Summary

PUL-042 is a combination of a lipopeptide Pam2CSK4 acetate (Pam2) and a phosphorothioate oligodeoxynucleotide (ODN) M362 sodium that act as agonists of Toll-like receptors (TLR)-2/6 and TLR-9, respectively. PUL-042 has been shown to have anti-viral, anti-bacterial, anti-fungal and immunomodulatory properties in studies using experimental animals. This Phase II study was designed to assess firstly; the safety and efficacy of inhaled PUL-042 on experimental rhinovirus (RV-A16) induced lower respiratory symptoms in GOLD stage 0 chronic obstructive pulmonary disease (COPD) participants that were active smokers. Secondly, the effects of PUL-042 (on peak and area under the curve [AUC] values) on other symptoms, including upper airways respiratory score (URSS) & the Wisconsin Upper Respiratory Symptom Score-11 (WURSS-11), and symptoms scored using the **EXA**cerbations of **C**hronic Pulmonary Disease **T**ool (EXACT)-RS and COPD assessment test (CAT) clinical assessments of COPD symptoms. The safety profile of PUL-042 was assessed by monitoring any effects on lung function (% predicted FEV1, PEF, % predicted FVC and FEV1/FVC ratio), blood biomarkers including differential white blood cell counts, serum C-reactive protein (CRP), adverse events (AEs) and severe adverse events (SAEs). Finally, the effects of treatment on rhinovirus load, serum, nasal and sputum pro-inflammatory cytokines (IP-10/CXCL10 and IL-6), sputum cell counts, exhaled nitric oxide (FeNO), sputum bacterial cultures and sputum bacterial 16S rRNA abundance were examined as exploratory endpoints.

This single centre study was conducted in London, the United Kingdom between December 2018 and December 2020, and was temporarily suspended during the 1st wave of the COVID19 pandemic of 2020 (March-August 2020). The study screened 143 individuals, 24 were enrolled into the study (1:1 placebo:PUL-042) and 22 of these received at least one dose of PUL-042 or placebo (safety population, n=11 each) and 20 that received two doses of PUL-042 or placebo and were subsequently infected with RV-A16, two of whom were excluded due to presence of virus RNA at baseline prior to experimental RV-A16 challenge (infected and evaluable population, n=9 each). Overall, in the placebo arm the lower respiratory symptom scores were variable between study subjects and were lower than expected (based on previous studies) post RV-A16 challenge. There was no significant difference between placebo and PUL-042 on peak or AUC lower respiratory symptom score. There was also no significant difference between placebo and PUL-042 on upper respiratory symptom scores, CAT or total EXACT-RS score. PUL-042 did however show a significantly higher AUC score for the EXACT-RS cough and sputum domain (P=0.0488) versus placebo and exhibited non-significant trends for lower scores in the EXACT-RS chest symptom domain (all P>0.05).

In the safety and biomarker analysis, PUL-042 was well tolerated, and AEs were not-significantly different between the two groups, scored both as number of events (25 placebo and 33 PUL-042) and in number of subjects (occurring in 10/11 individuals in each group). There were no SAEs recorded. There were 2 doses of study drug given: one dose on Study Day -1 pre-RV challenge (on Study Day 0) and the second dose on Study Day 2 (post RV challenge). Compared with placebo, PUL-042 caused a significant, yet temporary reduction in % predicted FEV1 on both dosing days, and reductions in % predicted FVC, but not PEF or FEV1/FVC ratio. In all cases, lung function

returned to within the pre-treatment range by the 8h post dosing time point on each dosing day and there was no accumulation of effect on FEV1 between Study Days -1 and 2. PUL-042 caused a significant increase in blood neutrophils at 4-8h post dosing on both dosing days (P<0.001) which returned to within the pre-treatment range within 24h post dose. PUL-042 also affected other blood biomarkers, including a temporary decrease in blood monocytes at 2h on both dosing days (P<0.001), and then a significant increase in blood monocytes at 6h on Study Day -1 (P<0.05). PUL-042 also showed trends for decreases in the number of blood lymphocytes on both dosing days, however these results were not significantly different from placebo (all P>0.05). PUL-042 significantly increased levels of serum CRP within 8h on Study Day -1 (P=0.0111) and within 24h on Study Day 2 (P=0.0011). CRP returned to pre-treatment levels 24h later on both Study Days.

In the exploratory endpoints, there was no significant difference between placebo and PUL-042 in peak values or AUC in either nasal or sputum virus load, however PUL-042 did cause a significant decrease in Study Day 6 virus load in nasal lavage (P=0.0314). No significant difference was seen on any other day. PUL-042 showed trends for increases in serum and sputum IL-6 compared to placebo on dosing days, however these were not significantly different from placebo (P>0.05). There was no significant difference in IL-6 or IP-10 in nasal or sputum compared to placebo. Sputum cells were also examined, PUL-042 showed trends for increases in total sputum cells and total immune cells on each dosing day, which were driven by increases in sputum neutrophils, and to a lesser extent lymphocytes and monocytes. PUL-042 also showed trends for increases in neutrophils and lymphocytes later during the time course of rhinovirus infection after Study Day 15 however, these results were not significantly different from placebo (P>0.05). There was no significant difference between PUL-042 and placebo regarding FeNO measurements at any time. The effects of PUL-042 on sputum microbiology cultures, including total bacterial counts (including normal flora) and respiratory pathogens were also examined. Only 4 subjects returned positive microbiology cultures, 3 of which were treated with placebo. There was no significant difference in either the amount of total bacterial cultures or respiratory pathogens between each group (P>0.05), or in the number of subjects returning positive tests for any bacteria, or respiratory pathogens. Respiratory bacteria were also assessed using 16S rRNA abundance, and there was no significant difference between PUL-042 and placebo at any time point analysed.

In conclusion, PUL-042 treatment did not significantly affect symptom responses to experimental rhinovirus challenge in GOLD 0 COPD patients who were active smokers. This may in part be influenced by lower than expected or variable lower respiratory symptoms reported in the placebo arm of the study population. PUL-042 may have caused an increase in cough and sputum scores in the EXACT-RS domain. These changes were negatively correlated with changes in FEV1 on Study Day 2 at 2h post dose, possibly suggesting a shared mechanism. The activity of PUL-042 as a direct anti-viral remain unclear, as no sustained suppressive effect was seen on rhinovirus replication, except for a significant decrease in nasal lavage virus load on Study Day 6 which may have been, at least in part, due to investigational drug being administration by facemask in the final seven subjects (4 subjects received PUL-042 and 3 subjects received placebo).

Overall, the data highlight the immunomodulatory effects of PUL-042, as PUL-042 caused a temporary, yet robust, change in systemic and to a lesser extent, local immune biomarkers following inhaled delivery. These increases were not correlated to changes in other biomarkers, safety markers or the tolerability of PUL-042 in the study population and were not correlated with the longitudinal responses to experimental rhinovirus challenge. The transient changes in % predicted FEV1 and FVC seen on the dosing days did not affect responses to RV challenge and were not correlated with the observed transient changes in systemic or local biomarkers.

The study further shows the potential of PUL-042 as an immunomodulator and underscores inhalation as a suitable delivery method for systemic biological activity.