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POSITIVE TOP-LINE RESULTS FROM PRECLINICAL STUDY INVESTIGATING PPS TREATMENT IN ARDS

KEY HIGHLIGHTS

- Top-line results from a preclinical proof-of-concept model of acute respiratory distress syndrome (ARDS) study.
- PPS treatment reduced lung inflammation, improved oxygen saturation and reduced weight loss compared to vehicle treated controls at 8 days post-infection (acute phase) in a mouse model of ARDS mediated by influenza infection.
- PPS treatment demonstrated statistically significant reductions in the levels of complement 3a, a marker of tissue damage in the lung, and in serum levels of inflammatory biomarkers IL-6, INF-gamma, IL-12p70 and CCL2 in influenza infected mice compared to vehicle treated controls in the acute phase of infection.
- PPS at the post-acute phase of infection (21-days post infection) demonstrated a statistically significant reduction in pulmonary fibrosis compared to vehicle treated controls based on histological staining of collagen.
- Proof of concept data supports the potential investigation of PPS for the treatment of acute lung inflammation such as ARDS with ensuing pulmonary fibrosis as a result of viral infection.
- Preclinical data supports potential to expand the PPS product pipeline from its use in the treatment of musculoskeletal indications to acute and chronic respiratory indications with unmet needs.
- People with ARDS have severe shortness of breath and may subsequently develop fibrosis (or lung scarring). This is a serious condition with unmet medical needs and limited therapeutic options. Paradigm has filed a patent for the treatment of ARDS with PPS.

Paradigm Biopharmaceuticals Ltd (ASX: PAR) (Paradigm or the Company) a clinical stage biopharmaceutical company focussed on repurposing existing molecules for new indications with unmet clinical needs, is pleased to announce top line summary of results of the actions of pentosan polysulfate sodium (PPS) in the influenza model of acute respiratory distress syndrome (ARDS). This preclinical study expands the PPS product pipeline from its use in the treatment of musculoskeletal indications in osteoarthritis (OA), Mucopolysaccharidosis (MPS) and alpha-viral induced arthralgia (RRV and CHIKV) into respiratory indications where acute inflammation in the lung is a potential therapeutic target of PPS. The preclinical study was performed at the Menzies Institute at Griffith University.

In order to determine the effects of PPS in ARDS the C57BL/6J mouse model of Influenza A virus infection was used as an exploratory proof-of concept model. The C57BL/6J mouse model of Influenza A virus (IAV) infection is well accepted as a model

of viral lung pathogenesis and has been used extensively to characterise mechanisms of immune response in the lung that led to severe respiratory disease, including antiviral studies, immunotherapy, and vaccination studies[1–5]. In C57BL/6J mice which are particularly susceptible to IAV infection and disease, clinical symptoms observed include anorexia, malaise, reduced blood oxygen saturation following a cytokine storm response[6]. In this preclinical model of IAV infection, the acute phase involving a cytokine 'storm' is followed by a post-acute phase of pulmonary fibrosis[7].

Top line Summary of Data:

The following data summarising the effects of PPS in the exploratory model are being further evaluated in additional experimental models to assess translational relevance of these findings to human studies. The complete data set of results including confirmatory studies will be prepared for peer review and publishing.

- PPS administered subcutaneously at 3 mg/kg and 6mg/kg resulted in a significant reduction in weight loss due to PR/8-induced infection and disease at days 6, 7, and 8 days post-infection compared to vehicle (phosphate-buffered saline) treated infected animals (**Figure 1**).
- PPS administered subcutaneously at Day 0 at 3mg/kg demonstrated a statistically significant improvement in blood oxygen saturation at 8 days post-infection compared to vehicle treated controls (98.1% (SD 1.0) vs 92.7% (4.3) $p<0.05$).
- PPS administered subcutaneously at Day 0 at 3mg/kg resulted in a statistically significant (reduction in inflammatory cell infiltrates in the lungs at 8 days post-infection. This observation is coupled with the presence of infiltrating cellular aggregates in the lung tissue where inflammation is most severe. These infiltrating cellular aggregates were reduced in PPS-treated mice (**Figure 2**).
- PPS administered subcutaneously at 6 mg/kg (N=6 animals) resulted in significant reductions compared to vehicle treated animals (N=6 animals) in the levels of IL-6 ($p<0.05$), IL-12p70 ($p<0.001$), IFN gamma ($p<0.05$) and CCL-2 ($p<0.0001$), in the serum, and a reduction in Complement C3a ($p<0.01$) in the lung at the acute-phase at Day 8 post-infection.
- PPS administered subcutaneously at a dose of 3 mg/kg showed a strong reduction in fibrosis consistently in the lungs of animals at 21 days post-infection compared to the vehicle control group (**Figure 3**). Quantitative analysis of fibrosis assessed by collagen staining (**Figure 4**) demonstrated a significant reduction in fibrosis with PPS treatment compared to the vehicle treated group $p=0.0018$.

Mechanisms of Action (MOA) associated with ARDS

The multiple MOA of PPS have provided the scientific rationale for repurposing the agent in other indications with unmet or poorly met medical needs such as osteoarthritis and in alpha-viral induced arthralgia[8–10].

In this mouse model of influenza-mediated ARDS the pathophysiological changes were demonstrated by weight loss and impaired lung function was demonstrated by reduction in oxygen saturation. Histopathology findings in the lung were associated with increased cellular infiltration and progressive pulmonary fibrosis. These pathogenic mechanisms are driven by the cytokine storm that ensues at the onset of viral infection. The effects mediated by PPS in reducing ARDS in this animal model

are potentially orchestrated by anti-inflammatory actions mediated via the inhibition of the transcription factor NF-κB and other inflammatory processes involving deposition of fibrin, complement-mediated lung injury, cytokine response, inflammation and immune cell infiltration. These actions of PPS require further validation and characterisation in additional experimental models to assess translational relevance of these findings to human studies.

Dr Ravi Krishnan, Paradigm's Chief Science Officer, commented: *"I am very pleased that the data reported in this exploratory preclinical study of influenza virus-induced ARDS has provided preliminary evidence that PPS administered subcutaneously in the acute phase of an inflammatory response has the possibility to target the cytokine 'storm'. Furthermore, the observation that PPS appears to have durable biological activity for an animal study, extending into the post-acute phase of the response to regress the progression of pulmonary fibrosis in the lung is compelling. These findings may have potential implications in halting the progression towards chronic lung disease by early intervention with PPS at the onset of acute lung inflammation where there is a significant unmet medical need. Paradigm's next steps are to consolidate and further validate these data to assess the preclinical translational relevance of these findings to identify the path to possible clinical trials in humans."*

Study Design

8–10-week-old female C57BL/6J mice were intranasally inoculated with the optimal pathogenic, sublethal dose (150 – 300 plaque forming units (PFU)) of H1N1 PR-8 influenza strain at day 0.

A dose of 3 mg/kg or 6 mg/kg of PPS was administered to the mice subcutaneously (s.c.) daily for 8 days. The PPS dose of 3 mg/kg/day corresponds to a human equivalent dose (HED) of 0.24 mg/kg/day (weekly total dose of 1.7 mg/kg); PPS dose of 6 mg/kg/day corresponds to a HED of 0.48 mg/kg/day (weekly total dose of 3.4 mg/kg). Control animals were administered phosphate-buffered saline as the vehicle daily. Experimental groups tested were a) uninfected animals b) infected animals treated with vehicle (phosphate buffered saline) and c) infected animals treated with PPS at 3 mg/kg or d) infected animals treated with PPS at 6 mg/kg.

Clinical Disease Monitoring: Mice were weighed daily to determine disease progression. Pulse oximetry was performed on days 6 and 8 post-infection to measure oxygen saturation (SpO2).

Biomarker analyses were performed on lung extracts as well as serum from animals and assayed to detect pro-inflammatory cytokines CCL-2, IL-6, IFN-γ, IL12p70, IL-10 and TNF-α and complement proteins C3a and C3b.

Histological Analyses: At termination, lung tissue was harvested and sections were stained for haematoxylin and eosin (H&E) to determine cellular infiltration and Masson's trichrome staining used to detect collagen.

Figure 1: PPS administered subcutaneously prevented significant weight loss in C57BL/6J mice caused by PR/8- infection.

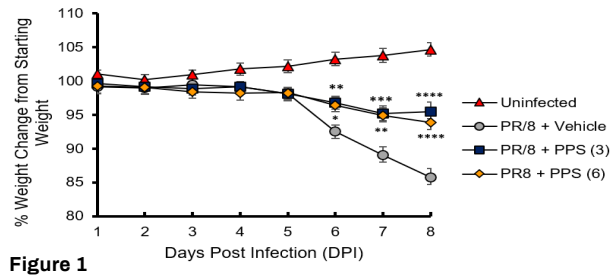


Figure 1

The figure shows the weight loss associated with PR/8 infection in C57BL/6J mice during the course of the acute infection phase of ARDS (statistical analysis is a one-way ANOVA with a Tukey's multiple comparisons test; ****$p < 0.0001$; ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$). These data show that PPS administered subcutaneously at doses of 3 mg/kg and 6 mg/kg in this mouse model of influenza induced ARDS prevents weight loss compared to vehicle treated animals that show excessive weight loss.

Figure 2: PPS administered subcutaneously reduces inflammatory cellular infiltrates in lungs of PR8-infected C57BL/6J mice at 8 days post-infection (acute phase).

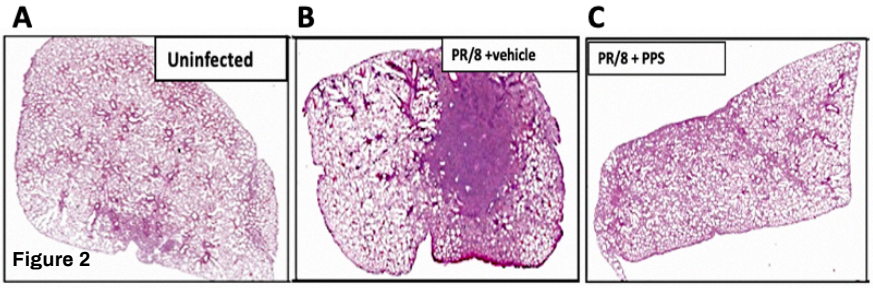


Figure 2

The panels A, B, and C are micrographs of lung sections obtained from mice stained with H&E Stain at Day 8 post-infection representing the acute phase of ARDS. The cell nuclei are stained purple. **Panel A** is a representative section from an uninfected animal; **Panel B** is a representative lung section from an animal infected with influenza virus PR-8 strain of H1N1 and treated s.c. with vehicle and **Panel C** is a representative lung section from an animal infected with influenza virus PR-8 strain of H1N1 and treated s.c. with PPS at dose of 3 mg/kg.

The micrographs demonstrate that PPS treatment resulted in a strong reduction in the interstitial area of the lung compared to the vehicle treated animals. In this set of analysis only the 3 mg/kg PPS group was compared against the vehicle group.

Further quantitative analysis of the H&E staining demonstrated that there was a statistically significant reduction in the number of infiltrates in the lungs of PPS treated animals (N=10 animals dosed at 3mg/kg dose) compared to the vehicle group (N=10 treated with vehicle) as measured by the mean (SD) nuclei/mm^2. (PPS: 5511 nuclei/mm^2 (SD=1114) vs Vehicle: 6573 nuclei/mm^2 (SD=1402); p= 0.0464 (Mann-Whitney U non-parametric test).

Figure 3: PPS administered subcutaneously demonstrated reduction in fibrosis in the lungs of PR/8 infected C57BL/6J mice at 21 days post-infection (post-acute phase).

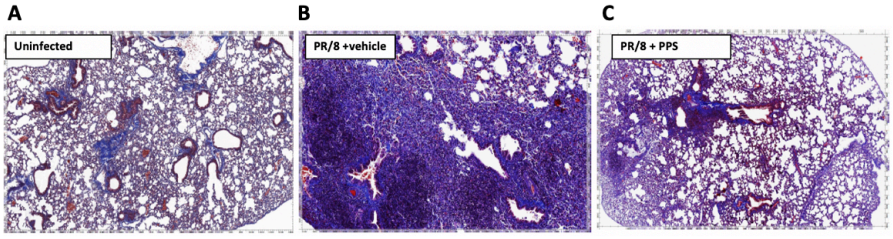
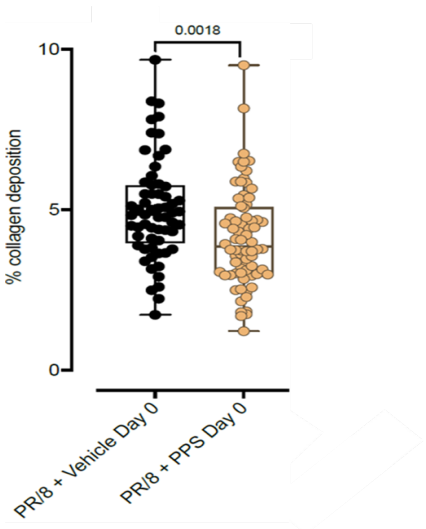


Figure 3

The panels A, B, and C are micrographs of lung sections obtained from mice stained with Masson's Trichrome stain at Day 21 post-infection representing the post-acute phase of ARDS. Collagen, the major component of the fibrotic response was stained blue as shown in the panels. **Panel A** is a representative section from an uninfected animal (normal lung) showing minimal interstitial collagen staining (blue); **Panel B** is a representative lung section from an animal infected with influenza virus PR-8 strain of H1N1 and treated s.c. with vehicle shows very extensive and strong collagen staining in the interstitial area of the lung and **Panel C** is a representative lung section from an animal infected with influenza virus PR-8 strain of H1N1 and treated s.c. with PPS at dose of 3 mg/kg demonstrating a comparative reduction in interstitial collagen staining. The micrographs demonstrate that PPS treatment resulted in a strong reduction in the collagen staining in the interstitial area of the lung compared to the vehicle treated animals. In this set of analysis only the 3 mg/kg PPS group was compared against the vehicle group.

Figure 4: PPS administered subcutaneously demonstrated a statistically significant reduction in fibrosis as measured by collagen deposition in the lungs of PR/8 infected C57BL/6J mice compared to vehicle treated mice at 21 days post-infection (post-acute phase).



Quantitative analysis of collagen by Masson's Trichrome staining demonstrated that there was a statistically significant reduction in the percentage of interstitial collagen in the lungs of PPS treated animals (N=7 animals dosed at 3 mg/kg dose) compared to the vehicle group (N=7 treated with vehicle) PR/8-infected PPS treated mice

displayed a significant reduction in percentage collagen deposition when compared to the untreated PR/8-infected untreated mice at 21 days post-infection (**p = 0.0018).

Mr Paul Rennie, Paradigm's Chief Executive Officer, commented:
"This proof-of-concept preclinical study has provided very exciting results for our drug, pentosan polysulfate sodium (PPS) and it again demonstrates PPS is a platform technology. Paradigm is investigating in late-stage clinical trials the use of PPS in subjects with painful osteoarthritis, joint pain in subjects with the rare genetic disorder mucopolysaccharidosis (MPS). Preclinical proof-of-concept has been completed in joint pain as a result of viral arthritis and now promising data in an ARDS model. These ARDS preclinical data suggest that PPS may have a role to play in the acute and chronic phases of this disease".

About ARDS / Pulmonary fibrosis

Acute respiratory distress syndrome (ARDS) is a life-threatening condition in which fluid collects in the alveoli (air sacs of the lungs), depriving organs of oxygen. People with ARDS have severe shortness of breath and often require ventilation. ARDS usually occurs in those who are critically ill or who have significant injuries. The mainstay interventions are supportive lung protective ventilation and prone positioning. The mortality rate for ARDS is 30-40%[11].

Development of fibrosis in lung tissue is common as ARDS progresses. Fibrosis in lung tissue hinders the ability of the lungs to expand and contract during breathing, resulting in low oxygen levels in the blood (hypoxia), frequently requiring ventilation in an attempt to normalise blood oxygen levels[12]. ARDS survivors may experience post-traumatic stress disorder, post-intensive care syndrome, long-term physical disability, neuromuscular weakness and persistent pulmonary fibrosis (PF) resulting in chronic lung dysfunction[11,13].

Pre-Covid, over 200,000 people were estimated to be living with PF in the US, with approximately 50,000 new cases diagnosed each year and as many as 40,000 deaths per year[14]. The full impact of chronic pulmonary fibrosis associated with the SARS-CoV-2 virus on the overall prevalence of PF is yet to be determined.

Unmet Need

Despite recent advances in therapeutics, supportive lung ventilation and prone positioning of the subject, remain the mainstay interventions for the management of ARDS[11]. Given the rapidity with which ARDS can cause fibrosis of the lung and the difficulty of reversing established fibrosis, an intervention is needed that prevents severe structural damage to the lung and thus tip the scales to facilitate repair to the injured lung[13].

About Paradigm Biopharmaceuticals

Paradigm Biopharmaceuticals LTD (ASX: PAR) is a late-stage drug development company with the mission to develop and commercialise pentosan polysulfate sodium for the treatment of pain associated with musculoskeletal disorders driven by injury, inflammation, ageing, degenerative disease, infection or genetic predisposition. Paradigm is also exploring proof-of-concept studies for the use of PPS in respiratory and heart failure indications.

Forward Looking Statements

This Company announcement contains forward-looking statements, including statements regarding anticipated commencement dates or completions dates of preclinical or clinical trials, regulatory developments and regulatory approval. These forward-looking statements are not guarantees or predictions of future performance, and involve known and unknown risks, uncertainties and other factors, many of which are beyond our control, and which may cause actual results to differ materially from those expressed in the statements contained in this presentation. Readers are cautioned not to put undue reliance on forward-looking statements.

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