CRISPR in therapeutics and diagnostics: Perspectives from landscape analysis

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Abstract

In this report, we examine the extensive research landscape of CRISPR with an emphasis on CRISPR therapeutics and showcase our results from an in-depth analysis of the most up-to-date scientific information consisting of more than 53,000 publications encompassing academic journal articles and patents, spanning nearly three decades, extracted from the CAS Content Collection. Our analysis indicates that cancer and infectious diseases are the most explored in the context of CRISPR. Identified gene targets associated with CRISPR-related publications are led by TP53, c-myc, and hemoglobin beta subunit (HBB). Among the many delivery methods, adeno-associated vectors (AAVs) appear to be highly explored. With >140 CRISPR-based therapeutics in the clinical development pipeline and billions of dollars in investment, the field of CRISPR continues to evolve rapidly. We also briefly discuss the ethical implications of CRISPR technology. While some fundamental challenges persist, the future of CRISPR is undoubtedly bright.

Keywords: CRISPR, landscape analysis, Casgevy, gene therapy, gene editing, CRISPR diagnostics

Introduction

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated proteins (Cas) have revolutionized the field of genetic engineering and therapeutic development.[1-4] Originally discovered as an adaptive immune mechanism in bacteria, CRISPR/Cas systems have been harnessed to enable precise and efficient genome editing in a variety of organisms.[5-7] This powerful technology offers unprecedented opportunities for advancing our understanding of genetic diseases, developing novel therapies, and potentially curing previously intractable conditions.

CRISPR/Cas systems were first identified in bacteria and archaea as a defense mechanism against viral infections.[8, 9] The system works by capturing snippets of DNA from invading viruses and storing them in the bacterial genome. When the same virus attacks again, the bacteria produce RNA segments from the CRISPR sequences to target the viral DNA, guided by the Cas proteins, which then cut the DNA, neutralizing the threat.

This natural mechanism has been adapted for use in gene editing.[1, 10] The most commonly used system, CRISPR/Cas9, involves a guide RNA (gRNA) that matches the target DNA sequence and the Cas9 enzyme, which acts as molecular scissors to cut the DNA at the desired location. This break in the DNA can then be repaired by the cell's natural repair mechanisms, allowing for the insertion, deletion, or modification of genes.[11, 12]

Since its adaptation for gene editing, CRISPR technology has rapidly advanced. Researchers have developed various modifications of the original CRISPR/Cas9 system to improve specificity, efficiency, and versatility. For example, CRISPR/Cas12 and CRISPR/Cas13 target different nucleic acids, expanding the range of possible applications.[13-15] Base editing techniques allow for precise conversion of single DNA bases without introducing double-strand breaks, reducing the risk of unwanted mutations.[16, 17] Prime editing represents a more recent advancement that combines aspects of CRISPR and reverse transcriptase to directly write new genetic information into a DNA site without causing double-strand breaks.[18, 19]

The potential therapeutic applications of CRISPR are vast and encompass a wide range of diseases. Monogenic disorders, i.e., diseases caused by mutations in a single gene, such as sickle cell anemia, cystic fibrosis, and Duchenne muscular dystrophy, are prime targets for CRISPR-based therapies. Early clinical trials have shown promise in correcting these genetic defects.[20, 21] CRISPR is being explored to enhance cancer immunotherapy by editing immune cells to better recognize and attack cancer cells. It is also being used to identify and validate new drug targets.[22-24] CRISPR has potential applications in combating viral infections, such as HIV, by targeting and disabling viral DNA within the host genome.[25-27]

The future of CRISPR therapeutics is bright, with ongoing research aimed at overcoming current limitations and expanding its applications. Innovations such as CRISPR-based diagnostics,[28, 29] CRISPRa/i (CRISPR activation/interference for gene regulation),[30, 31] and combination therapies hold promise for broadening the impact of this technology. CRISPR therapeutics represent a transformative advance in medical science, offering the potential to treat and even cure a wide array of diseases. As research progresses and challenges are addressed,

CRISPR-based therapies are poised to become a cornerstone of precision medicine, revolutionizing how we approach genetic disorders and complex diseases.

In this paper, we give an overview of the research progress in CRISPR therapeutics by analyzing data from the CAS Content Collection™.[32] The CAS Content Collection is the largest human-curated collection of published scientific information, supporting comprehensive quantitative analysis of global research across parameters including time, geography, scientific discipline, application, disease, chemical composition, etc. Covering scientific literature published around the world in more than 50 languages, the CAS Content Collection encompasses data and discoveries published in more than 50,000 scientific journals and by over 100 patent offices. A major advantage provided by the CAS Content Collection is that, along with the standard reference information, it also provides human curated data on major substances and concepts explored in scientific publications. The CAS REGISTRY,[33] the authoritative source for information on more than 250 million unique organic and inorganic substances and 70 million protein and nucleic acid sequences, is part of the CAS Content Collection. The CAS Content Collection is broadly accessible through CAS solutions including CAS SciFinder®[34] and CAS STNext®.[35]

Here we examine the publication landscape in the area in an effort to provide insights into current knowledge, advances and developments. We review the most discussed and emerging concepts and assess therapeutic strategies. Relying on the expertise and knowledge of our subject matter experts, we have analyzed the corpus of CRISPR-related publications to determine/highlight interesting trends in terms of protein targets often targeted using CRISPR, the co-occurrences between diseases and protein targets, prevalence of different CRISPR/Cas proteins, leading commercial and non-commercial entities engaged in research related to CRISPR. Finally, we inspect clinical applications of CRISPR therapeutics and diagnostics with details of their development. The objective of this review is to provide a broad overview of the evolving landscape of current knowledge regarding CRISPR application in therapeutics and diagnostics, to outline challenges that lie ahead, and evaluate growth opportunities to further efforts in this groundbreaking technology.

CRISPR/Cas: Biology and Mechanism

To fully understand CRISPR, it is essential to breakdown its components, and the mechanism of its natural function in prokaryotes to understand the way CRISPR can be exploited to achieve genome editing capabilities in humans and other organisms.

How CRISPR is employed by prokaryotic organisms as a natural defense

In 1987, the presence of CRISPR, in the *Escherichia coli* genome was reported by Nakata *et al.* from the Osaka University, Japan[36] while Cas was discovered in 2002 by Jansen *et al.*[37] Further discovery of in both Gram-positive and Gram-negative bacteria, along with archaea, led to the obvious questions regarding the relevance of CRISPR to these organisms.[38, 39] Later on, in the mid-2000s, the functionality and importance of CRISPR was first realized in prokaryotes wherein the CRISPR system is a key component of their adaptive immunity, which protects prokaryotes from attack by viruses, plasmids, and other invasive genetic elements.[40-42]

The CRISPR defense mechanism protects bacteria via three basic stages: spacer acquisition (adaptation), CRISPR RNA (crRNA) biogenesis (expression or maturation), and target interference[43-45] (Figure 1). CRISPR/Cas systems are composed of a *Cas* operon and a CRISPR array. The CRISPR array contains the unique sequences that are nestled in between the palindromic repeats (spacers) which are bits of foreign DNA, originated from mobile genetic elements such as bacteriophages, transposons, or plasmids that have previously infected the prokaryote. Cas gene (CRISPR-associated nuclease protein) is usually found adjacent to CRISPR which codes the nuclease protein (Cas protein) responsible for destroying or cleaving viral nucleic acid.[42, 46-48] In the first stage (spacer acquisition), upon phage infection, a sequence of the invading DNA (protospacer) is incorporated into the CRISPR array by the Cas1–Cas2 complex to form immune memory. In the second stage referred to as the CRISPR RNA (crRNA) biogenesis, when foreign nucleic acids invade again, the CRISPR array is transcribed to generate crRNA and a complementary trans-activating crRNA (tracrRNA), which form a double-stranded RNA structure. At the interference stage, crRNA-tracrRNA complex recruit Cas proteins to specifically destroy the invading nucleic acid.[49-52] Adjacent to the crRNA-targeted sequence on the invading DNA, a short sequence named as the protospacer adjacent motif (PAM) plays an essential role in the adaptation and interference stages, which the CRISPR/Cas complex recognizes target DNA binding. The absence of a PAM sequence can alter the affinity between Cas and the target DNA, since specific PAM sequence recognition serves to discriminate non-self-target sequences from non-target sequences.[49]

How this bacterial phenomenon was adapted to be used as a programmable toolkit for genome editing

In 2012, Jennifer Doudna and Emmanuelle Charpentier, a molecular biologist and microbiologist, respectively, were the first to propose that the bacterial CRISPR/Cas9 system could be used as a programmable toolkit for genome editing in humans and other animal species. For their path breaking work, they were eventually awarded the Nobel prize in chemistry in 2020.[50] Another breakthrough achieved the synthesis of single gRNA (sgRNA) which combines the role of crRNA and tracrRNA into a single molecule by fusing them together with a linker.[51, 52] The mechanism of CRISPR/Cas-9 genome editing contains three steps, recognition, cleavage, and repair[53] summarized in Figure 2. The designed sgRNA directs Cas9 and recognizes the target sequence in the gene of interest through its 5'crRNA complementary base pair component. The Cas9 protein remains inactive in the absence of sgRNA. The Cas9 nuclease makes double-stranded breaks (DSBs) at a site 3 base pair upstream to PAM.[54] A short 2–5 base-pair length sequence, PAMs are conserved DNA sequences downstream to the cleavage site and its size varies depending on the bacterial species. Once Cas9 has found a target site with the appropriate PAM, it triggers local DNA melting followed by the formation of RNA-DNA hybrid. Then, the Cas9 protein is activated for DNA cleavage. Finally, the DSB is repaired by either non-homologous end joining (NHEJ) or homology-directed repair (HDR) cellular mechanisms.[55, 56] The NHEJ pathway repairs DSBs in DNA by directly ligating through an enzymatic process without the need for an exogenous homologous DNA, which means a DNA strand with similar sequence that can act as a template. The NHEJ mechanism can also introduce insertion or deletion (indels) of specific sequences at the joining ends.[57] The other route of repair, the HDR pathway, is commonly found in bacterial and archaeal cells, while the NHEJ pathway is more common in a eukaryotic domain.

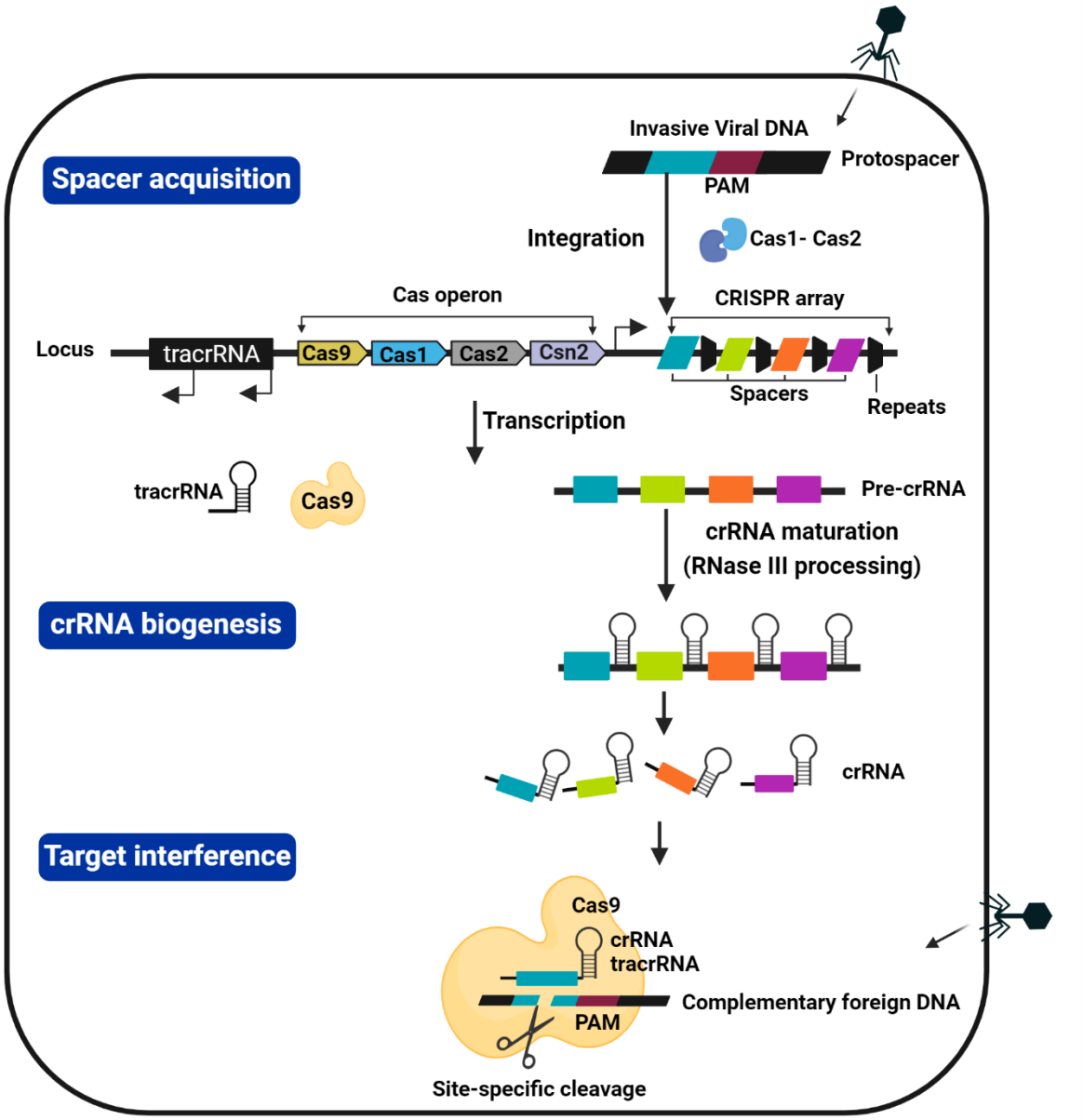


Figure 1. Mechanism of CRISPR/Cas9 adaptive immunity. The CRISPR/Cas systems are composed of a *Cas* operon and a CRISPR array that comprises identical repeat sequences that are interspersed by phage-derived spacers. The CRISPR/Cas mediated adaptive immunity consists of three stages, namely, spacer acquisition, crRNA biogenesis and target Interference. Abbreviations used: PAM – protospacer adjacent motif (PAM), crRNA – CRISPR RNA, tracrRNA – trans-activating crRNA. Figure was created using www.BioRender.com and adapted from Jiang and Doudna.[56]

The HDR process, although more complex than NHEJ, uses a homologous DNA template. The homologous DNA template has homology to the adjacent sequences surrounding the site of cleavage to incorporate new DNA fragments. The template guides the repair process and lowers the possibility of errors. Since there is no insertion or deletion of nucleotide sequences, the HDR

pathway maintains uniformity in the size of the resulting double-stranded DNA (dsDNA), unlike NHEJ.[58]

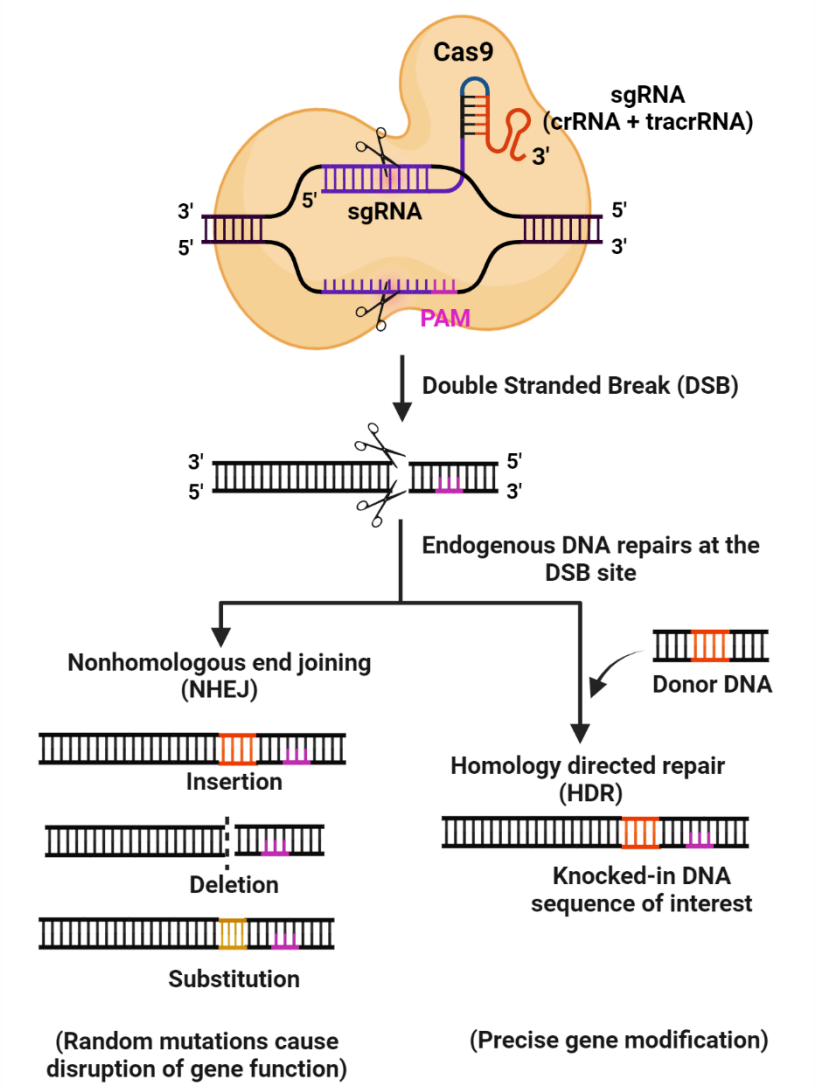


Figure 2. The mechanism of CRISPR–Cas9–mediated genome engineering. The synthetic sgRNA guides Cas9 to introduce a double-strand break (DSB) in targeted genomic DNA. The DSB generated by two distinct Cas9 nuclease domains is repaired by host-mediated DNA repair mechanisms. Figure was created using www.BioRender.com and adapted from Jiang and Doudna.[56]

Types of CRISPR/Cas systems

The bacterial and archaeal CRISPR/Cas systems of adaptive immunity show remarkable diversity of protein composition, effector complex structure, genome locus architecture, mechanisms of adaptation, pre-crRNA processing and interference. CRISPR/Cas systems have been divided largely into two classes, 6 types and 48 subtypes[59] (summarized in Figure 3 and Table 1). Class 1 systems include Types I, III, and IV, that use multiprotein complexes to destroy foreign nucleic acids, and class 2 systems include Types II, V, and VI, that use single proteins.[60]

In this section, we provide a brief overview of natural Cas nucleases and engineered variants that have been adopted for use as therapeutics (Figure 3 and Table 1).

Class 1 CRISPR/Cas systems

Class 1 systems require multiple Cas proteins to come together in a complex to mediate interference against foreign genetic elements making gene editing applications of class 1 systems limited. Class 1 systems are further divided into three CRISPR/Cas types based on the presence of a specific signature protein: Type I contains Cas3, Type III contains Cas10, and the Type IV contains Csf1, a Cas8-like protein.[61] Please see Table 1 for key differences and similarities between the different Class I CRISPR/Cas systems.

Type I is currently divided into seven subtypes, I-A to I-G; there are three distinct variants of the I-F subtype (I-F1, I-F2, and I-F3). A characteristic of Type I systems is CRISPR-associated complex for antiviral defense (Cascade), , which is a complex encompassing Cas molecules and crRNA.[62] The Cascade proteins of Type I systems typically encompass Cas5, Cas6, Cas7, and Cas8 (Cse1), with some subtypes also presenting Cas4 and/or Cas11(Cse2).[63] The pre-crRNA is processed by Cas6. In the interference stage of Type I systems, Cascade recognizes the PAM sequence, targets DNA in a sequence-specific manner through crRNA, and uses Cas3 to generates a single-strand nick on the unwinding target DNA thereby degrading the displaced strand through its 3′–5′ exonucleolytic activity.[64]

The overall composition and structure of Type III are highly similar to the Type I system.[65] It utilizes multi-subunit effector complexes to cleave both RNA and DNA and has been classified into six subtypes: III-A to III-F.[59] In the Type III system, the signature protein is multidomain Cas10 with an N-terminal histidine-aspartate (HD) nuclease domain (several subtypes) and two palm domains (a type of the RNA recognition motif). The HD domain takes part in nonspecific single-stranded DNA (ssDNA) cleavage activity, whereas the palm domain catalyzes the conversion of ATP to cyclic oligonucleotides (cOAs) when the type III crRNA-guided effector complex recognizes the target RNA. The cOAs activate the Csm6 protein, which nonspecifically degrades RNA molecules.[66, 67]

Type IV CRISPR/Cas system is divided into IV-A, IV-B, and IV-C subtypes, which mainly exist in plasmids however, their specific function is still poorly characterized.[59] Type IV lack certain hallmark components of other CRISPR–Cas systems, including the highly conserved adaptation module and an effector nuclease. All Type IV systems contain Cas7 protein (Csf2) and Cas5 protein (Csf3), which are part of the multi-subunit complex. Type IV-A CRISPR/Cas carry a DinG helicase instead of a nuclease component. Type IV CRISPR/Cas system may have the potential to be used in clinical drug-resistance gene therapy.[68, 69]

Class 2 CRISPR/Cas systems

Unlike Class 1 CRISPR/Cas system, the effector module of Class 2 CRISPR/Cas system is only a single protein with multiple domains and functions.[70] Class 2 CRISPR/Cas systems are further divided into following: Type II, Type V and Type VI. For details regarding differences and similarities between subtypes of Class 2 systems, please refer to Table 1.

Type II CRISPR/Cas system has been divided in II-A, II-B, and II-C subtypes. The II-C subtype has two variants (II-C1, II-C2).[59] The Type II system uses a single large multidomain Cas9 protein as the effector complex. Moreover, all Type II CRISPR/Cas loci contain *cas1* and *cas2* genes (essential for the CRISPR adaptation) and tracrRNA (noncoding RNA, required to mature the long pre-crRNA and for interactions with Cas9 protein). The crRNA–tracrRNA–Cas9 protein complex recognizes and cleaves the target DNA sequences. Cas9 nucleases recognize the PAM sequence (5'-NGG-3') and induce blunt-end DSBs at target sites.[71-73] Cas9 has two distinct nuclease domains, HNH and RuvC, which cleave the target and non-target strand, respectively[51, 70] (Figure 3B and Table 1). Among the different types of CRISPR/Cas systems, the Type II system is the most widely used in genetic engineering due to its simplicity, versatility, and efficiency.[74] The engineered CRISPR/Cas9 system encompasses two main components: a sgRNA and Cas9 protein. The sgRNA is a fusion of tracrRNA and crRNA, usually, 20 nucleotides long, complementary to the target sequence. The target DNA sequence should be adjacent to the PAM site in the genome. Inactivation of either nuclease domain of Cas9 creates a Cas9 nickase (nCas9), which cleaves only one DNA strand. Inactivation of both nuclease domains generates dead Cas9 (dCas9), which still binds to target DNA.[75] nCas9 is useful in base editors and prime editors, which perform precise genome editing without requiring DSBs,[76] and dCas9 serves as a scaffold for recruiting effectors proximal to specific genomic sites. dCas9 is widely used for regulating transcription, altering epigenetic controls, imaging living cells, and other purposes.[77, 78]

Type V CRISPR/Cas system can be divided into V-A to V-I, V-K, and V-U subtypes with diverse functions and its effectors are Cas12a (Cpf1), Cas12b (C2c1), Cas12c (C2c3), etc.[59] Type V CRISPR/Cas system requires Cas and crRNA to edit the target site. Once the crRNA recognizes the PAM site (5'-TTTN-3') and fully pairs with the target DNA base, Cas12a uses the RuvC domain to cut the target sequence in a cis manner to generate the 5' sticky end, and at the same time, uses the trans cutting activity to cut any adjacent ssDNA.[79] Cas12a shows RNase III activity, does not require a tracrRNA, and generates mature crRNA.[80] Like Cas12a, Cas14a belongs to the Class 2 system Type V family. Cas14a proteins have RNA-guided ssDNA-targeted endonuclease activity. Cas14a proteins are not required to recognize PAM sites in the DNA sequence.[81, 82]

Type VI CRISPR/Cas system can be divided into VI-A, VI-B, VI-C, and VI-D subtypes.[59] The signature protein is Cas13 (C2c2), which has higher eukaryotes and prokaryotes nucleotide-binding (HEPN) domains.[83] The uniqueness of this system is that Cas13 can recognize ssRNA molecular targets. Under the targeting effect of crRNA without the requirement of tracrRNA, the Cas13-crRNA complex recognizes the sequence of the protospacer flanking site (PFS) on the target nucleic acid, and at the same time cuts the target RNA, trans cuts the single-strand RNA (ssRNA).[84-86]

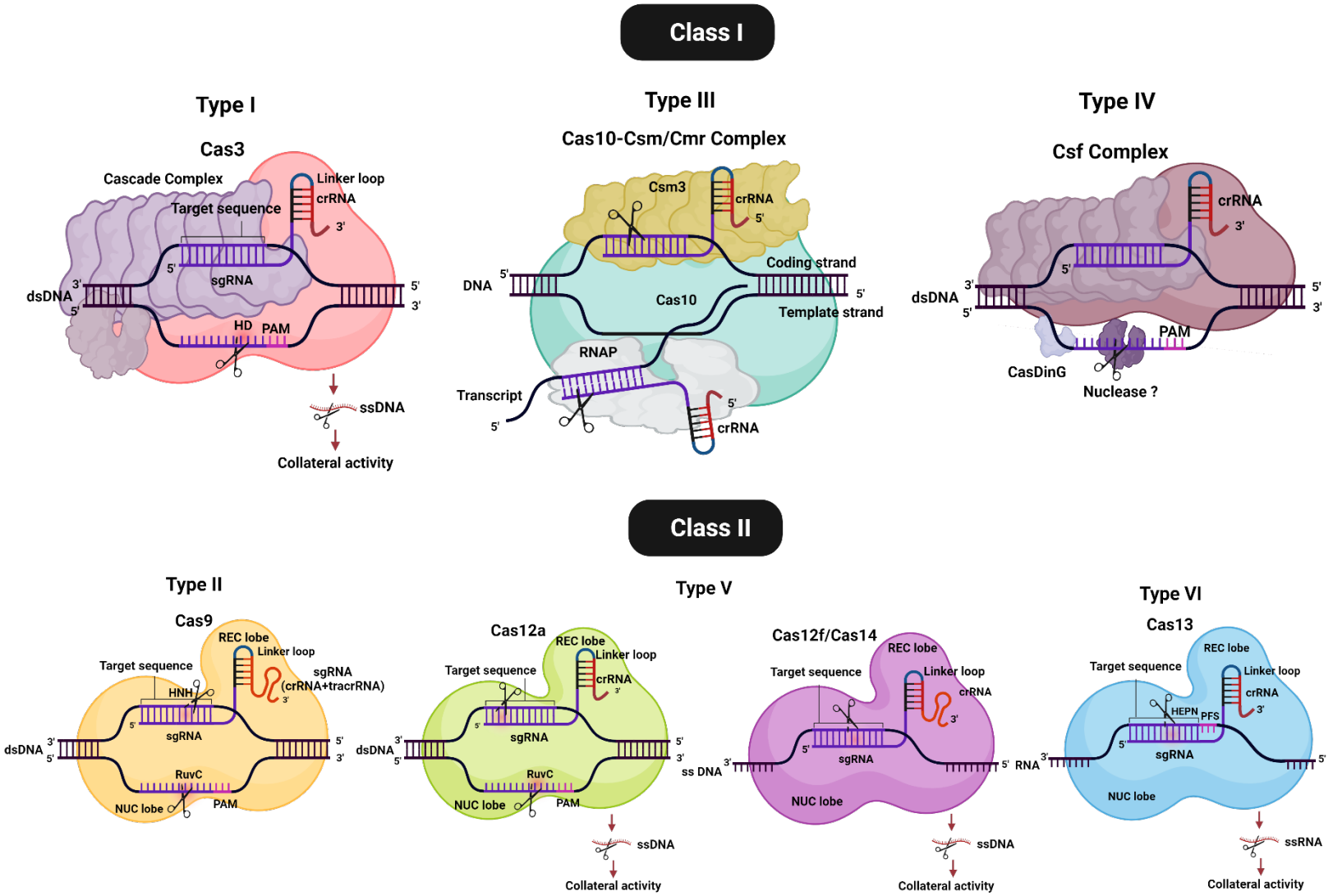


Figure 3. Schematics depicting various CRISPR/Cas system types and cleavage characteristics. The CRISPR/Cas system is classified into two classes based on the effector molecules involved. Class I is characterized by multi-unit effectors, while Class II exhibits a single effector. Each class is further divided into three types distinguished by their catalytic domain and target nucleotide specificity. The illustration was created using BioRender.com.

Table 1. Classification of CRISPR/Cas system with respect to various parameters including their class, types, subtypes, protein, nuclease domain, protospacer restriction, pre-crRNA processing, effectors, tracrRNA requirement, target and cleavage activity.

Class	Type	Sub-types	Signature Protein	Nuclease domain	Protospacer restrictions	Pre-crRNA Processing	Effectors of CRISPR system	tracrRNA	Target and Cleavage activity
I	I	A-G	Cas3	N-terminal histidine-aspartate (HD) domain	PAM-like NTT sequences	Cas6	Cas3, Cascade, crRNA	No	Nuclease activity to ssDNA
	III	A-F	Cas10	RRM	No PFS bias	Cas6	Cmr/Csm, crRNA, Cas10	No	Nuclease activity to ssDNA/RNA
	IV	A, B	Csf1	-	5′-GNAWN-3′ on the 5′-side of the target	Cas6	Cas7 (Csf2), Cas5 (Csf3) , Cas8 (Csf1), crRNA, CasDinG	No	Unknown
II	II	A-C	Cas9	HNH and RuvC domains	PAM, 3′ G-rich motif	RNase III, tracrRNA	Cas9, tracrRNA, crRNA	Yes	dsDNA blunt cleavage activity and no collateral cleavage activity
	V	A-I, K, U	Cas12	RuvC domain	PAM, 5′ T-rich motif	Cpf1	Cpf1, crRNA, tracr RNA	No	dsDNA overhang cleavage activity and ssDNA collateral cleavage activity
			Cas12f (also known as Cas14)	RuvC domain	PAM independent, Use T-rich sequences as PAMs	Yes	Cpf1, tracrRNA, crRNA	Yes	ssDNA and ssDNA collateral cleavage activity
	VI	A-D	Cas13	2 HEPN domains	PFS, 3’ Non-G PFS	No	C2c1, crRNA	No	ssRNA overhang cleavage activity and ssRNA collateral cleavage activity

General trends in CRISPR research: Insights from the CAS Content Collection

Querying the CAS Content Collection for publications related to CRISPR and it's role in therapeutic treatment, therapeutic development and therapeutic discovery (shortened to CRISPR therapeutics in this manuscript), while filtering out all agriculture related documents (see Methods section for query and details), resulted in over 39,000 academic journal articles and over 14,000 patents spanning from 1995 to June 2024. Publications on this topic sharply rose in 2008 and have steadily increased ever since with an average growth rate of 54% in the last decade (2014-2023) (Figure 4). This total rise in publications is primarily led by academic journal articles; however, patents showed a larger average yearly growth rate of 72% in the last decade when compared to journals (50%), demonstrating an increase in commercial interest.

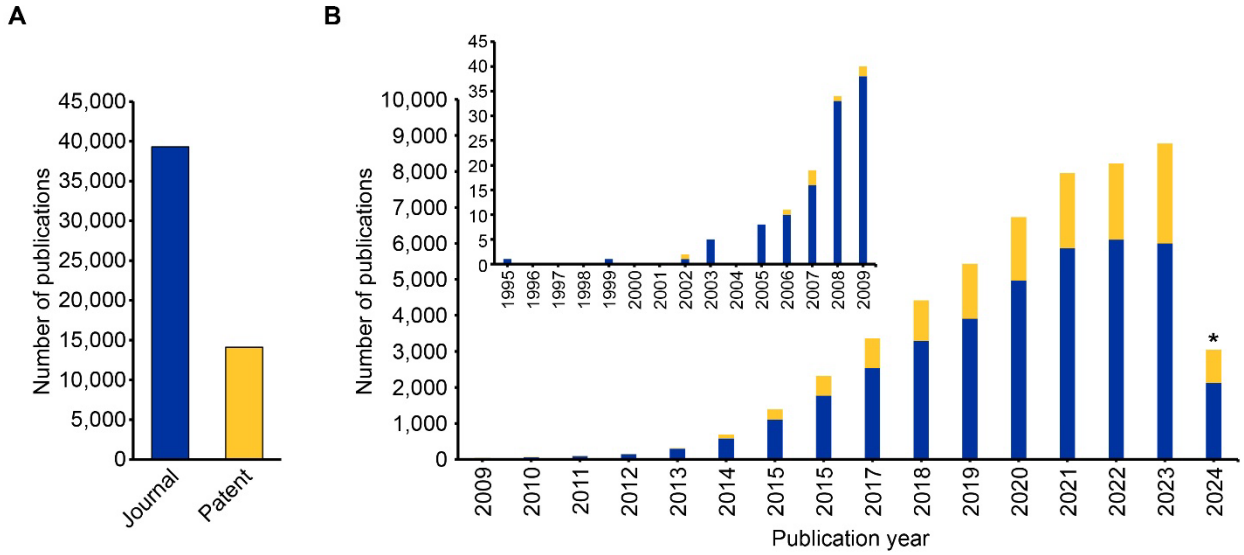


Figure 4. (**A**) Total number of journal and patent publications and (**B**) patent and journal publications through the years for the field of CRISPR including CRISPR therapeutics from 1995-2024. *Note that data for 2024 is incomplete due to time of data extraction and encompasses data for January to June.

We identified the top 100 journals containing the largest number CRISPR therapeutics documents between 1995 and 2024. We then filtered out of this set the journals with the highest average citation per publication to provide Figure 5. The journal Science, with 262 publications, has the highest average citation (253 citations/publication) out of the top 100 journals by total publication (Figure 5). Topics of recently published and highly cited articles from this journal explore: the use of CRISPR/Cas9 screens to identify genes that could protect against copper-induced cell killing;[87] the development of astrocyte-specific CRISPR/Cas9–based gene knockdown to reduce the expression of astrocyte morphology genes related to of Alzheimer's disease risk and other central nervous systems disorders;[88] and the combination of fluorescence image-enabled cell sorting with CRISPR-pooled screens to identify regulators of the nuclear factor κB (NF-κB) pathway, quickly completing genome-wide image-based screens (9 hours).[89]

Cell, the most known and oldest journal under Cell Press, comes in second place when it comes to citations with 220 citations/publications and 258 publications. Two recent publications in this journal with a high number of citations discuss the development and application of engineered DNA-free virus-like particles that efficiently package and deliver base editor or Cas9 ribonucleoproteins in vivo by overcoming cargo packaging, release, and localization bottlenecks;[90] and the use of genome-scale Perturb-seq targeting all expressed genes with CRISPRi across >2.5 million human cells for the generation of information-rich genotype-phenotype maps.[91]

Out of the top 15 journals shown in Figure 5, seven are owned by Springer Nature. The journals Nature Biotechnology, with a total of 353 publications, and Nature, with a total of 486 publications, come in third and fourth place with 161 citations/publication and 124 citations/publication respectively. In addition, our data also shows that Nature Communications is the journal with the most publications on the topic of CRISPR therapeutics with 1220 publications (Figure S1). Examples from Nature Biotechnology discuss things like: the design of an optimized Un1Cas12f1 and its application as a miniature CRISPR system that fits into the adeno-associated virus,[92] new technologies to address challenges and allow biologically targeted mRNA therapeutics,[93] and a prime editing-based method that achieves higher precision than CRISPR–Cas9 and sgRNA pairs in programming genomic deletions.[94] Some examples of recent highly cited publications from the journal Nature use CRISPR to conduct a genome-wide CRISPR knockout screen in glioblastoma to systematically identify potential resistance pathways to CAR T cell cytotoxicity in solid tumors,[95] use CRISPR-mediated targeting to identify mediators of Hopx induction (a transcriptional regulator) by β-hydroxybutyrate (BHB) and identify a BHB-triggered pathway regulating intestinal tumorigenesis,[96] and provide molecular insight into the underlying structural mechanisms that cause off-target effects of Cas9 and a proof of concept for the design of Cas9 variants that reduce off-target DNA cleavage while retaining efficient cleavage of on-target DNA.[97]

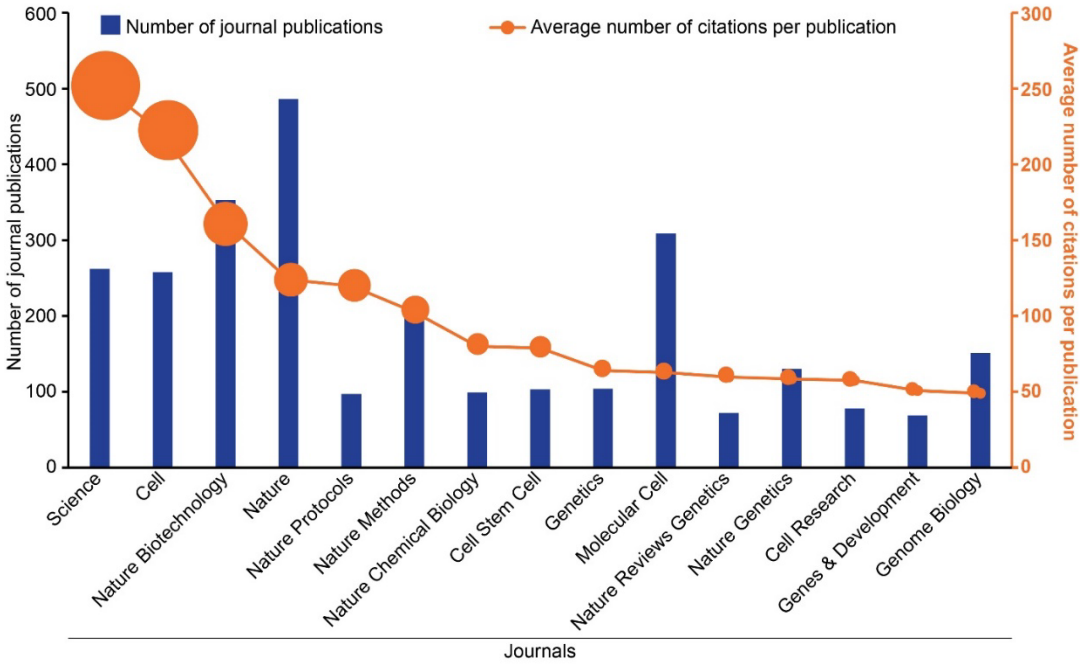


Figure 5. Leading scientific journals in the field of CRISPR based on journal publication and citation data from the CAS Content Collection for the period 1995-2024. Note that data for 2024 is incomplete due to time of data extraction and encompasses data for January to June.

We then took a look at which organizations are leading academic research in the field of CRISPR Therapeutics. If only taking into consideration number of publications (Figure S2), the University of California, the Chinese Academy of Sciences, and Harvard University take the lead. Combination of research output (number of journal publications) and its impact (average citation per publication) reveals a different list (Figure 6) with Massachusetts General Hospital, Massachusetts Institute of Technology (MIT) and Harvard University as the leaders. Analyzing the geographical distribution of these leading organizations indicate that a majority of them originate in the United States (Figure 6). To gain further insight into what these 3 organizations are researching we looked at some examples of recent publications.

Taking a look into recent publications from the Massachusetts General Hospital we can see the use of CRISPR: as a screening strategy to connect genes to detailed bioenergetic phenotypes in mitochondrias;[98] to elucidate how Galectin 3 (Gal3) contributes to uterine serous carcinoma by using CRISPR/Cas9-mediated Gal3-knockout (KO) alongside a Gal3 inhibitor to evaluate Gal3's impact on cell function;[99] and to target PMS1 to reduce somatic expansion of the Huntington's disease-associated CAG repeat.[100] Examples of recent publications by the MIT discuss using Cas9-assisted biological containment of a genetically engineered human commensal bacterium that could be used as a way to bring genetically modified microorganisms into biomedicine in a safe manner,[101] and to examine effects of several simultaneous gene expression perturbations on growth using an *Escherichia coli* model.[102] Recent publications from Harvard University that utilize CRISPR technology: for germline mutagenesis to achieve genetic sterilization of male *Anopheles gambiae,* species of malaria carrying mosquitoes;[103] to reveal a druggable pocket in STT3A, a subunit of oligosaccharyltransferase complex OST-A, whose inhibition blocks lipopolysaccharides signaling to NF-κB;[104] to investigate the role of the progesterone receptor membrane component 1 (PGRMC1) in progesterone signaling at the maternal-fetal interface by knocking out PGRMC1 in JEG3 cells;[105] and the use of CRISPR-corrected isogenic controls in research on human induced pluripotent stem cell lines.[106]

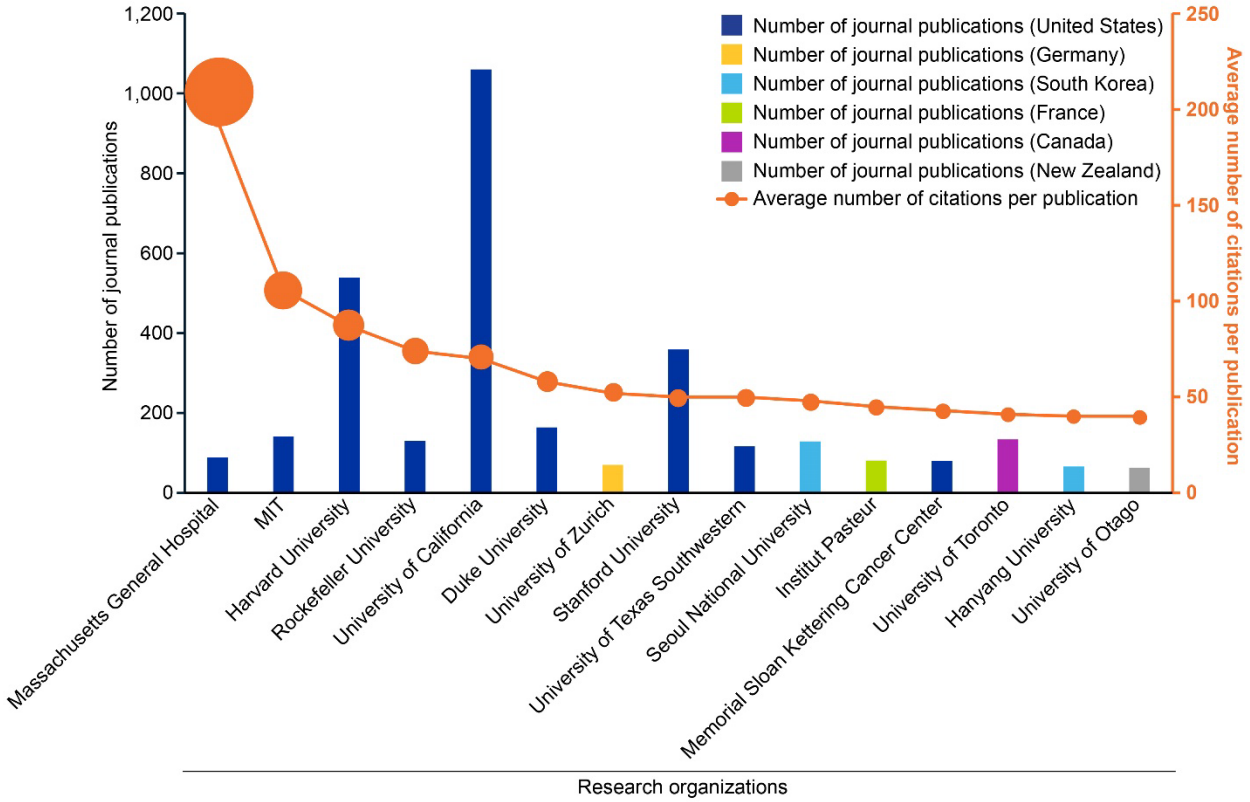


Figure 6. Leading research organizations in the field of CRISPR based on journal publication and citation data from the CAS Content Collection for the period 1995-2024. Note that data for 2024 is incomplete due to time of data extraction and encompasses data for January to June.

A look at patents in the field of CRISPR therapeutics, both submitted and approved patents, divided by commercial institution or a public institution are shown in Figures 7 and 8, respectively. When it comes to commercial assignees, Regeneron Pharmaceuticals in the U.S., CRISPR Therapeutics from Switzerland, and Shandong Shunfeng Biotechnology in China emerge as leaders among other key players. Overall, we observe that a majority (10 out of 15) commercial assignees among the top 15 are located in the US. Unlike commercial patents, Chinese and American research institutions have a closer ratio (9:6 respectively) of dominance.

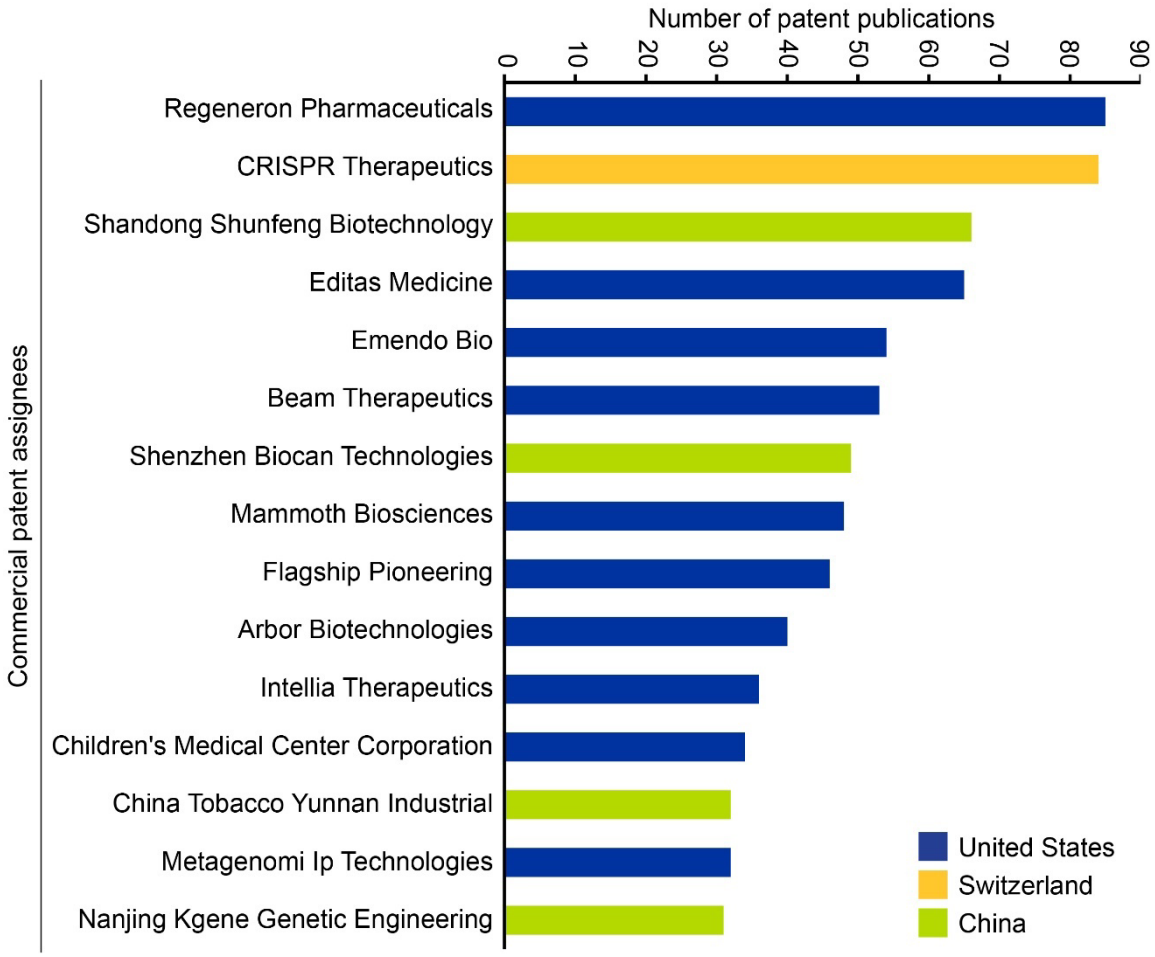


Figure 7. Leading commercial patent assignees in the field of CRISPR in terms of number of patent publications between 1995-2024 based on data from the CAS Content Collection. Note that data for 2024 is incomplete due to time of data extraction and encompasses data for January to June.

A more detailed look into the top 3 commercial assignees and their recent submitted patents was merited. Regeneron Pharmaceuticals, an American biotechnology company, has recently published various patents on the use of CRISPR for: the identification and treatment of liver disease,[107-110] as therapeutics for c9orf72 repeat expansion disease,[111, 112] and for the treatment of ophthalmic diseases[113, 114] and metabolic disorders.[115, 116] Some other examples of recent patents include a CRISPR SAM biosensor cell line and their methods of use,[117] and CRISPR/Cas methods and compositions for knocking out a C5 locus or gene.[118] The Swiss–American biotechnology company, CRISPR Therapeutics, is known for its collaboration with Vertex Pharmaceuticals in creating the first-ever approved CRISPR/Cas9 gene-edited therapy known as CASGEVY.[119] CASGEVY, also known as exagamglogene autotemcel, is a one-time therapy for sickle cell disease and β-thalassemia.[120] Recent patents by CRISPR Therapeutics include the use of CRISPR for producing: CAR-T cells,[121-124] genetically engineered immune cells,[125-130] methods for differentiating stem cells into NK cells,[131, 132] and for in vivo editing of stem cells.[133] Finally, Shandong Shunfeng Biotechnology, recently known for the development of the first gene-edited crop (soybean) approved by China also ranks high among commercial patent assignees/entities.[134] Some of their recent patents demonstrate various novel CRISPR/Cas

systems and enzymes for targeting, editing, detecting mutations in, and cleaving nucleic acids.[135-140] They have also recently published patents on efficient methods for detection of viruses[141, 142] based on CRISPR, including foot and mouth disease[143, 144] and African swine fever.[145]

A deeper look into recent patent publications from the leading non-commercial assignees (Figure 8) reveal the following:

- The Chinese Academy of Sciences, a group of 124 individual research institutions,[146] is a distinct leader with respect to the number of published patents in the field of CRISPR. A portion of their recent publications appear to be focused on: use of CRISPR/Cas13 systems for targeting and treating diseases, such as SOD1,[147, 148] UBE3a,[149] DMD,[150] and MECP2[151] associated diseases; nucleic acid detection based on CRISPR/Cas13a.[152-155]
- The Broad Institute of MIT and Harvard, a biomedical and genomic research organization in Massachusetts, has recently patented CRISPR-associated transposase systems,[156-159] CRISPR/Cas systems for gene editing mitochondria,[160, 161] and preparation of CRISPR/Cas systems comprising of adenine base editors,[162] small novel Type V Cas polypeptides,[163] and novel Cas5- HNH and Cas8-HNH polypeptides.[164]
- The University of California, who as previously mentioned has the highest amount of journal publications, comes in third place when it comes to patents from non-commercial institutions. Some examples of recent patents discuss: CRISPR/Cas effector proteins[165, 166] and polypeptides[167, 168] for gene editing, the use of CRISPR/Cas systems for modifying eukaryotic cells[169] and oocytes,[170] and CRISPR/Cas-mediated RNA targeting for treating Huntington's disease[171]

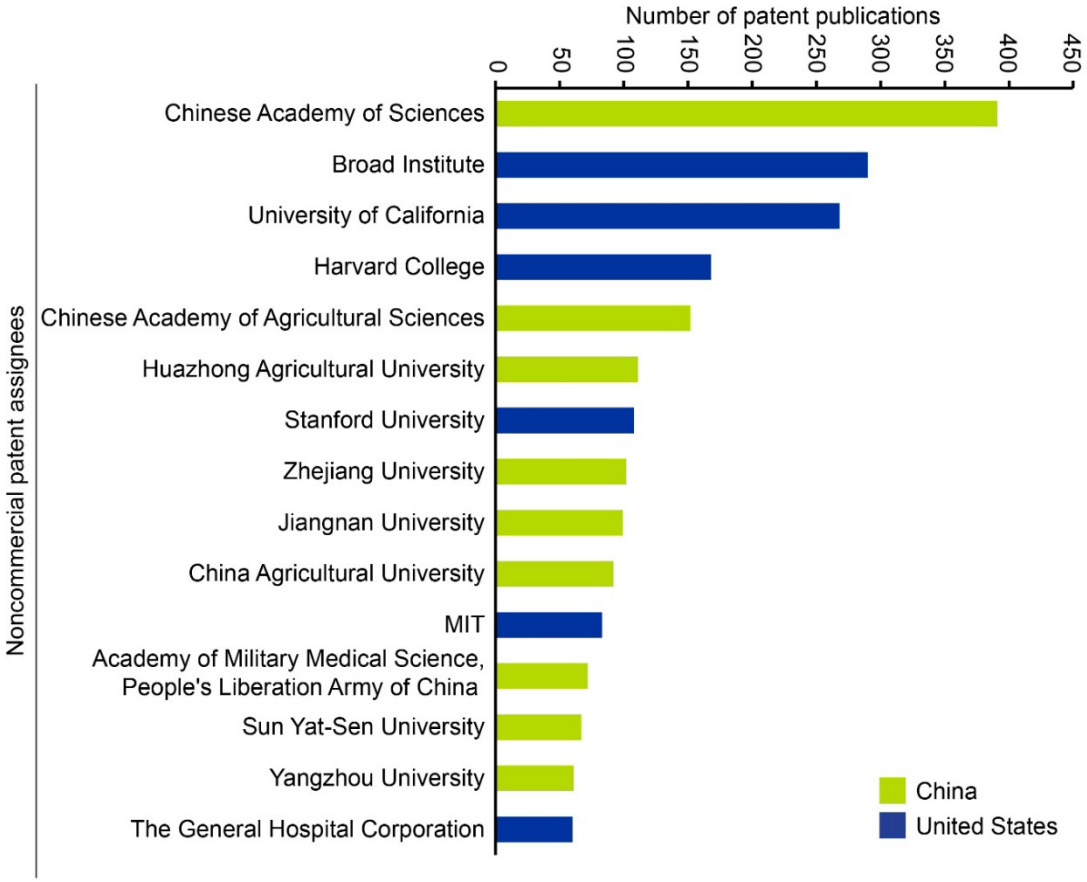


Figure 8. Leading non-commercial patent assignees in the field of CRISPR in terms of number of patent publications between 1995-2024 based on data from the CAS Content Collection. Note that data for 2024 is incomplete due to time of data extraction and encompasses data for January to June.

We next took a look at patent activity data in the field of CRISPR therapeutics; an activity is defined as an event that leads to the publication of a patent document, for example application publications, issuing of patents, and among other events. An analysis of patent family activity data in Figure 9 shows the flow from the patent assignee country (left) to the patent office where the first application in a given family is filed (center) and finally to the destination patent office for individual patent publications within the family. The United States leads in terms of sheer volume of patent publications, most of which are sent to the World Intellectual Patent Office (WIPO). Still, a decent sized portion of US patents go straight to the home office. For China, the second country/region leading in terms of sheer volume of patent publications, an overwhelming majority of patent applications appear to have been filed and granted at their home office. Most of the patents initially submitted to WIPO are subsequently submitted to US, the European patent Office, Canada, Japan, South Korea and China, with a smaller number being filed at patent offices in India, Brazil, Australia, and other smaller patent offices around the world.

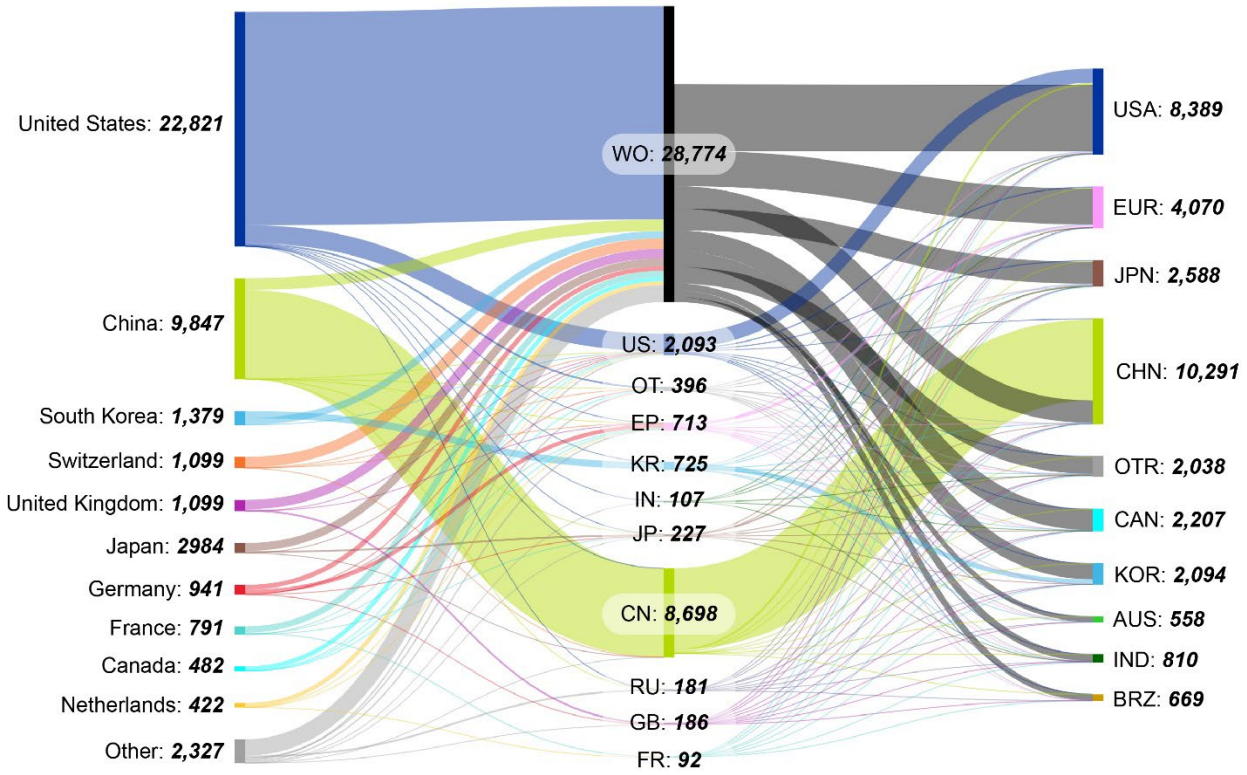


Figure 9. Patent family activity data related to patents in the field of CRISPR for the period 1995-2024 based on data from the CAS Content Collection. Note that data for 2024 is incomplete due to time of data extraction and encompasses data for January to June.

CRISPR Therapeutics

The concept of gene therapy was introduced by Friedmann and Roblin back in 1972.[172] ZFN (zinc finger nucleases) and TALEN (transcription activator-like effector nucleases) were then developed as mainstream tools to evaluate the possibility of targeting or editing genes to cure diseases. . Both these methods require complex design strategies and can tolerate only a small number of positional mismatches making development of successful and effective gene therapy challenging. With ZFN, it is difficult to target non-G-rich sites and for each TALEN monomer, 5' targeted base must be a T.[173-176] Later, CRISPR/Cas emerged as a new tool to edit genes and since its discovery, it has been explored tremendously by researchers as a potential therapeutic approach for disorders which were previously thought to be incurable or difficult to cure. These include certain types of cancers, infectious diseases, and various genetic disorders, among others. CRISPR/CAS is beneficial over earlier conventional gene therapy methods such as ZFN and TALENs as it is easy to engineer and can tolerate positional/multiple consecutive mismatches.[177]

CRISPR/Cas technology has various key applications in the field of therapeutics, the most apparent of which would be to correct or replace the mutated or disease-causing gene(s). CRISPR/Cas based gene therapy can be delivered in two modes – *in vivo* and *ex vivo*. In *in vivo* approach, any viral or non-viral vector with the packaged CRISPR/Cas system is injected directly into the patient body. Whereas, in *ex vivo* approach, first the cells are extracted from patient body, they are grown in the laboratory set-up where gene editing process is carried out and eventually the genetically altered cells are injected back into the patient body.[178]

Apart from this, it is often used in functional genomics field to identify gene targets associated with certain diseases. Researchers can create gRNA libraries that target different genes in cell lines or animals and can further note the disruptions leading to phenotypic changes. This allows identification of candidate target genes involved in disease mechanism as well as potential therapeutic targets. CRISPR also enables high-throughput screening of genes in a fast and efficient manner. It is possible to establish experiments using pooled CRISPR libraries to screen thousands of genes simultaneously to discover gene functions and understand their effects on various biological and pathological processes. Such high-throughput libraries are being constructed and explored particularly in cancers paving the way of using CRISPR in personalized medicine.[179] Furthermore, CRISPR can also be used to create animal models for many diseases helping researchers understand the molecular mechanisms of those diseases and eventually serving as an excellent tool during early-stage drug discovery by enabling identification of therapeutic targets.[180]

As of today, numerous CRISPR-based therapeutics are in the pre-clinical stage of development, and many are undergoing clinical trials to validate their safety and efficacy for diverse disease conditions, as discussed further in this article (*CRISPR Therapeutics: Candidates in the Developmental Pipeline)*). In December 2023, the first CRISPR/Cas9 based gene editing therapy got approval by the US Food and Drug Administration (FDA) for the treatment of patients with transfusion-dependent β-thalassemia. The same therapy was approved in Europe in November 2023 for sickle cell disease and transfusion-dependent β-thalassemia.[181-183]

To gain insight and to understand the current trend in CRISPR therapeutics research, we explored the data from CAS Content Collection™, and performed a quantitative analysis. Highlighted in Figure 10 are potential gene targets with the highest publication frequency in the CRISPR dataset (journals and patents from 1995 to 2024). TP53, c-myc and hemoglobin beta subunit (HBB) genes were the top 3 occurring genes identified. As per Figure 11, the publication trend for TP53 has particularly shown rapid increase over the past few years.

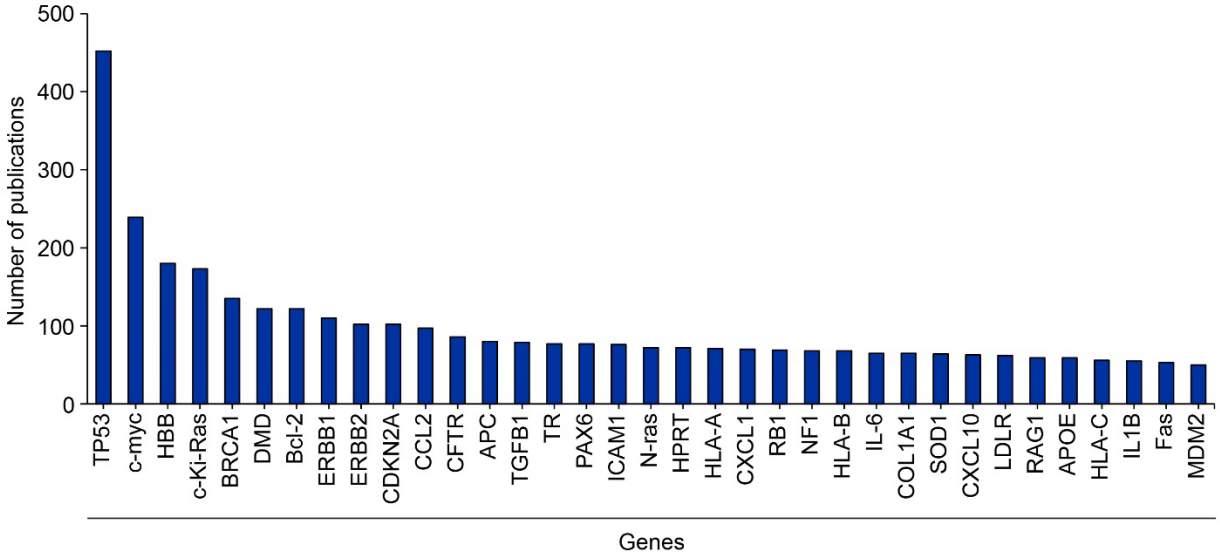


Figure 10. Publication frequencies of potential gene targets occurring in the CRISPR dataset retrieved from the CAS Content Collection. Data includes patent and journal publications for the period 1995-2024 and is based on CAS indexing. Note that data for 2024 is incomplete due to time of data extraction and encompasses data for January to June.

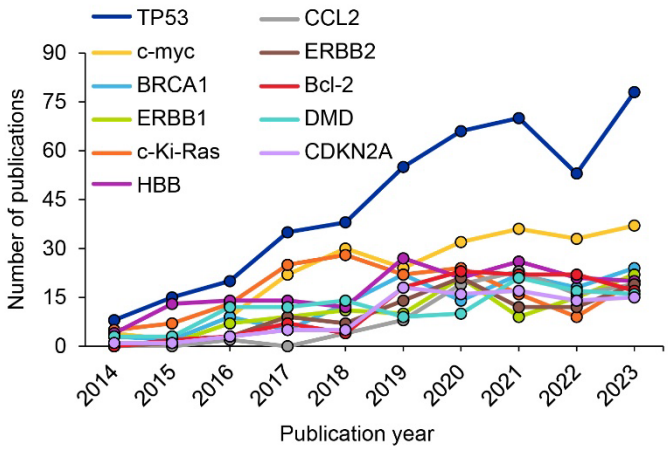


Figure 11. Time trends of some of the most highly occurring potential gene targets in the CRISPR dataset retrieved from the CAS Content Collection. Data includes patent and journal publications for the period 2014-2023 and is based on CAS indexing.

As shown in Figure 12, a majority of publications appear to be focused on cancer (35% of all journal articles and 24% of all patents), followed by infectious diseases (25% and 22% of journal articles and patents, respectively). Year-wise publication trends also shows remarkable and consistent increase in number of CRISPR articles focused on these two diseases after 2016. Other broader categories of disease conditions observed in the dataset were blood disorders, genetic disorders, nervous system disorders, cardiovascular diseases, respiratory diseases, immune diseases and metabolic disorders. In the following section we have discussed briefly how CRISPR/Cas technology is being utilized in the therapy targeted for these diseases with an emphasis on cancer, infectious diseases, blood disorders, genetic disorders (common as well as rare) and nervous system disorders.

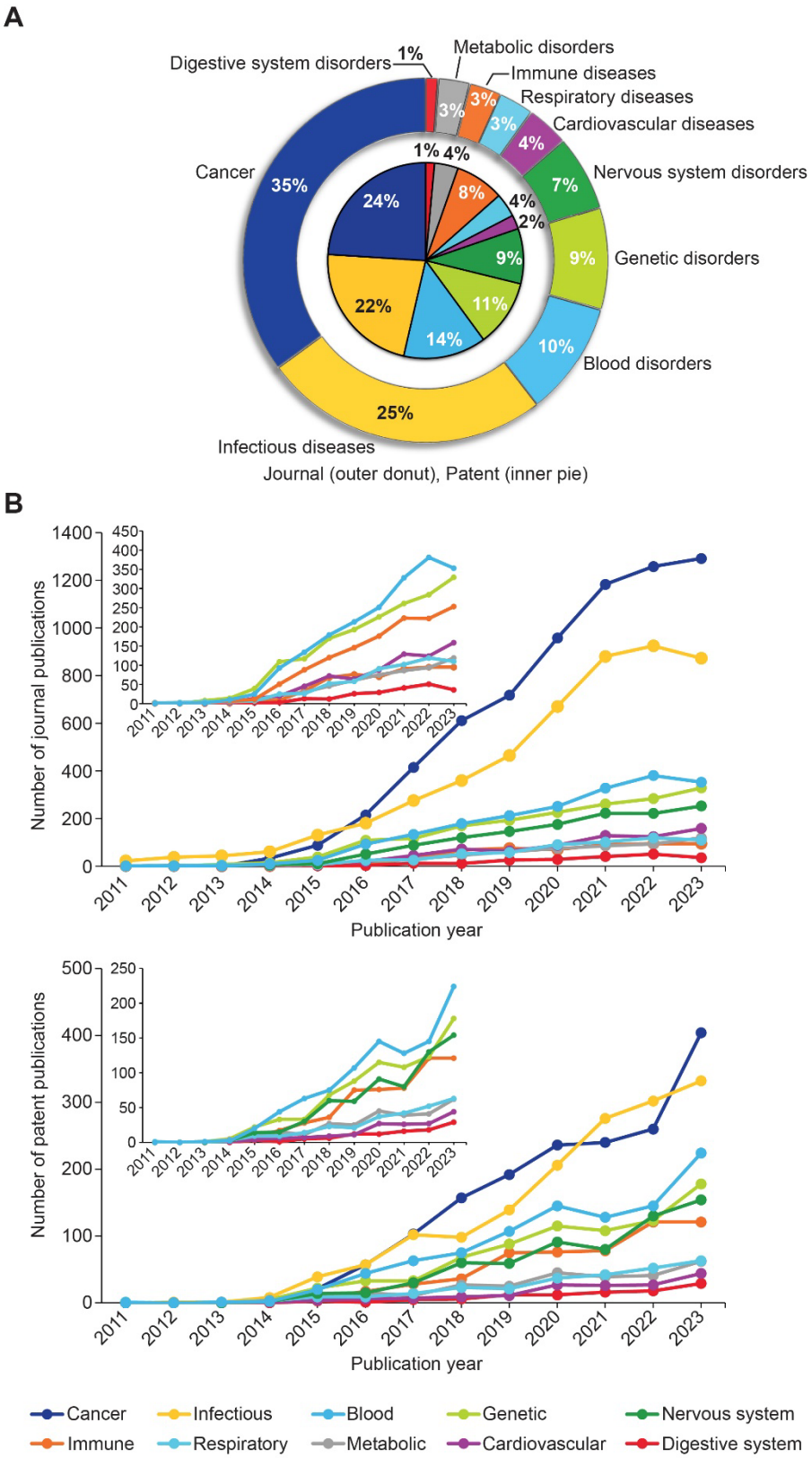


Figure 12. (**A**) Distribution and (**B**) time trends for CRISPR documents co-occurring with various disease conditions. Data includes journal and patent publications from the CAS Content Collection for the period 2011-2023.

I. Cancer

Cancer is a multifaceted disease involving changes at the genomic, cellular, and eventually at the organismic level. Fundamentally, cancer originates in the genome, by mutations that either activate oncogenes or inactivate tumor suppressors. Dysregulation of the epigenome is another feasible way by which cells can become cancerous due to altered expression of certain genes involved in DNA damage pathway or cell cycle pathway. At cellular level, cancer results in altered metabolism, altered cell structure and migration which enables growth of cancer cells in unfavorable environments. Eventually, in the affected organism, cancer cells circumvent the immune defense mechanism of the host and co-exist with normal cells. Understanding of all these complex genomic, cellular, and tissue level changes is crucial for the development of more effective treatment options and improving outcomes in cancer patients. CRISPR/Cas technology has had a significant impact on our understanding of cancer biology and is continuously driving new discoveries in the field.[184]

Supplementary Figure S3 shows the publication trend of CRISPR-related publications – journals and patents for different cancers subtypes (both solid cancer and hematological malignancies). Increase in journal publications was most prevalent/evident for breast cancer, acute myeloid leukemia (AML), liver, lung and rectal cancer. In line with the journal publications, patent publication trends show breast cancer, AML, lung and liver cancer related patents to be growing rapidly indicating potentially more commercialization efforts for these cancer types. Other cancer types with rapid increase in CRISPR patents include melanoma. Multiple gene candidates are being studied in cancers in context of CRISPR. Figure 13 below shows co-occurrences between cancer types and genes found in the CRISPR dataset retrieved from CAS Content Collection. A few key observations from this co-occurrence analysis are as follows:

- Cancers such as breast, lung, rectal, prostate and liver appear to co-occur more frequently with certain genetic targets than others.
- Overall, TP53 emerges as a highly explored target appearing to co-occur with each of the 7 cancer subtypes.
- Out of the more than 25 targets co-occurring frequently, ~10 of them co-occur with more than one cancer type.
- Besides TP53, other highly explored genes include c-Ki-Ras, c-myc, ERBB1 and BRCA1.
- In terms of diversity of genes co-occurring, breast, lung, rectal and prostate cancer lead the way.

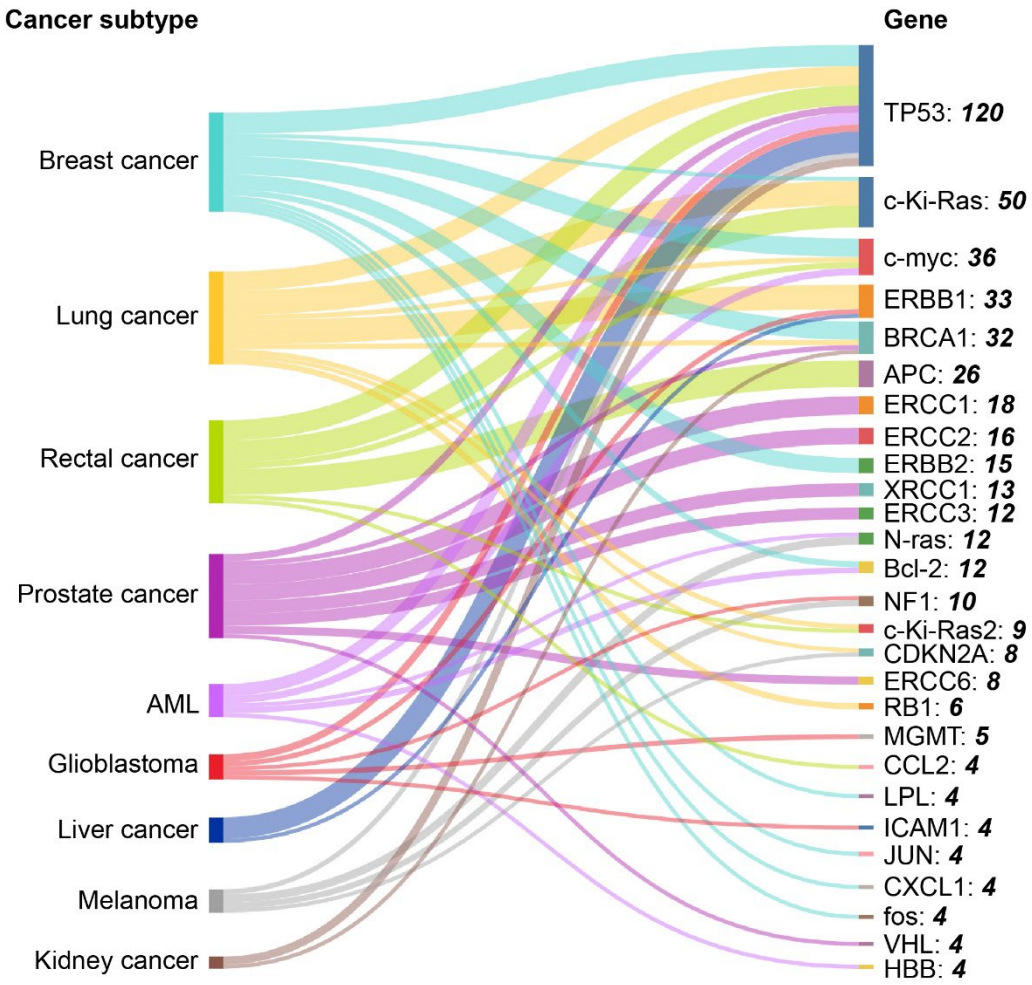


Figure 13. Co-occurrence of cancer subtypes with genes in the CRISPR dataset retrieved from the CAS Content Collection. Data includes patent and journal publications for the period 1995-2024. Note that data for 2024 is incomplete due to time of data extraction and encompasses data for January to June.

There are several approaches of using CRISPR/Cas technology in cancer therapy as discussed below:

Correcting driver mutations in oncogenes or tumor suppressor genes:

Oncogenes and tumor suppressor genes play a critical role in the process of tumorigenesis. There are known driver mutations which either activate the oncogenes or suppress the tumor suppressor genes, and both these phenomena disrupt the normal growth signaling pathways in cells, making them grow uncontrollably. Several studies have shown that by using CRISPR, it is possible to edit these mutations and revert the cancerous phenotype *in vitro* as well as *in vivo*.

Kim *et al.*[185] used CRISPR/Cas9 mediated gene editing to target mutations in c-Ki-Ras (KRAS) oncogene (KRAS G12C, G12D and G12V) in pancreatic cancer cells in mice and found

that it inhibited cancer cell proliferation without affecting wild type cells. In other studies, CRISPR/Cas9 was used to knock out another mutant oncogene, epidermal growth factor receptor (EGFR), resulting in the inhibition of proliferation of lung adenocarcinoma cell lines and considerable decline in tumor size and weight in xenograft mouse models.[186, 187]

TP53 gene codes for a transcription factor and a well-known tumor suppressor that regulates multitude intracellular pathways involved in DNA damage repair, cell cycle arrest, apoptosis, and senescence.[188, 189] Mutations in TP53 leading to its inactivation are involved in tumorigenesis and are found to be prevalent in more than 50% of human primary tumors.[190] Majority of TP53 mutations are missense mutations (around 80%) occurring due to G to A transitions, followed by C to T transitions. These are clustered in the central DNA-binding region consisting of exons 3-5. Other known TP53 mutations are truncating mutations, in-frame mutations, and slice site alterations. Since majority of mutations are missense, it opens great opportunities for the CRISPR/Cas9 system to correct single nucleotides.[191, 192]

In prostate cancer cell lines, the TP53 414delC mutation was corrected to the wild-type TP53 genotype by using the CRISPR/Cas9 system, thereby promoting apoptosis and preventing tumor proliferation.[192]

Zhan *et al*.[193] have designed and constructed a genetic sensor that specifically detects wt-p53 expression in cells. Furthermore, by combining the p53 sensor with diphtheria toxin using the CRISPR/Cas9 system they were able to specifically kill p53-deficient tumor cells.

Chira *et al*.[194] proposed a novel and highly tumor specific TP53 delivery system based on CRISPR/Cas9 genome editing technology which can be used to replace the mutant TP53 in the tumor genome with a functional copy by homologous recombination, leading to sustained expression of p53 protein and tumor regression.

Modifying or silencing epigenetic markers:

The epigenome is a complex framework through which precise gene expression takes place. Epigenome is one of the key regulators of cell fate, certain diseases, and aging. Editing the epigenome is a promising therapeutic approach in cancer.[77] For epigenome editing, a 'dead' Cas9 protein (dCas9) is used that lacks nuclease activity, and it is places alongside an epigenetic effector domain. Based on fusion partners of dCas9, an exact epigenetic status can be achieved.[195]

Granulin (GRN), is upregulated in hepatoma tissues and is associated with decreased tumor survival in patients with hepatoma. It is a growth factor and a potent pluripotent mitogen that promotes cancer progression by maintaining self-renewal of hepatic stem cancer cells. Wang *et al.* synthesized a set of dCas9 epi-suppressors to target GRN by tethering the C terminus of dCas9 with three epigenetic suppressor genes: DNMT3a (DNA methyltransferase), EZH2 (histone 3 lysine 27 methyltransferase), and KRAB (the Krüppel-associated box transcriptional repression domain). The epigenetic knockdown of GRN (by altering promoter methylation status) led to the inhibition of cell proliferation, decreased tumor sphere formation, and reduced cell invasion.[196]

The mutated transcription factor FOXA1 acts as an oncogene and is responsible for the onset and progression of prostate cancer. Zhou *et al*.[197] identified a group of six cis-regulatory elements in the FOXA1 regulatory plexus containing somatic single-nucleotide variants in primary prostate tumors. Deletion and repression of these cis-regulatory elements with the help of CRISPR/Cas technology, significantly decreases FOXA1 expression and prostate cancer cell growth.

Furthermore, CRISPR/Cas9-based epigenome editing was shown to successfully repress interleukin receptors (IL1R1) and tumor necrosis factor α receptor (TNFR1) in human adipose-derived stem cells and ovarian cancer cells, respectively.[198, 199] This approach may be used to control various kinds of inflammations that accelerate the growth of diverse types of cancers.

Assisting in cancer immunotherapy:

Cancer immunotherapy, or immuno-oncology, is an approach to treat cancer by stimulating the body's immune system to combat cancer cells. The major categories of immunotherapy include cytokine therapies, cancer vaccines, oncolytic virus therapies, immune checkpoint inhibitors, and adoptive cell transfer – which includes chimeric antigen receptor-T (CAR-T) cell therapy and natural killer (NK) cell therapy.[200] One of the most promising applications of CRISPR/Cas-9 mediated genome editing is the generation of CAR-T cells. To create CAR-T cells, autologous T cells are collected and genetically engineered to attack cancer antigens *ex vivo.* These cells are then transferred back into the patient. Zych et al. reported that CRISPR/Cas9 system could be able to improve CAR-T cell function via interrupting the genes that code T cell inhibitory receptors or signaling molecules.[201]

CRISPR/Cas9 can also be used to create allogenic CAR-T cells, which can overcome the major limitation of autologous CAR-T cells, which is mismatch of HLA typing.[202] Various studies have attempted to create allogenic CAR-T cells by knocking out genes like beta-2 microglobulin (B2M), T cell receptor α subunit constant (TRAC), and programmed-death 1 (PD-1).[203, 204] Using such approach, it will be possible to create universal CAR-T cells derived from healthy donors and use for multiple patients. This will also reduce the overall cost and time required to generate CAR-T based cell therapies.

Table 2 elaborates various applications of using CRISPR/Cas9 system in CAR-T cell therapy.

Table 2. Applications of CRISPR/Cas9 in CAR-T cell therapy

Approach to modify CAR-T cells	Modifications done in CAR-T cells	Major outcome of the study	Reference
Immune checkpoint blockade	Knock-out programmed death-ligand 1 (PD-L1) in primary T cells	Enhanced CAR-T cytotoxicity	Su *et al.*[205]
	Knock-out cyclin-dependent kinase 5 (CDK-5) in CAR-T cells	Reduced expression of PD-L1, and enhanced CAR-T cytotoxicity	Tu *et al.*[206]

Approach to modify CAR-T cells	Modifications done in CAR-T cells	Major outcome of the study	Reference
	Lymphocyte activation gene-3 (LAG-3) knock-out in CAR-T cells	Strengthened T cell response and increased cytokine production	Zhang *et al.*[207]
	Diacylglycerol kinase (DGK) knock-out in CAR-T cells	Stimulated CD3 signaling and increased resistance to the immunosuppressive factors TGF-β and prostaglandin E2	Jung *et al.*[208]
Editing CAR-T cells to improve efficiency	CD40 ligand (CD154) expressing CAR-T cell	Superior antitumor effects via NF-κB pathway	Kuhn *et al.*[209]
	Inducible interleukin-12 (IL-12) secreting CAR-T cells	IL-12 secreting CAR-T cells attracted activated macrophages and eliminated antigen-loss tumor cells via Tumor necrosis factor (TNF)-alpha mediated process	Chmielewski *et al.*[210]
	CXCR-2 expressing hepatocellular carcinoma (HCC)-targeting CAR-T cells	CXCR2 expression stimulated the cohesion of CAR-T cells at the tumor site and ensured their migratory effect to the tumor microenvironment in HCC	Jin *et al.*[211]
Improving durability and safety of CAR-T cells	Disrupted TET2 (Tet methylcytosine dioxygenase 2) promoter in CAR-T cells	TET2 disrupted CAR-T cells exhibited higher antitumor activity *in vivo*	Fraietta *et al.*[212]
	CD7 and T cell receptor alpha chain (TRAC) expression lacking CAR-T cells, targeting T cell malignancies	Modified CAR-T cells demonstrated efficacy against human T cell acute lymphoblastic leukemia (T-ALL) cell lines and primary T-ALL *in vitro* and *in vivo* without the induction of xenogeneic graft versus host disease (GvHD).	Cooper *et al.*[213]
	Granulocyte-macrophage colony-stimulating factor (GM-CSF) knock-out in CAR-T cells	GM-CSF is a major contributor in development of cytokine release syndrome (CRS) a well-known toxicity of CAR-T cell therapy. GM-CSF KO CAR-T cells retained anti-tumor activity while reducing CRS.	Sterner *et al.*[214]

Targeting mutations that determine drug response or susceptibility:

Cancer cells can acquire resistance to targeted drugs or chemotherapy drugs by several mechanisms. Several mutations, mainly pathogenic single nucleotide polymorphisms (SNPs), are known to develop during the course of therapy conferring resistance to cancer cells. One such example is the T315I mutation in the BCR-ABL kinase domain (threonine is substituted to

isoleucine) which confers resistance against imatinib, a tyrosine kinase inhibitor used in treatment of BCR-ABL positive hematological cancers. At the protein level, the mutation T315I results in a loss of a hydrogen bond, which is necessary for the binding of imatinib to the ATP-binding site of BCR-ABL, leading to significant reduction in efficacy of the drug.[215] CRISPR based editing offers novel approach to silence such mutations and thereby restore drug efficacy.

EGFR T790M and TP53 R273H mutations are associated with gefitinib (a tyrosine kinase inhibitor drug) resistance in lung cancer patients. Yoon *et al.* showed that co-delivery of adenine base editor (ABE) and EGFR- and TP53-SNP specific sgRNA via adenovirus resulted in accurate correction of the oncogenic mutations with high efficiency *in vitro* and *in vivo*. There was increased drug sensitivity and suppressed abnormal tumor growth in cells with altered EGFR and TP53 mutations as compared to control cells.[216]

In breast cancer cells, there are studies done which show that genetically modified T47D and MCF7 breast cancer cells containing mutations in estrogen receptor 1 (ESR1) (Y537S and D538G) showed estrogen-independent growth and resistance to fulvestrant, raloxifene and 4-hydroxytamoxifen (4-OHT) *in vitro*.[217-219] In addition to addressing existing drug resistances, CRISPR can also be used to identify newer drug resistance mechanisms and mutations. Chen *et al*. showed that, in triple negative breast cancer cells (HCC1937) the genetic ablation of ATPE1, a base excision repair enzyme led to resistance to olaparib, a poly (ADP-ribose) polymerase (PARP) inhibitor.[220] In another study, CRISPR based knockout of MAP3K1 in mutant PIK3CA breast cancer cells increased the proliferation rate and decreased sensitivity to AZD5363 (an AKT inhibitor) *in vitro* as well as *in vivo*.[221]

Inactivating carcinogenic viral infections:

The International Agency for Research on Cancer (IARC) has classified following viruses as carcinogens after comprehensive analysis: Epstein-Barr virus (EBV), hepatitis B virus (HBV), hepatitis C virus (HCV), Kaposi's sarcoma herpes virus (KSHV), human immunodeficiency virus, type-1 (HIV-1), human T cell lymphotropic virus, type-1 (HTLV-1), and human papillomavirus (HPV). EBV, HPV, HTLV-1, and KSHV are classified as direct carcinogens while HBV, HCV and HIV-1 are considered indirect carcinogens (HBV and HCV cause chronic inflammation and HIV-1 causes immune suppression).[222]

CRISPR/Cas technology has a promising role in targeting E6 or E7 genes in HPV, which are responsible for inducing cervical carcinoma. Kennedy *et al.* showed that the expression of a bacterial Cas9 RNA-guided endonuclease, together with sgRNAs specific for E6 or E7, induced cleavage of the HPV genome, resulting from inactivating mutations (deletions and insertions) into the E6 or E7 gene. This further induced p53 or retinoblastoma (Rb) protein, leading to cell cycle arrest and eventual cell death.[223] In another study, CRISPR/Cas9 was used to target the promoter of HPV 16 E6/E7 as well as E6, E7 transcript resulting in significant reduction in proliferation of cervical cancer cell line SiHa and reduced tumorigenesis in mouse models.[224]

The CRISPR/Cas9 system could successfully treat EBV-related cancers during the latent phase of EBV infections by targeting EBV viral genomes.[225] CRISPR/Cas9 was shown to cause direct cleavage of the JCV genome, a small circular dsDNA that encodes for the viral early protein, T-antigen. CRISPR/Cas9 was used to stop viral replication in transformed human glial cells

because of the inactivation of the T-antigen-coding genes, which are critical for directing viral reactivation and lytic infection.[226]

The following two approaches are not direct therapy approaches but are important tools in translational research. They both help in understanding molecular mechanisms of various cancerous phenotypes providing invaluable information during early phases of drug discovery:

Creating tumor models and organoids:

Transfecting of mouse embryonic stem cells with CRISPR/Cas9, sgRNA, and +/− donor template promotes HDR and enables development of efficient knockout or knock-in mouse models. CRISPR/Cas9 can also be used to develop inducible Cas9 mouse models to perform efficient somatic editing *in vivo*, with various organs as possible targets using either adeno-associated viruses (AAVs), lentivirus or nanoparticle mediated sgRNA delivery.[227-229]

Heckl *et al.* used CRISPR/Cas9 system via lentiviral delivery method to revive several inactivated oncogenes in primary hematopoietic stem and progenitor cells (HSPCs) to generate leukemia model. The targeted genes were: Tet2, Runx1, Dnmt3a, Nf1, Ezh2, and Smc3.[230]

CRISPR/Cas9 technology has also been adopted to develop organoid tumor models. For example, organoid models for colon cancer were constructed *in vitro* by using CRISPR to introduce mutations in tumor suppressor genes (APC, TP53, SMAD4, etc.) and modify oncogenes (KRAS, PI3K, etc.).[231] Roper *et al.*[232] established a protocol to induce site-directed tumors rapidly and efficiently in the distal colon of mice by utilizing colonoscopy-guided mucosal injection. This technique can be extrapolated to deliver viral vectors carrying Cre recombinase, CRISPR/Cas9 components, CRISPR-engineered mouse tumor organoids, or human cancer organoids to mice to model the adenoma-carcinoma-metastasis sequence of tumor progression.

Creating high-throughput genetic screens:

CRISPR based high-throughput screening is a large-scale genetic loss-of-function experimental approach that facilitates discovery of key genes or gene sequences that correlate with a specific function or phenotype for a cell type, for example, resistance or sensitivity to a drug, susceptibility to environmental toxins, components of a cellular pathway or novel pathogenic biomarkers.[233, 234]

Recently, using CRISPR screens a compelling lethal interaction between the helicase-encoding WRN gene and microsatellite instability was identified.[235, 236] In immuno-oncology, the molecular mechanism of tumor immune evasion was explored which included multiple factors like Ras signaling, antigen presentation, interferon, autophagy, and epigenetic remodeling.[237-239] In another study, a CRISPR based screening approach showed that depletion of neurofibromin, merlin and the mediator complex component MED12 conferred resistance to vemurafenib, a B-Raf enzyme inhibitor, in BRAF-mutant melanoma cells.[234]

In the future, CRISPR/Cas9 based efficient and precise cancer models and high-throughput screens are likely to significantly promote functional cancer genomics research and accelerate the development of novel cancer therapies.

II. Infectious diseases

Infectious diseases were the second largest subset of publications in the CRISPR dataset extracted from the CAS Content Collection. A total of 25% of all journal publications and 22% of all patent publications mentioned infectious diseases. There has been a steep increase over the past few years in number of publications on infectious diseases and CRISPR technology especially marked for bacterial and viral infectious diseases (Figure S4). A majority of these documents appear to focus on viral, followed by bacterial and lastly fungal studies.

CRISPR has emerged as a promising alternative to develop therapeutics against various pathogens by –

- Targeting the pathogen genes required for replication or entering or infecting the host cells or
- Altering the host genes required by the pathogen to cause infection or
- Modifying the genes responsible for drug resistance or susceptibility[180, 240, 241]

CRISPR-based antimicrobials have a unique advantage over other conventional antimicrobials because they can destroy microbes based on their genomic sequence. This is particularly useful in situations where only a small number of microbes within a genus must be targeted and eradicated, which is tough to do with existing antimicrobial strategies.[242, 243]

Table 3 enlists numerous studies conducted for exploring CRISPR based therapeutics as antimicrobial agents.

Table 3. Examples of CRISPR/Cas9-based therapeutics as antimicrobials

Pathogen	Target genes of the pathogen	Major outcome of the study	Reference
Cas9			
Herpes simplex virus 1 (HSV-1)	HSV-1 genome was targeted using *Streptococcus pyogenes* Cas9 (SpCas9) mRNA and viral gene-targeting gRNAs (designated HSV-1-erasing lentiviral particles, HELP)	HSV-1 replication was blocked	Yin *et al.*[244]
Hepatitis B virus (HBV)	The surface antigen (HBsAg)-encoding region of HBV, *in vitro* and *in vivo*	HBV replication and expression was inhibited	Zhen *et al.*[245]
Hepatitis C virus (HCV)	HCV 5′ untranslated region involved in both translation of the viral polyprotein and replication of the viral RNA	HCV RNA transcription was inhibited	Price *et al.*[246]

Pathogen	Target genes of the pathogen	Major outcome of the study	Reference
Cas9			
Human immuno-deficiency virus (HIV)	Edit integrated proviral DNA (long terminal repeats region)	HIV-1 expression was suppressed	Ebina *et al.*[247]
Staphylococcus aureus	Virulence genes and antibiotic resistance genes	Only the virulent *Staphylococcus aureus* was killed. By targeting antibiotic resistance genes, bacteria became more susceptible to treatment	Bikard *et al.*[248]
Mycobacterium tuberculosis	Multiple genes of *Mycobacterium tuberculosis*	Sequence-specific regulatory suppression in M. tb was observed	Choudhary *et al.*[249]
Aspergillus fumigatus	Multiple genes of *Aspergillus fumigatus,* like those involved in drug resistance or ribosomal RNA processing or other essential functions	Increased drug susceptibility, reduction in fungal growth was observed	Vyas *et al.*[250]
Candida albicans	CDR1 and CDR2 (members of the multigene drug efflux pump encoding family), responsible for drug resistance to azoles)	By knocking out CDR1 and CDR2, the clinical strain of Candida albicans did not show hyper-resistance to fluconazole or cycloheximide	Vyas *et al.*[250]
Cas3			
Clostridium difficile	The genome of *Clostridium difficile* to create long-range deletions (packaged in bacteriophages)	Bacteriophages containing the targeted CRISPR/Cas3 system killed *Clostridium difficile*	Selle *et al.*[251]

III. Blood disorders

The delivery of genome editing machinery by utilizing CRISPR/Cas technology to target blood cells possesses an interesting possibility to provide cure for patients with inherited monogenic blood diseases such as sickle cell anemia and β-thalassemia. The first US FDA approved CRISPR therapeutic, Casgevy™ is an autologous gene therapy which edits BCL11A gene which helps in production of fetal hemoglobin. Eventually, this stops red blood cells (RBCs) from adopting their characteristic sickle shape.[252] Other therapies for the treatment of sickle cell

anemia and β-thalassemia include targeting the erythroid-specific enhancer region of the BCL11A gene, and HBG1/HBG2 genes and are currently undergoing clinical trials.[253]

β-thalassemia is also associated with mutations in the HBB gene, particularly a point mutation in intron 2 that alters splicing. Xu *et al.* used TALENs and CRISPR/Cas9 to target the aberrant intron to restore HBB gene expression in induced pluripotent stem cells (iPSCs) *in vitro*, creating a potential opportunity for cell therapy through haemopoietic stem cell replacement.[254]

IV. Common and uncommon genetic disorders

Among the many promising possibilities of using CRISPR-based therapeutics, their translational use in monogenic human genetic diseases has the potential to provide long-term therapy after a single treatment. Genetic disorders can be treated with the help of CRISPR by editing the defective (disease causing) gene or by editing the enhancer or regulator of the defective gene. Numerous studies, which are summarized in the table below (Table 4), have shown promising results by using these two approaches.

Table 4. Examples of CRISPR-based therapeutics for the treatment of genetic disorders.

Disease	CRISPR Target	Approach	Major outcome of the study	Reference
Duchenne muscular dystrophy	Dystrophin gene (DMD)	Single or multiplexed sgRNAs were developed to restore the dystrophin reading frame by targeting the mutational hotspot at exons 45-55 and introducing shifts within exons or deleting one or more exons.	Dystrophin expression is restored in vitro.	Ousterout *et al.*[255]
Huntington disease	Huntingtin gene (HTT)	HTT 5′ UTR was targeted	Improper maturation of the transcript and reducing the expression of the disease-causing allele.	Kolli *et al.*[256]
		A dual sgRNA approach was used *in vitro* to excise a 44kb promoter region upstream of a mutant HTT gene to silence its expression	Expression of the Huntington disease-causing variant was ablated	Shin *et al.*[257]
Glaucoma	MYOC	Knocked down the expression of mutant MYOC in a mouse model of primary open-angle glaucoma.	Reduction of ER stress, lower intraocular pressure, and the preventability of further glaucomatous damage in mouse eyes was observed. The authors also demonstrated the feasibility of utilizing CRISPR/Cas9 in human eyes with glaucoma.	Jain *et al.*[258]
Hereditary tyrosinemia type I	FAH	HDR-mediated point mutation correction in mouse hepatocytes.	A significant proportion of alleles were corrected	VanLith *et al.*[259]

Disease	CRISPR Target	Approach	Major outcome of the study	Reference
Leber congenital amaurosis type 10 (LCA10)	CEP290 gene	AAV5-based therapy (EDIT-101) encapsulates *Staphylococcus aureus* Cas9 (SaCas9) and two sgRNAs targeting genomic locations upstream and downstream of the intronic CEP290 point mutation. The two sgRNAs enable cutting around the mutation to induce its removal or inversion	Normal splicing of CEP290 pre-mRNA was restored	Maeder *et al.*[260]
Noonan syndrome	LZTR1 gene	Intron 16 of LZTR1 was targeted	The gene editing process could overcome the disease phenotype associated with Noonan syndrome-associated cardiomyopathy in iPSC-derived cardiomyocytes *in vitro*	Hanses *et al.*[261]
Angelman syndrome	UBE3A-ATS lncRNA	UBE3A-ATS lncRNA was targeted in cultured human neurons and in a mouse model of the disease	Targeting of UBE3A-ATS ablated its function, leading to expression of the paternal UBE3A gene and rescuing the disease phenotype.	Wolter *et al.*[262]
Congenital muscular dystrophy type 1A (MDC1A)	Lama1	CRISPR activator mediated gene upregulation	3.6-fold upregulation of Lama1 was observed	Kemaladevi *et al.*[263]
Genetic Deafness	Tmc1	NHEJ-mediated mutant Tmc allele disruption	Deafness was prevented in mouse model up to one year post injection	György *et al.*[264]

V. Nervous system disorders:

While accounting for a smaller fraction of CRISPR publications (Figure 12), nervous system disorders still contribute about 7% and 6% of journal articles and patents in the field of CRISPR. Supplementary Figure S5 further shows the breakdown of publication trend across various nervous system disorders – a key takeaway is that the rate of growth of publications in the field of CRISPR co-occurring with Alzheimer's and Parkinson's diseases has increased over the last decade indicating interest from both academic researchers as well as commercial entities. CRISPR/Cas9 technology has gained popularity in the field of neurodegenerative diseases due to its short experimental duration and easy molecular engineering requirements. It is currently being extensively utilized for building disease models, identifying pathogenic genes through screening, and for targeted therapy.

Alzheimer's disease (AD) is the most prevalent neurodegenerative disease characterized by memory deficits and cognitive decline. It is mainly characterized by two neuropathological

features - the accumulation of extracellular amyloid β (Aβ) protein plaques and neurofibrillary tangles primarily composed of hyperphosphorylated Tau protein.[265, 266] Majority of cases of AD are known to be sporadic in nature, however, a small percentage of cases are familial (known as Familial AD or FAD), caused by dominant autosomal mutations found in one of three genes: presenilin-1 (PSEN1), and presenilin-2 (PSEN2), and amyloid precursor protein (APP).[267, 268]

Sun *et al.*[269] knocked out PSEN1 genes using CRISPER/Cas9 in mouse neuroblastoma cells and this decreased the production of Aβ42 and Aβ40. Konstantinidis *et al*.[270] suggests that the CRISPR/Cas9 approach can be used to selectively disrupt the PSEN1M146L allele responsible for AD, and partly switch the abnormal Aβ42/40 ratio that leads to the development of the disease in carriers of this mutation. Ortiz-Virumbrales *et al.*[271] demonstrated that CRISPR/Cas9 can correct neurons derived from the PSEN2N141I mutated individual fibroblasts and can further normalize the Aβ42/40 ratio. This was shown to effectively restore the associated electrophysiological deficits.

Parkinson's disease (PD) is the second most prevalent neurological disorder in humans, which is characterized by the progressive loss of dopaminergic neurons and significant decrease in dopamine levels. Subsquently, there is functional impairment of the motor circuit. Majorly, around 90% of PD cases are not linked to any known cause, while the remaining 10% have familial PD caused by mutations in specific genes like α-synuclein (SNCA), parkin RBR E3 ubiquitin protein ligase (PRKN), PTEN induced kinase 1 (PINK1), leucine rich repeat kinase 2 (LRRK2).[272, 273]

The missense mutation, Ala53Thr (A53T) in SNCA is considered to be one of the most prominent risk factors for early-onset PD. Yoon *et al.*[274] conducted a study where they deleted the A53T-SNCA gene using CRISPR/Cas9 which significantly improved conditions related to PD, such as the overproduction of α-synuclein, reactive microgliosis, dopaminergic neurodegeneration, and PD associated motor symptoms.

There is significant research still ongoing in identifying novel biomarkers and mutations involved in the onset of AD and PD. Developing disease models is critical in understanding disease biology and pathology, and CRISPR has shown promising utility in the same. Few of the examples are - cellular model of AD with disease-causing mutations in APP and PSEN1;[275] mouse model for AD with tau knock-out[276] and a monkey model for PD with PINK1 deletion.[277]

CRISPR Therapeutics: Candidates in the Developmental Pipeline

Over the last decade, CRISPR has made significant strides in clinical research, with numerous trials launched to explore its potential in therapeutics. As a result, in late 2023, the CRISPR-based therapeutic, Casgevy, was granted approval becoming the first-ever in just 11 years which is truly a remarkable achievement. Casgevy (exagamglogene autotemcel), the CRISPR/Cas9 gene editing therapy for the treatment of patients with transfusion-dependent β-thalassemia and the treatment of sickle cell disease in patients aged ≥12 years with recurrent vaso-occlusive crises was approved by the UK Medicines and Healthcare Products Regulatory Agency (MHRA) on 16 November 2023.[182] US FDA approved Casgevy and Lyfgenia (lovotibeglogene autotemcel) for patients with sickle cell disease on 8 December 2023.[181]

Casgevy has been approved by the European Medicines Agency (EMA) for sickle cell disease and transfusion-dependent β-thalassemia on 15 December 2023.[183]

To gain insights on ongoing preclinical and clinical trials on CRISPR technology, we retrieved data from Pharmaproject Citeline Clinical Intelligence (Figure 14). At present, there are 142 CRISPR therapeutics in different stages of development. Of which 10% are in phase I, 11% in phase II and 1% in phase III clinical trials. A vast majority of CRISPR therapeutics (77%) are still in the preclinical stage of development.

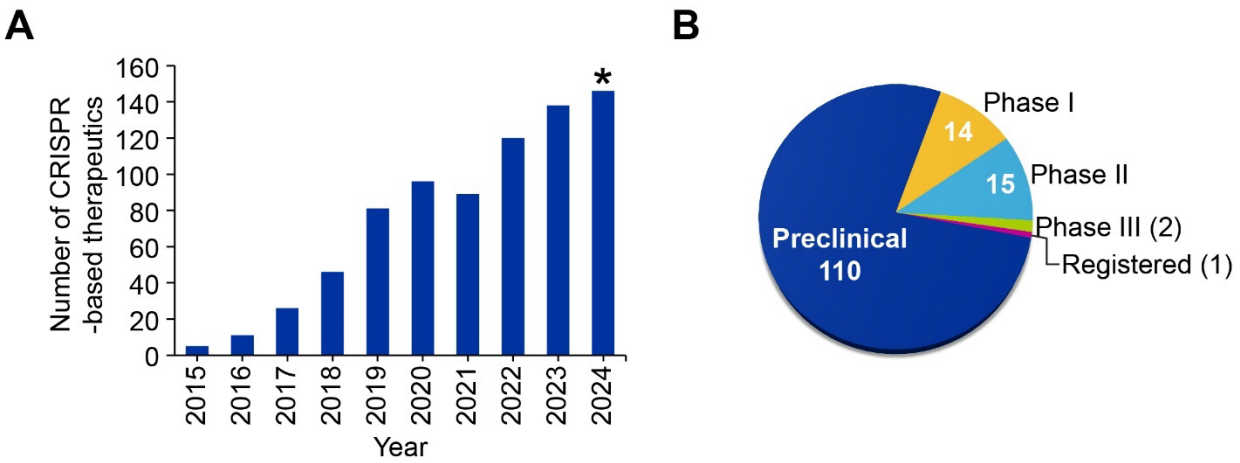


Figure 14. (**A**) Year-wise distribution of CRISPR-based therapeutics in preclinical and clinical trials. (**B**) Current status of CRISPR-based therapeutics in various stages of development. Data taken from Pharmaproject Citeline Clinical Intelligence. Data for 2024 is partial and includes data till June 2024.

The range of disease conditions targeted by CRISPR-based therapeutics currently in the preclinical stages of development are wide – from rare genetic disorders and blood diseases to various forms of cancer and even infectious diseases such as HIV, tuberculosis (TB) and COVID-19. The data reveals that 25% of these therapeutics are focused on cancer (Figure 15), which consist of treatment for solid tumors (60%) and hematological malignancies (34%). CRISPR-edited CAR-T therapies are leading (57%) against hematological malignancies. However, some CAR-T cell therapies are also being developed for solid tumors (43%) with the help of CRISPR technology. Nkarta in collaboration with CRISPR Therapeutics is developing an allogeneic chimeric antigen receptor-natural killer (CAR-NK) cell therapy targeting CD70, using its off-the-shelf NK cell-based technology for the treatment of solid and hematological cancers. Other major disease groups targeted by CRISPR-based therapeutics are shown in Figure 15; there are comparatively fewer CRISPR-based therapeutics that are currently under exploration for immunological (4%), respiratory (3%) and dermatological (1%) diseases (Figure 15).

CRISPR-based therapeutics in preclinical and clinical trials focused on the treatment of neurological conditions (Figure 15) such as transthyretin amyloidosis, amyotrophic lateral sclerosis, anxiety, mild cognitive impairment, depression, Alzheimer's disease, Angelman syndrome, small fiber neuropathy and primary erythromelalgia. Of these 92% are in preclinical research stage and 8% in clinical trial phases (Figure 16 and S6). Of the 9% of CRISPR-based therapeutics aimed at the treatment of alimentary or metabolic diseases, 92% are in the preclinical stage and include diseases such as hyperoxaluria, hepatic dysfunction, inflammatory bowel

disease , type 1 diabetes, Pompe's disease,[278, 279] radio/chemotherapy-induced GI injury and ulcerative colitis, and at present only one CRISPR-based therapy named CTX-211 has reached the phase II clinical trials for the treatment of type 1 diabetes (NCT05565248).

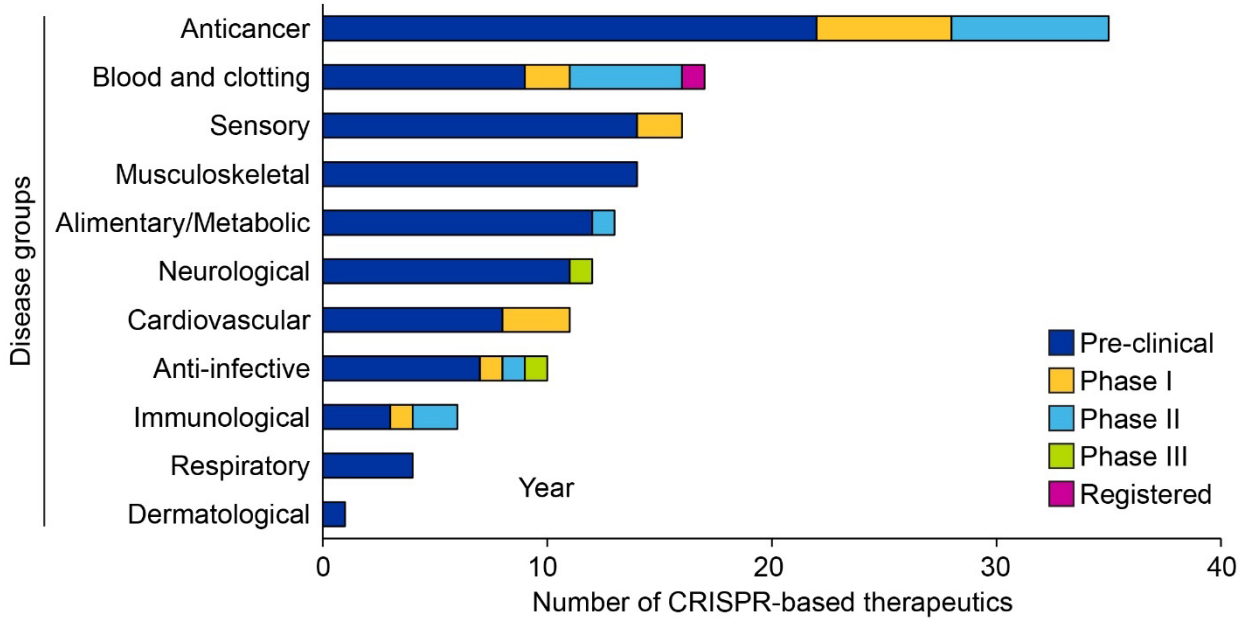


Figure 15. Distribution of CRISPR-based therapeutics development among different disease groups. The stacked bar shows the split of therapeutics among various stages of development for each disease group. The percentage distribution among disease groups has been mentioned in parentheses.

Several different CRISPR based therapies tackle infectious diseases (7%) (Figure 15), from CRISPR-enhanced bacteriophages to the excision of integrated retroviruses, and even epigenetic silencing of entire viral genomes. LBPEC-01, an anti-infective CRISPR based therapy in phase III clinical trial (NCT05488340), is a bacteriophage, under development by Locus Biosciences, using CRISPR/Cas3 (crPhage) technology for the treatment of urinary tract infections caused by the E coli and K pneumoniae.[280] The first ever CRISPR therapy for HIV, EBT-101, aims to cut the virus from the genome of human cells using CRISPR/Cas9 and two guide RNAs, delivered via AAV9 (NCT05144386). Data presented at the 27th American Society of Gene & Cell Therapy (ASGCT) meeting revealed that EBT-101 met the primary and secondary endpoints of safety and biodistribution/ immunogenicity, respectively, it did not prevent viral rebound in three individuals who stopped anti-retroviral medication in a phase 1/2 clinical trial.[281]

The sankey charts in Figures 16 and S6 depict the breakdown of CRISPR-based therapeutics across disease groups and phases of development. A few key takeaways from these Sankeys are as follows:

- A majority of CRISPR-based therapeutics currently in the developmental pipeline are aimed at treating cancers ranging from solid cancers to non-small cell lung cancer (NSCLC) and AML among others.
- Many of the targets (35%) currently explored in pre-clinical stages remain unspecified.

- Among the specified targets, gene editing via the CRISPR system of dystrophin is being explored to permanently correct DMD mutations and thus restore the reading frame, allowing for the production of functional dystrophin.[282]
- Similarly, CRISPR based strategies are also being investigated for facioscapulohumeral muscular dystrophy (FSHD) and merosin-deficient congenital muscular dystrophy type 1A (MDC1A) which are caused by the aberrant expression of the DUX4 gene in the muscle tissue[283] and mutation in the laminin alpha 2-chain (LAMA2) gene encoding laminin alpha 2 (Lama2) protein respectively.[284]

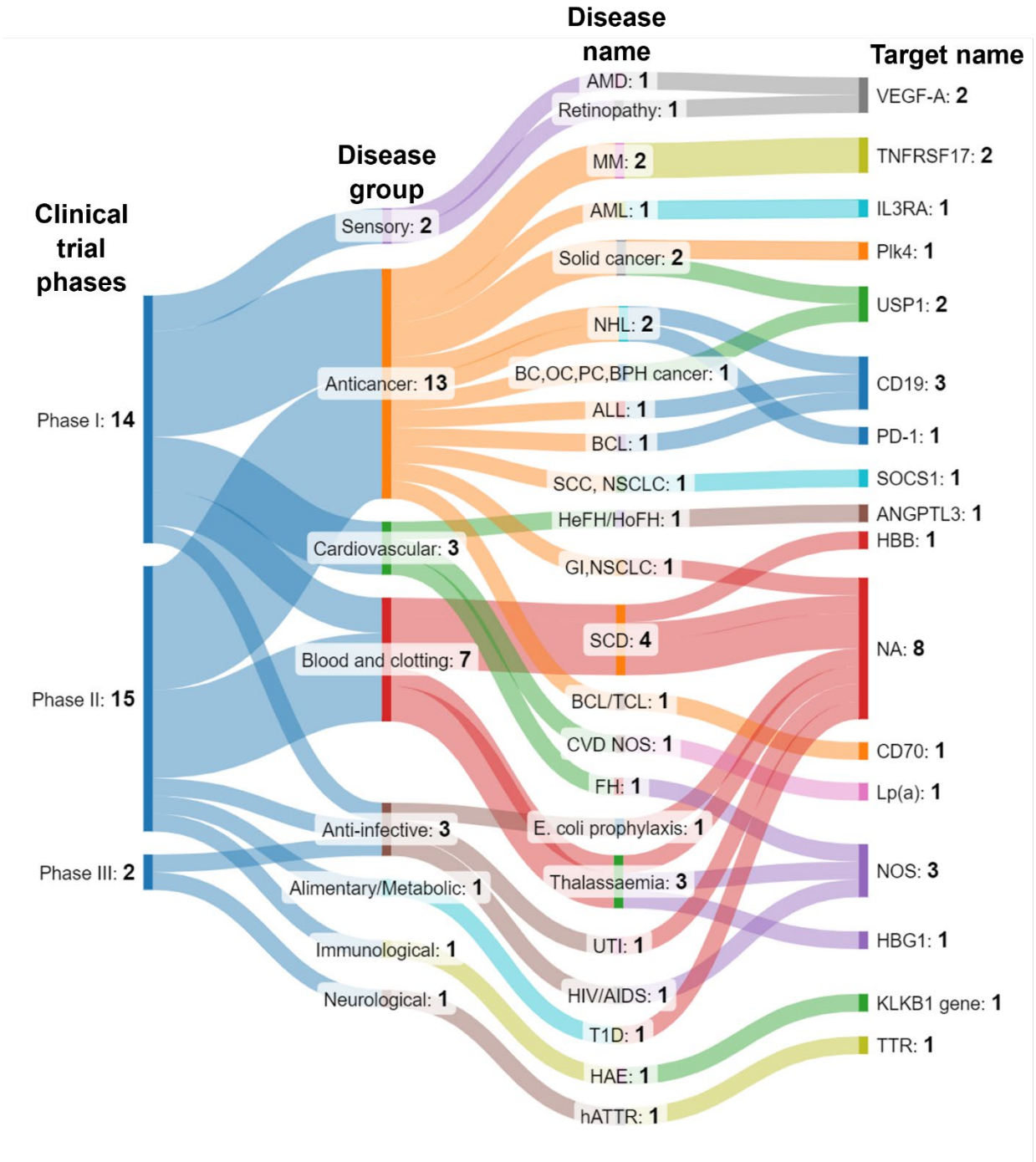


Figure 16. Distribution of CRISPR-based therapeutics in the clinical stages (Phase I, II and III) of development across disease groups, individual diseases, and their biological targets. Data retrieved from Pharmaproject Citeline Clinical Intelligence in June 2024. The names of the diseases and their targets are abbreviated here as: AMD, age-related macular degeneration; MM, multiple myeloma; AML, acute myeloid leukemia; NHL, non-Hodgkin's lymphoma; BC, breast cancer; OC, ovarian cancer; PC, pancreatic cancer; BPH, benign prostatic hyperplasia; ALL, acute lymphocytic leukemia; BCL, B-cell lymphoma; SCC, squamous cell carcinoma; NSCLC, non-small cell lung cancer; SCD, sickle cell disease; TCL, T-cell lymphoma; CVD, cardiovascular disease; NOS, not specified; FH, familial hypercholesterolemia; UTI, urinary tract Infection; HIV/AIDS, human immunodeficiency virus/acquired immunodeficiency syndrome; T1D, type 1 diabetes; HAE, hereditary angioedema; hATTR, hereditary transthyretin amyloidosis; VEGF-A,

vascular endothelial growth factor A; TNFRSF17, TNF receptor superfamily member 17; IL3RA, interleukin 3 receptor alpha; Plk4, polo-like kinase 4; USP1, ubiquitination-specific proteases; CD19, cluster of differentiation 19; PD-1, programmed-death 1; SOCS1, suppressor of cytokine signaling 1; ANGPTL3, angiopoietin-like protein 3; HBB, hemoglobin subunit beta; NA, not applicable; CD70, cluster of differentiation 70; Lp(a), lipoprotein (a); HBG1, hemoglobin subunit gamma 1; KLKB1, kallikrein B1; TTR, transthyretin.

In terms of sheer number of CRISPR-based therapeutics in the developmental pipeline, the leading organization is CRISPR Therapeutics contributing 17% of CRISPR-based therapeutics in preclinical and clinical development. With a focus on the development of transformative medicines using its proprietary CRISPR/Cas9 gene-editing platform, CRISPR Therapeutics in collaboration with Vertex Pharmaceuticals launched the first-ever US FDA approved CRISPR-based therapy Casgevy.[182] Other key players that are actively involved in developing CRISPR-based therapeutics include Intellia Therapeutics (10%), followed by Arbor Biotechnologies (8%), and Chengdu Gene Vector Biotechnology (6%) among others (Figure S7A). Geographical distribution of companies engaged in CRISPR-based research and development indicates that the United States is the leader accounting for 46%, followed by China (14%) and Switzerland (12%) (Figure S7B). American universities, research institutions, and biotech companies have spearheaded much of the work on CRISPR technology. Biotech companies such as CRISPR Therapeutics, Vertex Pharmaceuticals and Intellia Therapeutics are major players. China has been a pioneer (14%) in applying CRISPR technology in clinical settings.[285] The country has launched a variety of clinical trials, particularly focusing on cancer treatment using CRISPR-edited immune cells.[286]

CRISPR in Disease Diagnosis

CRISPR technology, originally harnessed for gene editing, has rapidly evolved into a powerful tool for disease diagnosis.[287-289] Its ability to detect specific genetic sequences is invaluable in identifying infectious diseases, genetic disorders, and even cancers. Although quantitative polymerase chain reaction (qPCR)-based nucleic acid detection is a gold standard method in routine clinical practice,[290, 291] it relies on optimizing numerous processes, such as DNA or RNA extraction, (RNA integrity control and cDNA synthesis in the case of RNA), primer design, amplicon detection, and data normalization.[292, 293] Isothermal amplification and next-generation sequencing (NGS) are also used in routine clinical diagnostics. For comparisons between the three most prevalent molecular diagnostic methods please see Table S2 in supporting information.

The CRISPR/Cas system can open a new window of possibilities for genetic diagnostics that integrates the ease of use and cost efficiency of isothermal amplification with the diagnostic accuracy of PCR for genotyping, detecting cancer mutations and mutations that confer resistance to antibiotics, antiviral medicines or cancer drugs. Additionally, CRISPR/Cas system can fulfill the ASSURED criteria (affordable, sensitive, specific, user-friendly, rapid, equipment-free, delivered) set by the World Health Organization[294] for infectious disease diagnostics.

The various Cas proteins, combined with other technologies such as biosensors, biochips, biomagnetic beads, isothermal amplification, lateral flow, protein aptamers, etc. have led to the development of new molecular diagnostic methods with high sensitivity, specificity, low cost, short turnaround time, and portability in complex biological specimens.[296] Most current CRISPR/Cas mediated diagnostic assays utilize Class 2 CRISPR/Cas systems that consist of type II (Cas9),

type V (Cas12 and Cas14), and type VI (Cas13) CRISPR/Cas systems employing single multidomain effectors. Class 1 type I CRISPR/Cas3 system is also emerging for nucleic acid detection.[297] The CRISPR/Cas12a, CRISPR/Cas13a, CRISPR/Cas14a and CRISPR/Cas3 systems depend on the measurement of *trans*-cleavage activity triggered by target sequence recognition[298, 299] (Figure 3) *trans*-cleavage activity may be inhibited or nonspecifically activated by target-independent factors.[300] The CRISPR/Cas9 system possesses excellent DNA recognition capability but does not possess *trans*-cleavage activity, and has been developed for biosensor-based diagnostics for its excellent DNA recognition capability.[301-303] Only the CRISPR/Cas12a and CRISPR/Cas9 systems are available for dsDNA recognition. In this section, we have discussed the publication landscape of CRISPR-based disease diagnostics and briefly described their mechanisms.

Publication Landscape on CRISPR-based Disease Diagnostics

Our data analysis indicates more than 6,600 and 2,900 journal articles and patent publications, respectively, on the application of CRISPR technology in disease diagnosis from 2004 to 2024 which accounts for 17% and 21% of total journal articles and patent publications, respectively, on CRISPR therapeutics in CAS Content collection (Figure 17). Publication trends of CRISPR in disease diagnosis has shown a remarkable increase in recent years, reflecting its growing importance as a diagnostic tool in molecular biology and medical research. Since the discovery of CRISPR's potential in 2012, there has been an exponential rise in journal and patent publications, especially after 2015. The COVID-19 pandemic coincides with accelerated use of CRISPR-based diagnostics with a notable increase in publications (44%) between 2020 and 2022. Patent publications on CRISPR-based disease diagnosis have surged in recent years, paralleling the technology's rapid adoption in research and clinical applications.

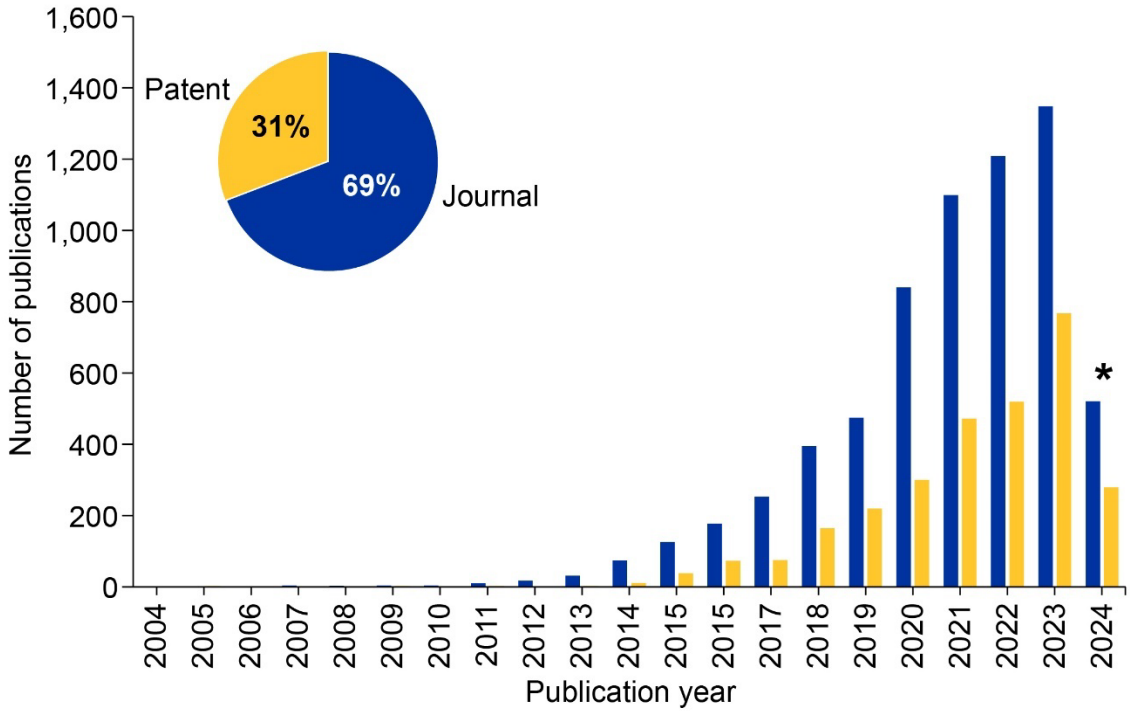


Figure 17. Journal and patent publication trends on CRISPR-based disease diagnostics from the CAS Content Collection for the period 2004 to 2024. *Note that data for 2024 is incomplete due to time of data extraction and encompasses data for January to June.

The publication trends on CRISPR technology and its various Cas proteins associated with diagnosis have evolved significantly over the last decade as the diversity of Cas systems has expanded (Figure 18). Each Cas protein has unique properties and has been adapted for various applications. Cas9 was the first and most widely studied protein in CRISPR research. Early studies predominantly focused on gene editing, but some initial exploration of Cas9's potential for diagnostics began in 2014 with a steady increase in publications (Figure 18B). The discovery of Cas12 (for DNA detection) and Cas13 (for RNA detection) led to major breakthroughs in diagnostics, especially with the development of the SHERLOCK (Cas13-based) (Sherlock Biosciences) and DETECTR (Cas12-based) (Mammoth Biosciences) platforms. Publications on CRISPR/Cas12 increased several-fold since 2019 indicating development of accurate, fast and scalable testing solutions. Similarly, publications on Cas12 and Cas13 surged due to their applications in infectious disease detection (e.g. Zika, Dengue and HPV), multiplexed diagnostics (e.g. Influenza, HIV, SARS-CoV-2), and cancer. Finally, publications associated with other Cas proteins such as Cas14 and Cas3, Cas14 appear to be a handful. Cas14 can detect single-stranded DNA thereby providing more versatility in diagnostic applications, while Cas3 is known for its ability to degrade long stretches of DNA.

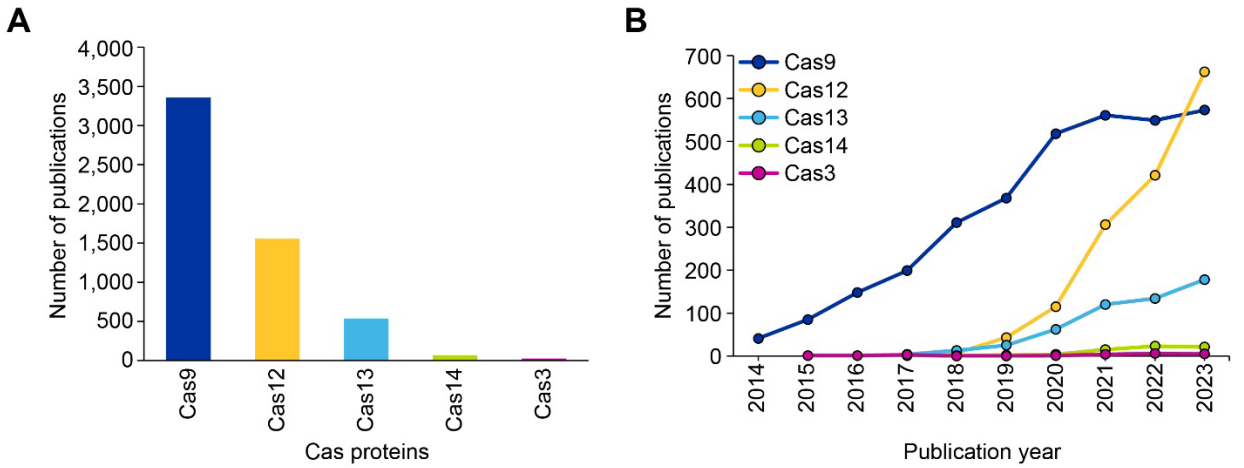


Figure 18. (**A**) Distribution of publications (journal and patent) based on Cas proteins - Cas9, Cas12, Cas13, Cas14 and Cas3 – in publications related to the application of CRISPR in diagnostics. (**B**) Year-wise distribution of publications (journal and patent) associated with various Cas proteins in the CRISPR diagnostics subset of publications. Data includes journal and patent publications from the CAS Content Collection for the period 2014 to 2023.

The analysis of CRISPR-based disease diagnostics documents co-occurring with various diseases (Figure 19) reflects a growing interest in both infectious and non-infectious diseases. Viral infections in infectious diseases and cancer in non-infectious diseases led the way with the highest number of documents, followed by bacterial, genetic, immune, and fungal diseases (Figure 19A). Publications on CRISPR-based disease diagnostics co-occurring with cancer show continuous and constant growth since 2014, whereas publications on viral diseases show a sudden spike in 2019 (Figure 19B).

The intersection of CRISPR technologies with preamplification methods for disease diagnosis is a dynamic and rapidly growing area of research, driven by the need for sensitive, specific, and rapid diagnostic tools for various diseases including infectious diseases and cancers. Many diagnostic methods based on CRISPR require preamplification to detect low-abundance nucleic acids. 56% of publications appear to be associated with PCR as a

preamplification technique in combination with CRISPR diagnostics to achieve low-cost and point-of-care solutions. This is followed by recombinase polymerase amplification (RPA) (18%) and loop-mediated isothermal amplification (LAMP) (7%) etc. Recent publications are also exploring non-amplification methods (4%), focusing on simpler, faster and more portable diagnostic systems (Figure 20A). Various readout methods are used to interpret the results of CRISPR diagnostics, ranging from simple colorimetric assays to more complex fluorescence-based systems. Fluorescence and sequencing readouts dominate the landscape (39% and 33% respectively), with growing interest in lateral flow, electrochemical, colorimetric, luminescence and optical (Figure 20B).

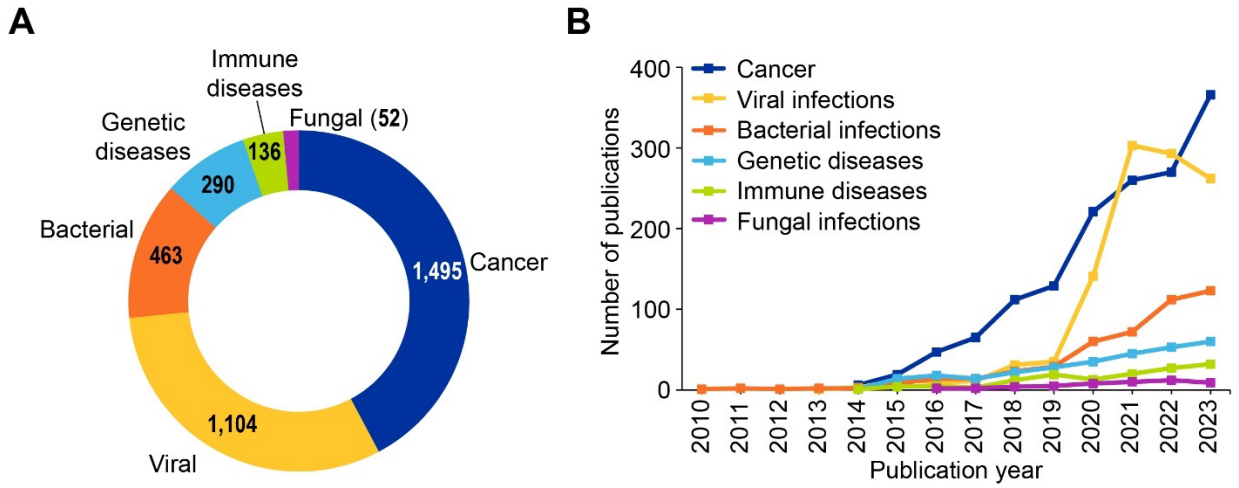


Figure 19. (A) CRISPR-based disease diagnostics documents co-occurring with various diseases (cancer, viral, bacterial, genetic, immune and fungal diseases. (**B**) Time trend for CRISPR-based disease diagnostics documents co-occurring with various diseases. Data includes journal and patent publications from the CAS content Collection for the period 2010-2023.

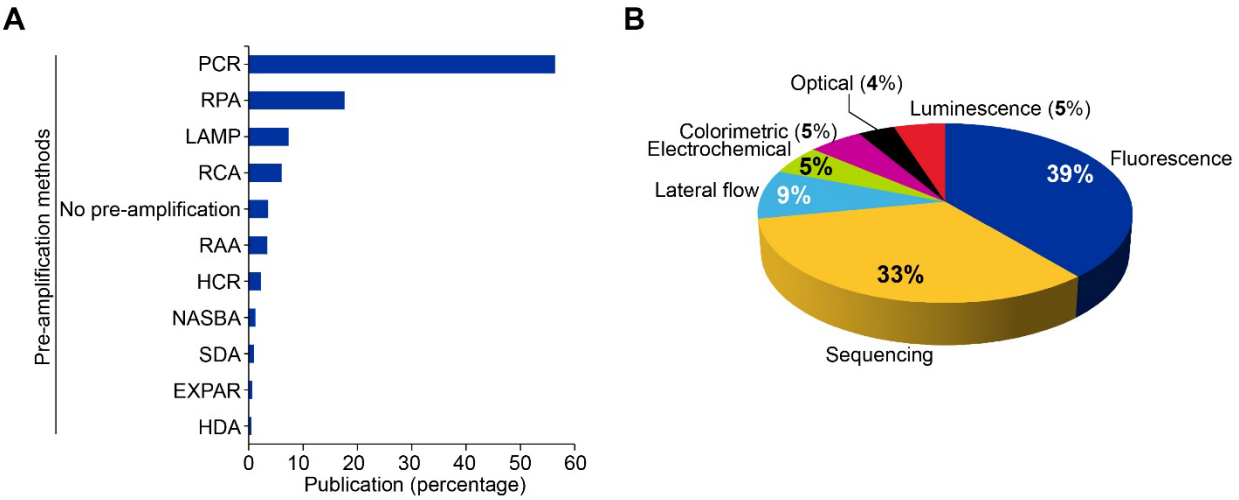


Figure 20. (**A**) Number of journal and patent publications on various preamplification methods used in CRISPR technology-based nucleic acid detection and diagnosis. (**B**) Number of journal and patent publications on methods of readout used for CRISPR-based diagnosis. Data includes journal and patent publications from the CAS Content Collection for the period 2004 to 2023.

Abbreviations used: PCR, polymerase chain reaction; RPA, recombinase polymerase amplification; LAMP, loop-mediated isothermal amplification; RCA, rolling circle amplification; RAA, recombinase-aided amplification; HCR, hybridization chain reaction; NASBA, nucleic acid sequence-based amplification; SDA, strand-displacement amplification; EXPAR, exponential amplification reaction; HAD, helicase-dependent amplification.

Mechanisms of CRISPR/Cas-based Diagnostics

CRISPR/Cas-based diagnostics leverage the precise targeting capabilities of the CRISPR/Cas system, particularly variants such as Cas9, Cas12, Cas13, Cas14 and Cas3, to recognize and bind to a target nucleic acid sequence followed by, cleavage used to generate a detectable signal. The key mechanisms of various CRISPR/Cas-based platforms developed for disease diagnosis have been described in Table 5 and details of individual detection platforms are summarized in Tables S3-S5.

Table 5. Key mechanisms, advantages/drawbacks, and detection platforms of CRISPR/Cas-based disease diagnostics.

Cas protein involved	Mechanism	Advantages / drawbacks	Detection platforms
Cas9	▪ sgRNA directs the Cas9 protein to a specific DNA sequence, where it creates a blunt DSB in DNA in vitro ▪ HNH and RuvC domains cleave complementary and non-complementary strands, respectively	▪ *Drawback*: - Cas9 protein is always used as an independent element to recognize, adhere to, or cut the target sequence, but does not participate in the whole detection process because it cannot easily indicate the result of the reaction without the support of other technologies	▪ **NASBACC**: Nucleic acid sequence-based amplification (NASBA)-CRISPR cleavage ▪ **CAS-EXPAR**: CRISPR/Cas9-triggered isothermal exponential amplification reaction ▪ **FLASH**: Finding low abundance sequences by hybridization) [313] ▪ **FELUDA**: FnCas9 editor linked uniform detection assay (Milenia Biotec) [309]
nCas9	▪ When the nCas9-sgRNA complex binds to the target DNA and forms an R-loop, the single-stranded DNA can be cleaved by the nickase, releasing 30 termini available for further manipulation,[315] which serve as ready substrates for strand extension and displacement reactions	▪ *Advantage:* - Efficient genome engineering with minimal off-target effects[295]	▪ **Cas9nAR**: Cas9 nickase-based amplification reaction[317] ▪ **CRISDA**: CRISPR/Cas9-triggered nicking endonuclease-mediated strand displacement amplification [318]
dCas9	▪ Incorporation of D10A and H840A mutations into Cas9 resulted in the generation of a nuclease-deficient Cas9 (dCas9) protein, which retains its DNA targeting ability while abolishing double-strand break formation and acquiring additional localization functionality[75]	▪ *Advantage:* - The frequency of off-target effects is lower than the conventional CRISPR[296]	▪ **CASLFA**: CRISPR/dCas9-mediated lateral flow nucleic acid assay[319] ▪ **dCas9-DNA-FISH**: CRISPR-mediated DNA-fluorescence in situ hybridization (FISH) [322] ▪ **Paired dCas9** platform[297] ▪ **RCasFISH**: CRISPR/dCas9-MS2-based RNA fluorescence in situ hybridization assay[324] ▪ **PICASSO**: Peptide immobilization by dCas9-mediated self-organization[323]

Cas protein involved	Mechanism	Advantages / drawbacks	Detection platforms
			▪ **Bio-SCAN**: Biotin-coupled specific CRISPR-based assay for nucleic acid detection[320]
Cas12a	▪ A single mature crRNA (40–44 nt in length) guides Cas12a and it binds upstream of a typical thymidine-rich PAM TTTV (where V = A, C and G) and cleaves DNA 18–23 nt distal to the PAM via 5 bp staggered DSBs [80, 325] resulting in DNA sticky ends (cis-acting), and it non-specifically cleaves ssDNA (trans-acting)[298]	▪ *Advantage*: - Potential to be used as a nucleic acid detection system through the incorporation of a ssDNA reporter molecule	▪ **DETECTR**: DNA endonuclease-targeted CRISPR trans reporter [298] ▪ **Cas12aVDet**: Cas12a-based visual detection[328] ▪ **POIROT**: Photo-initiated CRISPR–Cas12a system for robust one-pot testing[298] ▪ **HOLMES**: One-hour low-cost multipurpose highly efficient system[327] ▪ **SCAN**: Solid-state CRISPR/Cas 12a-assisted nanopores [330] ▪ **E-CRISPR**: CRISPR/Cas12a based electrochemical biosensor [331] ▪ **apta-HCR-CRISPR**: Aptamer-hybridization chain reaction- CRISPR[332] ▪ **CaT-SMelor**: CRISPR/Cas12a- and aTF (allosteric transcription factors) -mediated small molecule detector[333] ▪ fDNA-regulated CRISPR-Cas sensor[299] ▪ **UCAD**: Ultrasensitive CRISPR/Cas12a-based antibody detection assay[334]
Cas12b	▪ Recognizes the PAM sequence of 5′- TTN as Cas12a ▪ Upon cleaving DNA, it produces sticky ends and subsequently activates collateral cleavage	▪ *Advantages*: - smaller size of Cas12b makes it more suitable for clinical use - can react over a wide temperature range and pH range with lower off-target effects[337,338]	▪ **HOLMESv2**: One-hour low-cost multipurpose highly efficient system v2[339] ▪ **CDetection**: Cas12b-mediated DNA detection[342]
Cas12c	▪ Cas12c protein functions exclusively as an RNA-induced DNA-binding enzyme while lacking DNase activity ▪ Three types of Cas12c reported so far: Cas12c1, Cas12c2, and OspCas12c ▪ PAM of Cas12c1 and OspCas12c is 5′TG, while that of Cas12c2 is 5′TN[343]	▪ *Advantages*: - broad recognition range different from other Cas12 systems - Cas12c1 exhibited ssDNA trans-cleavage activity equivalent to the Cas12a system - higher sensitivity and specificity for nucleic acid detection compared to Cas12a	▪ **Cas12c-DETECTOR:** Cas12c-based nucleic acid detection platform[300]

Cas protein involved	Mechanism	Advantages / drawbacks	Detection platforms
Cas13	▪ Cas13 system, consists of a single effector, (Cas13) complexed with crRNA without the need for tracrRNA. ▪ Cas13 incorporates nucleotide-binding higher eukaryotes and prokaryotes nucleotide-binding ribonuclease domains enabling it to process precursor crRNA, cleave target RNA, and degrade nonspecific bystander RNA.	▪ *Advantages*: - targets RNA rather than DNA - besides cleaving specific RNA, Cas13 can target arbitrary RNA (collateral cleavage) when it is activated providing a new solution for nucleic acid detection - the Cas13 system, guided by crRNA, stands out for its ability to target RNA sequences without the need for PAMs, thereby broadening its range of targetability[84, 301]	▪ **SHERLOCK**: Specific high-sensitivity enzymatic reporter unlocking[348] ▪ **SHERLOCKv2**[299] ▪ **HUDSON**: Heating unextracted diagnostic samples to obliterate nucleases ▪ **CARMEN**: Combinatorial arrayed reactions for multiplexed evaluation of nucleic acids[350] ▪ **DESCS**: Dual methylation-sensitive restriction endonucleases coupling with an RPA-assisted CRISPR/Cas13a system[352] ▪ **CrisprZyme**: CRISPR–Cas-based reaction with a nanozyme-linked immunosorbent assay[351] ▪ **PADLOCK-CRISPR**: Picoinjection aided digital reaction unLOCKing [353] ▪ **APC-Cas**: Allosteric probe initiation catalysis and CRISPR/Cas13a[355] ▪ **SPRINT**-SHERLOCK-based profiling of *in vitro* transcription[354] ▪ **CLISA**: CRISPR/Cas13a signal amplification linked immunosorbent assay[358]
Cas14 (Cas12f)	▪ Cas14 binds to its target ssDNA and activates a collateral cleavage mechanism similar to Cas12 and Cas13	▪ *Advantages*: - detect and cleave ssDNA without requiring the presence of a PAM[360] - they are significantly more compact (400 to 700 amino acids) than Cas12a (about the half size) - Cas14, unlike Cas12, has lower tolerance to nucleotide mismatches between sgRNA and the target template, which greatly reduces on-target activity of Cas14 allowing use of Cas14 to detect SNPs in DNA[302]	▪ **HARRY**: Highly sensitive aptamer-regulated Cas14 R-loop for bioanalysis[363]
Cas3	▪ Operates with a CRISPR-associated complex called Cascade[364]	▪ *Advantage*: - can cleave long stretches of the target DNA, unlike Cas9 or Cas12, which only create DSBs	▪ **CONAN**: Cas3-operated nucleic acid detection[297]

Cas protein involved	Mechanism	Advantages / drawbacks	Detection platforms
	▪ Cascade complex first recognizes the target DNA based on sequence complementarity between the target and the crRNA ▪ Upon recognition, Cas3 is recruited to the site and degrades the target DNA in a helicase-nuclease fashion[365]	▪ *Drawback:* - requires multiple Cas proteins	

CRISPR: Delivery systems

The ability to target and modify specific genomic sequences holds promise for treating a myriad of genetic disorders, from monogenic diseases to complex, multifactorial conditions. In practice, however, CRISPR-based therapeutics must enter the desired cells without eliciting an unwanted immune response, so a delivery system is required. Thus, despite its transformative potential, the therapeutic application of CRISPR faces significant challenges, particularly in the realm of delivery systems.[303-305] Effective and safe delivery of CRISPR components — such as the Cas9 nuclease and sgRNA) — to target cells and tissues, is paramount for achieving desired therapeutic outcomes while minimizing off-target effects and immune responses. The choice of delivery method can significantly influence the efficiency, specificity, and safety of CRISPR-mediated gene editing.

Carriers currently used for delivery of gene editing system cargo fall into three general groups: (i) viral vectors, (ii) non-viral vectors, and (iii) physical delivery (Figure 21).[306-310] Viral vectors have been extensively studied and utilized due to their high efficiency in delivering genetic material. Among them, adeno-associated viruses (AAVs), lentiviruses, and adenoviruses are the most used. AAVs are particularly favored for their low immunogenicity and ability to infect both dividing and non-dividing cells, making them suitable for a wide range of tissues. Lentiviruses, derived from HIV-1, can integrate into the host genome, providing long-term expression of the CRISPR components. However, the potential for insertional mutagenesis remains a concern. Adenoviruses offer transient expression and can carry larger genetic payloads, but their high immunogenicity can limit their use in clinical settings. The unfavorable effects of the viral vectors such as genome integration, immunogenetic responses and limited cargo loading impede the further clinical applications.[311, 312]

Non-viral vectors, including lipid-, polymer-, or metal-based nanocarriers and cell-penetrating peptides (CPPs), offer an alternative approach in CRISPR delivery. Although considered not as prominent as viral-based delivery vectors, they possess the advantages of lower immunogenicity and toxicity, and huge cargo size, and are a proliferating area of research.[306, 313, 314]

Negatively charged nucleic acids can be electrostatically complexed to cationic materials with the complexes subsequently endocytosed by cells. The most successful classes of cationic materials applied so far for nucleic acid delivery are lipids, e.g., rationally designed lipids and lipid-like materials, and naturally occurring and synthetic polymers. Ideally, any non-viral delivery material for genome editing should be well tolerated – biocompatible, non-immunogenic, and capable of delivering payloads to the nucleus.[315]

Thus, lipid-based nanoparticles can encapsulate CRISPR components and facilitate their delivery into cells via endocytosis. Polymer-based systems, such as polyethyleneimine (PEI) and poly(lactic-co-glycolic acid) (PLGA) nanoparticles, provide customizable platforms for delivering CRISPR payloads with controlled release profiles. Nanoparticles offer unique advantages in terms of size, surface modification, and targeting capabilities. These nanocarriers can enhance cellular uptake and provide protection for CRISPR components from degradation. Exosomes, which are naturally occurring extracellular vesicles, have garnered interest due to their inherent

biocompatibility and ability to mediate intercellular communication. Engineering exosomes to deliver CRISPR components holds promise for achieving targeted and efficient gene editing with minimal immunogenicity.[316-318]

In some cases, delivery vectors are not necessary for genome editing. In *ex vivo* therapies, mechanical intervention can create transient holes in cell membranes, allowing nucleic acids and proteins to enter the cell. The most common physical delivery methods include microinjection and electroporation/sonoporation, while methods such as hydrodynamic delivery are currently under development. Optimization of the *in vivo* CRISPR delivery still faces multiple challenges, including encapsulation of large size CRISPR system, targeted delivery, and enhanced endocytosis.[319-321] In addition to gene editing, CRISPR systems have been developed for delivery of drugs, such as doxorubicin – e.g., CRISPR-dCas9.[322] Thus, based on the potent functions of CRISPR system for disease correction, efficient *in vivo* delivery systems are urgently needed.

With regards to CRISPR/Cas9 cargoes, three forms have been explored: (i) **plasmid** DNA encoding both Cas9 protein and the sgRNA; (ii) a mixture of Cas9 **mRNA** and a separate sgRNA; and (iii) a mixture of Cas9 protein and the sgRNA (Cas9 **ribonucleoprotein**, **Cas9 RNP**) (Figure 21).[323-325] It is now widely believed that the safest delivery method for CRISPR is to deliver it as a complete RNP. By delivering the Cas enzyme and gRNA as a preformed RNP complex, the amount of time of the complex spent in the cells is reduced, minimizing the risks of triggering an immune response or off-target editing of the genome.[326]

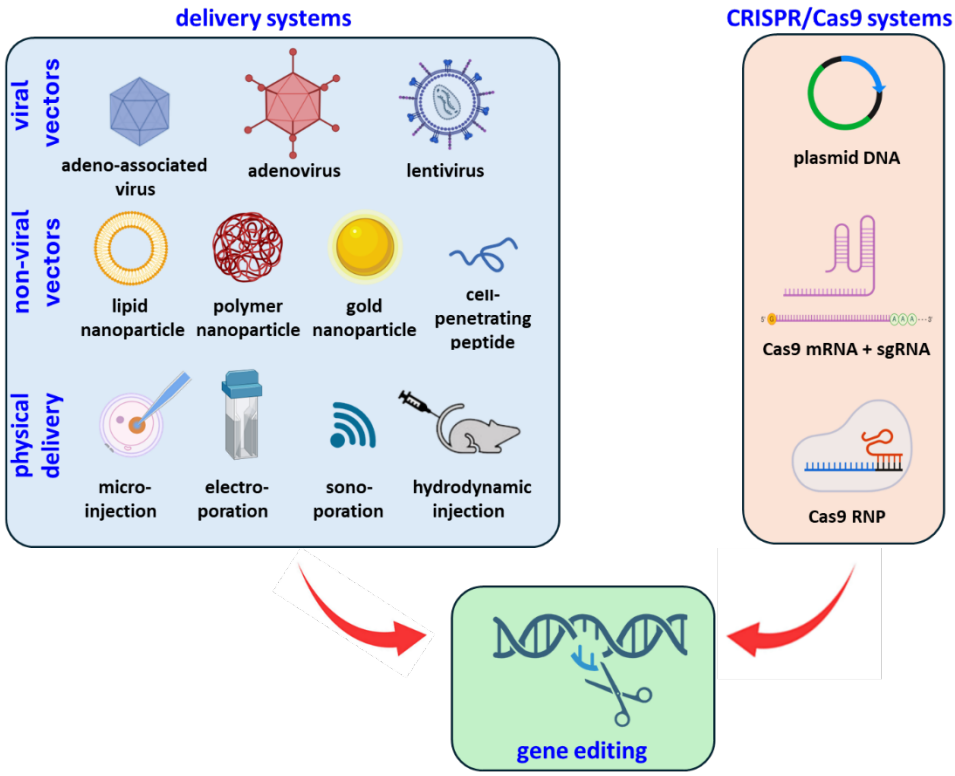


Figure 21. Scheme of the CRISPR/CAS9 delivery. Partially created with www.BioRender.com.

An outline of the various delivery systems for CRISPR therapeutics is summarized in Table 6.

Viral vectors

- **Adeno-associated viruses (AAVs)** are small viruses that infect humans and some other primate species. They are not known to cause disease and have a low immune response, making them suitable for gene therapy. AAVs can deliver genes by infecting cells and inserting the therapeutic gene into the cell's DNA. The limited cargo size is a significant challenge, often necessitating the use of smaller Cas9 variants or split Cas9 systems. Non-pathogenic, low immunogenicity, limited cargo capacity (~5 kb), stable expression in non-dividing cells.

- **Lentiviruses** are a type of retrovirus that can integrate their genetic material into the host cell genome, enabling long-term expression. They can infect both dividing and non-dividing cells and have a larger cargo capacity than AAVs, accommodating full-size Cas9. However, their integration into the host genome raises concerns about insertional mutagenesis and oncogenesis. High transduction efficiency, larger cargo capacity (~8 kb), long-term expression, potential safety risks due to genome integration.

- **Adenoviruses** are common viruses that cause mild infections in humans. They can deliver large DNA sequences and do not integrate into the host genome, which reduces the risk of insertional mutagenesis. However, they can elicit strong immune responses, which can be problematic for repeated treatments. Large cargo capacity (~8-10 kb), high efficiency, transient expression, potential for strong immune responses.

Non-viral vectors

- **Lipid nanoparticles (LNPs)** are tiny vesicles composed of lipids that can encapsulate nucleic acids, such as mRNA or small interfering RNA (siRNA), protecting them from degradation and facilitating cellular uptake. LNPs are widely used for delivering RNA-based CRISPR components and have been proven effective in recent mRNA vaccines. Protects RNA, facilitates uptake, low immunogenicity, potential toxicity at high doses.

- **Polymeric nanoparticles** are made from biodegradable polymers and can carry DNA, RNA, or protein cargoes. They can be engineered to release their payloads in a controlled manner, targeting specific cells or tissues. Their versatility allows for customization in design and functionality. Versatile, can carry various cargo types, tailored release profiles, potential toxicity.

- **Cell-penetrating Peptides (CPPs)** are short peptides that facilitate the delivery of various molecules, including nucleic acids and proteins, across cell membranes. They are versatile and can be conjugated with different cargoes, though their efficiency can vary. Can deliver a variety of cargoes, minimal toxicity, variable efficiency.

- **Gold nanoparticles** can be functionalized with nucleic acids and are used for their stability and ease of modification. They can deliver CRISPR components into cells effectively but are expensive and may be toxic at high concentrations. Biocompatible, easily functionalized, effective delivery, high cost.

Physical methods

- **Electroporation** involves applying an electric field to cells to create temporary pores in their membranes, allowing CRISPR components to enter. This method is highly efficient but can cause significant cell damage and is less suitable for in vivo applications. High efficiency, applicable to various cell types, potential cell damage.

- **Microinjection** involves directly injecting CRISPR components into individual cells using a fine needle. This method is precise and commonly used for creating genetically modified embryos but is labor-intensive and not scalable. Highly precise, suitable for single-cell applications, labor-intensive.

- **Hydrodynamic injection** involves rapidly injecting a large volume of solution into the bloodstream, usually targeting the liver. This creates transient pores in cell membranes, allowing CRISPR components to enter. It is mainly used in animal models. Simple, efficient for liver, potential tissue damage.

- **Particle bombardment (gene gun)** uses high-velocity particles (gold or tungsten) coated with CRISPR components to deliver them into target cells. When the particles penetrate the cell membrane, they deliver the CRISPR cargo directly into the cytoplasm. This is effective for plant cells and has some applications in mammalian tissues. Good for hard-to-transfect cells, potential cell damage, lacks precision.

- **Sonoporation** involves ultrasound waves creating temporary pores in the cell membrane, facilitating the uptake of CRISPR components. It has been used experimentally in tissues like muscle and tumor tissues. It has also shown promise in delivering therapeutics across the blood-brain barrier. Non-invasive, limited to tissues accessible by ultrasound.

Figure 22 shows the distribution of the documents related to the various types of CRISPR delivery systems in the CAS Content Collection. The largest part concern viral vectors, with adeno-associated vectors being most represented. From the physical delivery methods, electroporation and microinjection are largely represented.

Figure 23 represents a heatmap showing the relative co-occurrences of diseases targeted by CRISPR and the delivery vectors utilized. Here are some key takeaways:

- In general, viral vectors (AAV, lentivirus, adenovirus) and some non-viral vectors (LNPs, polymer nanoparticles) have been explored more than other methods of delivery.
- Among the physical methods of delivery, electroporation co-occurs to a higher extent as compared to all other methods for most diseases except for liver diseases.
- Some of the highest correlations are between ocular diseases and AAV, cancer and lentiviral vectors, and liver and cardiovascular diseases and lipid nanoparticles.

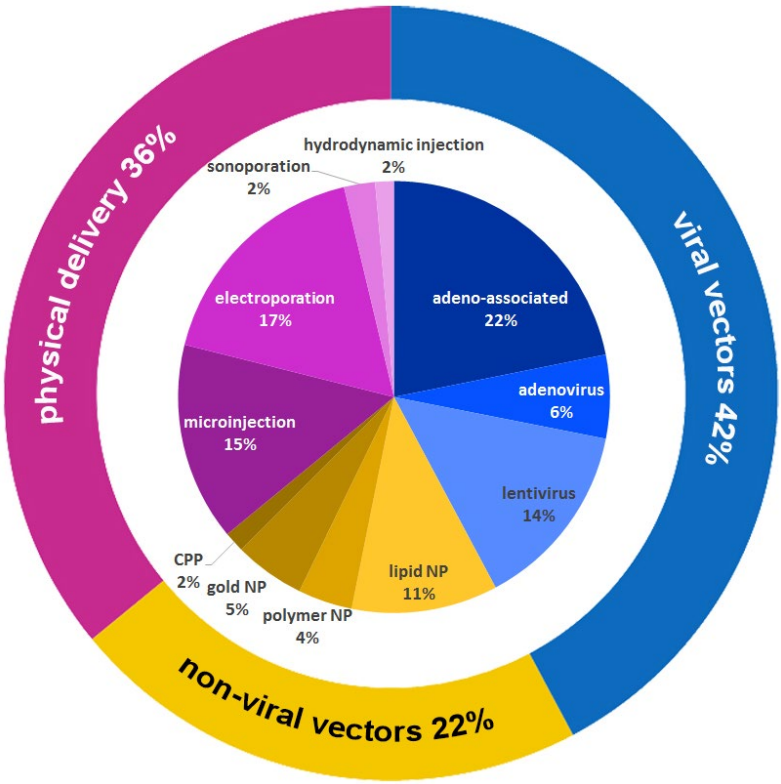


Figure 22. Distribution of the documents related to the various types of CRISPR delivery systems in the CAS Content Collection. Data includes journal and patent publications from the CAS Content Collection for the period 1995-2024.

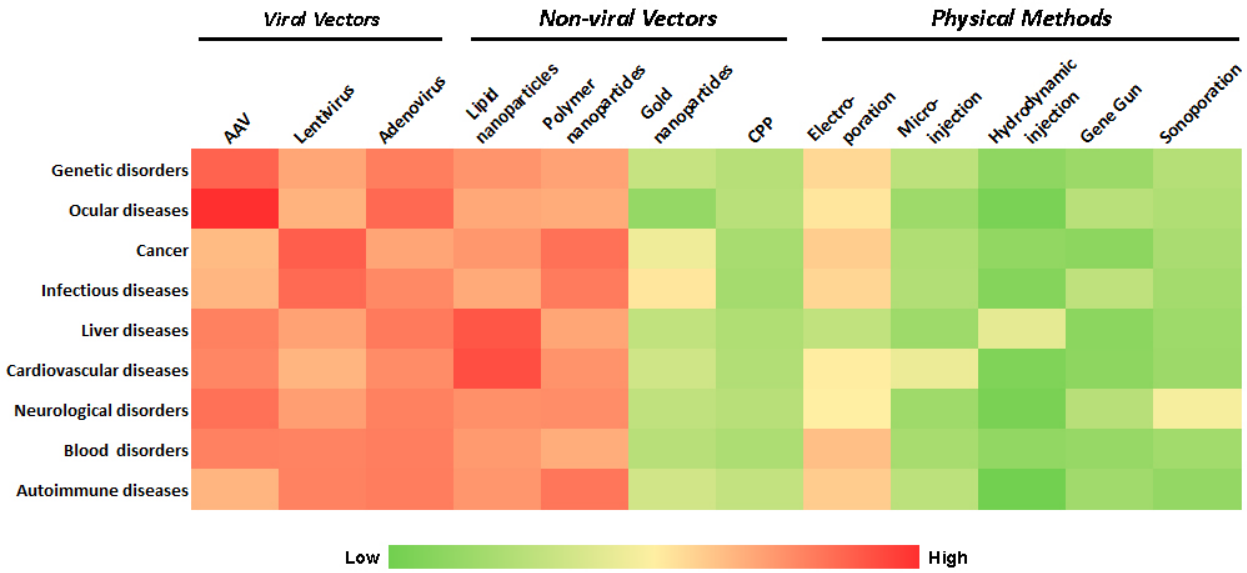


Figure 23. Heatmap showing relative co-occurrences of diseases targeted by CRISPR and the delivery vectors. Listed here are diseases included within each of the broader categories:

Genetic disorder - sickle cell disease, β-thalassemia, and cystic fibrosis, Duchenne muscular dystrophy, and Tay-Sachs disease; ocular diseases - Leber congenital amaurosis and retinitis pigmentosa; infectious diseases - HIV/AIDS and hepatitis B; liver diseases - antitrypsin deficiency and hereditary tyrosinemia; cardiovascular diseases - familial hypercholesterolemia and hypertrophic cardiomyopathy; neurological disorders - fragile X syndrome, autism spectrum disorders, amyotrophic lateral sclerosis, Huntington's disease and Alzheimer's disease; blood disorders - sickle cell disease and β-thalassemia. Data includes journal and patent publications over the period 1995-2024 from the CAS Content Collection. (AVV, adeno-associated virus; CPP, cell-penetrating peptide).

Table 6. Delivery systems/vectors for CRISPR therapeutics.

Delivery system / vector	Advantages	Disadvantages	Mechanism	Applications	Example
Viral Vectors					
Adeno-Associated Virus (AAV)	High transduction efficiency, low immunogenicity, ability to infect non-dividing cells	Limited packaging capacity (~5 kb), potential for pre-existing immunity	AAV vectors deliver CRISPR components by infecting target cells, where the viral DNA is expressed	Used in gene therapy for treating genetic disorders such as Duchenne muscular dystrophy and hemophilia	Luxturna (voretigene neparvovec): The first FDA-approved gene therapy for treating inherited retinal disease. Uses AAV to deliver a functional copy of the RPE65 gene.[327-329]
Lentivirus	Ability to integrate into the host genome, large packaging capacity (~8 kb), stable expression	Risk of insertional mutagenesis, potential for long-term effects	Lentiviral vectors integrate CRISPR components into the host genome, ensuring stable expression	Suitable for long-term gene therapy applications, such as treating HIV or genetic blood disorders	CAR-T Cell Therapy: Lentiviral vectors are used to modify T cells to express CARs for cancer immunotherapy.[330, 331]
Adenovirus	High transduction efficiency, large packaging capacity (~36 kb)	High immunogenicity, transient expression	Adenovirus vectors deliver CRISPR components as episomal DNA, resulting in transient expression	Used for transient gene editing applications and cancer gene therapy	Clinical trials for muscular dystrophy: Adenovirus vectors are being investigated for delivering CRISPR components to correct mutations in the dystrophin gene in muscle cells.[332-335]
Non-Viral Vectors					
Lipid Nanoparticles (LNPs)	Low immunogenicity, ability to carry large cargoes, adaptable for mRNA delivery	Potential for off-target effects, need for optimization of lipid composition	LNPs encapsulate CRISPR components, facilitating cellular uptake and endosomal escape	Widely used for mRNA-based CRISPR delivery, such as in liver-targeted therapies	Moderna's mRNA vaccine platform: while primarily used for mRNA vaccines, LNPs are also being explored for delivering CRISPR components for gene editing applications.[336-338]
Polymeric Nanoparticles	Versatility in design, ability to encapsulate various types of nucleic acids, biodegradable	Potential for toxicity, need for extensive optimization	Polymeric nanoparticles encapsulate CRISPR components and release them in a controlled manner	Used for sustained release applications and targeting specific tissues	Cationic polymers for gene editing: Researchers are developing PNPs based on cationic polymers like PEI to

					deliver CRISPR/Cas9 plasmids for cancer therapy.[339-341]
Gold Nanoparticles	High stability, ease of functionalization, low toxicity	Limited cargo capacity, need for complex surface modification	Gold nanoparticles are functionalized with CRISPR components for cellular uptake	Used in precision medicine for targeted gene editing in cancer cells	Targeted cancer therapy: AuNPs conjugated with CRISPR components are being investigated for targeted gene editing in cancer cells to knock out oncogenes.[308, 342-344]
Cell-Penetrating Peptides (CPPs)	Ability to deliver cargo directly into the cytoplasm, minimal toxicity	Limited cargo size, potential for off-target delivery	CPPs facilitate the direct delivery of CRISPR components into the cytoplasm	Used for intracellular delivery of nucleic acids and proteins	Tat peptide for protein delivery: The HIV-1 Tat peptide is used to deliver Cas9 protein and sgRNA into cells for efficient gene editing in vitro.[345-347]
Physical Methods					
Electroporation	High efficiency, ability to transfect a variety of cell types	Potential for cell damage, limited in vivo applicability	Electrical pulses create pores in the cell membrane, allowing CRISPR components to enter	Used for ex vivo gene editing in cell therapy applications	CRISPR-edited T cells for cancer immunotherapy: Electroporation is used to introduce CRISPR components into T cells ex vivo to knock out PD-1, enhancing their anti-tumor activity.[348-350]
Microinjection	High precision, direct delivery into the nucleus or cytoplasm	Labor-intensive, not suitable for high-throughput applications	Direct injection of CRISPR components into cells using a fine needle	Used in research for precise gene editing in embryos and zygotes	Gene editing in mouse embryos: Microinjection of CRISPR/Cas9 components into mouse zygotes is used to create genetically modified mice for research.[351-353]
Hydrodynamic Injection	Simple technique, effective for delivering plasmids to the liver	Limited to certain tissues, potential for tissue damage	Rapid injection of a large volume of CRISPR components into the bloodstream, creating transient pores in endothelial cells	Used primarily for liver-targeted gene therapy	Liver-specific gene editing: Hydrodynamic injection of CRISPR plasmids into mice for liver-specific gene editing to study metabolic diseases.[354, 355]

Gene Gun (Biolistics)	Can penetrate cell walls, effective for plant cells. Allows for direct delivery to tissues.	Potential tissue damage. Variable efficiency and low cell viability. Limited to accessible tissues.	The gene gun propels microscopic gold or tungsten particles coated with CRISPR components into target cells using a high-velocity helium pulse.	Primarily used for plant cells, but also applicable to certain animal tissues and cells. Useful for in vivo applications where other methods are less effective.	Plant genetic engineering: Biolistic delivery of CRISPR/Cas9 plasmids into plant cells to generate genetically modified crops with desired traits.[356-358]
Ultrasound (Sonoporation)	Non-invasive and can be targeted to specific tissues. Enhances membrane permeability.	Requires optimization to avoid tissue damage. Variable efficiency. Limited to certain tissues.	Ultrasound waves create cavitation bubbles that disrupt cell membranes, allowing CRISPR components to enter the cells.	Used for both in vitro and in vivo gene editing. Potential applications include targeted delivery to tumors and other tissues.	Targeted cancer gene therapy: Sonoporation is used to enhance the delivery of CRISPR components to tumor cells in animal models for gene knockdown studies.[359-361]
Laser-Induced Poration	High precision and control. Minimal invasiveness.	Requires specialized equipment. Potential thermal damage to cells and tissues. Limited throughput.	Lasers create transient pores in the cell membrane, facilitating the entry of CRISPR components.	Used for precise delivery to specific cells or tissues in research settings. Potential for applications in dermatology and ophthalmology.	Dermatology applications: Laser-induced poration is used to deliver CRISPR/Cas9 components into skin cells for potential treatments of skin disorders.[362-364]

CRISPR in Agriculture

CRISPR/Cas is also considered as the leading site-specific nuclease for plant genome editing, first reported in 2013 by Li *et al.*,[365] Shan *et al.*,[366] and Nekrasov *et al.*[367] Not only is it useful to develop crops that are high yielding and resistant to abiotic stresses (drought, salinity, flooding, etc.) exacerbated by climate change, but also to resist biotic stresses such as insects and pathogens that are also being exacerbated by climate change. Our original search query resulted in a considerable number of documents, specifically around 13%, related to the application of CRISPR in plants and for agricultural use. The use of CRISPR in agriculture is beyond the scope of this manuscript and the related documents were filtered out of our dataset. For more information on the application of CRISPR for the enhancement of disease and parasitic plant resistance in crops and/or for climate change resilience we suggest reviews by: Jhu *et al.*,[368] Paul *et al.*,[369] Khan *et al.*,[370] Zaidi *et al.*,[371] Karavolias *et al.*,[372] Ndudzo *et al.*,[373] Misra *et al.*,[374] Schenke and Cai,[375] Ahmad *et al.*,[376] and Maximiano and Franco.[377]

AI in CRISPR

With the recent and ongoing surge in artificial intelligence (AI) and its application in a wide range of fields, interest in using AI in CRISPR has also seen an increase as exhibited by the growth of publications over the last decade (Figure 24).

Discussed briefly below are a few examples of AI models developed for CRISPR:

- DeepCas9,[378] a deep convoluted neural network (CNN) model consisting of a combination of several other deep-learning models and capable of predicting on-target activity of sgRNAs based on DNA sequences potentially allowing high-throughput screening of sgRNAs.
- OpenCRISPR-1,[379] a protein language trained on several components of naturally occurring CRISPR systems with an emphasis on Class 2 systems (specifically the Types II system). The model was capable of designing diverse novel Cas9-like proteins not found in nature along with functional sgRNAs for said novel Cas9-like proteins. The model was generated using ProGen2, a protein large language model,[380] and trained using the CRISPR-Atlas (collection of Cas proteins, CRISPR arrays, tracrRNAs and PAMs from microbial genome).
- TIGER (Targeted Inhibition of Gene Expression via guide RNA design)[381] – A CNN-based model developed for predicting on- and off-target efficacy of Cas13d gRNAs. Potential applications of the TIGER model include reducing undesirable off target effects thereby improving safety profile of CRISPR therapeutics.
- EVO,[382] a genomic foundational model trained on >82,000 CRISPR/Cas loci capable of producing unique Cas proteins (Cas9, Cas12 and Cas13).
- Other models/tools include: CRISPick,[383-385] sgRNA Scorer,[386, 387] SSC,[388, 389] DeepCRISPR,[390, 391] CRISTA,[392] CRISPR MultiTargeter,[393, 394] DeepHF,[395, 396] CRISPR-A-I,[397] CRISPR-P[398, 399] and many more.

For a comprehensive review on the use of AI in CRISPR please see Dixit *et al.*[400] and Lee.[401]

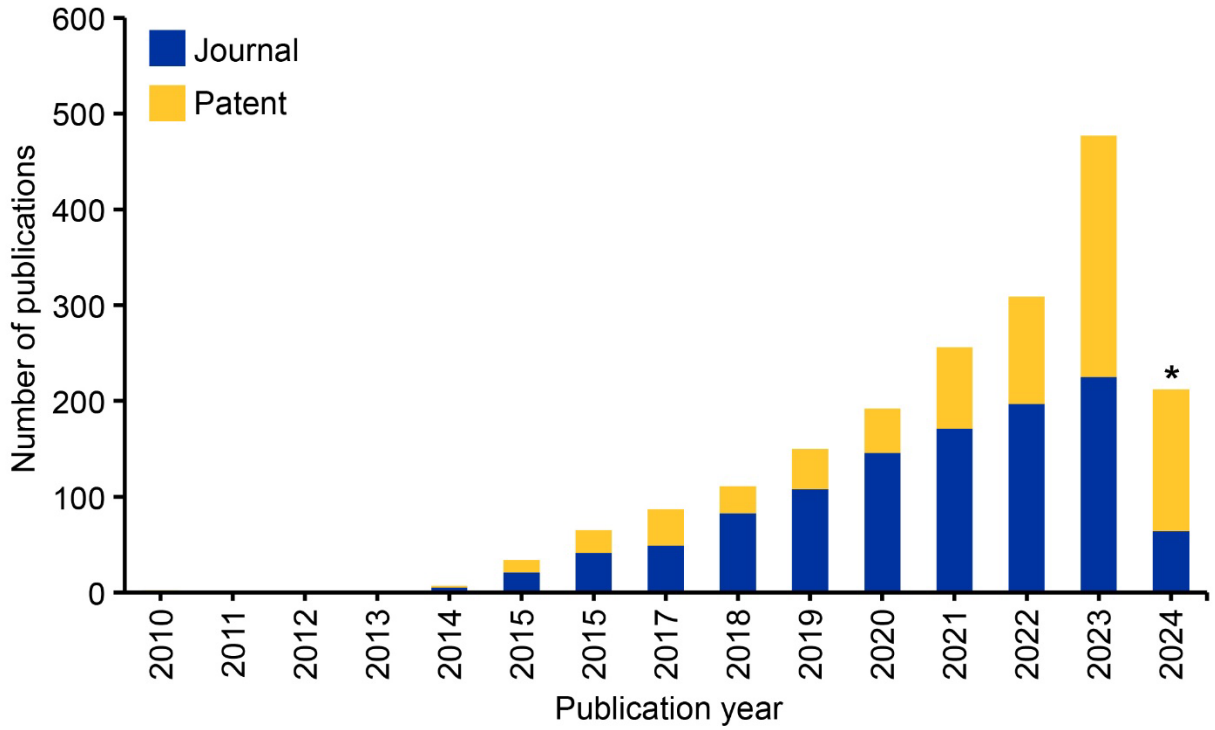


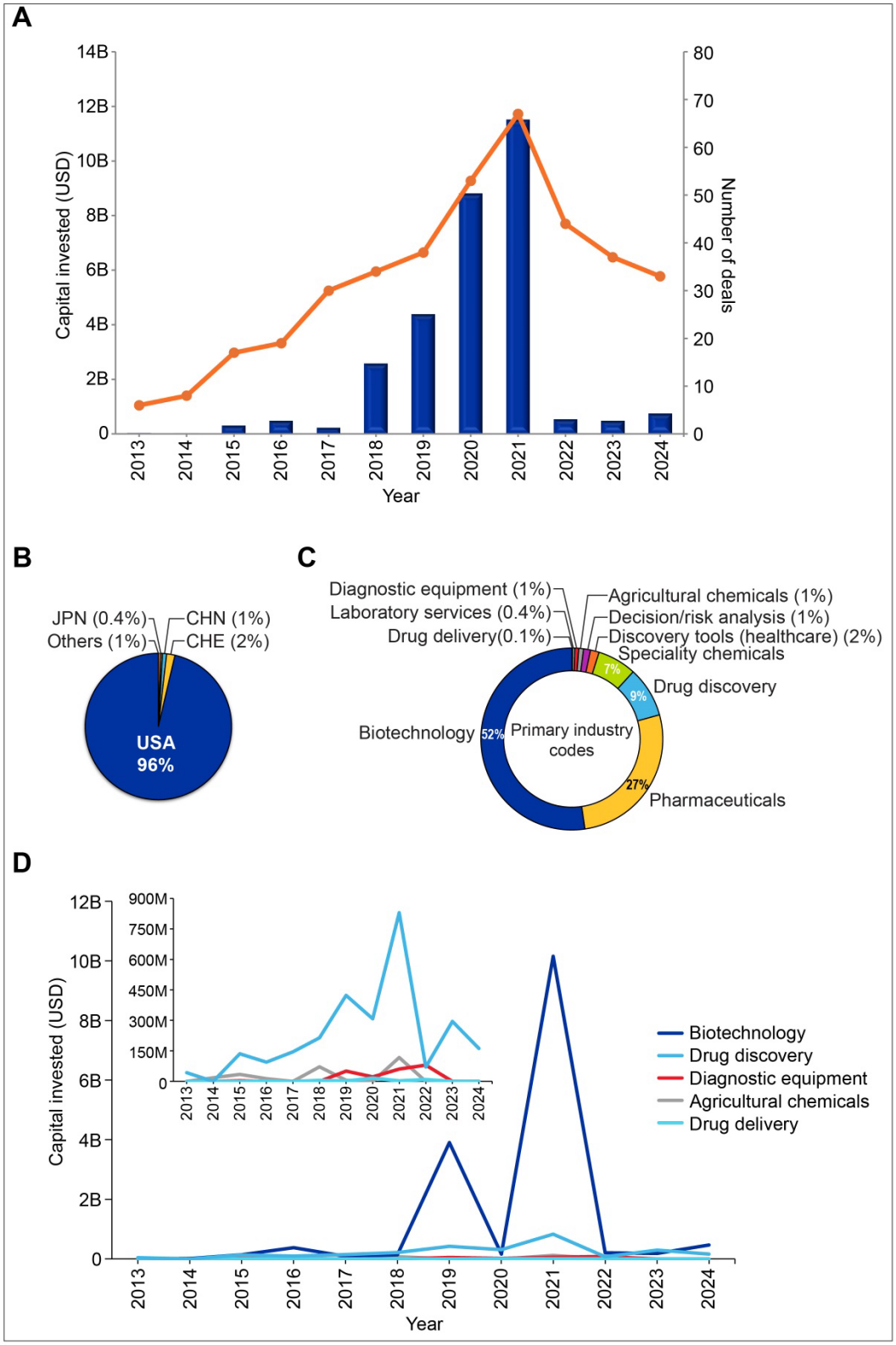
Figure 24. Time trends of publications related to artificial intelligence (AI) in the CRISPR dataset. Data includes journal and patent publications from the CAS Content Collection for the period 2010-2024. *Note that data for 2024 is incomplete due to time of data extraction and encompasses data for January to June.

Commercial Interest in CRISPR

In the past decade, capital investment in the field of CRISPR technology has seen a remarkable increase with a sharp increase starting in 2018 and persisting until 2021 with investments exceeded a staggering 11 billion USD in 2021 (Figure 25A). An overwhelming majority of these investments involved companies originating in the United States (USA, 96%). Other key players in terms of geographical distribution, though of much smaller magnitude, included Switzerland (CHE), China (CHN) and Japan (JPN) (Figure 25B).

Breakdown of the capital invested by primary industry code, a system of classification indicative of a business's main line of revenue, shows that as expected biotechnology, pharmaceuticals and drug discovery, together account for almost 90% of capital invested (Figure 25C). Other industries with smaller contributions include agricultural chemicals, diagnostic equipment and laboratory services among others (Figure 25C). This is in line with our analysis which also identified CRISPR therapeutics and CRISPR in agriculture as the major applications. Time trends of capital invested in these sectors indicate largest influx of capital in the biotechnology industry with two major spikes in 2019 and 2021. A record number of deals occurred in 2021 and few of the biggest (in terms of deal size) involved Century Therapeutics and Mammoth Biosciences (>150M USD) and, Caribou Biosciences and AgBiome (>100M USD). The most consistent influx in capital on the other hand appears to be in drug discovery until 2021 with a plunge in 2022 and uptick again after 2022 (Figure 25D).

A few key players in the biotechnology space with a high number of patents related to CRISPR are: CRISPR Therapeutics, Caribou Biosciences and Intellia Therapeutics, while those in pharmaceuticals and drug discovery space are Regeneron Pharmaceuticals, Editas Medicine, Beam Therapeutics and Metagenomi, among many others. Regeneron Pharmaceuticals, most well-known for its AAV vectors for gene delivery, in collaboration with Intellia Therapeutics is actively pursuing development of CRISPR/Cas9-based therapeutics for hemophilia A and B,[402] a type of rare bleeding disorder.[403] Regeneron Pharmaceuticals appears to also be engaged in collaboration with Mammoth Biosciences[404] co-founded by the Nobel Laureate and CRISPR pioneer Jennifer Doudna.



Source: PitchBook Data, Inc.

Figure 25. Commercial interest in CRISPR technology. (**A**) Capital invested in and number of deals in CRISPR. (**B**) Geographical distribution of capital invested in CRISPR. (**C**) Breakdown of capital invested as per primary industry codes and (**D**) their time trends over the last decade (2013-2024).

Ethics

Doudna, one of the inventors of the CRISPR technology, expressed in the 2016 American Association for the Advancement of Science Annual Meeting that one of her biggest fears is "waking up one morning and reading about the first CRISPR baby, and having that create a public backlash where people ban or regulators shut this down, and I think that could be very detrimental to the progress of the field."[405] In 2018, her fears were realized when Chinese researcher He Jiankui claimed that he used CRISPR to alter the DNA of seven embryos of couples where the males were HIV carriers to immunize the babies against the HIV virus. This resulted in the birth of two twin girls, the first CRISPR babies.[406, 407]

Beauchamp and Childress proposed four main principles of biomedical ethics: beneficence, nonmaleficence, respect for autonomy, and justice.[408] In summary: proposed "treatment" should result in a positive outcome/benefit (beneficence), avoid or minimize harm as much as possible (nonmaleficence), patients should not be treated without informed consent (autonomy), and equitable access to treatment (justice). When looking at applications and study of CRISPR/Cas genome editing, researchers should take these principles into consideration.[409] For example, under beneficence and nonmaleficence is the risk of unwanted effects such as genomic off target activity, immune response, age related or disease related challenges that should be considered,[410, 411] and natural genetic diversity that could alter on-target and off-target outcomes.[412, 413] Under justice an argument is the equitable distribution and accessibility of these expensive, but potentially lifesaving therapies.[414] In the case of autonomy there is the argument of embryonic and gamete targeting vs somatic cell targeting. There is less ethical argument when it comes to targeting somatic cells, but the possible human beings that result from any embryonic/gamete genetic modification would lack informed consent as the decision to be modified was not made by them yet would have to live with the consequences of the modification throughout their life.[415, 416]

Other ethical concerns are legal regulations, the use of the technology at home by communities without medical supervision (biohackers),[409] and the use of CRISPR for non-therapeutic purposes like enhancements, eugenics, and even gene terrorists. A survey of laws, regulations and governance principles on genome editing in humans was also published by the Scientific Foresight Unit of the European Parliamentary Research Service in 2022.[417] For more information and outlook on the ethical issues regarding the application of CRISPR technologies we suggest publications by Gonzalez-Avila *et al.*,[409] Lorenzo *et al*.,[415]Brokowski and Adli,[418] and Nada Kubikova *et al.*[419] as well as news articles and interviews published by NPR,[420] MIT Technology Reviews,[421] and the Harvard Gazette.[422]

Challenges

Despite the wide acceptance of CRISPR technology in gene editing owing to its versatility and ease of use, there remain certain challenges associated with it.

- ***Off-target effects:*** In natural setting, CRISPR/Cas systems tolerate mismatches between the gRNA and the target to a certain extent. This is a likely evolutionary consequence to overcome the high mutational rate of phages. However, this property is unsought for

genome engineering applications, as it may result in the targeting and editing of off-target sites. Numerous studies have reported off-target activity at sites ranging from a single base mismatch to sites containing multiple consecutive mismatches, or even nucleotide insertions or deletions.[423-426] Regardless of the mismatch tolerance of CRISPR/Cas9, most potential off-target sites do not result in dsDNA cleavage and gene editing. This might be due to existing intrinsic checkpoints in the DNA binding and cleavage mechanisms of Cas9.[97, 427, 428] Notably, high-throughput profiling studies exploring off-target effects have shown that their frequency is consistently lower *in vivo* as compared with isolated genomic DNA.[429, 430]

- ***PAM requirement:*** Another limitation of the technology is the requirement for a PAM near the target site, which restricts its targeting scope. SpCas9 is one of the most extensively used Cas9s with a relatively short PAM recognition site - 5′NGG3′ (N is any nucleotide). Theoretically, SpCas9 permits finding a suitable target site every 8 nucleotides on an average throughout the genome. However, some genomic regions are not easily targetable by SpCas9 due to a high A/T content. Several naturally occurring orthologs of Cas9 with alternative PAM specificities have been identified and adopted for gene editing; however, many of these have even more limiting PAM requirements.[431-433]
- ***Packaging and delivery:*** *In vivo* delivery of CRISPR/Cas9 into mammalian cells is generally accomplished using viral vectors. AAVs remain the preferred choice due to their low immunogenicity and high transduction efficiency. However, AAVs have limited packaging capacity and hence, it is difficult to package the genes encoding most used Cas9 (SpCas9) and its associated sgRNA into a single AAV vector unless compact promoters are used.[434, 435] Another limiting factor for most gene editing components is their safe, efficient, and targeted delivery to the specific organ or tissue. If CRISPR/Cas9 components are delivered *in vivo* via systemic approach, they can get degraded by circulating proteases or nucleases or get cleared by the mononuclear phagocyte system. Furthermore, other factors such as vascular permeability, diverse endocytosis mechanisms, and lysosomal degradation can result in variable efficacy, which may eventually result in suboptimal therapeutic outcomes.[436]
- ***DNA-damage toxicity:*** CRISPR-based gene editing relies on introduction of DSBs which can trigger apoptosis and growth inhibition rather than the intended gene edit.[437] Additionally, large deletions spanning few kilobases/megabases and complex genetic rearrangements have been reported in several studies highlighting a major biosafety issue for clinical applications of CRISPR therapy.[438, 439] Furthermore, multiple simultaneous off-target edits can ultimately result in genomic rearrangements such as inversions, deletions, and chromosomal translocations and trigger DNA damage and stress response pathways.[438, 440, 441]
- ***Immunotoxicity:*** Immunogenic toxicity is a known limitation of any gene editing technology, including CRISPR. Pre-existing antibodies against Cas9 and reactive T cells have been identified in humans and Cas9 immunity has been associated with compromised therapeutic outcomes in various disease models.[442-445]
- ***Regulatory hurdles:*** Different countries have varying regulations regarding CRISPR based gene editing and in some countries the guidelines are still under development. Also, in some countries one regulatory agency oversees gene therapy while other agencies

regulate genetically modified organisms, and this creates complex regulatory process for CRISPR based therapeutics. Additionally, the long-term effects and safety of these therapeutics are not yet fully understood. All of these factors may contribute to lengthy and complex approvals of CRISPR based therapeutics.

Conclusions and Looking Ahead

Since the first use of CRISPR-based gene editing, the field has evolved at an exceptional pace exhibting an average growth in publications of 54% in the last decade (2014-2023). This sustained and extensive interest has resulted in a plethora of publications exploring the use of CRISPR in treating hard to treat/cure diseases, disease diagnostics as well as identification of genes underlying various disorders.

A majority of leading commercial entities active in the CRISPR space originate in the United States, while patents filed by academic research institutions appears to slightly more evenly divided between organizations in China and United States. Among the various gene targets occurring in the CRISPR dataset, TP53 emerges as the clear leader, growing drastically after 2018. Perhaps unsurprising since mutations in TP53 have been linked to various types of cancer. These mutations tend to be missense mutations and present great opportunities for the use of CRISPR/Cas technology in correcting/rectifying them. Other notable gene targets appearing frequently include c-myc, HBB, c-Ki-Ras and BRCA1.

A considerable number of CRISPR related publications appear to be connected to cancer and infectious diseases, while other diseases such as blood, genetic and nervous system disorders are also explored in the context of CRISPR/Cas technology. Within the broader category of cancer, breast cancer, AML, liver, lung and rectal cancer exhibit a remarkable increase in journal publications in the CRISPR dataset indicating exploration of this technology in the treatment of these cancer or to establish critical genetic targets for these cancer types. Among nervous system disorders, the neurodegenerative diseases Alzheimer's and Parkinson's show a marked increase in publications, especially patents, related to CRISPR indicative of greater commercial interest.

The use of CRISPR/Cas technology in disease diagnostics has also seen a surge, most notably after 2019. Cas9 remains the Cas protein of choice in CRISPR/Cas-based diagnostics with the most number of publications associated with it, though in recent years Cas12 appears to be catching up managing to exceed Cas9 in 2023. CRISPR/Cas-based diagnostics have found application in detecting pathogens such as Zika virus and MRSA as well as cancer markers.

All of the research and development in the field has translated to considerable increase in commercial interest in CRISPR based diagnostics and therapeutics over the last few years. Currently there are >140 CRISPR-based therapeutics in various stages of clinical trials a quarter of which appear to be for a range of cancer subtypes. Despite the great strides that have occurred in this field, there remain quite a few challenges in using CRISPR/Cas technology for therapeutic purposes. Researchers are actively engaged in developing alternative and better approaches to overcome these limitations. Off-target effects of CRISPR/Cas technology are being addressed by the development of chemically modified gRNAs, high-fidelity nuclease variants, and controlled

expression of genome editor nucleases. The PAM sequence requirement of SpCas9 restricts the scope of targetable genomic sites, however this issue can be addressed using engineered variants of Cas9 with alternative or relaxed PAM requirements or other naturally derived Cas9 orthologs, and Cas12a enzymes. Second-generation CRISPR based technologies such as base editing or prime editing enable the introduction of precise modifications independently of DSBs. Newer packaging and delivery methods like electroporation/nucleofection and lipid nanoparticles have great potential to overcome existing targeted delivery problems.[446]

The ongoing refinement of existing CRISPR components continue to improve the efficiency and specificity of CRISPR based therapeutics. Expanding the targeting capabilities and optimizing delivery systems continue to aid in significant improvements in clinical outcomes. Ultimately, in the future CRISPR based therapeutics are likely to be developed successfully for myriads of diseases beyond cancer.

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Abbreviations used

4-OHT	4-hydroxytamoxifen
AaCas12b	*Alicyclobacillus acidiphilus* Cas12b
AAVs	adeno-associated viruses
ABE	adenine base editor
AD	Alzheimer's disease
AI	artificial intelligence
ALL	acute lymphocytic leukemia
AMD	age-related macular degeneration
AML	acute myeloid leukemia
ANGPTL3	angiopoietin-like protein 3
APP	amyloid precursor protein
ASGCT	American Society of Gene & Cell Therapy
aTFs	allosteric transcription factors
Aβ	amyloid β
B2M	beta-2 microglobulin
BC	breast cancer
BCL	B-cell lymphoma
BHB	β-hydroxybutyrate
BPH	benign prostatic hyperplasia
CARMEN	combinatorial arrayed reactions for multiplexed evaluation of nucleic acids
CAR-NK	chimeric antigen receptor-natural killer
CARP	CRISPR-associated reverse PCR
CAR-T	chimeric antigen receptor-T
Cas	CRISPR-associated proteins
Cas9 RNP	Cas9 ribonucleoprotein
Cas9nAR	Cas9 nickase-based amplification reaction
Cascade	CRISPR-associated complex for antiviral defense
CAS-EXPAR	CRISPR/Cas9-triggered isothermal exponential amplification reaction
Cas-G4EX	CRISPR/Cas9 system-mediated G4-EXPAR
CASLFA	CRISPR/dCas9-mediated lateral flow nucleic acid assay
CaT-Smelor	CRISPR/Cas12a- and aTF-mediated small molecule detector
CD19	cluster of differentiation 19
CD70	cluster of differentiation 70
CDK-5	cyclin-dependent kinase 5
CHE	Switzerland
CHN	China
CLISA	CRISPR/Cas13a signal amplification linked immunosorbent assay
CNN	convoluted neural network
cOAs	cyclic oligonucleotides
CONAN	Cas3-operated nucleic acid detection
CPPs	cell-penetrating peptides

CRISDA	CRISPR–Cas9-triggered nicking endonuclease-mediated strand displacement amplification
CRISPR	clustered regularly interspaced short palindromic repeats
CRISPRa/i	CRISPR activation/interference
crRNA	CRISPR RNA
CRS	cytokine release syndrome
ctPCR	CRISPR-typing PCR
CVD	cardiovascular disease
dCas13	deficient Cas13
dCas9	dead Cas9
ddRPA	droplet digital RPA
DETECTR	DNA endonuclease-targeted CRISPR trans reporter
DGK	diacylglycerol kinase
DNMT	DNA methyltransferase
DSBs	double-stranded breaks
dsDNA	double-stranded DNA
EBV	Epstein-Barr virus
EGFR	epidermal growth factor receptor
EMA	European Medicines Agency
ESR1	estrogen receptor 1
EZH2	histone 3 lysine 27 methyltransferase
FAD	familial AD
FDA	food and drug administration
fDNA	functional DNA
FELUDA	FnCas9 Editor Linked Uniform Detection Assay
FFPE	formalin-fixed, paraffin-embedded
FH	familial hypercholesterolemia
FISH	fluorescence in situ hybridization
FLASH	finding low abundance sequences by hybridization
FSHD	facioscapulohumeral muscular dystrophy
Gal3	galectin 3
GI	gastrointestinal cancer
GM-CSF	granulocyte-macrophage colony-stimulating factor
GRN	granulin
gRNA	guide RNA
GvHD	graft versus host disease
HAE	hereditary angioedema
HARRY	highly sensitive aptamer-regulated Cas14 R-loop for bioanalysis
hATTR	hereditary transthyretin amyloidosis
HBB	hemoglobin subunit beta
HBG1	hemoglobin subunit gamma 1
HBV	hepatitis B virus
HCC	hepatocellular carcinoma
HCR	hybridization chain reaction

HCV	hepatitis C virus
HD	histidine-aspartate
HDR	homology-directed repair
HeFH/HoFH	heterozygous /homozygous familial hypercholesterolaemia
HELP	HSV-1-erasing lentiviral particles
HEPN	higher eukaryotes and prokaryotes nucleotide-binding
HER2	human epidermal growth factor receptor 2
HIV/AIDS	human immunodeficiency virus/acquired immunodeficiency syndrome
HIV-1	human immunodeficiency virus, type-1
HPV	human papillomavirus
HSPCs	hematopoietic stem and progenitor cells
HTLV-1	human T cell lymphotropic virus, type-1
IARC	International Agency for Research on Cancer
IL-12	interleukin-12
IL1R1	interleukin receptors
IL3RA	interleukin 3 receptor alpha
indels	insertions or deletions
iPSCs	induced pluripotent stem cells
JPN	Japan
KLKB1	kallikrein B1
KO	knockout
KRAB	Krüppel-associated box transcriptional repression domain
KRAS	c-Ki-Ras
KSHV	Kaposi's sarcoma herpes virus
LAG-3	lymphocyte activation gene-3
LAMA2	laminin alpha 2-chain
Lama2	laminin alpha 2
LAMP	loop-mediated isothermal amplification
LCA10	leber congenital amaurosis type 10
LNPs	lipid nanoparticles
Lp(a)	lipoprotein (a)
LRRK2	leucine rich repeat kinase 2
MDC1A	merosin-deficient congenital muscular dystrophy type 1A
MHRA	Medicines and Healthcare Products Regulatory Agency
MIT	Massachusetts Institute of Technology
MM	multiple myeloma
MRSA	methicillin-resistant *Staphylococcus aureus*
NA	not applicable
NASBA	nucleic acid sequence-based amplification
NASBACC	nucleic acid sequence-based amplification-CRISPR cleavage
nCas9	Cas9 nickase
NF-κB	nuclear factor κB
NHEJ	non-homologous end joining

NHL	non-Hodgkin's lymphoma
NK	natural killer
NOS	not specified
NSCLC	non-small cell lung cancer
OC	ovarian cancer
PADLOCK	picoinjection aided digital reaction unlocking
PAM	protospacer adjacent motif
PARP	poly (ADP-ribose) polymerase
PB-19	parvovirus B19
PC	pancreatic cancer
PD	Parkinson’s disease
PD-1	programmed-death1
PD-L1	Programmed death-ligand 1
PEI	polyethyleneimine
PFS	protospacer flanking site
PGRMC1	progesterone receptor membrane component 1
PICASSO	CRISPR-based peptide display technology called peptide immobilization by dCas9-mediated self-organization
PINK1	PTEN induced kinase 1
PLGA	poly(lactic-co-glycolic acid)
Plk4	polo-like kinase 4
PNA	peptide nucleic acid
POIROT	photo-initiated CRISPR–Cas12a system for robust one-pot testing
PRKN	parkin RBR E3 ubiquitin protein ligase
PSEN1	presenilin-1
PSEN2	presenilin-2
qPCR	quantitative polymerase chain reaction
Rb	retinoblastoma
RBCs	red blood cells
RCasFISH	CRISPR/dCas9-MS2-based RNA fluorescence in situ hybridization assay
RNA-RBP	RNA-binding proteins
RPA	recombinase polymerase amplification
SaCas9	*Staphylococcus aureus* Cas9
SCAN	solid-state CRISPR/Cas12a-assisted nanopores
SCC	squamous cell carcinoma
SCD	sickle cell disease
sgRNA	single guide RNA
SHERLOCK	specific high-sensitivity enzymatic reporter unlocking
siRNA	small interfering RNA
SNCA	α-synuclein
SNPs	single nucleotide polymorphisms
SOCS1	suppressor of cytokine signaling 1
SpCas9	*Streptococcus pyogenes* Cas9

SPRINT	SHERLOCK-based profiling of in Vitro transcription
ssDNA	single-stranded DNA
ssRNA	single-strand RNA
STOP	Sherlock testing in one pot
T1D	type 1 diabetes
TALEN	transcription activator-like effector nucleases
T-ALL	T cell acute lymphoblastic leukemia
TCL	T-cell lymphoma
TET2	Tet methylcytosine dioxygenase 2
tgRNA	tuned guide RNA
TNF	tumor necrosis factor
TNFR1	tumor necrosis factor α receptor
TNFRSF17	TNF receptor superfamily member 17
TRAC	T cell receptor α subunit constant
tracrRNA	trans-activating crRNA
TTR	transthyretin
UCAD	ultrasensitive CRISPR/Cas12a-based antibody detection
UNIVERSE	universal nuclease for identification of virus empowered by RNA-sensing
USA	United States
USP1	ubiquitination-specific proteases
UTI	urinary tract Infection
VEGF-A	vascular endothelial growth factor A
WIPO	World Intellectual Patent Office
ZFN	zinc finger nucleases

TOC graphic