

Evaluation of the Efficacy of Lentiviral Vectors in Gene Therapy of Beta-thalassemia Patients: A Systematic Review

Saeed Dorgaleh¹, Ahmad Barahouie¹, Farzad Dastaviz¹, Zahrasadat Ghodsalavi¹, *Morteza Oladnabi^{2,3,4}

¹Student Research Committee, Golestan University of Medical Sciences, Gorgan, Iran.

²Gorgan Congenital Malformations Research Center, Golestan University of Medical Sciences, Gorgan, Iran.

³Stem Cell Research Center, Golestan University of Medical Sciences, Gorgan, Iran.

⁴Ischemic Disorders Research Center, Golestan University of Medical Sciences, Gorgan, Iran.

Abstract

Background

Beta thalassemia is a genetic blood abnormality identified through mutations, which reduce the synthesis of the β -globin chain. Gene therapy through Lentiviral vectors have cured many of genetic disorders. The purpose of this study was to investigate the efficacy of lentiviral vectors in treatment of β -thalassemia as a novel approach for sustained treatment and prevention of recurrent blood transfusion that has many adverse effects on the patients.

Materials and Methods

In this systematic review, a systematic search of online databases (Web of Science, Scopus, and Medline) with no language restriction between 2012 and 2020 using the combination related keywords of Mesh included (Thalassemia OR Beta thalassemia OR β _thalassemia OR BTM) AND (Lentivirus) AND (Genetic therapy OR Gene therapy). All valuable data was allocated, and two independent researchers considered all articles.

Results

Finally, 20 articles that met the criteria were selected. Articles were quantitative (n=16), and qualitative (n=4), their year of publication varied from 2012 to 2020. Results showed that the main outcome of the desired survey was therapeutic treatments for thalassemia worldwide. While highly compatible donors for allogeneic bone marrow transplantation are accessible to less than 30% of all patients, gene therapy has emerged as a holistic and practical approach for the remaining 70%.

Conclusion

Nowadays, with the development of gene therapy, there is hope for a cure for genetic diseases. The successes of gene therapy techniques can bring laboratory methods of gene therapy a step closer to clinical and general use as a common treatment for genetic defects.

Key Words: Beta-thalassemia, Lentiviral, Gene therapy, Vectors.

*Please cite this article as: Dorgaleh S, Barahouie A, Dastaviz F, Ghodsalavi Z, Oladnabi M. Evaluation of the Efficacy of Lentiviral Vectors in Gene Therapy of Beta-thalassemia Patients: A Systematic Review. Int J Pediatr 2020; 8(5): 11243-250. DOI: [10.22038/ijp.2020.47309.3839](https://doi.org/10.22038/ijp.2020.47309.3839)

*Corresponding Author:

Dr. Morteza Oladnabi, Department of Medical Genetics, School of Advanced Technologies in Medicine, Golestan University of Medical Sciences, Gorgan, Iran.

Email: oladnabidozin@yahoo.com.

Received date: Dec.10, 2019; Accepted date: Feb.12, 2020

1- INTRODUCTION

Beta-Globinopathies are genetic blood abnormalities identified through mutations that reduce the synthesis of the β -globin chain, as we see in beta-thalassemia and sickle cell disease that lead to polymerization of sickle cell hemoglobin (1). Beta-thalassemia is the result of at least 200 mutations in the HBB gene, which decrease the β -globin synthesis (2). More than 300,000 babies are born annually worldwide with a genetic Hemoglobin (Hb) disorder (3), leading to approximately 3.4% of deaths in infants, mostly in Africa (3, 4). The only effective therapeutic behavior is the transplantation of hematopoietic stem cells, however the majority of patients are not human leucocyte antigen (HLA)-matched to donors, consequently, patients are at risk of life threatening problems (5).

Thus, gene therapy through the modification of hematopoietic stem cells (HSCs) and their engraftment is a promising novel therapeutic approach. Gene therapy through lentiviral vectors (LVs) has cured many genetic disorders. For the first time LVs revealed their potency in the handling of problems of childhood cerebral adrenoleukodystrophy (CCALD) (6, 7), and β -thalassemia (8, 9). LVs last generation with β -globin gene have developed treatment of Beta-thalassemia via efficient hematopoietic stem cells (HSC) transduction, with no reported genotoxicity (10). Numerous trials based on various vectors are ongoing in the world and the primary outcomes are promising with regard to the predominant production of biological factors like hemoglobin, a lower transfusion requisition, and more expectancy of life. The improvement of patient life quality by HSC collecting and HSC transduction has confirmed the therapeutic capability of this method. Innovative lentiviral vectors based approaches with endogenous inducing fetal hemoglobin (HbF) are encouraging, since

increasing hemoglobin fetal (HbF) levels is capable of decreasing the severity of β -thalassemia and sickle cell disease (11, 12). Therefore, gene-editing methods are developing to edit thalassemia responsible mutations or reactivating HbF (11). The primary designed experiments on β -thalassemia gene therapy were established on murine leukaemia virus (MLV)-derived vectors, which expressed human β -globin genes. The identification of the long terminal repeat (LTR) as main protooncogene of MLV resulted in the design of self-inactivating (SIN) vectors lacking LTR controlling sequences and including cellular promoters to express the therapeutic gene. SIN vector safety and efficiency have been confirmed in an experiment of SCID-X1 gene therapy (13).

To diminish risk of additional tumorigenesis, (self-inactivating lentivirus vector (SIN LVs) derived from the human immunodeficiency virus (HIV)-replaced MLV-derived vectors in all clinical trials involving genetically modified HSCs. Gene therapy is a great approach to hemoglobinopathies. Globin genes are exposed to a complicated modulation based on collaboration of locus-control region with globin promoters that modulates gene expression as selection of different genes in various stages of growth (14, 15). The sequences of locus control region (LCR) in association with β -globin gene *al* vector. Although studies attempted to make this vector smaller, LVs, which express globin, are large and have a lower transduction efficacy toward simple vectors. Lentiviral vectors have been advanced and effectively confirmed in types of hemoglobinopathies; while recent clinical experiments are also demonstrating notable safety and efficacy. However, MLV specially targets transcription modulator factors (16-18). Lentiviral vectors integrate in transcribed genes (16) near the nuclear membrane (19). A naturally safer integration model as

showed in experiments (20). Lentiviral vectors are presently applied in different stages of trials of gene therapy for X-linked chronic granulomatous disease (X-CGD), adenosine deaminase severe combined immunodeficiency (ADA-SCID) and Wiskott-Aldrich syndrome. We intended to systematically review the approach of lentiviral vector in treatment of β -globinopathies. The outcomes of this systematic review may help our understanding about the benefits of using lentiviral vectors in patients with β -thalassemia. The purpose of this study was to investigate the efficacy of lentiviral vector in treatment of β -thalassemia as a novel approach for sustained treatment and prevention of recurrent blood transfusion that has many adverse effects on the patients (as it has been done for SCID and ADA deficiency).. The gap and limitations of our knowledge was access to both English and Farsi as well as the time constraints we considered from 2012 to 2020.

2- MATERIALS AND METHODS

2-1. Data sources

This study is a systematic review. The research population included articles in the field of thalassemia and lentiviral gene therapy that were indexed in one of the Internet databases. Web of Science, Scopus, and Medline (via PubMed) were searched with no language restriction between 2012 and 2020. All valuable data was allocated and two independent researchers considered all articles. Furthermore, all papers were presented at national seminars, congresses, and reports, as well as the dissertations connected to were assessed. If there were abstracts of related articles, access to full text articles was by contacting the corresponding author.

2-2. Search strategy

To find relevant articles in international databases, the combination related keywords of Mesh included (Thalassemia OR Beta thalassemia OR β _thalassemia OR BTM) AND (Lentivirus) AND (Genetic therapy OR Gene therapy) were searched. In order to avoid missing related papers, no limitations were set in the search strategy. Furthermore, all references of retrieved articles were also screened to find additional studies. Initially, the titles and abstracts were reviewed to find relevant papers and exclude irrelevant ones. Then the full-text of remaining papers were reviewed according to our inclusion and exclusion criteria.

2-3. Study selection

The arrangement of the process steps was done firstly by searching the relevant databases, 841 articles were found; after that, 180 repeated articles were eliminated because of duplication, titles and abstracts of 661 articles were reviewed; 626 articles were removed due to not being relevant with the aim of the research. The full texts of the rest of the articles from the previous steps (35 articles) were reviewed. Ultimately, 20 articles that contained inclusion criteria of our study were chosen. Two authors considered the quality of the articles independently. To organize the studies, Endnote software was used. Inclusion criteria involved publication of an article in Farsi or English languages, operation of research in thalassemia and gene therapy, key informants, access to the full text of articles and original academic articles (including quantitative and qualitative articles). Review articles and letters to the editor-in-chief were not selected, because they did not use the primary data. Through studying the titles and abstracts of articles, which had inclusion criteria by the researchers carefully identified, many of them were excluded due to not being relevant to the purpose of our study. If it was not possible to make a decision on the article after

studying the title and the abstract, the full text of it was read. To ensure the retrieval of all documents, a list of articles' references was searched as well. After

completing the articles' investigation, 20 final papers were selected by using flowchart (**Figure.1**).

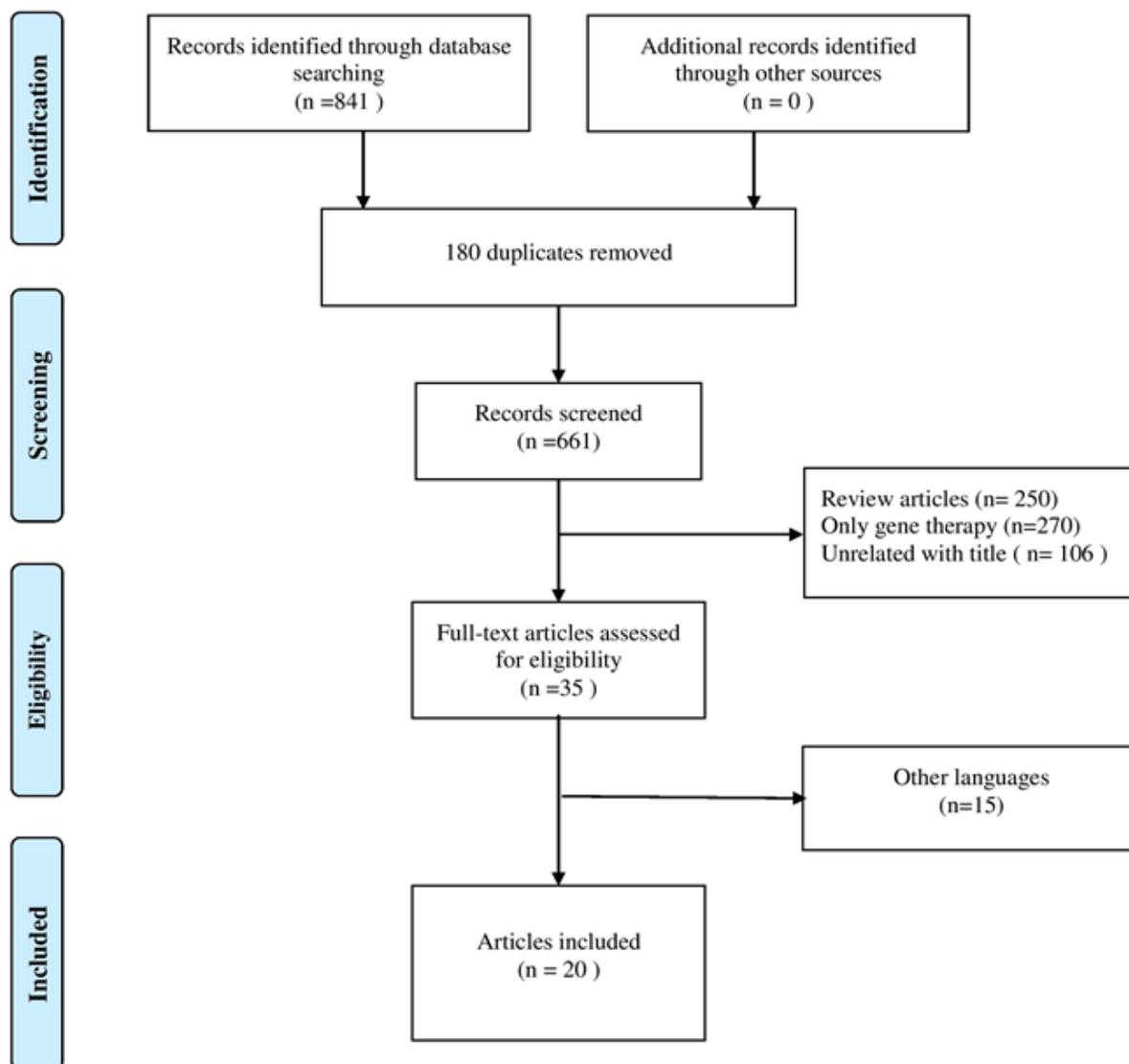


Fig.1: Flowchart for selection of studies.

2-4. Quality Assessment

Four reviewers considered the quality of the articles independently. Independent reviewers screened titles and abstracts. Any discrepancies were resolved through discussion after completion of screening and a fifth person's opinion sought. After obtaining full texts of screened studies, two independent reviewers selected eligible papers based on the inclusion/

exclusion criteria. Discrepancies were resolved with discussion at the end of this stage and where necessary, the fifth reviewer's opinion was sought. The full text of two to five of the included studies were selected randomly to evaluate the pilot data extraction questionnaire. Any further information that is not considered in the pilot form was added after being approved by the review team. Title, abstract and full text of the included

studies was then carefully read. Any existing discrepancies in data extraction was discussed and where necessary, a fifth reviewer was involved.

2-5. Data extraction

Finally, for extracting data from the text of these articles, four reviewers collected the data independently, collected data was combined and compared for accuracy, any discrepancies were resolved by a fifth reviewer. Data collected from the selected studies included: study design, study population (sample size, age, gender, etc.), the type of study (qualitative or quantitative), the place of research operation, the sample size and target group, the profile of participants (baseline characteristics), and the main results.

3- RESULTS

In this study, all published articles were deliberated based on the research purpose in electronic databases. In the first step, by the basic search with the relevant keywords, 841 articles (PubMed=141; Scopus=311; Web of Science=339 and other references=50) were extracted. Then, after removing repetitive articles by reviewing titles and considering the rest of them through the abstract and their full text, finally, 20 articles were assessed.

The articles were both quantitative (n=16), and qualitative (4). The year of release varied from 2012 to 2020. The sampling method in most qualitative studies was purpose-based and semi structured deep interviews from the target group; however, in seven qualitative studies, in addition to a personal interview method, group discussion (FGD) with thalassemia and key informants were applied to collect the data. In most qualitative and quantitative studies, the target group was both thalassemia and key informants. The study, which eventually included 20 articles, confirmed that by removing bone marrow stem cells from patients and

engineering them produced healthy hemoglobin before returning (21). As well as allogeneic cell transplantation, cell transplantation led to cell transformation and hemoglobin production. Gene Therapy with Genetically Modified Stem Cell (22). Autologous Transplantation Experimental gene transfer surgery was performed in the laboratory to insert the autologous stem cell genes of the participants using a lentiviral vector. In addition, lentivirus vectors were confirmed to be safe, safe and low in toxic effects (23, 24).

From 841 articles, 20 studies were included. Results showed that: the main outcome of the desired survey was therapeutic treatments for thalassemia worldwide. While highly compatible donors for allogeneic bone marrow transplantation are accessible to less than 30% of all patients, gene therapy has emerged as a holistic and practical approach for the remaining 70%. Beta-globin gene transfers clinical trials using lentiviruses (LVs) began in the new millennium and have since produced results that not only demonstrate the potential of this approach but also better strategies. In addition, gene therapy should be constructed in vectors with expression in each copy, so that a copy can be therapeutic

4- DISCUSSION

The purpose of this study was to investigate the efficacy of lentiviral vector in treatment of β -thalassemia as a novel approach for sustained treatment and prevention of recurrent blood transfusion that has many adverse effects on the patients. Gene therapy can broadly be defined as the transfer of genetic material to treat disease or at least to improve the clinical status of patients. One of the basic concepts of gene therapy is the conversion of viruses into genetic shuttles, which deliver the gene to insert target cells. Using several viral and non-viral vectors,

safe methods have been devised to do this. Two main approaches emerged: *in vivo* correction and *in vitro* correction. Retrovirus, Adenovirus, Adenovirus is based on the principles of gene therapy. Gene transformers have been approved for human use in hereditary diseases, cancers and aquatic disorders. Although existing vector systems are capable of delivering *in vivo* genes to cells, they have not been found to be an ideal means of transport. Gene therapy in blood-thalassemia patients requires a hematopoietic stem cell progenitor, like the erythroid that boosts the level of production of the globin. Beta-thalassemia is a genetic disorder that disrupts the body's ability to produce a key component of hemoglobin. Patients with severe forms of beta-thalassemia need to receive blood on a monthly basis to supply red blood cells with iron chelators to remove excess iron.

Our study examined lentiviral-based gene therapy techniques by Flip Leboulch et al., which once treated with lentiglobin BB305 vector gene therapy reduced or eliminated the need for blood transfusion in 22 patients with thalassemia major. They used this viral vector to transmit genetic instructions to their patient's own blood stem cells and to restore hemoglobin production by them. Our findings, which confirm the safety, were consistent with the finding that it was low risk and effective in this study. Analyses in this study showed that patients treated with HCST were three times more likely to be infected than gene therapy (25).

However, our findings were not in agreement with the results of the work of Dormiani and colleagues. In this method, they used the plasmid to deliver a healthy gene that was less susceptible to the virus, the healthy gene was introduced into a specific part of the cell's genome with precision techniques, and the gene segments that were involved in gene therapy techniques were programmed;

have been deleted. The results showed that the healthy gene in the cells tested correctly replaced the defective gene and the healthy beta - globin was maintained for months by the gene therapy cell (26).

4-1. Limitations of the study

Methods of LVs using in beta-thalassemia treatment in different studies varies and it can disturb the homogeneity of the outcomes and restrict reliability of quantitative analysis of the data. The conception of bias and accuracy could be different in various studies, thus, there may be restricted choices to designing a meta-analysis through this data.

5- CONCLUSION

There are a number of diseases that are caused by defects in a person's genes. These types of diseases, which are usually hereditary, are called genetic diseases. For years, these diseases were considered incurable. One of these diseases is thalassemia, the treatment of which is repeated blood transfusion. But nowadays, with the development of gene therapy, there is hope for a cure for these diseases. The successes of gene therapy techniques can bring laboratory methods of gene therapy a step closer to clinical and general use as a common treatment for genetic defects. Lentiviral vectors (LVs) last generation with β -globin gene have developed treatment of Beta- thalassemia via efficient Hematopoietic Stem Cell transduction, with no reported genotoxicity.

6- ACKNOWLEDGMENTS

The authors would like to express gratitude for faculty members at Department of Medical Genetics, School of Advanced Technologies in Medicine, Golestan University of Medical Sciences.

7- CONFLICT OF INTEREST: None.

8- REFERENCES

1. Steinberg MH, Forget BG, Higgs DR, Weatherall DJ. Disorders of hemoglobin: genetics, pathophysiology, and clinical management. Cambridge University Press; 2009.
2. Higgs DR, Engel JD, Stamatoyannopoulos G. *Thalassaemia*. *Lancet*. 2012; 379(9813):373-83.
3. Modell B, Darlison M. Global epidemiology of haemoglobin disorders and derived service indicators. *Bull World Health Organ*. 2008;86: 480-7.
4. Grosse SD, Odame I, Atrash HK, Amendah DD, Piel FB, Williams TN. Sickle cell disease in Africa: a neglected cause of early childhood mortality. *Am J Prev Med*. 2011; 41(6):S398-405.
5. Locatelli F, Kabbara N, Ruggeri A, Ghavamzadeh A, Roberts I, Li CK, et al. Outcome of patients with hemoglobinopathies given either cord blood or bone marrow transplantation from an HLA-identical sibling. *Blood*. 2013; 122(6):1072-78.
6. Cartier N, Hacein-Bey-Abina S, Bartholomae CC, Bougneres P, Schmidt M, Von Kalle C, et al. Lentiviral hematopoietic cell gene therapy for X-linked adrenoleukodystrophy. *InMethods in enzymology* 2012; 507: 187-98. Academic Press.
7. Cartier N, Hacein-Bey-Abina S, Bartholomae CC, Veres G, Schmidt M, Kutschera I, et al. Hematopoietic stem cell gene therapy with a lentiviral vector in X-linked adrenoleukodystrophy. *Science*. 2009; 326(5954):818-23.
8. Cavazzana-Calvo M, Payen E, Negre O, Wang G, Hehir K, Fusil F, et al. Transfusion independence and HMGA2 activation after gene therapy of human β -thalassaemia. *Nature*. 2010; 467(7313):318-22.
9. Payen E, Leboulch P. Advances in stem cell transplantation and gene therapy in the β -hemoglobinopathies. *Hematology Am Soc Hematol Educ Program*. 2012; 2012(1):276-83.
10. Naldini L. Gene therapy returns to centre stage. *Nature*. 2015; 526(7573):351-60.
11. Modlich U, Bohne J, Schmidt M, von Kalle C, Knöss S, Schambach A, Baum C. Cell-culture assays reveal the importance of retroviral vector design for insertional genotoxicity. *Blood*. 2006; 108(8):2545-53.
12. Magrin E, Miccio A, Cavazzana M. Lentiviral and genome-editing strategies for the treatment of β -hemoglobinopathies. *Blood*. 2019;134(15):1203-13.
13. Forget BG. Molecular basis of hereditary persistence of fetal hemoglobin. *Ann N Y Acad Sci*. 1998;850(1):38-44.
14. Hacein-Bey-Abina S, Pai SY, Gaspar HB, Armant M, Berry CC, Blanche S, et al. A modified γ -retrovirus vector for X-linked severe combined immunodeficiency. *N Engl J Med*. 2014; 371(15):1407-17.
15. Wilber A, Nienhuis AW, Persons DA. Transcriptional regulation of fetal to adult hemoglobin switching: new therapeutic opportunities. *Blood*. 2011;117(15):3945-53.
16. Smith EC, Orkin SH. Hemoglobin genetics: recent contributions of GWAS and gene editing. *Hum Mol Genet*. 2016; 25(R2):R99-105.
17. Cavazza A, Moiani A, Mavilio F. Mechanisms of retroviral integration and mutagenesis. *Hum Gene Ther*. 2013; 24(2):119-31.
18. Cattoglio C, Pellin D, Rizzi E, Maruggi G, Corti G, Miselli F, et al. High-definition mapping of retroviral integration sites identifies active regulatory elements in human multipotent hematopoietic progenitors. *Blood*. 2010;116(25):5507-17.
19. De Ravin SS, Su L, Theobald N, Choi U, Macpherson JL, Poidinger M, et al. Enhancers are major targets for murine leukemia virus vector integration. *J Virol*. 2014; 88(8):4504-13.
20. Marini B, Kertesz-Farkas A, Ali H, Lucic B, Lisek K, Manganaro L, et al. Nuclear architecture dictates HIV-1 integration site selection. *Nature*. 2015; 521(7551):227-31.
21. Harrison C. First gene therapy for β -thalassaemia approved. *Nat Biotechnol*. 2019; 37(10):1102.

22. Karponi G, Zogas N. Gene therapy for beta-thalassemia: updated perspectives. *Appl Clin Genet.* 2019;12:167.
23. Thompson AA, Walters MC, Kwiatkowski J, Rasko JE, Ribeil JA, Hongeng S, et al. Gene therapy in patients with transfusion-dependent β -thalassemia. *N Engl J Med.* 2018; 378(16):1479-93.
24. Mansilla-Soto J, Riviere I, Boulad F, Sadelain M. Cell and Gene Therapy for the Beta-Thalassemias: Advances and Prospects. *Hum Gene Ther.* 2016; 27(4):295-304.
25. Coquerelle S, Ghardallou M, Rais S, Taupin P, Touzot F, Boquet L, et al. Innovative Curative Treatment of Beta Thalassemia: Cost-Efficacy Analysis of Gene Therapy Versus Allogenic Hematopoietic Stem-Cell Transplantation. *Hum Gene Ther.* 2019;30(6):753-61.
26. Dormiani K, Mir Mohammad Sadeghi H, Sadeghi-Aliabadi H, Ghaedi K, Forouzanfar M, Baharvand H, et al. Long-term and efficient expression of human β -globin gene in a hematopoietic cell line using a new site-specific integrating non-viral system. *Gene Ther.* 2015; 22(8):663-74.