# EBT-101 achieves robust CRISPR-based editing of HIV proviral DNA without detectable off-target effects

Thomas J Cradick,[1] Vahan Simonyan,[2] Elaine E Thompson,[2] Eli J Fine,[3] Rafal Kaminski,[4] Matthew Hayden,[5] Sarmistha Bhattacharya,[5] Wenwen Huo,[1] Ethan Xu[1]

[1]Excision BioTherapeutics, San Francisco, CA; [2]Embleema, Metuchen, NJ; [3]Genome Engineering Consultant, Smyrna, GA; [4]Temple University, Philadelphia, PA; [5]Dartmouth-Hitchcock Medical Center, Lebanon, NH

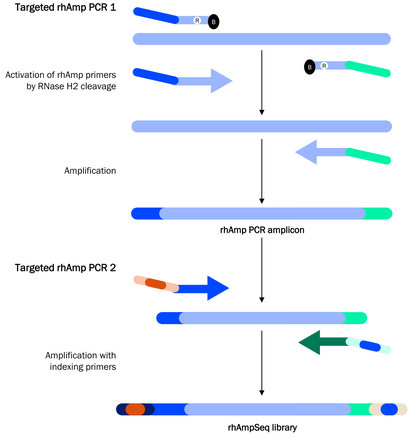
## INTRODUCTION

- According to recent estimates, ~1 million people in the US and 36.9 million people worldwide live with HIV/AIDS.[1] The current standard of care is a combination of antiretroviral therapies, which may control the disease but are not curative, as they are unable to target integrated HIV-1 genomes.[3] Gene editing to remove proviral DNA can provide the opportunity to eradicate the disease, advancing beyond the current standard of care
- Safe and effective viral excision requires pairs of CRISPR-Cas guide RNAs (gRNAs) that specifically cleave HIV but have minimal similarity to sites in the human genome
- Bioinformatics facilitate selection of HIV-specific gRNAs with fewer nominated (potential) sites output in the human genome than when targeting human sequences
- EBT-101 consists of an adeno-associated virus (AAV) vector expressing the SaCas9 nuclease and 2 gRNAs targeting 3 sites in HIV: 2 well-conserved regions in the 5' and 3' long terminal repeats (LTRs) and one in the Gag gene. Excising these large sections of the integrated HIV-1 genomes disrupts the viral life cycle and is intended to reduce latent HIV-1 patient reservoirs. The efficacy of EBT-101 has been demonstrated in humanized mouse models[2]
- Here, we describe the bioinformatics, as well as our results from multiplex amplicon sequencing that identified high levels of viral excision without detecting unintended indels or recombination

## METHODS

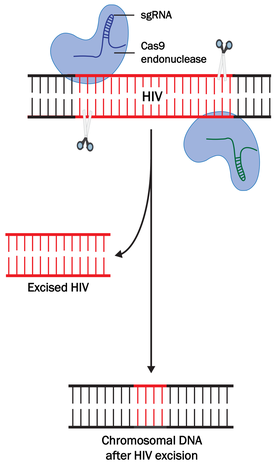
- The human reference genome was scanned for the 21-bp EBT-101 LTR-1 and GagD guide sequences with NNGRRN, the SaCas9 protospacer adjacent motif (PAM), using the bioinformatics program COTANA (CRISPR Off-TArget Nomination and Analysis) based on the Cas-OFFinder algorithm

### Figure 1: The use of rhAmpSeq[11] allows for the processing of tens of thousands of cells to achieve robust sequencing resolution




B, blocking moiety; PCR, polymerase chain reaction; R, base of RNA; rhAmp, RNase H-dependent PCR amplification; rhAmpSeq, rhAmp sequencing.

### Figure 2: CRISPR is a viral defense strategy




sgRNA, single guide RNA.

## RESULTS

- The COTANA bioinformatics search of the human genome found no identical matches, as there are no target sites in human cells without HIV. There were also no candidate off-target sites nominated with 1 or 2 differences (mismatches and/or bulges)

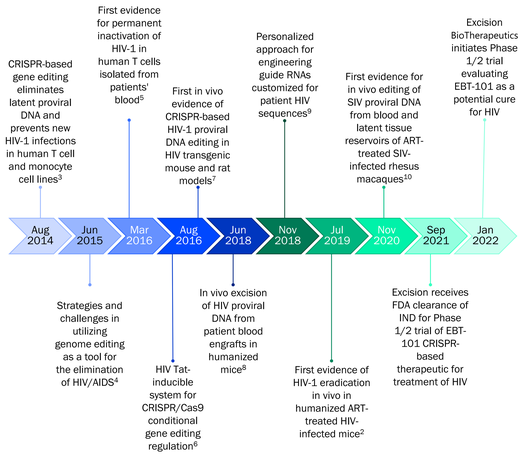
### Table 1: Only 1 human chromosomal site nominated with 3 differences from either EBT-101 guides

| Differences | Mismatches | Bulges | LTR-1 | GagD |
|---|---|---|---|---|
| Zero | 0 | 0 | – | – |
| One | 1 | 0 | – | – |
| One | 0 | 1 | – | – |
| Two | 2 | 0 | – | – |
| Two | 1 | 1 | – | – |
| Three | 3 | 0 | **1** | – |
| Three | 2 | 1 | – | – |
| Four | 4 | 0 | **13** | **24** |
| Four | 3 | 1 | **57** | **85** |

The human reference genome was searched using COTANA bioinformatics software to nominate locations with the fewest differences to the EBT-101 guide RNA sequences and the SaCas9 PAM (NNGRRN). Sites were then scored and assigned by the number of nucleotide mismatches and bulges.LTR, long terminal repeat; PAM, protospacer adjacent motif.

**High-throughput multiplex amplicon sequencing detected high levels of HIV editing and excision, but not unintended editing or recombination in U1 cells, a pro-monocytic, human myeloid leukemia cell line containing 2 integrated copies of the HIV genome that were treated with LTR-1 and GagD gRNAs coupled with SaCas9**

### Figure 3: Evolution of the multi-guide platform for excision of latent proviral HIV-1




FDA, food and drug administration; IND, investigational new drug; ART, antiretroviral therapy; SIV, simian immunodeficiency virus.

### Table 2: Bioinformatics output of nominated chromosomal sites with the fewest mismatches and bulges against the GagD and LTR-1 guides, respectively. Secondary ranking is by match score. Only one site was identified with 3 or fewer differences

| Rank | Chr. | PAM Location | GagD Alignment | MM + Bulges | Match Score |
|---|---|---|---|---|---|
| | | | G G A T A G A T G T A A A A G A C A C C A N N G R R N | | |
| 1 | 2 | 65655605 | • C • G • • - • • • T • • • • • • • • • • G G G A A T | 3 + 1 | 1.49 |
| 2 | 2 | 18127193 | • A • • • - • G • • C • • • • • • • • • • A G G A A T | 3 + 1 | 1.55 |
| 3 | 2 | 22163338 | • • • G G • • - • • C • • • • • • • • • • G G G A A T | 3 + 1 | 1.89 |
| 4 | 7 | 131867278 | • • • • G • • - • G • • G • • • • • • • • A G G G G T | 3 + 1 | 2.06 |
| 5 | 10 | 10888380 | • T C A • • • • • - • • • • • • • • • • • G T G G A T | 3 + 1 | 2.26 |
| | | | LTR-1 Alignment | | |
| 1 | 16 | 60092884 | • • • • C • • • • • • • T • • • • • • T A A G A A T | 3 + 0 | 7.27 |
| 2 | 6 | 22390293 | • • • A • C • C • • • G • • • • • • • • • T A G G G T | 3 + 1 | 1.77 |
| 3 | 7 | 15437814 | • • T • C • • - • • • • G • • • • • • • • T A G G A T | 3 + 1 | 1.84 |
| 4 | 5 | 95984632 | A • • • • T • • • C • - • • • • • • • • • T G G A A T | 3 + 1 | 2.10 |
| 5 | X | 8905548 | • G • • G C • • • • • G • • • • • • • • • C T G G G T | 3 + 1 | 2.18 |

chr, chromosome; LTR, long terminal repeat; PAM, protospacer adjacent motif

### Table 3: Bioinformatics output of nominated chromosomal sites with top 5 sites listed by match score for the GagD and LTR-1 guides respectively. Ranking by score models probability and may contain more mismatches, especially if they are outside the region near the PAM where mismatches are very poorly tolerated

| Rank | Chr. | PAM Location | GagD Alignment | MM + Bulges | Match Score |
|---|---|---|---|---|---|
| | | | G G A T A G A T G T A A A A G A C A C C A N N G R R N | | |
| 1 | 2 | 65655605 | • C • G • • - • • • T • • • • • • • • • • G G G A A T | 3 + 1 | 1.49 |
| 2 | 14 | 34248015 | A • • G • C • - • C • • • • • • • • • • C A G A A T | 4 + 1 | 1.53 |
| 3 | 3 | 12279106 | A • • A • • C A • G • • • • • • • • • • C A G A G T | 5 + 0 | 1.54 |
| 4 | 2 | 18127193 | • G • • • - • G • • C • • • • • • • • • • A G G A A T | 3 + 1 | 1.55 |
| 5 | 1 | 205588788 | T • T • T • T • T • T • • • • • • • • • • A C G A G T | 6 + 0 | 1.58 |
| | | | LTR-1 Alignment | | |
| 1 | 11 | 3368042 | • A G • C • • C • C C • • • • • • • • • • G G G G A T | 5 + 0 | 1.65 |
| 2 | 1 | 41908553 | • • • • G • G • • G G • • • • • • • • • • C T G A G T | 4 + 0 | 1.73 |
| 3 | 5 | 1116177 | C • • • G • G • C • - • • • • • • • • • • G A G A A T | 4 + 1 | 1.76 |
| 4 | 6 | 22390293 | • • • A • C • C • • • • • • • • • • • • • T A G G G T | 3 + 1 | 1.77 |
| 5 | 8 | 31584267 | C • • • G • • A • • T • T • • • • • • • • • T G G A A T | 5 + 0 | 1.80 |

chr, chromosome; LTR, long terminal repeat; PAM, protospacer adjacent moti

## CONCLUSIONS

- This work extends our previous specificity studies of EBT-101, a CRISPR-based therapeutic candidate entering clinical trials as a potential one-time cure for HIV and extends our validation of HIV excision in humanized mice and non-human primate studies that demonstrate biodistribution, safety, and simian immunodeficiency virus excision in relevant tissues

## References

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## Acknowledgments

- Thanks to the Bioinformatics and Gene editing teams
- Thanks to our collaborators Kamel Khalili, PhD, and Tricia H. Burdo, PhD, at Temple University
- The presentation was developed by Chuck Blajszczak, PhD, of AlphaScientia, LLC, with the support of Excision BioTherapeutics

## Disclosures

TJ Cradick reports commercial (salary and ownership) interest in Excision BioTherapeutics.

**Presented at ASGCT, May 16–18, 2022**