VIP-ELP FUSION MOLECULES PB1120 AND PB1046 CORRECT F508DEL-CFTR DYSFUNCTION

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Introduction

Rescuing highly functional F508del-CFTR in CF epithelial cells by correcting its misfolding to promote membrane targeting while increasing surface stability is the major goal of current therapeutic strategies using corrector molecules.

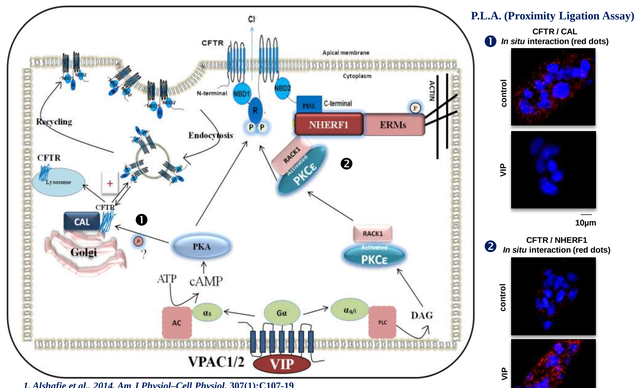
We previously reported the importance of prolonged exposure of airway epithelial cells to VIP (Vasoactive Intestinal Peptide), a 28-amino acid neuropeptide released from intrinsic neurons which controls multiple functions in exocrine tissues including inflammation, relaxation of airway and vascular smooth muscles and CFTR-dependent secretions.

VIP increases CFTR membrane insertion, stability and function in human airway epithelial cells[1] (Fig. I). Moreover, it corrects the molecular defects associated with the F508del mutation[2,3] (Fig. II). With VIP-knockout C57Bl/6 mice, a proven model of airway diseases, we have demonstrated *in vivo* that the absence of functional CFTR can be corrected by exogenous VIP administration (*Alcolado et al. 2014. Am J Physiol–Cell Physiol.* 307: C195-209). These results indicated the potential of VIP for the treatment of CF.

PhaseBio Pharmaceuticals has developed two VIP-ELP fusion proteins (Fig. III & IV): PB1120, which has similar potency to native peptide for both VPAC1 and VPAC2 receptors, and PB1046 (Vasomera™), which is relatively selective for VPAC2. PB1046 was well tolerated in 2 phase I clinical trials in subjects with hypertension and provided exposure of at least a week following a single subcutaneous injection (Fig. V).

Here we report F508del-CFTR corrector effects of PB1120 and PB1046 that are highly resistant to the peptidases that normally rapidly degrade VIP *in vivo* and preclude its use as a therapeutic.

Signaling cascade of CFTR regulation by VIP in airways epithelial cells



1. Alshafie et al., 2014. Am J Physiol–Cell Physiol. 307(1):C107-19

Figure I. VIP-dependent increase in CFTR membrane density involves two mechanisms:

1. Dissociation of CFTR from CAL in the cytoplasm to promote CFTR membrane insertion ❶.
2. Activation of the PKCε signaling cascade that potentiates NHERF1 / P-ERMs complex interaction with membrane CFTR to mediate its surface stability ❷.

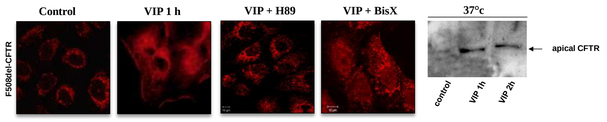


Figure II. Correction of F508del-CFTR.

Maturation and membrane expression of F508del-CFTR (here illustrated with immunofluorescence and immunoblotting data) were corrected by prolonged VIP treatment in human nasal epithelial cells (JME/CF15) and involved PKC and PKA signaling.

2. Alcolado N et al.,(2011). Am J Physiol–Cell Physiol. 301(1): C53-65.
3. Rafferty S. te al.(2009). J Pharmacol. Exp. Ther. 331: 2-13.

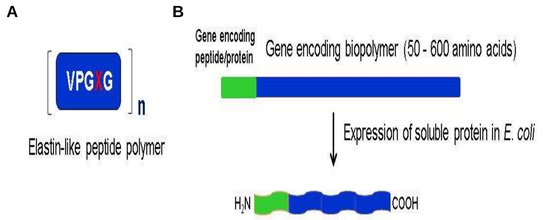


Figure III. Preparation of ELP fusion proteins.

(***A***), Elastin-Like Polypeptides (ELPs) individual subunit, derived from a five amino acid motif found in the human protein elastin, is repeated multiple times to form the ELP biopolymer. (***B***), The gene sequence of the therapeutic peptide or protein (in green) is fused at the level of DNA 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.

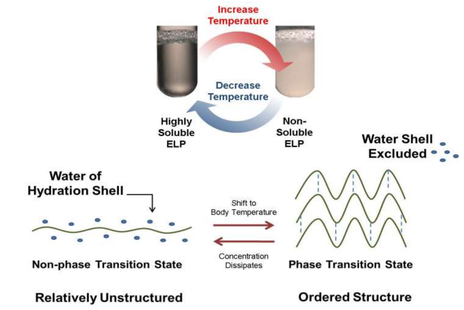


Figure IV. Flat PK profile: A unique dual mechanism of slow absorption and extended half-life.

A unique attribute that distinguishes PhaseBio's technology is the property of ELP fusion molecules to undergo a fully reversible phase transition (or coacervation) to form a material that provides a depot for sustained release. Phase transition is driven by thermodynamic forces involving highly reversible intermolecular hydrophobic and hydrogen bonding interactions.

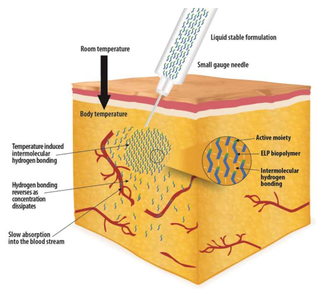


Figure V. Slow absorption & long half –Life.

Fusion to ELPs significantly improves the solubility, stability and bioavailability of peptides and proteins. An important benefit of the technology is that it 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.

For pharmaceutical application as illustrated above, phase transition from a solution to a coascervate is triggered by a specific temperature shift such as to body temperature following administration, and reversal occurs as the concentration of the ELP fusion dissipates at the periphery of the subcutaneous injection site. Once product enters the circulation, the extended polymeric structure of ELP provides a large hydrodynamic radius that confers a prolonged circulatory half-life. This dual mechanism of slow release and long half-life provides a shallow peak to trough ratio of drug levels in the circulation, which is key for maintaining therapeutic benefit between weekly or even up to monthly dosing intervals.

Results

Iodide effluxes. Briefly, cells are incubated with 136 mM NaI loading buffer for 1 h. Extracellular NaI solution is then removed and rapidly replaced with efflux buffer in which NaI is replaced with $NaNO_3$. Samples are taken and replaced at 1 minute intervals. The first 3 samples (time 0-2 min) are used to establish a stable baseline of efflux. CFTR activation cocktail (cpt-cAMP + IBM + Fsk) is included in the efflux buffer from time 3 min. NaI concentration is then measured using an iodide sensitive electrode moved over each sample by a computerised autosampler (Efflux Analysis Software © F. Chappe & V. Chappe) and NaI efflux rate constant k (min^{-1}) are calculated. Iodide efflux peaks (maximum efflux rate during stimulation – basal level) are compared.

Immunoblotting. Membrane proteins are solubilized in RIPA buffer and 100µg of proteins subjected to 6-7.5% SDS-PAGE, transferred to PVDF membranes and probed with M3A7 monoclonal anti-CFTR antibody.

Immunolocalization. JME/CF15 cells plated on glass coverslips at low density and maintained in culture at 27°C or 37°C for 24 to 48 hrs were used. Cells were fixed in 2% PFA, permeabilized in 0.1% Triton X-100/2% BSA /PBS. Primary Ab:M3A7, secondary: goat anti-mouse conjugated to Cy3. Negative controls were performed by omitting the primary Ab.

Statistics. Results are reported as the means ± S.E.M., for 3-5 independent experiments. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

Correction of F508del-CFTR chloride channels activity

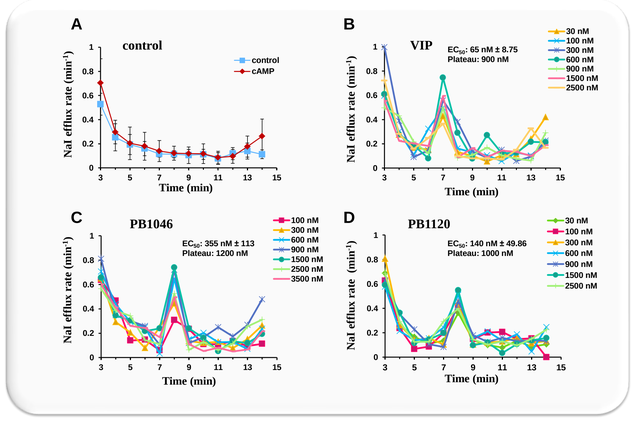


Figure 1. Iodide efflux rates were measured on JME/CF15 cells maintained at 37°C and incubated with VIP (***B***), PB1046 (***C***), or PB1120 (***D***) at the indicated concentrations for 2 hrs before the experiments. Rescued F508del-CFTR were stimulated by a cAMP activator cocktail added to the efflux buffer from time 3 to 15 min. (***A***) Iodide efflux rates from JME/CF15 cells maintained at 37°C in absence of correctors.
Calculated EC_{50} and plateau concentrations (n = 3-5) are indicated for each compound.

Time-course of ELP-VIP corrector effect

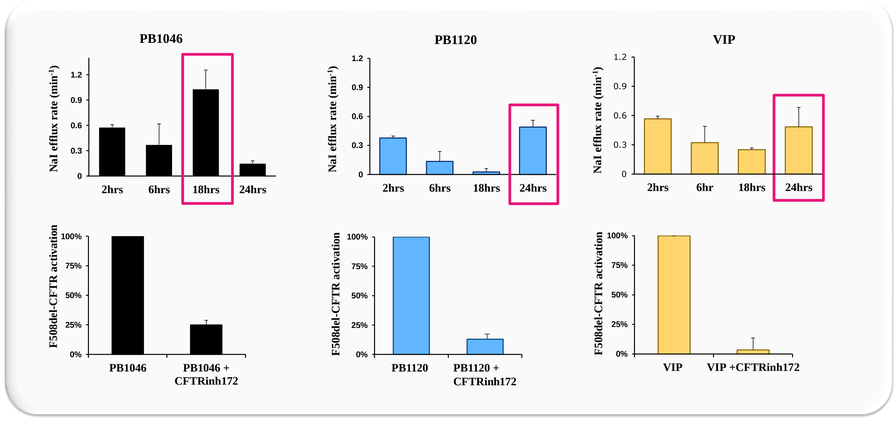


Figure 2. Iodide efflux rates were measured on JME/CF15 cells maintained at 37°C and incubated with VIP, PB1046, or PB1120 as indicated for 2 to 24 hrs before the experiments. Rescued F508del-CFTR were stimulated by a cAMP activator cocktail added to the efflux buffer from time 3 to 15 min. *Lower panels*: the CFTR inhibitor $CFTR_{inh172}$ (20µM) was added 30 min before and during the entire efflux experiments .

Correction of F508del-CFTR maturation and membrane expression

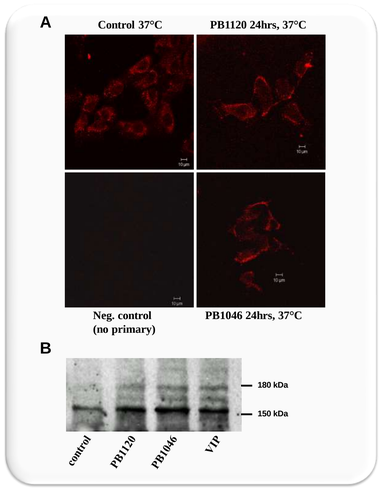


Figure 3. (***A***), JME/CF15 cells were immunostained for CFTR as described in methods.
(***B***), Immunoblotting of lysates from cells maintained at 37°C and incubated with each compound for 24 hrs.

Comparative corrector effects

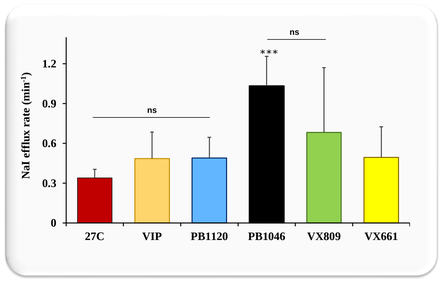


Figure 4. Iodide efflux rates measured on JME/CF15 cells treated with the following conditions before the experiments: (27C) 24 hrs at 27°C; (VIP) 37°C + VIP 900 nM for 24 hrs; (PB1120) 37°C + PB1120 1µM for 24 hrs; (PB1046) 37°C + PB1046 1,2µM for 18 hrs; (VX809) 37°C + VX809 1µM for 24 hrs; (VX661) 37°C + VX661 3µM for 24 hrs. Rescued F508del-CFTR was stimulated by a cAMP activator cocktail.
ns: not significantly different; ***significantly different from 27C.

Best corrector & potentiator combinations

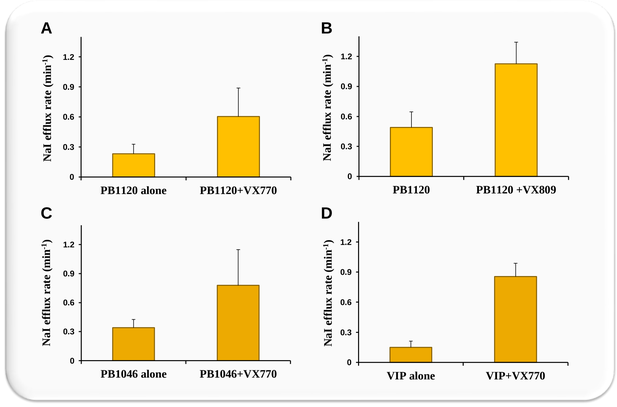


Figure 5. Iodide efflux rates measured on JME/CF15 cells maintained at 37°C. (***A***) PB1120 140 nM 24 hrs, alone or in combination with VX770 1µM (acute). (***B***), PB1120 1µM 24 hrs alone or in combination with VX809 1µM 24 hrs. (***C***), PB1046 350 nM 18 hrs alone or in combination with VX770 1µM (acute). (***D***), VIP 65 nM 24 hrs alone or in combination with 1µM VX770 (acute). Acute VX770 (1µM) @ 37°C did not produce any significant stimulation compared to basal ($p > 0.7$). Rescued F508del-CFTR was stimulated by a cAMP activator cocktail.

Conclusions

Our data indicate that PB1120 and PB1046 are strong candidate drugs for the treatment of CF patients with the F508del mutation.

- **Prolonged stimulation of human nasal CF cells with VIP-ELP corrects F508del-CFTR trafficking and function better than native VIP or other corrector treatment alone.**
- **PB1046 1µM 18 hrs alone produces the best functional correction.**
- **Combining PB1120 or PB1046 with VX770 or VX809 produces an additive positive effect on F508del-CFTR function.**

PhaseBio Pharmaceuticals proprietary platform technology uses elastin-like polypeptides (ELPs) genetically fused to peptides and proteins in order to modulate both 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, the ELP platform also provides several other distinct advantages over traditional half-life extension technologies:

- **ELP fusions are expressed as soluble proteins in *E. coli*, allowing for production at high yields and for facile purification.**
- **ELP fusions are stable in near physiologic aqueous buffers, which allows for pharmaceutically elegant, "ready-to-use" formulations (no reconstitution or mixing required).**
- **The company has had success in co-formulating combinations of ELP fusion versions of proteins that have historically been difficult to combine.**
- **To date, over 400 clinical trial participants have been exposed to one or more doses of ELP fusion proteins across three development programs (~75% exposed for 20 weeks).**

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