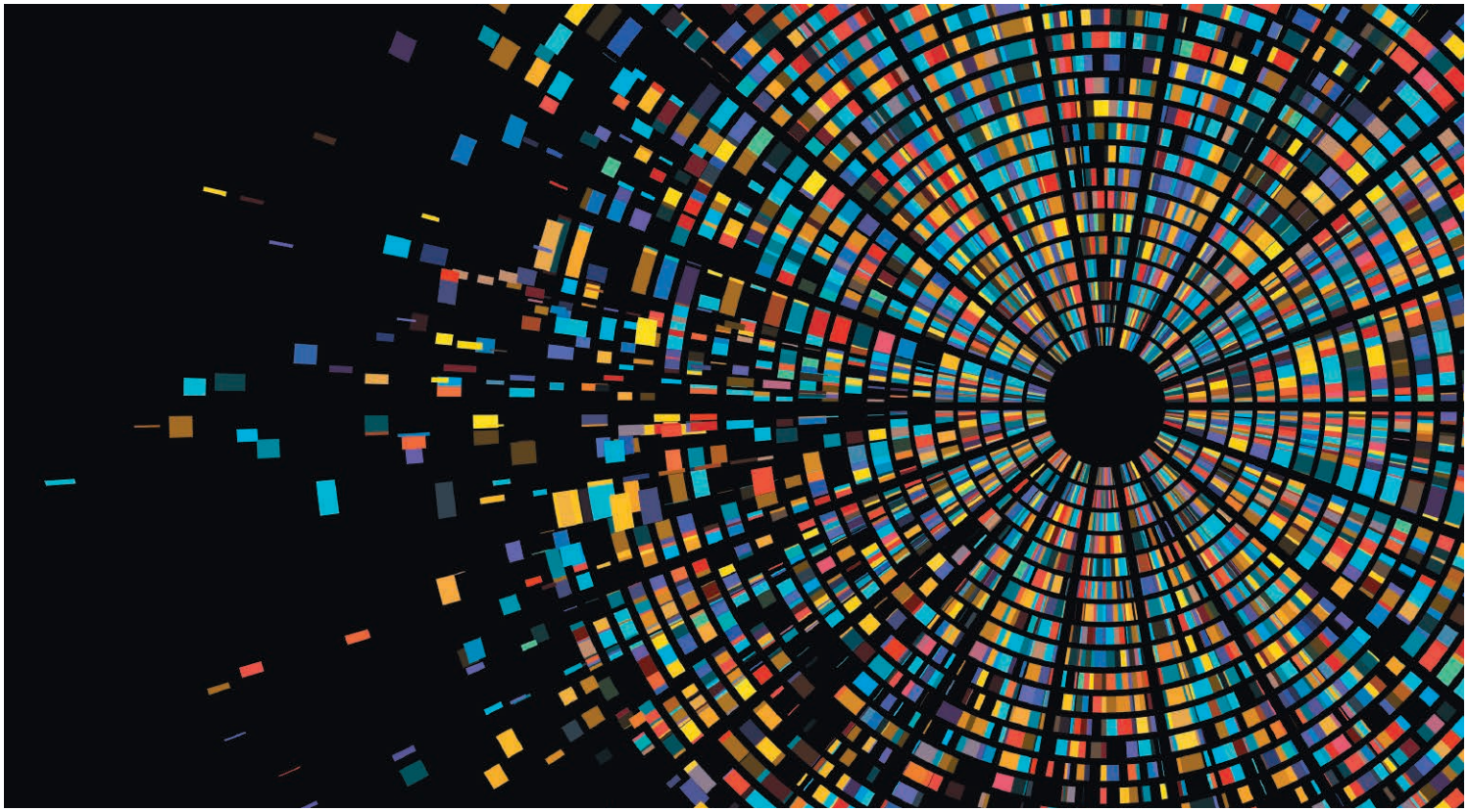

Drugging the Genome with a Novel and Scalable Therapeutic Modality to Address Diseases at the Base Level

By Dietrich A. Stephan, Ph.D.

MOST DISEASES REMAIN undruggable with current therapeutic modalities, leaving many patients with limited options. As many (if not most) diseases have a genetic component, the ability to target the genome to address disease at the base level could become a critical approach for the future of health.[1] Yet, the vast majority of therapeutic approaches focus on targets downstream of the genome, initially proteins and, more recently, RNA transcripts. The complexity associated with drugging proteins, each of which is a unique and often dynamic entity, too often results in an inefficient, time-consuming, and expensive drug development process with low probabilities of success. This years-long, high-risk strategy contributes to high drug pricing necessary to recapture these development costs; furthermore, the high price also makes these drugs unaffordable for the many patients across the globe. Proteins as a target class, in general, introduce complexities and inefficiencies that have resulted in many diseases remaining undruggable.

Among the reasons that targeting genes for therapeutic benefit has lagged is because the »

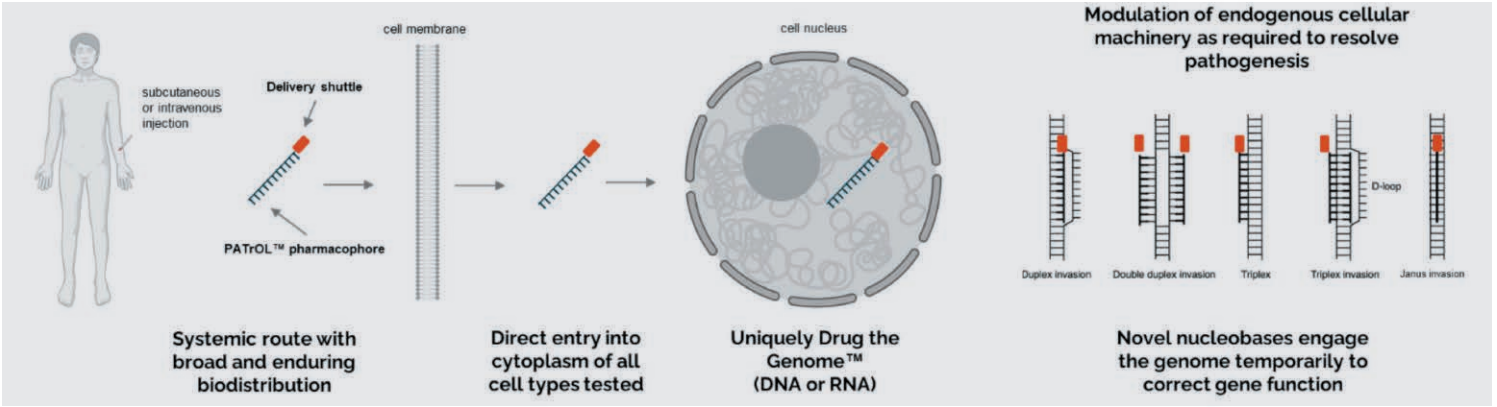

Figure 1: Conceptual overview of the process by which NeuBase compounds enter the cell after systemic administration, engage genes within the double-stranded target and resolve disease causality.

A proprietary delivery shuttle is coupled to the modified peptide-nucleic acid oligonucleobase to allow systemic routes of administration with broad bio-distribution including into the central nervous system. Cellular entry occurs via both direct membrane translocation and endocytosis in a non-cell type or species-specific manner. Compounds diffuse into the nucleus to engage the double-stranded target variety of binding modes which depend on the type of nucleobase that is used in the compound. Gene output or function is effected via steric interactions with cellular machinery such as RNA polymerase and transcription factors. Our gene editing solution utilizes duplex or triplex invasion to recruit endogenous high-fidelity human repair enzymes and does not require delivery of bacterial proteins. These endogenous high-fidelity enzymes have co-evolved with the human genome and do not induce double-stranded DNA breaks. Tropism for double-stranded RNAs can be induced through proprietary backbone modifications..

"Among the reasons that targeting genes for therapeutic benefit has lagged is because the genome has evolved a protective double-stranded structure to prevent insults and corruption of its existential information content. To overcome this hurdle, we have developed a novel therapeutic modality predicated on a synthetic polymer chemistry which can engage double-stranded genes of interest with exquisite sequence selectivity to modulate gene output as appropriate to resolve pathogenesis."

genome has evolved a protective double-stranded structure to prevent insults and corruption of its existential information content.[2] To overcome this hurdle, we have developed a novel therapeutic modality predicated on a synthetic polymer chemistry which can engage double-stranded genes of interest with exquisite sequence selectivity to modulate gene output as appropriate to resolve pathogenesis. For further background, see inset: **History and Summary of Nucleic Acid Therapeutic Modalities**.

PATrOL™ – Neubase's Platform Technology

To address the large gap in the marketplace in addressing disease causality, we have designed, built, and validated PATrOL™, a new platform technology that can uniquely drug the genome to address the three disease-causing mechanisms – gain-of- function, change-of-function, and loss-of-function of a gene – without the limitations of other early precision genetic medicines. Our technology is predicated on synthetic peptide-nucleic acid (PNA) chemistry which has the unique ability to directly engage double-stranded nucleic acids with exquisite sequence-selectivity and requiring no protein machinery to enable this engagement (see **Figure 1**). These PATrOL-enabled PNA compounds operate by temporarily binding the target and, through steric interference, modulate the cellular machinery that interacts with the nucleic acid to correct the pathogenic cascade.

Component of the PATrOL platform

The PATrOL platform is comprised of three main component modules: a neutral-charged synthetic polymer backbone, new nucleobases, and novel delivery shuttles (see **Figure 2**).

1. Neutral – Charged Polymer Backbone

Peptide-nucleic acids (PNA) use an amino ethyl glycine "monomer" unit, linked by peptidyl bonds, allowing for nucleobases to be conjugated to the monomer subunits in a known sequence at an appropriate spacing such that they can engage with an DNA target.[22] *The key differentiator between a PNA backbone and natural or modified backbones (like ASOs) is that they are neutral in*

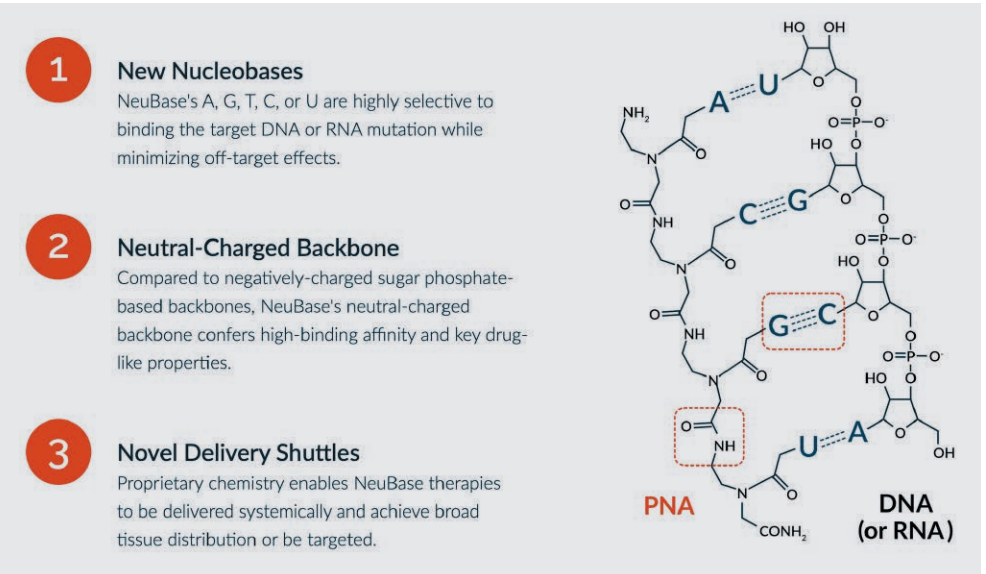

Figure 2: PATrOL: Peptite-Nuclec Acid Antisense Oligonucleobase Platform

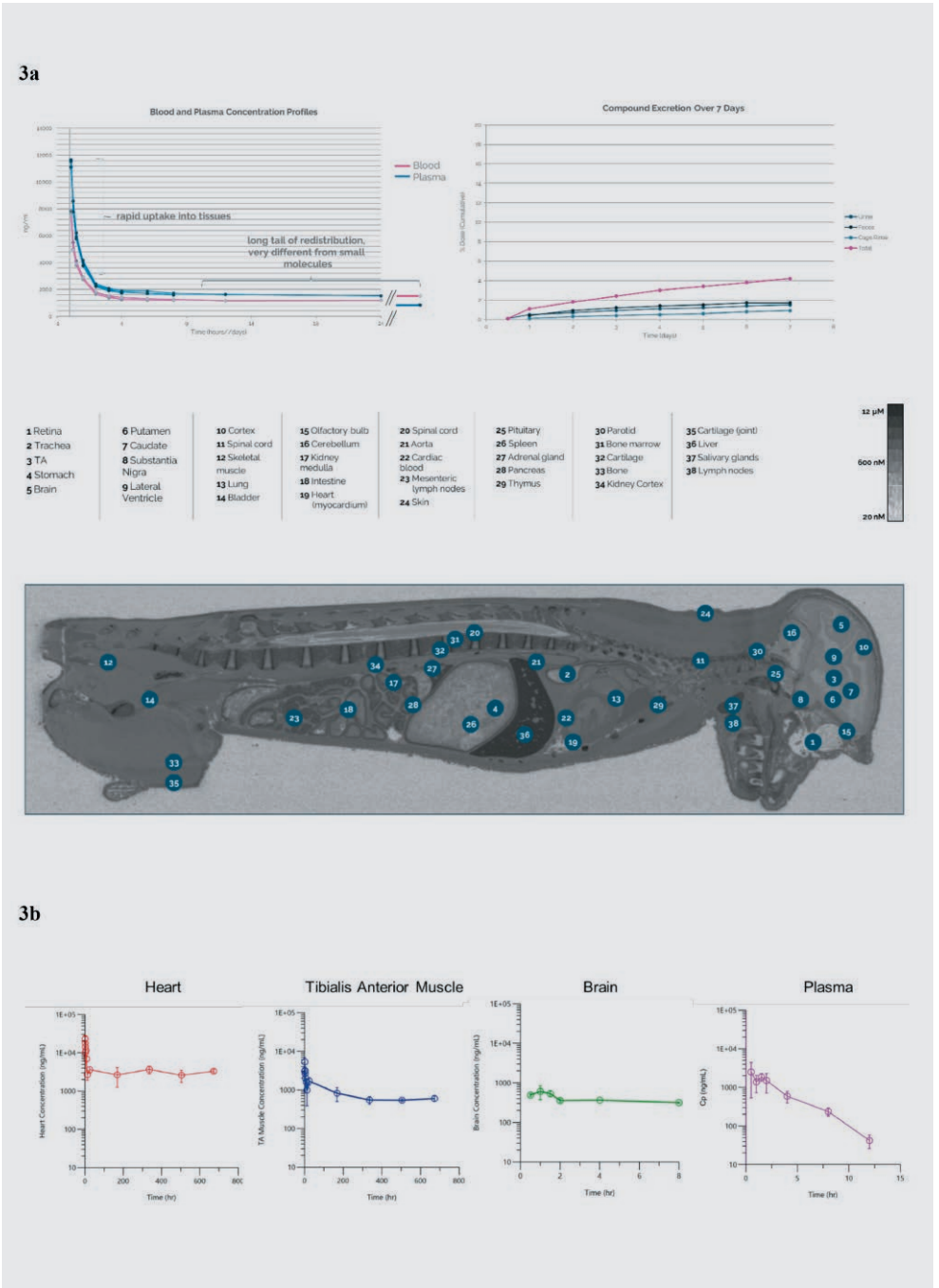

Figure 3:

a. PATrOL – delivery shuttle pharmacokinetics and biodistribution in non – human primates following a 5 mg/kg bolus intravenous administration illustrates rapid uptake from the circulation, broad biodistribution, and durable tissue residence time.

The delivery shuttle, without a pharmacophore payload, was radiolabeled with ^{14}C and injected intravenously into a series of cynomolgus non-human primates after which blood, plasma, urine and feces were collected at a variety of timepoints over the course of 7 days for quantification of the delivery shuttle and animals were sacrificed at 4 hours, 12 hours and 7 days followed by quantitative whole-body autoradiography to ascertain tissue biodistribution.

b. DM1 development candidate (NT – 0231.F; shuttle plus pharmacophore) pharmacokinetics and biodistribution into Balb/C wild-type mice following a 30 mg/kg bolus intravenous administration illustrate similarly rapid uptake from the circulation, broad biodistribution, and slow clearance from tissues.

DM1 NT – 0231.F development candidate was injected into mice at a 30 mg/kg dose demonstrates rapid distribution out of plasma into tissues. This is shown by the rapid uptake of NT-0231.F in Heart, Tibialis Anterior (TA) Muscle, and Brain. NT – 0231.F C_{max} concentrations vary with heart having 23400 ng/mL, TA Muscle having 5333 ng/mL, and Brain having 608 ng/mL. Although NT – 0231.F rapidly cleared the plasma ($T_{1/2}$ 2.0 hours), each tissue displayed an extended elimination phase with NT-0231.F in heart having a half – life of 2294 hrs., TA Muscle having a half – life of 1212 hrs., and brain having a half-life of 31 hrs.

charge (not anionic) and can be functionalized such that they become helical, which contributes to a high binding affinity and sequence selectivity. Helical features allow the PNA compounds to either invade the double stranded target and outcompete the complementary strand or form triplex structures by engaging through the major groove without the need to invade, all without repulsing the target.

Another major differentiator of our backbone technology is sequence selectivity of target engagement imparted by the "rigidity" of the backbone and conferring an intolerance for sequence mismatches. These rigidity manifests in an advantage to better differentiate mutant gene sequences from their wild-type counterparts, and in potentially reducing off-target engagements with highly similar sequences elsewhere in the genome. PNAs exhibit high stability in biological fluids and are resistant to protease and nuclease digestion, contributing to their enduring presence in tissues after systemic administration prior to renal excretion.[22,23] PNA backbones have been described in the literature to be "immunologically inert," eliminating a key issue of other precision genetic medicines, and thus potentially allowing for routine dosing.[24]

"Optimizations of the natural nucleobases have been made to strengthen or weaken certain positions of engagement with the target to further increase selectivity, and to reduce self-complementary binding."

2. New Nucleobases

A second differentiator is that Neubase has invented and assembled a kit of nucleobases which can be arranged in a specific order onto the polymer backbone so that they engage a genetic target. Natural nucleobases are often used as their properties are well understood and they provide for standard Watson-Crick hydrogen binding between complementary nucleobases. Optimizations of the natural nucleobases have been made to strengthen or weaken certain positions of engagement with the target to further increase selectivity, and to reduce self-complementary binding.

A second class of nucleobases are Hoogsteen nucleobases which allow the PATrOL compound to scan the outside of the double helix by querying nucleobase content through the major groove.[25] When sequence complementarity is found a triplex structure is formed. The kinetics of this »

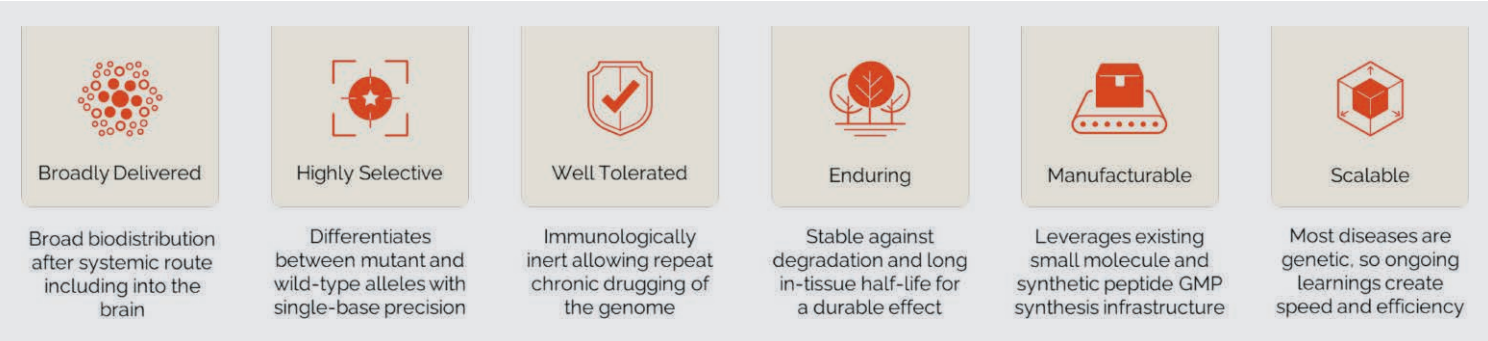

Figure 4: Overcoming the limitations of early genetic medicine technologies.

Delivery. Most early precision genetic medicine technologies are large and heavily negatively charged, making it difficult for them to broadly distribute throughout the body to address tissues that are affected by many diseases. This often requires them to be locally injected such as into the brain, likely limiting their ability for broad – based impact. We have designed and developed a proprietary delivery technology that allows our small, neutral – charge and water – soluble compounds to be administered using a patient-friendly route such as subcutaneous injection and achieve broad biodistribution, including into the deep brain and nuclei of cells. **Tolerability**. Most early precision genetic medicine technologies trigger the innate and/or acquired immune system, limiting their ability to achieve pharmacologic doses or to be used repeatedly. For example, delivery of negatively charged nucleic acid therapies often trigger the innate immune system and delivery of proteins often trigger the acquired immune system. Our technology is comprised of fully synthetic compounds that have been shown to be "immunologically inert", potentially allowing them to be administered chronically to temporarily drug the genome over a patient's lifetime. **Selectivity**. Many technologies in the early precision genetic medicines industry cannot discriminate between mutant gene sequences and their wild-type counterparts, nor between other highly similar target sequences in the cell. This potentially limits these technologies in their ability to address small disease-causing mutations such as single nucleotide changes, which account for a large fraction of disease-causing mutations and functional variants. Our technology can discriminate point mutations, which increases the opportunity space. This capability comes from the "rigid" nature of the backbone which does not tolerate imperfect target engagement. In addition, this single-base selectivity reduces the likelihood that our compounds will engage with genes elsewhere in the genome that are similar but not identical, potentially reducing any adverse events triggered by off-target engagement. **Manufacturability**. Many technologies in the early precision genetic medicines industry require significant investments in custom manufacturing infrastructure, and thus are limited in their potential impact and scalability. Our technology utilizes established and fully commoditized manufacturing processes, both for small molecule and synthetic peptide synthesis (the combination of which are required to manufacture our compounds) that are available with high redundancy and at commercial scale. **Durability**. Many technologies in the early precision genetic medicines industry can only be dosed a single time, are often cleared by the immune system, or are otherwise not durable in their efficacy. We have shown long in tissue residence time and associated pharmacology, promising an infrequent dosing regimen in the clinic. **Scalability**. Many technologies in the early precision genetic medicines industry are not truly scalable across a variety of indications, for the reasons described above. As our goal is to provide solutions to those suffering from a wide variety of diseases across the globe, we have purpose-built a scalable platform. We address a single target type for all therapeutic programs (the genome), utilize the same delivery shuttle enabling similar pharmacokinetics (PK), absorption distribution metabolism and excretion (ADME), dose, route and regimens across programs, utilize predominantly the same chemistry yielding similar therapeutic indices, are able to predict OTEs *a priori* using bioinformatics and engineer around them before beginning development, and leverage manufacturing process development across programs such that ongoing platform learnings have already created increasing speed and efficiency.

binding mode are more rapid than invasion and we overcome the early sequence restrictions with modified Hoogsteen nucleobases.

A third class of nucleobases are termed Janus nucleobases. Janus bases are bi-specific nucleobases that can be considered natural nucleobases with a second binding face chemically engineered onto them.[26] Janus bases can be used to invade a target and binding both the Watson and the Crick strands simultaneously which increases the stability of the complex and in certain situations boost selectivity for the target allele.

Importantly, all these nucleobases, which allow sequence-specific recognition of a gene within a target double-stranded nucleic acid, are required to be used in combination with our neutral-charged backbone. Without the backbone, the nucleobases do not have the ability to engage utilizing these various binding modes.

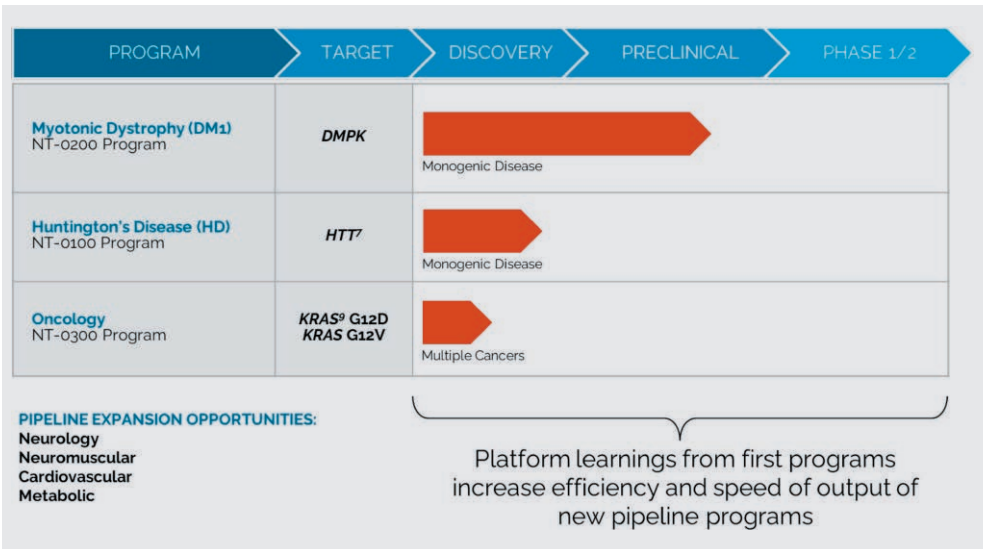

Figure 5: The currently disclosed NeuBase therapeutic pipeline in rare disease and oncology.

The modular design and platform learnings contribute to ongoing increases in efficiency and speed across additional undisclosed programs, with the goal being eventual "plug and play" capabilities for increasingly rare and ultimately private mutations.

3. Novel Delivery Shuttles

The third component of our modular platform is flexibility to couple the pharmacologic payload to a variety of delivery shuttles. This flexibility comes from the biophysical characteristics of the pharmacophore – small molecular weight, neutral charge and water solubility – that do not dominate the pharmacokinetics (PK) and biodistribution of the active pharmaceutical ingredient.

Neubase's proprietary delivery shuttle enables broad biodistribution after systemic routes of administration. The shuttle, conjugated to the

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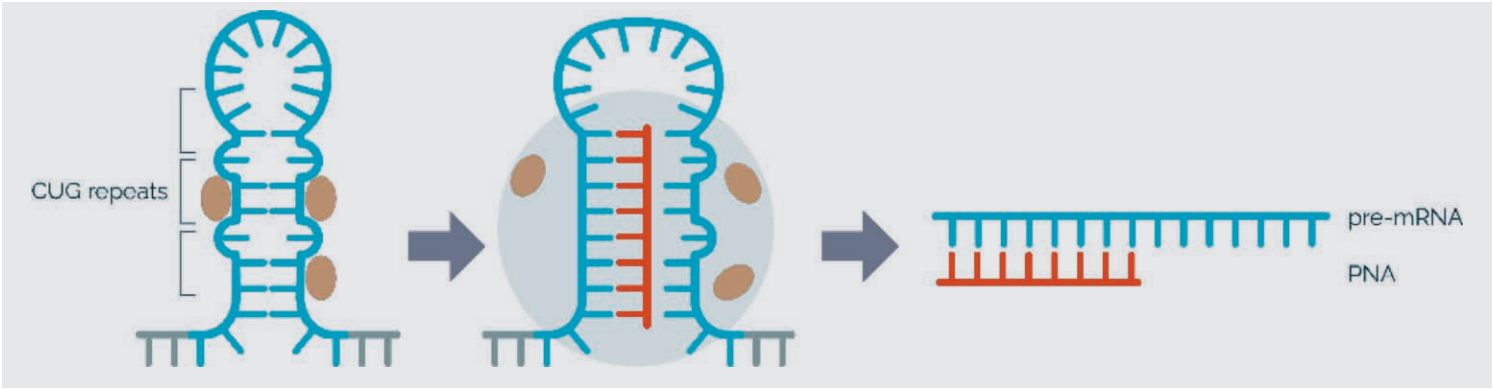


Figure 6: Targeting the toxic double stranded *DMPK* RNA secondary structure in the nucleus to sterically displace sequestered splice proteins to resolve the disease.

Our compounds are designed to drug the double stranded genome itself, but there are interesting use-cases of double-stranded RNA targets that can be targeted to resolve disease using our platform technology. In the case of myotonic dystrophy, a trinucleotide repeat expansion in the 3' UTR of the transcripts forms an imperfect hairpin secondary structure in the nucleus (blue) that inappropriately sequesters splice effectors (brown), resulting in a broad-based spliceopathy in patients. The DM1 development candidate named NT-0231.F (red) invades the double stranded structure, linearizes the hairpin and sterically displaced the sequestered splice effectors to resolve the pathogenic event.

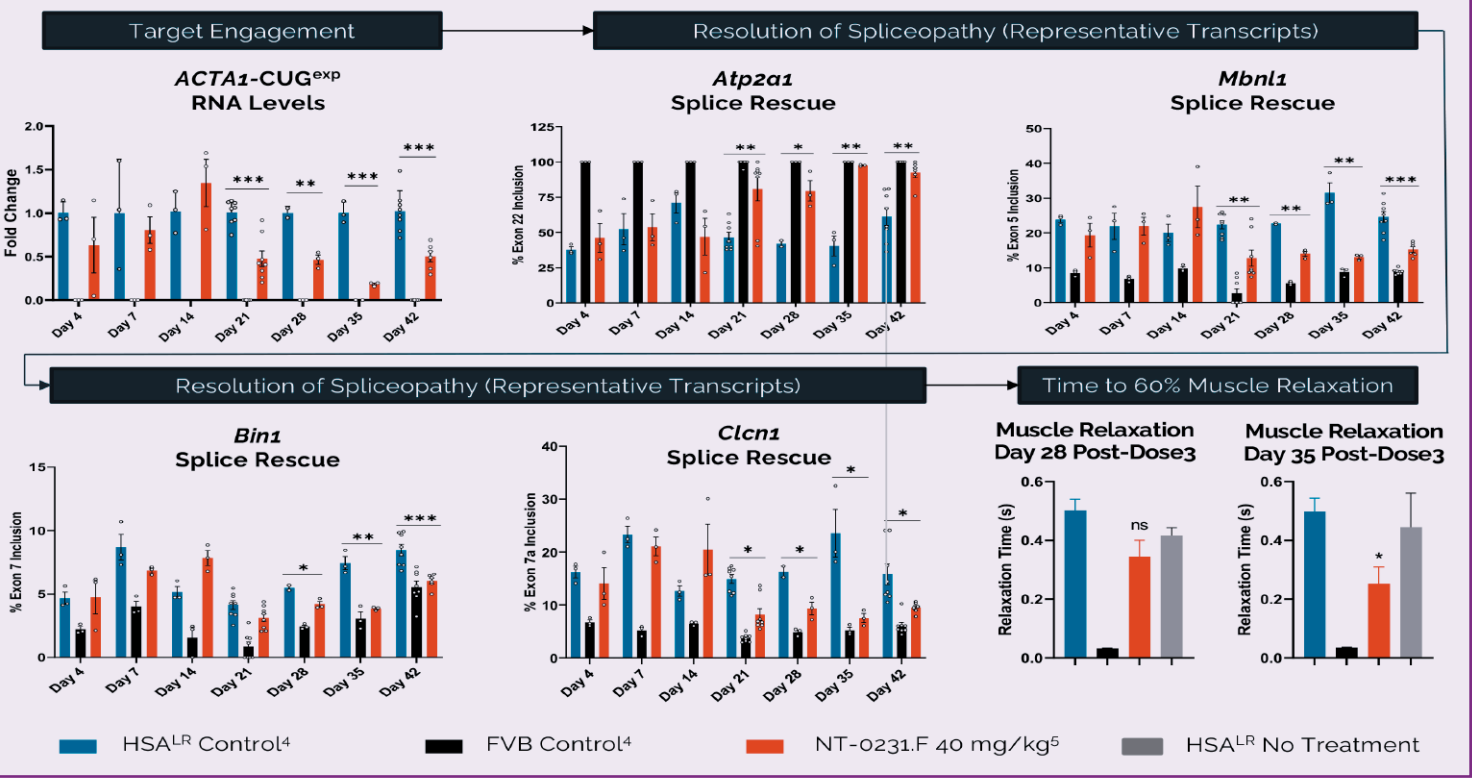
PATrOL-enabled PNA compound via a peptidyl bond, is also a modified PNA, but without nucleobases on the monomer subunits, and functionalized at specific positions in the backbone.

The delivery shuttle enables the PNA compounds to enter cells via endocytosis and also, importantly, through direct membrane translocation, then diffuse into the nucleus to engage the genetic target. We can drive tropism to various cell types by adding additional short peptide tags to the constructs.

PK and biodistribution of the delivery shuttle

Case Study: Adressing Double-Stranded Targets

This case study illustrates the ability of our DM1 development candidate NT-0231.F to be administered intravenously (IV) in the HSA^{LR} transgenic animal model of the disease, reach skeletal muscle groups, cross the myofiber membranes, enter the nuclei, invade and engage the CUG repeat in the double-stranded nucleic acid human skeletal muscle actin transgene (*ACTA1*), displace sequestered splice effectors (Mbnl1) to rescue correct exon use of a variety of transcripts which include complete correction of the skeletal muscle chloride channel splicing allowing functional channel protein to be produced resulting in functional recovery of the myotonic phenotype as measured by muscle relaxation. 40 mg/kg (oligo mass) of NT-0231.F was administered IV to HSA^{LR} mice (n=8/time group). Plantar-flexor heel press measurements we performed prior to sacrifice to measure muscle relaxation after maximal electrically induced contraction, and tibialis anterior muscles were collected at 7, 14, 21, 28, 35, 42 days post – dose to assay for *ACTA1* target engagement. Splice rescue and functional rescue of the phenotype.



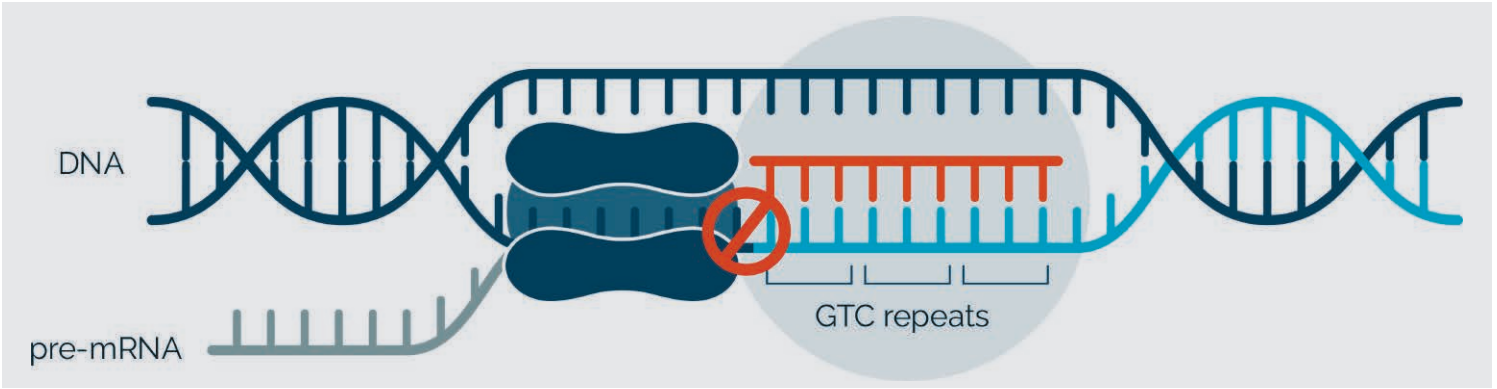


Figure 7: Targeting the double stranded *HTT* gene at the genomic level to inhibit transcription of the mutant allele in an allele-selective manner by blocking RNA polymerase.

Invasion by the compound (red) of double stranded locus likely occurs as the gene is being transcribed, followed by ultra-high affinity engagement to the expansion in exon 1 of HTT that then stably displaces the complementary strand of DNA. The stable duplex formed on the transcribed strand blocks the transcriptional complex from elongating the mutant mRNA (grey) and eliminates production of the toxic disease-causing protein.

alone in non-human primates (NHPs) after systemic administration via an intravenous route illustrates an in-circulation half-life of approximately 1.5 hours during which time the shuttle is taken up into every tissue in the body examined, including into the brain within 4 hours post single-dose (see **Figure 3a**). Thereafter, we see re-distribution across the body characterized by a plateau of circulating PATrOL-compound over long periods of time; for example, we see PATrOL-compound levels in the CNS approximately doubling over the course of 7 days. Elimination is primarily through the renal system and occurs slowly – we observe approximately 4% of the administered dose to be excreted over the course of 7 days after a single-dose injection. The long period of tissue elimination likely contributes to the durability of pharmacologic effect that we observe. This similar PK and biodistribution has been seen with one of our development compounds in wild-type mice as well (see **Figure 3b**). This delivery shuttle is currently being used for several of our therapeutic programs and allows us to anticipate the pharmacokinetics and biodistribution of the therapeutic agent in new pipeline programs for accelerated development.

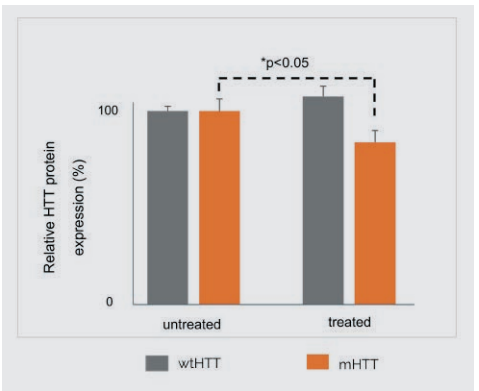


Figure 8: Subcutaneous administration of a PATrOL-compound that targets the trinucleotide expansion within exon 1 within the gene at the genome level, illustrating CNS penetration, target engagement, and statistically significant allele-selective reduction of mutant Huntingtin protein in the brain.

Twice weekly subcutaneous injections of a compound that is designed to invade the double-stranded genome and engage with the CAG repeat expansion in exon 1 were administered into the zQ175 transgenic animal model of Huntington's disease. At 7 days after first injection, whole brains were homogenized and western blotting used to quantify the levels of both mutant and wild-type Huntingtin protein.

"The components of the platform, and their biophysical characteristics have been proactively engineered to improve upon the limitations of early genetic medicines, while maintaining the unique ability to drug double-stranded targets such as the human genome."

The components of the platform, and their biophysical characteristics have been proactively engineered to improve upon the limitations of early genetic medicines, while maintaining the unique ability to drug double-stranded targets such as the human genome. **Figure 4** illustrates these engineering advances.

The Therapeutic Pipeline

We are initially developing precision genome medicines for two therapeutic needs: first, we are targeting rare, monogenic diseases, for which there are no approved therapies; second, we are developing treatments for more common genetic disorders (including cancers) that are resistant to current approaches. Our pipeline includes therapeutic candidates for the treatment of myotonic dystrophy, type 1 (DM1), Huntington's disease (HD), and cancer – driving point mutations in *KRAS*, G12V and G12D, which are involved in many tumor types and have historically been undruggable (**Figure 5**). We have recently finalized an analysis of the entire known mutational database and selected several additional high-value indications for screening and development.

Myotonic Dystrophy Type 1 (DM1)

DM1 is a multi – system progressive disorder characterized by weakness of smooth and skeletal muscles that can range from mild to

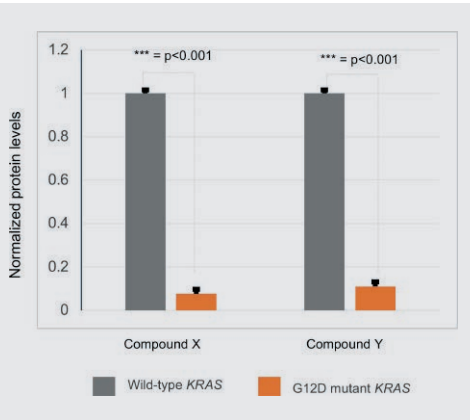
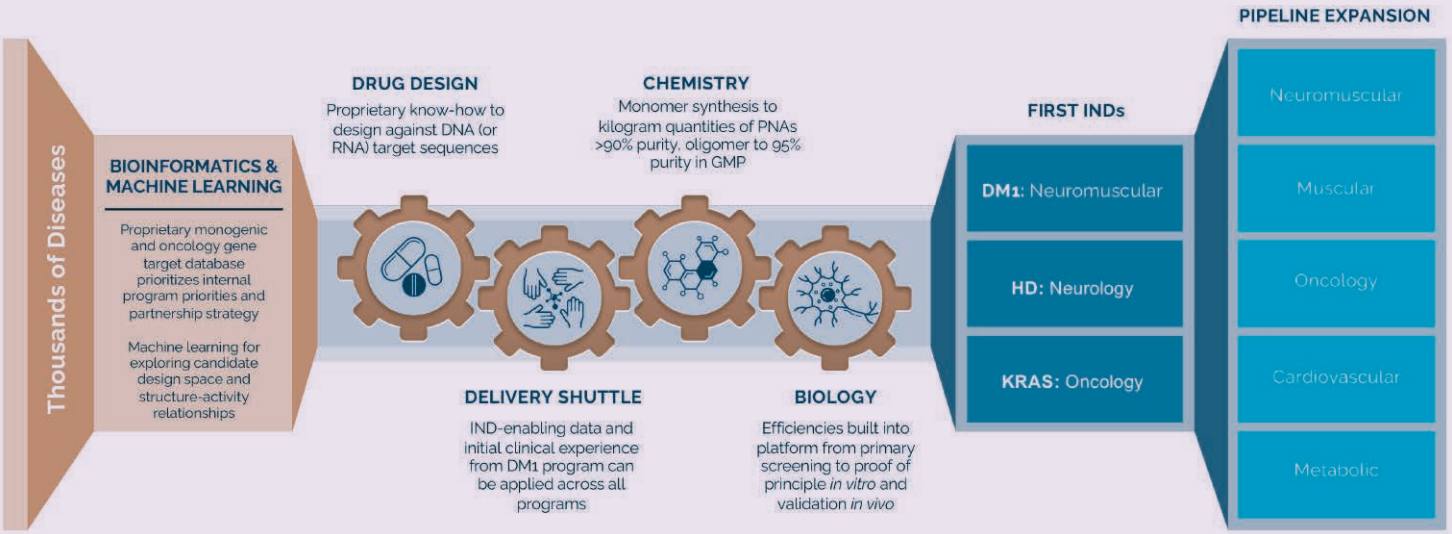


Figure 9: PATrOL-compounds targeting the activating point mutations G12D in KRAS exon 1 gene illustrate significant allele-selective inhibition of transcription and subsequent protein production.

Several PATrOL-compounds were designed to invade the *KRAS* gene and engage with single-nucleotide selectivity to the activated mutant allele (orange) versus the wild type allele (grey) in *in vitro* transcription and translation assays.

Corporate Case Study

This case study illustrates the discovery platform that has been established at NeuBase. Infrastructure to select targets with high unmet need that are not amenable to current therapeutic strategies, followed by computation design, manufacturing, conjugation to delivery shuttles, screening, GLP and GMP manufacturing, *in vivo* pharmacology and IND-enabling work have been established to support scalable output.



severe, myotonia, cardiac conduction defects, cognitive deficits, respiratory distress, and shortened lifespan.[27-28] It is estimated that the global prevalence of DM1 is approximately 1 in 8,000 individuals and is the most common neuromuscular disease.[29] Current treatment options include therapies to manage the symptoms, physical therapy, and assistive devices. No treatments that slow or stop the progression of DM1 are currently FDA approved.

DM1 is caused by a single inherited copy of a trinucleotide repeat expansion in the 3' untranslated region of the *DMPK* gene. Repeat length exceeding 34 repeats is abnormal and often patients have hundreds or thousands of repeat units. When transcribed into RNA, the expanded trinucleotide repeat creates a double-stranded hairpin structure that is toxic in that it sequesters critical splice effector proteins making them unavailable for normal developmentally appropriate splice regulation in the nucleus of affected cells. The resultant altered protein isoforms result in DM1's numerous symptoms.[27-28]

Our mechanism of action is to invade the double – stranded nucleic acid hairpin structure in the nucleus and release sequestered splice factors via steric displacement to restore normal splicing, without explicitly degrading the target. This is a departure from our focus on drugging the genome itself, and is our only program to do so, but is an excellent use-case for our ability to invade double-stranded nucleic acid targets of any type and sterically displace proteins that engage with these targets (see inset: **Case Study: Adressing Double-Stranded Targets**). **Figure 6** illustrates the mechanism of action of our PNA compound that targets double-stranded RNA in the nucleus of DM1 affected cells.

We see this therapeutic solution as having the potential to offer patients a differentiated option in the marketplace. Our approach would provide a whole – body solution to address brain pathologies (in addition to skeletal, cardiac and smooth muscle pathologies), and this approach is designed to maintain the target transcript, as opposed to other strategies that are designed to degrading it and possibly trigger secondary adverse events. We see this solution as not only important for long-term

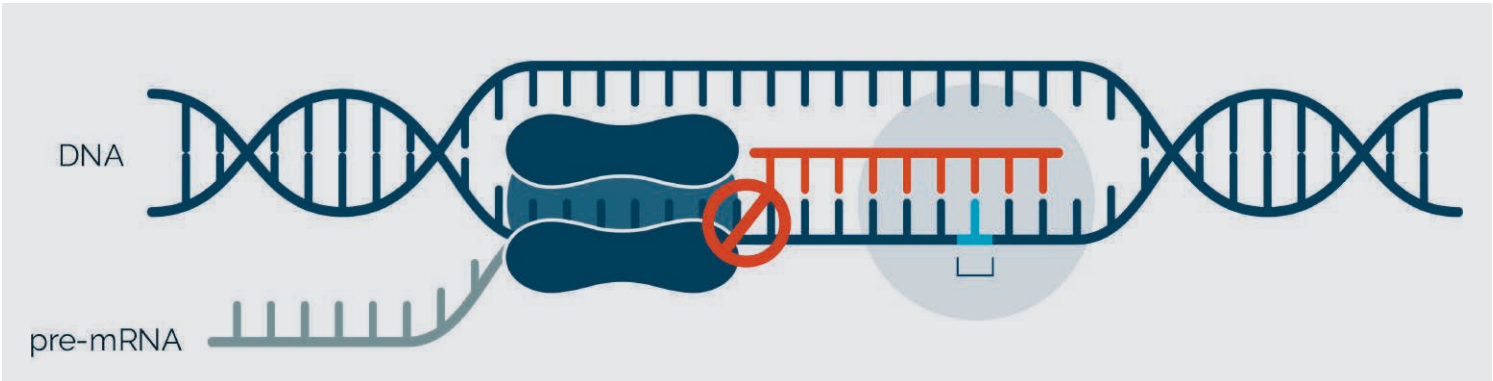


Figure 10: Targeting the double stranded *KRAS* gene with single-base selectivity at the genomic level to inhibit transcription of the mutant allele by blocking RNA polymerase.

Invasion by the compound (red) of double stranded locus likely occurs as the gene is being transcribed, followed by ultra-high affinity engagement to the mutant allele by discriminating the activating point mutation and stable displacement the complementary strand of DNA. The stable duplex formed on the transcribed strand blocks the transcriptional complex from elongating the mutant mRNA (grey) and eliminates production of the cancer-driving protein.

patient health, but also allows for a patient-friendly systemic route of administration.

Huntington's Disease (HD)

HD is a fatal neurodegenerative disease characterized by neuronal death in deep brain structures culminating in progressive impairments in movement and cognitive control followed by death. Current treatment options attempt to lessen involuntary movement and psychiatric symptoms, but disease-modifying treatments have yet to successfully advance through clinical trials.

HD is a dominantly inherited monogenic disease caused by a single inherited copy of a trinucleotide repeat expansion in the coding region of the Huntingtin gene (*HTT*). In the U.S. alone, there are likely more than 40,000 symptomatic patients and likely more than 200,000 at-risk of inheriting the disease.[30] The wild-type *HTT* gene has a region in which a three-base CAG is repeated.[31] When the CAG is 26 or fewer repeat units, the resulting protein behaves normally. When the CAG is repeated 40 times or more in this region, the resulting protein becomes toxic.[32,33] Current therapies for patients with HD can only manage individual symptoms. There is no approved therapy that has been shown to delay or halt disease progression.

> *"HD is a fatal neurodegenerative disease characterized by neuronal death in deep brain structures culminating in progressive impairments in movement and cognitive control followed by death."*

We are advancing drug candidates that invade the gene and selectively bind the mutant repeat expansion in exon 1 of the *HTT* gene to inhibit RNA polymerase from transcribing the gene and thus prevent production of the toxic protein (see **Figure 7**). We hypothesize it is important to maintain healthy wild-type HTT protein as an important contributor to normal function.

We have demonstrated the ability of our PATrOL-compounds to be administered subcutaneously, cross the blood-brain barrier, enter the parenchymal space, penetrate neuronal cell bodies and act in the nucleus to reduce the production of toxic protein while maintaining production of normal HTT protein (see **Figure 8**). We have shown that this systemic route of administration allows even exposures of the PATrOL compound across brain regions. We propose this is a superior approach over intrathecal injection which achieves high concentrations at the surface of the brain (cortex) which may become toxic while attempting to achieve therapeutic concentrations in the deep

History and Summary of Nucleic Acid Therapeutic Modalities

As a result of the publication of the genome sequence, identification of causal variants, establishment of variant databases, and global molecular diagnostics infrastructure to allow patients to access their genetic information, the field now has the information and infrastructure to be able to target nucleic acids.[3,4] Yet, technology to drug double-stranded structure of the genome has lagged. Thus, most precision genetic medicines today focus on drugging single-stranded RNA. RNA medicines generally fall into three categories: silencing, replacement and editing. No single technology can perform all three functions, and thus the nascent field remains fragmented. RNA silencing degrades the target transcript most often using antisense oligonucleotides (ASOs) or RNA interference (siRNA).[5,6] In these silencing strategies, a complementary sequence is used to identify single stranded RNA sequences of interest and induce degradation via mechanisms such as recruiting RNAseH to cleave the target RNA or by harnessing RISC-Argonaut complexes to do the same. Changing the function of an RNA can be done using an ASO to interfere with the spliceosome and induce exon skipping or via emerging RNA editing technologies that use ADAR.[7,8] Exogenous RNA can be introduced into a cell and leverage the endogenous translational machinery to express the protein of interest.[9] These RNA therapies, while limited to accessible single – stranded targets, have transformed outcomes for a handful of genetic and infectious diseases and proven that precision genetic medicines can resolve disease upstream of proteins.

There is complexity in addressing disease at the RNA level. Genes in the genome are transcribed into RNA molecules, which are processed by splicing events to remove introns and often combine various exons into mature mRNA to form a diversity of molecules from each gene.[10] In addition, the rate of transcription of each gene in each cell type and at each time in development and under different physiologic conditions can vary and often creates hundreds to thousands of target molecules.[11-13] In general, the larger size and negatively charged backbones of first-generation genetic medicines therapies have posed delivery challenges.[14] For example, the scavenger receptors in the liver clear these systemically administered circulating negatively charged PNA compounds, and tolerability issues such as immunogenicity can be seen with current RNA therapeutics due to innate immune responses such as complement activation and TLR9 receptor activation.[15] First generation genetic medicines have exhibited selectivity challenges as they are often unable to adequately discriminate between small mutations and normal gene sequences due to the backbone chemistry which can tolerates mismatches in localizing to a target sequence, thus resulting in less allele selective discrimination.[16] While delivery modalities of first generation genetic medicines continue to improve with, for example, antibody- or antibody fragment-mediated delivery, they also present new manufacturing complexities.[17] Replacement technologies also continue to improve, promising the potential to eliminate the need to encapsulate transcripts to protect them from degradation *in vivo*, such as by delivering circularized mRNA that are likely less accessible to nuclease digestion, but tissue targeting challenges, immunogenicity and liver clearance issues will likely persist.[18]

Medicines that target DNA are being developed in the community, with gene replacement and gene editing being the primary strategies. These approaches are meant to be permanent, which on the one hand would allow patients to live disease-free lives, but on the other hand pose challenges related to the level of rigor necessary to ensure mistakes do not occur. Gene replacement is generally currently done using viral vector-delivered gene therapies and does not entail drugging the duplex genome but rather delivering an exogenous sequence into a cell of interest and is generally constrained to addressing loss-of-function disorders. Gene therapy can generally only be administered because patients can generate an immune response against the viral delivery vectors.[19] Non-viral delivery technologies are improving, which may allow future DNA replacement strategies to be repeatedly delivered in the future and obviate concerns related to safety and durability.[20] Gene editing technologies have also recently shown dramatic human in vivo clinical activity and early tolerability and durability.[21] Issues related to delivery past the liver, off-target editing, proximal base deamination, and induction of double-stranded breaks warrant caution in developing gene editing solutions.[22] Most current gene editing strategies utilize modified bacterial enzymes which are likely antigenic and thus may obviate the ability to repeatedly dose to achieve therapeutic levels of editing in the tissues of interest. In short, gene therapy and gene editing, while promising and improving, have limitations related to delivery of high molecular weight molecules, accessing tissues at the sites of pathology, selectivity, durability, tolerability, manufacturability, and scalability.

brain (striatum) where HD neuropathology first manifests.

The potential differentiators of our HD program are a whole-body solution to address brain and body pathologies, allele-selective knock-down of the toxic protein, and a patient-friendly systemic route such as subcutaneous administration.

Oncology (KRAS G12D and G12V)

Our oncology program targets KRAS G12D and G12V gene mutations, which are the two most common and historically "undruggable" *KRAS* driver mutations. There are no approved therapies for *KRAS* G12D or G12V mutations, which account for approximately 55% of all KRAS mutations.[34,35]

We have designed PATrOL-enabled compunds to selectively engage with the mutant cancer driver mutation at the DNA level to inhibit downstream mutant KRAS protein production and reduce hyperactive mitotic signaling. We have achieved allele-selective engagement of the mutant allele with single-nucleotide precision (see **Figure 9**). **Figure 10** illustrates how the PNA compounds access the *KRAS* mutations in codon 12 at the genomic locus.

The potential advantages of our *KRAS* inhibitors include: a whole-body solution to address primary tumors and metastases across tumor types, maintenance of wild-type KRAS protein which is likely important for health, and a patient-friendly systemic route such as intravenous or subcutaneous administration.

"Finally, rather than temporarily increasing or decreasing a gene's output, we also have the ability using our proprietary technology to permanently edit a mutation out of a gene with exquisite fidelity without the use of base editors or primer editors."

Loss of function strategies

In addition to the ability to address toxic gain-of-function mutations at the genomic level with our platform technology, we are also able to address loss-of-function mutations with our platform. For example, we have internally shown that we are able to increase gene output significantly by targeting the non-transcribed strand proximal to a promotor to facilitate transcriptional elongation which becomes a strategy for diseases caused by haploinsufficiency or which generally require increased gene output.

Permanent genome editing for mutation deletion

Finally, rather than temporarily increasing or decreasing a gene's output, we also have the ability using our proprietary technology to permanently edit a mutation out of a gene with exquisite fidelity without the use of base editors or primer editors.[25,36,37] Our "triplex editing" is achieved by targeting a strand of the genome pharmacophore proximal to a mutation of interest that acts to recruit endogenous high-fidelity repair machinery to the locus. Concurrently, a donor DNA oligonucleotide is introduced into the same cell that is complementary to sequence containing the mutation, but which includes the corrected base and facilitates homology directed repair.

We are advancing on this strategy to include only a single invader molecule that can not only recruit base excision machinery but concurrently prime the repair. The advantage over base or primer editors is primarily related to fidelity of repair. We utilize multiple layers of selectivity to target a locus and recruit human repair machinery which has evolved over millennia to work with the human genome as opposed to engineered bacterial repair enzymes. No toxic double-stranded breaks are induced and there is not proximal deamination at positions around the editing position.

Dietrich A. Stephan, Ph.D.

DNA Therapeutics Expert Driving the Development of a New Class of Precision Genetic Medicines to Address Rare and Common Diseases, Redefining Medicine for Millions of Patients

Dr. Dietrich Stephan has built a reputation for transforming medicine with an extensive background in applied genomics research and commercialization. As a leading expert and one of the early pioneers in molecular diagnostics and precision genetic medicines, Stephan works to transform the treatment landscape with cutting-edge solutions. He is now focused on "drugging the genome" with the potential to address and cure a wide range of both rare and common diseases.

Stephan has pioneered innovation in drug development by:

- **Identifying, Developing, and Delivering Revolutionary Solutions to Cure Disease** – Stephan consistently sees novel scientific breakthroughs via his deep academic network, and has a unique ability to suspend disbelief and evaluate the market potential with the expertise and insight to project probabilities of success. Stephan has founded and run high-throughput genome institutes across the country; pioneered direct-to-consumer genomic testing at Navigenics; holds the first patent on sequencing cancer genomes and assigning targeted therapies; and has moved quickly into first-in-class therapeutic opportunities to build industry leading companies including microbiome-modulating therapies at Pendulum and peptide therapies via deep machine learning at Peptilogics.
- **Structuring Organizations for Scalable and Sustainable Growth** – With a laser focus on introducing therapies that address the needs of people who have run out of options, Stephan navigates and steers businesses around complex challenges. As a founder or co – founder of many companies, he structures organizations to minimize the cost of capital and maximize alignment between boards and investors, ensuring the first priority of improving patient outcomes is achieved.
- **Cultivating Cultures that Attract the Industry's Best Talent** – Stephan builds high-performing, fully engaged, mission – driven teams by enabling and empowering the leading experts in biotech to focus on their work. He sets clear objectives, clears the path of obstacles, and creates a great place to work where people from diverse backgrounds are valued for the different viewpoints they bring.

In December 2018, Stephan founded NeuBase Therapeutics, where he currently serves as the company's CEO and Chairman. He recruited exceptional talent to stand up and validate a new platform for its capability to address genetic diseases. He and his team are working to develop a pipeline of first-in-class ultra – precision genetic medicines to "drug the genome", targeting diseases for which there are no approved therapies. The first patient is projected to be dosed in 2023 with a modality never administered in humans before.

Beyond his work with NeuBase Therapeutics, Stephan identified the molecular basis of dozens of rare and common diseases, which advanced novel molecular diagnostics and drug development across the globe. He has trained a cadre of physician-scientists who now lead activities across the country and also worked alongside young scientists to launch 14 biotech companies that brought first-in-class therapeutics to market. Stephan's companies are routinely funded by top-tier investors.

Prior to his entrepreneurial pursuits, Stephan built a distinguished career in academia where he discovered many genes that cause a wide variety of disease and published routinely in journals such as the New England Journal of Medicine, Nature, Science, Cell and PNAS. He served as Deputy Director for Discovery Research and Chairman of the Neurogenomics Division at the Translational Genomics Research Institute (TGEN), as well as Professor and Chairman of the Department of Human Genetics at the University of Pittsburgh and UPMC. In addition, he held affiliations with Harvard Medical School, Johns Hopkins University, and Children's National Medical Center.

Stephan completed a fellowship at the National Human Genome Research Institute (NHGRI) of the National Institutes of Health (NIH). He holds a Ph.D. from the University of Pittsburgh and a Bachelor's degree from Carnegie Mellon University.

Early versions of our backbone technology have been shown to achieve extremely high fidelity *in vivo* editing in several genetic diseases. We are advancing the next generation of triplex editors that leverage the lack of immunogenicity of the chemical entity to allow repeat dosing to ensure editing efficiency in tissues achieves therapeutic levels and to a variety of tissues beyond the liver.[25,36,37] These advantages promise a pipeline of safe and permanent treatments for rare and common disease.

Summary

Healthcare needs are enormous and a scalable platform technology that can address diseases at a genetic level would afford a host of solutions. Globally, there are a plethora of genetic diseases, most of which lack any therapeutic options. The relatively new strategies of targeting nucleic acid targets upstream of proteins to address disease at the genetic level have achieved human proof-of-concept, yet these early technologies are fragmented in their ability to address the different causal mechanisms, and suffer from limitations related to delivery, tolerability, selectivity, manufacturability, durability, and scalability. We believe we have a unifying solution without the limitations of other precision genetic medicine technologies that will allow us to address many diseases, both rare and common (see inset: **Corporate Case Study**). JoPM

Summary Points

- The goal of our efforts is to significantly reduce the burden of untreatable morbidity and mortality across the globe caused by rare and common diseases.
- Our strategy is based on the observation that all diseases, with few exceptions, are caused by genetic variations resulting in a limited set of pathogenic events: increase, decrease, or change of gene function.
- To achieve our goal, we have designed, built, and validated a new precision genetic medicines platform technology able to uniquely drug the double-stranded human genome (and other double-stranded nucleic acid targets such as RNA secondary structures, miRNAs, ecDNA, etc.) to address disease at the base level.
- The resultant PATrOL-enabled compounds are comprised of three components including a synthetic poly-amide backbone, a suite of novel nucleobases and a delivery shuttle that confer the requisite capabilities.
- Concurrently, we have engineered in advantages over early precision genetic medicine technologies including the ability to have single nucleotide selectivity, broad biodistribution, allele selectivity, tolerability including lacking immunogenicity, manufacturable using existing manufacturing infrastructure, and be truly scalable.

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