Stealth Editing™ With Peptide Nucleic Acids: A New Class of In Vivo Gene Editors

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neubase

Introduction

Stealth Editors™, a new in vivo gene editing technology, is designed to provide pronounced effects that are safe, non-immunogenic, and broadly applicable

NeuBase believes that the future of in vivo gene editing is Stealth Editing™. Stealth Editing is a new type of gene editing designed to provide pronounced effects that are safe, are non-immunogenic editors that are delivered with nonviral technologies, and that are broadly applicable across different mutation types and species.

Stealth Editors are completely synthetic and do not use bacterial enzymes; they are nuclease-free. This new type of technology selectively tags a locus in the genome using a synthetic oligo that recruits the cell's own machinery to edit the sequence of interest. The technology has been previously validated in animal models via systemic routes of administration to edit multiple disease-causing mutations and is now being developed by NeuBase for the clinic in liver and blood diseases[1-3]. These pronounced in vivo effects have been shown to be safe in preclinical models[1-3]. Two key elements of the safety profile are that of the editing fidelity and the lack of immunogenicity. Fidelity is equal to that of the cellular machinery that performs the edits, we believe well below the measured error rates of the base and prime editors[1-3]. Stealth Editors use a synthetic chemistry known as peptide nucleic acids (PNAs), which do not trigger the innate or acquired immune system and thus avoid preconditioned acquired immune responses upon first dose that patients may have, and we believe enable multiple-dose regimens to ensure durable pharmacology to account for reaching clinical thresholds in editing efficiency and address possible tissue turnover. Delivery of these editors is achieved using nonviral delivery technologies such as lipid nanoparticles, which avoid the safety and immune issues that are well characterized with viral vector-based delivery modalities. We believe these editors can address an estimated >90% of all known mutations in ClinVar as they are PAM-sequence unrestricted and because they can likely edit all mutation types (including transitions, transversions, insertions, and deletions). Taken together, we believe these capabilities make this technology broadly applicable.

NeuBase is focused on its internal programmatic pipeline and on supporting co-development partnerships with other pharmaceutical and agricultural partners in areas of mutual interest.

1. *Nat. Commun.* 2016, 26 (7), 13304; **2.** *Sci. Adv.* 2022, 8, eabo0522; **3.** *Bioeng. Trans. Med.* 2023, 8, e10458

Key Differentiators of Stealth Editors™

Immunogenicity from viral delivery systems and bacterial proteins presents a potential safety issue for patients receiving gene editing therapies

Stealth Editor™, flying under the radar of the immune system to effect high-fidelity gene editing

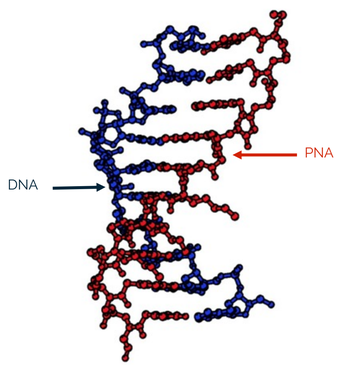
Peptide Nucleic Acid and Oligonucleotide Donor

Peptide Nucleic Acid (PNA) strands **invade** double-stranded DNA in a highly **selective** way to form **stable** PNA–DNA complexes.

These complexes are recognized by **DNA repair machinery** and are excised.

An oligonucleotide donor (ODN) acts as a template for DNA repair to correct a genetic mutation.

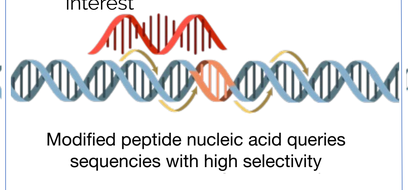
PNA and ODN go **undetected** by the immune system



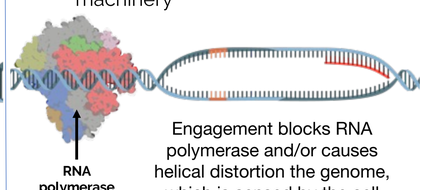
How Stealth Editors™ Work

1. A pathogenic variant in a gene sequence is selected for correction

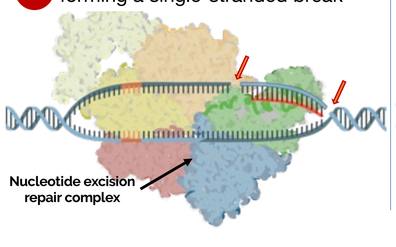
2. A Stealth Editor™ scans the genome to identify the locus of interest



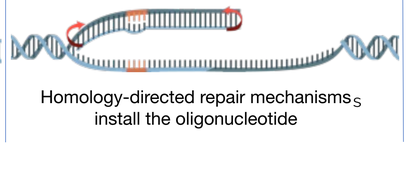
3. The editor engages proximal to the mutation and recruits repair machinery



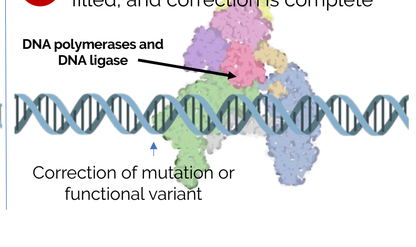
4. The target sequence is excised forming a single-stranded break



5. A donor sequence engages in the flap and installs the donated sequence

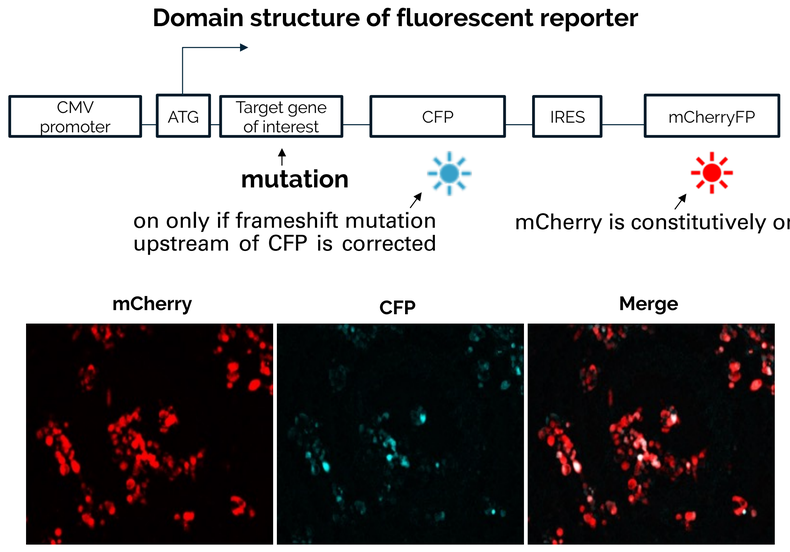


6. The single-stranded break is filled, and correction is complete



Rapid Target to Hit in Human Cells Ex Vivo

Stealth Editors™ are titratable, and editing efficiency is in range for clinical benefit in various conditions and continues to increase with optimization

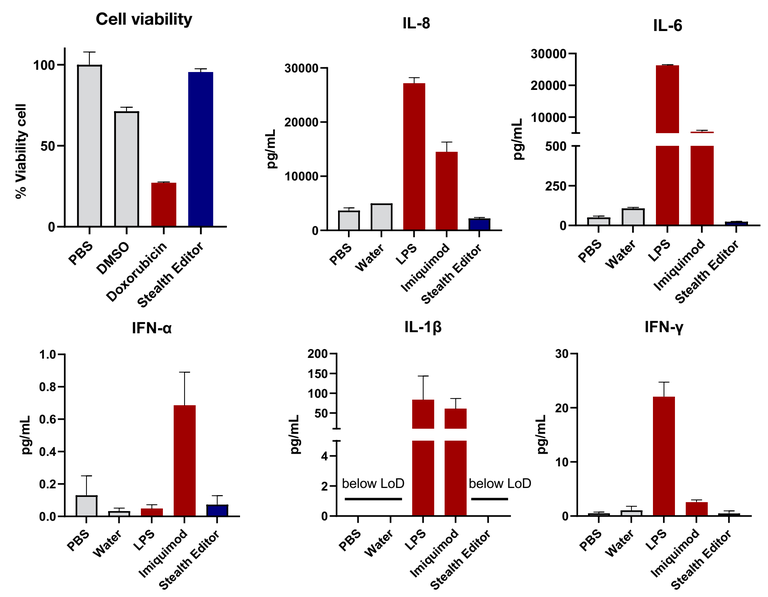


ATG, adenine-thymine-guanine; **CFP,** cyan fluorescent protein; **CMV,** cytomegalovirus; **IRES,** internal ribosome entry site; **mCherry,** monomer cherry; **ODN,** oligonucleotide.

Graph represents the mean ±S D. Statistics were calculated using a one-way ANOVA with a multiple comparison post hoc test to the untreated control. ***, $p < 0.001$; ****, $p < 0.0001$.

Stealth Editors™ Are Non-Immunogenic in Human Peripheral Blood Mononuclear Cells

Stealth Editors™ do not affect cell viability and are non-immunogenic, as measured by a cytokine panel.

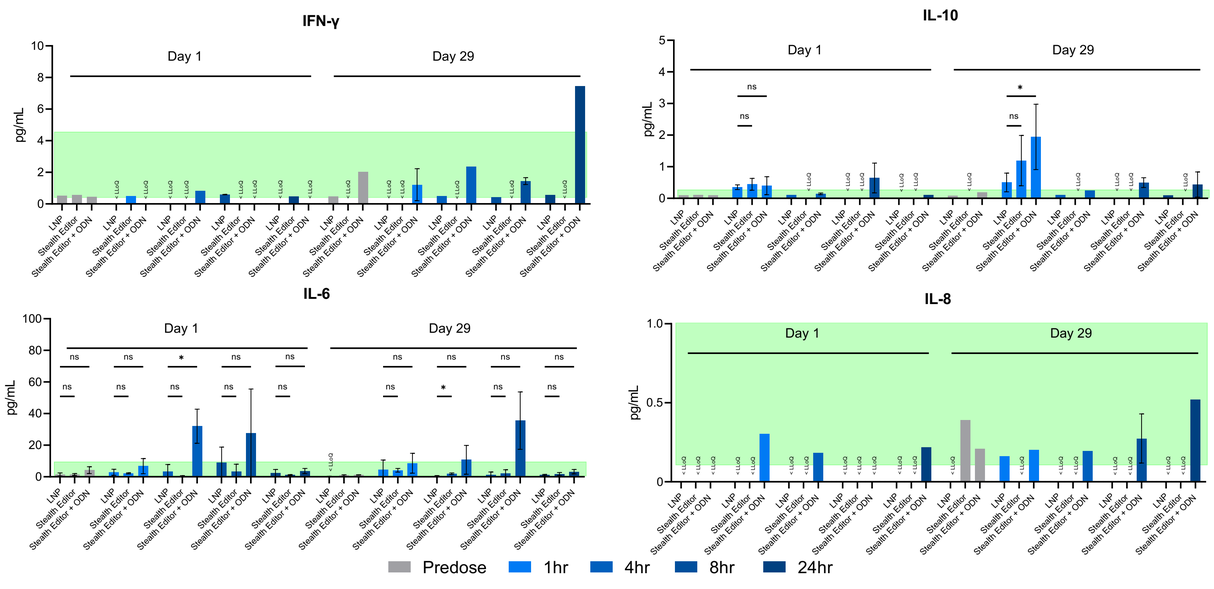


DMSO, dimethylsulfoxide; **IFN,** interferon; IL, interleukin; **LoD,** limit of detection; **LPS,** lipopolysaccharide; **PBS,** phosphate-buffered saline.

Data is representative of a panel of donors.
Immune activator-positive control: LPS = 5 ng/mL or imiquimod = 4 uM.
Stealth Editor = 5 uM.

Stealth Editors™ are Non-Immunogenic in Non-Human Primates

Stealth Editors™ do not elicit an innate nor acquired immune response in non-human primates as measured by a cytokine panel.



Non-human primates (NHP) (n=3 per group) were given an IV administration of MC_3 lipid nanoparticles (LNP), 0.10 mg/kg Stealth Editor encapsulated in LNP, or 0.16 mg/kg Stealth Editor with ODN encapsulated in LNP. At the indicated time points, blood was drawn and serum was assessed for cytokine induction using an MSD multi-spot assay. Green boxed area represents the natural variation in cytokine expression in healthy cynomolgous monkeys (n=30) as determined by Meso Scale Discovery. Similarly, serum levels of IL-2 and IL-1β were measured but no effect was observed upon treatment with any compound. Data are representative of mean ± SD. A two-way ANOVA with multiple comparisons was used to determine statistical significance. *, $p < 0.05$; ns, non-significant

Contact

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