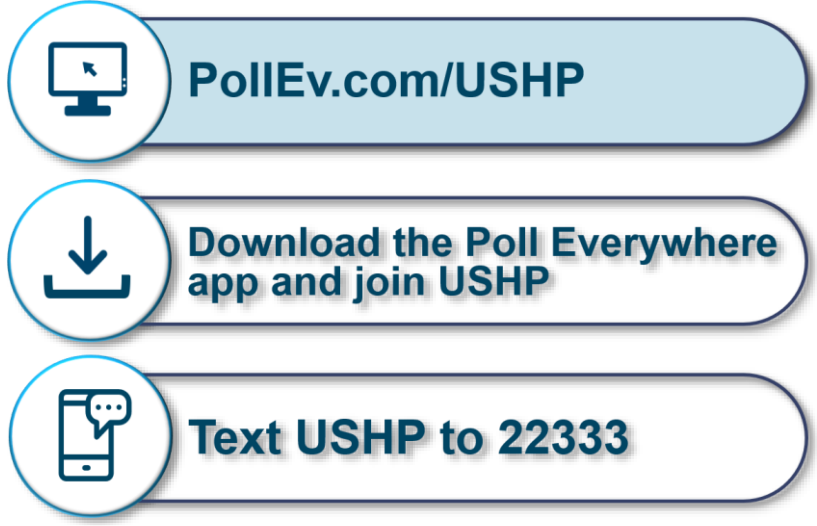
Question: Delivery of CRISPR/Cas9 modified T-cells for treatment of solid tumors utilizes:

A. In vivo modification and administration

B. Ex vivo modification and administration

C. In vitro modification and administration

D. Ex vitro modification and administration



CRISPR-Cas9 in vivo gene editing for transthyretin amyloidosis

Gillmore J, Gane E, Taubel J, Kao J, Fontana M, Maitland M, Seitzer J, O'Connell D, Walsh K, Wood K, Phillips J, Xu Y, Amaral A, Boyd A, Cehelsky J, McKee M, Schiermeir A, Harari O, Murphy A, Kryatsous C, Zambrowicz B, Soltys R, Gutstein D, Leonard J, Sepp-Lorenzino L, and Lebwohl D.

CRISPR-Cas9 in vivo gene editing for transthyretin amyloidosis

Design	Phase I open-label, multicenter study, proof of concept
Population	18-80yr with a diagnosis of polyneuropathy due to hATTR amyloidosis (w/wo cardiomyopathy), 50-90 kg, and without access to approved ATTR amyloidosis treatments. • Previous use of TTR stabilizers was permitted (washout of 3 days for diflunisal)
Intervention/ Comparison	Infusion of a single NTLA-2001 at a total RNA dose of 0.1mg/kg or 0.3mg/kg
Outcomes	**Primary Outcome:** Change in Serum TTR concentration **Safety Outcomes:** Off-target effects, adverse event monitoring (in-progress)
Study Time	November 2020 – April 2021
Statistical Analysis	Descriptive analysis only. Measurements of serum TTR protein levels at baseline compared with those at day 28 presented as mean percentage change and range

TTR protein as an ideal CRISPR target

Limited functionality of TTR

TTR Production

>99% hepatic

Lipid nanoparticle delivery

Lipid Nanoparticle product: Storage and preparation

- Product supplied in frozen liquid single-use vials
- Stored ≤ -65 °C to -85 °C
- Product diluted in 250 mL 0.9% normal saline infusion bag
- Time from freezer removal through completion of infusion not to exceed 4-hours

Lipid Nanoparticle product: Premedication

8-24 hours prior to administration of LNP

- Oral dexamethasone 8 mg or equivalent

1-2 hours prior to administration of LNP

- Intravenous steroid (e.g., dexamethasone 10mg or equivalent)
- Intravenous H1 blocker (e.g., diphenhydramine 50mg or equivalent) or oral H1 blocker (e.g., cetirizine 10mg or equivalent)
- Intravenous or oral H2 blocker (e.g., famotidine 20mg or equivalent)

Lipid Nanoparticle product: Administration

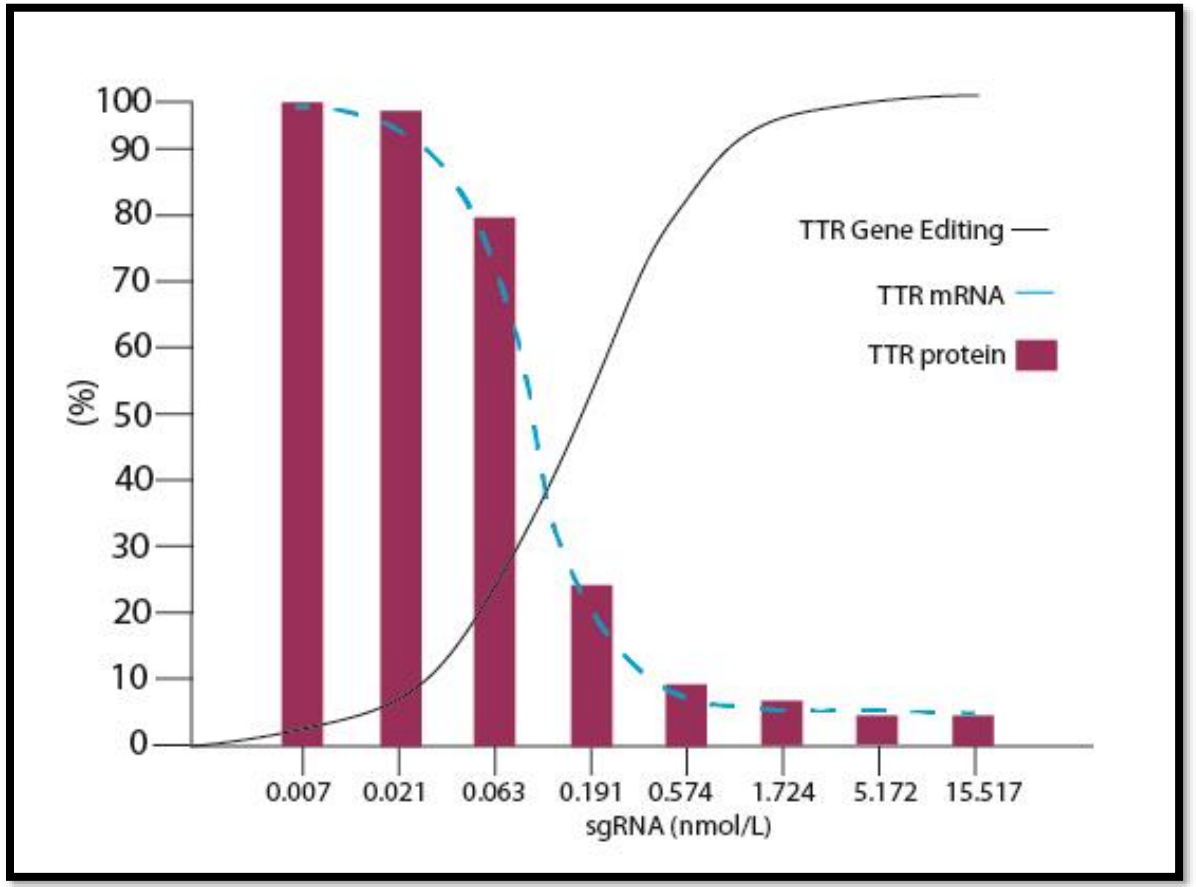
- Product administered over a 2-hour infusion (maximum of 4-hours)
- Patient monitored for a minimum of 96-hours post-infusion
  - Infusion related reactions
  - Signs and symptoms of CRS
  - Liver function tests
  - Hematologic symptoms

Gillmore JD et al., *N Engl J Med*. 2021

hATTR: pharmacokinetics

- Primary hepatocytes
  - EC_{50}: 0.05 – 0.15 nmol/L
  - EC_{90}: 0.17 – 0.67 nmol/L

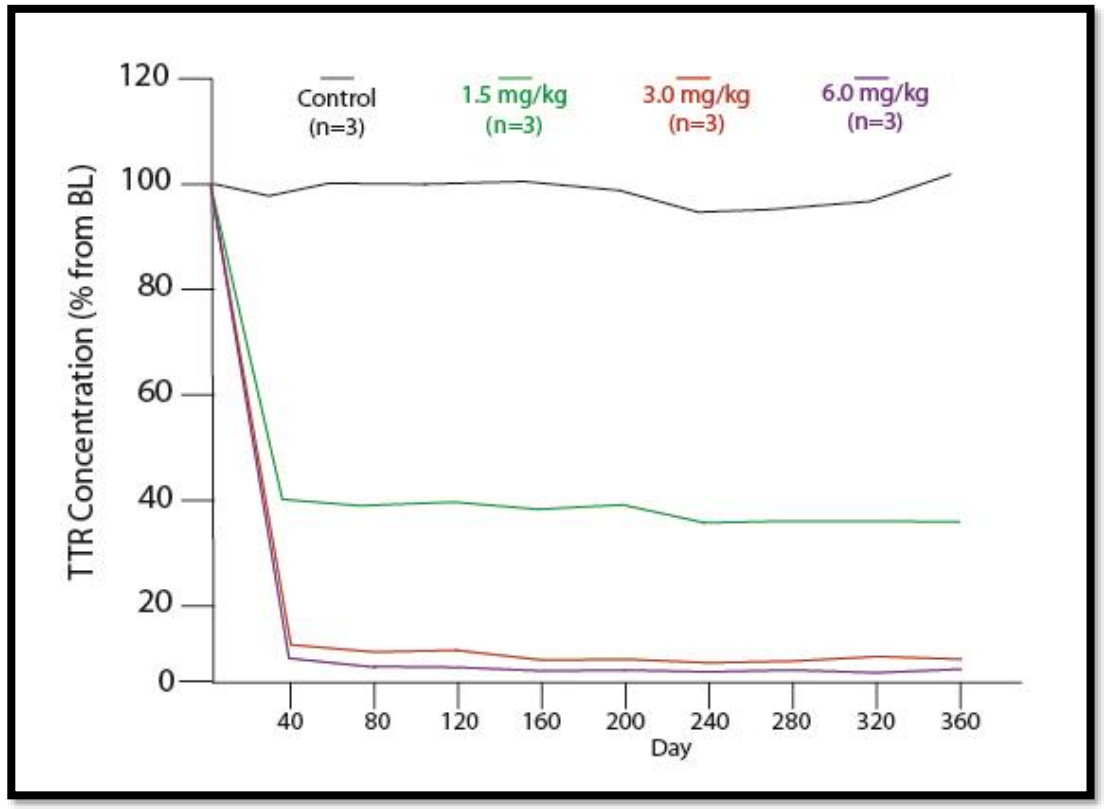
- *TTR* editing: 93.7%
- *TTR* mRNA: ≥91% reduction
- TTR protein: ≥95% reduction



Gillmore JD et al., *N Engl J Med*. 2021

hATTR: time to response

- Transgenic mouse model
- Decrease in circulating serum TTR protein
  - Nadir at **4-weeks** with a durable response at **12-months**
  - Resection of two-thirds of the liver resulted in durable response
- Cynomolgus monkey model of disease
  - 3-6 mg/kg single-dose Cyn-LNP
    - 73% gene-editing
    - >94% reduction in serum TTR protein over 12-months



Gillmore JD et al., *N Engl J Med*. 2021

hATTR Gene-editing *in vivo:* Patient characteristics

	Patients (n=6)
Age, range	46 – 64
Male, %	67%
Weight, range (kg)	70-90
p.T80A mutation	3/6 (50%)
p.S97Y mutation	2/6 (33%)
p.H110D mutation	1/6 (17%)
Previous therapy	3/6 (50%)
Previous diflunisal	3/6 (50%)
Sensory polyneuropathy	6/6 (100%)
NYHA- Class I	6/6 (100%)
N-terminal pro-B-type NP, range, ng/L	50 – 596

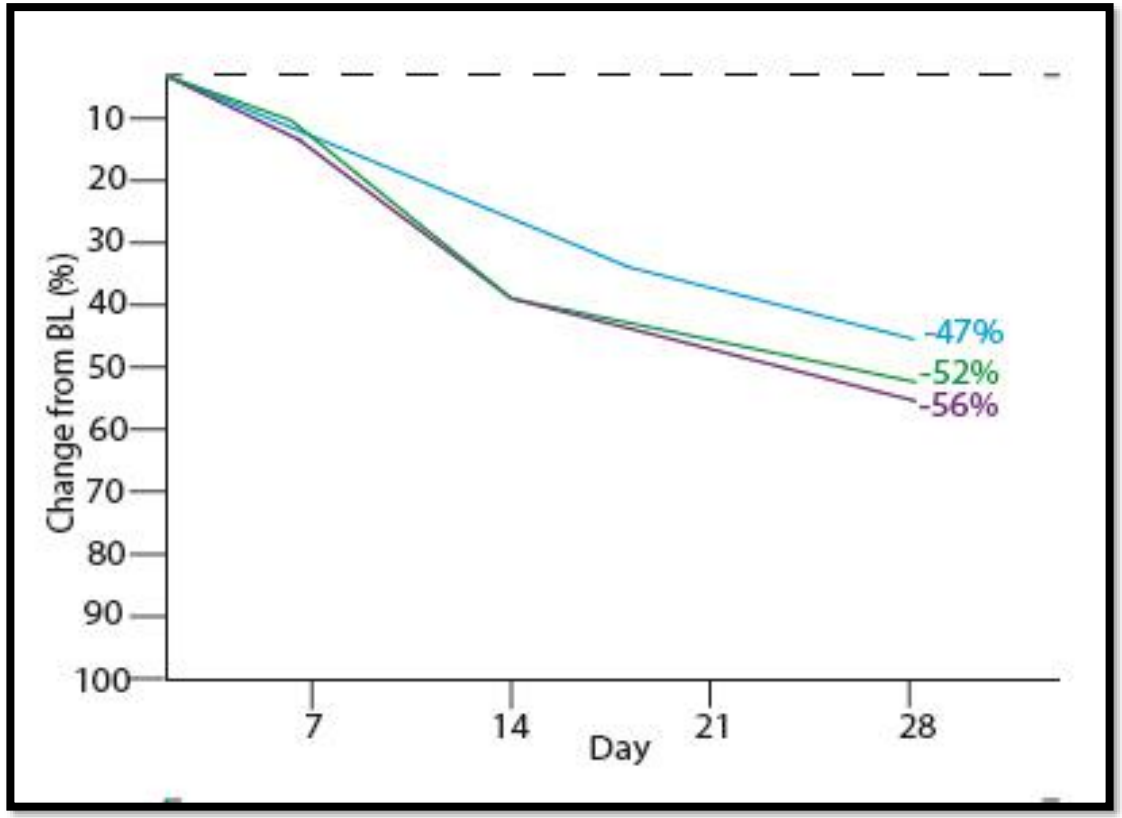
Gillmore JD et al., *N Engl J Med*. 2021

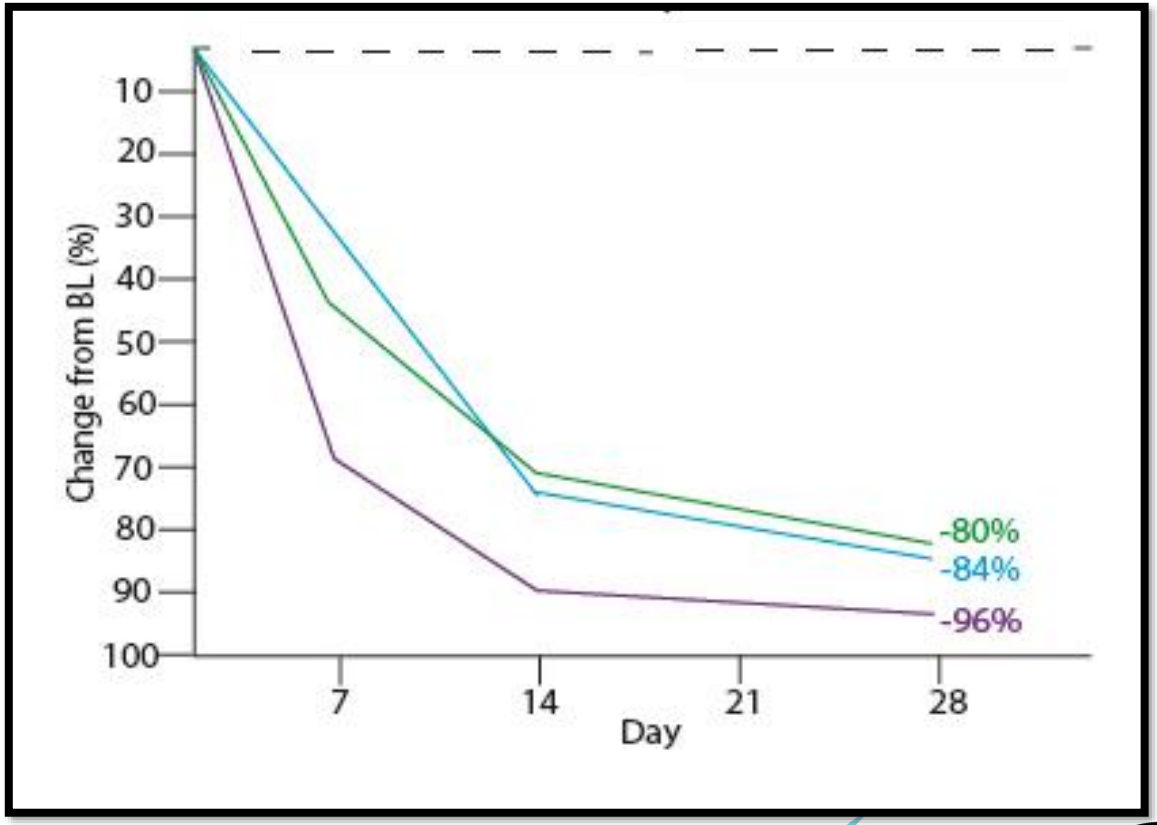
hATTR Gene-editing *in vivo:* (safety: Adverse events)

	Patients receiving 0.1mg/kg (n=3)	Patients receiving 0.3mg/kg (n=3)
Diarrhea	1	0
Nausea	1	0
Infusion-reaction	1	0
Skin abrasion	0	1
Headache	2	0
Vertigo	1	0
Foreign body sensation (eyes)	1	0
Catheter site swelling	1	0
Acute sinusitis	1	0
Thyroxine decreased	1	0
Rhinorrhea	1	0
Pruritis	1	0
Rash	1	0

Gillmore JD et al., *N Engl J Med*. 2021

hATTR Gene-editing *in vivo:* Time to response





CRISPR/Cas9 in oncology:

Where do we go from here?

CRISPR/Cas9 as a screening tool

- CRISPR/Cas9 is faster and cheaper to produce than other current offerings
- Plated into wells and produced as an array:
  - Large scale production using gene libraries for distinct gene knockout or insertion
  - Cancer cell lines transfected, and cell growth analyzed
  - Identification of molecular targets based on inhibition of growth
- Li et al., identified Npm1 for deletion in NSCLC
  - Significant reduction in tumor growth (*in vitro* and *in vivo*)
  - Larger effect in KRAS+ cells

CRISPR/Cas9 in Glioblastoma (GBM)

- Characterized by epidermal growth factor receptor (EGFR), vascular endothelial growth factor receptor (VEGF), and mammalian target of rapamycin (mTOR)
- Exon 17 of EGFR in glioma cells
- Huang et al., used CRISPR/Cas9 for knockout of exon 17
  - Increased expression of UBX domain containing protein-1 (UBXN1)
  - Suppressed nuclear factor-κβ (NFκβ)
  - Inhibition of GBM cell growth

CRISPR/Cas9 in Breast Cancer (BC)

- Triple-negative metastatic breast cancer is associated with poor outcomes
- Rattanasinchai et al., investigated MAP3K and ERK pro-survival pathway
  - Down-regulated mixed-lineage kinase-3 (MLK3) leading to decreased migration and invasion of TNBC-4T1 cells
- Protein tyrosine phosphatase N23 (PTPN23) and FYN (member of Src family kinases)
  - Zhang et al., knocked-out FYN and PTPN23 in both Cal-51 cell line and xenograft mouse model demonstrating reduced cancer cell growth in both models
  - FYN may play a roll in chemoresistance
  - Low PTPN23 a suppressor of BC motility and migration

Rattanasinchai C, et al., *Oncogenesis*. 2017
Zhang S, et al., *Genes Dev*. 2017

Concerns and limitations

- Off-target effects
- Adequate delivery to specific sites of disease
- Increasing editing efficiency
- Cas9 protein immune-response
- The morality of gene-editing

Future directions

What is to come in the next few decades?

Knock-out specific therapy before insertion

Adjuvant CRISPR/Cas9 to more traditional therapy

Whole gene replacement and correction of disease of genetic basis?

Conclusion

THE BASICS OF CRISPR/CAS9 GENE EDITING

SOME CURRENT USES OF CRISPR/CAS9 IN HUMAN DISEASE

POTENTIAL OF CRISPR/CAS9 IN ONCOLOGY AND AS A VEHICLE FOR DISCOVERY

LIMITATIONS AND CONCERNS

References

- Daisy PS, Shreyas KS, Anitha TS. Will CRISPR-Cas9 Have Cards to Play Against Cancer? An Update on its Applications. *Mol Biotechnol*. 2021;63(2):93-108.
- Gaj T, Gersbach CA, Barbas CF 3rd. ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. *Trends Biotechnol*. 2013;31(7):397-405.
- Miri SM, Tafsiri E, Cho WCS, Ghaemi A. CRISPR-Cas, a robust gene-editing technology in the era of modern cancer immunotherapy [published correction appears in Cancer Cell Int. 2020 Oct 28;20:521]. *Cancer Cell Int*. 2020;20:456. Published 2020 Sep 15.
- Behr M, Zhou J, Xu B, Zhang H. *In vivo* delivery of CRISPR-Cas9 therapeutics: Progress and challenges. *Acta Pharm Sin B*. 2021;11(8):2150-2171.
- Uddin F, Rudin CM, Sen T. CRISPR Gene Therapy: Applications, Limitations, and Implications for the Future. *Front Oncol*. 2020;10:1387. Published 2020 Aug 7.
- Zhang S, Shen J, Li D, Cheng Y. Strategies in the delivery of Cas9 ribonucleoprotein for CRISPR/Cas9 genome editing. *Theranostics*. 2021;11(2):614-648. Published 2021 Jan 1.
- Lu Y, et al., Safety and feasibility of CRISPR-edited T cells in patients with refractory non-small-cell lung cancer [published correction appears in Nat Med. 2020 Jul;26(7):1149]. *Nat Med*. 2020;26(5):732-740.
- Lee DW, Santomasso BD, Locke FL, et al. ASTCT Consensus Grading for Cytokine Release Syndrome and Neurologic Toxicity Associated with Immune Effector Cells. *Biol Blood Marrow Transplant*. 2019;25(4):625-638.
- Harris AC, Young R, Devine S, et al. International, Multicenter Standardization of Acute Graft-versus-Host Disease Clinical Data Collection: A Report from the Mount Sinai Acute GVHD International Consortium. Biol Blood Marrow Transplant. 2016;22(1):4-10.

References

- Gillmore JD et al., Gane E, Taubel J, et al. CRISPR-Cas9 In Vivo Gene Editing for Transthyretin Amyloidosis. *N Engl J Med*. 2021;385(6):493-502.
- Li F, Ng WL, Luster TA, et al. Epigenetic CRISPR Screens Identify *Npm1* as a Therapeutic Vulnerability in Non-Small Cell Lung Cancer. *Cancer Res*. 2020;80(17):3556-3567
- Huang K, Yang C, Wang QX, et al. The CRISPR/Cas9 system targeting EGFR exon 17 abrogates NF-κB activation via epigenetic modulation of UBXN1 in EGFRwt/vIII glioma cells. *Cancer Lett*. 2017;388:269-280.
- Rattanasinchai C, Llewellyn BJ, Conrad SE, Gallo KA. MLK3 regulates FRA-1 and MMPs to drive invasion and transendothelial migration in triple-negative breast cancer cells. *Oncogenesis*. 2017;6(6):e345. Published 2017 Jun 12
- Zhang S, Fan G, Hao Y, Hammell M, Wilkinson JE, Tonks NK. Suppression of protein tyrosine phosphatase N23 predisposes to breast tumorigenesis via activation of FYN kinase. *Genes Dev*. 2017;31(19):1939-1957