BioEssays
www.bioessays-journal.com

Latest Advances for the *Sleeping Beauty* Transposon System: 23 Years of Insomnia but Prettier than Ever

Refinement and Recent Innovations of the *Sleeping Beauty* Transposon System Enabling Novel, Nonviral Genetic Engineering Applications

*Maximilian Amberger and Zoltán Ivics**

The *Sleeping Beauty* transposon system is a nonviral DNA transfer tool capable of efficiently mediating transposition-based, stable integration of DNA sequences of choice into eukaryotic genomes. Continuous refinements of the system, including the emergence of hyperactive transposase mutants and novel approaches in vectorology, greatly improve upon transposition efficiency rivaling viral-vector-based methods for stable gene insertion. Current developments, such as reversible transgenesis and proof-of-concept RNA-guided transposition, further expand on possible applications in the future. In addition, innate advantages such as lack of preferential integration into genes reduce insertional mutagenesis-related safety concerns while comparably low manufacturing costs enable widespread implementation. Accordingly, the system is recognized as a powerful and versatile tool for genetic engineering and is playing a central role in an ever-expanding number of gene and cell therapy clinical trials with the potential to become a key technology to meet the growing demand for advanced therapy medicinal products.

1. Introduction

Recognized as a Nobel Prize-worthy discovery by Barbara McClintock, transposable elements (TEs), or transposons in short, have been described as genomic parasites, as selfish DNA sequences or as their commonly known epithet "jumping genes" due to their defining characteristic of being able to move from one genomic location to another. Found across all domains of life, they make up significant portions of the genetic information of their hosts including humans.[1] But while appearing to lack any functions beyond multiplying within the organism in which they reside, the impact of TEs on their hosts' genome has been proven to be a strong driving force of evolution. This profound impact is due to the ability of TEs to increase genetic variability by causing genome reorganization,[2] crossing species boundaries via horizontal gene transfer,[3] and increasing their hosts' fitness by evolving mutualistic relationships in a process known as domestication, in which TE-derived proteins and nucleic acids are repurposed for key cellular and organismic functions.[4] Furthermore, it is highly likely that numerous additional functions of TEs are still unknown.

Eukaryotic transposons can be generally classified according to the nature of their mobile nucleic acid molecule: RNA (Class I TEs) or DNA (Class II TEs).[5] Class I TEs or retrotransposons move via a replicative copy-and-paste mechanism, and their transposition involves a transcription as well as a reverse transcription step followed by insertion into the target DNA, whereas transposition of Class II TEs or DNA transposons does not rely on an intermediate RNA molecule; instead, it occurs directly through DNA.

A prominent member within Class II transposons is the Tc1/*mariner* superfamily,[6] which is thought to have the widest distribution throughout nature.[7] These transposons are comprised of a gene encoding the transposase, the enzyme catalyzing the transposition reaction, flanked by transposon-specific terminal inverted repeat (TIR) sequences containing binding sites for the transposase. Tc1/*mariner* elements transpose through what is best described as a nonreplicative cut-and-paste mechanism. Upon expression and binding to sequences at the TIRs, the transposase induces double-stranded breaks (DSBs) at both ends, thereby excising and liberating the transposon. During excision of Tc1/*mariner* elements one of the DNA strands is cleaved a few nucleotides within the transposon, thereby leaving behind 3'-overhangs, which are processed by DNA repair mechanisms of the cells, generating a characteristic transposon footprint at

M. Amberger, Prof. Z. Ivics
Division of Medical Biotechnology
Paul Ehrlich Institute
Langen D-63225, Germany
E-mail: zoltan.ivics@pei.de

The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/bies.202000136

DOI: 10.1002/bies.202000136

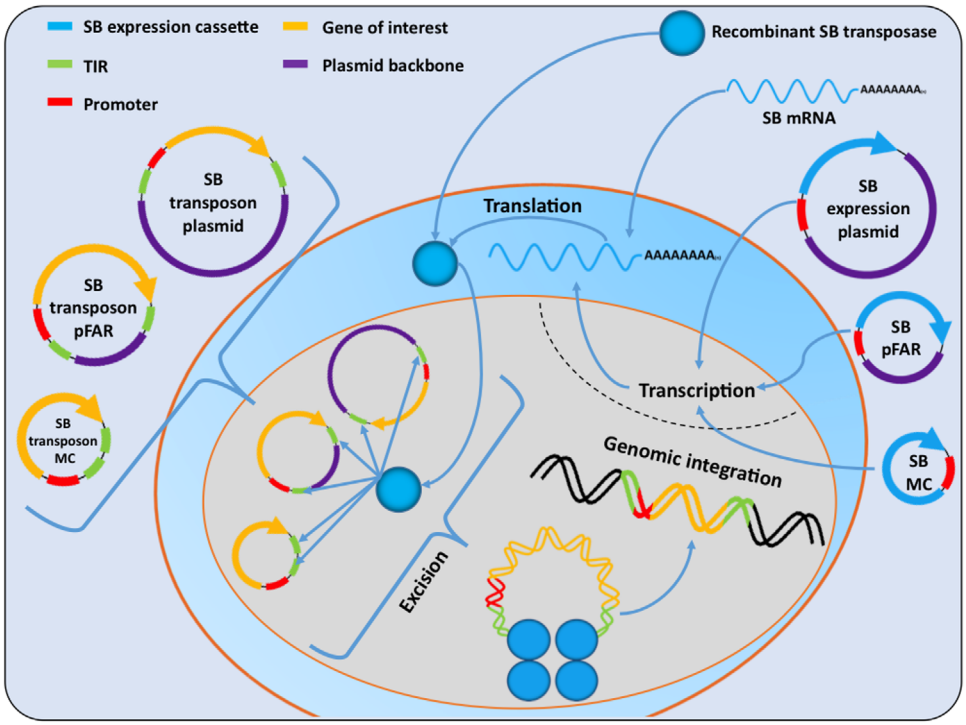

Figure 1. Stable transgene integration using the *Sleeping Beauty* transposon system. Transposon-carrying plasmid/MC/pFAR is transferred into target cells alongside the SB transposase via transfection/electroporation. SB transposase is either directly available when using recombinant protein or requires synthesis when using SB mRNA or SB expression cassettes. Once both components needed for transposition (transposon and transposase) are present in the nucleus, SB transposase binds to the TIRs, excises the transposon, and integrates it into the cell's genome.

the transposon donor site.[8] Integration of the excised transposon into a different location occurs when the transposase in the DNA-transposase complex finds a suitable target site, the only requirement for it being a TA dinucleotide,[9] and performs the reinsertion of its own genetic code[7]. Key similarities within the Tc1/*mariner* superfamily also include their basic transposase protein structure, namely, an N-terminal DNA-binding domain[10] through which the transposase interacts with the transposon TIRs, a nuclear localization signal,[11] and a C-terminal catalytic domain[8] mediating excision and integration.[12] Research on TEs, however, did not stop at being of exclusive interest for playing an important role to decipher questions in evolutionary biology. Indeed, the cut-and-paste transposition mechanism lends itself highly useful for the general purpose of genetic engineering.

2. The *Sleeping Beauty* Transposon System

Since its reconstruction from fossil DNA sequences within fish genomes into the first DNA transposon displaying activity in vertebrates,[13] the synthetic *Sleeping Beauty* (SB) transposon system has risen to be a well-established genetic tool displaying a wide gamut of applications ranging from wet lab to clinic. Just like any Tc1/*mariner* TE, the natural version of SB is composed of a single gene encoding its transposase flanked by specific TIRs, and its transposition follows the same basic mechanism outlined previously.[13,14] However, both components needed for transposition (transposase gene and TIRs) can be separated and supplied in a *trans*-arrangement. By constructing an artificial transposon replacing the SB transposase open reading frame between the TIRs with a sequence of interest and introducing it into a cell along with the transposase encoded on a separate expression plasmid, as messenger RNA or as recombinant protein, the transposase is able to stably integrate said sequence into the cell's genome (**Figure** 1) enabling sustained transgene expression, with which the doors were opened for simplified and efficient transgenesis culminating in gene therapy with ongoing clinical trials today. In the following sections, we describe the basic characteristics of the SB transposon system, including the latest developments in vectorology for advanced genetic engineering.

2.1. Classical Transposon Vector Setup: Double Plasmid Delivery

SB's inherent simplicity lies in that it only requires two components to function: transposon DNA and transposase enzyme. In which form these components are delivered into the target cell is largely irrelevant for the system's function, the only variable being efficiency, toxicity and cost. The most cost-efficient and least work-intensive delivery method is the classical two-plasmid configuration: an expression cassette for the SB transposase and an artificial transposon (gene of interest flanked by TIRs) sitting on two separate plasmids (**Figure** 1) that also contain the necessary components for propagation and manufacturing in bacterial cells, including an origin of replication and an antibiotic resistance gene of choice. Plasmid production is easily scalable and long-term storage of DNA or as a bacterial stock is possible. For use in target cells, the plasmids can be either transfected by common transfection reagents or alternatively electroporated.[15] As

such, the SB system represents a solid transgenesis tool for easy implementation in most BSL1 laboratories with standard equipment.

2.2. Miniature Plasmids and mRNA

Enhancing stable gene transfer efficiency in the context of clinical applications is often challenging due to overt toxicity of DNA transfection into primary human cell types. The current state-of-the-art delivery method is the combination of the SB transposase in the form of in vitro transcribed messenger RNA (mRNA) and the transposon supplied in the form of a DNA minicircle (MC).[16] This entails a number of advantages, including safety features relevant in regulatory assessment of potential biological medicinal products:

i. Toxicity caused by the cellular machinery sensing foreign DNA in its cytoplasm is greatly reduced by administering the transposase as mRNA.[17] mRNA can be directly translated once entering the cell, thereby eliminating the need for transcription. Therefore, transposase expression occurs faster and reaches levels required for transposition sooner, while at the same time the transient nature of transposase expression is enhanced due to reduced intracellular half-life of mRNAs when compared to DNA. This constitutes a critical safety aspect when applying for clinical trial approval, as prolonged transposase expression can cause remobilization of the transgene, potentially leading to genotoxicity. Additionally, the risk of spontaneous integration of the transposase gene potentially causing lifelong expression and remobilization is completely circumvented with the use of mRNA, as it is not subject to chromosomal integration.
ii. Overall transposon transfer into the target cell is facilitated and an immunogenic reaction is decreased by the recent use of MCs as transposon vehicles. MCs, essentially circular genes, are plasmids devoid of bacterial sequences (mainly bacterial origin of replication and antibiotic resistance gene).[18] Accordingly, their size is greatly reduced, facilitating membrane crossing, decreasing innate immune responses to unmethylated CpG motifs typically associated with bacterial DNA[19] and reducing overall cellular toxicity post-electroporation.[20]

In conjunction, MCs and mRNA have been efficiently employed in clinically relevant scenarios such as for the generation of chimeric antigen receptor (CAR)-T cells, and for genetic engineering of hematopoietic stem and progenitor cells (HSPCs).[16,20] Importantly, the MC/mRNA combination is planned to be employed in a phase I/IIa clinical trial (EudraCT No. 2019-001264-30/CARAMBA-1) to tackle multiple myeloma with SB-generated CAR-T cells targeting the signaling lymphocytic activation molecule family member 7 (SLAMF7) antigen[21] after being reported to satisfy regulatory criteria.[22] Moreover, an intermediate between regular transposon-harboring plasmid and MC called pFAR (plasmid free of antibiotic resistance markers)[23] (**Figure** 1) delivering both transposon and transposase in trans is currently being evaluated by the Swissmedic regulatory agency for an approval of a phase Ib/IIa trial to treat age-related macular degeneration (AMD). In this trial, autologous transgenic pigment epithelium-derived factor (PEDF)-expressing iris pigment epithelium (IPE) cells are planned to be transplanted, countering vascular endothelial growth factor (VEGF) overexpression causing AMD disease progression (https://www.targetamd.eu/). The CARAMBA and TargetAMD clinical applications are discussed in detail in sections 4.1 and 4.3.

2.3. Hyperactive Transposase Variants

Since its resurrection, the SB transposon system has experienced substantial optimizations in terms of transposition efficiency and flexibility. Multiple hyperactive variants of the SB transposon system (both transposase and transposon components) have been generated since its discovery, culminating with the current placeholder hyperactive transposase SB100X,[24] named after a 100-fold increase in transposition activity in comparison to the first-generation enzyme. This variant was generated through molecular evolution in an effort to match the leading viral vectors regarding efficiency of transgene delivery. Determining the crystal structure of the SB100X catalytic domain led to a further increase of its hyperactivity by 30% by enabling the rational optimization of DNA binding characteristics through mutation I212S.[25] In addition to viral-vector-like efficiency of gene integration, the SB transposon system has an extended capacity to deliver large transgenes. This is because transposition efficiency of SB in general is largely dependent on physical distance between the TIRs (which can be defined by the size of the vector backbone) instead of the length of the transposon itself[26] enabling transposition of over 100-kb sequences.[27]

2.4. High-Solubility *Sleeping Beauty* Transposase

A novel addition to SB's repertoire of advantages concerning ease of use and flexibility was reported by Querques et al.[28] by introducing the mutations I212S and C176S to the SB100X transposase. SB's low protein stability, low solubility, and aggregational properties were a major roadblock for working with a recombinant protein. Through these two mutations however, a novel transposase variant termed high-solubility SB (hsSB) was created displaying remarkable solubility and stability properties. Increased solubility enables purification of biologically active recombinant protein, electroporation of the protein into human cells, freeze–thaw cycles without compromising transposase activity and resistance to heat shock. Electroporation of hsSB was shown to be an efficient method to mediate transposition in a variety of human and mammalian cell types including difficult-to-transfect, clinically relevant models such as embryonic stem cells, HSPCs, induced pluripotent stem cells (iPSCs), and primary human cells including T cells, last of which were modified using hsSB to express an anti-CD19 CAR displaying a biological activity and antitumor potency in xenograft mice comparable to approved viral vector-based commercial products.[29]

Surprisingly, hsSB also displayed self-penetrating properties. Adding the recombinant protein to the cell culture medium was sufficient to enable transposition events in HeLa, iPSCs and T cells. Transgenesis efficiency was comparably low, ranging from

3% for iPSCs to 5–7% for T cells and 12% for HeLa cells, but as the mechanism of this phenomenon remains unknown, additional improvements coupled to the ease of use could turn hsSB self-penetration into a valuable tool for even simpler genetic manipulation.

When envisioning a potential product for SB-based gene therapy, providing the transposase in protein form could prove to have valuable advantages over the current DNA- or RNA-based methods: i) recombinant hsSB production in *E. coli* cultures is simple, affordable, scalable, and standardizable, ii) long-term storage and transport is possible as freezing is available, iii) short half-life under physiological conditions limits the presence of the transposase in cells to under 72 h, guaranteeing transposase-free cell products and lowering the risk of transgene remobilization, as ex vivo cell culture extends beyond said timeframe, and iv) titration of hsSB enables fine-tuning the number of insertions, limiting vector copy number (VCN) to a range acceptable by regulatory agencies worldwide.[30]

2.5. Excision$^+$/Integration$^-$ *Sleeping Beauty* Transposase Mutant

The obvious advantage of integrating gene transfer vectors over transient methods, that is, their ability to confer sustained transgene expression capabilities to a target cell, can in some scenarios prove unwanted or even detrimental. A prominent example is the generation of iPSCs, where reprogramming of somatic cells is achieved by forcing the overexpression of a defined set of transcription factors.[31] This can be done by stably integrating expression cassettes encoding the necessary transcription factors into a target cell's genome, as using transient expression methods is possible yet either inefficient[32,33] or labor-intensive.[34] The SB transposase system has been successfully used to generate iPSCs of multiple mammalian species including human.[35–38] Once reprogramming is complete, overexpression of the transcription factors is no longer a requirement, and the transgenes expressing the reprogramming factors are usually transcriptionally silenced by the cells. However, sustained residual transgene expression is regarded to be a considerable safety risk, as it was reported to alter differentiation potential,[39] to cause genomic instabilities elevating the risk of tumorigenesis,[40] and to cause dysplasia.[41,42] Thus, eliminating the reprogramming source after completion of the reprogramming process has increasingly become a major goal to reduce iPSC-derived risks for regenerative medicine.

An effective method to achieve this goal is to add loxP sites flanking the expression cassette, enabling Cre recombinase-mediated excision.[43] However, Cre recombinase-mediated toxicity[44] as well as pseudo-loxP sites in the human genome[45] pose a threat to clinical applications. Excision of transgenes including reprogramming factors used for the generation of iPSCs is theoretically possible using the SB transposon system[35] by reexpressing the SB transposase, as genomically integrated transgenes remain flanked by the TIRs needed for transposition. Yet, up to 75% of excised transposons end up reintegrating somewhere else into the genome,[46] reducing the utility for the generation of factor-free iPSCs.

This limitation was addressed in a recent study by Kesselring et al.,[47] who reported the generation of an SB transposase mutant that is restricted to catalyzing transposon excision while simultaneously generating extrachromosomal transposon circles that cannot undergo repeated integration. The mutant was designed based on previous observations highlighting the critical role of amino acid (aa) K248 in transposon reintegration, identified by alanine-scan,[48] and strengthened by comparing the locations of integration-deficient aa substitutions in structural models of the target capture complex of SB and the bacterial Tn10 transposase.[49] Saturation mutagenesis of K248 was performed to screen for mutants exhibiting not only an excision$^+$/insertion$^-$ phenotype but also relatively efficient excision at the same time, leading to K248T, which displayed no measurable insertion rate while maintaining 21% excision efficiency compared to the hyperactive SB100X transposase. In a follow-up experiment, K248T enabled efficient deletion of reprogramming factors and successful generation of transgene-free murine iPSCs. This constitutes a proof-of-concept for an alternative method to generate factor-free iPSCs (**Figure 2**), exhibiting the attractive efficiency, safety, and accessibility features of the SB transposon system platform.

2.6. Close-to-Random Genomic Insertion Site Distribution

Clearly, introducing foreign genetic material into a cell's genome will always go hand-in-hand with potential risks that the disruption of a genome entails. For all potential gene therapy applications, this means that a risk-benefit assessment has to take place in order to choose the proper method among available options. Since, tragically, one in five patients developed acute lymphoblastic leukemia (ALL)[50,51] following initial clinical trials to treat X-linked severe combined immunodeficiency (X-SCID)[52,53] due to insertional oncogenesis caused by first generation γ-retroviral vectors as therapeutic gene delivery vehicles, it soon became clear that insertion site preferences play a vital role when considering vectors for potential clinical applications.

The probability of insertional mutagenesis by vectors developed from γ-retroviruses based on the murine leukemia virus (MLV), similar to the ones employed in the initial X-SCID trials, is relatively high, because these viruses display a clear integration preference toward transcriptional start sites (TSSs) of actively expressed genes.[54] Lentiviral vectors based on the human immunodeficiency virus (HIV) show biased integration toward active transcription units,[55] and were also observed to cause leukemia in preclinical models.[56] *piggyBac*, a transposon displaying high transposition activity in human cells,[57] shows preferential integration near TSSs[58] similar to the MLV integrase, which is hypothesized to be mediated by the same mechanism. This can lead to gain-of-function mutations in oncogenes or loss-of-function mutations in tumor suppressor genes (TSGs), driving insertional oncogenesis. The human genome is largely composed of sequences considered as junk DNA, only about 1.2% of its sequence codes for all known proteins,[59] and latest estimations place the overall number of genes in the human genome including protein-coding and noncoding sequences at just over 43 000.[60] Out of this portion just over 1200 are known to be TSGs (1018 protein-coding and 199 noncoding genes, https://bioinfo.uth.edu/TSGene/),[61] therefore chances

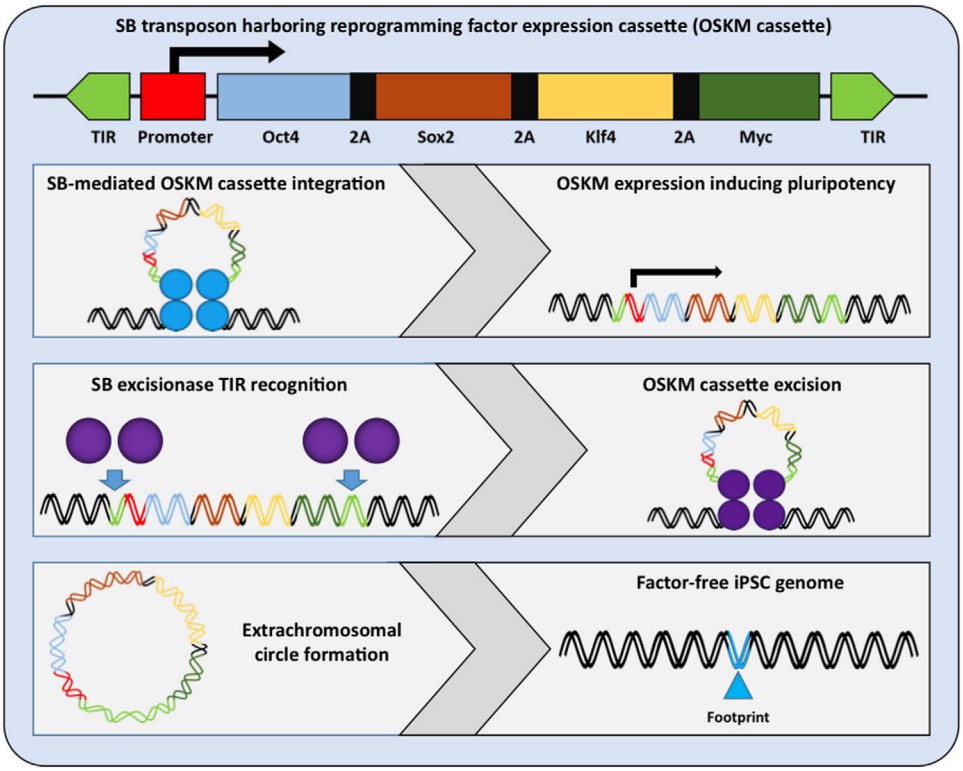

Figure 2. *Sleeping Beauty*-mediated pluripotency induction and reprogramming cassette excision. The reprogramming cassette is flanked by SB-specific TIRs (light green), and includes a suitable promoter (red) and OSKM coding sequences for Oct4 (light blue), Sox2 (light brown), Klf4 (yellow), and Myc (dark green), each separated by self-cleaving 2A peptide sequences (black). The black arrow indicates transcription initiated at the promoter. SB transposase (light blue circles) mediates cassette transposition leading to OSKM expression and pluripotency induction. Following pluripotency induction SB excisionase (violet circles) removes the OSKM cassette, leading to extrachromosomal circle formation and leaving a factor-free iPSC genome.

of disrupting TSGs leading to a loss-of-function are relatively low, further strengthened by the Knudson two-hit hypothesis. Insertional oncogenesis caused by disrupting the cell's regulatory mechanisms, however, is more likely, as enhancer/repressor functions can act over vast genomic distances through chromosomal looping.[62] Most recent evaluations for clinical trial applications using SB100X show an average VCN of 6–12 per genome,[22] which may sound a bit higher than one would instinctively aim for in a clinical setting. However, unlike the γ-retroviral, lentiviral and *piggyBac* transposon-derived vectors discussed previously, SB integrates its cargo in an almost random distribution across the genome.[9,58] Thus, the theoretical chance of an SB insertion hitting a cancer gene is significantly lower than with vectors that are specifically biased toward transcriptional regulatory elements and transcription units. In fact, SB has a significantly higher probability of integrating its cargo into a genomic safe harbor when compared to alternative viral and nonviral transposon-based vectors,[58] contributing to a favorable risk-benefit assessment by regulatory agencies when approving clinical trials that make use of the SB system. Until the present day no clinical trial using SB-mediated transgene delivery has reported insertional oncogenesis. Nevertheless, working toward minimizing the likelihood of adverse events caused by a misplaced integration remains a major research focus.

2.7. Targeted Transposition

Although integrating viruses, retrotransposons, and DNA transposons often exhibit target site preferences and in some cases even strict specificities,[63] these are mechanisms that evolved over the course of millions of years through natural selection to function as an evolutionary advantage to the respective element in order to optimize their vertical or horizontal transfer. Artificial generation of gene vehicles with target specificities beneficial in a gene therapy scenario, meaning avoiding disruption of host genes and regulatory elements that could lead to potentially harmful loss-of-function or gain-of-function mutations while ideally hitting genomic safe harbors, has become a major goal and simultaneously a substantial challenge. This is because significant and complex modifications are expected to be necessary to achieve said goal due to the above-mentioned evolutionary background.

Gene editing systems, including CRISPR/Cas9,[64] have the unique and powerful capacity to introduce targeted DSBs into the genome, but they lack the ability to integrate nucleic acids. Instead, integration of foreign genes can be potentiated by supplying transgenic DNA flanked by sequences corresponding to the genomic locus at the DSB introduced by Cas9. Ideally, cells repair said DSB using the homologous DNA as template, thereby introducing a transgene into a desired locus. This is, however,

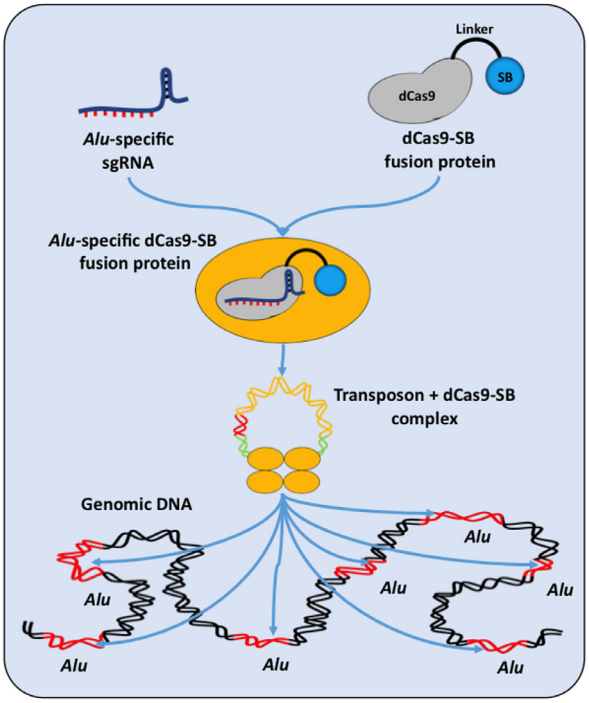

Figure 3. Targeted transposition. Fusion protein comprised of dCas9 and the SB transposase, joined by a linker peptide, associates with *Alu* element-specific sgRNA. SB denotes the SB100X hyperactive transposase. As a result, transposition is tethered toward the repetitive *Alu* sequences in the human genome, providing multiple targets increasing efficiency of targeted transposition.

not very efficient. In an effort to combine the exceptional targeting efficiencies of the CRISPR/Cas9 system[64] with the power to insert genetic material into the genome by the SB transposon system, Kovač et al.[65] recently presented an approach by fusing the SB100X transposase to a catalytically inactive Cas9 (dCas9), guided with single guide RNAs (sgRNAs) to copies of the human *Alu* retrotransposon. In comparison to previous work fusing DNA binding domains with transposases[63] that were either of low efficiency,[66,67] worked only in interplasmid settings,[68,69] in vitro,[70] or in the context of bacterial cells,[71,72] this study showed that SB-mediated transposition could be biased toward predetermined genomic locations in an sgRNA-dependent manner in human cells. Despite its comparably low efficiency, this study presented a proof-of-concept and highlighted a potentially viable strategy counteracting the strong and unspecific DNA binding characteristics of transposases, which complicates targeted insertion into single-copy sites. Instead, tethering the transposase toward a target that is overrepresented in the human genome greatly increases the number of possible hits and thereby the probability of targeted transposition with a flexible and easy-to-use RNA-guided system (**Figure 3**).

3. Preclinical and Basic Research Applications Using the *Sleeping Beauty* Transposon Platform

Developments and improvements of the SB transposon platform have led to it being adopted by numerous groups for studies in which integration into chromosomal DNA is required. Beginning with preclinical and basic research applications, it is of great advantage that SB has been shown to be highly active and reliable in an extensive number of models, virtually not compromising its transposition activity within germline versus somatic cells, tissue types, and animal species.[73] Accordingly, implementations have been reported for a wide range of fields, which will be discussed in the following sections.

3.1. Germline Transgenesis and Generation of Animal Models

The generation of genetically engineered animals is undergoing a significant growth in recent times due to the constantly growing interest in potential applications that can be divided into three categories: i) to create animal models for basic and preclinical research, ii) to produce valuable substances for which bacterial or in vitro production is unsuitable or lacks efficiency, and iii) to add value to livestock by altering its phenotype to showcase economically desirable traits. The SB transposon system has been used to generate a variety of transgenic vertebrates including fishes, amphibians and mammals of all sizes ranging from mice to cattle.[73] Protocols for their generation are efficient and solid, body-wide transgene expression is observed as expected based on in vitro studies indicating that SB transposon insertions are rarely subject to epigenetic silencing.[74] Adding SB's yet-to-be-determined upper cargo-size limit enabling the introduction of multiple transgenes simultaneously, potentially coupled to tissue-specific promoters, adoption of the SB system into all three categories previously mentioned has the potential to become a reality.

3.2. Insertional Mutagenesis Screens to Discover Genes and Decipher Gene Function

In contrast to reverse genetics, which sets out to induce mutations expecting a phenotype change that then can be linked to said mutation, forward genetics represents a different approach based on identifying phenotypes first, and then searching for the responsible mutation. Forward genetics provides a more focus-oriented approach, as the result is already identified, the challenge being finding the cause. For a long time, this has been an overwhelming challenge as the technology to identify and map unknown mutations among billions of nucleotides has only recently become reasonably viable for broad application. These technologies include next-generation sequencing (NGS) and transposon-based genetic screens. SB transposons have been designed to harbor gain-of-function and loss-of-function inducing cassettes.[75] As SB transposes randomly across the genome, inducing sustained transposition of these cassettes in vitro or in vivo enables high-throughput generation of phenotypic changes originating from unbiased genome-wide mutations. Identifying the responsible insertions as a follow-up is fairly simple, as transposons contain known sequences that can be used for PCR-based amplification and identification of the genomic neighborhood following NGS-based mapping. In addition, this technique has been of special interest for cancer research, as it was and still is successfully being employed to discover cancer-causing driver mutations[76] since the conception of the first oncogenic SB transposons suitable for large-scale screens in 2005.[77,78]

3.3. *Sleeping Beauty* Mediated Gene Transfer in Preclinical Studies

As a tool for integrating genes into a host's genome, SB can adopt a variety of roles in preclinical studies as transgenesis can either be used to treat a disease by introducing therapeutic genes or on the other hand to cause a disease in order to generate a suitable disease model. In regards to disease treatment, SB has been employed in several preclinical models successfully.[73,79] These include the metabolic disorders tyrosinemia type I,[80–82] diabetes type I,[83] hypercholesterolemia,[84] hyperbilirubinemia,[85] cystic fibrosis,[86] and mucopolysaccharidosis;[87,88] pulmonary disorders such as pulmonary fibrosis[89] and pulmonary hypertension;[90] hematological disorders including Hemophilia A and B[91–95] and sickle cell disease;[96,97] dermatological disorders including junctional epidermolysis bullosa[98] and dystrophic epidermolysis bullosa;[99] the neurologic disorders Huntington disease[100] and Alzheimer's disease;[101] the muscle disorder muscular dystrophy;[102,103] the eye disorder AMD;[104,105] and cancer, either through direct targeting of tumor cells[106–108] or indirectly via adoptive immunotherapy approaches.[109–114]

On the other hand, it is possible to generate disease models by stably introducing genes that cause disorders. This has been directly done for various cancer models induced by SB-mediated oncogene transposition including sarcomatoid carcinoma,[115,116] prostate cancer,[117] hepatocellular carcinoma,[118] peritoneal carcinomatosis,[119] glioma,[120] HPV16$^+$ oral tumors[121] but also for non-cancer-related disorders such as spinocerebellar ataxia type-1,[122] age-related renal pathologies,[123] and nephronophthisis-related ciliopathies.[124] Additionally, with increasing demand of gene therapies and a climbing number of successful transitions of preclinical research into clinical trials and approved medicinal products, SB's attractive features have further widened its implementation in the fields of basic research and preclinical studies (**Table 1**).

4. Clinical Applications

With recently obtained marketing authorization by the FDA and EMA, the CAR-T cell products Kymriah (lentiviral vector-based) and Yescarta (γ-retroviral vector-based) have proven to be a breakthrough for treatment of CD19$^+$ B-cell malignancies. However, costs revolving around manufacturing are expected to become a major bottleneck, preventing widespread access to these novel drugs for entire countries and social classes. SB holds significant potential to lower the costs associated with manufacturing of gene therapy products such as CAR-T cells, and thus enhancing availability for the general public. Current estimations range around a 90% reduction in vector manufacturing costs on a per patient basis,[143] as GMP-grade manufacturing of naked nucleic acids as required for the SB system is easily scalable and does not require work-intensive and time-costly additional quality control.

Accordingly, SB successfully entered the clinical stage in 2011 with two clinical trials as the first nonviral vector being used to generate CD19-specific CAR-T cells for adjuvant immunotherapy targeting minimal residual disease of non-Hodgkin lymphoma and ALL after autologous/allogeneic hematopoietic stem cell transplantation.[144] These pilot studies that proved SB to be a safe and effective tool to manufacture therapeutic CAR-T cells opened the door to several clinical trials that followed the lead using SB as a nonviral vector in their protocols to a current total of 14 clinical trials (**Table 2**) with additional ones being planned.

4.1. The CARAMBA Project

With significant advances being made both in viral and SB-based nonviral CAR-T cell therapy against CD19$^+$ hematological disorders, searching for novel antigens expressed specifically on additional cancer types to incorporate into the therapeutic repertoire has become a major focus of translational medicine research. In said effort, SLAMF7 has been identified as a robust marker of multiple myeloma,[145] a hematological malignancy of high medical relevance accounting for around 100 000 deaths globally each year (https://vizhub.healthdata.org/gbd-compare/, updated 2017). Pilot clinical trials of CAR-T cell therapy directed against the B cell maturation antigen (BCMA) proved effective and served as proof of concept.[146] However, antigen loss and reemergence of myeloma cells has been described, strengthening the need for alternative targets. As xenograft mouse models treated with T cells engineered to express a CAR derived from the targeting domain of a SLAMF7-specific monoclonal antibody[147] showed exquisite clearance of medullary and extramedullary myeloma cells, and relapsed/refractory patient-derived plasma cells were efficiently lysed in vitro by SLAMF7-CAR-T cells,[21] a premium candidate for adoptive immune therapy of multiple myeloma was potentially found.

In order to test SLAMF7-CAR-T efficacy against multiple myeloma, a phase I/IIa clinical trial with centers in Germany, Spain, Italy and France (EudraCT No. 2019-001264-30/CARAMBA-1) is being planned. The CARAMBA project, funded by the EU's Horizon2020 research and innovation program (http://www.caramba-cart.eu/), will not only deliver the world's first clinical trial to test the novel SLAMF7-CAR but also the first to employ the state-of-the-art hyperactive SB100X transposase delivered as mRNA along with the CAR transgene encoded on an MC via electroporation to generate the cell product. A preliminary analysis of the SB-generated cell product fulfilled requirements in regard to cell product safety,[22] namely, i) no measurable SB transposase could be detected in cell products, guaranteeing absence of unwanted transposon remobilization; ii) an average transgene VCN of ≈8, limiting potential genotoxicity and matching VCNs of CAR-T cells generated by viral vectors; and iii) a close-to-random insertion profile of the therapeutic gene in the T cell genome reducing the risk of insertional oncogenesis. Achieving regulatory approval recently, the CARAMBA trial has started this year in Germany.

4.2. Nonviral TCR-T Gene Therapy

While incredibly successful for certain types of cancer, CAR-T cell therapy is restrained in a sense that it is limited to target surface-expressed tumor antigens, potentially overlooking the vast majority of cancers impeding therapy where no specific surface antigen is available. Advances in sequencing

Table 1. Recent basic research and preclinical studies using the *Sleeping Beauty* transposon system to mediate gene transfer.

Background	Description	Transposon/transposase	Year	Reference
Acute lymphoblastic leukemia	Preclinical evaluation of allogeneic, SB-generated cytokine-induced killer cells expressing CD19 CAR shows proof-of-concept tumor eradication in ALL patient-derived xenograft mouse models leukemia	Plasmid/SB11 expression plasmid	2018	[125]
von Willebrand disease	SB transposon-mediated sustained expression of murine von Willebrand factor gene via hydrodynamic gene delivery in mice	Plasmid/SB100X expression plasmid//pFAR/SB100X expression pFAR4	2018	[126,127]
Age-related macular degeneration	Improved SB-mediated PEDF gene integration and expression in primary human RPE cells using miniplasmids	pFAR4/SB100X expression pFAR4	2018	[128]
Prostate cancer	Development of a mouse model of prostate cancer through in vivo electroporation of SB to stably integrate tumor-inducing expression cassettes	Plasmid/SB13 expression plasmid	2018	[117]
Various	Endoscopic retrograde cholangiopancreatography guided hydrodynamic injection for liver-targeted, SB-mediated gene delivery in pigs	Plasmid/SB expression plasmid	2018	[129]
$HPV16^+$ oral tumors	Generation of an $HPV16^+$ buccal tumor mouse model by SB-mediated transposition of oncogenes after submucosal injection and in vivo electroporation	Plasmid/SB13 expression plasmid	2018	[121]
Skeletal muscle development	SB-mediated generation of a mouse model overexpressing muscle-specific insulin-like growth factor 1 to study its effect on skeletal muscles and body weight	Plasmid/SB11 expression plasmid	2018	[130]
Glioma	Generation of a glioma mouse model by SB-mediated transposition of a single transposon harboring an oncogenic cassette	Plasmid/SB11 expression plasmid	2018	[131]
Age-related macular degeneration	SB-induced ex vivo modification of RPE cells to stably overexpress PEDF to reduce choroidal neovascularization in rats	pFAR4/SB100X expression pFAR4	2019	[132]
Cystic fibrosis	Peptide-poloxamine/SB nanoparticle vector-mediated resaturation of the cystic fibrosis transmembrane regulator gene in cystic fibrosis mice	Plasmid/SB100X mRNA	2019	[86]
Cancer	Silica-based nanoparticle platform for SB transposition of asparaginase gene into human lung adenocarcinoma cells inducing apoptosis	Plasmid/SB100X expression plasmid	2019	[133]
Hepatocellular carcinoma	Development of a mouse model for hepatocellular carcinomas by hydrodynamic tail vein injection to induce SB-mediated transposition of oncogenes to murine hepatocytes in vivo	Plasmid/SB13 expression plasmid	2019	[118]
Glioma	Shortened protocol to generate EGFRvIII targeting CAR-T cells in two weeks using the SB transposon system showcasing superior therapeutic efficacy in mice	Plasmid/SB11 expression plasmid	2019	[134]
Pancreatic cancer	Generation of mesothelin CAR NK-92MI cells using hybrid SB minicircle technology showcasing improved cytotoxicity against pancreatic cancer cell lines in vitro	MC/SB11 expression plasmid	2019	[135]
Glomerular diseases	Improved SB-mediated CXCR4 gene delivery to urine-derived progenitor cells leads to enhanced cell migration capacities in the context of potential renal regenerative therapies	Plasmid/SB100X expression MC	2019	[136]
Glioblastoma	AAV/SB/CRISPR hybrid vector development to perform in vivo screens for potential membrane targets for CAR-T cell therapy of glioblastoma	AAV/SB hybrid particles	2019	[137]
Metastatic colorectal cancer	SB-based forward genetic screen to identify pro-metastatic traits for colorectal cancer	Plasmid/SB100X expression plasmid	2019	[138]
Erythroleukemia	SB-based forward genetic screen in mice to identify drivers of erythroleukemia	T2/Onc2 + SB11 transgenic mice	2019	[139]
$CD19^+$ B-cell malignancies	Novel protocol to generate anti-CD19 CAR-T cells in 8 days yields effective antitumor response in xenograft mice	Plasmid/SB100X expression plasmid	2020	[140]

(*Continued*)

Table 1. Continued.

Background	Description	Transposon/transposase	Year	Reference
Peritoneal carcinomatosis	SB-induced peritoneal carcinomatosis mouse model by in vivo electroporation and transposition of tumor inducing expression cassettes	Plasmid/SB13,SB100X expression plasmid	2020	[119]
Age-related renal pathology	Generation of a transgenic rat model overexpressing Heme Oxygenase-1 in podocytes to study its effect on age-related kidney pathology	Plasmid/SB100X mRNA	2020	[123]
Hepatocellular carcinoma	Murine liver tumors induced by SB transposition after hydrodynamic tail vein injection to study the role of Ableson tyrosine-protein kinase 1 in hepatocellular carcinoma	Plasmid/SB expression plasmid	2020	[141]
Spinocerebellar ataxia type-1	Generation of an in vitro model to study the role of intracellular inclusion bodies in Spinocerebellar ataxia type-1 by SB-mediated overexpression of the ATXN1(Q82) gene in human mesenchymal stem cells	Plasmid/SB100X expression plasmid	2020	[122]
Central nervous system tumors	Identification of putative proto-oncogenes driving central nervous system tumors via SB-mediated forward genetic screen	T2/Onc//Onc2 + SB11 transgenic mice	2020	[142]

technology for rapid and economically viable transcriptome screening have enabled efficient identification of tumor-specific neoantigens,[148] derived from genetic alterations within tumor cells or virus-derived proteins within virus-associated tumors, presented as non-self-peptides on the major histocompatibility complex (MHC) molecules of cancerous cells, where they can be recognized by T cell receptors (TCRs). These neoantigens represent ideal immunotherapy targets, which is being explored successfully in preclinical models and clinical trials.[149]

The recent development of a platform to screen for tumor-specific neoantigens by whole transcriptome sequencing and identifying the respective, specifically matching TCRs via single cell RNA-sequencing[150] was significantly aided by SB, as expression cassettes encoding tumor-reactive TCRs could quickly be cloned into transposons and expressed in T cells to manufacture therapeutic cell products. Accordingly, SB will be used to generate genetically modified T cells expressing neoantigen-specific TCRs in an upcoming phase II clinical trial (NCT04102436) set out to treat patients with metastatic cancer, highlighting SB's value as a gene transfer tool, as viral vectors are outclassed when it comes to cost and manufacturing speed, which are essential criteria in truly personalized therapies.

4.3. The Target AMD Project

With accumulating victories being documented in nonviral adoptive immunotherapy, clinical trials directed against non-cancer-related disorders are still scarce but slowly emerging. One example is neovascular AMD, a pathology elicited by overproduction of VEGF. This manifests in form of abnormal neovascularization behind the retina, raising the macula potentially progressing to fluid leakage, hemorrhage, and retinal pigment epithelium (RPE) detachment[151] which can lead to severe tissue damage and progressive vision loss. Current treatments revolve around periodic intravitreal injections of VEGF inhibitors to block its angiogenic effects; these are effective, but transient, costly, linked to side effects[152–154] and of pronounced discomfort for patients.

The TargetAMD project supported by the EU's FP7 research program (https://www.targetamd.eu/) set out to test an innovative nonviral gene therapy approach in a phase Ib/IIa clinical trial. IPE cells will be extracted, genetically modified ex vivo using the SB system to express PEDF, whose anti-angiogenic effect directly counters VEGF's angiogenic effect, and transplanted into the subretinal compartment aiming at providing lifelong disease correction. Using the hyperactive SB100X transposase and the expression cassette of PEDF within an SB transposon both encoded on pFAR4 miniplasmids, gene delivery, sustained PEDF expression, and choroidal neovascularization inhibition has been confirmed in animal disease models,[104,132] while transfection efficiency and safety requirements concerning random genomic transposon insertion in human cells have been met.[105] The recent development of a manufacturing process of small-scale GMP-grade plasmid DNA production of the necessary SB components formulated in ready-to-use electroporation buffer[155] will enable an unprecedented surgical procedure in which IPE cells are isolated after iridectomy, electroporated and transplanted back into the patient within 60 minutes. This first-in-human study tackling AMD with transposon-based technology is pending approval by the Swissmedic regulatory agency.

5. Outlook

Integrating vector technology is heavily dominated by virus-based methods. Arguably, however, due to a head start on the availability of this technology. SB was close to a decade away from its discovery when the first clinical trials using first generation γ-retroviral vectors started and more than another decade from its clinical debut. Today, as gene therapy takes its first steps away from academic research toward marketing authorization with products intended for the general public, discussions are needed about a potential future in which the question may not be about

Table 2. Description of current and pending clinical trials using the *Sleeping Beauty* transposon system as indexed in the clinical trial database of *The Journal of Gene Medicine* with corresponding trial IDs (http://www.abedia.com/wiley/vectors.php). *not yet indexed (clinicaltrials.gov identifier: NCT04102436).

Trial ID	Title	Indication	Target cells	Cell source	Phase
US-0922	Adoptive immunotherapy for CD19$^+$ B-lymphoid malignancies using *Sleeping Beauty* transposition to express a CD19-specific CAR in autologous ex vivo expanded T cells	B-cell malignancies	T lymphocytes	Autologous	I
US-1003	CD19-specific T cell infusion in patients with B-lineage lymphoid malignancies after allogeneic hematopoietic stem-cell transplantation	B-cell malignancies	HLA matched T lymphocytes	Allogenic	I
US-1022	Adoptive Immunotherapy for CD19$^+$ B-cell malignancies using *Sleeping Beauty* transposition to express a CD19-specific CAR in allogeneic neonatal ex vivo expanded T cells	B-cell malignancies	Umbilical cord blood-derived lymphocytes	Allogenic	I
US-1142	Adoptive Immunotherapy for B-cell chronic lymphocytic leukemia using *Sleeping Beauty* transposition to express a CD19-specific CAR in autologous ex vivo expanded T cells	B-cell chronic lymphocytic leukemia	CD4$^+$ and CD8$^+$ T lymphocytes	Autologous	I
US-1192	A study to infuse ROR1-specific autologous T cells for patients with CLL	B-cell chronic lymphocytic leukemia	CD4$^+$ and CD8$^+$ T lymphocytes	Autologous	I
US-1203	A study of autologous CD19-specific CAR-T cells signaling via CD137 and CD3 zeta in patients with CLL	B-cell chronic lymphocytic leukemia	CD4$^+$ and CD8$^+$ T lymphocytes	Autologous	I
US-1225	CD19-specific T cell infusion in patients with B-lineage lymphoid malignancies after allogeneic hematopoietic stem cell transplantation	B-cell chronic lymphocytic leukemia	CD4$^+$ and CD8$^+$ T lymphocytes	Autologous	I
US-1236	Infusion of allogeneic, 3rd party CD19-specific T cell (CD19RCD137$^+$ T cells) in patients with refractory CD19$^+$ B-lineage malignancies	B-cell malignancies	Umbilical cord blood-derived lymphocytes	Allogenic	I
US-1353	Adoptive immunotherapy for B-cell malignancies in patients with low and high burdens of disease using *Sleeping Beauty* transposition to express CD19-specific CAR-positive T cells	B-cell malignancies	Primary CD3$^+$ lymphocytes	Autologous	I
US-1360	CAR MUC1 T cell immunotherapy for metastatic breast cancer	Metastatic breast cancer	T lymphocytes	Autologous	I/II
US-1710	Infusion of minimally expanded CD19$^+$ specific chimeric antigen receptor T cells for patients with advanced lymphoid malignancies	B-cell malignancies	CD4$^+$ and CD8$^+$ lymphocytes	Autologous	I
US-1801	*Sleeping Beauty* transposon-engineered plasmablasts for expression and delivery of alpha-L-iduronidase in patients with Hurler syndrome that have previously undergone allogeneic transplantation	Hurler syndrome	Plasmablasts	Autologous	I
*	A phase II study using the administration of autologous T-cells engineered using the *Sleeping Beauty* transposon/transposase system to express T-cell receptors reactive against mutated neoantigens in patients with metastatic cancer	Metastatic solid tumors	T lymphocytes	Autologous	II
XX-0098	A phase I/IIa clinical trial to assess feasibility, safety, and antitumor activity of autologous SLAMF7 CAR-T cells in multiple myeloma	Multiple myeloma	CD4$^+$ and CD8$^+$ T lymphocytes	Autologous	I/II

whether or not certain diseases can be treated, but rather if people can afford to be treated.

SB has been developed into a refined method that represents an alternative to viral vectors. Past studies have shown a dramatic reduction in manufacturing cost of SB-generated cell products, that SB transposition matches transgene integration efficiency of viral vectors, and that SB provides superior safety features concerning integration profile, genotoxicity and immunogenicity. Now, as the first clinical trials utilizing SB yield positive results,[144] additional trials are following making use of its

advantages. Concerning future perspectives, the accelerating pace of SB-based research and the development of next-generation SB technologies, as outlined in this review, will enable a second wave of potential clinical applications such as targeted transposition, regenerative medicine using factor-free iPSCs, and the transposition of plus-sized, complex therapeutic gene cassettes well exceeding viral cargo capacity. Here, the goal will not be to compete and provide an alternative to viral vectors, but to venture into novel areas where they are not an option.

Acknowledgements

The authors were supported by funding from the European Union's Horizon 2020 program for research and innovation under Grant Agreement No. 754658.

Open access funding enabled and organized by Projekt DEAL.

Conflict of Interest

Z.I. is inventor on patents relating to *Sleeping Beauty* transposon technology.

Keywords

clinical trial, gene therapy, genome engineering, nonviral, *Sleeping Beauty* transposon, transgenesis, transposition

Received: June 5, 2020
Revised: July 29, 2020
Published online: September 16, 2020

[1] E. S. Lander, L. M. Linton, B. Birren, C. Nusbaum, M. C. Zody, J. Baldwin, K. Devon, K. Dewar, M. Doyle, W. FitzHugh, R. Funke, D. Gage, K. Harris, A. Heaford, J. Howland, L. Kann, J. Lehoczky, R. LeVine, P. McEwan, K. McKernan, J. Meldrim, J. P. Mesirov, C. Miranda, W. Morris, J. Naylor, C. Raymond, M. Rosetti, R. Santos, A. Sheridan, C. Sougnez, International Human Genome Sequencing Consortium, *Nature* **2001**, *409*, 860.
[2] R. Cordaux, M. A. Batzer, *Nat. Rev. Genet.* **2009**, *10*, 691.
[3] C. Gilbert, C. Feschotte, *Curr. Opin. Genet. Dev.* **2018**, *49*, 15.
[4] M. Naville, I. A. Warren, Z. Haftek-Terreau, D. Chalopin, F. Brunet, P. Levin, D. Galiana, J.-N. Volff, *Clin. Microbiol. Infect.* **2016**, *22*, 312.
[5] D. J. Finnegan, *Trends. Genet.* **1989**, *5*, 103.
[6] H. M. Robertson, *J. Insect Physiol.* **1995**, *41*, 99.
[7] R. H. Plasterk, Z. Izsvák, Z. Ivics, *Trends Genet.* **1999**, *15*, 326.
[8] H. G. van Luenen, S. D. Colloms, R. H. Plasterk, *Cell* **1994**, *79*, 293.
[9] T. J. Vigdal, C. D. Kaufman, Z. Izsvák, D. F. Voytas, Z. Ivics, *J. Mol. Biol.* **2002**, *323*, 441.
[10] S. Pietrokovski, S. Henikoff, *Mol. Gen. Genet.* **1997**, *254*, 689.
[11] Z. Ivics, Z. Izsvak, A. Minter, P. B. Hackett, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 5008.
[12] M. Muñoz-López, J. L. García-Pérez, *Curr. Genomics* **2010**, *11*, 115.
[13] Z. Ivics, P. B. Hackett, R. H. Plasterk, Z. Izsvák, *Cell* **1997**, *91*, 501.
[14] Z. Ivics, Z. Izsvák, *Microbiol. Spectr.* **2015**, *3*, MDNA3-0042-2014.
[15] A. Hamm, N. Krott, I. Breibach, R. Blindt, A. K. Bosserhoff, *Tissue Eng.* **2002**, *8*, 235.
[16] R. Monjezi, C. Miskey, T. Gogishvili, M. Schleef, M. Schmeer, H. Einsele, Z. Ivics, M. Hudecek, *Leukemia* **2017**, *31*, 186.
[17] J. M. Wiehe, P. Ponsaerts, M. T. Rojewski, J. M. Homann, J. Greiner, D. Kronawitter, H. Schrezenmeier, V. Hombach, M. Wiesneth, O. Zimmermann, J. Torzewski, *J. Cell. Mol. Med.* **2007**, *11*, 521.
[18] A. M. Darquet, B. Cameron, P. Wils, D. Scherman, J. Crouzet, *Gene Ther.* **1997**, *4*, 1341.
[19] H. Hemmi, O. Takeuchi, T. Kawai, T. Kaisho, S. Sato, H. Sanjo, M. Matsumoto, K. Hoshino, H. Wagner, K. Takeda, S. Akira, *Nature* **2000**, *408*, 740.
[20] M. Holstein, C. Mesa-Nuñez, C. Miskey, E. Almarza, V. Poletti, M. Schmeer, E. Grueso, J. C. Ordóñez Flores, D. Kobelt, W. Walther, M. K. Aneja, J. Geiger, H. B. Bonig, Z. Izsvák, M. Schleef, C. Rudolph, F. Mavilio, J. A. Bueren, G. Guenechea, Z. Ivics, *Mol. Ther.* **2018**, *26*, 1137.
[21] T. Gogishvili, S. Danhof, S. Prommersberger, J. Rydzek, M. Schreder, C. Brede, H. Einsele, M. Hudecek, *Blood* **2017**, *130*, 2838.
[22] C. Miskey, M. Amberger, M. Reiser, S. Prommersberger, J. Beckmann, M. Machwirth, H. Einsele, M. Hudecek, H. Bonig, Z. Ivics, *bioRxiv* **2019**.
[23] C. Marie, G. Vandermeulen, M. Quiviger, M. Richard, V. Préat, D. Scherman, *J. Gene Med.* **2010**, *12*, 323.
[24] L. Mátés, M. K. L. Chuah, E. Belay, B. Jerchow, N. Manoj, A. Acosta-Sanchez, D. P. Grzela, A. Schmitt, K. Becker, J. Matrai, L. Ma, E. Samara-Kuko, C. Gysemans, D. Pryputniewicz, C. Miskey, B. Fletcher, T. VandenDriessche, Z. Ivics, Z. Izsvák, *Nat. Genet.* **2009**, *41*, 753.
[25] F. Voigt, L. Wiedemann, C. Zuliani, I. Querques, A. Sebe, L. Mátés, Z. Izsvák, Z. Ivics, O. Barabas, *Nat. Commun.* **2016**, *7*, 11126.
[26] Z. Izsvák, Z. Ivics, R. H. Plasterk, *J. Mol. Biol.* **2000**, *302*, 93.
[27] M. Rostovskaya, J. Fu, M. Obst, I. Baer, S. Weidlich, H. Wang, A. J. H. Smith, K. Anastassiadis, A. F. Stewart, *Nucleic Acids Res.* **2012**, *40*, e150.
[28] I. Querques, A. Mades, C. Zuliani, C. Miskey, M. Alb, E. Grueso, M. Machwirth, T. Rausch, H. Einsele, Z. Ivics, M. Hudecek, O. Barabas, *Nat. Biotechnol.* **2019**, *37*, 1502.
[29] S. L. Maude, T. W. Laetsch, J. Buechner, S. Rives, M. Boyer, H. Bittencourt, P. Bader, M. R. Verneris, H. E. Stefanski, G. D. Myers, M. Qayed, B. de Moerloose, H. Hiramatsu, K. Schlis, K. L. Davis, P. L. Martin, E. R. Nemecek, G. A. Yanik, C. Peters, A. Baruchel, N. Boissel, F. Mechinaud, A. Balduzzi, J. Krueger, C. H. June, B. L. Levine, P. Wood, T. Taran, M. Leung, K. T. Mueller, Y. Zhang, K. Sen, D. Lebwohl, M. A. Pulsipher, S. A. Grupp, *N. Engl. J. Med.* **2018**, *378*, 439.
[30] Y. Zhao, H. Stepto, C. K. Schneider, *Hum. Gene Ther. Methods* **2017**, *28*, 205.
[31] K. Takahashi, S. Yamanaka, *Cell* **2006**, *126*, 663.
[32] M. Stadtfeld, M. Nagaya, J. Utikal, G. Weir, K. Hochedlinger, *Science* **2008**, *322*, 945.
[33] W. Zhou, C. R. Freed, *Stem Cells* **2009**, *27*, 2667.
[34] L. Warren, P. D. Manos, T. Ahfeldt, Y.-H. Loh, H. Li, F. Lau, W. Ebina, P. K. Mandal, Z. D. Smith, A. Meissner, G. Q. Daley, A. S. Brack, J. J. Collins, C. Cowan, T. M. Schlaeger, D. J. Rossi, *Cell Stem Cell* **2010**, *7*, 618.
[35] I. Grabundzija, J. Wang, A. Sebe, Z. Erdei, R. Kajdi, A. Devaraj, D. Steinemann, K. Szuhai, U. Stein, T. Cantz, A. Schambach, C. Baum, Z. Izsvák, B. Sarkadi, Z. Ivics, *Nucleic Acids Res.* **2013**, *41*, 1829.
[36] T. R. Talluri, D. Kumar, S. Glage, W. Garrels, Z. Ivics, K. Debowski, R. Behr, H. Niemann, W. A. Kues, *Cell. Reprogram.* **2015**, *17*, 131.
[37] W. A. Kues, D. Herrmann, B. Barg-Kues, S. Haridoss, M. Nowak-Imialek, T. Buchholz, M. Streeck, A. Grebe, I. Grabundzija, S. Merkert, U. Martin, V. J. Hall, M. A. Rasmussen, Z. Ivics, P. Hyttel, H. Niemann, *Stem Cells Dev.* **2013**, *22*, 124.
[38] R. P. Davis, C. Nemes, E. Varga, C. Freund, G. Kosmidis, K. Gkatzis, D. de Jong, K. Szuhai, A. Dinnyés, C. L. Mummery, *Differentiation* **2013**, *86*, 30.
[39] C. A. Sommer, C. Christodoulou, A. Gianotti-Sommer, S. S. Shen, B. S. Sailaja, H. Hezroni, A. Spira, E. Meshorer, D. N. Kotton, G. Mostoslavsky, *PloS One* **2012**, *7*, e51711.
[40] M. Yoshihara, Y. Hayashizaki, Y. Murakawa, *Stem Cell Rev. Rep.* **2017**, *13*, 7.

[41] K. Hochedlinger, Y. Yamada, C. Beard, R. Jaenisch, *Cell* **2005**, *121*, 465.
[42] K. W. Foster, Z. Liu, C. D. Nail, X. Li, T. J. Fitzgerald, S. K. Bailey, A. R. Frost, I. D. Louro, T. M. Townes, A. J. Paterson, J. E. Kudlow, S. M. Lobo-Ruppert, J. M. Ruppert, *Oncogene* **2005**, *24*, 1491.
[43] F. Soldner, D. Hockemeyer, C. Beard, Q. Gao, G. W. Bell, E. G. Cook, G. Hargus, A. Blak, O. Cooper, M. Mitalipova, O. Isacson, R. Jaenisch, *Cell* **2009**, *136*, 964.
[44] A. Loonstra, M. Vooijs, H. B. Beverloo, B. A. Allak, E. van Drunen, R. Kanaar, A. Berns, J. Jonkers, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 9209.
[45] B. Thyagarajan, M. J. Guimarães, A. C. Groth, M. P. Calos, *Gene* **2000**, *244*, 47.
[46] G. Luo, Z. Ivics, Z. Izsvák, A. Bradley, *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 10769.
[47] L. Kesselring, C. Miskey, C. Zuliani, I. Querques, V. Kapitonov, A. Laukó, A. Fehér, A. Palazzo, T. Diem, J. Lustig, A. Sebe, Y. Wang, A. Dinnyés, Z. Izsvák, O. Barabas, Z. Ivics, *Nucleic Acids Res.* **2020**, *48*, 316.
[48] Y. Wang, D. Pryputniewicz-Dobrinska, E. É. Nagy, C. D. Kaufman, M. Singh, S. Yant, J. Wang, A. Dalda, M. A. Kay, Z. Ivics, Z. Izsvák, *Nucleic Acids Res.* **2017**, *45*, 311.
[49] D. B. Haniford, A. R. Chelouche, N. Kleckner, *Cell* **1989**, *59*, 385.
[50] S. Hacein-Bey-Abina, A. Garrigue, G. P. Wang, J. Soulier, A. Lim, E. Morillon, E. Clappier, L. Caccavelli, E. Delabesse, K. Beldjord, V. Asnafi, E. MacIntyre, L. Dal Cortivo, I. Radford, N. Brousse, F. Sigaux, D. Moshous, J. Hauer, A. Borkhardt, B. H. Belohradsky, U. Wintergerst, M. C. Velez, L. Leiva, R. Sorensen, N. Wulffraat, S. Blanche, F. D. Bushman, A. Fischer, M. Cavazzana-Calvo, *J. Clin. Invest.* **2008**, *118*, 3132.
[51] S. J. Howe, M. R. Mansour, K. Schwarzwaelder, C. Bartholomae, M. Hubank, H. Kempski, M. H. Brugman, K. Pike-Overzet, S. J. Chatters, D. de Ridder, K. C. Gilmour, S. Adams, S. I. Thornhill, K. L. Parsley, F. J. T. Staal, R. E. Gale, D. C. Linch, J. Bayford, L. Brown, M. Quaye, C. Kinnon, P. Ancliff, D. K. Webb, M. Schmidt, C. von Kalle, H. B. Gaspar, A. J. Thrasher, *J. Clin. Invest.* **2008**, *118*, 3143.
[52] M. Cavazzana-Calvo, S. Hacein-Bey, G. de Saint Basile, F. Gross, E. Yvon, P. Nusbaum, F. Selz, C. Hue, S. Certain, J. L. Casanova, P. Bousso, F. L. Deist, A. Fischer, *Science* **2000**, *288*, 669.
[53] H. B. Gaspar, K. L. Parsley, S. Howe, D. King, K. C. Gilmour, J. Sinclair, G. Brouns, M. Schmidt, C. von Kalle, T. Barington, M. A. Jakobsen, H. O. Christensen, A. Al Ghonaium, H. N. White, J. L. Smith, R. J. Levinsky, R. R. Ali, C. Kinnon, A. J. Thrasher, *Lancet* **2004**, *364*, 2181.
[54] X. Wu, Y. Li, B. Crise, S. M. Burgess, *Science* **2003**, *300*, 1749.
[55] A. R. W. Schröder, P. Shinn, H. Chen, C. Berry, J. R. Ecker, F. Bushman, *Cell* **2002**, *110*, 521.
[56] D. Heckl, A. Schwarzer, R. Haemmerle, D. Steinemann, C. Rudolph, B. Skawran, S. Knoess, J. Krause, Z. Li, B. Schlegelberger, C. Baum, U. Modlich, *Mol. Ther.* **2012**, *20*, 1187.
[57] J. E. Doherty, L. E. Huye, K. Yusa, L. Zhou, N. L. Craig, M. H. Wilson, *Hum. Gene Ther.* **2012**, *23*, 311.
[58] A. Gogol-Döring, I. Ammar, S. Gupta, M. Bunse, C. Miskey, W. Chen, W. Uckert, T. F. Schulz, Z. Izsvák, Z. Ivics, *Mol. Ther.* **2016**, *24*, 592.
[59] ENCODE Project Consortium, *Nature* **2012**, *489*, 57.
[60] M. Pertea, A. Shumate, G. Pertea, A. Varabyou, Y.-C. Chang, A. K. Madugundu, A. Pandey, S. L. Salzberg, *bioRxiv* **2018**.
[61] M. Zhao, P. Kim, R. Mitra, J. Zhao, Z. Zhao, *Nucleic Acids Res.* **2016**, *44*, D1023.
[62] J. Dekker, *Science* **2008**, *319*, 1793.
[63] A. Kovač, Z. Ivics, *Cell Gene Ther. Insights* **2017**, *3*, 103.
[64] M. Jinek, K. Chylinski, I. Fonfara, M. Hauer, J. A. Doudna, E. Charpentier, *Science* **2012**, *337*, 816.
[65] A. Kovač, C. Miskey, M. Menzel, E. Grueso, A. Gogol-Döring, Z. Ivics, *Elife* **2020**, *9*, 53868.
[66] B. E. Hew, R. Sato, D. Mauro, I. Stoytchev, J. B. Owens, *Synth. Biol.* **2019**, *4*, ysz018.
[67] K. Voigt, A. Gogol-Döring, C. Miskey, W. Chen, T. Cathomen, Z. Izsvák, Z. Ivics, *Mol. Ther.* **2012**, *20*, 1852.
[68] S. R. Yant, Y. Huang, B. Akache, M. A. Kay, *Nucleic Acids Res.* **2007**, *35*, e50.
[69] C. Kettlun, D. L. Galvan, A. L. George, A. Kaja, M. H. Wilson, *Mol. Ther.* **2011**, *19*, 1636.
[70] S. Bhatt, R. Chalmers, *Nucleic Acids Res.* **2019**, *47*, 8126.
[71] J. Strecker, A. Ladha, Z. Gardner, J. L. Schmid-Burgk, K. S. Makarova, E. V. Koonin, F. Zhang, *Science* **2019**, *365*, 48.
[72] S. E. Klompe, P. L. H. Vo, T. S. Halpin-Healy, S. H. Sternberg, *Nature* **2019**, *571*, 219.
[73] S. A. Narayanavari, S. S. Chilkunda, Z. Ivics, Z. Izsvák, *Crit. Rev. Biochem. Mol. Biol.* **2017**, *52*, 18.
[74] I. Grabundzija, M. Irgang, L. Mátés, E. Belay, J. Matrai, A. Gogol-Döring, K. Kawakami, W. Chen, P. Ruiz, M. K. L. Chuah, T. VandenDriessche, Z. Izsvák, Z. Ivics, *Mol. Ther.* **2010**, *18*, 1200.
[75] Z. Ivics, M. A. Li, L. Mátés, J. D. Boeke, A. Nagy, A. Bradley, Z. Izsvák, *Nat. Methods* **2009**, *6*, 415.
[76] A. Guimaraes-Young, C. R. Feddersen, A. J. Dupuy, *Front. Oncol.* **2019**, *9*, 611.
[77] L. S. Collier, C. M. Carlson, S. Ravimohan, A. J. Dupuy, D. A. Largaespada, *Nature* **2005**, *436*, 272.
[78] A. J. Dupuy, K. Akagi, D. A. Largaespada, N. G. Copeland, N. A. Jenkins, *Nature* **2005**, *436*, 221.
[79] M. Hudecek, Z. Izsvák, S. Johnen, M. Renner, G. Thumann, Z. Ivics, *Crit. Rev. Biochem. Mol. Biol.* **2017**, *52*, 355.
[80] E. Montini, P. K. Held, M. Noll, N. Morcinek, M. Al-Dhalimy, M. Finegold, S. R. Yant, M. A. Kay, M. Grompe, *Mol. Ther.* **2002**, *6*, 759.
[81] A. Wilber, K. J. Wangensteen, Y. Chen, L. Zhuo, J. L. Frandsen, J. B. Bell, Z. J. Chen, S. C. Ekker, R. S. McIvor, X. Wang, *Mol. Ther.* **2007**, *15*, 1280.
[82] X.-J. Pan, Z.-Z. Ma, Q.-J. Zhang, L. Fan, Q.-H. Li, *J. Int. Med. Res.* **2012**, *40*, 1850.
[83] C.-X. He, D. Shi, W.-J. Wu, Y.-F. Ding, D.-M. Feng, B. Lu, H.-M. Chen, J.-H. Yao, Q. Shen, D.-R. Lu, J.-L. Xue, *World J. Gastroenterol.* **2004**, *10*, 567.
[84] T. A. K. Turunen, J. Kurkipuro, T. Heikura, T. Vuorio, E. Hytönen, Z. Izsvák, S. Ylä-Herttuala, *Mol. Ther.* **2016**, *24*, 620.
[85] X. Wang, D. P. Sarkar, P. Mani, C. J. Steer, Y. Chen, C. Guha, V. Chandrasekhar, A. Chaudhuri, N. Roy-Chowdhury, B. T. Kren, J. Roy-Chowdhury, *Hepatology* **2009**, *50*, 815.
[86] S. Guan, A. Munder, S. Hedtfeld, P. Braubach, S. Glage, L. Zhang, S. Lienenklaus, A. Schultze, G. Hasenpusch, W. Garrels, F. Stanke, C. Miskey, S. M. Johler, Y. Kumar, B. Tümmler, C. Rudolph, Z. Ivics, J. Rosenecker, *Nat. Nanotechnol.* **2019**, *14*, 287.
[87] E. L. Aronovich, J. B. Bell, L. R. Belur, R. Gunther, B. Koniar, D. C. C. Erickson, P. A. Schachern, I. Matise, R. S. McIvor, C. B. Whitley, P. B. Hackett, *J. Gene Med.* **2007**, *9*, 403.
[88] E. L. Aronovich, J. B. Bell, S. A. Khan, L. R. Belur, R. Gunther, B. Koniar, P. A. Schachern, J. B. Parker, C. S. Carlson, C. B. Whitley, R. S. McIvor, P. Gupta, P. B. Hackett, *Mol. Ther.* **2009**, *17*, 1136.
[89] J. Xiao, X.-M. Meng, X. R. Huang, A. C. Chung, Y.-L. Feng, D. S. Hui, C.-M. Yu, J. J. Sung, H. Y. Lan, *Mol. Ther.* **2012**, *20*, 1251.
[90] L. Liu, H. Liu, G. Visner, B. S. Fletcher, *FASEB J.* **2006**, *20*, 2594.
[91] S. R. Yant, L. Meuse, W. Chiu, Z. Ivics, Z. Izsvak, M. A. Kay, *Nat. Genet.* **2000**, *25*, 35.
[92] J. R. Ohlfest, J. L. Frandsen, S. Fritz, P. D. Lobitz, S. G. Perkinson, K. J. Clark, G. Nelsestuen, N. S. Key, R. S. McIvor, P. B. Hackett, D. A. Largaespada, *Blood* **2005**, *105*, 2691.

[93] L. Liu, C. Mah, B. S. Fletcher, *Mol. Ther.* **2006**, *13*, 1006.
[94] B. T. Kren, G. M. Unger, L. Sjeklocha, A. A. Trossen, V. Korman, B. M. Diethelm-Okita, M. T. Reding, C. J. Steer, *J. Clin. Invest.* **2009**, *119*, 2086.
[95] M. A. Hausl, W. Zhang, N. Müther, C. Rauschhuber, H. G. Franck, E. P. Merricks, T. C. Nichols, M. A. Kay, A. Ehrhardt, *Mol. Ther.* **2010**, *18*, 1896.
[96] J. Zhu, B. T. Kren, C. W. Park, R. Bilgim, P. Y.-P. Wong, C. J. Steer, *Biochemistry* **2007**, *46*, 6844.
[97] J. D. Belcher, J. V. Vineyard, C. M. Bruzzone, C. Chen, J. D. Beckman, J. Nguyen, C. J. Steer, G. M. Vercellotti, *J. Mol. Med.* **2010**, *88*, 665.
[98] S. Ortiz-Urda, Q. Lin, S. R. Yant, D. Keene, M. A. Kay, P. A. Khavari, *Gene Ther.* **2003**, *10*, 1099.
[99] M. C. Latella, F. Cocchiarella, L. de Rosa, G. Turchiano, M. A. F. V. Gonçalves, F. Larcher, M. de Luca, A. Recchia, *J. Invest. Dermatol.* **2017**, *137*, 836.
[100] Z. J. Chen, B. T. Kren, P. Y.-P. Wong, W. C. Low, C. J. Steer, *Biochem. Biophys. Res. Commun.* **2005**, *329*, 646.
[101] H. Eyjolfsdottir, M. Eriksdotter, B. Linderoth, G. Lind, B. Juliusson, P. Kusk, O. Almkvist, N. Andreasen, K. Blennow, D. Ferreira, E. Westman, I. Nennesmo, A. Karami, T. Darreh-Shori, A. Kadir, A. Nordberg, E. Sundström, L.-O. Wahlund, A. Wall, M. Wiberg, B. Winblad, Å. Seiger, L. Wahlberg, P. Almqvist, *Alzheimer's Res. Ther.* **2016**, *8*, 30.
[102] S. Muses, J. E. Morgan, D. J. Wells, *PLoS Curr.* **2011**, *3*, RRN1296.
[103] H. Escobar, V. Schöwel, S. Spuler, A. Marg, Z. Izsvák, *Mol. Ther. Nucleic Acids* **2016**, *5*, e277.
[104] L. Garcia-Garcia, S. Recalde, M. Hernandez, J. Bezunartea, J. R. Rodriguez-Madoz, S. Johnen, S. Diarra, C. Marie, Z. Izsvák, Z. Ivics, D. Scherman, M. Kropp, G. Thumann, F. Prosper, P. Fernandez-Robredo, A. Garcia-Layana, *Mol. Ther. Nucleic Acids* **2017**, *9*, 1.
[105] G. Thumann, N. Harmening, C. Prat-Souteyrand, C. Marie, M. Pastor, A. Sebe, C. Miskey, L. D. Hurst, S. Diarra, M. Kropp, P. Walter, D. Scherman, Z. Ivics, Z. Izsvák, S. Johnen, *Mol. Ther. Nucleic Acids* **2017**, *6*, 302.
[106] L. R. Belur, K. M. Podetz-Pedersen, B. S. Sorenson, A. H. Hsu, J. B. Parker, C. S. Carlson, D. A. Saltzman, S. Ramakrishnan, R. S. McIvor, *Mol. Cancer* **2011**, *10*, 14.
[107] J. R. Ohlfest, Z. L. Demorest, Y. Motooka, I. Vengco, S. Oh, E. Chen, F. A. Scappaticci, R. J. Saplis, S. C. Ekker, W. C. Low, A. B. Freese, D. A. Largaespada, *Mol. Ther.* **2005**, *12*, 778.
[108] J. S. Song, C. W. Kim, E. R. Ochoa, *Biosci. Biotechnol. Biochem.* **2009**, *73*, 165.
[109] X. Huang, H. Guo, J. Kang, S. Choi, T. C. Zhou, S. Tammana, C. J. Lees, Z.-Z. Li, M. Milone, B. L. Levine, J. Tolar, C. H. June, R. S. McIvor, J. E. Wagner, B. R. Blazar, X. Zhou, *Mol. Ther.* **2008**, *16*, 580.
[110] H. Singh, P. R. Manuri, S. Olivares, N. Dara, M. J. Dawson, H. Huls, P. B. Hackett, D. B. Kohn, E. J. Shpall, R. E. Champlin, L. J. N. Cooper, *Cancer Res.* **2008**, *68*, 2961.
[111] Z. Jin, S. Maiti, H. Huls, H. Singh, S. Olivares, L. Mátés, Z. Izsvák, Z. Ivics, D. A. Lee, R. E. Champlin, L. J. N. Cooper, *Gene Ther.* **2011**, *18*, 849.
[112] C. F. Magnani, N. Turazzi, F. Benedicenti, A. Calabria, E. Tenderini, S. Tettamanti, G. M. P. G. Attianese, L. J. N. Cooper, A. Aiuti, E. Montini, A. Biondi, E. Biagi, *Oncotarget* **2016**, *7*, 51581.
[113] P. D. Peng, C. J. Cohen, S. Yang, C. Hsu, S. Jones, Y. Zhao, Z. Zheng, S. A. Rosenberg, R. A. Morgan, *Gene Ther.* **2009**, *16*, 1042.
[114] D. C. Deniger, A. Pasetto, E. Tran, M. R. Parkhurst, C. J. Cohen, P. F. Robbins, L. J. Cooper, S. A. Rosenberg, *Mol. Ther.* **2016**, *24*, 1078.
[115] J.-S. Park, B.-H. Kim, S. G. Park, S. Y. Jung, D. H. Lee, W.-C. Son, *Biochem. Biophys. Res. Commun.* **2013**, *434*, 589.
[116] S. Jung, S. W. Ro, G. Jung, H.-L. Ju, E.-S. Yu, W.-C. Son, *Oncol. Rep.* **2013**, *29*, 1293.
[117] H.-J. Choi, H.-B. Lee, S. Jung, H.-K. Park, W. Jo, S.-M. Cho, W.-J. Kim, W.-C. Son, *Molecules* **2018**, *23*.
[118] M. Yamamoto, B. Xin, Y. Nishikawa, *Methods Mol. Biol.* **2019**, *1905*, 221.
[119] S.-H. Tseng, S.-T. Park, B. Lam, Y.-C. Tsai, M. A. Cheng, E. Farmer, D. Xing, C.-F. Hung, *J. Immunother. Cancer* **2020**, *8*, e000480.
[120] S. M. Wiesner, S. A. Decker, J. D. Larson, K. Ericson, C. Forster, J. L. Gallardo, C. Long, Z. L. Demorest, E. A. Zamora, W. C. Low, K. SantaCruz, D. A. Largaespada, J. R. Ohlfest, *Cancer Res.* **2009**, *69*, 431.
[121] Y.-H. Lin, M.-C. Yang, S.-H. Tseng, R. Jiang, A. Yang, E. Farmer, S. Peng, T. Henkle, Y.-N. Chang, C.-F. Hung, T.-C. Wu, *Cancer Immunol. Res.* **2018**, *6*, 305.
[122] S. Laidou, G. Alanis-Lobato, J. Pribyl, T. Raskó, B. Tichy, K. Mikulasek, M. Tsagiopoulou, J. Oppelt, G. Kastrinaki, M. Lefaki, M. Singh, A. Zink, N. Chondrogianni, F. Psomopoulos, A. Prigione, Z. Ivics, S. Pospisilova, P. Skladal, Z. Izsvák, M. A. Andrade-Navarro, S. Petrakis, *Redox Biol.* **2020**, *32*, 101458.
[123] E. Poulaki, M. G. Detsika, E. Fourtziala, E. A. Lianos, H. Gakiopoulou, *Sci. Rep.* **2020**, *10*, 5719.
[124] K. Weihbrecht, W. A. Goar, C. S. Carter, V. C. Sheffield, S. Seo, *PloS One* **2018**, *13*, e0192755.
[125] C. F. Magnani, C. Mezzanotte, C. Cappuzzello, M. Bardini, S. Tettamanti, G. Fazio, L. J. N. Cooper, G. Dastoli, G. Cazzaniga, A. Biondi, E. Biagi, *Hum. Gene Ther.* **2018**, *29*, 602.
[126] I. Portier, K. Vanhoorelbeke, S. Verhenne, I. Pareyn, N. Vandeputte, H. Deckmyn, D. S. Goldenberg, H. B. Samal, M. Singh, Z. Ivics, Z. Izsvák, S. F. de Meyer, *J. Thromb. Haemostasis* **2018**, *16*, 592.
[127] M. Quiviger, A. Giannakopoulos, S. Verhenne, C. Marie, E. F. Stavrou, K. Vanhoorelbeke, Z. Izsvák, S. F. de Meyer, A. Athanassiadou, D. Scherman, *Eur. J. Med. Genet.* **2018**, *61*, 723.
[128] M. Pastor, S. Johnen, N. Harmening, M. Quiviger, J. Pailloux, M. Kropp, P. Walter, Z. Ivics, Z. Izsvák, G. Thumann, D. Scherman, C. Marie, *Mol. Ther. Nucleic Acids* **2018**, *11*, 57.
[129] V. Kumbhari, L. Li, K. Piontek, M. Ishida, R. Fu, B. Khalil, C. M. Garrett, E. Liapi, A. N. Kalloo, F. M. Selaru, *Gastrointest. Endosc.* **2018**, *88*, 755.
[130] B. Gao, W. Wang, H. Wu, C. Chen, D. Shen, S. Wang, W. Chen, L. Zhang, S. Chan, C. Song, *Biochem. Genet.* **2018**, *56*, 341.
[131] K. Sumiyoshi, H. Koso, S. Watanabe, *Cancer Sci.* **2018**, *109*, 1513.
[132] M. Hernandez, S. Recalde, L. Garcia-Garcia, J. Bezunartea, C. Miskey, S. Johnen, S. Diarra, A. Sebe, J. R. Rodriguez-Madoz, S. Pouillot, C. Marie, Z. Izsvák, D. Scherman, M. Kropp, F. Prosper, G. Thumann, Z. Ivics, A. Garcia-Layana, P. Fernandez-Robredo, *Mol. Ther. Methods Clin. Dev.* **2019**, *15*, 403.
[133] J.-H. Chang, K. Y. Mou, C.-Y. Mou, *Sci. Rep.* **2019**, *9*, 11457.
[134] H. G. Caruso, R. Tanaka, J. Liang, X. Ling, A. Sabbagh, V. K. Henry, T. L. Collier, A. B. Heimberger, *J. Neurooncol.* **2019**, *145*, 429.
[135] R. B. Batchu, O. V. Gruzdyn, P. S. Tavva, B. K. Kolli, R. Dachepalli, D. W. Weaver, S. A. Gruber, *Surgery* **2019**, *166*, 503.
[136] G. W. Liu, S. L. Johnson, R. Jain, D. J. Peeler, S. J. Shankland, S. H. Pun, *J. Biomed. Mater. Res. A* **2019**, *107*, 2718.
[137] L. Ye, J. J. Park, M. B. Dong, Q. Yang, R. D. Chow, L. Peng, Y. Du, J. Guo, X. Dai, G. Wang, Y. Errami, S. Chen, *Nat. Biotechnol.* **2019**, *37*, 1302.
[138] E. Grisard, M. Coan, L. Cesaratto, I. Rigo, L. Zandonà, A. Paulitti, E. Andreuzzi, G. L. R. Vinciguerra, E. Poletto, F. Del Ben, G. Brisotto, E. Biscontin, M. Turetta, E. Dassi, A. Mirnezami, V. Canzonieri, A. Vecchione, G. Baldassarre, M. Mongiat, R. Spizzo, M. S. Nicoloso, *EBioMedicine* **2019**, *46*, 79.

[139] K. R. Loeb, B. T. Hughes, B. M. Fissel, N. J. Osteen, S. E. Knoblaugh, J. E. Grim, L. J. Drury, A. Sarver, A. J. Dupuy, B. E. Clurman, *Sci. Rep.* **2019**, *9*, 5488.
[140] L. Chicaybam, L. Abdo, M. Viegas, L. V. C. Marques, P. de Sousa, L. R. Batista-Silva, V. Alves-Monteiro, S. Bonecker, B. Monte-Mór, M. H. Bonamino, *Gene Ther.* **2020**, *27*, 85.
[141] F. Wang, W. Hou, L. Chitsike, Y. Xu, C. Bettler, A. Perera, T. Bank, S. J. Cotler, A. Dhanarajan, M. F. Denning, X. Ding, P. Breslin, W. Qiang, J. Li, A. J. Koleske, W. Qiu, *Gastroenterology* **2020**.
[142] P. J. Beckmann, J. D. Larson, A. T. Larsson, J. P. Ostergaard, S. Wagner, E. P. Rahrmann, G. A. Shamsan, G. M. Otto, R. L. Williams, J. Wang, C. Lee, B. R. Tschida, P. Das, A. M. Dubuc, B. S. Moriarity, D. Picard, X. Wu, F. J. Rodriguez, Q. Rosemarie, R. D. Krebs, A. M. Molan, A. M. Demer, M. M. Frees, A. E. Rizzardi, S. C. Schmechel, C. G. Eberhart, R. B. Jenkins, R. J. Wechsler-Reya, D. J. Odde, A. Huang, M. D. Taylor, A. L. Sarver, D. A. Largaespada, *Cancer Res.* **2019**, *79*, 905.
[143] M. Hudecek, Z. Ivics, *Curr. Opin. Genet. Dev.* **2018**, *52*, 100.
[144] P. Kebriaei, H. Singh, M. H. Huls, M. J. Figliola, R. Bassett, S. Olivares, B. Jena, M. J. Dawson, P. R. Kumaresan, S. Su, S. Maiti, J. Dai, B. Moriarity, M.-A. Forget, V. Senyukov, A. Orozco, T. Liu, J. McCarty, R. N. Jackson, J. S. Moyes, G. Rondon, M. Qazilbash, S. Ciurea, A. Alousi, Y. Nieto, K. Rezvani, D. Marin, U. Popat, C. Hosing, E. J. Shpall, H. Kantarjian, M. Keating, W. Wierda, K. A. Do, D. A. Largaespada, D. A. Lee, P. B. Hackett, R. E. Champlin, L. J. N. Cooper, *J. Clin. Invest.* **2016**, *126*, 3363.
[145] E. D. Hsi, R. Steinle, B. Balasa, S. Szmania, A. Draksharapu, B. P. Shum, M. Huseni, D. Powers, A. Nanisetti, Y. Zhang, A. G. Rice, A. van Abbema, M. Wong, G. Liu, F. Zhan, M. Dillon, S. Chen, S. Rhodes, F. Fuh, N. Tsurushita, S. Kumar, V. Vexler, J. D. Shaughnessy, B. Barlogie, F. van Rhee, M. Hussein, D. E. H. Afar, M. B. Williams, *Clin. Cancer Res.* **2008**, *14*, 2775.
[146] S. A. Ali, V. Shi, I. Maric, M. Wang, D. F. Stroncek, J. J. Rose, J. N. Brudno, M. Stetler-Stevenson, S. A. Feldman, B. G. Hansen, V. S. Fellowes, F. T. Hakim, R. E. Gress, J. N. Kochenderfer, *Blood* **2016**, *128*, 1688.
[147] D. M. Benson, J. C. Byrd, *J. Clin. Oncol.* **2012**, *30*, 2013.
[148] T. N. Schumacher, R. D. Schreiber, *Science* **2015**, *348*, 69.
[149] T. Jiang, T. Shi, H. Zhang, J. Hu, Y. Song, J. Wei, S. Ren, C. Zhou, *J. Hematol. Oncol.* **2019**, *12*, 93.
[150] S. Zong, T. Mi, L. G. Flores, A. Alpert, S. Olivares, K. Patel, S. Maiti, G. Mcnamara, L. J. N. Cooper, H. Torikai, *PloS One* **2020**, *15*, e0228112.
[151] R. Velez-Montoya, S. C. N. Oliver, J. L. Olson, S. L. Fine, N. Mandava, H. Quiroz-Mercado, *Retina* **2013**, *33*, 1487.
[152] R. A. Adelman, Q. Zheng, H. R. Mayer, *J. Ocul. Pharmacol. Ther.* **2010**, *26*, 105.
[153] R. Krishnan, S. Goverdhan, J. Lochhead, *Clin. Exp. Ophtalmol.* **2009**, *37*, 384.
[154] A. M. Carneiro, D. Barthelmes, M. S. Falcão, L. S. Mendonça, S. L. Fonseca, R. M. Gonçalves, F. Faria-Correia, F. M. Falcão-Reis, *Ophthalmologica* **2011**, *225*, 211.
[155] N. A. M. Bakker, R. de Boer, C. Marie, D. Scherman, J. B. A. G. Haanen, J. H. Beijnen, B. Nuijen, J. H. van den Berg, *J. Biotechnol.* **2019**, *2*, 100007.

