

Elastin-like Polypeptide (ELP) Platform: Creation of Differentiated Products

1.0 Overview

PhaseBio Pharmaceuticals is a clinical-stage biopharmaceutical company developing novel drugs to treat metabolic and specialty cardiopulmonary diseases. Our proprietary platform technology uses elastin-like polypeptides (ELPs) genetically fused to peptides and proteins to modulate the rate of release of the therapeutic from the subcutaneous injection site and its half-life in circulation.

In addition to this unique dual mechanism of enhancing pharmacokinetics, PhaseBio's ELP platform provides distinct advantages over traditional half-life extension technologies. First, ELP fusions are expressed as soluble proteins in *E. coli*, allowing for production at high yields and for facile purification. Second, ELP fusions are stable in near physiologic aqueous buffers, which allows for pharmaceutically elegant, "ready-to-use" formulations (no reconstitution or mixing required). Finally, further exploiting this property, PhaseBio has had success in co-formulating combinations of ELP-fusion versions of proteins that have historically been difficult to combine.

For our long-acting products, the combination of slow subcutaneous absorption and long circulating half-life creates a flat pharmacokinetic (PK) profile, resulting in extended time in the therapeutic range and a low peak-to-trough ratio. This has enabled PhaseBio to create very long acting versions of otherwise rapidly cleared or degraded peptides. Our clinical development pipeline includes PE0139, a monomeric, super-long-acting native insulin-ELP fusion targeted at once-weekly administration for basal-insulin therapy; PB1046, a stable long-acting vasoactive intestinal polypeptide (VIP) ELP fusion for the treatment of acute and chronic heart failure, the treatment and prevention of cardiomyopathy associated with Duchenne muscular dystrophy (DMD), Becker muscular dystrophy (BMD) and X-linked dilated cardiomyopathy, and other indications.

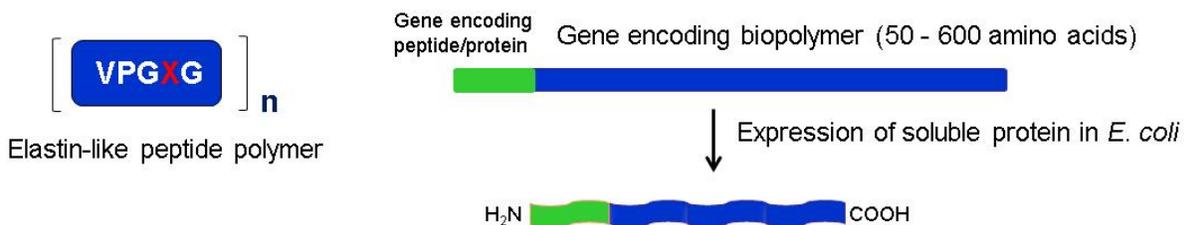
2.0 Technology platform

2.1 Production and biochemical attributes

PhaseBio's proprietary technology is based on recombinant biopolymers, called ELPs, to which peptides and proteins can be genetically fused (ELP-fusion proteins). The individual subunit or building blocks of ELPs are derived from a five amino acid motif found in nature in the human protein elastin. This five amino acid motif is repeated multiple times to form the ELP biopolymer. Fusion to ELPs significantly improves the solubility, stability, and bioavailability of peptides and

proteins. An important benefit is that the technology enables PhaseBio to use natural or minimally altered peptide sequences, since fusion of the peptides to ELP protects against degradation by enzymes in the circulation and the fusion protein retains similar potency to the native peptide or protein. These constructs are produced in the soluble fraction of *E. coli*, allowing for ease of scale-up and purification.

Modifying the sequence of the individual subunits of the ELP biopolymer (at the X position) and varying its overall length are used to optimize the physical and chemical properties of each ELP-fusion protein. As depicted below, the gene sequence of the therapeutic peptide or protein (in green) is fused to the gene encoding the biopolymer (in blue) and is expressed as one continuous fusion protein. The resulting fusion protein retains the activity of the therapeutic domain but assumes the solubility, stability, and long half-life of the ELP. These ELP fusion proteins bind and activate the relevant receptor as intact molecules and are not dependent on a drug release mechanism.



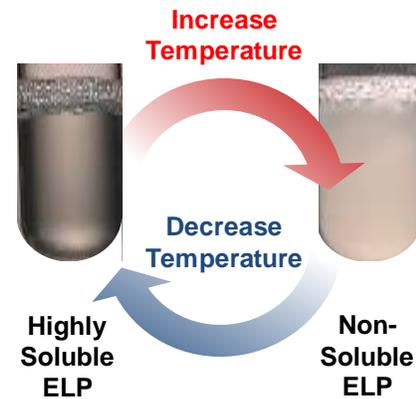
Fusion of target peptide/protein and ELP Biopolymer created at the DNA level.
Results in stable, highly active protein with reversible transition properties and long half-life

Fusing peptides or proteins to ELPs also confers resistance to degradation by enzymes in the body, resulting in enhanced stability at the injection site and significantly longer circulating half-life than the native peptide or protein. Alternative approaches to achieve this resistance to inactivation involve altering the natural sequence of the peptide or protein, thus increasing the risk of immunogenicity and potentially altering biological function.

In summary, PhaseBio's ELP biopolymer technology is a simple, one-step solution to the development of improved biologic drugs that are economical to manufacture and have superior pharmacological characteristics. Our goal is to leverage the optimized PK profile provided by ELPs to enable the differentiation of our products in the market place.

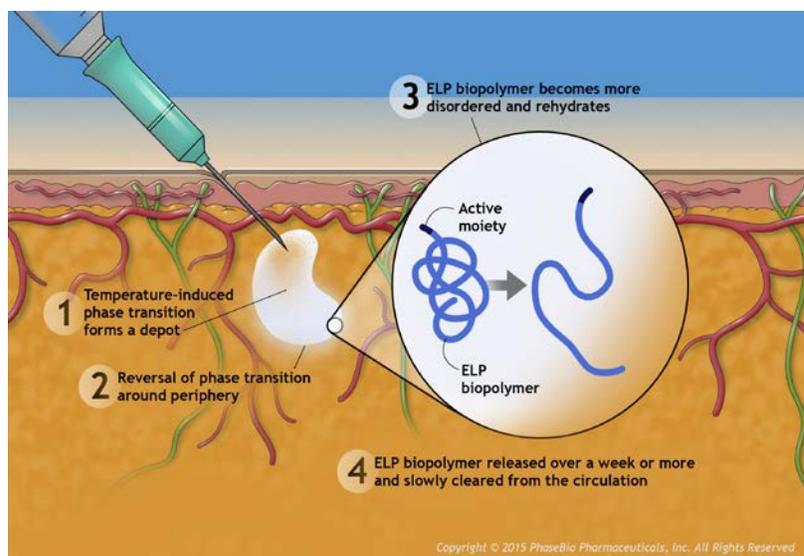
2.2 Prolonged drug exposure through a unique dual mechanism of slow absorption and extended half-life

A unique attribute that distinguishes PhaseBio's biopolymer technology is the ability of ELP fusion molecules to undergo a fully reversible phase transition (or coacervation) to form a material that provides a subcutaneous depot for sustained release. Phase transition occurs as a solution of the protein is raised above a specific transition temperature and is fully reversible as the temperature is lowered. Due to the nature of the five amino acid repeat, and particularly the presence of proline and glycine amino acids, ELPs are relatively unstructured, leading to a large hydrodynamic radius. As the temperature is raised through the transition temperature, the ELPs become more ordered and compact which, together with the hydrophobic nature of the ELP sequence, leads to exclusion of water and a liquid-liquid phase separation.

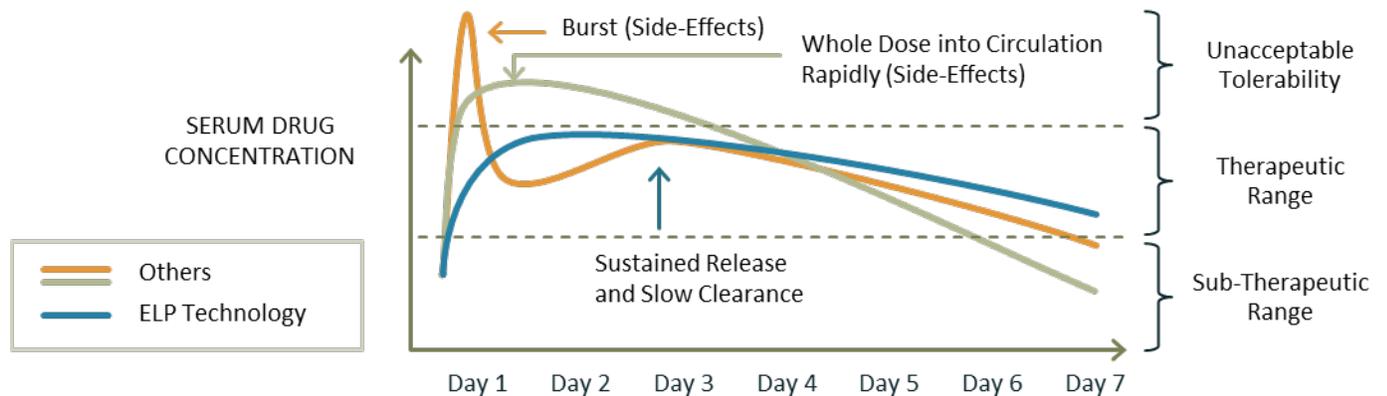


We have utilized this phenomenon by engineering an ELP biopolymer to undergo phase transition just below body temperature. Products based on this ELP biopolymer are soluble at room temperature in a vial or syringe, but on injection subcutaneously, the increase in temperature rapidly leads to phase transition and the formation of coacervate that behaves like a depot at the injection site.

Since phase transition is a concentration-dependent process, at the periphery of the subcutaneous depot there is a gradual reversal of the phase transition as the ELP biopolymer rehydrates and becomes more disordered. Molecules released in this way are then taken up into the circulation, but subsequently cleared slowly due to the large hydrodynamic radius and the protection against degradation of the active peptide afforded by fusion to ELP.



This dual mechanism of slow release and long circulatory half-life provides a shallow peak-to-trough ratio of drug levels in the blood, which is key for maintaining therapeutic benefit between weekly or even up to monthly dosing intervals. The goal is to administer enough drug to provide robust therapeutic effect for the entire week or month's duration, up to the point of receiving the next dose. Many other technologies utilized for providing a depot for protracted release of peptides have an initial burst or release into the circulation that creates undesirable side effects. This limitation of tolerability caused by a high transient concentration of drug compromises the full potential of many products, as illustrated below for a typical microsphere-based depot formulation. Technologies that rely solely on extension of circulating half-life still require substantial initial loading of drug to provide sufficient exposure between injections. Since initial drug uptake in these instances is relatively rapid, levels frequently rise to levels that result in side effects.



2.3 ELP technology overcomes limitations of peptide/protein drugs

In the case of PB1046, the ELP technology enabled the creation of the only stable, long-acting form of VIP, a molecule with great potential for treating cardiovascular, cardiopulmonary, and other diseases. Historically, clinical development of VIP as a pharmaceutical product has been severely hampered by rapid inactivation and clearance of the peptide following administration. Native VIP has been shown to have a half-life in the circulation of less than two minutes. In contrast, using PhaseBio's ELP technology, PB1046 is highly resistant to enzymatic degradation and offers prolonged activity in humans that correlates with maintenance of therapeutic drug levels over the course of one week.

With PE0139, our super-long-acting basal insulin, a slightly lower transition temperature provides for even slower uptake from the injection site and, consequently, an extremely flat PK profile that allows for 24/7 glycemic control with minimal risk of hypoglycemia

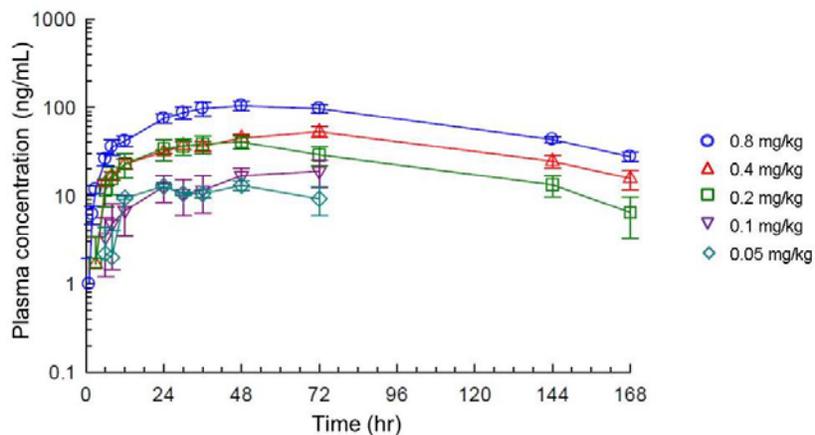
All products in PhaseBio's pipeline are stable in liquid formulations and are administered from a simple device using a fine-gauge needle. Furthermore, we have demonstrated that different ELP fusion molecules can be co-formulated while retaining their full individual characteristics in terms of PK and activity.

3.0 Examples of the application of ELP technology

Note that details of PhaseBio's clinical development pipeline can be found on our website: www.phasebio.com.

3.1 VIP-ELP: PB1046 and PB1120

VIP activates two different receptors, VAPC₁ and VPAC₂, which differ in their tissue distribution. PhaseBio has developed two fusions of VIP to the ELP-1 series, PB1120, which has similar potency to native peptide for both receptors, and PB1046, which is relatively selective for VPAC₂. Exposure to neutral endopeptidase, which is known to rapidly inactivate VIP in the body, showed that the VIP-ELP fusion protein is resistant to this protease, retaining the ability to activate the VPAC₂ receptor. PB1046 PK data from animal models and from a single ascending dose phase 1 trial indicated prolonged exposure following a subcutaneous injection, confirming protection against proteases and depot formation by ELP to provide a PK profile compatible with weekly administration.



Pharmacokinetic profiles following single subcutaneous injection of PB1046 in human subjects

The development of PB1046 and PB1120 enables the exploration of the therapeutic potential of this peptide and provides the first viable VIP-based therapies. PhaseBio's initial focus is the development of PB1046 for the treatment of cardiomyopathy in patients with muscular dystrophy.

VIP peptide and PB1046 have been shown to have positive inotropic and lusitropic effects on the heart which, together with VIP's anti-inflammatory and anti-fibrotic activity, peripheral and coronary vasodilation effects, indicate a potential to delay or reverse cardiomyopathy associated with DMD. Such treatment would likely have a significant impact on life expectancy, since cardiomyopathy is the leading cause of death in DMD patients and deterioration is progressive from an early age. Evaluation of PB1046 in *mdx* knockout mice and *mdx/utr* double knockout mice demonstrated positive effects on cardiac function and skeletal muscle.

VIP has also been shown to play a role in the processing and regulation of the cystic fibrosis transmembrane conductance regulator (CFTR), which is mutated in cystic fibrosis (CF). Furthermore, VIP has been shown to enable normal processing and to stabilize CFTR with the most common mutation, F508del, which is present in approximately 75 percent of CF patients. PhaseBio has confirmed that PB1046 and PB1120 are able to correct the F508del defect in cell culture and is currently exploring the potential of these products as disease-modifying treatments for this orphan disease.

3.2 Insulin-ELP: PE0139

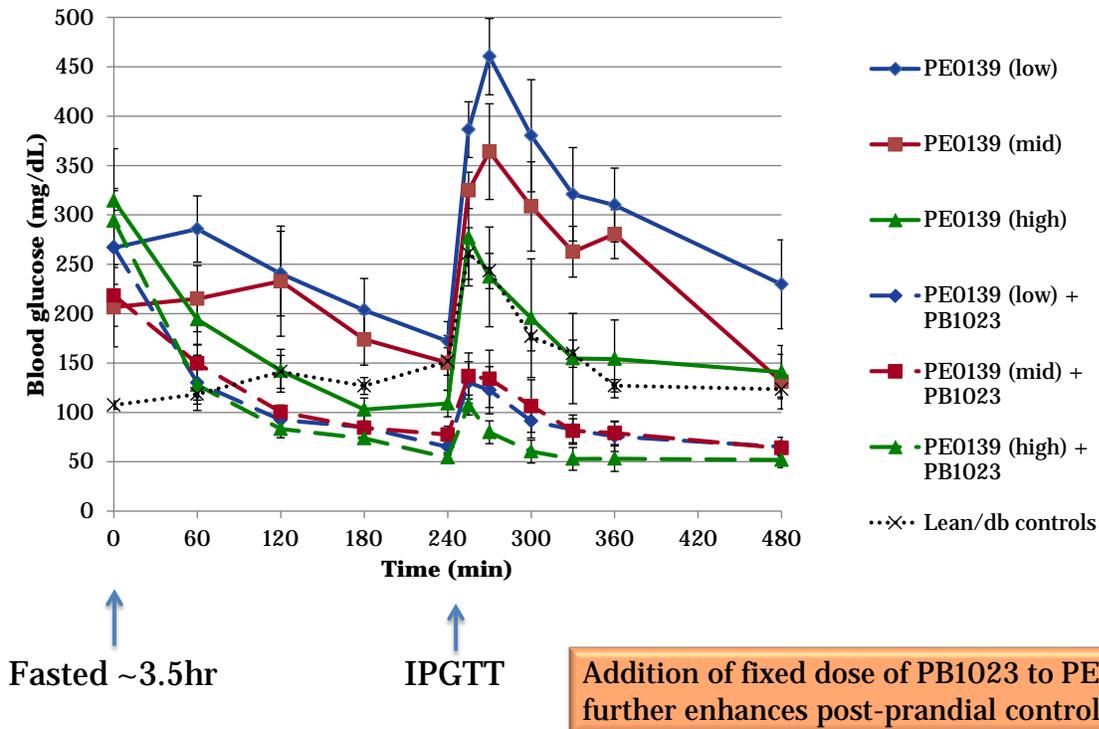
PE0139 is a fusion of fully native insulin to an ELP in order to form a super-long-acting basal insulin product. The fusion protein is produced in *E. coli* and does not form inclusion bodies, but is recovered as a soluble protein with correct disulphide bridging between the A and B chains of the insulin component.

The product has a slightly lower transition temperature than PB1023 or PB1046, which results in the formation of a more stable coacervate in the subcutaneous space and, consequently, even slower uptake from the injection site and an even flatter PK profile. Such a low peak-to-trough ratio is particularly important for a basal insulin product and is supported by prolonged pharmacodynamic activity compared to insulin glargine in animal models.

3.3 Co-formulation of PE0139 and PB1023 (once-weekly GLP-1)

PE0139 and PB1023 comprise the same ELP-1 series biopolymer fused to the active moiety (insulin or GLP-1) and are stable in the same simple aqueous formulation. There is increasing interest in developing combination products of basal insulin and GLP-1, but the formulation

requirements of the components generally makes this a major challenge, e.g. the low pH requirements for insulin glargine formulation, the microsphere formulation for Bydureon®. In contrast, PhaseBio has demonstrated the compatibility and stability of co-formulated PE0139 and PB1023, and we have also demonstrated a synergistic effect in an animal model of type 2 diabetes.

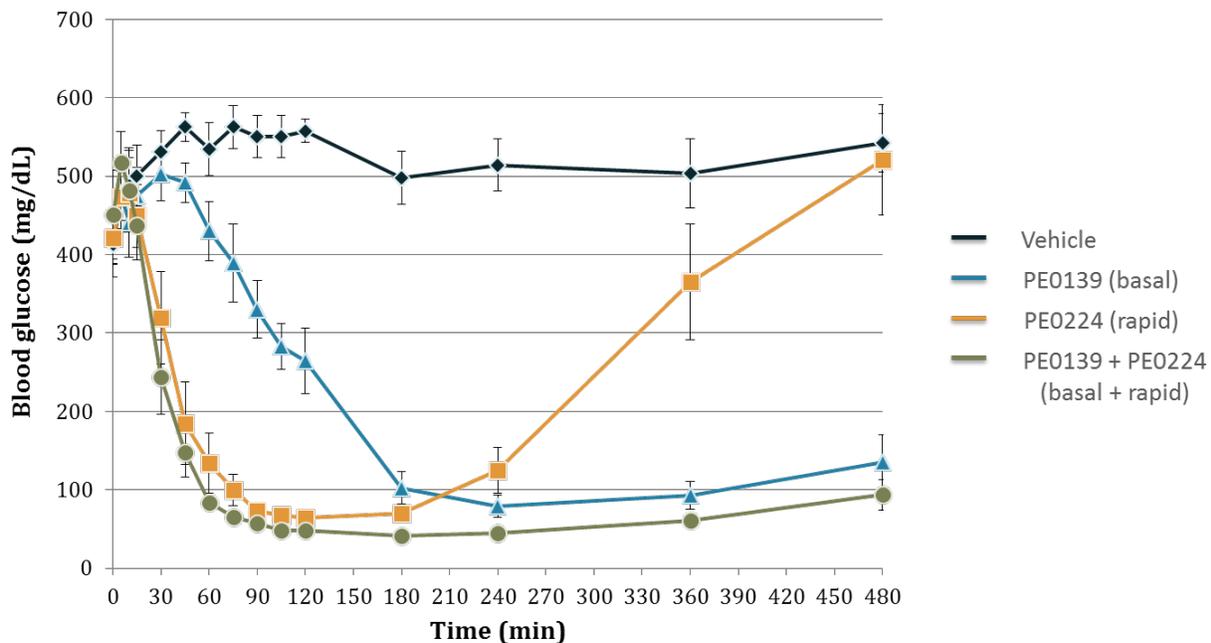


The synergistic effect of PE0139 and PB1023 on blood glucose control in diabetic mice (db/db).

3.4 Co-formulation of PE0139 and rapid-acting insulin ELP fusion

Insulin is normally stabilized as a hexamer, which has to breakdown into monomers following subcutaneous injection before it can be absorbed into the blood stream. For hexamers of native insulin this is relatively slow, but mutated forms of insulin (such as insulin lispro, insulin aspart, and insulin glulisine) convert to monomers faster due to decreased stability of the hexameric form. Such mutated rapid-acting insulins are currently the preferred form for prandial use because of their faster onset of action compared to native insulin. PhaseBio's ELP technology enables the stabilization of native insulin in the monomeric form. We have constructed insulin molecules with short ELP biopolymers that still confer monomer stability in aqueous solution but allow for rapid uptake from the injection site because they do not form a coacervate.

We have taken this one step further to demonstrate the flexibility of the technology by co-formulating this rapid-acting insulin with the basal insulin, PE0139. In an animal model of type 1 diabetes, we have shown that PE0139 and rapid-acting insulin components behave the same way in the co-formulation as they do when administered separately. The rapid-acting insulin is taken up into the circulation very fast and has an immediate effect on blood glucose levels, while the PE0139 basal component forms a depot and is released more slowly into the circulation, providing a long-lasting effect on blood glucose.



The effect of injection of PE0139 and a rapid-acting insulin construct (PE0224), alone and in combination, on blood glucose in streptozotocin-treated mice.

3.5 Human experience with ELPs and safety

To date, ELPs have been utilized in three separate classes of products – insulin, GLP-1, and VIP – and have been evaluated in multiple clinical trials involving hundreds of patients. In all cases, the product has been determined to be safe and well-tolerated and there have been no instances of neutralizing antibodies.

3.6 Other potential applications

While PhaseBio is initially focused on the application of the ELP technology in cardiopulmonary and metabolic diseases, the technology has broad potential in many other areas. PhaseBio is currently evaluating a number of pipeline candidates encompassing a range of therapeutic areas.

4.0 Summary

- Fusion of ELP to peptides and proteins modulates the rate of the therapeutic's release from the injection site and its half-life in circulation.
- Preclinical and clinical studies have demonstrated that ELPs can be utilized to convert peptides with very short half-lives of only minutes into products that can be dosed once a week or even less frequently.
- Fusion to ELP enables production of soluble protein fusions in *E. coli*, confers stability in aqueous formulations and facilitates co-formulation of ELP fusions with different active components.
- The flexibility of the ELP platform has enabled production of both a rapid-acting insulin in which the molecule is stable in monomeric form and a super-long-acting basal insulin based on the native insulin sequence. Further modulation of the ELP provides a PK profile to enable once-monthly dosing.
- PhaseBio's current focus is on the application of ELP technology to develop products in cardiopulmonary and metabolic diseases, but ELPs can also be the basis for products to treat orphan diseases.