

پژوهشکده فناوری زیستی
The Research Institute of Biotechnology

CRISPR in Diagnostic and Therapy of Cancer

Dr. Lena Goshayeshi

Postdoc in medicine biotechnology
The Research Institute of Biotechnology | Ferdowsi University of Mashhad, Mashhad, Iran
Goshayeshilena@gmail.com

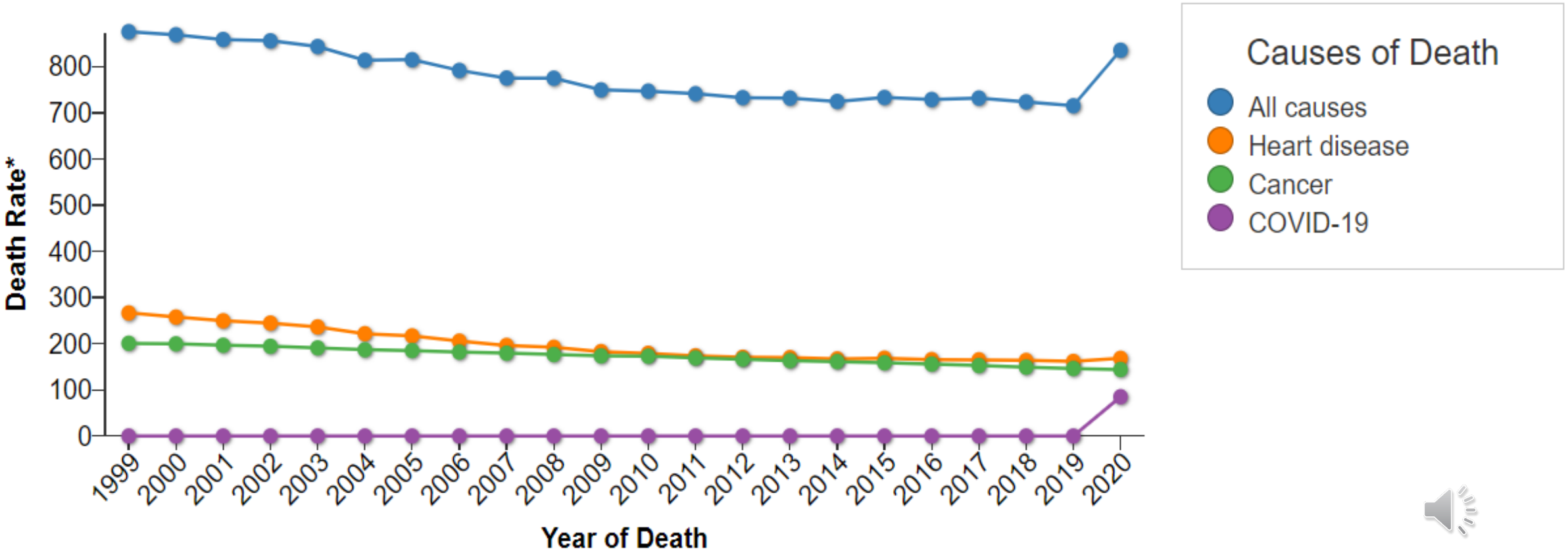
outline

1. **Cancer related Deaths and Main causative factors for human cancers**
2. **Cancer therapy approaches**
3. **Driver genes in cancer**
4. **Genome Editing**
5. **What is CRISPR Cas?**
6. **CRISPR genome editing & Immunotherapy of cancer**



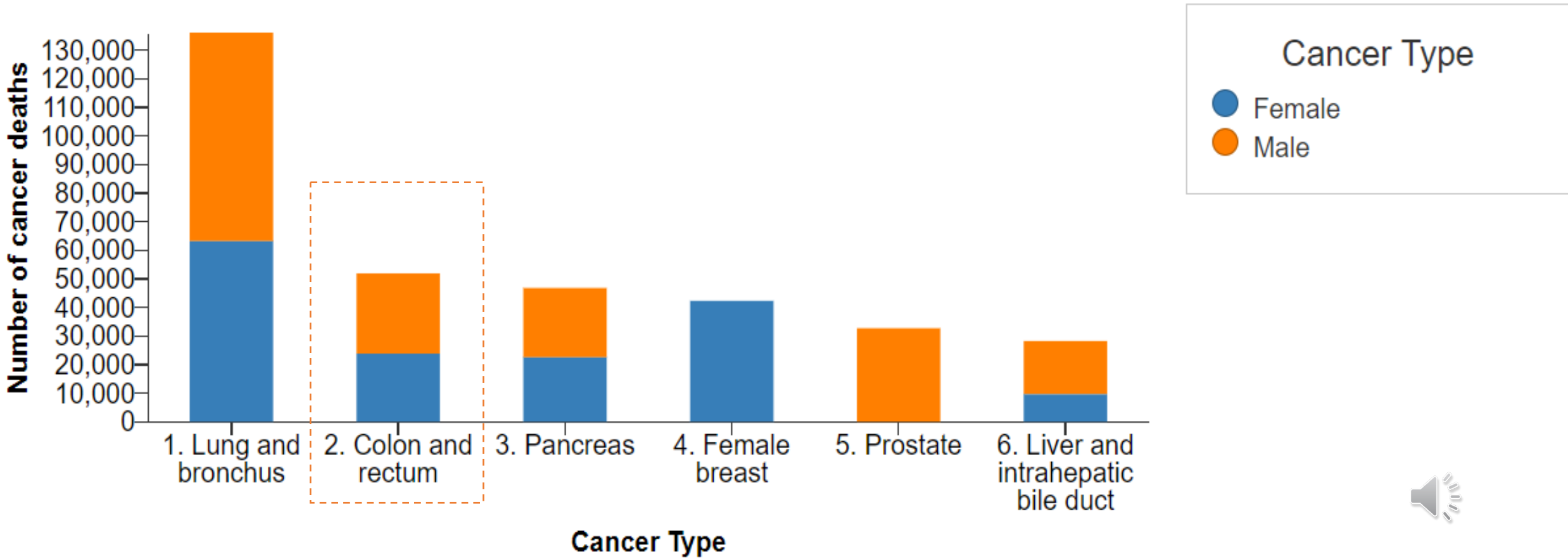
Cancer related Deaths

✓ WHO estimates that by the year 2030 cancer cases could surpass 20 million cases per year worldwide



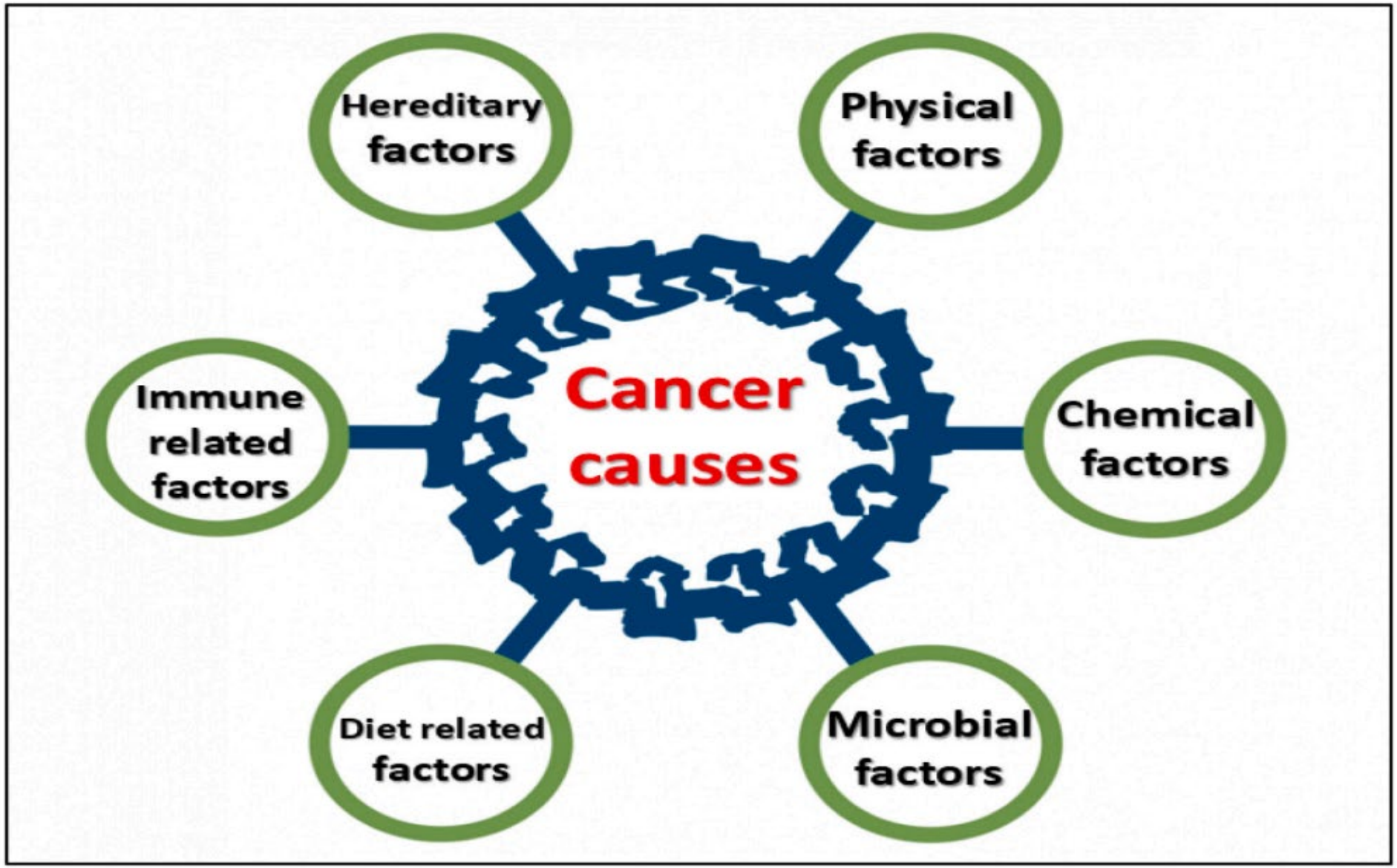
https://www.cdc.gov/

✓ Colon and rectum cancer is the second most common cancer among cancers



https://www.cdc.gov/

✓ Cancer is a complex and multifaceted disease



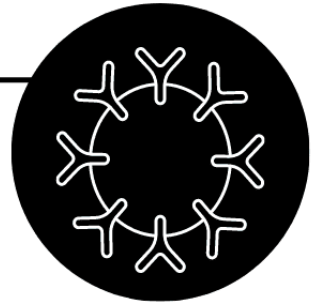
Review article Life Sciences 15 March 2021

CANCER TREATMENT OPTIONS

HORMONE THERAPY



IMMUNOTHERAPY



SURGERY



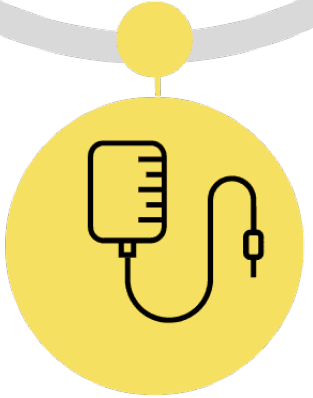
RADIATION THERAPY



BONE MARROW TRANSPLANTATION



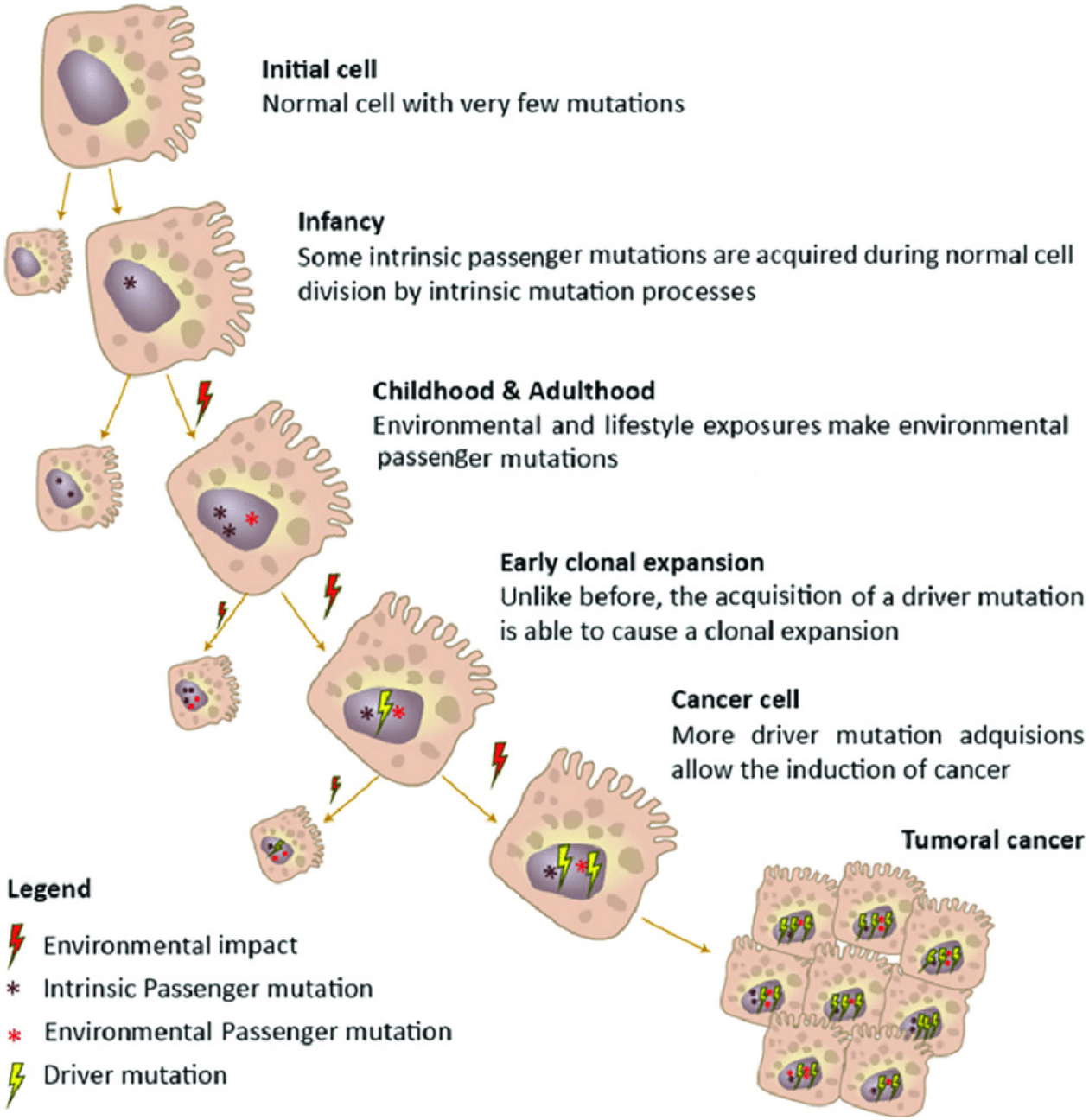
CHEMOTHERAPY



TARGETED THERAPY



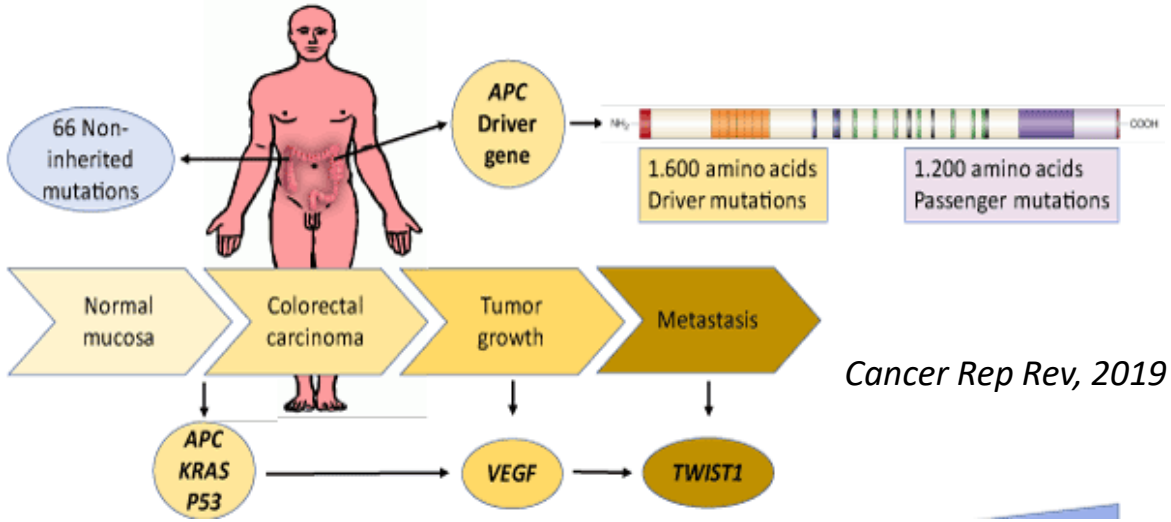
Driver genes in cancer



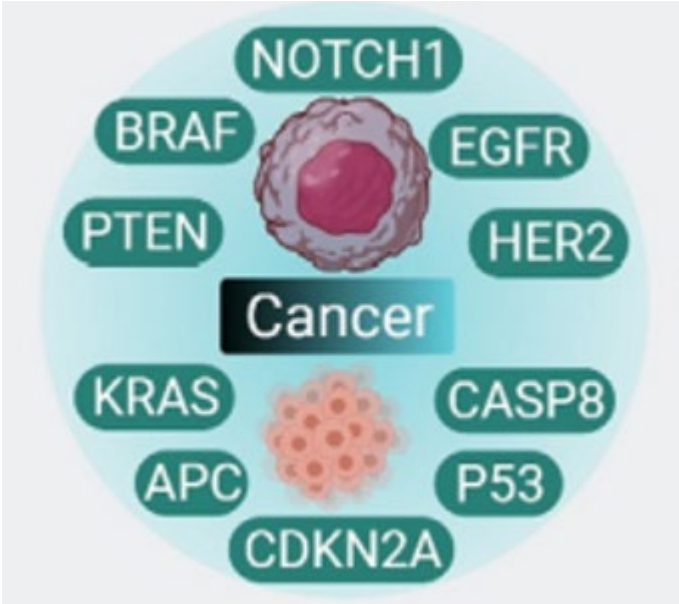
PNS, 2020

Role of Next-Generation Sequencing in Biomarker Discovery

- ❖WES - Whole Exome Sequencing
- ❖WGS - Whole genome Sequencing
- Identification of genetic and epigenetic biomarkers in gastrointestinal cancers
- Examples:
- mutations in BRCA, KRAS, TP53, MMR genes



Cancer Rep Rev, 2019



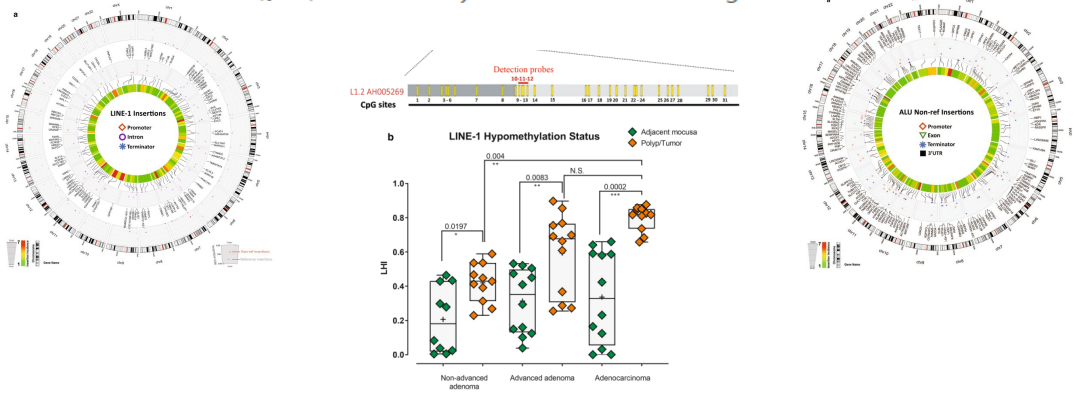
Shademan et al. Cancer Cell Int (2020) 20:426
https://doi.org/10.1186/s12935-020-01511-5

Cancer Cell International

PRIMARY RESEARCH Open Access

Promoter methylation, transcription, and retrotransposition of LINE-1 in colorectal adenomas and adenocarcinomas

Milad Shademan[1], Khadijeh Zare[2], Morteza Zahedi[1], Hooman Mosannen Mozaffari[3,4], Hadi Bagheri Hosseini[3,4], Kamran Ghaffarzadegan[5], Ladan Goshayeshi[3,6*] and Hesam Dehghani[2,7,8*]



Shademan et al. Cancer Cell Int (2019) 19:278
https://doi.org/10.1186/s12935-019-1000-1

Cancer Cell International

PRIMARY RESEARCH Open Access

Expression profile analysis of two antisense lncRNAs to improve prognosis prediction of colorectal adenocarcinoma

Milad Shademan[1†], Azam Naseri Salanghuch[1†], Khadijeh Zare[1], Morteza Zahedi[1], Mohammad Ali Foroughi[2], Kambiz Akhavan Rezayat[3,5], Hooman Mosannen Mozaffari[3,5], Kamran Ghaffarzadegan[6], Ladan Goshayeshi[3,4*] and Hesam Dehghani[1,2,7*]

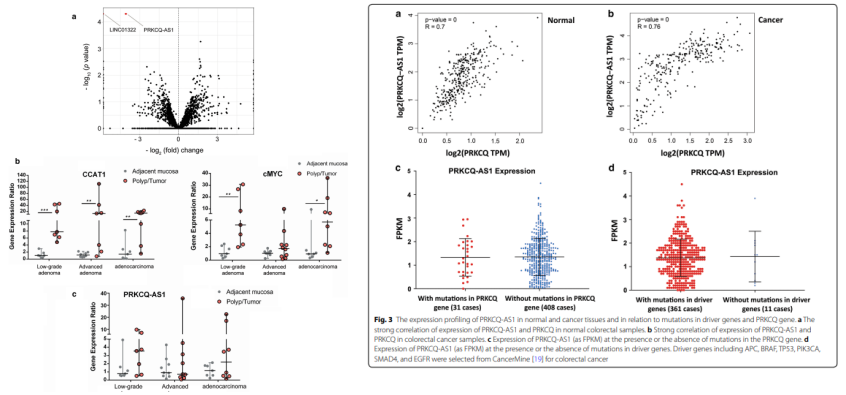


Fig. 3 The expression profiling of PRKCQ-AS1 in normal and cancer tissues and in relation to mutations in driver genes and PRKCQ gene. **a** The strong correlation of expression of PRKCQ-AS1 and PRKCQ in normal colorectal samples. **b** Strong correlation of expression of PRKCQ-AS1 and PRKCQ in colorectal cancer samples. **c** Expression of PRKCQ-AS1 (as FPKM) at the presence or the absence of mutations in the PRKCQ gene. **d** Expression of PRKCQ-AS1 (as FPKM) at the presence or the absence of mutations in driver genes. Driver genes including APC, BRAF, TP53, PIK3CA, SMAD4, and EGFR were selected from CancerMine [19] for colorectal cancer

پژوهشکده فناوری زیستی
The Research Institute of Biotechnology

❖ **ACMG SF v3.1 list for reporting of secondary findings in clinical exome and genome sequencing:**
The American College of Medical Genetics and Genomics (ACMG) has published recommendations for reporting of known pathogenic or likely pathogenic variants in the exons of a list of 78 genes as follow: *APC, RET, BRCA1, BRCA2, PALB2, SDHD, SDHAF2, SDHC, SDHB, MAX, TMEM127, BMPR1A, SMAD4, TP53, MLH1, MSH2, MSH6, PMS2, MEN1, MUTYH, NF2, STK11, PTEN, RB1, TSC1, TSC2, VHL, WT1, FBN1, TGFBR1, TGFBR2, SMAD3, ACTA2, MYH11, PKP2, DSP, DSC2, TMEM43, DSG2, RYR2, CASQ2, TRDN, TNNT2, LMNA, FLNC, TTN, BAG3, DES, RBM20, TNNC1, COL3A1, LDLR, APOB, PCSK9, MYH7, MYBPC3, TNNI3, TPM1, MYL3, ACTC1, PRKAG2, MYL2, KCNQ1, KCNH2, SCN5A, CALM1, CALM2, CALM3, BTD, GLA, OTC, GAA, HFE (p.C282Y homozygotes only), ACVRL1, ENG, RYR1, CACNA1S, HNF1A, RPE65, ATP7B, TTR..*

Goshayeshi *et al. Cancer Cell International* (2025) 25:140
https://doi.org/10.1186/s12935-025-03773-3

Cancer Cell International

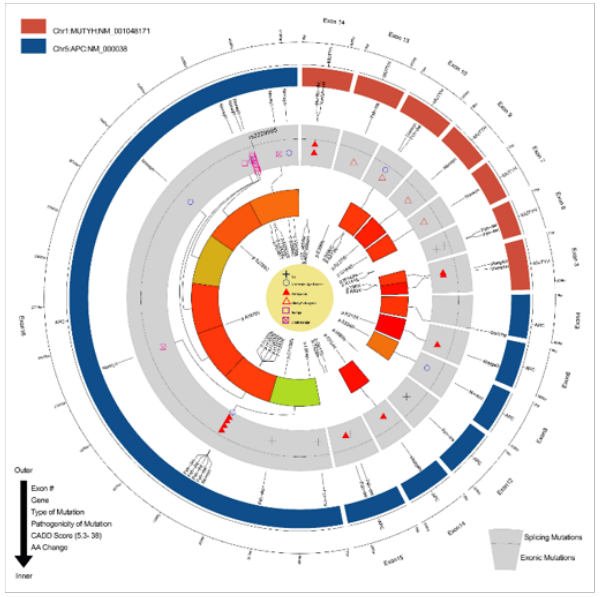
پژوهشکده فناوری زیستی
The Research Institute of Biotechnology

RESEARCH Open Access

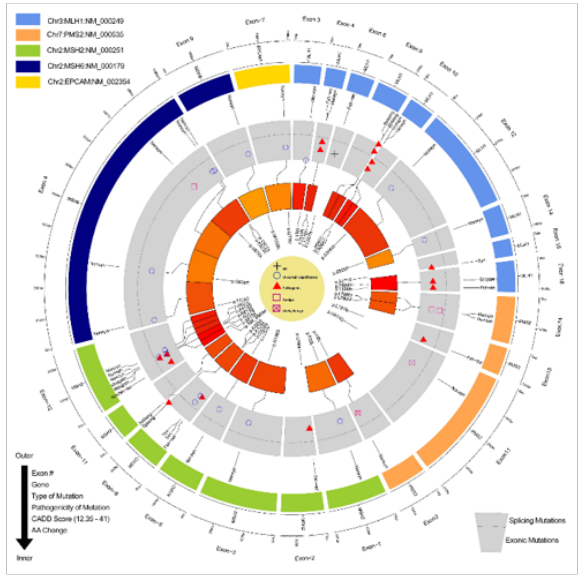
Germline variants in patients from the Iranian hereditary colorectal cancer registry

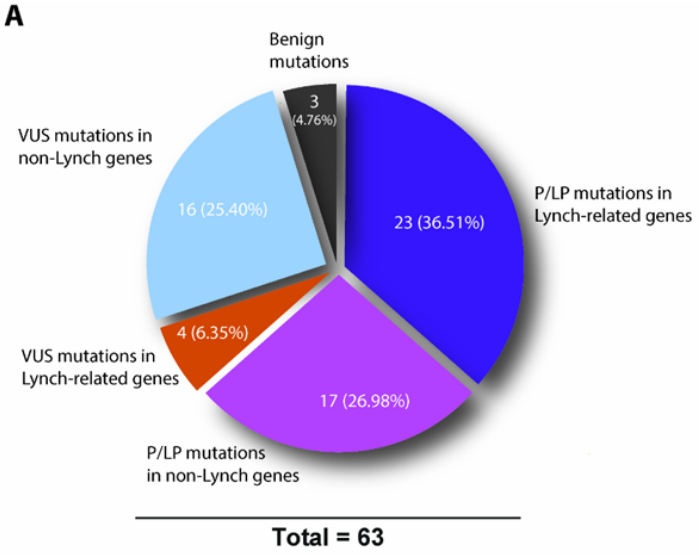
Lena Goshayeshi[1,2], Saeed Hoorang[3], Benyamin Hoseini[4], Mohammad Reza Abbaszadegan[5], Maryam Afrazeh[6], Maliheh Alimardani[5], Fatemeh Maghool[7], Milad Shademan[8], Morteza Zahedi[2], Mehrdad Zeinalian[9], Foroogh Alborzi[10], Mohammad Reza Keramati[11], Ashkan Torshizian[12], Hassan Vosoghinia[13], Farnood Rajabzadeh[14], Alireza Bary[15], Massih Bahar[16], Ali Javadmanesh[17,18], Jamshid Sorouri-Khorashad[19], Mohammad Hassan Emami[7], Nasser Ebrahimi Daryani[10], Hans F.A. Vasen[20], Ladan Goshayeshi[1,13*] and Hesam Dehghani[2,21*]

A

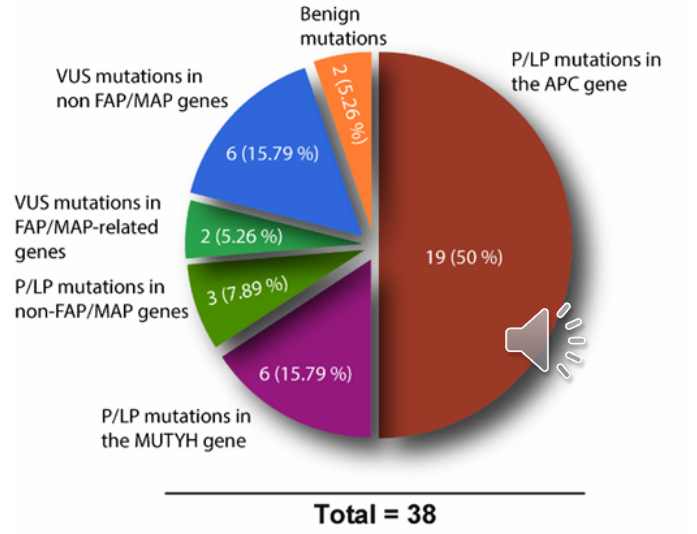


B





C



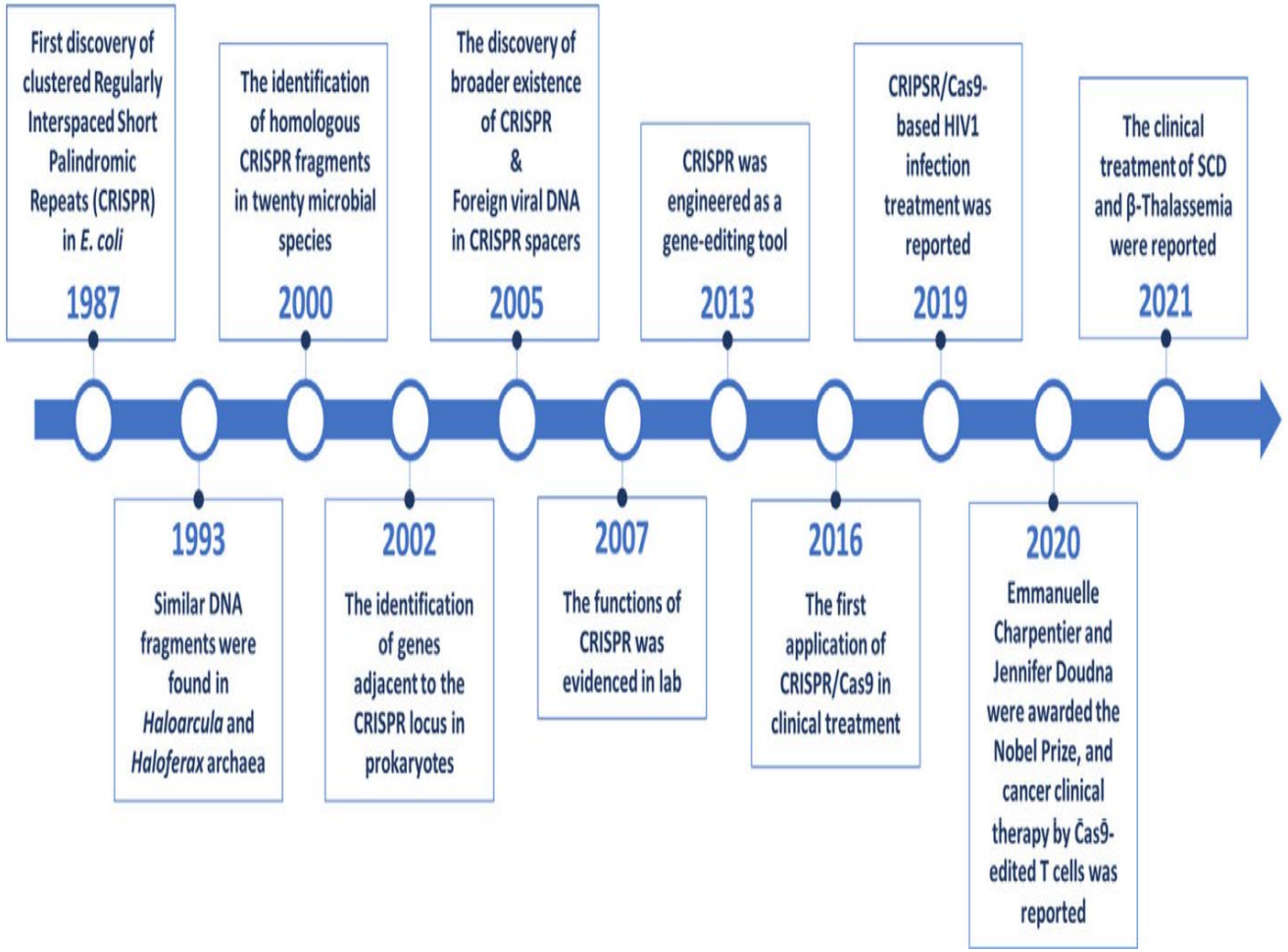
Pathogenic/likely pathogenic mutations			Germline variants of uncertain significance (VUS)				
Lynch-related genes	Non-lynch genes		Lynch-related genes	Non-lynch genes			
MLH1	**ABCA4**	**HPGD**	**MLH1**	**MSH3**	**DIAPH1**	**RNF6**	**KCNQ4**
rs63751194 [2]	rs761867791	rs121434480	p. L207fs	c.T569C	rs781577050	c.A1931G:p.K644R	rs760023398
rs267607789[2]	rs1800553 [5]	**ITGA7**	p.S368W	**PMS1**	**CREBBP**	**RNF43**	**LRRK2**
rs267607739	**ADAMTS18**	rs17854600	**MSH2**	rs147084726	rs200566758	rs766439784[2]	rs202009639
rs1064793177	c.C212G:p.S71X	**ITGB6**	rs878853827	**MLH3**	**CHL1**	c.T257C:p.I86T	**NOTCH1**
rs63751657	**ALDOB**	rs730880298	p. A615S [2]	rs151133595	rs775757574	**TTN**	c.G5972A:p.R1991H
rs63750217	rs1800546 [2]	**MSH3**	**MSH6**	c.G3884A:p.G1295E	**CFTR**	rs755956493	**PHGDH**
pT116P	**ASXL1**	c.1897-1G>A [2]	p.I22G	**GXYLT1**	rs193922516	**TTK**	c.G1117A:p.A373T
p. S529P	c.C2728T:p.Q910X	**MUTYH**	p.K793E	rs77044712 [5]	rs578029902	c.G323C:p.S108T	**NT5C**
P.Y684X	**ATM**	rs587778541		**BCR**	**CDC73**	**TLR2**	c.G28A:p.D10N
MSH2	rs763470424	**NPC1**		rs8792553796 [8]	p.P341L	rs767943819	**PLOD1**
rs63750493 [2]	**BCHE**	rs372445155		**DIS3L2**	**CDH1**	**ABCA4**	rs138698098
rs63751044	rs1799807	**PKHD1**		rs374080052	rs876659503	rs138359497	**POC1A**
rs267607940 [2]	**BCKDHA**	rs149798764		rs755574750	**EPHA3**	**CACNA1S**	rs146976547
rs63749831	rs373336888	c.C10780T:p.Q3594X		rs775360586	c.A2071G:p.K691E	rs200224590	**POLE2**
rs63749848	**C7**	**PMS1**		**APC**	**LRP2**	**COL6A6**	rs141483427
rs587779075	rs121964920	rs768246348 [2]		rs876660453	rs138269726	rs111457392	**POLG**
rs759263820	**CD36**	**SLC7A9**		c.C7477A:p.L2493I	**LMNA**	**DLL3**	rs751130039
c.1662-2A>C	c.C816A:p.C272X	rs121908486		**ALDOA**	rs142000963	rs142597040	**PTCH2**
MSH6	**CFTR**	**SOS1**		rs145582724 [2]	**MCM4**	**DOCK8**	rs140845138
rs587779926	rs397508761	rs397517150		**POLQ**	c.A802G:p.T268A	c.C2146T:p.R716C	**RYR2**
PMS2	**CNGB1**	**TCTN2**		rs757184831	**RB1**	**FASN**	rs769219555
rs63750695	rs183443649	rs374349989		rs572635997	c.T688C:p.S230P	rs748571162	**STARD9**
	COL6A3	**TGM1**		**ATAD2B**	**PALLD**	**GRID2**	rs202074007
	c.C1039T:p.Q347X	rs121918723		c.C1408T:p.R470X	c.A38G:p.Q13R	rs192450353	**SENP5**
	CTSA	**TP53**		**ERCC1**	**POLD1**	**IFT172**	rs996308782
	rs757912971	rs121912664		rs766869924	c.C1229G:p.A410G	rs144868723	**SUFU**
	DDOST	**TTN**		**ERCC4**	**PDGFRL**	**INPP5E**	rs141359583
	c.G696C:p.Q232H	rs748689777		rs374978891	rs35346456	rs199956627	**SMAD4**
	DNAH5	**USH2A**		**DDR2**	**APC**	**ITGA3**	c.T977C:p.I326T
	c.C5198A:p.S1733X	rs80338902		rs371333028	rs876660453	rs201830820	**VPS13B**
	ECHS1	c.T8271G:p.Y2757		**DLC1**	**WWOX**	**JAK2**	rs371325199
	rs754609693	**WRN**		rs774778702	rs200815431	rs139504737	**ZNF677**
	FH	rs878854131		**DICER1**	**DNAH5**		rs566714089
	rs587782618	**WNK1**		c.A4499T:p.D1500V	rs113742238		
	GALT	rs910119961		**DLG5**			
	rs75391579			c.C418T:p.Q140X			
	G6PD						
	rs5030868 [2]						

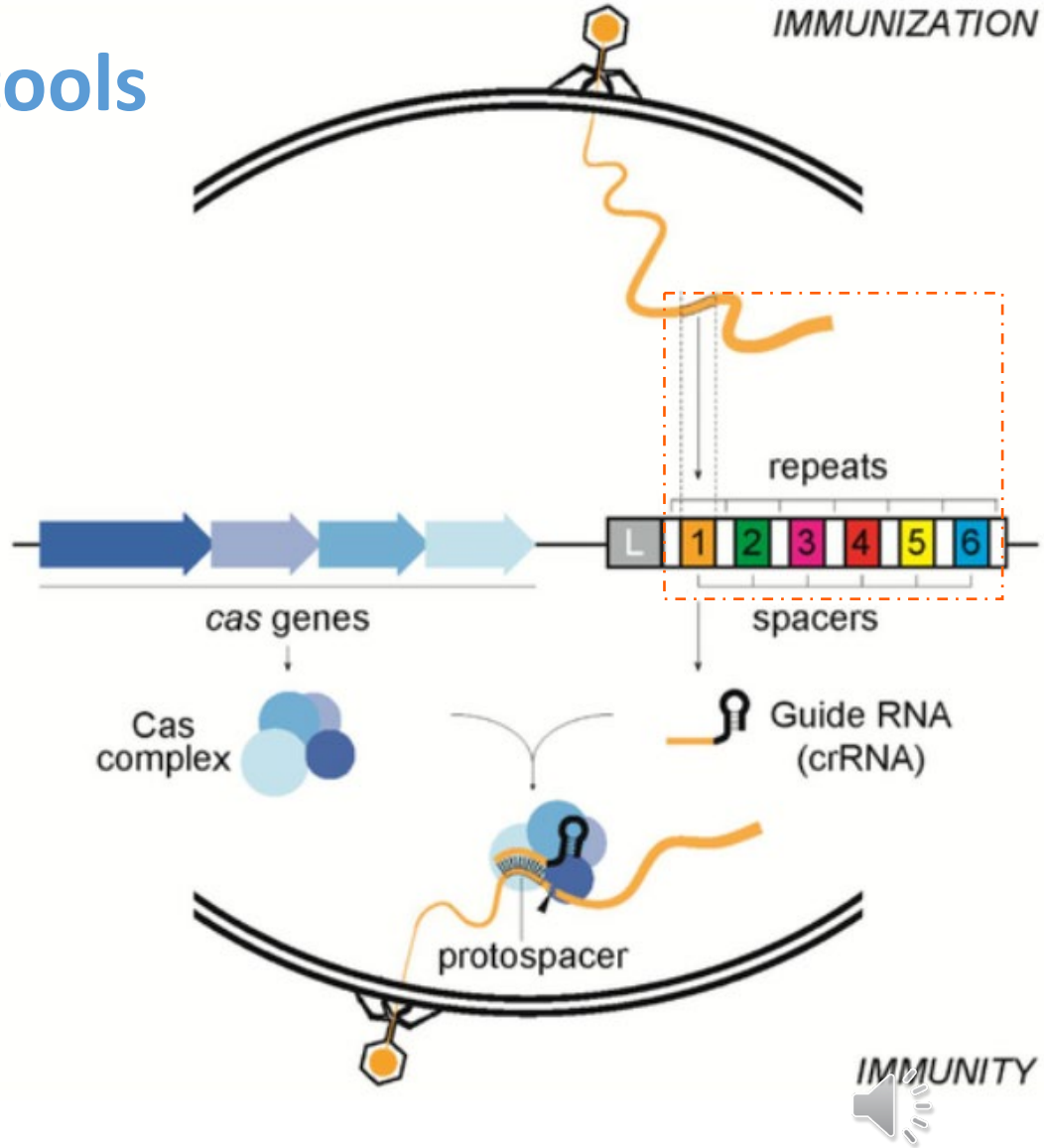
Table S2. List of genes with germline variants identified by whole-exome sequencing of blood samples in patients at risk for polyposis syndrome. All variants have been selected based on the minor allele frequency (MAF) of less than 0.01. *Numbers in superscript indicate the number of identified patients with the mutation. Gene names without any numbers have been observed only in one patient.

Pathogenic/likely pathogenic mutations			**Germline variants of uncertain significance (VUS)**		
FAP/MAP-related genes	Non-FAP/MAP genes		FAP/MAP-related genes	Non-FAP/MAP genes	
APC	**BCHE**	**IQCE**	**APC**	**MSH6**	**KDM5C**
rs1219132244	rs1799807	rs773701437	rs759307079	rs587779939	rs782205045
rs587781392	**BRCA2**	**MCM9**	rs770912433	rs63750832	**MSH2**
rs1064794227	rs80359200	rs587777872	**MUTYH**	rs587781616	rs151129360
rs876659647	c.1040delA: p. Q347fs	**PTEN**	rs199840380	**MSH3**	**CLK2**
rs137854573	**PADI3**	rs121909219		c.C214T: p. P72S	c.C13T: p.R5X
rs587779352	rs1421294093	**NEB**		**MCPH1**	**UBAP2**
rs5877822932	**SERPINA7**	c.C13537T p. Q4513X		rs572671721	c.C802T: p. Q268X
rs1801155	rs617544902	**RAD50**		**MLH3**	**MAP3K9**
p. K542fs	**EXOSC3**	rs397507178		rs151133595	c.C787T: p. Q263X
c.1483_1484insTT	rs797045567	**PRRT2**		rs375623365	**POLE2**
c.422+1G>A	**BLM**	rs730882071		**BRCA2**	c.C653T: p. P218L
p. G611fs2	rs587783037	**SBDS**		c.G9184A: p. D3062N	**FGFR4**
p. L964fs	**EIF2B2**	rs120074160		**BRCA1**	rs760787865
p. I1307K	p. Q72X	**TTN**		rs431825403	**POLK**
MUTYH	**CD36**	rs794729301		**TOPBP1**	rs148960463
Biallelic	rs747098468	**GALNT12**		rs774702840	
rs761468459	rs56381858	c.1063_1064 del p.		**BRIP1**	
rs587778541	**FCGR1A**	T355fs		rs765314472	
rs121908380	rs74315310	**PADI3**		**FCN3**	
c.424_425del: p. W142fs	**FLG**	rs144080386		rs532781899	
c.421_421del: p. K141fs	rs145119819	**TNNI2**		**MLH1**	
Monoallic	**GUCY2C**	c.C353A:p.S118X		rs926905165	
rs138775799	rs762829348			**POLE**	
rs34126013	**ILDR1**			rs778190944	
rs587781864	rs142746163				
rs587780078					



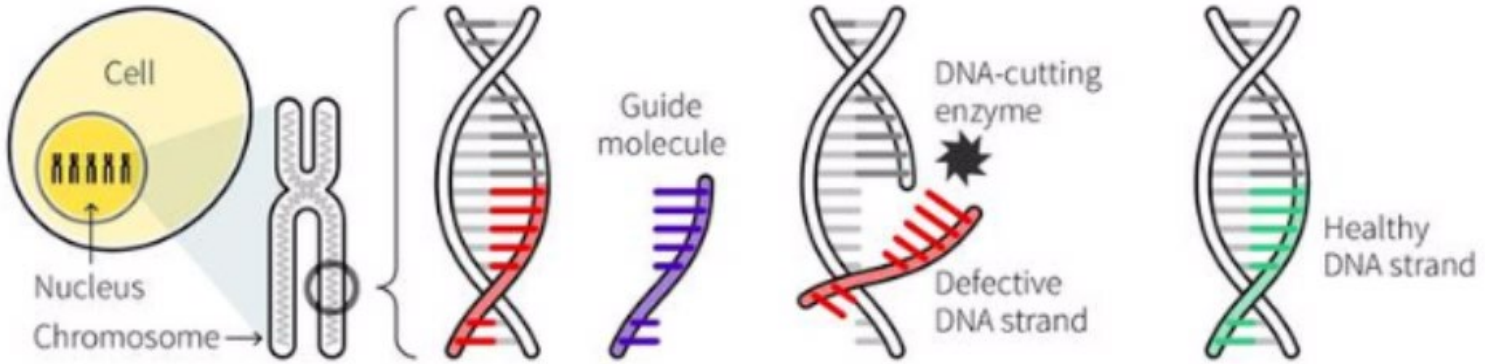
What is CRISPR/Cas9 genome editing tools

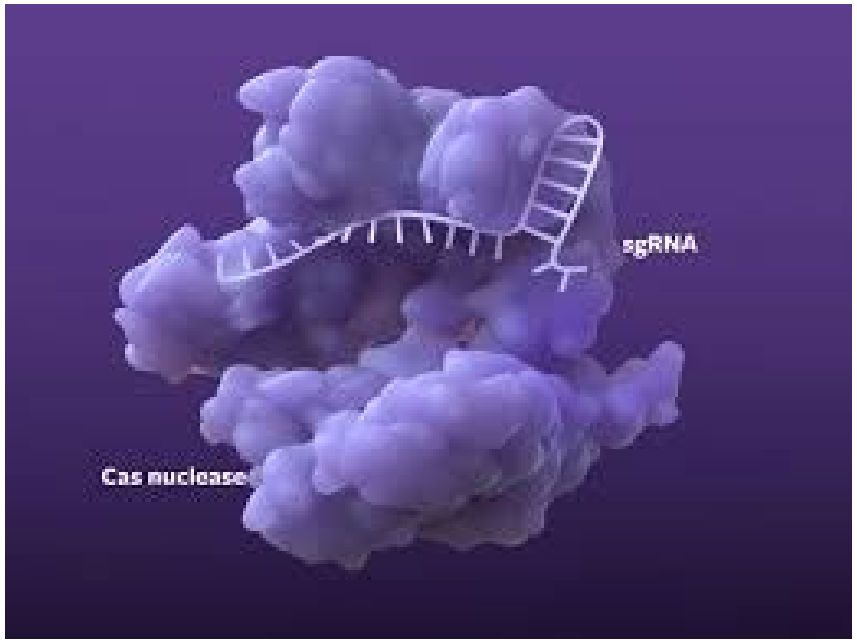


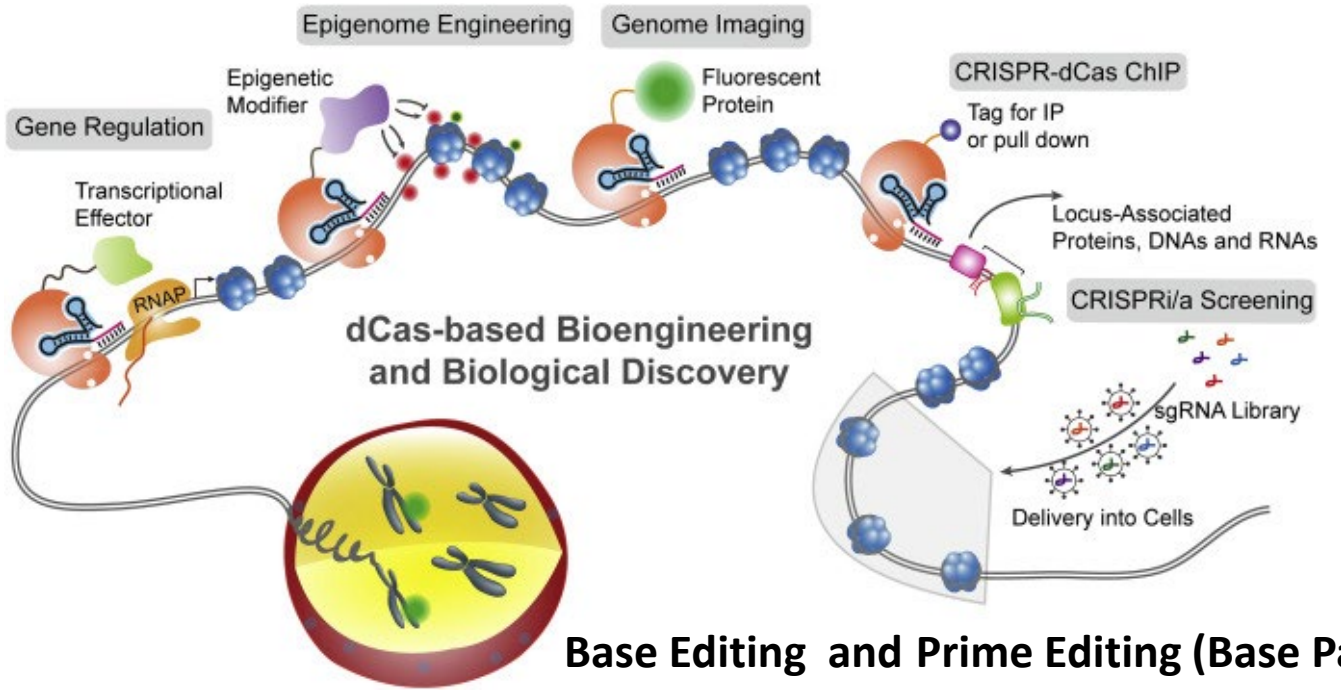


Advanced Research2021---FASEB,2016

Genome editing and mutation correction with CRISPR





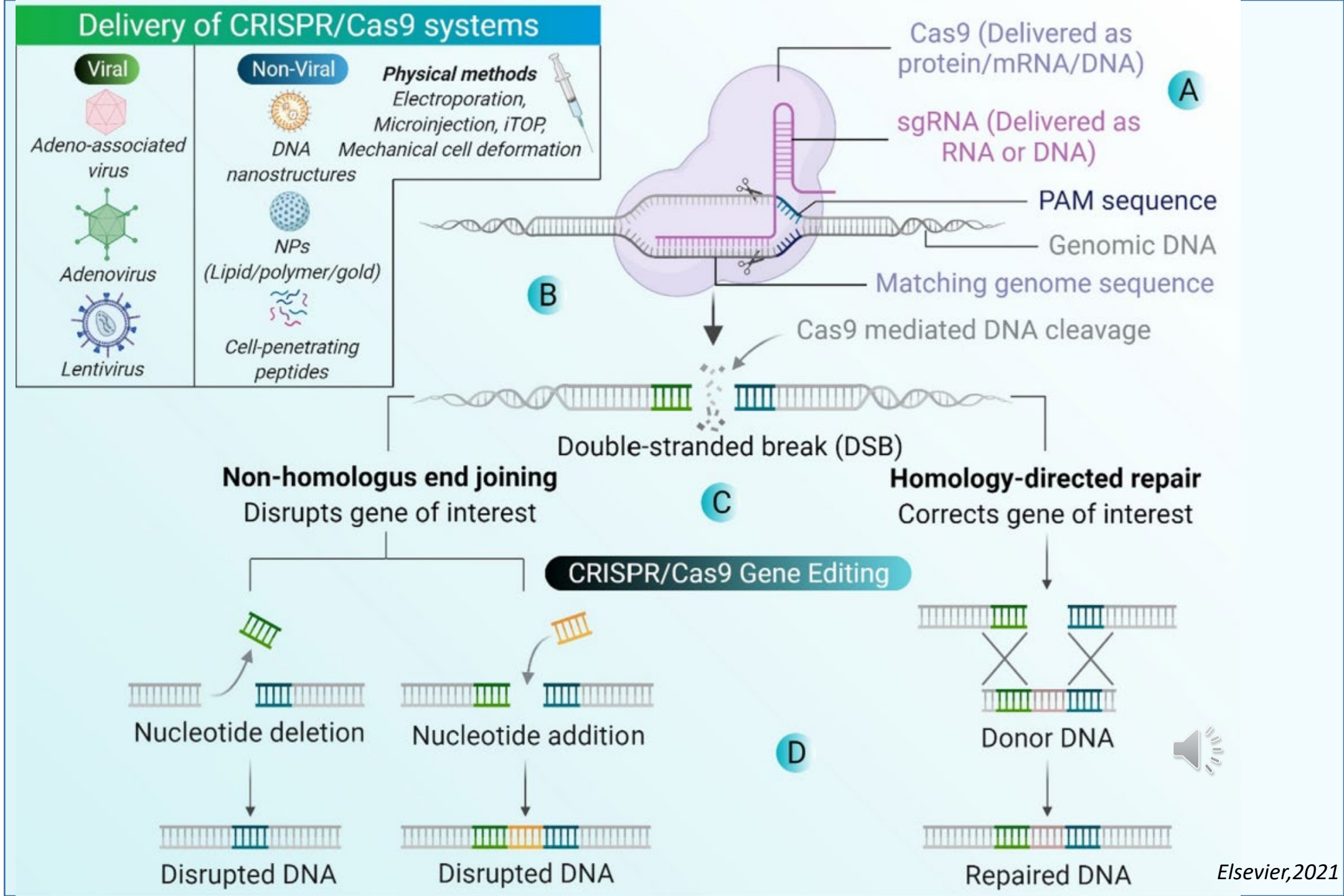


Base Editing and Prime Editing (Base Pair Editing)

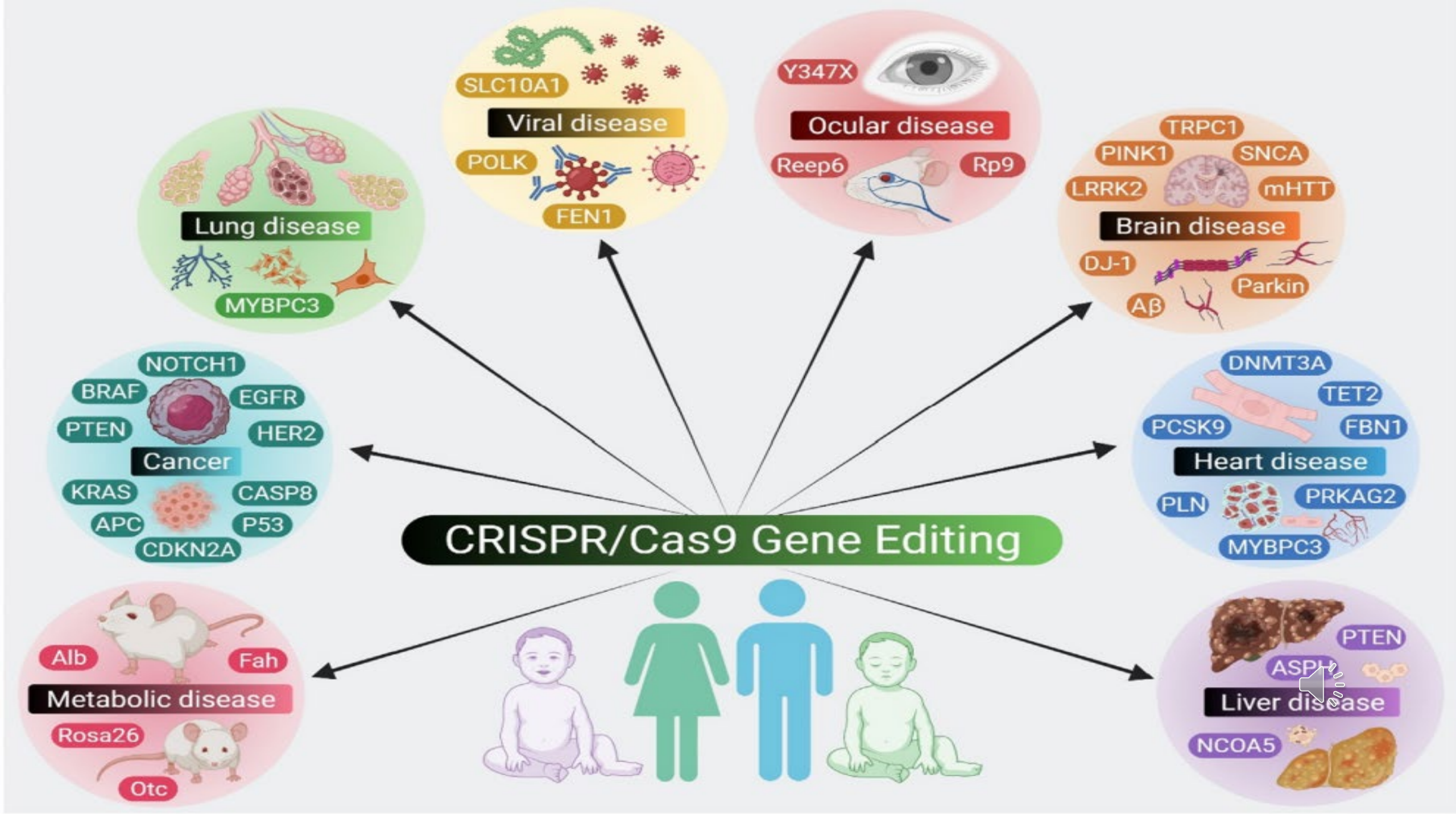
CRISPR/Cas9

1. Simple design
2. Rapid operation
3. Low cost
4. Robust scalability
5. **one-time** dosage
6. **higher specificity**
7. better safety with **tolerable adverse effects**

Mechanism of the classical CRISPR/Cas9 system

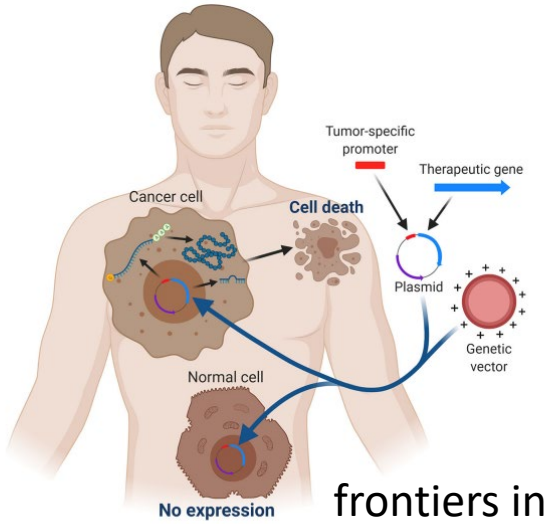


Elsevier,2021


SLC10A1
Viral disease
POLK
FEN1
Y347X
Ocular disease
Reep6
Rp9
Lung disease
MYBPC3
TRPC1
PINK1
SNCA
LRRK2
mHTT
Brain disease
DJ-1
Parkin
Aβ
NOTCH1
BRAF
EGFR
PTEN
HER2
Cancer
KRAS
CASP8
APC
P53
CDKN2A
DNMT3A
TET2
PCSK9
FBN1
Heart disease
PLN
PRKAG2
MYBPC3
CRISPR/Cas9 Gene Editing
Alb
Fah
Metabolic disease
Rosa26
Otc
PTEN
ASPH
Liver disease
NCOA5

Cancer treatment

- ✓Disrupt/knock out/inhibit gene
- ✓Correct tumor suppressor gene
- ✓Insert suicide gene
- ✓Cancer target screening



frontiers in oncology2021

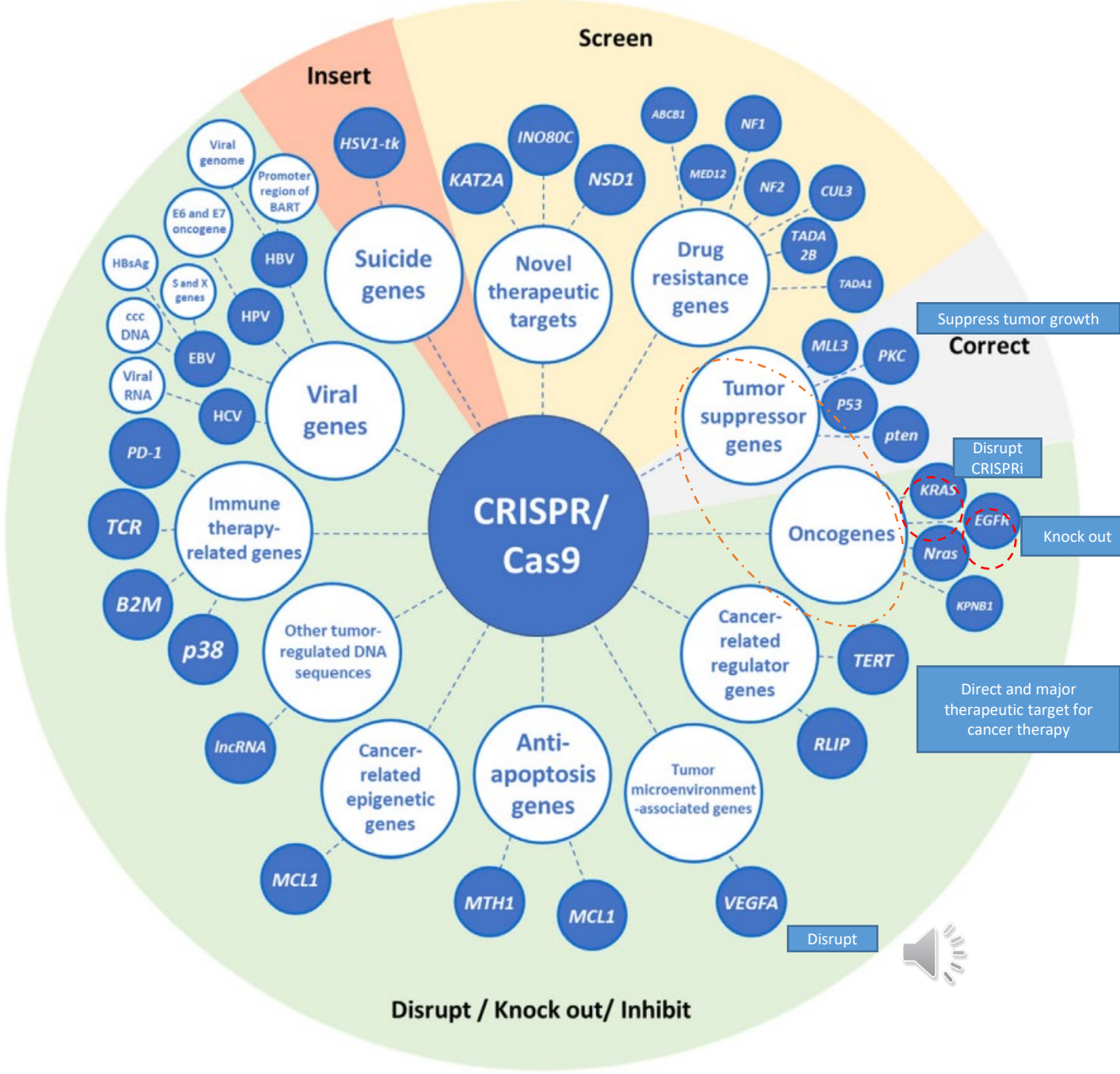


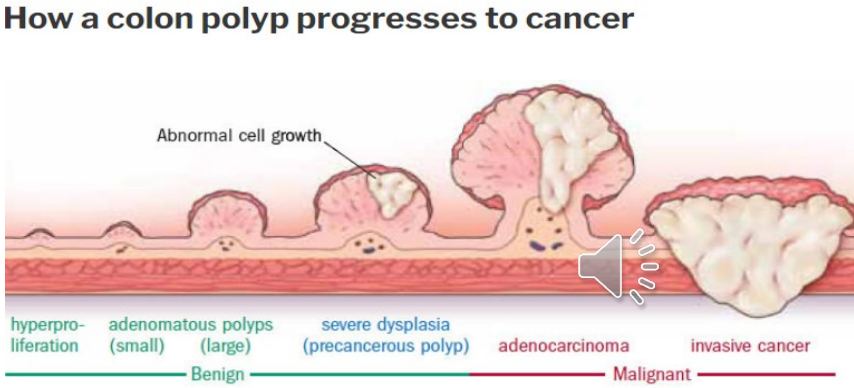
FIGURE 1 | The principle of targeted gene expression controlled by a tumor-specific promoter. Specific transcription factors in cancer cells are able to induce therapeutic gene expression. On the other hand, expression of the therapeutic gene is not obtained in normal cells because they lack the specific transcription

Advanced Research2021-

CRISPR/Cas9 applications in CRC treatment

Table 3 CRISPR/Cas9 applications in cancer treatment

Cancer	Target gene	KO/KI	Cell line/animal	Delivery method	Result	Ref.
CRC	*CCAT1*	KO	HT-29 and SW-480	Plasmid (pX459, pX460-1, pX461-1)	Lowering the anchorage-independent growth	[115]
CRC	*MUC5AC*	KO	HCT-8 and LS174T cell lines Mice	Lipofectamine 2000	Reducing tumorigenesis and chemoresistance by targeting CD44/β-catenin/p53/p21 signaling	[121]
CRC	*DACH1*	KO	HCT116 and SW620 cell lines	Lentivirus	Decreasing organoid formation and size	[127]
CRC	*Par3L*	KO	CaCO-2 cell lines	Plasmid	Attenuation of proliferation and increasing cell apoptosis by caspase-3 activation Enhanced susceptibility to chemotherapy and radiotherapy	[123]
CRC	*LSD1*	KO	T29, SW480, HCT116, LoVo, and RKO cell lines	Lentivirus	Inhibition of Akt-mediated EMT and migration	[129]
CRC	*PP2A*	KO	HCT-116 and HT-29 lines Mice	Lentivirus	Inducing AMPK signaling to avert cell proliferation	[299]

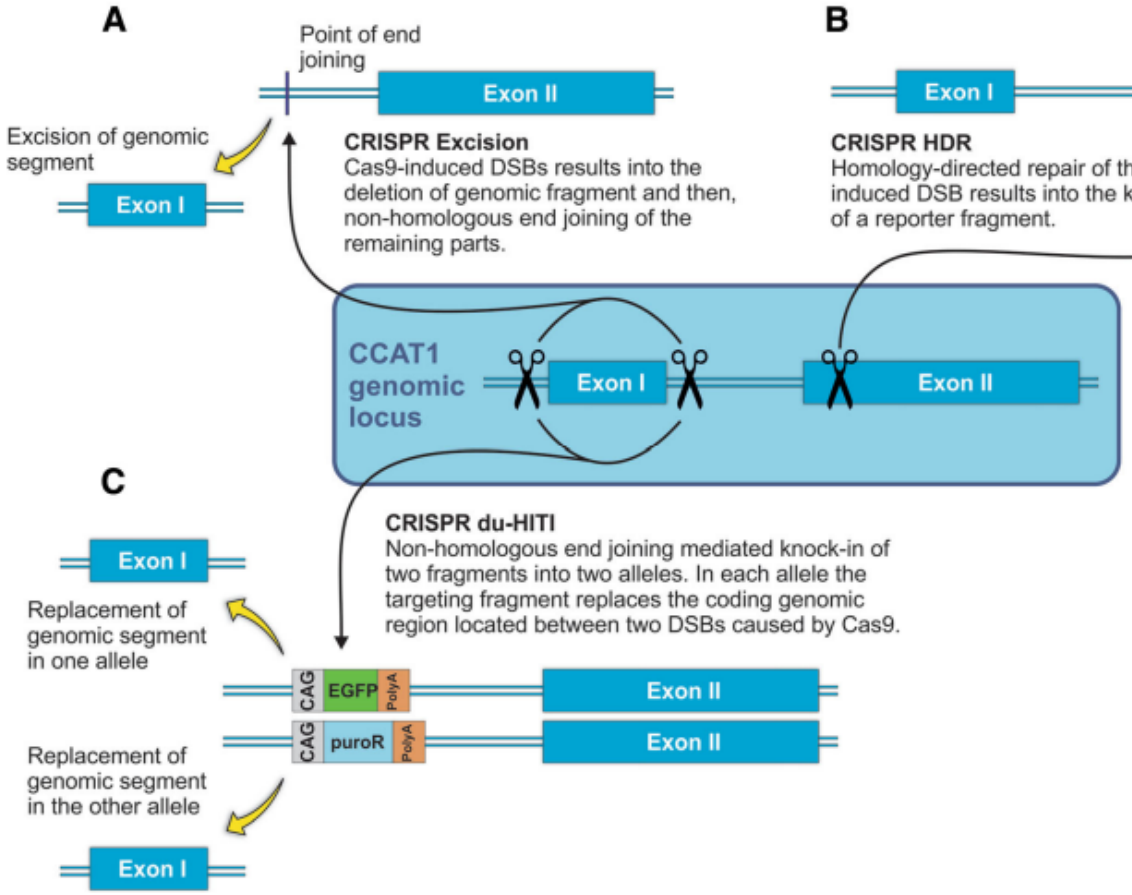


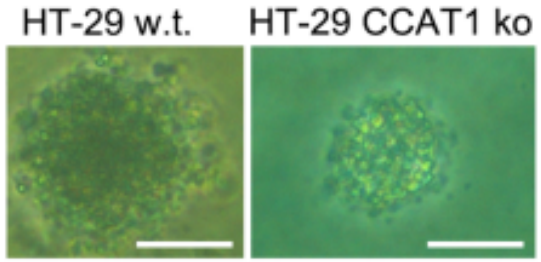
Cellular & Molecular Biology Letters (2022)

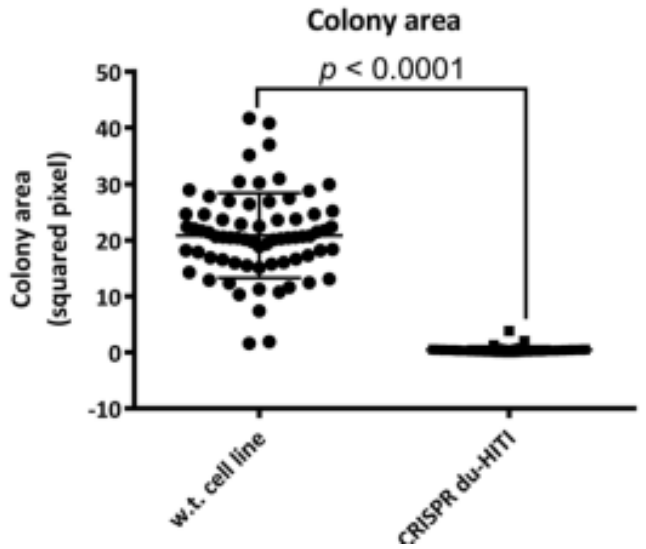
RESEARCH Open Access

CRISPR/Cas9 Knockout Strategies to Ablate *CCAT1* lncRNA Gene in Cancer Cells

Khadijeh Zare[1], Milad Shademan[1], Mohammad M. Ghahramani Seno[1,2] and Hesam Dehghani[1,2,3*]







Biological Procedures-2018

Received: 31 July 2020 | Revised: 24 December 2020 | Accepted: 28 December 2020

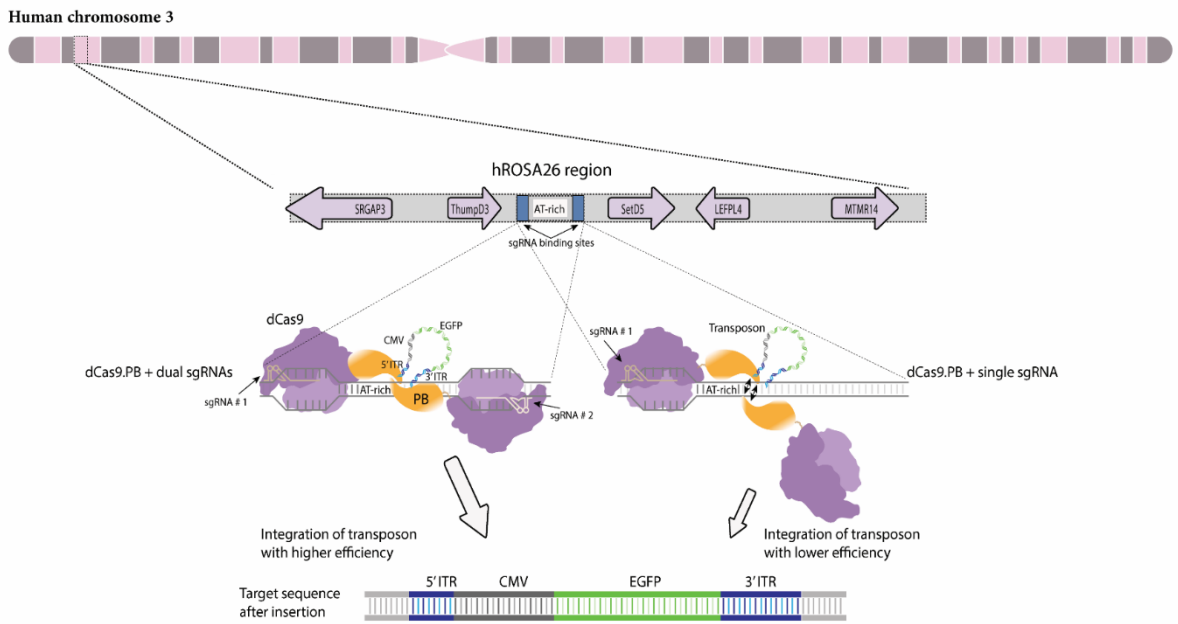
DOI: 10.1096/fj.202001830RR

RESEARCH ARTICLE

THE FASEB JOURNAL

CRISPR/dCas9-mediated transposition with specificity and efficiency of site-directed genomic insertions

Lena Goshayeshi[1,2] | Sara Yousefi Taemeh[1,2] | Nima Dehdilani[1,2] | Mohammadreza Nasiri[3,4] | Mohammad M. Ghahramani Seno[1,5] | Hesam Dehghani[1,2,5]



2020

www.nature.com/scientificreports

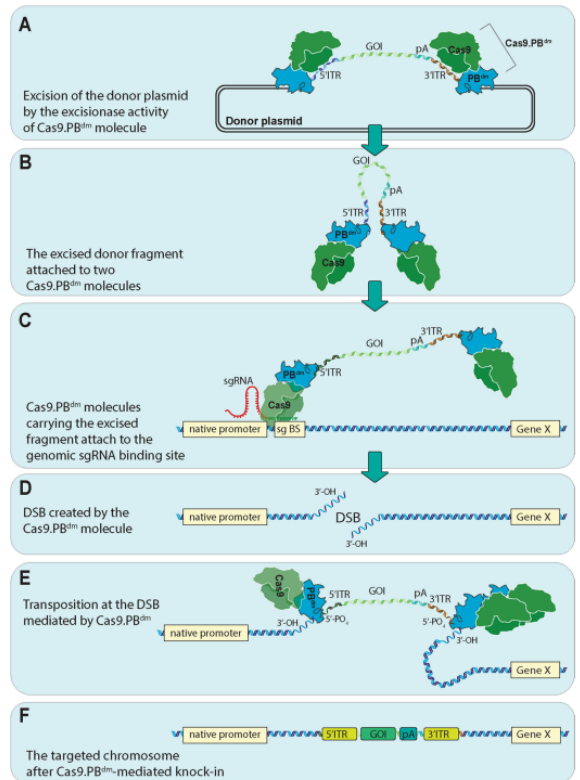
scientific reports

Check for updates

OPEN

Transposase-CRISPR mediated targeted integration (TransCRISTI) in the human genome

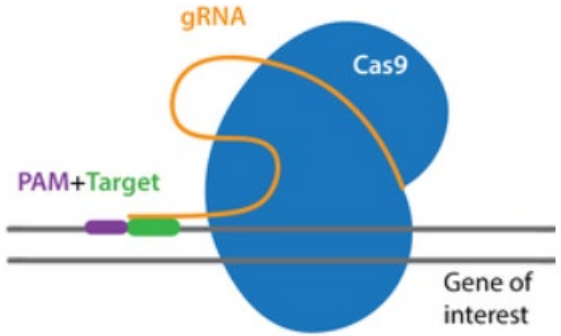
Mahere Rezazade Bazaz[1,2], Mohammad M. Ghahramani Seno[1,3] & Hesam Dehghani[1,2,3]

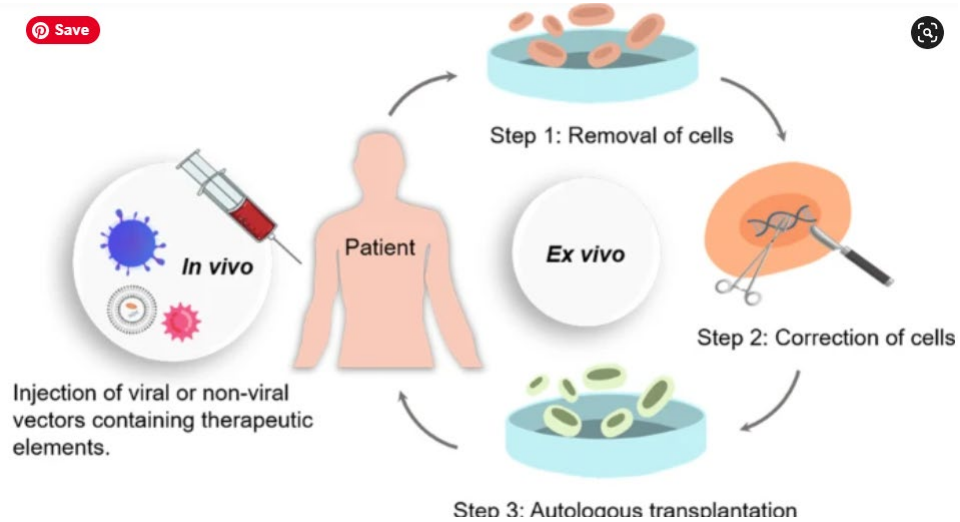


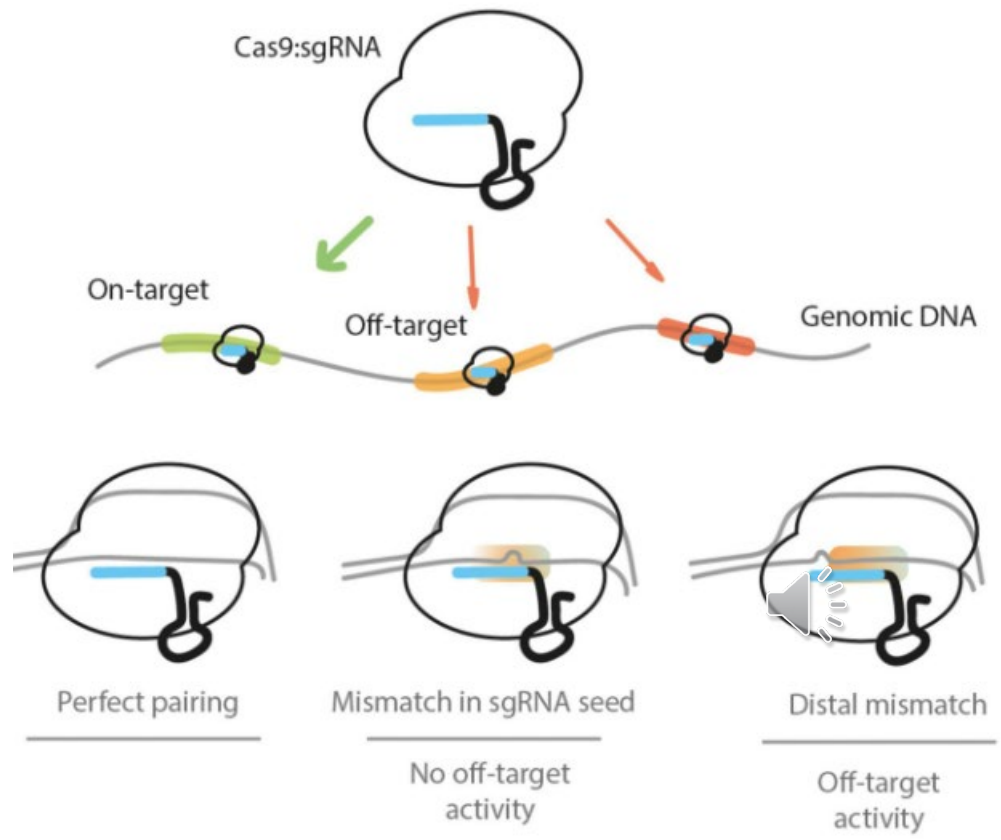
2022

Bottle neck of CRISPR/Cas9

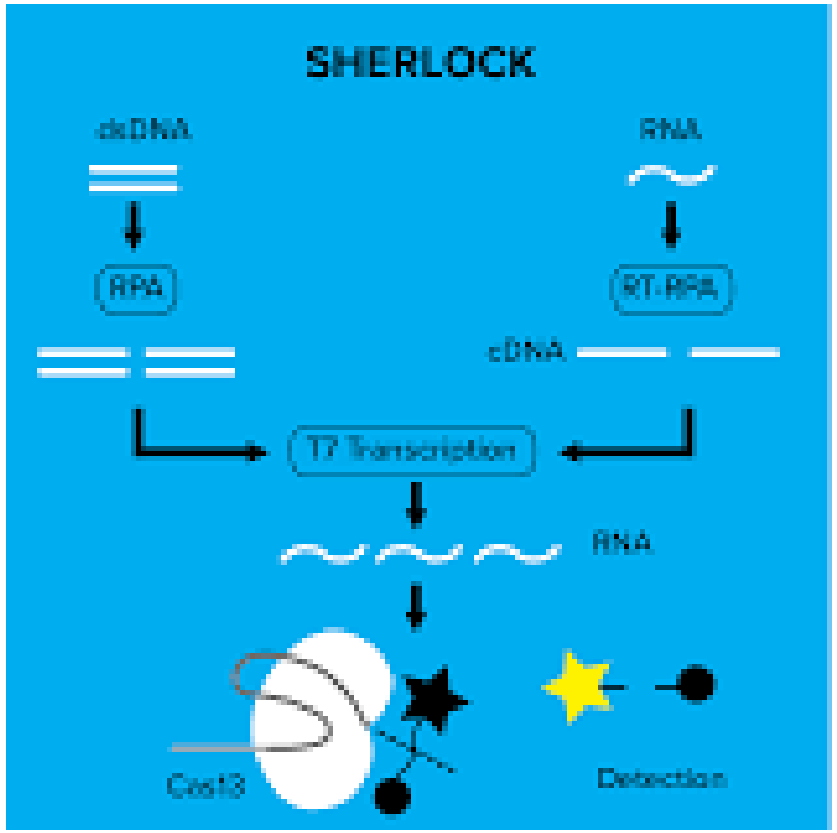
- ✓Delivery challenge — ✓ AAV+ Liposome
- ✓Off-target effect — ✓ SpCas9-HF; ✓ eSpCas9
- ✓Pam limitation
- ✓Immune response
- ✓Multiple gene-editing — ✓ Cas12

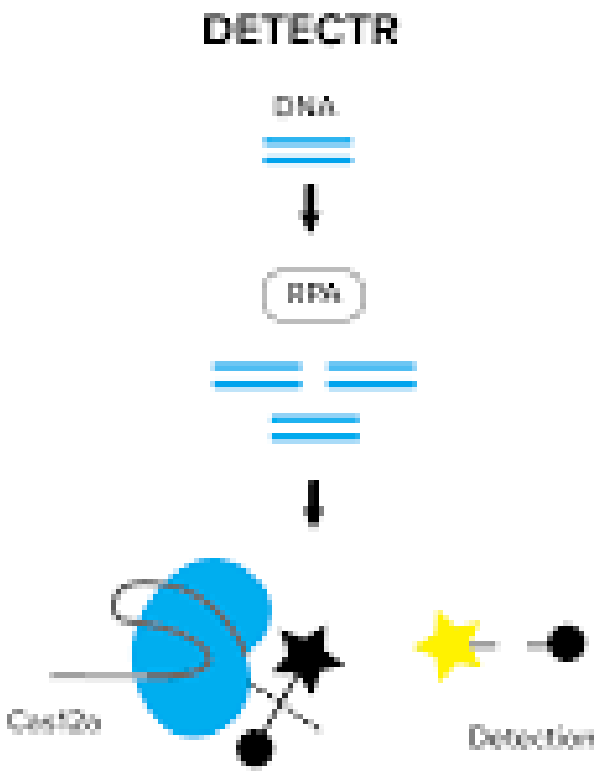


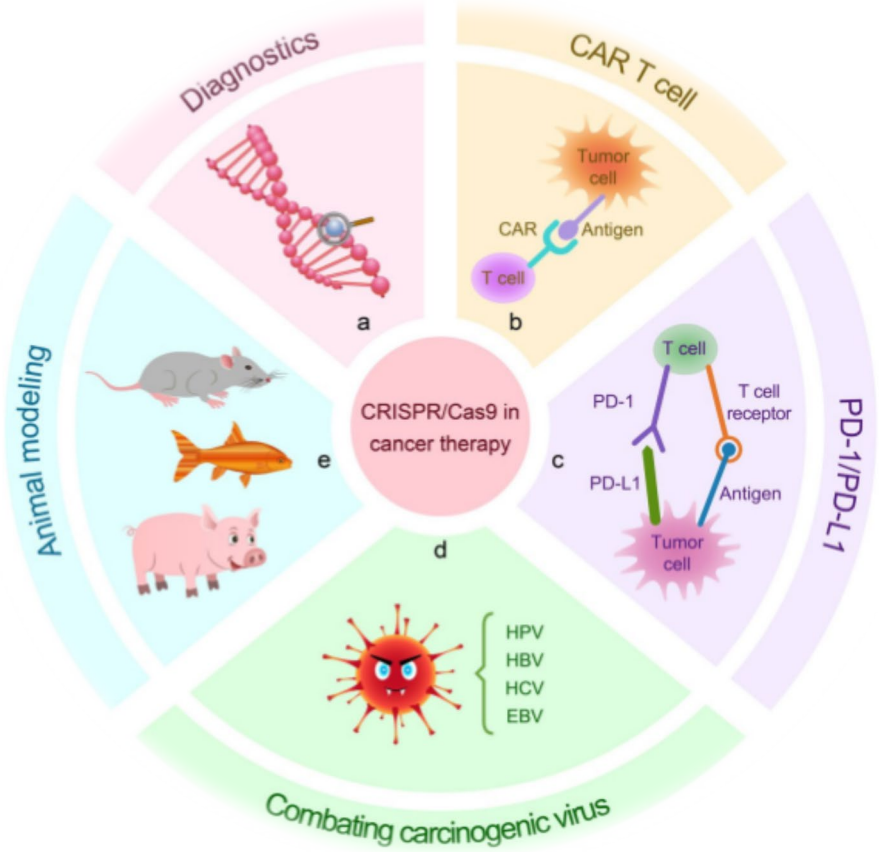




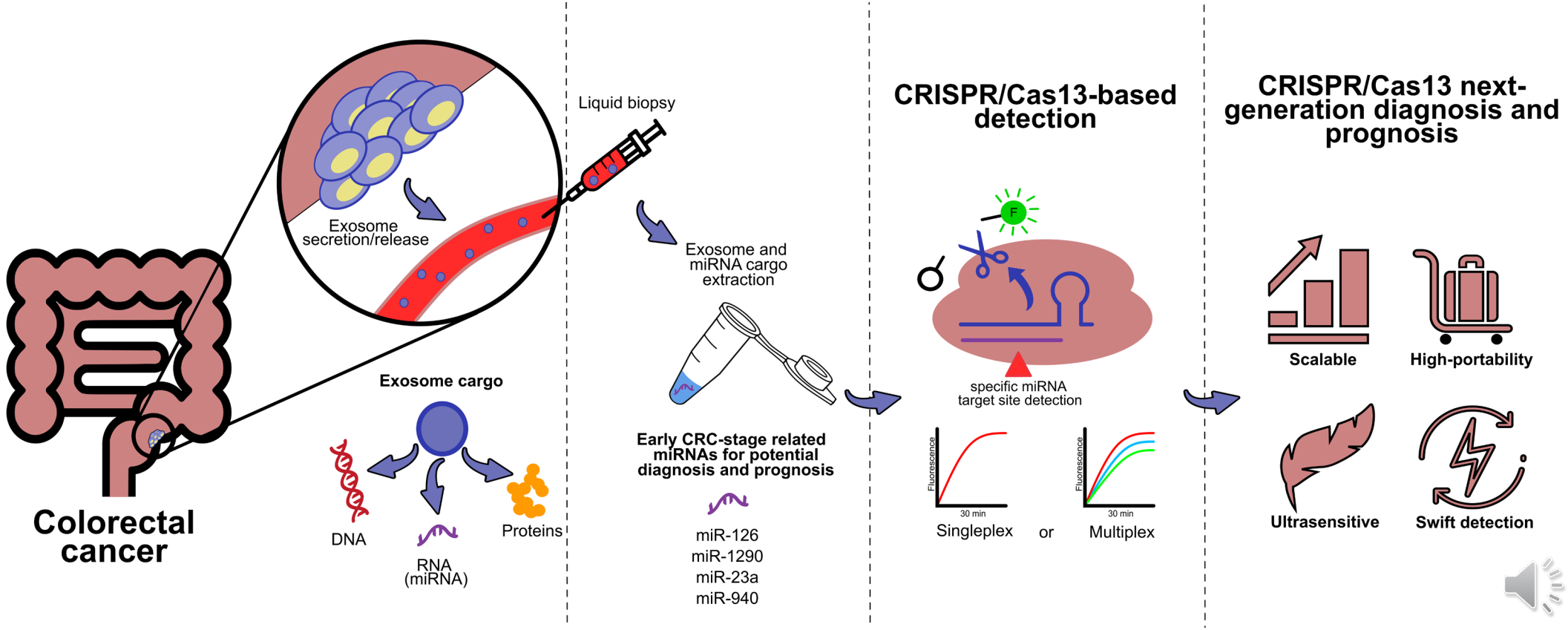
Diagnostic


SHERLOCK
dsDNA
RNA
RPA
RT-RPA
cDNA
T7 Transcription
RNA
Cas13
Detection


DETECTR
DNA
RPA
Cas12a
Detection


Diagnostics
a
CAR T cell
Tumor cell
CAR
Antigen
T cell
b
Animal modeling
e
CRISPR/Cas9 in cancer therapy
d
c
PD-1
T cell
T cell receptor
PD-L1
Antigen
Tumor cell
PD-1/PD-L1
HPV
HBV
HCV
EBV
Combating carcinogenic virus

CRISPR/Cas13-Based Platforms for a Potential Next-Generation Diagnosis of Colorectal Cancer through Exosomes Micro-RNA Detection



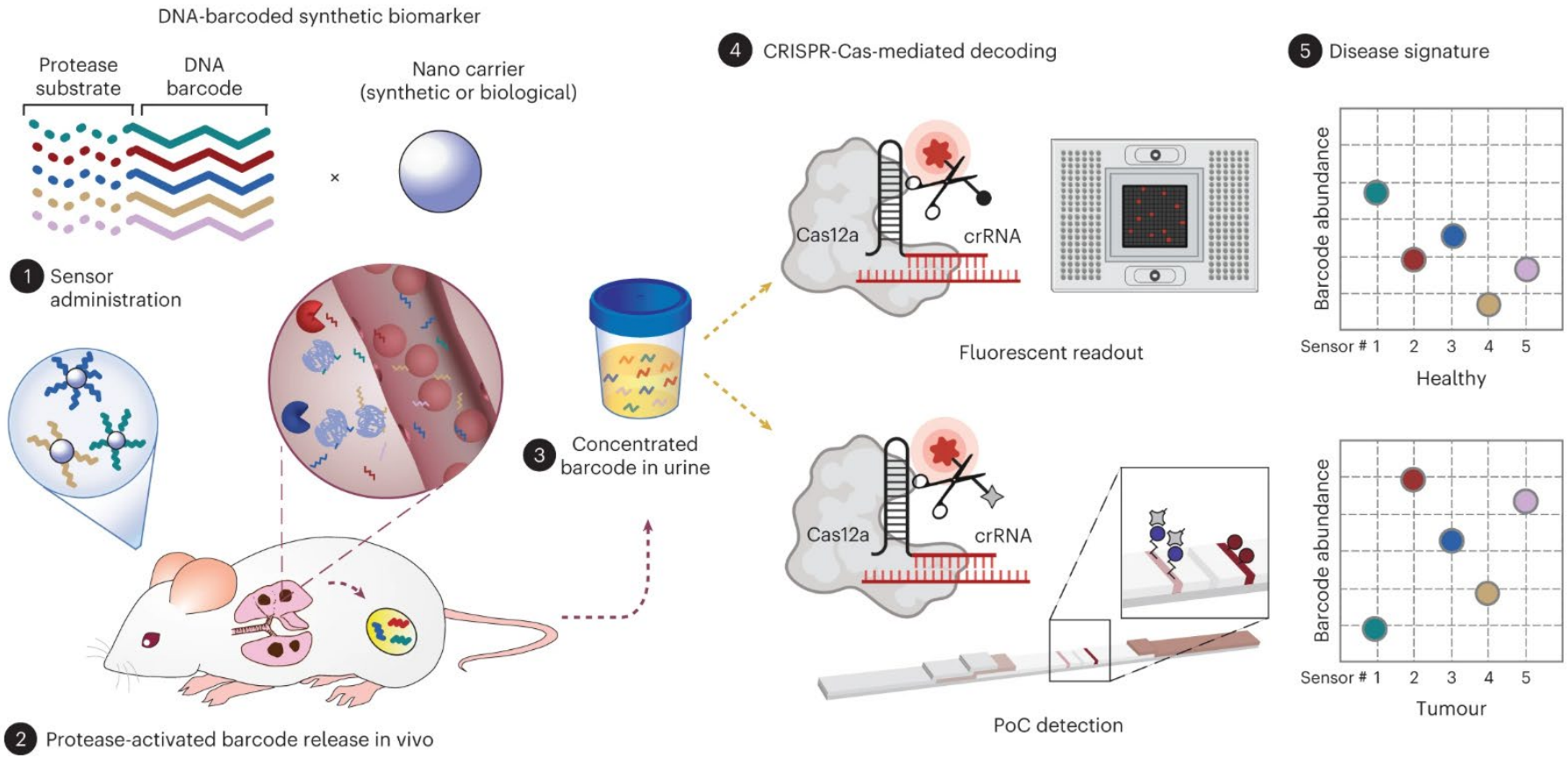
Cancers **2021**, *13*(18), 4640; https://doi.org/10.3390/cancers13184640

nature nanotechnology

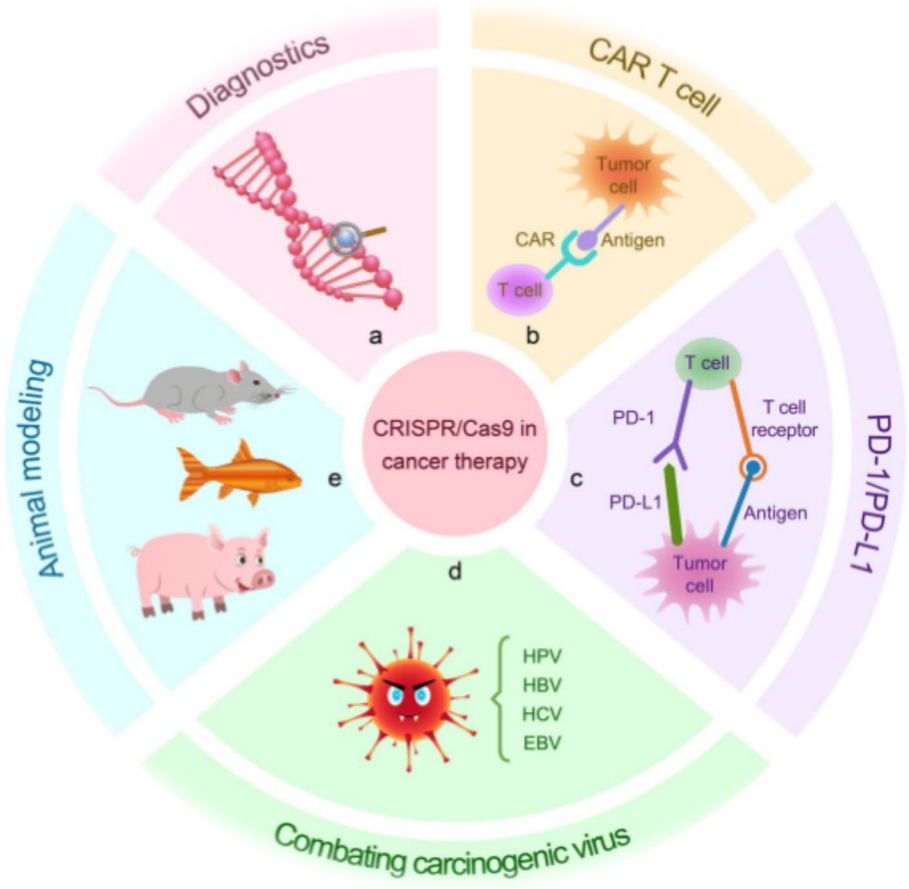
Article

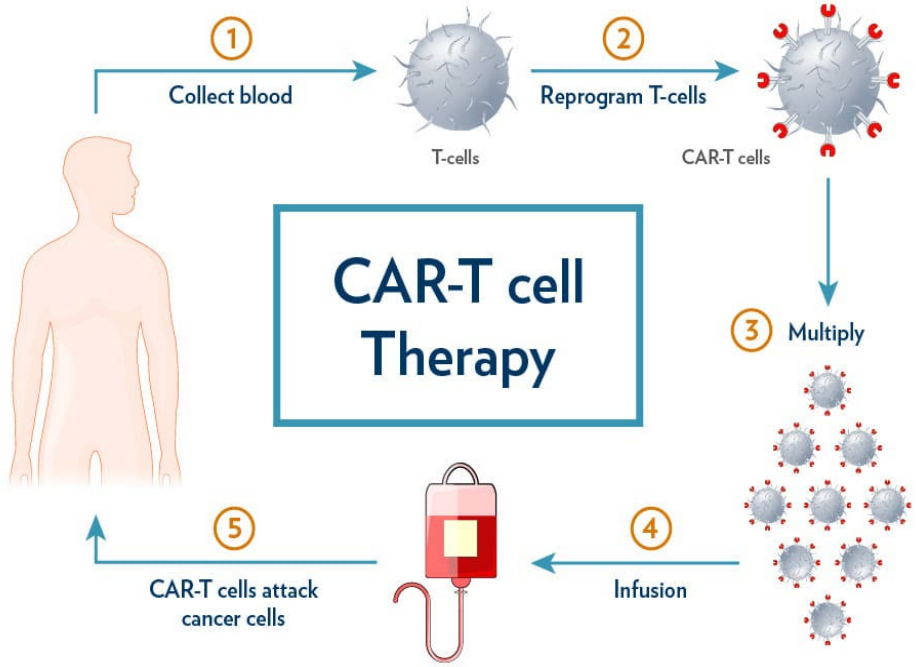
https://doi.org/10.1038/s41565-023-01372-9

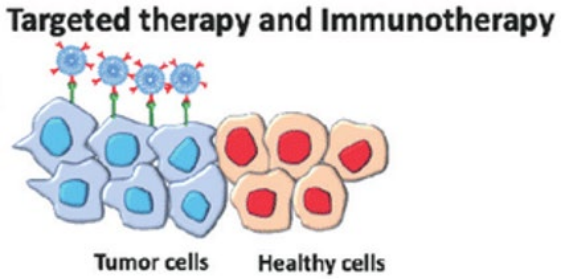
CRISPR-Cas-amplified urinary biomarkers for multiplexed and portable cancer diagnostics

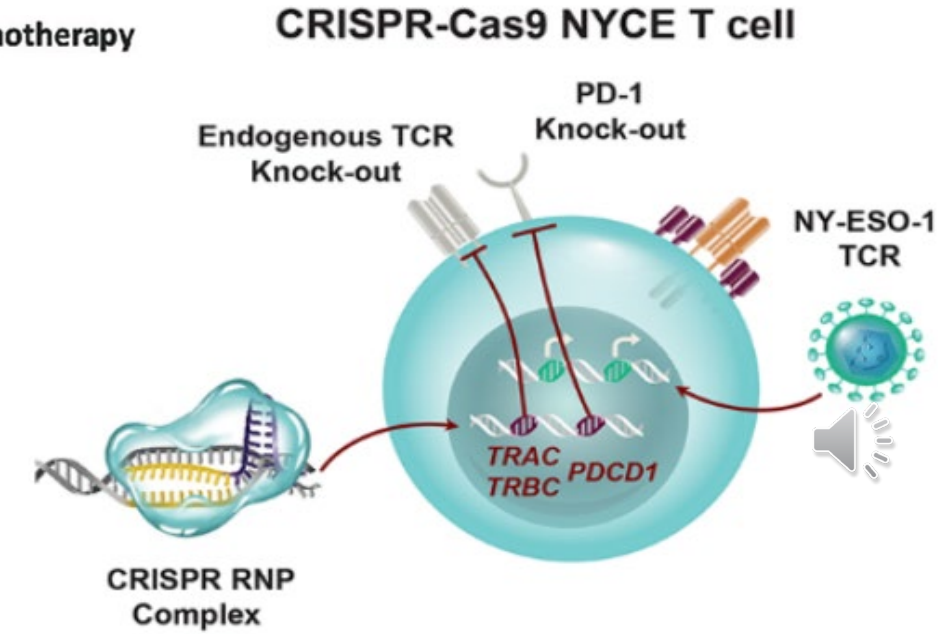


Immunotherapy



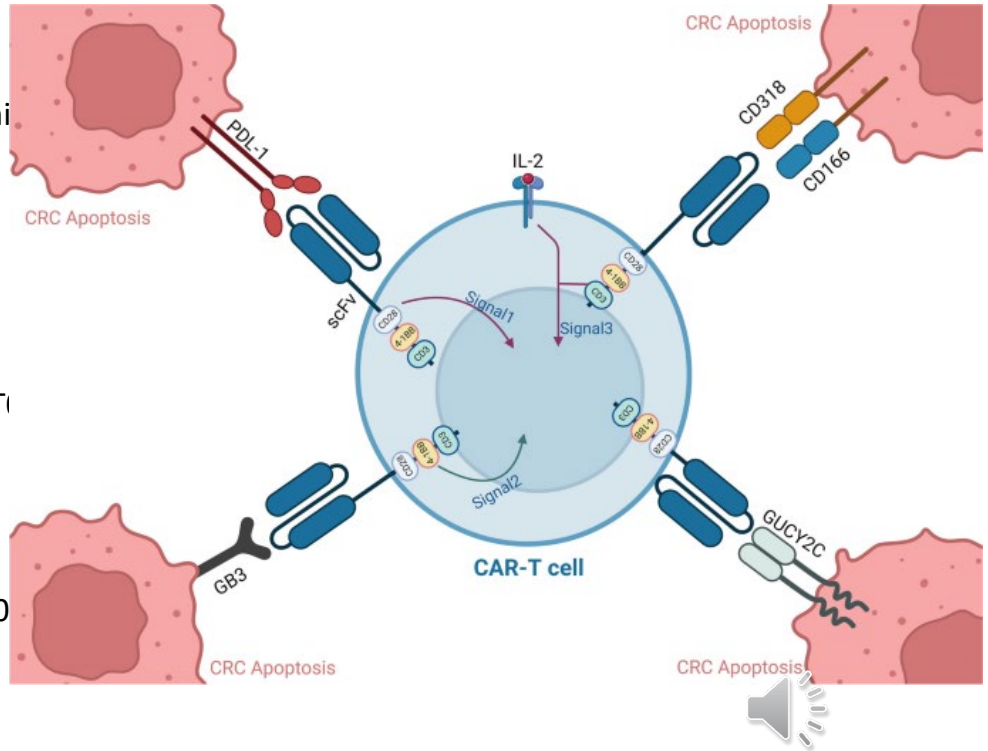






New CAR-T cell strategies, such as specific CAR-T cells against PDL-1, CD166/CD318, GB3, and GUCY2C, can be new immunotherapeutic approaches for CRC apoptosis

- **CEA-targeted**
  - NA (China) – Phase I trial in metastatic CRC (NCT02349724)
  - A2B530 (USA) – Logic-gated CAR-T targeting CEA in CRC (EVEREST-1, NCT05736731)
- **GUCY2C-targeted**
  - NA (China) – Phase I escalating-dose trial (ChiCTR2100044831)
  - IM96 (China) – Tested in advanced CRC (NCT05287165)
  - GCC19CART (China) – Phase I escalating-dose trial in relapsed/refractory metastatic CRC (Chi
  - GCC19CART (USA) – Phase I dose escalation in refractory metastatic CRC (NCT05319314)
- **LGR5-targeted**
  - CNA3103 (Australia) – Phase I/IIa first-in-human in metastatic CRC (NCT05759728)
- **NKG2D-targeted**
  - CYAD-101 (Belgium) – Allogeneic CAR-T in metastatic CRC (alloSHRINK, NCT03692429)
  - CYAD-101 + pembrolizumab (USA) – Allogeneic CAR-T in metastatic CRC (KEYNOTE-B79, NCT
- **EpCAM-targeted**
  - IMC001 (China) – CAR-T in advanced GI cancers (NCT05028933)
- **CDH17-targeted**
  - CHM-2101 (USA) – Phase I/II CAR-T in colorectal, gastric, and neuroendocrine cancers (NCT0
- **MUC1-C-targeted**
  - P-MUC1C-ALLO1 (USA) – Phase I allogeneic CAR-T in epithelial cancers (NCT05239143)
- **MSLN-targeted**
  - A2B694 (USA) – Logic-gated CAR-T in solid tumors (EVEREST-2, NCT06051695)

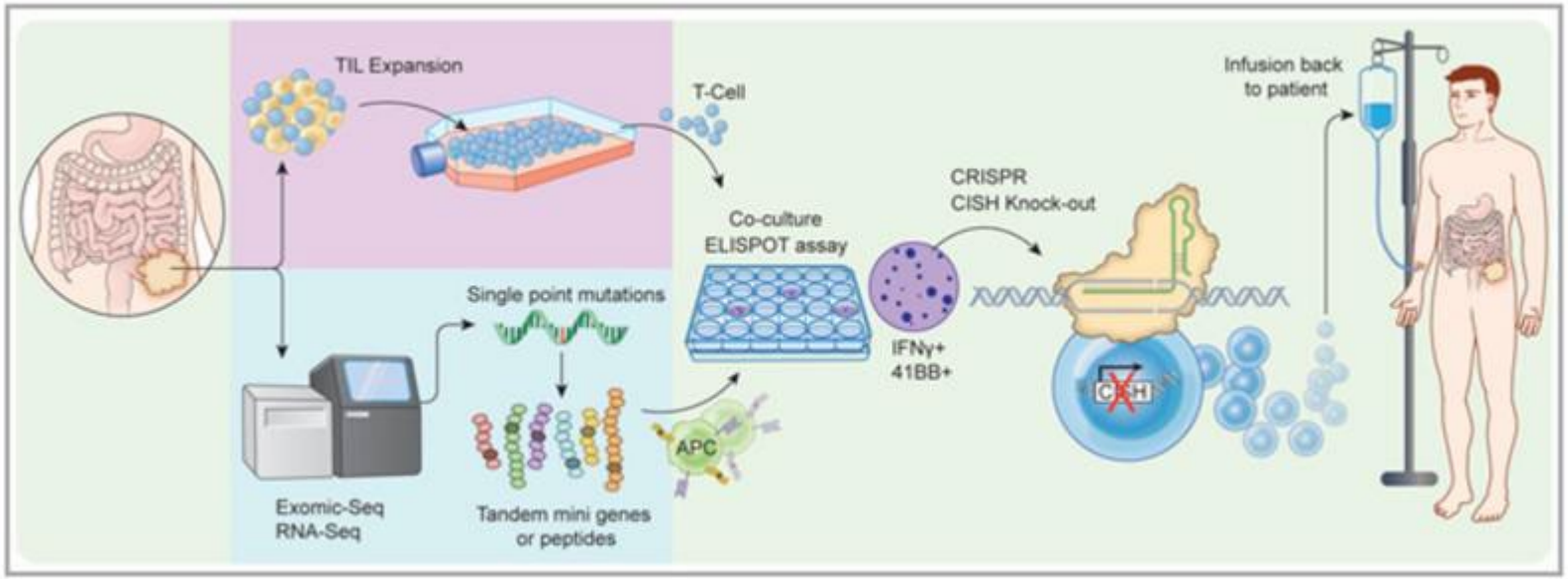


New CRISPR-Based Therapy in Advanced GI Cancers

•**Trial:** First-in-human, University of Minnesota (Lancet Oncology, 2025)

•**Method:** CRISPR/Cas9 editing of *CISH* (cytokine-induced SH2 protein) gene in tumor-infiltrating lymphocytes (TILs) → enhances T-cell anti-tumor activity

•**Patients:** 12 with metastatic, end-stage GI cancers



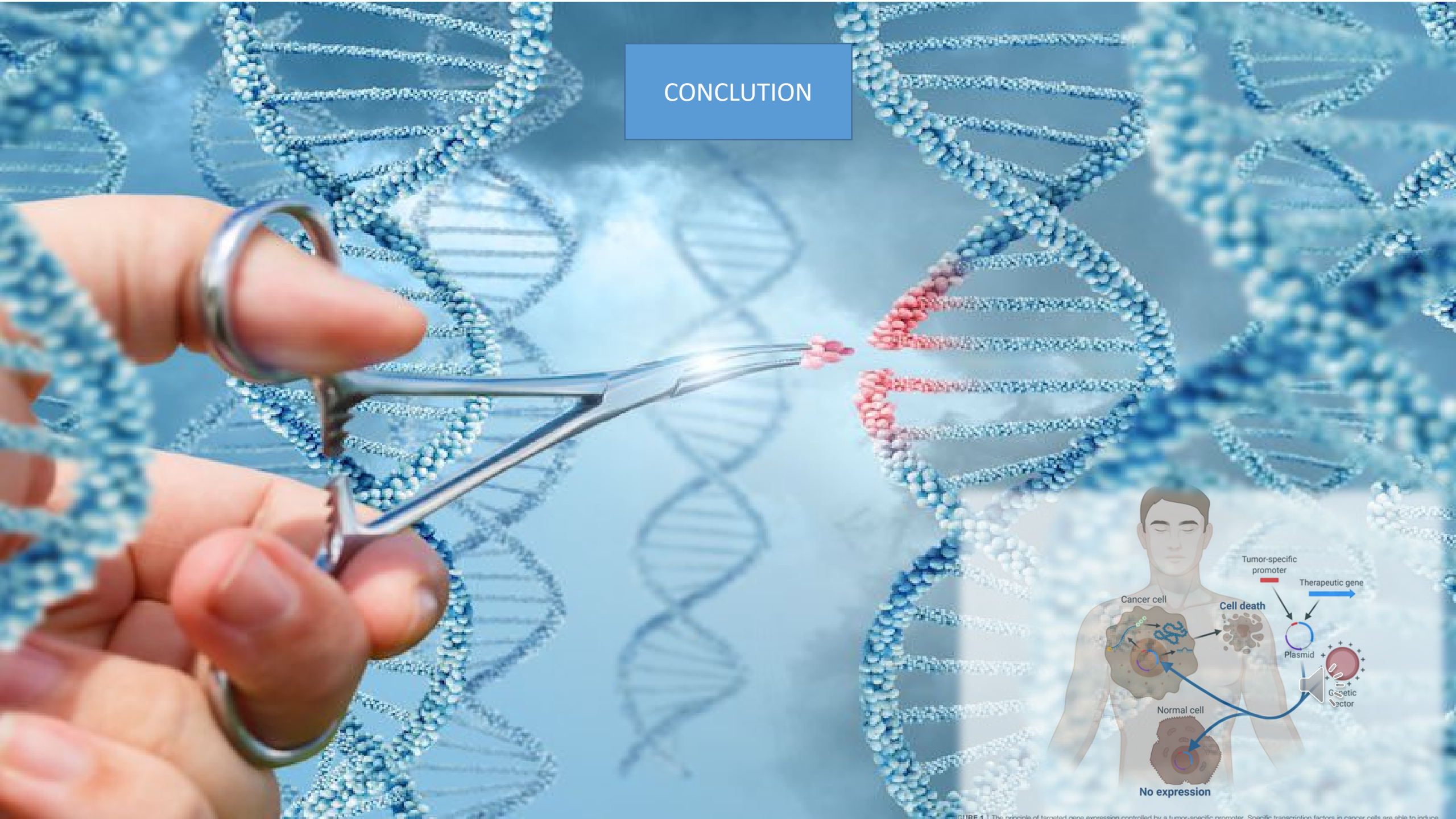
•**Results:**

- Safe, no serious side effects
- Several cases of halted tumor growth
- 1 complete response → tumors vanished, disease-free >2 years

•**Innovation:** Permanent checkpoint inhibition "hardwired" into T cells (vs. repeated dosing in conventional therapies)

•**Challenges:** Complex, costly; need to optimize production & understand variability in responses

CONCLUTION


Tumor-specific promoter
Therapeutic gene
Cancer cell
Cell death
Plasmid
Normal cell
No expression

ممنون از توجه شما











به امید روزهای روشن برای ایران زیبا...







