

Codexis, Inc.
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CODEXIS
PROTEIN ENGINEERING EXPERTS®

Better than natural: next-generation therapeutics through advanced protein engineering

With cutting-edge protein-engineering tools, Codexis designs transformative therapeutics tailored to patient needs.

Darwin's great achievement was to show how natural selection could fashion unimaginable biological complexity and adaptive functionality, without the need for a creative designer. Today, Codexis harnesses the power of evolutionary processes in the lab to create highly desired molecules never seen in nature. With scientists in the driving seat, these selective approaches are enhanced with artificial intelligence (AI) that guides the creation of new biological molecules for a wide range of therapeutic indications. Codexis is at the forefront of directed, evolutionary protein engineering and is applying this expertise to develop optimal protein and gene therapies.

Codexis therapeutic discovery

With the exception of antibodies created by the adaptive immune system, proteins in nature do not evolve to explicitly treat disease. Thus, most natural proteins administered as therapeutics to patients have suboptimal properties for treating the target disease. Natural proteins are not only less efficacious and less safe than desired, but their manufacture also often creates additional risks and complications. Codexis uses its CodeEvolver technology platform to overcome the suboptimal properties and limitations of natural proteins by generating novel biological variants with safety and efficacy profiles specifically tailored to target diseases (Fig. 1). As CodeEvolver acts on the DNA level, the resulting variants can also be developed for mRNA and gene therapy applications. Codexis's belief is that by applying advanced protein-engineering principles, every biologic can be improved for the benefit of patients.

Non-invasive protein therapies

The power to precisely engineer proteins with desired properties opens new possibilities in their application to treat disease. For instance, proteins can be engineered to function in previously unexplored harsh conditions and environments. Codexis has pursued this path with the development of CDX-6114, an orally administered enzyme for the potential treatment of the orphan metabolic disorder phenylketonuria (PKU).

PKU is an inborn error of metabolism caused by a mutation in the gene encoding the phenylalanine hydroxylase enzyme that converts the essential amino acid phenylalanine (Phe) into tyrosine. Without a functional version of this enzyme, Phe builds up to toxic levels, causing neurological damage in the brain that leads to intellectual disability, seizures, and cognitive and behavioral disabilities. As Phe is present in almost all dietary

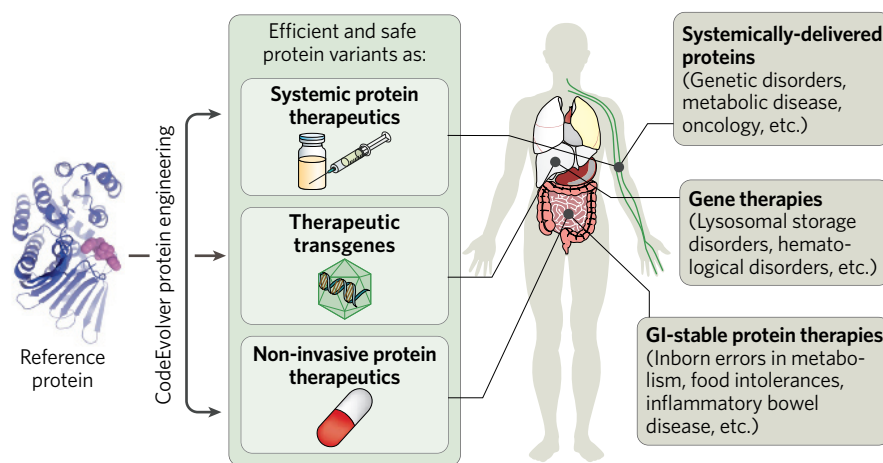


Fig. 1 | Engineering next-generation therapeutics with the CodeEvolver technology. Suboptimal properties of natural proteins are resolved to generate novel variants with safety and efficacy profiles specifically tailored to target diseases. GI, gastrointestinal.

protein, the estimated 50,000 PKU patients in the developed world must adhere to a strict, lifelong, low-Phe diet consisting of designated medical foods and nutritional supplements. However, as their caloric requirements increase with age, many patients are unable to continue adhering to PKU diets.

To address this critical need, Codexis has developed CDX-6114, an orally delivered, gastrointestinal (GI)-active enzyme that degrades Phe in the upper intestines before it can be taken up systemically. CDX-6114 compensates for the defective liver enzyme as a GI-specific enzyme, working in tandem with natural proteases in the duodenum and jejunum. From proprietary libraries of a natural Phe-degrading enzyme, Codexis scientists screened approximately 27,000 variants before identifying CDX-6114 as an effective variant that, as a result of 22 introduced mutations, is tolerant of the low pH conditions of the stomach, resistant to intestinal proteases, and can be readily manufactured.

Codexis reported top-line data from a phase 1a single ascending-dose study evaluating the safety and tolerability of CDX-6114 in healthy volunteers in November 2018. This study found that CDX-6114 was well tolerated at all doses, with no reported serious adverse events, GI symptoms, or evidence of systemic exposure. In February 2019, Nestlé Health Sciences—which entered a development, option and licensing agreement for CDX-6114 in 2017—exercised its option to acquire an exclusive license for its global development and triggered a \$3 million milestone payment.

The success of CDX-6114 and the recognition that Codexis-designed proteins could function in the harsh environment of the GI-tract, subjected to low pH, proteases, and detergents, provided the impetus for Codexis to further bolster its pipeline of non-invasive proteins. Expanding on its development of GI-active enzymes for inborn errors of metabolism, Codexis is also advancing similar candidates for more widespread diet-related indications, as well as other GI-stable proteins to locally address disorders such as inflammatory bowel disease.

Gene therapy

Since the first approval in the 1990s, enzyme-replacement therapies (ERTs) for genetic disorders such as Gaucher, Fabry, and Pompe disease initiated rapid industry growth to develop therapies for rare diseases. These ERTs were based on the natural sequences of the active human enzymes deficient in patients. Yet, because the natural sequences were not innately designed to be used as disease treatments, their pharmacological properties are suboptimal. For example, the natural maturation of α -galactosidase A (GLA; for Fabry disease) takes place within the cells in which it is active. However, the ERT manufactured through biotechnology is administered intravenously and must be taken up from the bloodstream and into affected tissue cells deficient in the enzyme, all while maintaining functional stability.

Realizing these considerable challenges with ERTs, Codexis sought to engineer GLA variants

that are highly stable at neutral pH (to increase plasma half-life and simplify CMC characteristics) as well as within the lysosome, more effectively taken up by impacted tissue, and with a greatly reduced 'predicted' immunogenicity profile. One of its lead GLA variants, CDX-6311, has 19 mutations and was identified after screening ~20,000 variants. CDX-6311 has a 7-fold longer serum half-life and demonstrated greater efficacy in removing the disease-causing Gb3 substrate in the cardiac tissue of Fabry mice.

CDX-6311 formed the basis of a strategic collaboration and license agreement with Takeda Pharmaceuticals announced in March 2020. Under this agreement, Takeda combines these optimized transgenes with its gene-therapy research capabilities and manufacturing infrastructure, to generate highly efficacious, next-generation gene therapies, which has expanded the use of CodeEvolver into the gene therapy field.

Not only is Codexis using CodeEvolver to engineer novel human protein variants that are more stable, better taken up by target cells, and more efficacious; CodeEvolver is also being applied to further improve other components of gene therapies, including the viral vectors (Fig. 2). In the past, better vectors have generally been identified via selection procedures that are limited by their general lack of actionable information output. With CodeEvolver technology it is possible to parse out various aspects of gene therapy, such as tropism, efficacy, and manufacturability to improve these characteristics in tandem. As a result, Codexis's pipeline has further expanded to include both novel transgenes and vector development programs.

The opportunities of the CodeEvolver technology in discovering highly effective therapies are limitless

The technology

With a long history and deep experience in designing and developing nearly 100 enzyme products used in various clinical and commercial applications, Codexis can develop a desired protein variant generally containing tens of mutations. While that number may seem small, there are more than 10^{75} ways of making 20 amino acid changes in a protein that has 400 amino acid residues. Exploring such an enormously diverse sequence space is possible with the CodeEvolver platform technology via the use of disease-relevant high-throughput screens, intention-inspired protein libraries, proprietary next-generation sequencing technologies, and AI.

Where some protein-engineering approaches attempt to construct proteins from scratch, CodeEvolver begins exploring the vast sequence space by identifying 'beneficial diversity' via the generation and screening of focused sets of variants containing amino-acid changes that are likely to lead to improvements for the desired attributes (for instance, by targeting the protein surface, active site, predicted epitopes, hydrophobic patches and so on). Such beneficial amino acid diversity is then iteratively recombined, while the

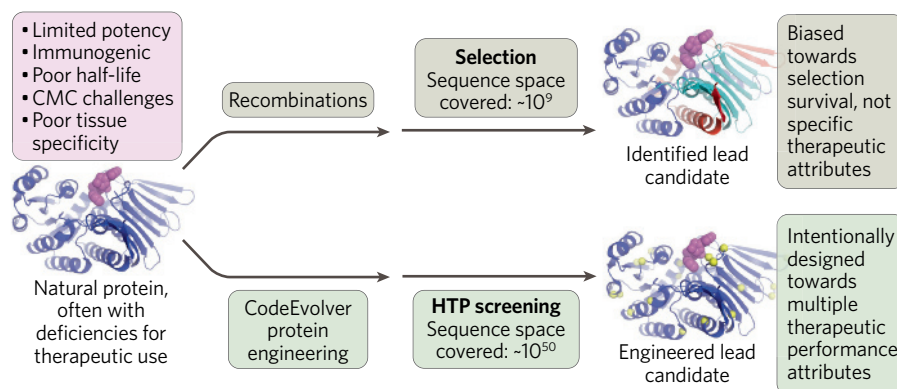


Fig. 2 | Efficiently searching sequence space for intentionally designed protein therapeutics. The iterative nature of the CodeEvolver technology that includes high-throughput (HTP) experimentation and artificial intelligence elements, enables efficient searching of sequence space. CMC, chemistry, manufacturing and controls.

introduction of new diversity continues in parallel, eventually arriving at the optimal combination of mutations that bestows the protein with the pharmacological, efficacy, safety, and CMC characteristics that make for an ideal therapy. This process is executed at high pace through proprietary workflows, information management systems, and AI tools that Codexis has continuously practiced and improved over more than 20 years, during which it evolved these systems for a variety of other industrial applications.

Built on deep experience

Codexis's therapeutic discovery business and CodeEvolver are built on a directed protein evolution platform that has delivered major contributions to industrial chemical processes.

Using chemoenzymatic processes (enzymes for the synthesis of organic compounds) has become widespread for the sustainable manufacture of pharmaceutical products. Codexis, in partnership with Merck, continues to be a pioneer in this field. A breakthrough came with a collaboration between the two companies 10 years ago that developed a novel chemoenzymatic route that is now implemented at commercial scale for the synthesis of Merck's type 2 diabetes drug Januvia (sitagliptin).

Since then, Codexis has continued to push advances in industrial biocatalysis. In December 2019, the company reported another landmark collaboration with Merck for the synthesis of the investigational HIV drug islatravir (MK8591). In this case, the goal was not to simply replace one catalytic step, but to engineer an entire biocatalytic cascade of nine enzymatic steps, in which the product of one reaction becomes the substrate for the next.

Codexis has also applied its expertise to create a portfolio of high-performance enzymes for life sciences. In 2019, Codexis engineered a DNA ligase that was licensed exclusively to Roche, Inc. In June 2020, Codexis entered a collaboration with Alphazyme LLC to produce and co-market three enzymes for life science applications: a high-fidelity DNA polymerase that delivers uniformity of coverage and provides an accurate and representative DNA diagnostic result; a T7 RNA polymerase for the efficient manufacture and capping of mRNA vaccines; and a reverse transcriptase for RNA-directed, point-of-care viral diagnostics.

In addition, Codexis is working with Molecular Assemblies, Inc. to improve enzymatic DNA synthesis that can potentially overcome limitations of phosphoramidite chemistry, which date back to the 1980s and have been a bottleneck in expanding the applications of synthetic DNA. The two companies announced their partnership in June 2020 to further this goal by using the CodeEvolver platform to create new, more cost-effective enzymes for enzymatic DNA synthesis.

Discovering biologics

Relying on natural, Darwinian evolution—the algorithmic process of random variation and selective retention—is too slow to support the discovery of biologic therapeutics that patients urgently need today. Codexis has addressed this through application of its technology platform and laboratory capabilities to exponentially accelerate Darwinian evolution, and continues to advance a pipeline of intentionally engineered protein therapeutics for systemic and oral administration, either as protein, mRNA, or gene therapies.

The opportunities of the CodeEvolver technology in discovering highly effective therapies are limitless. As Codexis pursues new protein therapeutics for non-invasive administration as well as building novel, differentiated gene therapies, alone, and in collaboration with its partners at Nestlé Health Science and Takeda, the company continues searching for new partners to add fuel to its discovery engine. With the high likelihood that any protein of interest can be improved for pharmaceutical properties and therapeutic use, Codexis welcomes discussions with potential partners who want to advance novel biologic therapies and contribute to a better future for patients.

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