PORTLAND PRESS

Review Article

CRISPR base editing applications for identifying cancer-driving mutations

Martin Pal[1,2] and ⓘ Marco J. Herold[1,2]

[1]WEHI (Walter and Eliza Hall Institute of Melbourne), Melbourne, Victoria 3052, Australia; [2]Department of Medical Biology, University of Melbourne, Melbourne, Victoria 3052, Australia

Correspondence: Marco J. Herold (herold@wehi.edu.au)

OPEN ACCESS

CRISPR base editing technology is a promising genome editing tool as (i) it does not require a DNA template to introduce mutations and (ii) it avoids creating DNA double-strand breaks, which can lead to unintended chromosomal alterations or elicit an unwanted DNA damage response. Given many cancers originate from point mutations in cancer-driving genes, the application of base editing for either modelling tumour development, therapeutic editing, or functional screening is of great promise. In this review, we summarise current DNA base editing technologies and will discuss recent advancements and existing hurdles for its usage in cancer research.

Introduction

The advent of RNA-guided programmable clustered regularly interspaced short palindromic repeats (CRISPR)-associated (Cas) nucleases has revolutionized genome editing applications [1–4]. The ability of CRISPR–Cas9 to introduce desired edits in the genome with ease and great precision has accelerated the pace of genetic research. However, introducing precise point mutations remains technically challenging. As a large number of diseases, including many cancers, are caused by single nucleotide variants (SNVs), the ability to either install disease-driving point mutations for disease modelling and drug discovery, or reverse those point mutations for therapeutic applications, is of critical importance. Hence, the development of CRISPR base editors represent a potential solution for the ability to install point mutations and to correct disease-changing mutations [5].

CRISPR base editors avoid DNA double-strand breaks generated by nucleases, and therefore undesired chromosomal alterations. In addition, base editing technology circumvents the need for homology-directed repair, which relies on the addition of a DNA template and is inefficient in non-dividing cells [6]. Owing to its potential to introduce point mutations at ease, base editing enjoys great popularity, which is reflected by the pace of development ever since the first base editors were described. Those advancements and optimisations are crucial to facilitate the therapeutic applications for many diseases, including cancer [7].

While this review discusses base editing in mouse and human cells, base editing has been successfully applied in plants [8,9] — including cotton [10], rice [11], soybean [12] — zebrafish [13,14], pigs [15,16], rats [17], rabbits [18–20], sheep [21,22], and more recently monkeys [23,24].

In this review, we will highlight some of the recent advancements that have been made towards the application of CRISPR base editing in mammalian cells using different delivery methods of base editing reagents for disease modelling, therapeutic applications and for functional genetic screens. While the principles can be translated to other research and disease backgrounds, this review focuses on DNA base editing applications for introducing cancer-specific gene variants.

The chemistry of CRISPR base editing

Originally DNA base editors were designed by creating Cas9 fusion proteins with enzymatic domains of proteins known to induce deamination of bases in DNA [25]. Directed to its target site in the genome by a specifically designed guide RNA, base editors install point mutations without the need for a template or

introducing DNA double-strand breaks. There are two main DNA base editors developed to date enabling the conversion of all four DNA base pairs: Cytosine base editors (CBE) and Adenine base editors (ABE) [5].

Cytosine base editors

Komor et al. [25] from the Liu laboratory developed the first class of CBEs containing a fusion protein of rat Apobec1 protein linked with a catalytically dead version of Cas9, capable of introducing point mutations (BE1). A major hurdle to overcome for cytosine base editing is the cellular base excision repair (BER) pathway as deamination of cytosine to uracil leads to the excision of uracil by uracil DNA glycosylase (UDG). Therefore a uracil glycosylase inhibitor (UGI) from the *Bacillus subtilis* bacteriophage PBS1 was fused to the first CBE to protect the UG intermediate [25,26]. This next-generation base editor was called BE2. Indeed, the importance of BER for editing outcomes has recently been shown in cells with high BER function, which exhibit low base editing efficiency [27]. In a subsequent optimisation step, a nickase version of Cas9 fused to Apobec 1 and two copies of the UGI was used to create the commonly used BE3 CBE. The nicking of the unedited strand initiates mismatch-repair (MMR) as G is now flagged as a mismatch to U (which is read as T). This ensures the use of the edited strand as template for the repair, thereby introducing a permanent C-to-T mutation [25].

Adenine base editors

Adenine deaminating enzymes do not exist in mammalian cells. However, Liu and colleagues [28] identified a prokaryotic enzyme called TadA, which is known for its deaminating capability. Hence, to develop ABEs bacterial TadA deaminase domains were fused to a nickase version of Cas9. Subsequent optimisation rounds found the presence of one WT and one evolved TadA domain fused to a nickase version of Cas9 known as the common ABE7.10 base editor to be most efficient for A-to-G editing [28]. As the deamination of adenine results in inosine, and given inosine is not readily excised and therefore reversed by BER or any other cellular repair mechanisms, additional components to aid adenine base editing are not needed. Indeed, adenine base editing is achieved with great efficiency accompanied with very low rates of InDel production when compared with CBEs [29].

Mode of action

The ability of both classes of base editors to introduce point mutations relies on protospacer-adjacent motif (PAM) and single-guide RNA (sgRNA) mediated binding of the DNA by Cas9, which leads to denaturing of the DNA creating the typical R-loop thereby exposing a short stretch of single-stranded DNA on the non-complementary strand. The single exposed DNA strand is now accessible for deamination and subsequent introduction of the desired point mutation. For the widely used BE3 editor the editing window is 5 nucleotides in length, usually from positions 4 to 8 within the protospacer sequence, when position 1 is counted as first position 5′ upstream of the PAM site. The editing window for the commonly ABE7.10 editor is slightly tighter, ranging from positions 4 to 7 within the protospacer sequence, when the PAM is counted as positions 21–23 (Figure 1).

It should be noted that since its first inception, the design and efficiency of base editors has been continuously improved based on the original design of CBEs and ABEs mentioned above. For example, narrowing or widening the targeting window and minimising off-target effects have been a major focus of base editor engineering. While the description of those variants is beyond the scope of this manuscript, we would like to direct the readers to a recently published overview by the Liu laboratory [30].

Delivery options of base editor proteins into cells for introducing cancer-driving mutations

The first generation of base editors have been established in standard cell lines, such as human embryonic kidney (HEK) cells [25,28]. However, delivery of CRISPR base editing reagents into primary cells or whole organisms remains challenging and presents the bottleneck of many base editing applications. Here, we summarise current attempts to install cancer-driving mutations in disease-relevant cells (Figure 2).

Nucleic acid and RNP approaches

Because of its ease (cost and stability), plasmid encoded base editors are a convenient tool to achieve the expression of base editors after transfection into cells. Indeed, plasmids have been used in initial studies to
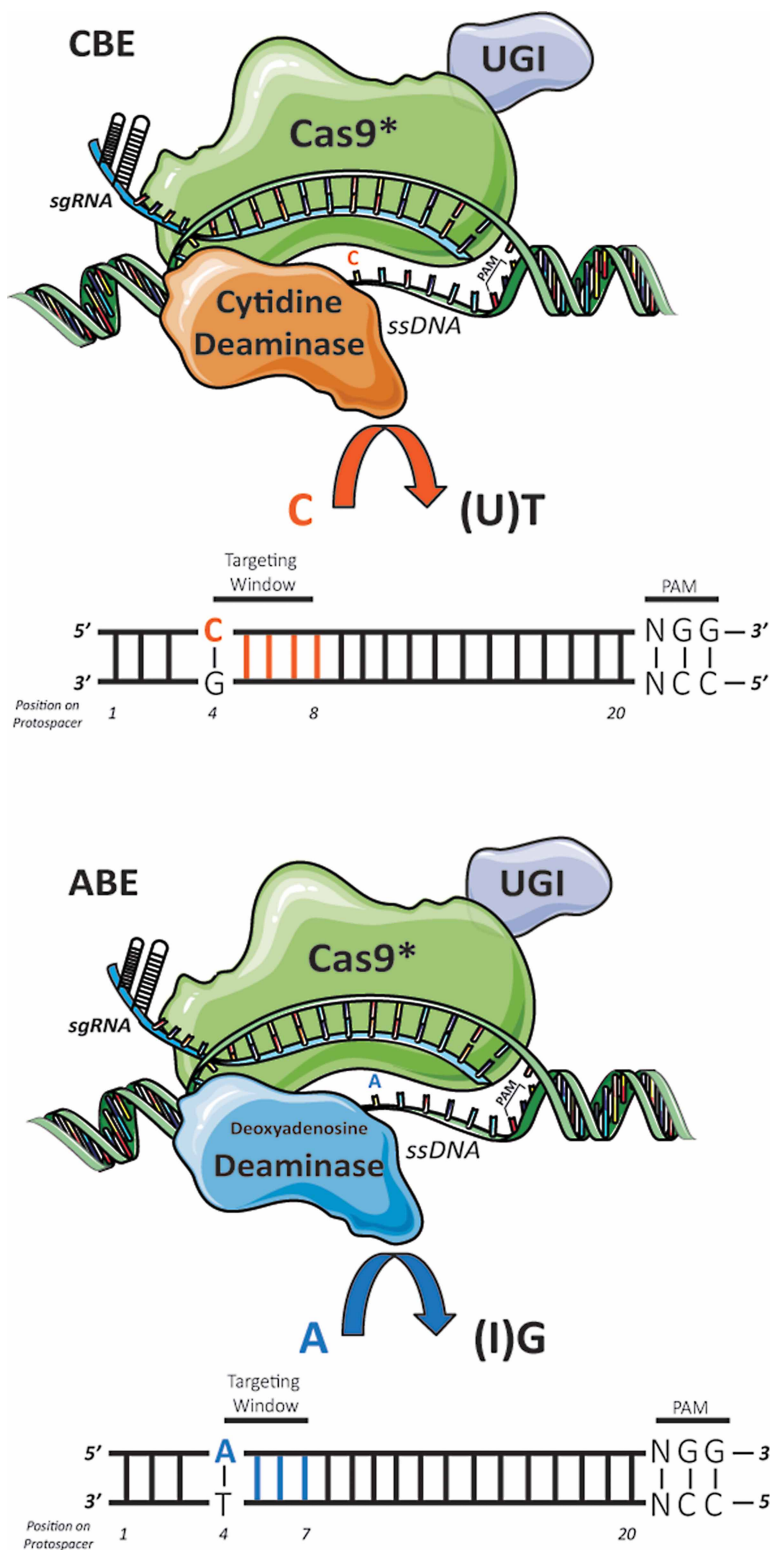
Figure 1. Composition of DNA base editors.   Part 1 of 2

Schematic of the basic structure of CRISPR DNA base editors. Cytosine base editors (CBE) consist of a nickase Cas9 variant (Cas9*) fused with a cytidine deaminase enzyme. In addition, one or two copies of an uracil glycosylase inhibitor (UGI) are fused with the protein complex to prevent cellular base excision repair (BER) mechanisms. CBEs introduce a C-to-T point mutation into DNA with a C:U intermediate. For adenine base editors (ABE) Cas9* is fused with an deoxyadenosine deaminase

Figure 1. Composition of DNA base editors.

enzyme enabling a permanent A-to-G point mutation in DNA with a A:I intermediate. The target base is shown in the single-stranded R-loop. Diagram indicating the targeting window for CBE or ABE, with deamination positions between position 4–8 (CBE) and 4–7 (ABE) on the protospacer sequence, when the PAM site is counting as positions 20–23.

establish base editing in cells and are used to enable *in vivo* editing by hydrodynamic tail vein injection. However, plasmids are toxic for some cell types, and, prolonged expression from plasmids can lead to undesired edits [31,32]. Another suitable delivery method is complexing Cas9 protein with synthesised sgRNAs to generate ribonucleoprotein (RNP) complexes [33]. Indeed, base editor RNP complexes have been successfully used for therapeutic editing in primary cells, such as human CD34+ cells [34]. Instead of RNPs, base editor constructs can be delivered as mRNA. Using mRNA offers the advantage that it can be readily translated into the base editor protein, and while mRNA can be chemically stabilised, it usually is degraded quickly. This reduces potential off-target effects while maintaining precise on-target editing [32]. Indeed, transfection of base editor mRNA together with sgRNAs targeting one or multiple genes by electroporation or lipofection appears to be a promising approach to achieve precise editing in primary cells, such as T cells or human induced pluripotent stem cells (hiPSCs) [29,35,36].

In addition to transfection techniques, gene editing components can be directly delivered into cells by microinjection. Indeed, to date fertilised oocytes have been successfully used for the generation of disease models by injection of base editor mRNA or RNP alongside gene targeting sgRNAs [37,38].

Viral delivery

Viral vectors have been the gold standard delivery vehicle for *in vivo* genome editing. In this regard, Adeno-associated viruses (AAVs) are widely used for local tissue delivery of genome editing tools [39]. However, owing to the packaging limits of AAVs (~4.7 kb), delivery of Cas9 base editors via AAVs remains challenging.
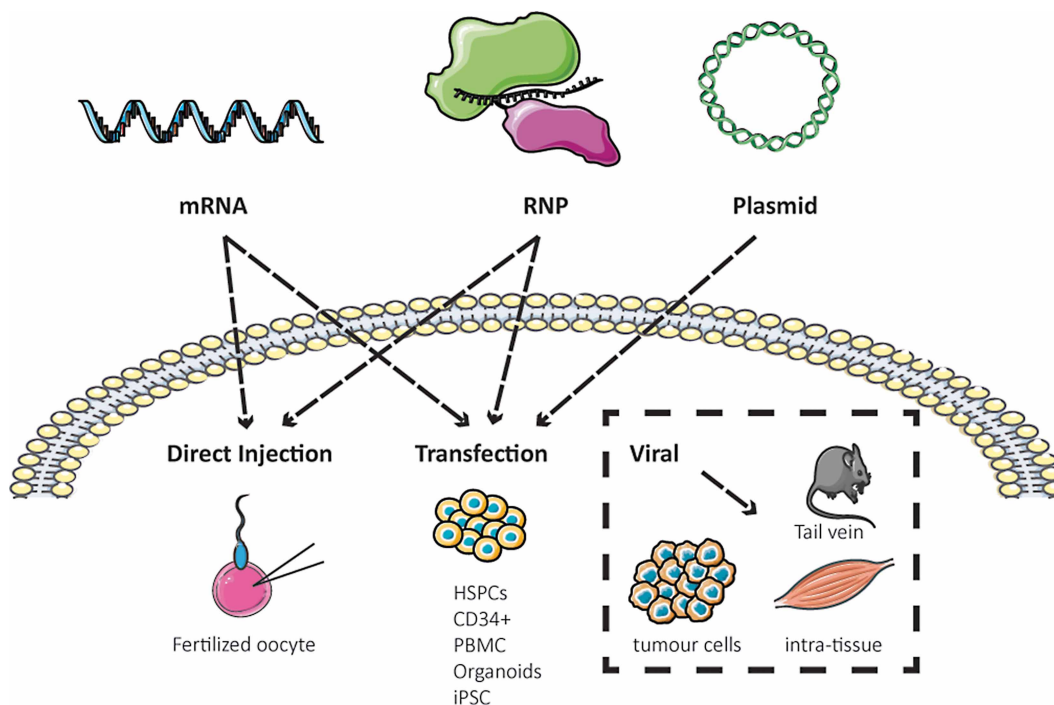
Figure 2. Overview of the different base editor delivery options useful for diverse applications.
Schematic showing different delivery options for base editors. Delivery of base editors into cells can be achieved by either direct injection or transfection of base editor constructs in form of mRNA, ribonucleoprotein (RNP) or plasmid. In addition, viral delivery options can be employed for either hard-to-transfect cells or intra-tissue delivery of base editors.

Recently, split AAV approaches have been described in which the coding sequence for base editors is split up in two AAV vectors and hence viral particles [40–43]. By utilising split-intein protein moieties base editor expression is achieved after viral delivery and trans-splicing of the protein products encoded by the dual AAVs [40]. In addition to AAVs, lentiviral vectors are commonly used to deliver CRISPR–Cas9 complexes into cells to generate cancer-driving mutations *in vivo* [44]. In this regard, Zafra et al. [45] recently delivered optimised versions of the BE3 CBE into human cancer cell lines. Similarly, in a recent pre-print publication, lentiviral vectors were used to achieve CBE expression in cancer cells for large-scale functional screening [46].

While viral delivery techniques ultimately might reach packaging capacities, the recently discovered novel Cas Θ enzyme presents an exciting avenue, as this novel Cas protein is much smaller in size compared with Cas9 while maintaining its ability to bind DNA [47].

An overview of how base editors are used with different delivery methods can be found in Table 1.

Disease modelling to characterise cancer-driving mutations using base editing technology

Base editing technologies represent a breakthrough for the characterisation of cancer-driving mutations *in vivo*. Recently, colleagues from the Jonkers laboratory established a transgenic Cre recombinase inducible BE3 base editor system to test critical tumour-driving mutations in a mouse model of triple-negative breast cancer [48]. Using their cancer model which is driven by loss of p53 and BRCA1 in mammary tissue, cancer-driving point mutations, such as in the AKT/PI3K pathway, can be readily introduced after lentiviral intraductal delivery of sgRNAs.

In a different study, Zafra et al. [45], explored the importance of point mutations in driving hepatocellular carcinoma. By tail vein injecting plasmids of CBEs, BE3 or an optimised version with an additional N-terminal flag-tagged nuclear localisation signal (FNLS-BE3), together with plasmids encoding the *Myc* oncogene and sgRNA targeting the activating S45F mutation of the catenin-beta 1 (CTNNB1) gene to drive cancer development by increased WNT-signalling, tumours were driven in livers of adult mice. Interestingly, analysis of tumour nodules revealed near-complete editing for the activating S45F mutation [45].

In another attempt to improve disease modelling and therefore potential modes of drug discovery, the Kuehn laboratory tested the possibility of introducing tumour-driving point mutations in hiPSCs to generate a pool of readily available isogenic cells from healthy donors with the same genetic background [29]. Of particular importance are mutations in the TP53 gene for which more than 5000 mutations are currently described. Indeed, Surun and colleagues successfully introduced TP53 point mutations by lipofection of mRNA for CBE and ABE base editors alongside sgRNAs targeting seven missense and two nonsense mutations of the TP53 gene into hiPSCs. Functional analysis of those introduced mutations revealed that iPSCs with mutated p53 protein are more resistant to cell cycle arrest when treated with the small-molecule murine double minute (MDM2) inhibitor Nutlin-3a, thereby demonstrating the oncogenic potential of p53 mutations [29]. While they achieved editing efficiencies of up to 90% (Y163H mutation), they found ABE editing more efficient and accurate when compared with CBE editing [29].

In addition to those ascribed studies pointing to the ability of base editing technologies to drive cancer progression, the feasibility of *in vivo* base editing to correct a tumour-driving mutation has been tested [49]. Li et al. targeted the activating hotspot −124C > T mutation within the TERT promoter region driving glioblastoma development. Intracranial AAV-delivery of a nickase version of *Campylobacter jejuni* (Cj) Cas9 fused to the commonly used ABE ABE7.10, creating CjABE together with a sgRNA targeting the −124C > T mutation, impaired tumour growth in PDX models in the brains of mice treated with sgRNA targeting the −124C > T mutation [49]. This was accompanied by a reduction in TERT activity, increased apoptosis and interestingly *in vivo* editing efficiencies matched the ones established *in vitro* [49]. In summary, this elegant study establishes the possibility for targeting cancer-driving mutations in patients.

Generation of pre-clinical mouse models using base editing technology

Another critical tool for mimicking disease, are murine models generated by direct injection of CRISPR reagents into fertilised oocytes [50]. While traditional HDR methods using Cas9 nucleases are successfully applied for the generation of mouse models, base editing technology might be advantageous if introducing a point mutation in a critical exon. Indeed, several studies used base editors to generated novel disease models by

Table 1. Summary of base editors and delivery modes for clinically relevant cells

Delivery type	Base editor	Delivery route	Model	Reference
AAV	CjABE (Campylobacter)	Intracranial	Glioblastoma	[49]
AAV	BE3, ABEmax	Retro-orbital	Neurodegeneration	[74]
AAV	ABE7.10, extended sgRNAs	Intramuscular	Muscular Duchenne Dystrophy	[75]
AAV	BE3	Intravenous *in utero*; retro-orbital; vitellein vein for fetuses	Therapeutic editing of hereditary tyrosinemia type1	[76]
AAV	SaKKH-BE3	tail vein injection	Metabolic liver disease	[40]
AAV	AID-CBEmax	Intracochlear	Mouse model of deafness (Baringo mouse)	[41]
AAV	BE3	Tail vein injection	Therapeutic editing of cardiovascular disease mouse model	[77]
AAV	BE3	Intratracheal	Therapeutic editing of ALS mouse model	[42]
AAV, plasmid	BE4max	Tail vein injection	Metabolic liver disease	[78]
lentivirus	BE3	Cell transduction	BRCA1/2 screening	[61]
lentivirus	BE3.9max	Cell transduction	Gene variant screening	[46]
RNP	ABE	Electroporation	CAR-T cell engineering	[56]
RNP	A3A (N57Q)-BE3	Electroporation	Therapeutic SCD editing	[34]
RNP	BE3	Zygote electroporation	Embryo targeting	[37]
mRNA	BE3, ABE7.10	2-cell stage injection	Embryo targeting	[52]
mRNA	BE4, ABE7.10; BE2, BE3, SaBE3, VQR-BE3, BE4, ABE	Zygote injection	Embryo targeting	[51,37,53]
mRNA	ABEmax	Transfection	hiPSCs, patient-derived fibroblasts	[79]
mRNA	ABE8	Electroporation	CAR-T cell	[57]
mRNA	BE4, ABE7.10	Lipofection	hiPSCs	[29]
mRNA	RA6.3	Lipid nanoparticle (LNP) tail vein injection	Tyrosinemia mouse model	[73]
mRNA, plasmid	ABE6.3, RA6.3	Tail vein injection	Hereditary tyrosinemia type 1	[80]
mRNA, RNP	BE3, BE4, coBE4	Electroporation	CAR-T cell	[36]
Plasmid	BE3, BE3-FLNS	Tail vein injection	Hepatocellular carcinoma	[45]
Plasmid	ABEmax, xCas9-ABEmax	Transfection	Human intestinal organoids, CF	[81]
Plasmid	ABEmax-NG	Electroporation	Patient-derived liver and intestinal organoids	[72]
Transgenic expression	BE3	Intraductal lentiviral sgRNA	Triple negative breast cancer	[48]

Table showing different base editors for usage in clinically relevant cells. AAV: Adeno-associated virus; RNP: ribonucleoprotein; ALS: amyotrophic lateral sclerosis; BRCA1/2: breast cancer genes 1 and 2; CAR: chimeric antigen receptor; SCD: sickle cell disease; hiPSCs: human induced pluripotent stem cells; CF: cystic fibrosis.

injecting either CBE or ABE encoding mRNA together with sgRNAs into the one-cell stage mouse embryos to introduce specific point mutations [51,52]. Detailed analysis of injected mouse embryos revealed substantial off-targets for CBE but not for ABE editing, probably owing to spurious deamination of random single-stranded DNA by the Apobec molecules of the CBEs [53].

PORTLAND
PRESS

Genetic engineering of CAR-T cells for cancer immunotherapy using base editing technology

Base editing holds great promise for genetic engineering of CAR-T cells aiding cancer immunotherapy. In contrast to conventional HDR methods using WT Cas9 nucleases, base editing can efficiently disrupt gene expression without leading to Cas9-mediated DNA double-strand breaks and subsequent chromosomal rearrangements [54].

In a pilot study, Webber et al. [36] used a codon-optimised 4th generation cytosine base editor (coBE4) for efficient multiplex base editing in primary CD3^{+}T cells for genes relevant for the generation of allogenic CAR-T cells. Current CAR-T cell approaches for cancer therapy are hampered by the immune escape of tumour cells by up-regulating the immune checkpoint protein programmed death ligand 1 (PD-L1) to counteract programmed cell death 1 (PD1) receptor expression on CAR-T cells, thereby stalling its cytotoxic tumour-killing potential [55]. In the recent study, delivering coBE4 mRNA by electroporation together with sgRNAs targeted to disrupt splice sites for the T-cell receptor alpha (TCAR), beta-2 microglobulin (B2M) and PD-1 genes, led to an efficient reduction in protein levels in cells engineered with the CAR T cell receptor by lentiviruses. Importantly, those multiplex-edited T cells retained their functionality enabling target cell killing and due to the lack of PD-1 surface expression were able to overcome PD-1/PD-L1 induced tumour cell resistance [36].

Another example of overcoming PD-1/PD-L1 mediated tumour resistance, was shown by using adenine base editing to enhance cytotoxic function of CAR-T cells. Base Editing was used to reduce the N-linked glycosylation of PD-1 thereby decreasing its expression levels [56]. Similar to a genetic disruption of PD-1 expression in CAR-T cells, decreasing protein stability by altering the post-translational glycosylation pattern of PD-1 offers a robust avenue to overcome the inhibitory effect of tumour cells on immune cells. To this end, lentiviral co-delivery of sgRNA targeting the codon of amino acid N47 of the PD-1 protein (PDCD1 gene) and the coding sequence of the mesothelin-directed CAR into T cells was followed by electroporation of ABE protein into these cells. Shi et al. [56] investigated the anti-tumour functions of edited CAR-T cells *in vivo* and found that tumour growth was reduced leading to improved survival, which was due to greater expansion of base edited CAR-T cells with decreased surface expression of PD-1.

Recently, Gaudelli et al. [57] generated a newer edition of ABE editors (ABE8s) and tested their potential to induce precise point mutations in primary T cells from healthy donors. Electroporating ABE8 mRNA into CD3^{+}CD28^{+} activated T cells together with sgRNAs designed to target splice sites to disrupt expression of genes augmenting CAR-T cell therapy, the authors were able to demonstrate efficient multiplex gene editing using base editor technology [57].

In summary, the ability to genetically engineer CAR-T cells carrying receptors for tumour-specific antigens holds great promise for the future of anti-cancer therapy.

Discovery of novel cancer-driving gene variants through functional screens using base editing technology

More than 90% of human gene variants are caused by single nucleotide variants (SNVs). However, the vast majority of these SNVs lack functional characterisation, and the causal relationship between a variant and disease progression remains unclear.

Previously, assessing this functional relationship between SNVs and phenotype remained technically challenging. Usually performed at small-scale only addressing a few variants at a time to identify their effect on disease progression, high-throughput screening measures remain critical for disease research and cancer genetics.

While a few technologies exist that can screen gene variants in large-scale, for example, saturation mutagenesis, or saturation genome editing (SAGE), they either rely on exogenous overexpression of a variant or inefficient HDR technologies. Therefore, CRISPR-mediated base editor screening offers great promise in overcoming those challenges.

Indeed, recent advancements have been made to establish CRISPR base editors as large-scale screening tools [58–60]. Excitingly, cancer-driving gene variants are a particular focus of base editing screening attempts. Kweon et al. [61] performed a targeted base editing screen to find novel functional variants of the BRCA1 gene. Using the BE3 CBE and 745 sgRNAs targeting all exons of the BRCA1 gene to introduce loss-of-function mutants, they discovered previously unknown variants important for BRCA1 function [61].

In an exciting pre-print publication, Hanna et al. [46] employ large-scale pooled functional screens using cytosine CRISPR base editors. In a first step, they set a benchmark for their base editor screens by validating pathogenic variants of the BRCA1/2 genes. Hanna et al. even went a step further and established base editor screening to identify interaction sites of common chemo-therapeutic drugs, such as BH3 mimetics and PARP inhibitors. This will help identify novel point mutations that render cells resistant to treatment with those drugs. Finally, they created a large library of >68k sgRNAs targeting >50k clinically relevant gene variants via C > T mutation corresponding to around 3500 genes, which identified novel loss-of-function variants required for DNA damage repair and cell stress triggered by low-doses of cisplatin and hygromycin, respectively, in two independent cancer cell lines (HT29, colorectal and MELJUSO, melanoma) [46].

While base editing technology for functional genetic screens is very promising, caution is warranted for the interpretation of results. Gene deletions can be induced by multiple guides targeting different regions of the same gene in both conventional knockout and base editor screens. However, base editor screens used to introduce specific point mutations often rely on the suitability of the guide RNA or cell to cell variability of editing. Therefore, particular importance is placed on validation experiments of primary screens, clearly establishing which edit is causal to the observed phenotype. In the future base editor screens can be further optimised by application of novel Cas variants or base editor architecture, and it will be interesting to see, whether functional genetic screens with ABE editors can recapitulate findings observed with CBE, thereby further strengthening the validation of base editor screens for functional genomic experiments of different gene variants.

Outlook for therapeutic editing

Advances in base editor design have greatly improved editing efficiencies [30]. Minimising off-target effects of CBEs remain a critical task to facilitate therapeutic genome editing. While it is beyond the scope of this review to expand on details on the off-target effects associated with base editing experiments, we would like to direct the readers to the landmark report by Rees and Liu [5]. Specific mutations have been made to the Apobec deaminase sequence to reduce random deamination events of RNA and DNA and to improve on-target C-to-T editing [62,63]. In a very promising improvement, the addition of the RAD51 DNA binding domain into the linker sequence between Apobec and Cas9n increases the affinity to bind the exposed single-stranded DNA and therefore improves editing efficiency and reduces editing of neighbouring cytosines ('Bystander editing') [64]. In addition, on-target activity remains critical for therapeutic base editing as many SNVs are currently not accessible due to PAM incompatibility. Therefore, advancements to improve the range of CRISPR base editors by widening PAM requirements present a promising avenue [65,66]. In this regard, dual base editors have recently been described capable of introducing both C-to-T and A-to-G mutations, thereby increasing editing potential [67,68]. In addition to those dual base editors, recently Kurt et al. [69] reported the generation of C-to-G base editors (CGBE) capable of introducing transversion mutations into human cells. Recently, attempts have been made to predict base editing outcomes to create disease-associated gene variants [70].

Limitations of current base editor technologies could potentially be overcome by the recently described prime editing technology [71]. Similar to base editors, prime editors are Cas9 fusion proteins, but instead of a deaminase enzyme a nickase version of Cas9 is fused to a reverse transcriptase enzyme which incorporates the desired edit by copying from a template encoded within the designed prime editing guide RNA (pegRNA) [71]. Indeed, prime editing was already successfully used to correct disease-causing mutations in patient-derived intestinal and liver organoids [72]. In another advancement, the first *in vivo* delivery of RNA-encoded ABE was described to correct a disease-causing mutation in hepatic cells of a tyrosinemia mouse model [73].

Taken together, the ability of genome editing technologies, such as base editing, offer great therapeutic potential to correct disease-causing point mutations.

Perspectives

- *Importance of the field*: CRISPR base editing technology offers the unique advantage of introducing precise point mutations without the need of DNA templates. Given the majority of currently know SNVs, including many of the cancer-driving gene variants, remain functionally uncharacterised, base editing tools represent crucial assets for pre-clinical and therapeutic applications.

PORTLAND PRESS

- *Summary of the current thinking*: Since their first development CRISPR base editors are continuously optimised to reduce off-target and increase on-target editing. Base editors show great potential for cancer immunotherapy by multiplex engineering of CAR-T cells.

- *Future directions*: Current limitations will be addressed by optimised base editor design aiding the therapeutic potential of this novel genome editing tool. In addition, *in vivo* base editor screens offer a great advantage for drug discovery to counteract cancer-driving gene variants.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Author Contribution

M.P. and M.J.H. planned, discussed and wrote the manuscript.

Open Access

Open access for this article was enabled by the participation of Walter and Eliza Hall Institute in an all-inclusive *Read & Publish* pilot with Portland Press and the Biochemical Society under a transformative agreement with CAUL.

Acknowledgements

Abbreviations

AAVs, Adeno-associated viruses; ABE, Adenine base editor; BER, base excision repair; CAR-T cells, chimaeric antigen receptor - T cells; CBE, Cytosine base editor; CGBE, C-to-G base editors; CRISPR, clustered regularly interspaced short palindromic repeats; HDR, homology directed repair; hiPSCs, human induced pluripotent stem cells; iPSCs, induced pluripotent stem cells; PAM, protospacer-adjacent motif; RNP, ribonucleoprotein; sgRNA, single-guide RNA; SNVs, single nucleotide variants; UGI, uracil glycosylase inhibitor.

References

1   Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J.A. and Charpentier, E. (2012) A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science* **337**, 816–821 https://doi.org/10.1126/science.1225829
2   Sapranauskas, R., Gasiunas, G., Fremaux, C., Barrangou, R., Horvath, P. and Siksnys, V. (2011) The *Streptococcus thermophilus* CRISPR/Cas system provides immunity in *Escherichia coli. Nucleic Acids Res.* **39**, 9275–9282 https://doi.org/10.1093/nar/gkr606
3   Cong, L., Ran, F.A., Cox, D., Lin, S., Barretto, R., Habib, N. et al. (2013) Multiplex genome engineering using CRISPR/Cas systems. *Science* **339**, 819–823 https://doi.org/10.1126/science.1231143
4   Mali, P., Yang, L., Esvelt, K.M., Aach, J., Guell, M., DiCarlo, J.E. et al. (2013) RNA-guided human genome engineering via Cas9. *Science* **339**, 823–826 https://doi.org/10.1126/science.1232033
5   Rees, H.A. and Liu, D.R. (2018) Base editing: precision chemistry on the genome and transcriptome of living cells. *Nat. Rev. Genet.* **19**, 770–788 https://doi.org/10.1038/s41576-018-0059-1
6   Lin, S., Staahl, B.T., Alla, R.K. and Doudna, J.A. (2014) Enhanced homology-directed human genome engineering by controlled timing of CRISPR/Cas9 delivery. *eLife* **3**, e04766 https://doi.org/10.7554/eLife.04766
7   Hess, G.T., Tycko, J., Yao, D. and Bassik, M.C. (2017) Methods and applications of CRISPR-mediated base editing in eukaryotic genomes. *Mol. Cell* **68**, 26–43 https://doi.org/10.1016/j.molcel.2017.09.029
8   Zong, Y., Wang, Y., Li, C., Zhang, R., Chen, K., Ran, Y. et al. (2017) Precise base editing in rice, wheat and maize with a Cas9-cytidine deaminase fusion. *Nat. Biotechnol.* **35**, 438–440 https://doi.org/10.1038/nbt.3811

9   Shimatani, Z., Kashojiya, S., Takayama, M., Terada, R., Arazoe, T., Ishii, H. et al. (2017) Targeted base editing in rice and tomato using a CRISPR-Cas9 cytidine deaminase fusion. *Nat. Biotechnol.* **35**, 441–443 https://doi.org/10.1038/nbt.3833

10   Qin, L., Li, J., Wang, Q., Xu, Z., Sun, L., Alariqi, M. et al. (2020) High-efficient and precise base editing of C*G to T*A in the allotetraploid cotton (*Gossypium hirsutum*) genome using a modified CRISPR/Cas9 system. *Plant. Biotechnol. J.* **18**, 45–56 https://doi.org/10.1111/pbi.13168

11   Xu, Y., Lin, Q., Li, X., Wang, F., Chen, Z., Wang, J. et al. (2020) Fine-tuning the amylose content of rice by precise base editing of the Wx gene. *Plant Biotechnol. J.* https://doi.org/10.1111/pbi.13433

12   Cai, Y., Chen, L., Zhang, Y., Yuan, S., Su, Q., Sun, S. et al. (2020) Target base editing in soybean using a modified CRISPR/Cas9 system. *Plant Biotechnol. J.* **18**, 1996–1998 https://doi.org/10.1111/pbi.13386

13   Qin, W., Lu, X., Liu, Y., Bai, H., Li, S. and Lin, S. (2018) Precise A*T to G*C base editing in the zebrafish genome. *BMC Biol.* **16**, 139 https://doi.org/10.1186/s12915-018-0609-1

14   Zhang, Y., Qin, W., Lu, X., Xu, J., Huang, H., Bai, H. et al. (2017) Programmable base editing of zebrafish genome using a modified CRISPR-Cas9 system. *Nat. Commun.* **8**, 118 https://doi.org/10.1038/s41467-017-00175-6

15   Yuan, H., Yu, T., Wang, L., Yang, L., Zhang, Y., Liu, H. et al. (2020) Efficient base editing by RNA-guided cytidine base editors (CBEs) in pigs. *Cell Mol. Life Sci.* **77**, 719–733 https://doi.org/10.1007/s00018-019-03205-2

16   Xie, J., Ge, W., Li, N., Liu, Q., Chen, F., Yang, X. et al. (2019) Efficient base editing for multiple genes and loci in pigs using base editors. *Nat. Commun.* **10**, 2852 https://doi.org/10.1038/s41467-019-10421-8

17   Ma, Y., Yu, L., Zhang, X., Xin, C., Huang, S., Bai, L. et al. (2018) Highly efficient and precise base editing by engineered dCas9-guide tRNA adenosine deaminase in rats. *Cell Discov.* **4**, 39 https://doi.org/10.1038/s41421-018-0047-9

18   Liu, Z., Chen, M., Shan, H., Chen, S., Xu, Y., Song, Y. et al. (2019) Expanded targeting scope and enhanced base editing efficiency in rabbit using optimized xCas9(3.7). *Cell Mol. Life Sci.* **76**, 4155–4164 https://doi.org/10.1007/s00018-019-03110-8

19   Liu, Z., Chen, S., Shan, H., Jia, Y., Chen, M., Song, Y. et al. (2020) Efficient base editing with high precision in rabbits using YFE-BE4max. *Cell Death Dis.* **11**, 36 https://doi.org/10.1038/s41419-020-2244-3

20   Liu, Z., Shan, H., Chen, S., Chen, M., Song, Y., Lai, L. et al. (2020) Highly efficient base editing with expanded targeting scope using SpCas9-NG in rabbits. *FASEB J.* **34**, 588–596 https://doi.org/10.1096/fj.201901587R

21   Zhou, S., Cai, B., He, C., Wang, Y., Ding, Q., Liu, J. et al. (2019) Programmable base editing of the sheep genome revealed No genome-wide off-target mutations. *Front. Genet.* **10**, 215 https://doi.org/10.3389/fgene.2019.00215

22   Zhou, S., Ding, Y., Liu, J., Liu, Y., Zhao, X., Li, G. et al. (2020) Highly efficient generation of sheep with a defined FecB(B) mutation via adenine base editing. *Genet. Sel. Evol.* **52**, 35 https://doi.org/10.1186/s12711-020-00554-6

23   Zhang, W., Aida, T., Del Rosario, R.C.H., Wilde, J.J., Ding, C., Zhang, X. et al. (2020) Multiplex precise base editing in cynomolgus monkeys. *Nat. Commun.* **11**, 2325 https://doi.org/10.1038/s41467-020-16173-0

24   Wang, F., Zhang, W., Yang, Q., Kang, Y., Fan, Y., Wei, J. et al. (2020) Generation of a Hutchinson-Gilford progeria syndrome monkey model by base editing. *Protein Cell* **11**, 809–824 https://doi.org/10.1007/s13238-020-00740-8

25   Komor, A.C., Kim, Y.B., Packer, M.S., Zuris, J.A. and Liu, D.R. (2016) Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. *Nature* **533**, 420–424 https://doi.org/10.1038/nature17946

26   Mol, C.D., Arvai, A.S., Sanderson, R.J., Slupphaug, G., Kavli, B., Krokan, H.E. et al. (1995) Crystal structure of human uracil-DNA glycosylase in complex with a protein inhibitor: protein mimicry of DNA. *Cell* **82**, 701–708 https://doi.org/10.1016/0092-8674(95)90467-0

27   Lee, K.Y., Lee, H.J., Choi, H.J., Han, S.T., Lee, K.H., Park, K.J. et al. (2020) Highly elevated base excision repair pathway in primordial germ cells causes low base editing activity in chickens. *FASEB J.* **24**, 15907–15921 https://doi.org/10.1096/fj.202001065RRR

28   Gaudelli, N.M., Komor, A.C., Rees, H.A., Packer, M.S., Badran, A.H., Bryson, D.I. et al. (2017) Programmable base editing of A*T to G*C in genomic DNA without DNA cleavage. *Nature* **551**, 464–471 https://doi.org/10.1038/nature24644

29   Surun, D., Schneider, A., Mircetic, J., Neumann, K., Lansing, F., Paszkowski-Rogacz, M. et al. (2020) Efficient generation and correction of mutations in human iPS cells utilizing mRNAs of CRISPR base editors and prime editors. *Genes (Basel)* **11**, 511 https://doi.org/10.3390/genes11050511

30   Anzalone, A.V., Koblan, L.W. and Liu, D.R. (2020) Genome editing with CRISPR-Cas nucleases, base editors, transposases and prime editors. *Nat. Biotechnol.* **38**, 824–844 https://doi.org/10.1038/s41587-020-0561-9

31   Merkle, F.T., Neuhausser, W.M., Santos, D., Valen, E., Gagnon, J.A., Maas, K. et al. (2015) Efficient CRISPR-Cas9-mediated generation of knockin human pluripotent stem cells lacking undesired mutations at the targeted locus. *Cell Rep.* **11**, 875–883 https://doi.org/10.1016/j.celrep.2015.04.007

32   Han, H.A., Pang, J.K.S. and Soh, B.S. (2020) Mitigating off-target effects in CRISPR/Cas9-mediated in vivo gene editing. *J. Mol. Med (Berl).* **98**, 615–632 https://doi.org/10.1007/s00109-020-01893-z

33   Vakulskas, C.A., Dever, D.P., Rettig, G.R., Turk, R., Jacobi, A.M., Collingwood, M.A. et al. (2018) A high-fidelity Cas9 mutant delivered as a ribonucleoprotein complex enables efficient gene editing in human hematopoietic stem and progenitor cells. *Nat. Med.* **24**, 1216–1224 https://doi.org/10.1038/s41591-018-0137-0

34   Zeng, J., Wu, Y., Ren, C., Bonanno, J., Shen, A.H., Shea, D. et al. (2020) Therapeutic base editing of human hematopoietic stem cells. *Nat. Med.* **26**, 535–541 https://doi.org/10.1038/s41591-020-0790-y

35   Porto, E.M., Komor, A.C., Slaymaker, I.M. and Yeo, G.W. (2020) Base editing: advances and therapeutic opportunities. *Nat. Rev. Drug Discov.* **19**, 839–859 https://doi.org/10.1038/s41573-020-0084-6

36   Webber, B.R., Lonetree, C.L., Kluesner, M.G., Johnson, M.J., Pomeroy, E.J., Diers, M.D. et al. (2019) Highly efficient multiplex human T cell engineering without double-strand breaks using Cas9 base editors. *Nat. Commun.* **10**, 5222 https://doi.org/10.1038/s41467-019-13007-6

37   Kim, K., Ryu, S.M., Kim, S.T., Baek, G., Kim, D., Lim, K. et al. (2017) Highly efficient RNA-guided base editing in mouse embryos. *Nat. Biotechnol.* **35**, 435–437 https://doi.org/10.1038/nbt.3816

38   Li, G., Liu, Y., Zeng, Y., Li, J., Wang, L., Yang, G. et al. (2017) Highly efficient and precise base editing in discarded human tripronuclear embryos. *Protein Cell* **8**, 776–779 https://doi.org/10.1007/s13238-017-0458-7

39   Xu, C.L., Ruan, M.Z.C., Mahajan, V.B. and Tsang, S.H. (2019) Viral delivery systems for CRISPR. *Viruses* **11**, 28 https://doi.org/10.3390/v11010028

40   Villiger, L., Grisch-Chan, H.M., Lindsay, H., Ringnalda, F., Pogliano, C.B., Allegri, G. et al. (2018) Treatment of a metabolic liver disease by in vivo genome base editing in adult mice. *Nat. Med.* **24**, 1519–1525 https://doi.org/10.1038/s41591-018-0209-1

41   Yeh, W.H., Shubina-Oleinik, O., Levy, J.M., Pan, B., Newby, G.A., Wornow, M. et al. (2020) In vivo base editing restores sensory transduction and transiently improves auditory function in a mouse model of recessive deafness. *Sci. Transl. Med.* **12**, eaay9101 https://doi.org/10.1126/scitranslmed.aay9101

42   Lim, C.K.W., Gapinske, M., Brooks, A.K., Woods, W.S., Powell, J.E., Zeballos, C.M. et al. (2020) Treatment of a mouse model of ALS by in vivo base editing. *Mol. Ther.* **28**, 1177–1189 https://doi.org/10.1016/j.ymthe.2020.01.005

43   Chen, Y., Zhi, S., Liu, W., Wen, J., Hu, S., Cao, T. et al. (2020) Development of highly efficient dual-AAV split adenosine base editor for in vivo gene therapy. *Small Methods* **4**, 2000309 https://doi.org/10.1002/smtd.202000309

44   Aubrey, B.J., Kelly, G.L., Kueh, A.J., Brennan, M.S., O'Connor, L., Milla, L. et al. (2015) An inducible lentiviral guide RNA platform enables the identification of tumor-essential genes and tumor-promoting mutations in vivo. *Cell Rep.* **10**, 1422–1432 https://doi.org/10.1016/j.celrep.2015.02.002

45   Zafra, M.P., Schatoff, E.M., Katti, A., Foronda, M., Breinig, M., Schweitzer, A.Y. et al. (2018) Optimized base editors enable efficient editing in cells, organoids and mice. *Nat. Biotechnol.* **36**, 888–893 https://doi.org/10.1038/nbt.4194

46   Hanna, R.E., Hegde, M., Fagre, C.R., DeWeirdt, P.C., Sangree, A.K., Szegletes, Z. et al. (2020) Massively parallel assessment of human variants with base editor screens. *bioRxiv* https://doi.org/10.1101/2020.05.17.100818

47   Pausch, P., Al-Shayeb, B., Bisom-Rapp, E., Tsuchida, C.A., Li, Z., Cress, B.F. et al. (2020) CRISPR-CasPhi from huge phages is a hypercompact genome editor. *Science* **369**, 333–337 https://doi.org/10.1126/science.abb1400

48   Annunziato, S., Lutz, C., Henneman, L., Bhin, J., Wong, K., Siteur, B. et al. (2020) In situ CRISPR-Cas9 base editing for the development of genetically engineered mouse models of breast cancer. *EMBO J.* **39**, e102169 https://doi.org/10.15252/embj.2019102169

49   Li, X., Qian, X., Wang, B., Xia, Y., Zheng, Y., Du, L. et al. (2020) Programmable base editing of mutated TERT promoter inhibits brain tumour growth. *Nat. Cell Biol.* **22**, 282–288 https://doi.org/10.1038/s41556-020-0471-6

50   Kueh, A.J., Pal, M., Tai, L., Liao, Y., Smyth, G.K., Shi, W. et al. (2017) An update on using CRISPR/Cas9 in the one-cell stage mouse embryo for generating complex mutant alleles. *Cell Death Differ.* **24**, 1821–1822 https://doi.org/10.1038/cdd.2017.122

51   Lee, H.K., Smith, H.E., Liu, C., Willi, M. and Hennighausen, L. (2020) Cytosine base editor 4 but not adenine base editor generates off-target mutations in mouse embryos. *Commun. Biol.* **3**, 19 https://doi.org/10.1038/s42003-019-0745-3

52   Zuo, E., Sun, Y., Wei, W., Yuan, T., Ying, W., Sun, H. et al. (2019) Cytosine base editor generates substantial off-target single-nucleotide variants in mouse embryos. *Science* **364**, 289–292 https://doi.org/10.1126/science.aav9973

53   Lee, H.K., Willi, M., Miller, S.M., Kim, S., Liu, C., Liu, D.R. et al. (2018) Targeting fidelity of adenine and cytosine base editors in mouse embryos. *Nat. Commun.* **9**, 4804 https://doi.org/10.1038/s41467-018-07322-7

54   Cullot, G., Boutin, J., Toutain, J., Prat, F., Pennamen, P., Rooryck, C. et al. (2019) CRISPR-Cas9 genome editing induces megabase-scale chromosomal truncations. *Nat. Commun.* **10**, 1136 https://doi.org/10.1038/s41467-019-09006-2

55   Sharma, P. and Allison, J.P. (2015) The future of immune checkpoint therapy. *Science.* **348**, 56–61 https://doi.org/10.1126/science.aaa8172

56   Shi, X., Zhang, D., Li, F., Zhang, Z., Wang, S., Xuan, Y. et al. (2019) Targeting glycosylation of PD-1 to enhance CAR-T cell cytotoxicity. *J. Hematol. Oncol.* **12**, 127 https://doi.org/10.1186/s13045-019-0831-5

57   Gaudelli, N.M., Lam, D.K., Rees, H.A., Sola-Esteves, N.M., Barrera, L.A., Born, D.A. et al. (2020) Directed evolution of adenine base editors with increased activity and therapeutic application. *Nat. Biotechnol.* **38**, 892–900 https://doi.org/10.1038/s41587-020-0491-6

58   Kuscu, C., Parlak, M., Tufan, T., Yang, J., Szlachta, K., Wei, X. et al. (2017) CRISPR-STOP: gene silencing through base-editing-induced nonsense mutations. *Nat. Methods* **14**, 710–712 https://doi.org/10.1038/nmeth.4327

59   Jun, S., Lim, H., Chun, H., Lee, J.H. and Bang, D. (2020) Single-cell analysis of a mutant library generated using CRISPR-guided deaminase in human melanoma cells. *Commun. Biol.* **3**, 154 https://doi.org/10.1038/s42003-020-0888-2

60   Despres, P.C., Dube, A.K., Seki, M., Yachie, N. and Landry, C.R. (2020) Perturbing proteomes at single residue resolution using base editing. *Nat. Commun.* **11**, 1871 https://doi.org/10.1038/s41467-020-15796-7

61   Kweon, J., Jang, A.H., Shin, H.R., See, J.E., Lee, W., Lee, J.W. et al. (2020) A CRISPR-based base-editing screen for the functional assessment of BRCA1 variants. *Oncogene* **39**, 30–35 https://doi.org/10.1038/s41388-019-0968-2

62   Koblan, L.W., Doman, J.L., Wilson, C., Levy, J.M., Tay, T., Newby, G.A. et al. (2018) Improving cytidine and adenine base editors by expression optimization and ancestral reconstruction. *Nat. Biotechnol.* **36**, 843–846 https://doi.org/10.1038/nbt.4172

63   Gehrke, J.M., Cervantes, O., Clement, M.K., Wu, Y., Zeng, J., Bauer, D.E. et al. (2018) An APOBEC3A-Cas9 base editor with minimized bystander and off-target activities. *Nat. Biotechnol.* **36**, 977–982 https://doi.org/10.1038/nbt.4199

64   Zhang, X., Chen, L., Zhu, B., Wang, L., Chen, C., Hong, M. et al. (2020) Increasing the efficiency and targeting range of cytidine base editors through fusion of a single-stranded DNA-binding protein domain. *Nat. Cell Biol.* **22**, 740–750 https://doi.org/10.1038/s41556-020-0518-8

65   Hu, J.H., Miller, S.M., Geurts, M.H., Tang, W., Chen, L., Sun, N. et al. (2018) Evolved Cas9 variants with broad PAM compatibility and high DNA specificity. *Nature* **556**, 57–63 https://doi.org/10.1038/nature26155

66   Huang, T.P., Zhao, K.T., Miller, S.M., Gaudelli, N.M., Oakes, B.L., Fellmann, C. et al. (2019) Circularly permuted and PAM-modified Cas9 variants broaden the targeting scope of base editors. *Nat. Biotechnol.* **37**, 626–631 https://doi.org/10.1038/s41587-019-0134-y

67   Grunewald, J., Zhou, R., Lareau, C.A., Garcia, S.P., Iyer, S., Miller, B.R. et al. (2020) A dual-deaminase CRISPR base editor enables concurrent adenine and cytosine editing. *Nat. Biotechnol.* **38**, 861–864 https://doi.org/10.1038/s41587-020-0535-y

68   Zhang, X., Zhu, B., Chen, L., Xie, L., Yu, W., Wang, Y. et al. (2020) Dual base editor catalyzes both cytosine and adenine base conversions in human cells. *Nat. Biotechnol.* **38**, 856–860 https://doi.org/10.1038/s41587-020-0527-y

69   Kurt, I.C., Zhou, R., Iyer, S., Garcia, S.P., Miller, B.R., Langner, L.M. et al. (2020) CRISPR C-to-G base editors for inducing targeted DNA transversions in human cells. *Nat. Biotechnol* https://doi.org/10.1038/s41587-020-0609-x

70   Arbab, M., Shen, M.W., Mok, B., Wilson, C., Matuszek, Z., Cassa, C.A. et al. (2020) Determinants of base editing outcomes from target library analysis and machine learning. *Cell* **182**, 463–480.e30 https://doi.org/10.1016/j.cell.2020.05.037

71   Anzalone, A.V., Randolph, P.B., Davis, J.R., Sousa, A.A., Koblan, L.W., Levy, J.M. et al. (2019) Search-and-replace genome editing without double-strand breaks or donor DNA. *Nature* **576**, 149–157 https://doi.org/10.1038/s41586-019-1711-4

72   Schene, I.F., Joore, I.P., Oka, R., Mokry, M., van Vugt, A.H.M., van Boxtel, R. et al. (2020) Prime editing for functional repair in patient-derived disease models. *Nat. Commun.* **11**, 5352 https://doi.org/10.1038/s41467-020-19136-7

73 Jiang, T., Henderson, J.M., Coote, K., Cheng, Y., Valley, H.C., Zhang, X.O. et al. (2020) Chemical modifications of adenine base editor mRNA and guide RNA expand its application scope. *Nat. Commun.* **11**, 1979 https://doi.org/10.1038/s41467-020-15892-8

74 Levy, J.M., Yeh, W.H., Pendse, N., Davis, J.R., Hennessey, E., Butcher, R. et al. (2020) Cytosine and adenine base editing of the brain, liver, retina, heart and skeletal muscle of mice via adeno-associated viruses. *Nat. Biomed. Eng.* **4**, 97–110 https://doi.org/10.1038/s41551-019-0501-5

75 Ryu, S.M., Koo, T., Kim, K., Lim, K., Baek, G., Kim, S.T. et al. (2018) Adenine base editing in mouse embryos and an adult mouse model of Duchenne muscular dystrophy. *Nat. Biotechnol.* **36**, 536–539 https://doi.org/10.1038/nbt.4148

76 Rossidis, A.C., Stratigis, J.D., Chadwick, A.C., Hartman, H.A., Ahn, N.J., Li, H. et al. (2018) In utero CRISPR-mediated therapeutic editing of metabolic genes. *Nat. Med.* **24**, 1513–1518 https://doi.org/10.1038/s41591-018-0184-6

77 Carreras, A., Pane, L.S., Nitsch, R., Madeyski-Bengtson, K., Porritt, M., Akcakaya, P. et al. (2019) In vivo genome and base editing of a human PCSK9 knock-in hypercholesterolemic mouse model. *BMC Biol.* **17**, 4 https://doi.org/10.1186/s12915-018-0624-2

78 Yang, L., Wang, L., Huo, Y., Chen, X., Yin, S., Hu, Y. et al. (2020) Amelioration of an inherited metabolic liver disease through creation of a de novo start codon by cytidine base editing. *Mol. Ther.* **28**, 1673–1683 https://doi.org/10.1016/j.ymthe.2020.05.001

79 Osborn, M.J., Newby, G.A., McElroy, A.N., Knipping, F., Nielsen, S.C., Riddle, M.J. et al. (2020) Base editor correction of COL7A1 in recessive dystrophic epidermolysis bullosa patient-derived fibroblasts and iPSCs. *J. Invest. Dermatol.* **140**, 338–47.e5 https://doi.org/10.1016/j.jid.2019.07.701

80 Song, C.Q., Jiang, T., Richter, M., Rhym, L.H., Koblan, L.W., Zafra, M.P. et al. (2020) Adenine base editing in an adult mouse model of tyrosinaemia. *Nat. Biomed. Eng.* **4**, 125–130 https://doi.org/10.1038/s41551-019-0357-8

81 Geurts, M.H., de Poel, E., Amatngalim, G.D., Oka, R., Meijers, F.M., Kruisselbrink, E. et al. (2020) crispr-based adenine editors correct nonsense mutations in a cystic fibrosis organoid biobank. *Cell Stem Cell* **26**, 503–510.e7 https://doi.org/10.1016/j.stem.2020.01.019