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REVIEW

The role of the CRISPR-Cas system in cancer drug development: Mechanisms of action and therapy

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Abstract

Background: The recent emergence of gene editing using Clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR associated system (Cas) tools and advances in genomics and proteomics has revolutionized drug discovery and personalized medicine.

Purpose and Scope: The CRISPR-Cas system has enabled gene and cell-based therapies, screening for novel drug targets, a new generation of disease models, elucidation of drug resistance mechanisms, and drug efficacy testing. Here, we summarized recent investigations and strategies involved in cancer-related drug discovery using the CRISPR-Cas system.

Conclusion: CRISPR-Cas-mediated gene editing has shown great potential in the development of next generation drugs for treatment of Mendelian disorders and various cancer types. In this review, we focused on the impact of the CRISPR-Cas system in drug discovery and its application to biomarker identification and validation, high-end target genes, and breakthrough anticancer cell therapies. We also highlighted the role of CRISPR-Cas in precision disease modeling and functional drug screening.

KEYWORDS
cancer, CRISPR-Cas, drug discovery, gene editing, immunotherapy

1 | INTRODUCTION

Recently, genome-editing techniques have made an impact on human health by modifying genetic material. Accurate editing of genetic sequences of any organism is essential to elucidate gene function. The processes of insertion, deletion, and modification of sequences to alter gene function are fundamental to biomedical research. Clustered regularly interspaced short palindromic repeats (CRISPR) have been adapted from the bacterial immune system to manipulate the genome. CRISPR-Cas is an RNA-guided tool to target and modify a gene of interest in diverse organisms.[1]

In recent years, the CRISPR-Cas system has become the tool of choice for molecular geneticists. CRISPR-Cas-based genome editing has been implemented in various applications from cancer research to plant biology (Figure 1). The CRISPR screening technology employs a dynamic library system, and hit validation by combinatorial drug screening gives a strong new strategy, which is especially well adapted to the diverse experimental needs of each screening campaign, with the potential for quick clinical translation. This suggests that these techniques will be ready to help with robust hit finding in future positive

Abbreviations: CRISPR, clustered regularly interspaced short palindromic repeats; Cas, CRISPR associated system; hESCs, human embryonic stem cells; hiPSCs, human induced pluripotent stem cells; PDX, patient-derived xenograft; DMD, Duchenne muscular dystrophy; TSS, transcription start sites; HSPCs, hematopoietic stem and progenitor cells; PD-1, programmed cell death protein-1; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; MHC, major histocompatibility complex; PRC2, polycomb repressive complex 2; LAG-3, lymphocyte-activation gene 3; TIM-3, T-cell membrane protein 3; GITR, glucocorticoid induced TNFR-related protein; CAR-T, chimeric antigen receptor T-cells; TCR-T, T-cell receptor T-cells

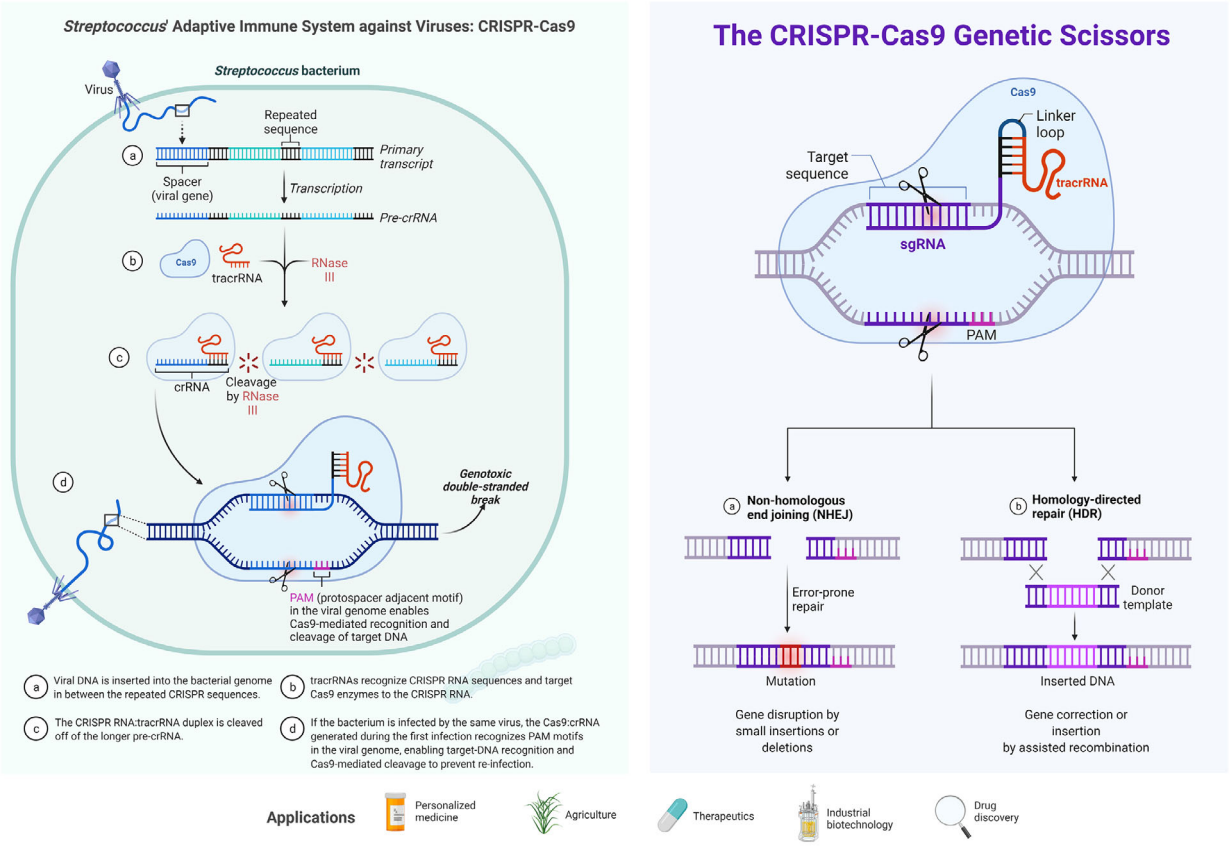

FIGURE 1 (A) CRISPR-Cas-mediated genome editing. CRISPRs are sections of the bacterial genome that aid in viral defense. These are made up of short DNA repetitions and spacers. When a previously unknown virus infects a bacterium, a novel viral-derived spacer is integrated amid existing spacers. The CRISPR sequence is transcribed and decoded to produce small CRISPR RNA molecules. The CRISPR RNA binds to and directs bacterial molecular machinery to a target sequence in the invading virus. The invading viral genome is broken up and destroyed by the molecular machinery. (B) CRISPR-based gene editing. Guided RNA tailored to match the specific DNA region enables molecular machinery to cleave both strands of the targeted DNA. A repair template with a defined sequence alteration is inserted into the cell and integrated into the DNA during the repair process, resulting in a targeted DNA region that carries the new sequences. Reprinted from "2020 Nobel Prize in Chemistry: A Tool for Genome Editing (CRISPR-Cas9)," by BioRender.com (2021). Retrieved from https://app.biorender.com/biorender-templates

selection screening approaches.[2] Although CRISPR is widely used to correct point-mutation diseases, it also holds promise for development of therapies to treat complex heritable and somatic disorders. Currently, the CRISPR-Cas system is being used toward novel drug development and new therapeutic breakthroughs.

2 | CRISPR-CAS IN DISEASE MODELING AND NOVEL DRUG SCREENING

A drug screening system consists of targeted drugs, screening methods, and, most importantly, a model that closely recapitulates the physiology and clinical relevance of the disease condition. An integrated study based on publicly accessible resources including The cancer genome atlas, the encyclopedia of DNA elements, the UCSC cancer genomics browser, the cancer cell line encyclopedia, and other similar databases provides an important insight into genetic variations across the global population.[3] Recent advances in medicine integrate personal genetic information and publicly available genetic data to design individual treatment strategies and drug development. However, hypotheses generated by large-scale genomic or proteomics data need to be tested using precision cell-based or animal-based genetic models to confirm the efficacy and sustainability of approved drugs before designing treatment paradigms.

2.1 | CRISPR-Cas in the generation of cellular models and large-scale screens

The advent of CRISPR-Cas-based genome editing has transformed the dynamics of disease modeling for pharmacological research and development of innovative therapeutic strategies. The development of human isogenic gene-knockout cell lines for relative genomic studies has proved efficacious in almost all cell lines.[4] The efficiency of CRISPR-Cas-mediated gene knockouts in cancer cell lines, human embryonic stem cells (hESCs), human induced pluripotent stem cells

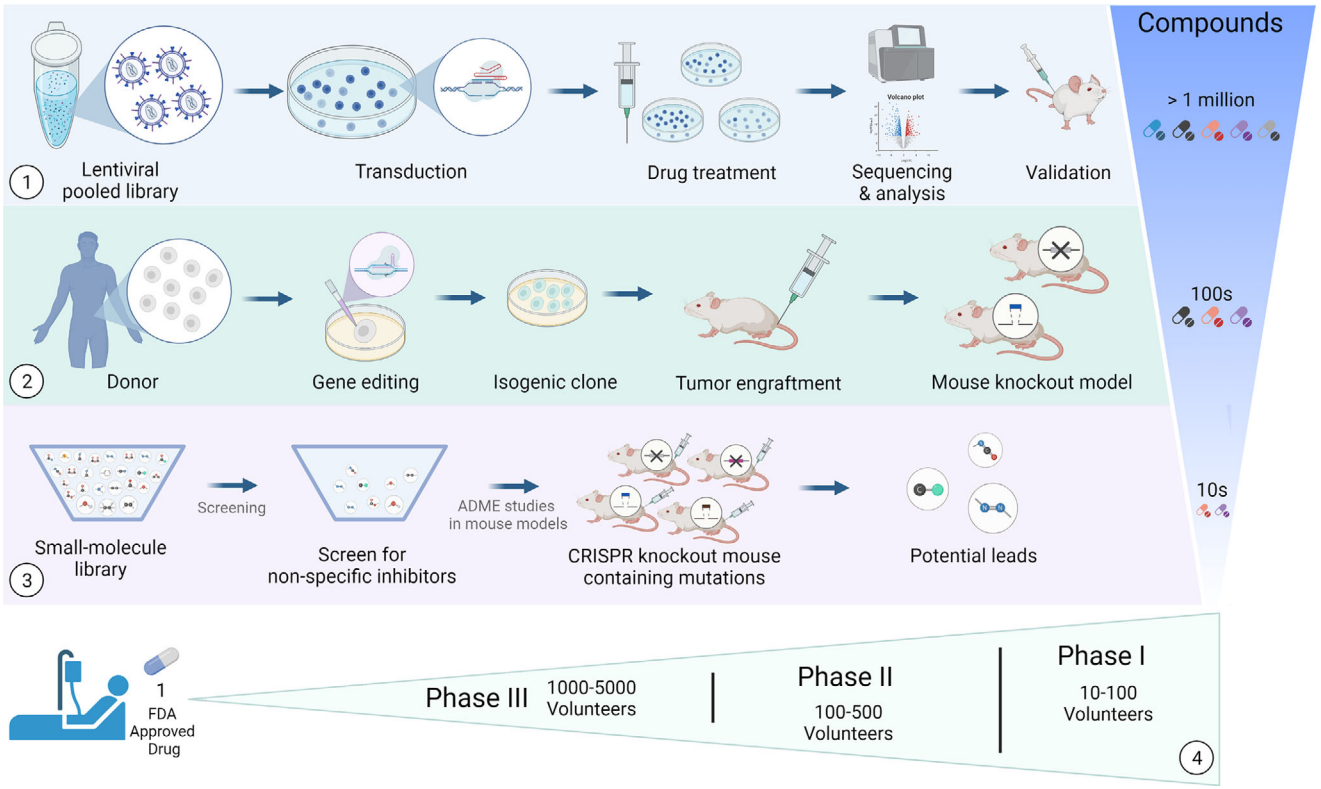

FIGURE 2 CRISPR-powered drug discovery process. CRISPR has shown potential to address several technological problems associated with drug discovery and early drug development through the following steps: Step 1. Multiple mutations can be generated simultaneously using CRISPR during chemical screening to simulate disease genotypes. With improved cellular models, these preliminary screenings accurately remove inefficient compounds and identify the most effective ones early in the drug discovery process pipeline. Hit molecules that pass this screening step are subjected to more rigorous inspection in the pipeline's following stage. Step 2: CRISPR has had a significant influence on this stage of preclinical development by increasing the creation of models that properly replicate diseases during hit validation. Step 3: CRISPR facilitates progress during lead identification and optimization by improving development of cellular and animal models used to assess the efficacy and safety of candidate drugs. For example, instead of crossing single-mutant animals, mouse models with numerous mutations may now be produced in a single step by directly altering zygotes. Furthermore, CRISPR will most likely allow for the generation of a broader spectrum of animal models. Step 4: Phases of clinical trials. Created with BioRender.com

(hiPSCs), normal immune cells, and organoids has helped to generate clinically relevant models for drug screening and development.[5] CRISPR-Cas-based genetic manipulation is relatively less laborious and highly efficient for knockout-based target discovery, testing hypotheses about genetic lethality, and identifying proto-oncogenes, oncogenes, tumor suppressors, and other cancer-associated factors.[6] For example, the CRISPR-Cas9 system was used in recent clinical trials to generate in vitro cancer models by silencing MELK and NAMPT, which are targets for novel chemotherapy drugs, OTS167 and KPT-9274, respectively.[7] To achieve this, they designed seven guides against MELK and transduced into Cal51 and MDA-MB-231 cell lines. The combination of CRISPR-Cas-mediated gene knockout and high-throughput sequencing along with computational mutation discovery approaches have helped to identify physiological targets of drugs and bioactive small molecules and have also unraveled mechanisms of drug resistance in cancer (Figure 2).[8]

Recent developments in CRISPR-Cas9 technology and identification of other Cas9 proteins has not only improved the success rate for generating cellular models but has also addressed issues such as efficiency, off-target effects, production cost, and high-throughput screening. Among such models, iPSC-based disease models are one of the most valuable tools in regenerative medicine. The CRISPR-Cas9 system has been widely utilized to generate iPSCs disease models with different gene KOs or to introduce disease-specific mutations through HDR-mediated gene editing, thus providing an excellent platform for disease modeling, autologous cell therapies, and drug discovery.[9] We have summarized different available cellular models that are important in drug discovery set up in Table 1. Furthermore, iPSC-based disease models unravel primary-cell-level disease phenotypes, but to recapitulate tissue-level or organ-level phenotypes more complex three-dimensional systems are required.

2.2 | CRISPR-Cas in organoid-based disease modeling for drug discovery

Three dimensional organoid models have been critical to personalized anticancer therapy development, especially for targeted drug

TABLE 1 CRISPR/Cas based disease models for drug discovery

Disease model type	Disease	Animal/Cell/ tissue description	Gene	Modification	Reference
Cellular models	Epilepsy	iPSCs-derived neurons	*SCN1A*	Fluorescently labelled iPSC's derived GABAergic neurons.	[53]
	Huntington's Disease	iPSCs-derived neurons	*HTT*	Replacement of expanded 97 CAG repeats in exon 1.	[54]
	β-thalassemia	iPSCs	*HBB*	Correction of intron 2 mutation site IVS2-654 in β-globin gene	[55]
	Fragile X syndrome	iPSCs-derived neurons	*FMR1*	Reversal of FMR1 silencing and methylation by deletion of CGG triplet repeats at 5′UTR of FMR1.	[56]
	T cell leukemia	Jurkat cells	*XPO1*	Generation of homozygous C528S *XPO1* mutant.	[57]
	Friedreich's ataxia	HEK293	*FXN*	Inducible expression of *FXN* gene.	[58]
	Hematopoietic malignancies	Hematopoietic stem and progenitor cells	*B2M, CCR5*	Deletion of B2M and CCR5 genes.	[59]
	Burkitt lymphoma (BL)	Mouse FDC-P1, human BL-derived cells	*BIM, MCL-1*	Inducible deletion of MCL-1 and BCl-2 family genes	[60]
Organoid models	Autism	Cerebral organoids	*CHD8*	Heterozygous CHD8+/- KO cells.	[61]
	Cystic fibrosis	Intestinal stem cell organoids	*CFTR*	Functional repair of CFTR by transfecting donor plasmid encoding wild type CFTR gene sequence.	[62]
	Alzheimer's Disease	Cerebral organoids	*p35*	Inhibition of p25/Cdk5 by replacing mutant p35 with endogenous p35.	[63]
	Colorectal cancer	Intestinal tumor organoids	*APC, SMAD4, KRAS, PIK3CA and TP53*	Generation of $KRAS^{G12V/D}$ and $PI3KCA^{E545K}$ CRISPR knock-in organoid models	[5]
			ACVR1b, ACVR 2a, and *ARID2*	Targeted disruption of *ACVR1b/2a*, and *ARID2 genes*	[64]
	Esophageal adenocarcinoma	Barrett esophagus (BE) organoids	*APC*	Introduction of APC gene mutation in human BE organoids	[65]
	Colorectal cancer	Human colon organoids	*TGFBR2*	Pooled CRISPR-Cas9 library screening	[66]
	Breast cancer	Human breast organoids	*TP53, PTEN, RB1 and NF1*	Targeted knockout of breast cancer associated tumor suppressor genes.	[67]
	Microvillus inclusion disease	Human intestinal epithelial organoids	*MYO5B*	Loss-of-function mutation	[68]

(Continues)

TABLE 1 (Continued)

Disease model type	Disease	Animal/Cell/ tissue description	Gene	Modification	Reference
Animal models	Duchenne muscular dystrophy	Rat, mouse, Rhesus monkey	*DMD*	Correction of dystrophin mutations at exon 45–55 using multiplexed sgRNAs.	[69]
	Hereditary tyrosinemia	Mouse	*FAH*	Correction of causative Fah-splicing mutation using combined viral and non-viral delivery of sgRNA, repair template and Cas9.	[70]
	Cataract	Mouse	*CRYGC*	Correction of dominant mutation in *Crygc* gene into zygotes of mutant mouse disease models.	[71]
	Lung adenocarcinoma	Mouse	*KRAS*, *p53 and LKB1*	Generation of mutant $Kras^{G12D}$ and loss of function mutation in *p53* and *Lkb1*.	[72]
	Urea cycle disorder	Mouse	*OTC*	Correction of G→A point mutation in spf^{ash} mouse	[73]
	Acute myeloid leukemia	Mouse	*TET2*, *DNMT3A*, *RUNX1*, *NF1*, and *EZH2/Mll3*	Generation of in vivo myeloid malignancy models	[74]
	X linked adrenal hypoplasia congenital and hypogonadotrophic hypogonadism	Cynomolgus monkey	*DAX1*	Generation of DAX1 deficient monkey	[75]
	Parkinson's disease	Baba miniature pigs	*PRKN*, *DJ-1*, *PINK1*	Multiplexed sgRNA's targeting *PRKN*, *DJ-1*, *and PINK1* genomic loci	[76]
	Burkitt lymphoma	Mouse	*TP53*	Generation of hematopoietic-cell restricted p53 knockout mice.	[60]
	Hepatocellular carcinoma and intrahepatic cholangiocarcinoma	Mouse	*APC*, *ARID1A*, *BRCA1*, *BRCA2*, *CDKN2A*, *PTEN*, *SMAD4*, *TET2* and *TRP53*	Multiplexed sgRNAs targeting ARID family proteins.	[77]
	Colon cancer	Mouse	*TP53*, *APC*, *PTEN*, *COL1A1*	Conditional deletion	[78]
	Medulloblastoma and glioblastoma	Mouse	*PATCH1/Pten*, *TP53*, *NF1/EOMES/TBR2*	Multiplexed loss-of-function mutation in tumor suppressor genes in brain	[79]
	Rett syndrome	Mouse, Rabbit embryos	*Mecp2*, *Dnmt1*, *Dnmt3a/b*	Multiplexed targeting of by AAV mediated in vivo delivery spCAS9	[80]
	Pancreatic cancer	Mouse	*Lkb1*, *Apc*, *Arid1a*,*1b*,*5b*, *Atm*, *Brca1*,*2*, *Cdkn2a1b*,*2a2*,*2b*, *Trp53*, *Pten*, *Smad4*	Pancreatic cancer modeling using Cre regulated Cas9.	[81]

screening, genetic and epigenetic marker discovery, and prognosis based on related hallmarks.[10] Compared with two-dimensional cell culture methods, the organoid culture method has shown far better representation of key biomarkers of disease histopathology. The three-dimensional organoids models are more efficient for high-throughput screening of candidate compounds than animal studies, thereby increasing the success rate and significantly reducing the cost of production. Recent studies have demonstrated that patient-derived tumor organoids can recapitulate gene expression, the genetic mutational spectrum, gene-drug association, histopathology, and heterogeneous therapeutic response, making them suitable model for personalized drug screening.[11,12] This novel approach can help prioritize and design personalized therapeutic interventions needed to improve quality of life and survival in patients. The wide range of organoids that include cerebral, stomach, intestinal, kidney, prostate, liver, and retinal organoids have been generated from pluripotent stem cells or cancerous tissue. The organoids are promising models for in vitro disease and drug-testing efficacy prior to in vivo studies.[13]

Recent developments in organoid models have significantly influenced the clinical drug discovery pipeline.[14] Three dimensional organoid models bridge gaps between physiologic microarchitecture and diffusion parameters by integrating cell-cell and cell-drug interactions and stromal components for more efficient testing in the human organ system.[15] Genetically engineered glioma organoids generated by integration of pluripotent stem-cell-derived organoids and CRISPR-Cas9-based gene editing provides a system for in vitro disease modeling and high throughput screening. Recently, studies have reported on an engineered glioma cerebral organoid model by disruption of TP53 and simultaneous addition of activated oncogene $HRas^{G12V}$.[16] Similarly, Bian et al. established neoplastic cerebral organoids via CRISPR-Cas9-mediated mutagenesis and transposons. These glioblastoma organoids provide a preclinical model platform for investigating human brain cancer biology and for evaluating drug efficacy and toxicity in context with specific genetic alterations.[17] Overall, the incorporation of organoid-based systems along with conventional two-dimensional cell culture and animal models will revolutionize conventional drug discovery and will produce cost-effective benefits in clinical disease modeling. We have summarized different available organoid models with potential application in fast-tracking drug discovery in Table 1.

2.3 | CRISPR-Cas in conventional animal-based disease modeling for drug discovery

Since its discovery, genetic engineering has dramatically influenced the ability to generate animal disease models. The CRISPR-Cas9-based genome-editing tool has been used to generate mouse, rat, and primate gene knockout models carrying single or multiple mutations or knock-in models of reporter genes in mouse zygotes.[18] The CRISPR-Cas9 system allows a one-step editing method to simultaneously target single or multiple genes carrying mutations in zygotes, thus eliminating the need for crossing mutant strains by breeding. Modern drug discovery systems utilize CRISPR-Cas9 to rapidly generate specific animal models, thereby greatly impacting the drug discovery pipeline.[19]

Animal applications of the CRISPR-Cas system have been challenging due to the large transgene size. However, use of viral vectors for CRISPR-Cas9 pairing have allowed researchers to introduce somatic mutations into lung and liver tissues. Recent studies using CRISPR-Cas9 gene editing have not only targeted mice but has also successfully been applied in preclinical drug development models such as monkeys, rats, guinea pigs, and dogs.[20] Studying gene function through gene knockouts and conditional transgenesis is an important application of CRISPR-Cas9 in drug discovery and disease modeling in rodents. However, these preclinical animal models may fail to predict the efficacy of novel drugs. Patient-derived xenograft (PDX) cancer models generated by surgically engrafting a patient xenograft onto immunodeficient mice may maintain histological heterogeneity of a patient tumor. PDX models provide proof of concept to determine clinically equivalent drug doses, toxicity, and drug activity.[21] Several studies have demonstrated the efficiency of PDX models for evaluating efficiency of immune checkpoint-based immunotherapy, such as CTLA-4 and PDL1/PD1 inhibitors.[22]

Many mutant animal models have been developed through the CRISPR-Cas9 system that recapitulate various infectious diseases including tuberculosis, influenza, malaria, and leptospirosis.[19] Recent studies have reported on isogenic stable zebrafish mutant models carrying loss-of-function mutations that resulted in neurological, kidney, or cardiovascular disorders.[23] Despite various challenges, zebrafish have been one of the most reliable experimental models for phenotypic drug discovery pipeline. CRISPR-Cas9-edited zebrafish models have been successfully employed in preclinical drug screening, including dorsomorphin, a BMP inhibitor, and a PDE5A inhibitor, which are both under chemical trials as therapy for Duchenne muscular dystrophy (DMD).[24] Similarly, CRISPR-Cas9-based humanized models have been generated that carry a spectrum of the genetic mutations found in patients suffering from DMD, neurological, or cardiovascular disorders.[25] We have summarized available animal models in Table 1. Animal models are primarily used in preclinical evaluation of personalized regenerative medicine and designing therapeutic strategies. Although discovery of the CRISPR-Cas9 tool has been a boon to cell and animal disease model generation, condition optimization for genetic manipulation in primates and other higher mammals remains a major challenge. We have summarized different available animal models with potential application in fast-tracking drug discovery in Table 1.

2.4 | Screening for novel drugs using the CRISPR-Cas system

The use of CRISPR-Cas tools for functional genomic screening provides a unique method for monitoring phenotypic changes. However, it is often necessary to carefully analyze trial results and perform error analysis, especially for negative-selection screening, which is the most experimentally necessary screening method but has always been a challenge in drug discovery. The pooled CRISPR-Cas gene knockout

technique is an effective method to identify biomarkers for drug sensitivity where increased drug toxicity is likely to occur.[26] Negative selection screening should identify the loss of key genotypes or phenotypes in experimental datasets. This is a more challenging screening setup, because a trial aims to find the rarer event, which is dependent upon high penetrance of gene disruption and usually results in complex datasets where the signal-to-noise ratio is high.[26]

CRISPRi-mediated screening is generally used in drug discovery and can mimic the effects of small-molecule drugs better than complete ablation. The CRISPRi screening method can also recognize the influence of transcripts from different transcription start sites (TSS). Alternatively, RNAi-based screening can uniquely differentiate between different splice variants.[27] Similarly, CRISPRa screening is used to assess genetic targets whose overexpression leads to a given phenotype and is a developing field.[28] Compared with previous cDNA-based screening, CRISPR-based screening has many advantages. Due to the complex nature of cDNA, the construction of cDNA screening resources is arduous. In contrast, the resources needed to perform a CRISPRa screen are similar to CRISPRn- or CRISPRi-based screens. Additionally, cDNA expression screening depends on the transcripts available in the library, and these transcripts may lack some variants or genes. Conversely, by activating endogenous site expression, CRISPRa can trigger the expression of other secondary TSS transcripts as easily as primary transcript expression, and sgRNAs can be delivered to target each TSS. However, CRISPRa screening has its own false-negative outcomes. For example, if a gene in the cell line of interest comprises a loss-of-function, CRISPRa will not work. However, combined screening strategies improve datasets for negative selection screening.[29] These screens entail an extra arm to deliver functional gain data, are experimentally complex, and may not offer the high sensitivity loss-of-function genetic intervention needed for rapid drug development.

The recent development in screening strategies has significantly improved the application of CRISPR-Cas in in vivo gene editing, however, safety aspects of the CRISPR-Cas system in context of the in vivo gene editing in humans is unknown. The recent human preclinical and clinical trials have been crucial in providing proof of concept of the practical application of CRISPR-Cas9 based gene editing in humans. Using CRISPR-Cas9 based genotype-driven approach Beck et al. recently reported for the first time that postzygotic deleterious somatic mutation in UBA1 gene results in previously unknown adult-onset inflammatory syndrome called VEXAS syndrome.[30] In another recently published case study, Gillmore et al. evaluated safety and pharmacodynamics effect of "NTLA-2001", a new CRISPR-Cas9 based in vivo gene editing therapy in 6 patients with hereditary Transthyretin amyloidosis (ATTR) (NCT04601051). The preclinical studies demonstrated that in vivo knockout of mutant *TTR* gene using at different concentration of NTLA-2001 resulted in decrease in misfolded TTR protein in the serum after single dose. However, serial safety assessment showed mild adverse effect during first 28 days of administration. The recent clinical trials reported the successful long-term transplantation of CD34+ hematopoietic stem and progenitor cells (HSPCs) in patients suffering from acute lymphoblastic leukemia and HIV infection.[31] A similar study demonstrated the reactivation of fetal hemoglobin in two patient transplanted with CRISPR-Cas9 edited CD34+ HSPCs by targeting BCL11A erythroid specific enhancer (NCT03655678 and NCT03745287).[32]

3 | ROLE OF CRISPR-CAS IN CANCER THERAPY

3.1 | CRISPR-Cas-based screening for cancer-related candidates

CRISPR has been used successfully to find actionable targets in cancer drug discovery. Several high-throughput genetic screening experiments with CRISPR have been conducted in a variety of cell types. Furthermore, genome-wide lentiviral CRISPR libraries have been produced in high throughput settings. Several readings were used in these screening studies as follows: (i) sgRNA from key genes or genes used as therapeutic targets will be minimally expressed in the library because they lead to undesirable growth and (ii) sgRNAs targeting tumor suppressors will be in the library because they have growth advantages. Cells carrying sgRNA genes that alter drug sensitivity will be selected or exhausted during drug treatment depending on whether the target gene is favorable or unfavorable. Additionally, antitumor immune response elements, such as T-cell effector or TNF signaling activities, can be investigated and all relations between various elements in the regulatory network can be delineated. Along with genome-scale screening, specific sgRNA libraries can be implemented, such as kinases involved in epigenetic regulation.

Recent screening studies on two types of libraries to identify multiple therapeutic agents, such as BRAF inhibitor (vemurafenib), MEK inhibitor (trametinib), ALK inhibitor (crizotinib), EGFR inhibitor (erlotinib), and ATR inhibitor (AZ-20) have been investigated.[33,34] These investigations identified new candidate genes linked to drug resistance. For instance, KEAP1 depletion promotes MEK inhibition resistance. In another recent studies published by Lee et al. scanned 431 patients with for *WWP1* germline variants at early-onset colon cancers without *PTEN* germline mutation. They further established gain-of-function in vitro and in vivo murine models to characterize *WWP1* variants using CRISPR-Cas9 system. The study confirmed the function of WWP1 as a cancer susceptible gene in patients without PTEN germline mutation.[35]

Although, many CRISPR tools are successful, this application still has some challenges. First, next-generation sgRNA libraries are required to increase CRISPR screening accuracy. Recently, new tools for improving sgRNA design have been developed, and sgRNA lentiviral libraries with better efficiency and less off-target activity have been produced. Second, aberrant copy numbers of the genome in CRISPR screening investigations might result in misleading positive outcomes. In comparison to sgRNAs that target genes away from these regions, sgRNAs targeting genes with multiple copies might produce replication pressure and G2/M cell cycle arrest because of induced DNA damage. We anticipate that by addressing these issues, CRISPR-based detection technology will continue to increase our insights into cancer maintenance-related

genes in a single tumor, opening the path to discover novel therapeutic targets.

3.2 | Cancer immunotherapy

Cancer immunotherapy, a therapeutic method in which highly specific and potent immune responses are generated against a wide range of cancers, is an emerging field of cancer therapeutics.[36] Cell reprogramming is facilitated by basic mechanisms of immune regulation response and immune cell function, combined with genome-editing tools. Immune response modulation via monoclonal antibodies and adaptive cellular therapy has yielded unprecedented responses in otherwise fatal advanced-stage tumors.[37] Despite recent advancements in immunotherapy, treatment efficiency varies across cancer subtypes. However, CRISPR-Cas-mediated genetic manipulation along with conventional methods have improved the efficacy of immunotherapy and facilitated discovery of novel immunotherapeutic strategies.

3.2.1 | Immune checkpoint blockades against cancer immunotherapy

The antibody-mediated blockade of immune checkpoint molecules in cancer elicits potent T-cell responses against cancer cells.[38] CRISPR-based loss-of-function screens have identified various genes that regulate immune responses in humans. Immune checkpoint regulation has improved response to immunotherapy. CRISPR-Cas9-mediated disruption of immunomodulatory regulators, such as programmed cell death protein-1 (PD-1), programmed death ligand 1, cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), major histocompatibility complex (MHC) class I, and CD47 can affect specific immune checkpoints or control immune response, thereby revealing new potential therapeutic approaches.[39] Immune checkpoint disruption by targeting genes on tumor cells and T-cells is a safer approach then targeting systemic administration blockade antibodies.[40] Ongoing CRISPR-Cas9-based clinical trials aim to generate allogenic universal T-donor cells by disrupting PD-1 in autologous T-cells for bladder cancer (NCT02863913), renal cell carcinoma (NCT02867332), and prostate cancer (NCT03867345) or by simultaneous knockout of PD-1 and CTLA-4. Recent studies have hypothesized reversible transcriptional repression of surface expression of MHC class I via polycomb repressive complex 2 (PRC2). The pharmacological inhibition of EZH2 by tazemetostat and CRISPR-Cas9-mediated disruption of EED2 and SUZ12, the components of PRC2, resulted in increased surface expression of MHC class I and cytotoxic T-cell response.[41] The latest advances in immunotherapy and CRISPR-Cas9-mediated genetic screening have demonstrated good predictive value for other immune checkpoint blockade targets, such as lymphocyte-activation gene 3 (LAG-3), T-cell membrane protein 3 (TIM-3), CD40, T-cell antigen 4-1BB homologue (4-1BB), OX40, and glucocorticoid induced TNFR-related protein (GITR). Various studies have argued for integration of pharmacological interventions and the CRISPR-Cas9 system for T-cell-mediated anticancer therapy. However, it is important to gain detailed knowledge about molecular mechanisms involved in immune checkpoint regulation, transcriptional and translational control of signaling pathways, and off-target effects during novel therapeutic strategy development.

3.2.2 | Engineering T-cells for cancer immunotherapy

A second-line immunotherapeutic approach involves use of adoptive T-cell therapy including chimeric antigen receptor T-cells (CAR-T), T-cell receptor T-cells (TCR-T), and tumor-infiltrating lymphocytes to create a robust and specific antitumor immune response.[42] The field of T-cell immunoengineering involves a novel immune cell modification approach targeting TCRs or CARs along with immunosuppressive genes and epigenetic enzymes with the CRISPR-Cas9 system to overcome cell therapy limitations.[42] The engineered T-cells expressing TCRs with specificity to tumor-associated antigen, when transplanted in patients, can generate a more potent and powerful immune response to overcome an inhibitory tumor environment. Preclinical studies of CRISPR-Cas9-edited T-cells have shown high efficacy and low toxicity, encouraging first-in-human clinical trials of CRISPR-Cas9-edited anti-NY-ESO-1 TCR-T cells harboring endogenous TCR and PD-1 genetic disruption to treat multiple myeloma, melanoma, and synovial sarcoma patients (NCT03399448; NCT01967823; NCT02869217).[42,43] Because engineered TCR-T cells can virtually display any type of tumor-associated intracellular peptides, TCR-T cell-based therapy exclusively targeting large pools of neoantigens (produced because of mutations only in cancer cells) may be a safe and effective resource for personalized cancer immunotherapy. Thus, adoptive cell therapy using a cocktail of neoantigen TCR-T cells in combination with CRISPR screens may be an effective immunotherapeutic option.

Among adoptive cell-based therapies, engineered CAR-T cells have been successful for treating cancer cells. The main difference between CAR-T cell-based therapy and TCR therapy is that CAR-T uses single chain variable fragments targeting either a variable heavy chain and a light chain domain of an antibody (first generation CAR-T cells) or costimulatory receptors (second and third generation CAR-T cells) to target extracellular antigen independent of MHC restrictions.[44] CAR-T-cell-mediated cancer interventions have shown promising results in various clinical trials. Since its first approval in 2017, anti-CD19 CAR-T-cell-based clinical trials for treating acute lymphoblastic leukemia (NCT03366350; NCT02546739), B-cell lymphoma (NCT03366350; NCT03448393), leukemia (NCT02546739), and non-Hodgkin lymphoma (NCT03467256) have been approved.[45] The application of CRISPR-Cas system in various ongoing anticancer clinical trials is shown in Table 2. Meanwhile, a series of CAR-T-cells targeting an array of antigens, such as BCMA, CD133, CD22, CD33, and HER2, have demonstrated profound effects in clinical settings.[46] However, long-term treatment appeared resistant to CAR-T cell therapies. Therefore, further characterization and evaluation of CAR signaling are needed to elicit a powerful immune response.[47]

TABLE 2 Application of CRISPR-Cas system in anticancer clinical trials (www.clinicaltrials.gov)

Clinical trial number	Title of the study	Cancer type	Drug interventions	Status
NCT03545815	Study of CRISPR-Cas9 mediated PD-1 and TCR gene-knocked out mesothelin-directed CAR-T cells in patients with mesothelin positive multiple solid tumors	Mesothelin positive solid tumors	anti-mesothelin CAR-T cells	Recruiting
NCT04426669	A study of metastatic gastrointestinal cancers treated with tumor infiltrating lymphocytes in which the gene encoding the intracellular immune checkpoint CISH is inhibited using CRISPR genetic engineering	• Gastrointestinal cancer • Colorectal cancer • Pancreatic cancer • Bladder cancer • Esophageal cancer • Stomach cancer	• Cyclophosphamide • Fludarabine • Aldesleukin • Tumor-infiltrating lymphocytes(TIL)	Recruiting
NCT04035434	A safety and efficacy study evaluating CTX110 in subjects with relapsed or refractory B-cell malignancies (CARBON)	• B-cell malignancy • Non-Hodgkin lymphoma • B-cell lymphoma • adult B cell ALL	CTX110	Recruiting
NCT03081715	PD-1 knockout engineered T cells for advanced esophageal cancer	Esophageal cancer	PD-1 knockout T cells	Completed
NCT04976218	TGFβr-KO CAR-EGFR T cells in advanced biliary tract cancer	Advanced biliary tract cancer	TGFBR-KO CAR-EGFR T cells	Not yet recruiting
NCT02793856	PD-1 knockout engineered T cells for metastatic non-small cell lung cancer	Metastatic non-small cell lung cancer	CyclophosphamidePD-1 knockout T cells	Completed
NCT04037566	CRISPR (HPK1) edited CD19-specific CAR-T cells (XYF19 CAR-T cells) for CD19+ leukemia or lymphoma.	• Lymphocytic acute leukemia (ALL) • B-cell Lymphoma • CD19 + B-cell lymphoma	• XYF19 CAR-T cell • Cyclophosphamide • Fludarabine	Recruiting
NCT04767308	Safety and efficacy of CT125A cells for treatment of relapsed/refractory CD5+ hematopoietic malignancies	• Chronic lymphocytic leukemia • Mantle cell lymphoma • Diffuse large B-cell lymphoma • Follicular lymphoma • Peripheral T-cell lymphomas	• CT125A cells • Cyclophosphamide • Fludarabine	Not yet recruiting
NCT03044743	PD-1 knockout EBV-CTLs for advanced stage Epstein-Barr Virus (EBV) associated malignancies	• Gastric carcinoma • Nasopharyngeal carcinoma • T-cell lymphoma • adult Hodgkin lymphoma • Diffuse large B-cell lymphoma	• Fludarabine • Cyclophosphamide • Interleukin-2	Recruiting
NCT04637763	CRISPR-edited allogeneic anti-CD19 CAR-T cell therapy for relapsed/refractory b cell non-hodgkin lymphoma	• Non-Hodgkin lymphoma • B-cell lymphoma	• CB-010 • Cyclophosphamide • Fludarabine	Recruiting

(Continues)

TABLE 2 (Continued)

Clinical trial number	Title of the study	Cancer type	Drug interventions	Status
NCT04557436	TT52CAR19 therapy for B-cell acute lymphoblastic leukemia (B-ALL)	• B-cell acute lymphoblastic leukemia	PBLTT52CAR19	Recruiting
NCT03398967	A feasibility and safety study of universal dual specificity CD19 and CD20 or CD22 CAR-T cell immunotherapy for relapsed or refractory leukemia and lymphoma	• B- cell leukemia • B- cell lymphoma	Universal dual specificity CD19 and CD20 or CD22 CAR-T cells	Recruiting

Although, adoptive cell therapy focuses on autologous T-cell transplantation owing to MHC restrictions, elimination of MHC by genetic engineering in allogenic "universal T-cells" provides immunological advantages over developing resistance to immunotherapy. Use of CRISPR-Cas9 to target MHC and related genes in universal CAR-T cells derived from healthy donors can overcome immunological defects associated with cancer treatment.[48] Torikai et al. first reported use of gene-editing technology to generate CD19-specific CAR-T cells with deletions in endogenous TCR.[49] Another recent study demonstrated that exogenous expression of HLA-E in MHC class-I-deficient universal CAR-T cells could prevent NK-cell-mediated immune rejection.[50] Elimination by gene editing of immunosuppressive genes like CTLA-4 and PD-1 or CAR targets expressed on T-cells may also prevent toxicity and transplantation efficiency associated with CAR immunotherapy.[51] Taken together, these studies demonstrate that CRISPR-Cas9-mediated gene editing is a crucial tool for discovering potential drug targets involved in immune checkpoints and T-cell-mediated therapy, yielding a new potential paradigm for cancer-immunity therapeutics.

4 | CONCLUSION AND FUTURE PERSPECTIVES

In summary, use of CRISPR-Cas-mediated genome editing has allowed genetic manipulation in previously intractable cells and organisms. Although scientists use RNAi technology in either pooled or arrayed formats for several applications. Both CRISPR and RNAi-based techniques offer advantages and potential limitations that should be assessed based on the biology of the genes under investigation. The genome-wide pooled CRISPR library has the ability to strengthen negative selection CRISPR screening and offer value across the drug development process. However, one of the major drawbacks of CRISPR screening is heterogeneity. The prevalence of in-frame indels, which create wild-type and heterozygous subpopulations and have been reported to interfere with screening results, is one possible source of this variability.

Current use of both viral and non-viral-based vectors has allowed successful delivery of genetic engineering machinery in almost all types of cells, including hESCs. In particular, use of patient-derived cells and animal models to generate a precise mammalian model system in combination with functional genomics has helped unravel cellular mechanisms and support discovery of novel drug targets. Furthermore, use of ever-expanding patient-derived organoid biobanks has helped to establish a representative collection of diverse cancer subtypes, whose use in a systematic drug discovery pipeline has enabled researchers to determine drug effects in similar tumor subtypes.[52]

The recent enhancement of homologous recombination methods and identification of Cas9 homologues or base editors have further reduced off-target effects and improved genome-editing precision. Improvements in CRISPR-Cas-aided high-throughput drug screening, disease modeling, testing, and validation will further allow acceleration and improvement of the drug discovery process. CRISPR-Cas-based therapeutic approaches will be key in next-generation translational drug discovery and futuristic medicine. CRISPR screening has more specificity to its genetic target than previous methods like RNA interference, which means we can get a lot accurate output. We can target the endogenous gene at the transcriptional start point using CRISPRi and CRISPRa tools. This enables us to change the genetic expression in the cellular state, providing us with greater biological data. The rate at which CRISPR has accelerated clinical research is astounding.

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CONFLICT OF INTEREST

The authors declare no financial or commercial conflict of interest

DATA AVAILABILITY STATEMENT

Not applicable.

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REFERENCES

1. Mali, P., Yang, L., Esvelt, K. M., Aach, J., Guell, M., DiCarlo, J. E., Norville, J. E., & Church, G. M. (2013). RNA-Guided Human Genome Engineering via Cas9. *Science (80-.)*, *339*, 823, https://doi.org/10.1126/science.1232033.
2. Chandrasekaran, A. P., Song, M., & Ramakrishna, S. (2017). Genome editing: a robust technology for human stem cells. *Cellular and Molecular Life Sciences*, *74*, 3335, https://doi.org/10.1007/s00018-017-2522-0.
3. Yang, Y., Dong, X., Xie, B., Ding, N., Chen, J., Li, Y., Zhang, Q., Qu, H., & Fang, X. (2015). Databases and Web Tools for Cancer Genomics Study. *Genomics. Proteomics Bioinformatics*, *13*, 46, https://doi.org/10.1016/j.gpb.2015.01.005.
4. Grobarczyk, B., Franco, B., Hanon, K., & Malgrange, B. (2015). Generation of Isogenic Human iPS Cell Line Precisely Corrected by Genome Editing Using the CRISPR/Cas9 System. *Stem Cell Review Reports*, *11*, 774, https://doi.org/10.1007/s12015-015-9600-1.
5. Matano, M., Date, S., Shimokawa, M., Takano, A., Fujii, M., Ohta, Y., Watanabe, T., Kanai, T., & Sato, T. (2015). Modeling colorectal cancer using CRISPR-Cas9 mediated engineering of human intestinal organoids. *Nature Medicine*, *21*, 256, https://doi.org/10.1038/nm.3802
6. Shi, J., Wang, E., Milazzo, J. P., Wang, Z., Kinney, J. B., & Vakoc, C. R. (2015). Discovery of cancer drug targets by CRISPR-Cas9 screening of protein domains. *Nature Biotechnology*, *33*, 661, https://doi.org/10.1038/nbt.3235.
7. Neggers, J. E., Kwanten, B., Dierckx, T., Noguchi, H., Voet, A., Bral, L., Minner, K., Massant, B., Kint, N., Delforge, M., Vercruysse, T., Baloglu, E., Senapedis, W., Jacquemyn, M., & Daelemans, D. (2018). Target identification of small molecules using large-scale CRISPR-Cas mutagenesis scanning of essential genes *Nature Communication*, *9*, 502, https://doi.org/10.1038/s41467-017-02349-8.
8. Kasap, C., Elemento, O., & Kapoor, T. M. (2014). DrugTargetSeqR: a genomics- and CRISPR-Cas9-based method to analyze drug targets *Nature Chemical Biology*, *10*, 626, https://doi.org/10.1038/nchembio.1551.
9. Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J. A., & Charpentier, E. (2012). A Programmable Dual-RNA-Guided DNA Endonuclease in Adaptive Bacterial Immunity *Science (80-.)*, *337*, 816, https://doi.org/10.1126/science.1225829.
10. Hay, M., Thomas, D. W., Craighead, J. L., Economides, C., & Rosenthal, J. (2014). Clinical development success rates for investigational drugs *Nature Biotechnology*, *32*, 40, https://doi.org/10.1038/nbt.2786.
11. Li, J., Stamato, M., Velliou, E., Jeffryes, C., & Agathos, S. N. (2014). Design and characterization of a scalable airlift flat panel photobioreactor for microalgae cultivation *Journal of Applied Phycology*, *75*, https://doi.org/10.1007/s10811-014-0335-1.
12. Mignot, L., a Junter, G., & Labbe, M. (1989). A new type of immobilized-cell photobioreactor with internal illumination by optical fibres *Biotechnology Techniques*, *3*, 299, https://doi.org/10.1007/BF01875625.
13. Rae, C., Amato, F., & Braconi, C. (2021). Patient-Derived Organoids as a Model for Cancer Drug Discovery *International Journal of Molecular Sciences*, *22*, 3483, https://doi.org/10.3390/ijms22073483.
14. Skardal, A., Devarasetty, M., Forsythe, S., Atala, A., & Soker, S. (2016). A reductionist metastasis-on-a-chip platform for in vitro tumor progression modeling and drug screening *Biotechnology and Bioengineering*, *113*, 2020, https://doi.org/10.1002/bit.25950.
15. Byrne, H. (2020). Three-dimensional biological cultures and organoids. *10*, 20200014, https://doi.org/10.1098/rsfs.2020.0014.
16. Ogawa, J., Pao, G. M., Shokhirev, M. N., & Verma, I. M. (2018). Glioblastoma Model Using Human Cerebral Organoids *Cell reports*, *23*, 1220, https://doi.org/10.1016/j.celrep.2018.03.105.
17. Bian, S., Repic, M., Guo, Z., Kavirayani, A., Burkard, T., Bagley, J. A., Krauditsch, C., & Knoblich, J. A. (2018). Genetically engineered cerebral organoids model brain tumor formation *Nature Methods*, *15*, 631, https://doi.org/10.1038/s41592-018-0070-7.
18. Wang, H., Yang, H., Shivalila, C. S., Dawlaty, M. M., Cheng, A. W., Zhang, F., & Jaenisch, R. (2013). One-Step Generation of Mice Carrying Mutations in Multiple Genes by CRISPR/Cas-Mediated Genome Engineering *Cell*, *153*, 910, https://doi.org/10.1016/j.cell.2013.04.025.
19. Fellmann, C., Gowen, B. G., Lin, P. C., Doudna, J. A., & Corn, J. E. (2017). Cornerstones of CRISPR-Cas in drug discovery and therapy *Nature Reviews Drug Discovery*, *16*, 89, https://doi.org/10.1038/nrd.2016.238.
20. Li, D., Qiu, Z., Shao, Y., Chen, Y., Guan, Y., Liu, M., Li, Y., Gao, N., Wang, L., Lu, X., Zhao, Y., & Liu, M. (2013). Heritable gene targeting in the mouse and rat using a CRISPR-Cas system *Nature Biotechnology*, *31*, 681, https://doi.org/10.1038/nbt.2661.
21. Kerbel, R. S., *Cancer Biology & Therapy 2*, S134.
22. Buchbinder, E. I., & Desai, A. (2016). CTLA-4 and PD-1 Pathways *American Journal of Clinical Oncology*, *39*, 98, https://doi.org/10.1097/COC.0000000000000239.
23. Cornet, C., Di Donato, V., & Terriente, J. (2018). Combining Zebrafish and CRISPR/Cas9: Toward a More Efficient Drug Discovery Pipeline *Frontiers in Pharmacology*, *9*, https://doi.org/10.3389/fphar.2018.00703.
24. Yu, P. B., Hong, C. C., Sachidanandan, C., Babitt, J. L., Deng, D. Y., Hoyng, S. A., Lin, H. Y., Bloch, K. D., & Peterson, R. T. (2008). Dorsomorphin inhibits BMP signals required for embryogenesis and iron metabolism *Nature Chemical Biology*, *4*, 33, https://doi.org/10.1038/nchembio.2007.54.
25. Echigoya, Y., Lim, K. R. Q., Trieu, N., Bao, B., Miskew Nichols, B., Vila, M. C., Novak, J. S., Hara, Y., Lee, J., Touznik, A., Mamchaoui, K., Aoki, Y., Takeda, S., Nagaraju, K., Mouly, V., Maruyama, R., Duddy, W., & Yokota, T. (2017). Quantitative Antisense Screening and Optimization for Exon 51 Skipping in Duchenne Muscular Dystrophy *Molecular Therapy*, *25*, 2561, https://doi.org/10.1016/j.ymthe.2017.07.014.
26. Blanck, M., Budnik-Zawilska, M. B., Lenger, S. R., McGonigle, J. E., Martin, G. R. A., le Sage, C., Lawo, S., Pemberton, H. N., Tiwana, G. S., Sorrell, D. A., & Cross, B. C. S. (2020). A Flexible, Pooled CRISPR Library for Drug Development Screens *Cris Journal*, *3*, 211, https://doi.org/10.1089/crispr.2019.0066.
27. Rock, J. (2019). Tuberculosis drug discovery in the CRISPR era *Plos Pathogens*, *15*, e1007975, https://doi.org/10.1371/journal.ppat.1007975.
28. Horlbeck, M. A., Gilbert, L. A., Villalta, J. E., Adamson, B., Pak, R. A., Chen, Y., Fields, A. P., Park, C. Y., Corn, J. E., Kampmann, M., & Weissman, J. S. (2016). Compact and highly active next-generation libraries for CRISPR-mediated gene repression and activation *Elife*, *5*, https://doi.org/10.7554/eLife.19760.
29. Sharma, S., & Petsalaki, E. (2018). Application of CRISPR-Cas9 Based Genome-Wide Screening Approaches to Study Cellular Signalling Mechanisms *International Journal of Molecular Sciences*, *19*, 933, https://doi.org/10.3390/ijms19040933.
30. Huang, H., Zhang, W., Cai, W., Liu, J., Wang, H., Qin, T., Xu, Z., Li, B., Qu, S., Pan, L., Huang, G., Gale, R. P., & Xiao, Z. (2021). *Experimental Hematology & Oncology*, *10*, 23, https://doi.org/10.1186/s40164-021-00217-2.
31. Tsukamoto, T. (2020). Hematopoietic Stem/Progenitor Cells and the Pathogenesis of HIV/AIDS *Frontiers in Cellular and Infection Microbiology*, *10*, https://doi.org/10.3389/fcimb.2020.00060.
32. Park, S. H., & Bao, G. (2021). CRISPR/Cas9 gene editing for curing sickle cell disease *Transfusion and Apheresis Science*, *60*, 103060, https://doi.org/10.1016/j.transci.2021.103060.
33. Nagler, A., Vredevoogd, D. W., Alon, M., Cheng, P. F., Trabish, S., Kalaora, S., Arafeh, R., Goldin, V., Levesque, M. P., Peeper, D. S., & Samuels, Y. (2020). *Pigment Cell & Melanoma Research*, *33*, 334, https://doi.org/10.1111/pcmr.12825.
34. Li, Z., Wang, B., Gu, S., Jiang, P., Sahu, A., Chen, C. - H., Han, T., Shi, S., Wang, X., Traugh, N., Liu, H., Liu, Y., Wu, Q., Brown, M., Xiao, T., Boland,

G. M., & Liu, X. S (2020). CRISPR Screens Identify Essential Cell Growth Mediators in BRAF Inhibitor-resistant Melanoma *Genomics. Proteomics Bioinformatics*, *18*, 26, https://doi.org/10.1016/j.gpb.2020.02.002.

35. Lee, Y. - R., Yehia, L., Kishikawa, T., Ni, Y., Leach, B., Zhang, J., Panch, N., Liu, J., Wei, W., Eng, C., & Pandolfi, P. P. (2020). WWP1 Gain-of-Function Inactivation of PTEN in Cancer Predisposition *New England Journal of Medicine*, *382*, 2103, https://doi.org/10.1056/NEJMoa1914919.
36. Khalil, D. N., Smith, E. L., Brentjens, R. J., & Wolchok, J. D. (2016). The future of cancer treatment: immunomodulation, CARs and combination immunotherapy *Nature reviews Clinical oncology*, *13*, 273, https://doi.org/10.1038/nrclinonc.2016.25.
37. Yin, H., Xue, W., & Anderson, D. G. (2019). CRISPR-Cas: a tool for cancer research and therapeutics *Nature reviews Clinical oncology*, *16*, 281, https://doi.org/10.1038/s41571-019-0166-8.
38. Hodi, F. S., O'Day, S. J., McDermott, D. F., Weber, R. W., Sosman, J. A., Haanen, J. B., Gonzalez, R., Robert, C., Schadendorf, D., Hassel, J. C., Akerley, W., van den Eertwegh, A. J. M., Lutzky, J., Lorigan, P., Vaubel, J. M., Linette, G. P., Hogg, D., Ottensmeier, C. H., Lebbé, C., … Urba, W. J. (2010). Improved Survival with Ipilimumab in Patients with Metastatic Melanoma *New England Journal of Medicine*, *363*, *711*, https://doi.org/10.1056/NEJMoa1003466.
39. Kleinovink, J. W., Marijt, K. A., Schoonderwoerd, M. J. A., van Hall, T., Ossendorp, F., & Fransen, M. F. (2017). PD-L1 expression on malignant cells is no prerequisite for checkpoint therapy *Oncoimmunology*, *6*, e1294299, https://doi.org/10.1080/2162402X.2017.1294299.
40. Lloyd, A., Vickery, O. N., & Laugel, B. (2013). Beyond the Antigen Receptor: Editing the Genome of T-Cells for Cancer Adoptive Cellular Therapies *Frontiers in Immunology*, *4*, https://doi.org/10.3389/fimmu.2013.00221.
41. Italiano, A., Soria, J. - C., Toulmonde, M., Michot, J. - M., Lucchesi, C., Varga, A., Coindre, J. - M., Blakemore, S. J., Clawson, A., Suttle, B., McDonald, A. A., Woodruff, M., Ribich, S., Hedrick, E., Keilhack, H., Thomson, B., Owa, T., Copeland, R. A., Ho, P. T. C., & Ribrag, V. (2018). Tazemetostat, an EZH2 inhibitor, in relapsed or refractory B-cell non-Hodgkin lymphoma and advanced solid tumours: a first-in-human, open-label, phase 1 study *The Lancet Oncology*, *19*, 649, https://doi.org/10.1016/S1470-2045(18)30145-1.
42. Paucek, R. D., Baltimore, D., & Li, G. (2019). The Cellular Immunotherapy Revolution: Arming the Immune System for Precision Therapy *Trends in Immunology*, *40*, 292, https://doi.org/10.1016/j.it.2019.02.002.
43. Rapoport, A. P., Stadtmauer, E. A., Binder-Scholl, G. K., Goloubeva, O., Vogl, D. T., Lacey, S. F., Badros, A. Z., Garfall, A., Weiss, B., Finklestein, J., Kulikovskaya, I., Sinha, S. K., Kronsberg, S., Gupta, M., Bond, S., Melchiori, L., Brewer, J. E., Bennett, A. D., Gerry, A. B., … June, C. H. (2015). NY-ESO-1-specific TCR-engineered T cells mediate sustained antigen-specific antitumor effects in myeloma *Nature Medicine*, *21*, 914, https://doi.org/10.1038/nm.3910.
44. Gross, G., Waks, T., & Eshhar, Z. (1989). Expression of immunoglobulin-T-cell receptor chimeric molecules as functional receptors with antibody-type specificity, *86*, 10024, https://doi.org/10.1073/pnas.86.24.10024
45. June, C. H., O'Connor, R. S., Kawalekar, O. U., Ghassemi, S., & Milone, M. C. (2018). CAR T cell immunotherapy for human cancer *Science (80-.)*, *359*, 1361, https://doi.org/10.1126/science.aar6711
46. Yadav, R. K., Ali, A., Kumar, S., Sharma, A., Baghchi, B., Singh, P., Das, S., Singh, C., & Sharma, S. (2020). CAR T cell therapy: newer approaches to counter resistance and cost *Heliyon*, *6*, e03779, https://doi.org/10.1016/j.heliyon.2020.e03779
47. Shah, N. N., & Fry, T. J. (2019). Mechanisms of resistance to CAR T cell therapy *Nature reviews Clinical oncology*, https://doi.org/10.1038/s41571-019-0184-6
48. Zakrzewski, J. L., Suh, D., Markley, J. C., Smith, O. M., King, C., Goldberg, G. L., Jenq, R., Holland, A. M., Grubin, J., Cabrera-Perez, J., Brentjens, R. J., Lu, S. X., Rizzuto, G., Sant'Angelo, D. B., Riviere, I., Sadelain, M., Heller, G., Zúñiga-Pflücker, J. C., Lu, C., & van den Brink, M. R. M. (2008). Tumor immunotherapy across MHC barriers using allogeneic T-cell precursors *Nature Biotechnology*, *26*, 453, https://doi.org/10.1038/nbt1395
49. Torikai, H., Reik, A., Liu, P. - Q., Zhou, Y., Zhang, L., Maiti, S., Huls, H., Miller, J. C., Kebriaei, P., Rabinovitch, B., Lee, D. A., Champlin, R. E., Bonini, C., Naldini, L., Rebar, E. J., Gregory, P. D., Holmes, M. C., & Cooper, L. J. N. (2012). A foundation for universal T-cell based immunotherapy: T cells engineered to express a CD19-specific chimeric-antigen-receptor and eliminate expression of endogenous TCR *Blood*, *119*, 5697, https://doi.org/10.1182/blood-2012-01-405365
50. Gornalusse, G. G., Hirata, R. K., Funk, S. E., Riolobos, L., Lopes, V. S., Manske, G., Prunkard, D., Colunga, A. G., Hanafi, L. - A., Clegg, D. O., Turtle, C., & Russell, D. W. (2017). HLA-E-expressing pluripotent stem cells escape allogeneic responses and lysis by NK cells *Nature Biotechnology*, *35*, 765, https://doi.org/10.1038/nbt.3860.
51. Eyquem, J., Mansilla-Soto, J., Giavridis, T., van der Stegen, S. J. C., Hamieh, M., Cunanan, K. M., Odak, A., Gönen, M., & Sadelain, M. (2017). Targeting a CAR to the TRAC locus with CRISPR/Cas9 enhances tumour rejection *Nature*, *543*, 113, https://doi.org/10.1038/nature21405.
52. Ho, B., Pek, N., & Soh, B. - S. (2018). Disease Modeling Using 3D Organoids Derived from Human Induced Pluripotent Stem Cells *International Journal of Molecular Sciences*, *19*, 936, https://doi.org/10.3390/ijms19040936.
53. Liu, J., Gao, C., Chen, W., Ma, W., Li, X., Shi, Y., Zhang, H., Zhang, L., Long, Y., Xu, H., Guo, X., Deng, S., Yan, X., Yu, D., Pan, G., Chen, Y., Lai, L., Liao, W., & Li, Z. (2016). CRISPR/Cas9 facilitates investigation of neural circuit disease using human iPSCs: mechanism of epilepsy caused by an SCN1A loss-of-function mutation *Translational Psychiatry*, *6*, e703, https://doi.org/10.1038/tp.2015.203.
54. An, M. C., Zhang, N., Scott, G., Montoro, D., Wittkop, T., Mooney, S., Melov, S., & Ellerby, L. M. (2012). Genetic Correction of Huntington's Disease Phenotypes in Induced Pluripotent Stem Cells *Cell Stem Cell*, *11*, 253, https://doi.org/10.1016/j.stem.2012.04.026.
55. Xu, P., Tong, Y., Liu, X., Wang, T., Cheng, L., Wang, B., Lv, X., Huang, Y., & Liu, D., Both TALENs and CRISPR/Cas9 directly target the HBB IVS2-654 (C->-T) mutation in Î²-thalassemia-derived iPSCs *Science Reports*, 2015, *5*, 12065, https://doi.org/10.1038/srep12065.
56. Park, C. - Y., Halevy, T., Lee, D. R., Sung, J. J., Lee, J. S., Yanuka, O., Benvenisty, N., & Kim, D. - W., Reversion of FMR1 Methylation and Silencing by Editing the Triplet Repeats in Fragile X iPSC-Derived Neurons *Cell reports*, 2015, *13*, 234, https://doi.org/10.1016/j.celrep.2015.08.084.
57. Neggers, J. E., Vercruysse, T., Jacquemyn, M., Vanstreels, E., Baloglu, E., Shacham, S., Crochiere, M., Landesman, Y., & Daelemans, D., *Chemistry & Biology*, 2015, *22*, 107, https://doi.org/10.1016/j.chembiol.2014.11.015.
58. Vannocci, T., Faggianelli, N., Zaccagnino, S., della Rosa, I., Adinolfi, S., & Pastore, A. (2015). *Disease Models & Mechanisms*, *8*, 711, https://doi.org/10.1242/dmm.020545.
59. Mandal, P. K., Ferreira, L. M. R., Collins, R., Meissner, T. B., Boutwell, C. L., Friesen, M., Vrbanac, V., Garrison, B. S., Stortchevoi, A., Bryder, D., Musunuru, K., Brand, H., Tager, A. M., Allen, T. M., Talkowski, M. E., Rossi, D. J., & Cowan, C. A., Efficient Ablation of Genes in Human Hematopoietic Stem and Effector Cells using CRISPR/Cas9 *Cell Stem Cell*, 2014, *15*, 643, https://doi.org/10.1016/j.stem.2014.10.004.
60. Aubrey, B. J., Kelly, G. L., Kueh, A. J., Brennan, M. S., O'Connor, L., Milla, L., Wilcox, S., Tai, L., Strasser, A., & Herold, M. J., An Inducible Lentiviral Guide RNA Platform Enables the Identification of Tumor-Essential Genes and Tumor-Promoting Mutations InÂ Vivo *Cell reports*. 2015, *10*, 1422, https://doi.org/10.1016/j.celrep.2015.02.002.
61. Wang, P., Mokhtari, R., Pedrosa, E., Kirschenbaum, M., Bayrak, C., Zheng, D., & Lachman, H. M. (2017). CRISPR/Cas9-mediated heterozy-

gous knockout of the autism gene CHD8 and characterization of its transcriptional networks in cerebral organoids derived from iPS cells *Molecular Autism*, *8*, 11, https://doi.org/10.1186/s13229-017-0124-1.
62. Schwank, G., Koo, B. - K., Sasselli, V., Dekkers, J. F., Heo, I., Demircan, T., Sasaki, N., Boymans, S., Cuppen, E., van der Ent, C. K., Nieuwenhuis, E. E. S., Beekman, J. M., & Clevers, H. (2013). Functional Repair of CFTR by CRISPR/Cas9 in Intestinal Stem Cell Organoids of Cystic Fibrosis Patients *Cell Stem Cell*, *13*, 653, https://doi.org/10.1016/j.stem.2013.11.002.
63. Seo, J., Kritskiy, O., Watson, L. A., Barker, S. J., Dey, D., Raja, W. K., Lin, Y. - T., Ko, T., Cho, S., Penney, J., Silva, M. C., Sheridan, S. D., Lucente, D., Gusella, J. F., Dickerson, B. C., Haggarty, S. J., & Tsai, L. - H. (2017). Inhibition of p25/Cdk5 Attenuates Tauopathy in Mouse and iPSC Models of Frontotemporal Dementia *Journal of Neuroscience*, *37*, 9917, https://doi.org/10.1523/JNEUROSCI.0621-17.2017.
64. Takeda, H., Kataoka, S., Nakayama, M., Ali, M. A. E., Oshima, H., Yamamoto, D., Park, J. - W., Takegami, Y., An, T., Jenkins, N. A., Copeland, N. G., & Oshima, M. (2019). CRISPR-Cas9-mediated gene knockout in intestinal tumor organoids provides functional validation for colorectal cancer driver genes *Proceedings of the National Academy of Sciences of the United States of America*, *116*, 15635, https://doi.org/10.1073/pnas.1904714116.
65. Liu, X., Cheng, Y., Abraham, J. M., Wang, Z., Wang, Z., Ke, X., Yan, R., Shin, E. J., Ngamruengphong, S., Khashab, M. A., Zhang, G., McNamara, G., Ewald, A. J., Lin, D., Liu, Z., & Meltzer, S. J. (2018). Modeling Wnt signaling by CRISPR-Cas9 genome editing recapitulates neoplasia in human Barrett epithelial organoids *Cancer Letters*, *436*, 109, https://doi.org/10.1016/j.canlet.2018.08.017.
66. Michels, B. E., Mosa, M. H., Streibl, B. I., Zhan, T., Menche, C., Abou-El-Ardat, K., Darvishi, T., Członka, E., Wagner, S., Winter, J., Medyouf, H., Boutros, M., & Farin, H. F. (2020). Pooled In-Vitro and InÂ Vivo CRISPR-Cas9 Screening Identifies Tumor Suppressors in Human Colon Organoids *Cell Stem Cell*, *26*, 782, https://doi.org/10.1016/j.stem.2020.04.003.
67. Dekkers, J. F., Whittle, J. R., Vaillant, F., Chen, H. - R., Dawson, C., Liu, K., Geurts, M. H., Herold, M. J., Clevers, H., Lindeman, G. J., & Visvader, J. E. (2020). Modeling Breast Cancer Using CRISPR-Cas9-Mediated Engineering of Human Breast Organoids *JNCI: Journal of the National Cancer Institute*, *112*, 540, https://doi.org/10.1093/jnci/djz196.
68. Müller, T., Hess, M. W., Schiefermeier, N., Pfaller, K., Ebner, H. L., Heinz-Erian, P., Ponstingl, H., Partsch, J., Röllinghoff, B., Köhler, H., Berger, T., Lenhartz, H., Schlenck, B., Houwen, R. J., Taylor, C. J., Zoller, H., Lechner, S., Goulet, O., Utermann, G., Ruemmele, F. M., … Janecke, A. R. (2008). MYO5B mutations cause microvillus inclusion disease and disrupt epithelial cell polarity *Nature Genetics*, *40*, 1163, https://doi.org/10.1038/ng.225.
69. Ousterout, D. G., Kabadi, A. M., Thakore, P. I., Majoros, W. H., Reddy, T. E., & Gersbach, C. A. (2015). Multiplex CRISPR/Cas9-based genome editing for correction of dystrophin mutations that cause Duchenne muscular dystrophy *Nature Communication*, *6*, 6244, https://doi.org/10.1038/ncomms7244
70. Yin, H., Song, C. - Q., Dorkin, J. R., Zhu, L. J., Li, Y., Wu, Q., Park, A., Yang, J., Suresh, S., Bizhanova, A., Gupta, A., Bolukbasi, M. F., Walsh, S., Bogorad, R. L., Gao, G., Weng, Z., Dong, Y., Koteliansky, V., Wolfe, S. A., … Anderson, D. G. (2016). Therapeutic genome editing by combined viral and non-viral delivery of CRISPR system components in vivo *Nature Biotechnology*, *34*, 328, https://doi.org/10.1038/nbt.3471.
71. Wu, Y., Liang, D., Wang, Y., Bai, M., Tang, W., Bao, S., Yan, Z., Li, D., & Li, J. (2013). Correction of a Genetic Disease in Mouse via Use of CRISPR-Cas9 *Cell Stem Cell*, *13*, 659, https://doi.org/10.1016/j.stem.2013.10.016.
72. Platt, R. J., Chen, S., Zhou, Y., Yim, M. J., Swiech, L., Kempton, H. R., Dahlman, J. E., Parnas, O., Eisenhaure, T. M., Jovanovic, M., Graham, D. B., Jhunjhunwala, S., Heidenreich, M., Xavier, R. J., Langer, R., Anderson, D. G., Hacohen, N., Regev, A., Feng, G., … Zhang, F. (2014). CRISPR-Cas9 Knockin Mice for Genome Editing and Cancer Modeling *Cell*, *159*, 440, https://doi.org/10.1016/j.cell.2014.09.014.
73. Yang, Y., Wang, L., Bell, P., McMenamin, D., He, Z., White, J., Yu, H., Xu, C., Morizono, H., Musunuru, K., Batshaw, M. L., & Wilson, J. M. (2016). A dual AAV system enables the Cas9-mediated correction of a metabolic liver disease in newborn mice *Nature Biotechnology*, *34*, 334, https://doi.org/10.1038/nbt.3469.
74. Heckl, D., Kowalczyk, M. S., Yudovich, D., Belizaire, R., Puram, R. V, McConkey, M. E., Thielke, A., Aster, J. C., Regev, A., & Ebert, B. L. (2014). Generation of mouse models of myeloid malignancy with combinatorial genetic lesions using CRISPR-Cas9 genome editing *Nature Biotechnology*, *32*, 941, https://doi.org/10.1038/nbt.2951.
75. Kang, Y., Zheng, B., Shen, B., Chen, Y., Wang, L., Wang, J., Niu, Y., Cui, Y., Zhou, J., Wang, H., Guo, X., Hu, B., Zhou, Q., Sha, J., Ji, W., & Huang, X. (2015). CRISPR/Cas9-mediated Dax1 knockout in the monkey recapitulates human AHC-HH *Human Molecular Genetics*, *24*, 7255, https://doi.org/10.1093/hmg/ddv425.
76. Wang, X., Cao, C., Huang, J., Yao, J., Hai, T., Zheng, Q., Wang, X., Zhang, H., Qin, G., Cheng, J., Wang, Y., Yuan, Z., Zhou, Q., Wang, H., & Zhao, J. (2016). One-step generation of triple gene-targeted pigs using CRISPR/Cas9 system *Science Reports*, *6*, 20620, https://doi.org/10.1038/srep20620.
77. Barrangou, R., & Doudna, J. A. (2016). Applications of CRISPR technologies in research and beyond *Nature Biotechnology*, *34*, 933, https://doi.org/10.1038/nbt.3659.
78. Dow, L. E., Fisher, J., O'Rourke, K. P., Muley, A., Kastenhuber, E. R., Livshits, G., Tschaharganeh, D. F., Socci, N. D., & Lowe, S. W. (2015). Inducible in vivo genome editing with CRISPR-Cas9 *Nature Biotechnology*, *33*, 390, https://doi.org/10.1038/nbt.3155.
79. Zuckermann, M., Hovestadt, V., Knobbe-Thomsen, C. B., Zapatka, M., Northcott, P. A., Schramm, K., Belic, J., Jones, D. T. W., Tschida, B., Moriarity, B., Largaespada, D., Roussel, M. F., Korshunov, A., Reifenberger, G., Pfister, S. M., Lichter, P., Kawauchi, D., & Gronych, J. (2015). Somatic CRISPR/Cas9-mediated tumour suppressor disruption enables versatile brain tumour modelling *Nature Communication*, *6*, 7391, https://doi.org/10.1038/ncomms8391.
80. Swiech, L., Heidenreich, M., Banerjee, A., Habib, N., Li, Y., Trombetta, J., Sur, M., & Zhang, F. (2015). In vivo interrogation of gene function in the mammalian brain using CRISPR-Cas9 *Nature Biotechnology*, *33*, 102, https://doi.org/10.1038/nbt.3055.
81. Chiou, S. - H., Winters, I. P., Wang, J., Naranjo, S., Dudgeon, C., Tamburini, F. B., Brady, J. J., Yang, D., Grüner, B. M., Chuang, C. - H., Caswell, D. R., Zeng, H., Chu, P., Kim, G. E., Carpizo, D. R., Kim, S. K., & Winslow, M. M. (2015). *Genes & development*, *29*, 1576, https://doi.org/10.1101/gad.264861.115.

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