CRISPR–Cas: a tool for cancer research and therapeutics

Hao Yin[1,2]*, *Wen Xue*[3,4,5]* and *Daniel G. Anderson*[6,7,8,9]*

Abstract | In the past decade, the development of a genome-editing technology mediated by CRISPR has made genetic engineering easier than ever, both in vitro and in vivo. CRISPR systems have enabled important advances in cancer research by accelerating the development of study models or as a tool in genetic screening studies, including those aiming to discover and validate therapeutic targets. In this Review, we discuss these applications as well as new potential uses of CRISPR to assist in cancer detection or the development of anticancer therapies.

CRISPR–Cas is an RNA-guided, targeted genome-editing platform with great potential in both basic research and clinical applications[1–5]. These premises are particularly true in cancer research and oncology drug development, and CRISPR–Cas (referred to as CRISPR for brevity in this article) systems have been used to establish cellular and animal models of cancer, validate drug targets and develop cellular therapeutics[6–13]. For example, focused and genome-wide CRISPR-based genetic screens in cellular and animal models have been performed to discover new drug targets in oncology[7,14–17]. CRISPR systems also hold great potential to improve patient outcomes with immunotherapies by enhancing the potency and reducing the toxicity of these agents, as well as potentially decreasing the cost of manufacture of adoptive T cell therapies[18–20].

Genome-editing proteins recognize and modify specific sequences in the genome. Typical genome-editing nucleases generate double-strand breaks (DSBs), which can be repaired by enzymes via two pathways: homology-directed repair (HDR) or, more frequently, non-homologous end joining (NHEJ)[21] (FIG. 1). NHEJ is an error-prone process that often introduces small insertions and/or deletions (indels), which can disrupt target genes by shifting the reading frame[22]. By contrast, using template DNA with homologous arms, HDR can enable precise modification of genomic sequences[21].

More than a decade ago, technologies using zinc-finger nucleases (ZFNs) were developed as the first practical tools for genome editing[23]. A zinc-finger protein consists of three or more zinc-finger domains, each of which interacts with a 3 bp DNA sequence with high specificity[23]. The fusion of zinc-finger proteins with the FokI nuclease creates ZFNs[23] (FIG. 1a). The efficiency of genome editing by ZFNs can be high, but a fairly complicated process of protein engineering is required in order to target specific DNA sequences[23]. Several years ago, transcription activator-like effector nucleases (TALENs) were developed for efficient genome editing[24] (FIG. 1a). TALENs also comprise a FokI nuclease domain fused to a DNA-binding domain, which in this case are highly conserved repeats derived from transcription activator-like effectors (TALEs) produced by different species of *Xanthomonas*. Both ZFNs and TALENs can introduce DSBs, which are repaired by NHEJ or HDR, enabling deletion and addition of DNA sequences[23,24]. Although both ZFNs and TALENs recognize DNA sequences through protein–DNA interactions, the design and assembly process of TALENs is faster than those of ZFNs, and the potency and specificity of TALENs are potentially higher too[24].

Distinct from ZFN and TALEN platforms, CRISPR systems are predicated on RNA-guided nucleases[1–4]. The currently used class 2 CRISPR systems consist of a Cas endonuclease and at least one target-specific CRISPR RNA (crRNA)[1]. The Cas enzyme recognizes DNA sequences through base pairing between the guide sequence of the crRNA and the target DNA in the presence of a 2–6 bp protospacer adjacent motif (PAM) next to the target DNA[1–4] (FIG. 1a). The PAM sequence, as part of the target DNA, enables distinction between self DNA versus foreign DNA[1–4].

The most widely used *Streptococcus pyogenes* Cas9 (SpCas9) system requires a *trans*-activating CRISPR RNA (tracrRNA), which is fused with the crRNA to form a single-guide RNA (sgRNA)[1]. The tracrRNA sequences within the sgRNA adopt a specific conformation through internal base pairing, enabling its interaction with Cas and a precise positioning in the target DNA[1–4]. The simplicity of the canonical PAM sequence (NGG) and the high efficiency of SpCas9 make CRISPR a popular method for mammalian genome editing. Using bacterial selection-mediated directed evolution and structural information, SpCas9 can be engineered to recognize PAM sequences different from NGG[25]. Through phage-assisted protein evolution, an SpCas9

*e-mail: haoyin@whu.edu.cn; wen.xue@umassmed.edu; dgander@mit.edu

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Key points

- CRISPR systems have been extensively applied to edit genes and genomic sequences in order to develop cancer models, providing a rapid, simple and low-cost system with which to identify and study genetic determinants of cancer and therapeutic targets.
- CRISPR systems have been widely adapted in cancer research to facilitate the discovery of new targets; many high-throughput in vitro and in vivo genetic screening studies have been performed with CRISPR.
- CRISPR systems are being robustly adapted to improve the efficacy of immunotherapies by enhancing their potency, mitigating toxicity, reducing manufacturing cost and facilitating the discovery and development of new immunotherapeutic strategies.
- The delivery of CRISPR to tumours might inhibit tumour growth directly and indirectly. As a diagnosis platform, CRISPR could be used to detect low numbers of cancer cells or rare mutations in clinical samples.

variant was engineered to recognize less-restrictive PAM sequences, including GAT, GAA and NG, with greater specificity for DNA than that of SpCas9 (REFS[26,27]). Alternatively, the *Staphylococcus aureus* Cas9 analogue (SaCas9) is smaller in size than SpCas9 and thus more amenable to delivery with adeno-associated virus (AAV), which has a limited payload capacity[28]. Other Cas9 analogues, such as *Streptococcus thermophilus* Cas9 (StCas9) or *Neisseria meningitidis* Cas9 (NmCas9), have also been developed for mammalian genome editing[4,29]. Besides CRISPR–Cas9, platforms involving CRISPR–Cpf1, another member of the class 2 CRISPR family, have been explored as tools for genome editing of mammalian cells[30]. While Cas9 endonucleases require dual RNAs (the crRNA and the tracrRNA) and a PAM at the 3′ end of the DNA strand homologous to the crRNA sequence, resulting in blunt-end DNA cleavage[1–4], Cpf1 endonucleases require only a crRNA and a 5′ PAM and have a staggered DNA cleavage site[30]. These CRISPR systems all have the potential for off-target activities during genome editing[31]. Thus, several strategies to improve the specificity of CRISPR editing have been pursued. These include truncated sgRNAs[32], structure-guided engineering of the Cas9 proteins[33–35], fusion of deactivated Cas9 (dCas9; a variant of Cas9 engineered to bind to, but not cut, a specific DNA sequence) with FokI enzymes[36,37], fusion of Cas9 with programmable DNA-binding domains such as zinc-fingers proteins or TALENs[38], use of a pair of Cas9 nickases (with single-strand as opposed to double-strand nuclease activity)[39] and chemical modification of the guide sequences (to optimize the binding affinity between the guide sequence and the target DNA)[40].

Targeting of a new genomic locus for gene deletion, mutation and targeted insertion can be done rapidly through the generation of a sgRNA, making CRISPR a powerful tool for research and drug development (FIG. 1b). With a single sgRNA, Cas9 can destroy the open reading frame by inducing a frameshift mutation[5]. With two sgRNAs, deletions of the sequence between two DSBs can be created or induce chromosome translocation when targeting different chromosomes[41–45]. Efficient target insertion (1–90%) can be achieved when DSBs are repaired by either HDR or NHEJ in the presence of a donor DNA[46–48]. In contrast, the efficiency of conventional gene targeting by homologous recombination without DSBs is extremely low (<0.1%)[49]. Importantly, the time required to obtain genetically engineered animals using conventional homologous recombination can be ~2 years[49]. ZFNs and TALENs are less popular than CRISPR in research and development because engineering new pairs that target a genomic locus is complicated, expensive and time-consuming[23,24].

In addition to modifying genomic DNA sequences, CRISPR can be used to regulate the expression of a target gene[50–56]. Different CRISPR activators (CRISPRa) or inhibitors (CRISPRi) of gene expression have been developed. For example, a CRISPRa consisting of a fusion of dCas9 with the transcriptional activation domain VP64 has been used to induce the expression of targeted genes[50,51] (FIG. 2). Other versions of CRISPRa have been developed, including the tripartite activator VP64–p65–Rta[56–58] (FIG. 2). CRISPRi systems can involve either dCas9 binding alone or fused with the Kruppel-associated box (KRAB) transcriptional repressor domain[50]. Base editor systems in which Cas9 or Cpf1 proteins are fused with a cytidine deaminase effectively convert cytidine to uridine at specific locations, resulting in C-to-T or G-to-A substitutions within a window of approximately five nucleotides in a particular position[59–62]. Adenine base editors via fusing Cas9 nickase with an engineered adenosine deaminase have also been successfully developed to convert A–T base pairs into G–C[63].

Genome-editing platforms have clear implications in cancer research and, potentially, anticancer therapy. In this Review, we describe how these principles of genome editing and CRISPR systems have been used for cancer modelling and genetic screening. Moreover, we discuss genome editing for immunotherapy and current preclinical advances and efforts to develop clinical applications involving CRISPR efforts. Finally, we outline other potential applications of CRISPR in oncology and the challenges associated with these approaches.

CRISPR for cancer modelling

The identification of genes that drive cancer progression and maintenance in genetically tractable models is an important step in the development of therapeutics. However, this effort has been limited by the high cost and slow pace associated with the development of traditional gene knockout and knock-in models, which require laborious gene targeting in the case of cell models and

Author addresses

[1]Department of Urology, Zhongnan Hospital of Wuhan University, Wuhan, China.
[2]Medical Research Institute, Wuhan University, Wuhan, China.
[3]RNA Therapeutics Institute, University of Massachusetts Medical School, Worcester, MA, USA.
[4]Department of Molecular, Cell and Cancer Biology, University of Massachusetts Medical School, Worcester, MA, USA.
[5]Department of Molecular Medicine, University of Massachusetts Medical School, Worcester, MA, USA.
[6]David H. Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology (MIT), Cambridge, MA, USA.
[7]Department of Chemical Engineering, MIT, Cambridge, MA, USA.
[8]Harvard–MIT Division of Health Sciences & Technology, Cambridge, MA, USA.
[9]Institute of Medical Engineering and Science, MIT, Cambridge, MA, USA.

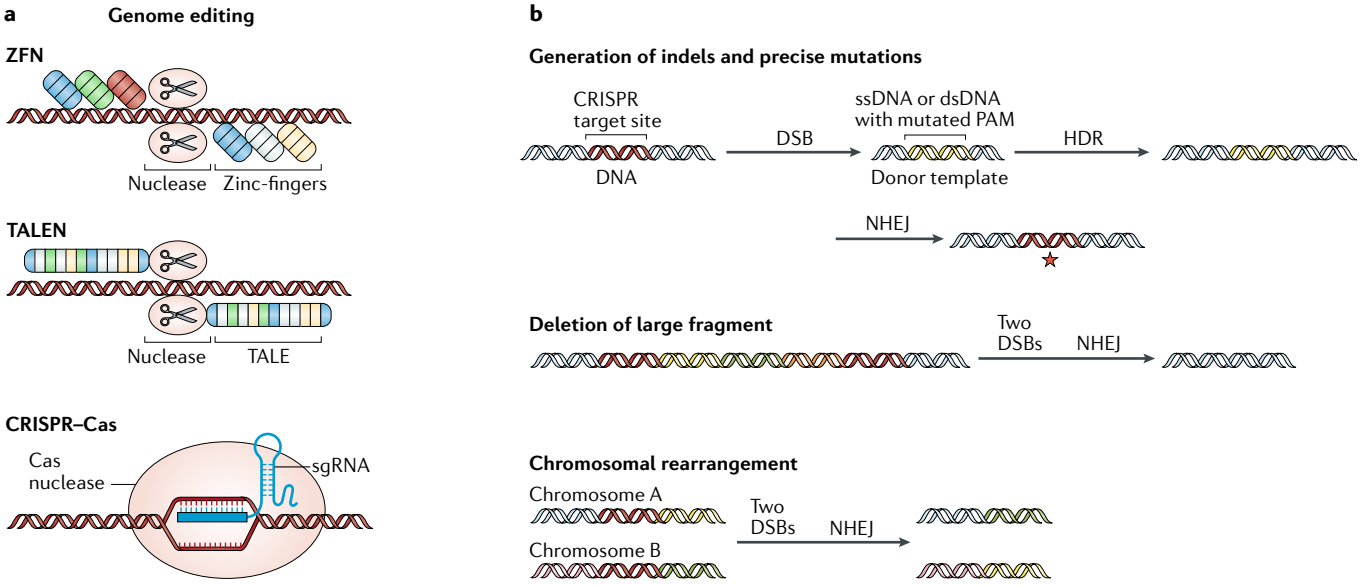

Fig. 1 | **Mechanisms of gene editing. a** | Zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and CRISPR–Cas systems induce double-strand breaks (DSBs) in specific loci in the genome. **b** | Homology-directed repair (HDR) or non-homologous end joining (NHEJ)-mediated insertion of donor template enables insertion of DNA sequences (including mutations) in DNA. In mammalian cells, CRISPR-induced DSBs are usually repaired via NHEJ, which introduces small insertions and/or deletions (indels), resulting in gene inactivation via frameshift processes. The introduction of two DSBs in the same chromosome can induce deletion of a large fragment; in contrast, the introduction of DSBs in different chromosomes can induce chromosomal rearrangements. dsDNA, double-stranded DNA; PAM, protospacer adjacent motif; sgRNA, single-guide RNA; ssDNA, single-stranded DNA; TALE, transcription activator-like effector.

additional breeding steps for the generation of mouse models[64]. CRISPR systems have been extensively used to edit genes in order to develop cancer models, providing a rapid and simple genetic system with which to identify and study genetic determinants of cancer.

Cellular modelling using CRISPR

With CRISPR, the generation of mammalian cell lines with single (or even multiple) gene deletions is feasible[65,66], and, thus, pharmacological studies of targeted therapies can be substantially accelerated. For example, MELK was identified as a cancer target in studies using RNA interference and small-molecule inhibitors, resulting in the subsequent development of OTS167, a MELK inhibitor that is currently being evaluated in several clinical trials[13]. However, CRISPR-mediated silencing of MELK showed no effect on the fitness of cell lines derived from seven cancer types[13]. Cancer cells with genetic inactivation of MELK remain sensitive to OTS167, suggesting that this drug inhibits cell growth through off-target mechanisms[13]. CRISPR–Cas9 was used to validate the p53-reactivating small molecules nutlin and RITA[67]; nutlin was confirmed to inhibit tumour proliferation via a p53-dependent mechanism, but the activity of RITA was found to be p53-independent[67]. Besides validating or invalidating the role of genes identified in cancer studies, the use of CRISPR to disrupt functional alleles or introduce point mutations can result in drug resistance in cultured cells. Indeed, specific mutations or candidate genes associated with drug resistance can be identified and quickly validated with next-generation sequencing and CRISPR-based approaches[68]. For example, CRISPR screening studies have been performed to identify therapeutic resistance mutations in essential genes for drug target identification. One example is *NAMPT*, encoding nicotinamide phosphoribosyltransferase, which was identified as the main target for the anticancer agent KPT-9274 (REF.[69]). In a different study, CRISPR-mediated mutagenesis led to the identification of variants of *MEK* and *BRAF* resistant to the inhibitors selumetinib and vemurafenib, respectively[70]. Finally, rare drug-resistant alleles generated using CRISPR have enabled rapid validation of the on-target anticancer effect of existing inhibitors of DOT1-like protein or EZH2 (REF.[71]).

CRISPR can also be used to manipulate multiple genes in order to explore the genetic complexity of human malignancies. Examples include the engineering of human cell lines with oncogenic chromosomal translocations generated by CRISPR-mediated DSBs followed by a selection process[41–43,72]. Fusion of *RUNX1T1* (on chromosome 8) and *RUNX1* (on chromosome 21) occurs in a subgroup of patients with acute myeloid leukaemia[72]. Using a pair of sgRNAs, one for *RUNX1T1* intron 1 and the other for *RUNX1* intron 5, a gene fusion was induced in 1–4% of CRISPR-transfected cells[72]. Another example is a study of myeloid malignancies, which are driven by alterations in multiple genes including *Tet2*, *Runx1*, *Dnmt3a*, *Ezh2*, *Nf1*, *Smc3*, *Trp53* and *Asxl1*. Combinations of up to five genes were modified in single mouse haematopoietic stem cells to induce outgrowth and myeloid malignancies in mice, recapitulating different combinations of mutations in cancer[73]. Moreover, several elements in a signalling pathway can be simultaneously modified

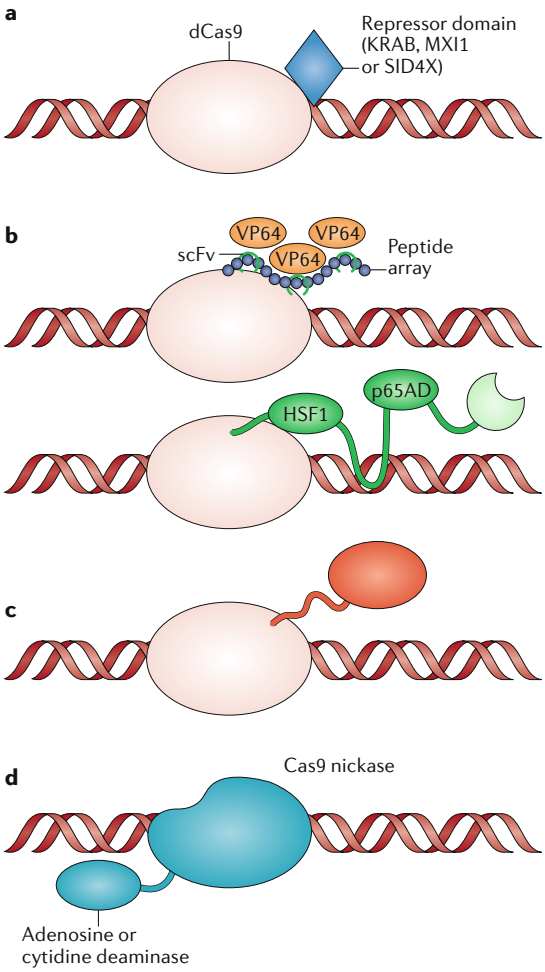

Fig. 2 | **Applications of CRISPR in cancer research.** **a** | Deactivated Cas9 (dCas9) can be fused with repressor domains, leading to inhibition of the gene of interest. **b** | Fusion of dCas9 with activation domains can induce the expression of a gene of interest. Furthermore, additional transcription activators can be bound to a single-guide RNA or dCas9 to enhance the expression of target exons. **c** | Fusion of dCas9 with epigenetic regulators leads to either transcriptional repression or activation. **d** | Fusion of dCas9 with adenosine deaminase or cytidine deaminase can enable focused introduction of point mutations in the genome. KRAB, Kruppel-associated box; scFv, single-chain variable fragment.

with CRISPR in the same cell; for example, many of the upstream regulators and components of the Hippo pathway were simultaneously inactivated by CRISPR to explore their relative effects in downstream YAP–TAZ-mediated regulation of gene expression in different cell lines[74]. In addition to inactivation of genes, CRISPR can be used to invert DNA sequences in cells. CTCF binding sites are frequently mutated in several types of cancer[75]. CRISPR-mediated inversion of the binding sites for CTCF, an insulator-binding protein, in two model genes was shown to alter the function of enhancers and promoters, as well as the genome topology[76].

Besides cultured cell lines, organoids have been genome edited to study tumour biology. Human colon organoids were edited by CRISPR to inactivate key DNA repair genes[9] (FIG. 3a), and the mutation profiles of organoids deficient in *MLH1*, the gene encoding DNA mismatch repair protein MLH1, were consistent with those identified in patients with colorectal tumours deficient in this mechanism[9]. In a different study, key cancer driver genes including *KRAS*, *CDKN2A* and *SMAD4* were edited using CRISPR in a pancreatic tumour organoid library to reveal that WNT niche independency might occur through epigenetic mechanisms rather than as a result of mutations in driver genes[77]. *KRAS*, *CDKN2A*, *SMAD4* or *TP53*-edited primary human pancreas duct cells induced lesions resembling pancreatic intraepithelial neoplasia upon transplantation in mice[78]. Finally, CRISPR genome-edited organoids have also been used in drug screening studies[68] or to understand the role and interactions between genes involved in the initiation and progression of cancer[11,79,80]. The commonly used delivery systems for cultured cells and organoids include lentiviral infection[78], electroporation[77] and lipid reagents[76].

In vivo modelling using CRISPR

CRISPR has been used to establish cancers in animal models[12,81,82]. The advantage of the CRISPR systems is that they can be used to edit the genome of somatic cells to introduce driver mutations, and, therefore, time-consuming manipulation of germline cells is not required. Moreover, genetic manipulation of somatic cells to introduce driver mutations can recapitulate oncogenesis initiated from a small number of somatic cells that have acquired such mutations[82]. The choice of a delivery system is a key factor for the efficient introduction of genetic alterations in somatic cells; lentivirus, AAV, adenovirus and plasmid delivery can be used to deliver CRISPR in vivo to establish mouse cancer models (TABLE 1).

Lentivirus and retrovirus. Lentiviral vectors enable stable expression of Cas9 and sgRNA and can therefore enable efficient gene deletions in transduced cells in vivo[83–86]. Notably, integrase-defective lentiviral vectors can also serve as HDR donor templates during CRISPR-dependent genome editing[87]. Lentiviral vectors have been widely used to deliver CRISPR locally in the target organs of interest in order to create animal models of brain[88], breast[89], colon[83,90], lung[84,85,91,92] or pancreatic[86] cancer (FIG. 3b). For example, lentivirus was used to deliver both CRISPR construct and Cre recombinase to the lung by intratracheal injection[75]. Using this method, CRISPR-mediated loss of *Nkx2-1*, *Apc* or *Pten* by random indels was demonstrated to accelerate lung tumorigenesis in both $Kras^{LSL-G12D/+}$ and $Kras^{LSL-G12D/+}$;$Trp53^{fl/fl}$ mice[84]. Quantitative methods for assessing the outcomes of in vivo CRISPR-mediated experiments have been developed to identify tumour suppressor genes in a specific genetic context[85,91]. Similarly to lentivirus, retrovirus has been used to generate ex vivo cancer models[93,94].

Despite their utility, lentiviral vectors have limitations. Surgery is required to deliver these vectors to organs such as the brain or the pancreas. In addition, control experiments with sgRNAs need to be performed to account for the random genomic integration of lentivirus in order to exclude lentivirus-induced off-target effects[83–86].

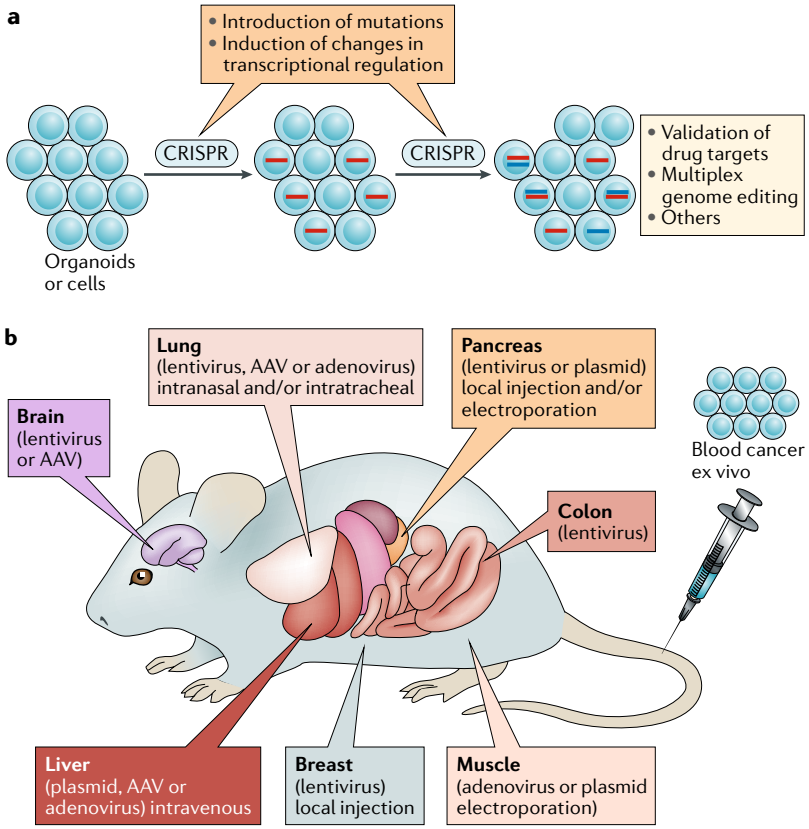

Fig. 3 | **CRISPR for cancer modelling in cells and mice. a** | Cultured cells or organoids can be genome edited (in one or more steps) with CRISPR to introduce mutations or induce changes in transcriptional regulation. **b** | CRISPR systems can be delivered to animal models with different methods, reaching several tissues and organs. AAV, adeno-associated virus.

Adeno-associated virus and adenovirus. AAV and adenovirus vectors have been used for genome editing to generate animal models of cancer because different AAV and adenovirus serotypes have broad-ranging tissue tropisms[10,14,87,95]. For example, intravenous injection of AAV8 selectively and efficiently targets liver[87,96]. Of note, adenovirus can induce a strong immune response in liver, which might compromise the disease phenotype[97]. Adenovirus can also target the lung when delivered by intranasal or intratracheal injection; chromosomal rearrangements in lung cancers, such as *EML4–ALK* inversion, have been modelled in mice using CRISPR delivered by adenoviral vectors[44,45]. Similarly, intramuscular injection of adenovirus has been used to generate animal models of sarcoma[98]. Of note, Winslow and colleagues have developed elegant strategies for multiplexed cancer modelling in vivo using an AAV HDR template carrying *Kras* mutations such as G12D. In their study, these researchers evaluated the contribution of different *Kras* mutations to pancreatic or lung tumorigenesis[95].

The major limitation of AAV-based vectors is that their cargo capacity (<5 kb) is smaller than that of other viral vectors. The large size of Sp*Cas9* (>4 kb) limits the achievable titres of lentivirus and precludes effective single AAV-based delivery of the CRISPR components; therefore, several *Cas9*-modified mouse strains have been generated to facilitate in vivo genome editing[10,86,99]. These mice have stable or conditional Cas9 expression in the germ line or in organs of interest[10,86,99]. Similarly, doxycycline-inducible *Cas9* and *Cas9*^{D10A} mouse models[100] have been developed to enable inducible genome editing in mice.

Hydrodynamic injection or electroporation of plasmids. Hydrodynamic injection, a high-volume and/or high-pressure tail vein injection, is a well-established method of delivering plasmids to the liver in rodents[87]. Hydrodynamic injection does not require viral manufacturing, although the delivery efficiency is lower than that of AAV and can result in liver damage[6,87]. For example, hydrodynamic injection of DNA plasmids encoding Cas9 and sgRNAs can result in transient expression of Cas9 in 20–30% of mouse hepatocytes and induce indels in ~10% of these transfected cells (~3% of total hepatocytes)[6]. In this study, CRISPR-induced frameshifting indels in *Pten* led to increased phosphorylation of AKT and lipid accumulation in genome-edited hepatocytes compared with mice injected with plasmids encoding sgRNA targeting a control gene[6]. The injected DNA can serve as an HDR donor. Co-injection of CRISPR plasmids and a single-stranded DNA oligonucleotide donor carrying specific activating point mutations in *CTNNB1* resulted in activation of β-catenin-mediated signalling in the liver[6]. Although the efficiency of HDR-mediated genome editing is low (usually <5–10% of the transfected cell pool in vivo), this method has been shown to reliably model point mutations of tumour suppressor genes and oncogenes in the liver[6].

Transposon plasmids have also been delivered by high-pressure injection to target genes in cells of the liver[101,102]. Unlike the plasmids discussed above, transposons such as *Sleeping Beauty* or *piggyBac* can stably integrate in the genome of a subset of hepatocytes. Several proof-of-concept studies demonstrated CRISPR-mediated somatic mutagenesis of tumour suppressor genes in mice using this approach[101,102]. Hydrodynamic injection can also be used to deliver plasmid DNA to the pancreas[103] or muscle[98]. For example, a pool of 15 different sgRNAs encoded in the same vector was delivered to the pancreas in a *Kras*^{G12D}-driven mouse model for combinatorial gene editing[103]. In addition to single gene indels, large chromosomal deletions and translocations between two sgRNA sites can generate gene fusions[103].

Overall, in vivo modelling using CRISPR has simplified and accelerated the generation of cancer animal models.

Future directions

In summary, CRISPR provides a flexible approach to developing models of cancer for studies of gene function or drug targets. Currently, both viral and non-viral vectors are useful tools to deliver genome-editing systems into animals for the development of cancer models. One limitation in the generation of cancer models is how to precisely manipulate specific cell types within the tissue. Improvements in the delivery of CRISPR in vivo such as developing cell type-specific AAVs or nanoparticles for CRISPR delivery might enable this hurdle to be overcome.

Cancer modelling using CRISPR can be expanded using new tools such as base editing[104]. By converting a few codons into stop codons, base editing enables

Table 1 | **In vivo cancer mouse models using CRISPR**

Delivery strategy	Integration	Cancer type	Advantages	Limitations	Refs
Lentivirus	Yes	Brain, breast, colon, lung and pancreatic cancer	Stable expression	• Random integration • Need surgery for delivery to certain organs (such as in the pancreas)	83–86,88–92
AAV	Low	Brain, lung, liver and pancreatic cancer and sarcoma	• AAV serotypes have broad tissue tropism • AAV can serve as HDR donor	Small cargo size (<5 kb)	10,14,86,95
Adenovirus	NA	Lung and liver cancer and sarcoma	Good efficiency for lung	Strong immune response in liver	45,97,98
Plasmid (hydrodynamic delivery or electroporation)	Low	Liver and pancreatic cancer and sarcoma	Do not need to package virus	• Low efficiency for hydrodynamic delivery • Need surgery for pancreas	6,101–103
Retrovirus (for example, MSCV)	Yes	Blood cancer (manipulated ex vivo)	Stable expression	Random integration	94

AAV, adeno-associated virus; HDR, homology-directed repair; MSCV, murine stem cell virus; NA, data not available.

inactivation of cancer-related genes and studies of the role of cancer-associated nonsense mutations[104]. Using this approach, mouse models harbouring cancer-relevant mutations were generated via microinjection of base editors into mouse zygotes[105]. In cells and organoids derived from adult mice, base editors that were optimized for codon usage to enhance protein translation and contained nuclear localization signals (fused to the base editors) were used to induce cancer-related mutations[59].

CRISPR for target discovery in oncology

Screening approaches

Genome-wide and focused in vitro screening. CRISPR has been successfully adapted to facilitate the discovery of actionable targets in cancer. Many high-throughput genetic screening studies performed with CRISPR in a variety of cell types have been reported[16,106–109] (FIG. 4a). Indeed, genome-scale lentiviral CRISPR libraries have been established[16,106–109]. Several readouts have been applied to these screening studies: sgRNAs to essential genes or genes that are potential targets of a therapeutic agent will be underrepresented in the library because they confer a growth disadvantage[7,14–17]; sgRNAs targeting candidate tumour suppressor genes will be enriched in the library because they confer a growth advantage[7]; cells expressing sgRNAs of genes that influence drug sensitivity will be depleted or selected for upon drug treatment if the target gene provides an advantage or disadvantage[15], respectively; and, finally, genes involved in the antitumour immune response can be identified, such as genes involved in TNF signalling[110] or in the effector function of T cells[111], enabling the characterization of all the interactions between elements in a regulatory network. Besides genome-wide libraries, specific sgRNA libraries are available, for example, targeting panels of kinases or proteins involved in epigenetic regulation[112]. Screening studies have been performed with both types of libraries to identify genes involved in sensitivity to multiple therapeutic agents (such as the nucleotide analogue 6-thioguanine[16] and inhibitors of BRAF (vemurafenib)[17], MEK (trametinib), EGFR (erlotinib), ALK (crizotinib)[113] and ATR (AZ-20)[114]). These studies have revealed new candidate genes that are involved in drug resistance. For example, loss of *KEAP1* mediates resistance to MEK inhibition[113].

Ex vivo and in vivo CRISPR screening. Besides using cell lines, CRISPR-based screening studies have been performed ex vivo and in vivo (FIG. 4b). For example, studies have been performed wherein libraries of genes are modified in a pool of cells ex vivo, which are then transplanted into mice. Using this approach, modified mouse fetal liver cells were injected in the tail vein of immunocompetent mice[115,116], and modified SKOV3 ovarian cancer cells were injected intraperitoneally[117] or as xenografts[7,118] in immunocompromised mice. Subsequently, the cells making up the tumour can be isolated and genetically characterized through screens in order to determine the effects of different gene aberrations on tumour growth or treatment responses. sgRNAs targeting genes essential for tumour growth will be depleted in tumour cells compared with the initial cell pool. Using a similar approach, several genes that mediate the response to anticancer immunotherapy have been identified in a study using a melanoma xenograft model[8].

In vivo CRISPR screens have also been performed by introducing a library of mutations directly into non-transformed tissues[14,101]. Examples of such proof-of-concept studies include screens identifying genes mediating liver tumorigenesis in mice[90] or identifying tumour suppressor genes in a mouse model of glioblastoma[14], both of which involve direct mutation of genes in somatic cells by CRISPR.

Advanced screening. Besides the gene inactivation CRISPR screens mentioned above, other genetic studies can be performed using CRISPR: screens can be developed to enable the identification of pairs of genes with synthetic lethal interactions[119,120]; genetic interactions between gene pairs can be mapped in combinatorial CRISPR screens (using lentiviral vectors encoding two sgRNAs)[121–123]; genome-scale CRISPRa or CRISPRi screening studies can

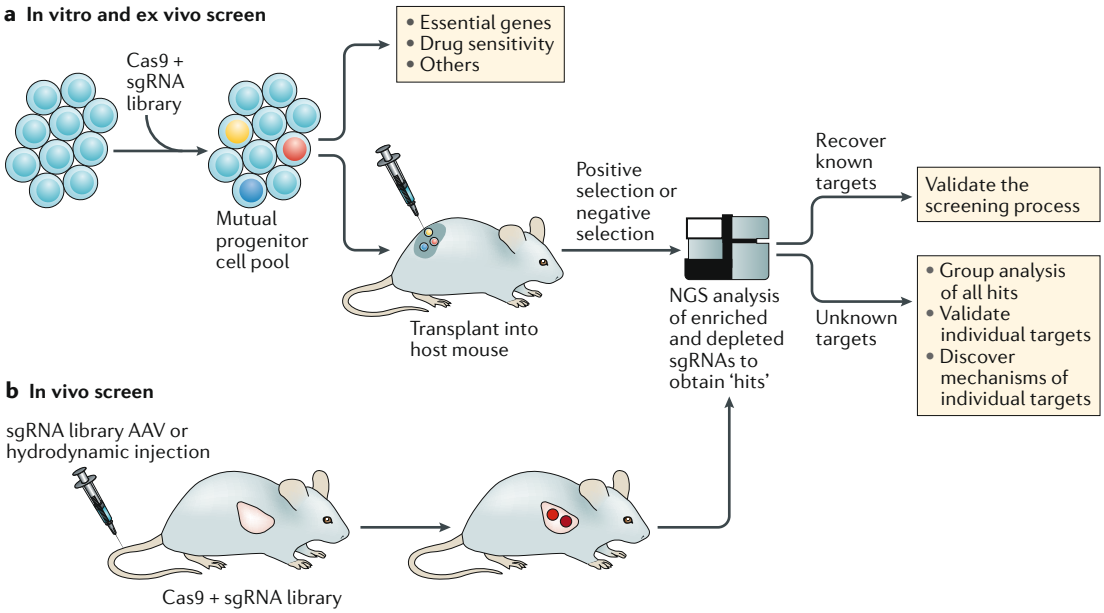

Fig. 4 | **CRISPR for genetic screening. a** | Cultured cells can be transfected with different types of CRISPR libraries and incubated in vitro in the required experimental conditions or transplanted into animals (ex vivo). **b** | Animals can be infected with types of CRISPR libraries for in vivo screening. In vitro, ex vivo and in vivo screening all require performing selection assays and next-generation sequencing (NGS) to identify 'hits'. These hits are evaluated to recover known targets and identify unknown targets. Analysis of patterns among those hits helps to identify the mechanisms (for example, a particular pathway can be the major hit of a screen). The unknown targets can be validated individually through loss-of-function and gain-of-function assays. The roles of individual target genes can be further investigated using various biological assays. AAV, adeno-associated virus; sgRNA, single-guide RNA.

be used[50–56]; or screening studies incorporating single-cell RNA-sequencing data as a readout can be used[124]. CRISPR sgRNA libraries have also been used to map non-coding regions of the genome[125–129]. Together, these studies demonstrate that CRISPR can be adapted in a range of creative ways to accelerate the identification of novel drug target genes in oncology.

Challenges in screening and solutions

Despite the efficiency of many CRISPR systems, several challenges remain in their application to cancer target discovery. First, next-generation sgRNA libraries are needed to improve the specificity of CRISPR screens. In the past few years, new software for improved sgRNA design has been developed[130], and lentiviral sgRNA libraries with improved efficacy and reduced off-target activity have been generated[131]. Second, aberrant genomic copy number can cause false-positive results in CRISPR screening studies[132]. sgRNAs targeting genes of which multiple copies exist can lead to replication stress and G2–M cell cycle arrest when compared with sgRNAs targeting genes outside these regions, likely owing to excessive DNA damage induced by CRISPR cutting[132]. Aguirre et al.[133] and Munoz et al.[134] reported that sgRNAs targeting genomic amplifications (including non-expressed genes or intergenic regions) in human cancer cell lines reduce proliferation, suggesting that gene-independent false-positive results in CRISPR screens can be obtained in cells with high levels of copy number aberrations. A method to computationally correct such copy number effects was developed in a study involving genome-wide CRISPR screens in 342 cancer cell lines[135]. Finally, as a general important consideration in screening, CRISPR-mediated targeting of exons encoding functional protein domains increases the occurrence of null mutations and might increase the likelihood of functional gene disruption[15]. By developing solutions to these challenges, we expect that CRISPR-based screening technology will continue to improve our understanding of genes involved in cancer maintenance in individual tumours, paving the road for identifying new therapeutic targets.

CRISPR in immuno-oncology

Cancer immunotherapy, the therapeutic modality in which an antitumour immune response is generated or potentiated, is emerging as a new direction in the treatment of a wide range of cancers[136,137]. The high specificity and potency of the immune system make this therapeutic approach an attractive method to target cancer[138]. Immunotherapies have been explored for >100 years, although only in the past decade have such agents been associated with improved survival in patients with advanced-stage cancers[136,137]. Despite these important advances, for almost all cancer types, a large proportion of patients do not derive sustained responses from the currently available immunotherapies[136,137]. CRISPR systems are being adapted to improve the efficacy of immunotherapies through enhancing potency, reducing toxicity and manufacturing cost and facilitating the discovery of new immunotherapeutic strategies.

Immunotherapy approaches

Discovery of immune checkpoints. Antibodies that block or activate surface receptors can stimulate T cell function, leading to tumour regression in some patients[139,140]. For example, blockade of the immune checkpoints mediated by programmed cell death 1 (PD-1) or cytotoxic T lymphocyte antigen 4 (CTLA-4) on T cells can activate the immune response against tumours[136]. In the past few years, several antibodies against PD-1 and CTLA-4 have been approved for the treatment of several types of cancer, such as melanoma and non-small-cell lung cancer[139,140]. Despite the successes with these immune-checkpoint inhibitors (ICIs), a response to treatment is not observed in the majority of patients, suggesting that additional immunotherapeutic strategies remain to be discovered[136]. In 2017, the results of CRISPR-based screens of new genetic targets for immunotherapy expressed by tumour cells were published[8,111]. In one study, >2,000 genes expressed in melanoma cells were evaluated through a loss-of-function approach[8]; loss of tyrosine-protein phosphatase non-receptor type 2 in melanoma cells was found to sensitize mice to PD-1 inhibition[8]. This study also confirmed that deficiency in IFNγ signalling promoted resistance to immunotherapy[8]. Using a genome-scale CRISPR library, another study identified genes whose loss enables escape from ICIs[111]. For example, loss of apelin receptor in tumour cells reduced the efficacy of ICI and adoptive T cell therapy in mice[111].

CRISPR for engineering of chimeric antigen receptor T cells. The aim of using adoptive T cell therapy is to create a robust antitumour response by infusing T cells that have typically been manipulated ex vivo to increase their anticancer potency. This manipulation can include gene transfer of a chimeric antigen receptor (CAR) or an engineered T cell receptor (TCR) or purification and expansion of tumour-infiltrating lymphocytes (TILs)[141]. A common form of CAR consists of single-chain variable fragments (scFvs) that recognize an extracellular antigen fused with a transmembrane and intracellular signalling region (generally derived from the TCR CD3ζ chain) and usually with one or two co-stimulatory domains[141]. CAR T cells have been proven effective in a number of clinical trials[142,143]. Treatment with CD19-targeted CAR T cells can induce a complete response in 70–90% of patients with relapsed and/or refractory B cell acute lymphoblastic leukaemia (B-ALL). In contrast, the same patients would have a 30% response rate and 5-year overall survival of ~7% with chemotherapy[136,142,144]. Two CD19-directed CAR T cell therapies were approved by the FDA in 2017 (REF.[145]). Meanwhile, many CAR T cells are under clinical evaluation in patients with solid tumours or haematological cancers[136]. Despite the early success with CAR T cell therapy, several limitations remain, and genome-editing platforms, in particular, CRISPR, can be a powerful tool to mitigate these limitations (FIG. 5).

Most trials of CAR T cells have used autologous T cells, which are collected from a patient, engineered to express a CAR construct, expanded and infused back into the same patient[146]. This long process is extremely expensive — the first approved CAR T cell therapy costs US$475,000 for a single infusion[147]. Moreover, patient-to-patient variation of the quality and quantity of T cells might substantially hamper the therapeutic outcome of such cell-based therapy[136,146]. Finally, autologous T cells are very difficult to manufacture for infants or heavily pretreated patients[148]. Thus, allogeneic CAR T cells — to be used as 'universal' or 'off-the-shelf' agents — hold great potential to simplify product development and reduce costs[146]. A key challenge to using allogeneic CAR T cells, however, is that donor T cells can recognize antigens from the recipients through their native TCRs, leading to severe toxicity owing to graft-versus-host disease (GVHD)[149]. Furthermore, the immune system of the recipient can reject allogeneic donor T cells rapidly[150]. The genes encoding TCRα constant (TRAC) and CD52 were disrupted in off-the-shelf donor T cells using TALENs; the resulting cell product was used to successfully treat two infants with relapsed B-ALL[148]. One patient developed low-grade GVHD, and the other one did not have GVHD, likely owing to the inactivation of *TRAC*, whereas inactivation of *CD52* enabled survival of donor T cells after treatment with anti-CD52 as a conditional therapy to deplete the host T cells before stem cell transplantation[148] (FIG. 5). The expression of the HLA histocompatibility antigen on the surface of donor T cells can also cause rejection by the host immune system[19]. Using genome-editing tools to delete β_2-microglobulin (β_2M), which is essential for the expression and function of HLA on the surface of T cells, might reduce the possibility of rejection[19].

The antitumour functions of T cells can be enhanced through antibodies that block or activate key regulatory receptors on the surface of T cells, as emphasized by the efficacy of ICIs in the treatment of cancer[136]. Similarly, the activity of CAR T cells can be boosted by deletion of inhibitory signalling molecules such as PD-1 and CTLA-4 using genome-editing tools. Indeed, the disruption of PD-1 in CAR T cells using CRISPR enhanced their antitumour activity in animal models[19,151]. Rapidly multiplexed genome editing with CRISPR has enabled gene editing of PD-1, TCR and β_2M to create universal CAR T cells with enhanced antitumour activity[19]. Nevertheless, severe immune-mediated adverse events can occur after treatment with ICIs or CAR T cells[152,153]. Future work will be needed to maximize tumour targeting of allogeneic cells while minimizing off-target immune-mediated toxicity.

Besides being used to modulate T cell signalling, CRISPR has been applied to precisely insert CARs into the *TRAC* locus[18]. This approach resulted in CD19-specific CAR T cells with improved performance compared with T cells with CARs delivered by randomly integrating vectors[18]. Targeted insertion of CARs enables uniform CAR expression, reducing the potential of transcription silencing and/or variable transgene expression[18]. Moreover, the risk of clonal expansion and oncogenic transformation owing to random gene integration in T cells is reduced by the absence of off-target hot spots associated with the use of such CRISPR-mediated gene modification approaches[18]. Furthermore, directing the CAR to the *TRAC* locus has been reported to improve antitumour potency in a mouse model of

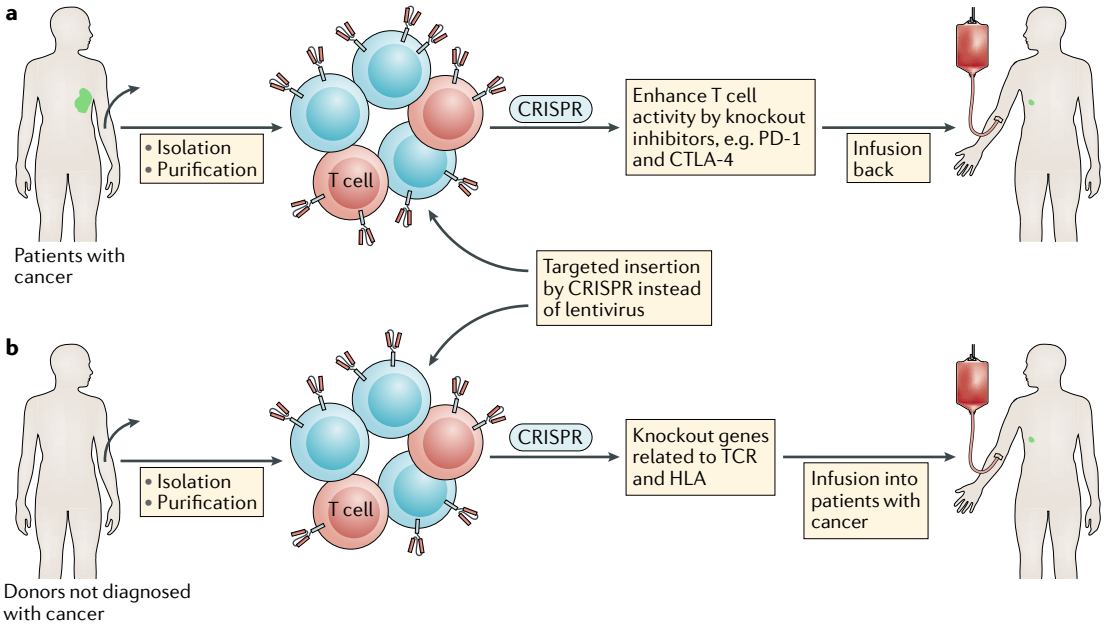

Fig. 5 | **CRISPR in immuno-oncology. a** | Primary T cells from patients with cancer can be isolated and purified. Lentiviral-mediated transduction of a chimeric antigen receptor (CAR) can be replaced with CRISPR-mediated targeted insertion of a CAR. CRISPR can be used to inactivate immune-checkpoint genes in primary T cells, such as those encoding programmed cell death 1 (PD-1) and cytotoxic T lymphocyte antigen 4 (CTLA-4). **b** | Alternatively, primary T cells can be isolated and purified from donors not diagnosed with cancer. A CAR can be introduced into those cells using CRISPR systems, which can also be used for inactivation of the genes encoding T cell receptor (TCR) and the HLA components to generate 'universal' allogeneic CAR T cells to be infused into patients with cancer.

B-ALL compared with conventionally generated CAR T cells because tight regulation of CAR expression delays T cell differentiation and exhaustion[18].

CRISPR for T cell receptor-based and tumour-infiltrating lymphocyte-based therapies. TIL-based adoptive immunotherapy has been shown to mediate cancer regression in animal models and in ~50% of patients with metastatic melanoma[154], but the complicated TIL manufacturing procedures have limited the clinical application of this approach. In addition, T cell exhaustion might compromise the antitumour activity of TILs[155]. In mice, CRISPR-mediated inactivation of the gene encoding the zinc-finger transcription factor GATA3 (*Gata3*) led to reduced T cell exhaustion of $CD8^+$ TILs[156]. The aim of the first clinical trial using CRISPR reported was to inactivate *PD1* in isolated T cells from patients with refractory metastatic non-small-cell lung cancer, which were then infused back into patients[157]. The safety of this approach and three different dosage regimens are currently being evaluated (NCT02793856); results of an interim analysis indicate that *PD1*-null T cells might be safely used in patients[157,158].

To enhance the activity of TILs and reduce the adverse events caused by nonspecific target recognition, cancer-reactive TCRs have been delivered by integrating viral vectors into primary T cells[159]. In contrast to CAR T cells, which recognize cellular surface molecules only, TCRs can recognize both surface and intracellular proteins through digestion of those proteins and presentation of peptide epitopes by HLA molecules[137]. A T cell product with an engineered TCR targeting the human tumour antigen NY-ESO-1 has demonstrated antitumour activity in patients with multiple myeloma[160].

Despite the transduction of tumour antigen-specific TCRs, engineered TCRs compete with endogenous TCRs for CD3 binding, a process required for T cell activation. Transgenic and endogenous TCRs might even hybridize to form mismatched TCR dimers[20]. Correspondingly, CRISPR-mediated inactivation of endogenous TCR genes has been shown to enhance the reactivity of T cells harbouring transduced TCRs against haematological cancer cells[20]. Such manipulation might also reduce the incidence of fatal autoimmunity owing to unwanted TCR dimers[20].

CRISPR versus ZFN or TALEN

ZFN, TALEN and CRISPR systems are currently being used as tools to develop cellular immunotherapies[18,19,137,148]. The primary advantage of CRISPR over the other two genome-editing platforms is that, owing to targeting genetic sequences with an sgRNA instead of protein recognition, CRISPR can be rapidly retargeted to new genomic sequences. Given the complexity of genetic modifications required to develop a specific and efficacious universal cell therapy for cancer, multiple genome-editing events might be required during product development[18,19,137,148]. The delivery of a single form of Cas9 protein and multiple sgRNAs might require a lower dosing of macromolecules than that associated with the delivery of multiple ZFNs or TALENs, thus minimizing the associated cellular toxicity[18,19,87,137,148,161]. In addition, the reported

genome-editing efficiency of CRISPR is likely higher than that of other systems[87,161].

CRISPR immune-related adverse events

The host immune responses towards CRISPR-mediated genome editing need to be considered in therapeutic approaches. The use of CRISPR in immuno-oncology could generate an immune response to both components: the delivery system and the genome-editing payload. Indeed, Cas9 exposure via adenoviral and AAV delivery in mice evokes Cas9-specific antibodies[97,162]. Pre-existing antibodies against SaCas9 and SpCas9 were detected in 79% and 65%, respectively, of humans in the populations tested, and anti-SaCas9 T cells were identified in 46% of these individuals[163]. While the interactions of innate or acquired immunity with Cas9 in CRISPR systems administered with a therapeutic intent remain unclear, a concern is that innate immunity or pre-existing Cas9-targeting antibodies could interfere with delivery systems that use ribonucleoprotein particles (RNPs). Immune recognition is not expected with systems that rely only on nucleic acids to encode Cas9 or systems in which RNPs are masked from the immune system via nano-encapsulation. A second concern is that cells expressing Cas9 could ultimately present Cas9 antigens, thereby leading to targeted destruction of engineered cells by the immune system[164]. Further studies are needed to determine whether or not Cas9 immunity is an important hurdle to therapeutic genome editing.

Approaches to reduce the immunogenicity of Cas9 have included Cas production in mammalian cells rather than in *Escherichia coli* to adjust the pattern of post-translational modifications. The masking of epitopes on Cas proteins via protein engineering might also facilitate escape from innate immunity in humans. Another immunogenicity challenge is related to the hairpins of sgRNA, which are important for binding with the Cas9 proteins[1]: the secondary structures of sgRNA might be detected by pattern recognition receptors and generate an immune response. Chemical modifications of sgRNA, such as 2′-*O*-methylation, hold the potential to mitigate such stimulatory effects[161].

DSBs generated by CRISPR can activate p53, resulting in cell cycle arrest and cellular death[165,166]. Thus, one can reasonably speculate that efficient genome editing might reduce the viability of immune cells and enrich *TP53*-mutant cells owing to selection[165,166]. Hence, monitoring of T cell viability and p53 function of all edited cells after genome editing is crucial.

On-target mutagenesis by CRISPR (including large deletions and genomic rearrangements) has been detected in mouse stem cells and progenitor cells, as well as in an immortalized human epithelial cell line[167]. The frequencies and pathogenic consequences of such genetic alterations should be carefully examined for an optimal clinical development of genome-editing-aided immunotherapy[167]. Considering the potential adverse effects of CRISPR and the unleashing of inflammatory responses with T cell therapy, a 'safety switch' can be inserted into T cells[168]. Such a switch consists of an extracellular binding domain (for example, human FK506-binding nuclear protein) with a small-molecule drug ligand linked to an intracellular apoptosis-inducing domain (for example, modified human caspase 9); a single dose of such a drug ligand can result in elimination of >99% of transferred T cells[168]. A safety switch could be introduced into T cells by targeted CRISPR-mediated insertion together with a CAR or TCR in order to perform multiplexed deletion of several key regulatory receptors in a one-step genome-editing approach[169].

Direct tumour targeting with CRISPR

CRISPR can be used to directly modify tumour cells. In one study, DSBs were specifically generated in cancer cells, but not in non-transformed cells, using a pair of Cas9 nickases targeting two cancer-specific gene fusions, *TMEM135–CCDC67* (also known as *DEUP1*) and *MAN2A1–FER*[170]. A suicide gene (encoding a prodrug-converting enzyme) with homology to the sequences surrounding the breakpoints was delivered with an adenoviral vector to enable introduction via HDR[170]. The approach consisting of CRISPR modification and later administration of the prodrug ganciclovir led to partial remission of xenograft tumours[170]. In another study, CRISPR-dependent specific targeting of mutant *KRAS*, but not wild-type *KRAS*, alleles was reported to inhibit growth of cancer cells in vitro and in vivo[171]. CRISPR systems might also be used to treat or prevent virally driven cancers through the targeted elimination of the oncogenic viruses from the genome[172].

Despite progress in therapeutic approaches based on the direct modification of cancer cells, we can expect the mutagenic nature of cancer to challenge such strategies. Cancer cells in which in-frame shifting or inefficient gene editing have occurred will be resistant to CRISPR-based treatment. Furthermore, additional work is needed to develop delivery systems optimal for these approaches in order to ensure safe and efficient targeting of all tumour cells[87].

CRISPR for diagnostics

Cas13a is an RNA-guided and RNA-targeting CRISPR enzyme[173,174] that exhibits indiscriminate ribonuclease activity upon target recognition as a 'collateral effect', leading to cleavage of non-targeted single-stranded RNAs nearby. Combining the collateral effect of Cas13a with isothermal amplification enables detection of DNA or RNA with single-base mismatch specificity and at attomolar sensitivity[175]. This platform provides the possibility of detecting specific cancer mutations, which has led to efforts to use CRISPR for early detection for cancer[175] (FIG. 6). Indeed, this system has been reported to be sensitive enough to detect cancer mutations present in a concentration as low as 0.1% of total DNA[175]. Using CRISPR proteins and lateral flow for visual readout (simple paper-based devices, similar to pregnancy tests), this technology has been further improved for portable, rapid, highly sensitive and multiplexed quantitative detection[176]. This improved technology, along with a sample preparation protocol, creates an ultrasensitive and field-deployable platform for the detection of viral infection in clinical settings[177]. In parallel, the combination of the nonspecific single-stranded deoxyribonuclease activity of Cpf1 with isothermal amplification

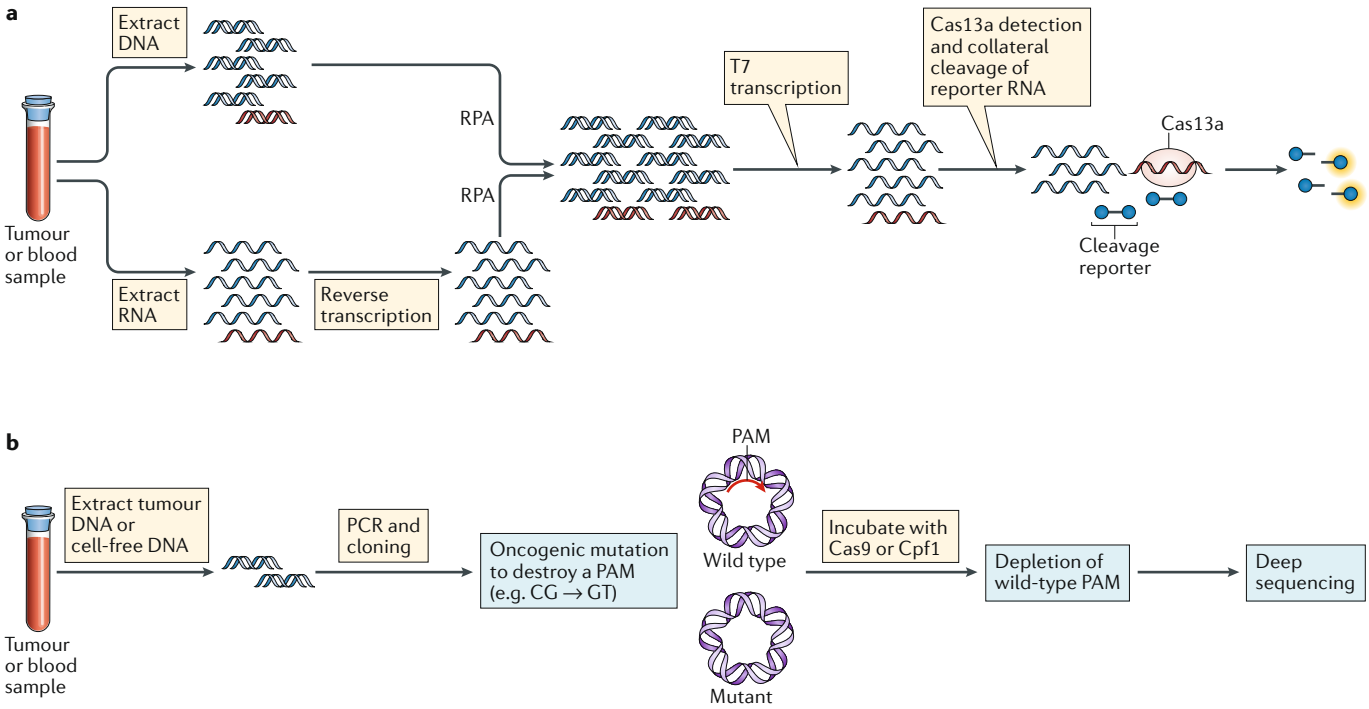

Fig. 6 | **CRISPR for cancer detection. a** | Cas13a can enable the detection of RNA and DNA. RNA first needs to be reverse-transcribed to DNA, which is then amplified by recombinase polymerase amplification (RPA) and then transcribed to RNA. Only the second and third steps are performed with DNA[175]. Upon activation, Cas13a engages in 'collateral' unspecific cleavage in nearby regions of a quenched fluorescent non-targeted RNA that acts as a reporter. **b** | Owing to the error rate of next-generation sequencing (NGS), low numbers of cells containing gene mutations (for example, 100 tumour cells out of 1 million non-malignant cells) are hard to detect in tissues or blood. The genomic sequences of a mutated oncogene and the wild-type variant can be amplified and cloned into a plasmid. The mutated sequence lacks the protospacer adjacent motif (PAM), which is present in the wild-type version, and, thus, the mutant sequence cannot be targeted by *Streptococcus pyogenes* Cas9 (SpCas9), but the wild-type sequence is cut by SpCas9, enabling enrichment of mutant sequences that can be detected by NGS with high sensitivity.

has resulted in a rapid and specific method for molecular diagnostics of viral infection with attomolar sensitivity[178]. CRISPR enzymes have been used in PCR analysis to specifically eliminate wild-type DNA sequences and improve the sensitivity of detecting mutated DNA sequences[179]. PCR amplification and deep sequencing of the CRISPR-targeted locus enable higher sensitivity (<0.01%) than conventional targeted deep sequencing (0.1–0.5%)[179]. The platforms described have not yet been translated into clinical applications, but CRISPR might be used in the near future to detect low numbers of cancer cells or rare mutations in human samples.

Technical considerations

Off-target genome editing with CRISPR

Studies indicate that SpCas9 might lead to off-target genome editing, which can be mitigated through careful selection of the guide sequences, the use of improved methods of delivery or even re-engineering of the Cas9 proteins and sgRNA[32–40]. A systematic comparison of the off-target effects of CRISPR with those associated with other genome-editing systems is difficult to establish, but the results of genome-wide off-target analysis suggest that the experimental conditions can be optimized to obtain a low level of off-target activity with substantial on-target editing[32–40]. The potential high fidelity of genome editing with CRISPR suggested in these studies can pave the road for CRISPR to be used in anticancer therapeutic approaches[32–40,180,181].

The target sites of CRISPR genome editing can be specific, but the downstream events after the generation of DSBs could lead to unwanted results[42,72]. For example, in current approaches for the generation of enhanced or universal CAR T cells, multiple DSBs might be induced, which could potentially induce chromosomal translocation[42,72]. Those events need to be carefully examined during development of CRISPR-engineered CAR T cells.

Delivery of CRISPR

As described, both viral and non-viral vectors have successfully been used to create cellular and animal cancer models using CRISPR systems. For example, electroporation of Cas9 protein–sgRNA complexes can efficiently induce indel formation in human primary T cells[182,183]. For targeted insertion of gene cassettes in primary T cells and $CD34^+$ haematopoietic stem cells, AAV6 is often used as a vector for donor sequences[184]. Other transfection methods of T cells, such as lentivirus, adenovirus or physical methods, have been used[87]. A study with results published in 2018 reported efficient targeted insertion in primary T cells using non-viral delivery of CRISPR machinery in an RNP format and a DNA donor[169]. The optimization of non-viral vectors might enable genome-editing of CAR T cells, TILs and other immune cells

independently of the lengthy and expensive manufacturing processing associated with the use of viral vectors, although the toxicity associated with electroporation of DNA into T cells remains substantial[169].

To ensure efficient antitumour activity with CRISPR systems delivered in vivo, the improvement of the delivery platforms and CRISPR enzymatic activities will be crucial[87]. The methods currently available target only a fraction of tumour cells, either because of the low frequency of HDR or owing to in-frame shifting after indel formations. Inefficient genomic targeting or the limitations associated with the delivery platforms might lead to genome editing in only a limited fraction of tumour cells and the subsequent failure of CRISPR-based treatments[170,171].

CRISPR: genetic tools in cancer research

Besides the technical aspects related to the use of CRISPR systems, the consideration of several biological aspects might be crucial when using CRISPR systems as genetic tools in cancer research[185]. In 2017, Housden et al.[185] highlighted the need for caution when interpreting the results of genome-editing approaches. In zebrafish, silencing of *egfl7* (which encodes an extracellular matrix protein) via morpholinos led to severe vascular defects, an effect also observed when CRISPRi was used to suppress the mRNA of this gene[186]. By contrast, no obvious phenotype was observed with TALEN-mediated deletion of *egfl7* (REF.[186]). A set of genes was found to be upregulated after gene inactivation but not after gene silencing, suggesting the presence of a network to compensate the effect of genetic mutations[186]. Similar observations have been obtained for other genes, such as *vegfa* and *bem1* (REF.[186,187]).

The compensation of loss-of-function via homeostatic regulation can vary depending on whether it has been achieved with small-molecule inhibitors or genetic inactivation. One striking example is that CDK9 kinase inhibitors, but not deletion of *CDK9*, induce *MYC* expression, likely because inactivated CDK9 but not the absence of *CDK9* indirectly induced the expression of primary response genes[188]. Thus, for characterization of genes with unknown function, investigations of different modes of inhibition can provide complementary information, and therefore approaches that yield gene silencing (RNA interference), gene inactivation (CRISPR systems) or protein inhibition (small molecules) should be combined.

Conclusions

Despite the somewhat short history of CRISPR-based genome editing, CRISPR systems have been applied in many areas of cancer research. CRISPR systems have been used to generate cells and animal models, dramatically improving the ease and speed with which genetic modifications can be made. With previous methods, years were required to generate a genetically modified mouse model that mimics tumour progression, with a high possibility of failure during that long process. The same animal model can be generated in <1 year by injecting the CRISPR machinery into mouse zygotes; alternatively, direct injection of CRISPR systems into animals can create similar somatic models[81,87]. With CRISPR, the generation of cellular models with various targeted genome modifications is feasible and results in accelerated functional genomic analysis to discover cellular mechanisms[185]. CRISPR-edited organoids, as self-organized 3D tissues with specific genomic modifications, closely mimic many features of human tumours and are suitable to explore mechanisms of tumour initiation and progression[11,79,80]. Various types of CRISPR-based screening systems have been developed, enabling genome-wide or focused, loss-of-function (CRISPR and CRISPRi) or gain-of-function (CRISPRa), in vitro, ex vivo and/or in vivo screening studies. CRISPR-mediated approaches to base editing and epigenetic editing have also been developed, enabling a better understanding of cancer biology[189].

CRISPR systems can also enable accelerated discovery and development of targeted therapies[65]: therapeutic targets can be discovered in CRISPR screening studies; and the targets and the efficiency and safety of cognate small-molecule inhibitors can be quickly validated or invalidated in CRISPR-mediated cellular and animal models[65]. Moreover, CRISPR systems have enabled the development of new cellular immunotherapies. In addition to a number of breakthrough discoveries in immuno-oncology, allogeneic T cells with superior activity and less toxicity could be generated through the manipulation of primary human T cells with CRISPR or other genome-editing tools. Indeed, two infants have been treated with TALEN-modified CAR T cells, and several dozen patients in China have been treated with CRISPR-mediated T cells with *PD1* inactivation[157,190]. In a number of trials, the use of CRISPR to modify human T cells to treat patients with cancer has been proposed[190]. CRISPR might even be directly delivered to tumour cells in vivo. Finally, the application of CRISPR systems that enable detection of mutant DNA or RNA could provide a new method for the early detection of cancer[175,179]. In conclusion, CRISPR systems have already had an immediate and important effect on cancer research ranging from fundamental mechanistic studies to drug development; we expect that CRISPR will continue to have an important role in the generation of the next transformative cancer therapies.

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H.Y. and W.X. researched different sections of the manuscript, and D.G.A. provided key opinions and oversaw data research. All authors discussed content and reviewed and edited the manuscript before submission.

Competing interests
H.Y., W.X. and D.G.A. have applied for CRISPR-related patents, one of which has been issued. D.G.A. is a scientific co-founder of CRISPR Therapeutics.

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