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Interim Analysis of a Phase 1 Study of PRTX007: Safety, PK and PD Response

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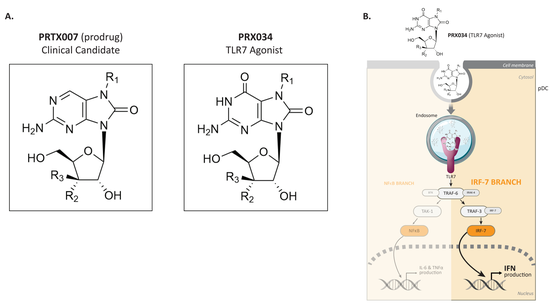
Background

Toll-like receptor 7 (TLR7) is a key sensor of viral infection by ssRNA viruses.[1] Engagement leads to direct activation of plasmacytoid dendritic cells (pDCs) and B cells, and indirect, orchestrated engagement of many other immune cells.[1,2] Furthermore, pDCs form an interferogenic synapse with virally infected cells,[2] targeting local interferon (IFN) production at the site of infection via paracrine transfers. A major limitation in targeting TLR7 is inflammation accompanying activation of 2 key intracellular pathways in pDCs, leading not only to biosynthesis of (a) all human Type I/III IFNs but also to (b) proinflammatory factors like IL-6, TNFα, and IL-1β via NF-κB. In COVID-19, cytokine release syndrome is a major driver of inflammation mediated in part by hyperactivation of the NF-κB pathway. IL-6 and TNFα are key mediators of hyperinflammation.[3,4] Therefore, any effective systemic TLR7 agonist must avoid aggravating this pathology.

PRTX007 is a prodrug of PRX034, a novel TLR7 agonist, for treatment of respiratory viral diseases, including those caused by SARS-CoV-2 (**Figure 1A**). PRX034 activates pDCs to preferentially synthesize poly subtype IFNs while minimizing NF-κB–mediated proinflammatory factors (**Figure 1B**). Preclinical studies with PRX034, for the first time demonstrated the ability to decouple these two normally linked processes in human PBMCs in vitro and in cynomolgus monkeys in vivo. Antiviral activity is preserved, as demonstrated in vitro (inhibition of cytopathicity and viral replication in RNA viruses by conditioned media from PRX034-treated PBMCs) and in vivo (efficacy and safety in a murine model of RSV infection) [unpublished data].



BTK=Bruton's tyrosine kinase; IL-6=interleukin 6; IRF7=interferon regulatory factor 7; IFN=interferon; IRAK-4=interleukin-1 receptor-associated kinase 4; NF-κB= nuclear factor-κB; pDC=plasmacytoid dendritic cell; TAK1=TGF-β-activated kinase 1; TRAF=tumor necrosis factor receptor (TNFR)-associated factor 6.

Figure 1. PRX034 induction of innate immunity without production of proinflammatory factors
A. Structures of PRTX007 and PRX034 **B.** Signalling pathways activated by PRX034 binding to TLR7 in pDCs

Methods

Study Design

- This is a first-in-human, phase 1, single-center, prospective, randomized, double-blind, placebo-controlled study of 9 single-ascending dose (SAD) cohorts and 4 multiple-ascending dose (MAD) cohorts of PRTX007 administered orally to adult healthy volunteers (HVs; **Figure 2**). This phase 1 trial is ongoing in Sydney, Australia
- The primary objective is to assess the safety and tolerability of PRTX007 following SAD and MAD in normal HVs as measured by adverse events, vital signs, clinical laboratory data, electrocardiograms, and physical examinations
- Secondary objectives are to (1) assess the pharmacokinetic (PK) characteristics of PRX034, the active metabolite of prodrug PRTX007, (2) determine the effect of a high-fat meal on a single oral dose of PRTX007 and its metabolite in normal HVs, and (3) assess the pharmacodynamics (PD) of PRTX007 given as single and multiple doses in normal HVs, including reproducibility of key immune response markers
  - Blood was collected from HVs and used for PD analysis of cytokines/chemokines, mRNA expression over 24 hours after dosing for all SAD cohorts
  - For PK analysis, blood was collected over 48 hours
- This interim analysis focuses primarily on the SAD portion of the study of 8 dose levels from 50 mg to 600 mg (**Figure 2**)
  - Safety, PK, and PD are presented for the first 8 SAD cohorts
  - Safety data are presented from 300 mg to 400 mg MAD cohorts
  - 64 of the 84 HVs in this interim analysis received drug

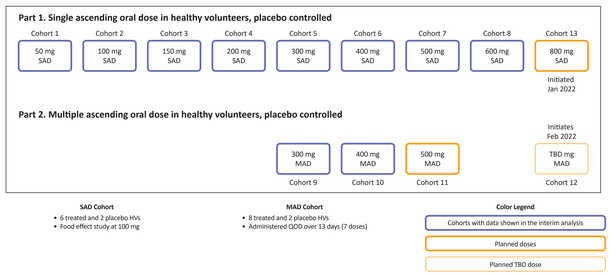


Figure 2. Study design for phase 1 SAD and MAD trial in healthy volunteers

Results

Favorable Safety Profile and Tolerability

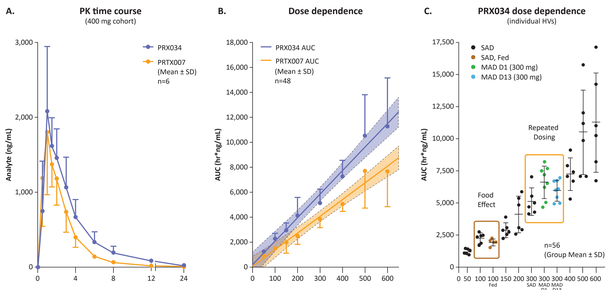
Total Group (N=84)	Mild		Moderate		Severe		Total	
	Related	Not Related	Related	Not Related	Related	Not Related	Related	Not Related
Nervous System Disorders								
Headache	8 (9.5%)	6 (7.1%)	2 (2.4%)	12 (14.3%)	-	-	10 (11.9%)	18 (21.4%)
Cardiac Disorders								
Tachycardia	1 (1.2%)	-	-	-	-	-	1 (1.2%)	-
Sinus Tachycardia	1 (1.2%)	-	-	-	-	-	1 (1.2%)	-
Gastrointestinal Disorders								
Loose Stool	1 (1.2%)	1 (1.2%)	-	-	-	-	-	2 (2.4%)
Diarrhea	1 (1.2%)	-	-	-	-	-	-	1 (1.2%)
Hepatobiliary Disorders								
Elevated ALT	4 (4.8%)	-	-	-	-	-	4 (4.8%)	-
Musculoskeletal and Connective Tissue Disorders								
Lower Back Pain	-	3 (3.6%)	1 (1.2%)	1 (1.2%)	-	-	1 (1.2%)	4 (4.8%)
Immune System Disorders								
Thrombocytopenia	1 (1.2%)	-	-	-	-	-	1 (1.2%)	-

ALT=alanine aminotransferase; MAD=multiple-ascending dose.

Table 1. PRTX007 treatment-related adverse events (up through the 400 mg MAD cohort)

- Treatment-related adverse events include mild to moderate headache
- Severity or frequency is not dose related, is of short duration, and is not associated with systemic symptoms
- One HV in the 400 mg and one HV in the 600 mg SAD cohorts had asymptomatic mild tachycardia attributed to PRTX007
- Two HVs in both the 300 mg MAD, and 400 mg MAD had mild increases in ALT that rapidly resolved after treatment
  - There were no associated changes in aspartate transaminase, bilirubin, or alkaline phosphatase
  - No stopping or dose modifications were required

Well-behaved PK of PRX034 Following Oral Administration of PRTX007



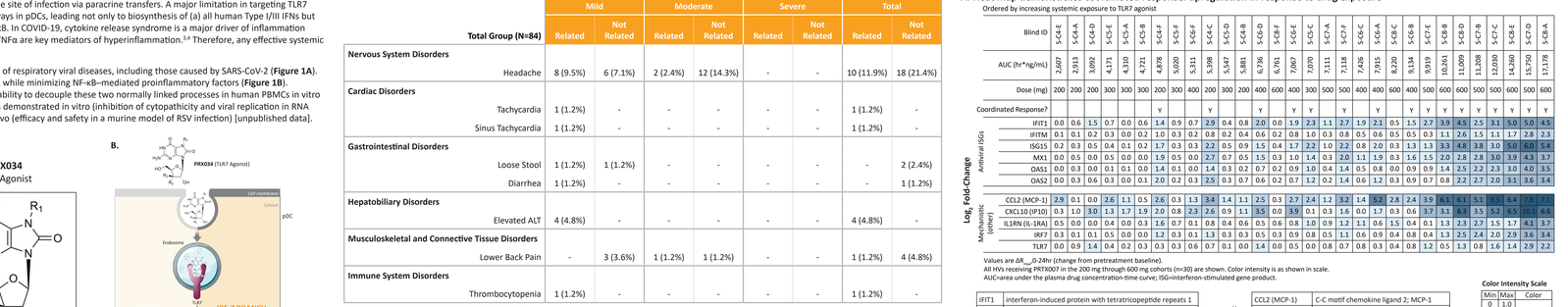
AUC=area under the plasma drug concentration-time curve; D=day; HV=healthy volunteer; MAD=multiple-ascending dose; PK=pharmacokinetics; SD=standard deviation; SAD=single-ascending dose.

Figure 3. Human pharmacokinetics (SAD cohorts 1-8, 50-600 mg; MAD 9, 300 mg PRTX007)

- Rapid absorption and conversion of prodrug PRTX007 to agonist PRX034 following oral administration (**Figure 3A**)
- Targeted short duration of pulsatile exposure to PRX034 (**Figure 3A**)
  - Duration of systemic exposure to PRX034 at pharmacologically active levels is consistent with activation of innate immune response without counter-regulation
- Dose-proportional increase in exposure to prodrug and active agonist (**Figure 3B**)
- Agonist/prodrug AUC ~1.7 for all HVs (**Figure 3B**)
- Minimal change in exposure with high-fat meal (modest delay in absorption; brown box; **Figure 3C**)
- Exposure unchanged between first (D1, green) MAD and seventh (D13, light blue) MAD doses (gold box; **Figure 3C**)

Induction of IFN-Stimulated Gene Products (ISG) and Other TLR7-Associated Cytokines Without NF-κB-Mediated Inflammatory Cytokines (IL-18, IL-6, TNFα)

Figure 4. Expression analysis (mRNA) from whole blood
A. Heat map demonstrates coordinated response: upregulation in response to drug exposure



Values are $2R_{-0.24hr}$ (change from pretreatment baseline).
All HVs receiving PRTX007 in the 200 mg through 600 mg cohorts (n=30) are shown. Color intensity is as shown in scale.
AUC=area under the plasma drug concentration-time curve; ISG=interferon-stimulated gene product.

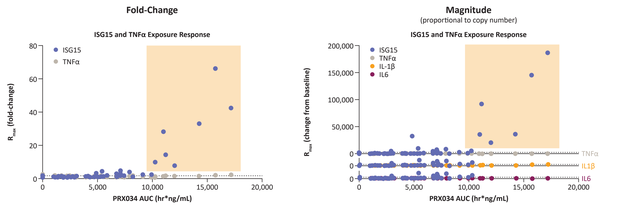
Antiviral ISGs	IFIT1	interferon-induced protein with tetratricopeptide repeats 1
	IFITM	interferon-induced transmembrane protein 1
	ISG15	ISG15 ubiquitin-like modifier
	MX1	MX dynamin-like guanosine triphosphate (GTP)ase 1
	OAS1	2′-5′-oligoadenylate synthetase 1
	OAS2	2′-5′-oligoadenylate synthetase 2

Mechanistic (other)	CCL2 (MCP-1)	C-C motif chemokine ligand 2; MCP-1
	CXCL10 (IP-10)	C-X-C motif chemokine ligand 10; IP-10
	IL1RN (IL-1RA)	interleukin 1 receptor antagonist; IL-1RA
	IRF7	interferon regulatory factor 7
	TLR7	toll-like receptor 7

Color Intensity Scale		
Min	Max	Color
0	1.0	
1	2.0	
2	3.0	
3	4.0	
4	5.0	
5	6.0	
6	7.0	
7	10.5	

- The induction of coordinated response was observed in the 200-600 mg dose groups; coordinated response rate increases with dose and is highly correlated with exposure to PRX034
- No HVs receiving placebo, or 50 mg, 100 mg, or 150 mg doses of PRTX007 in the SAD cohorts (n=34) exhibited coordinated induction; therefore, they were omitted from the graphic

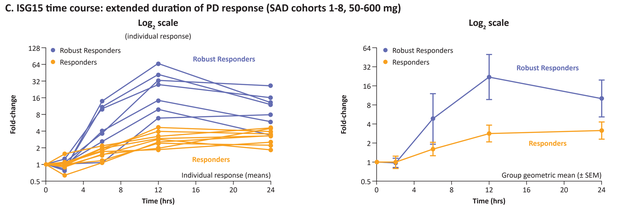
B. Robust IFN-mediated response without induction of proinflammatory factors



AUC=area under the plasma drug concentration-time curve; IFN=interferon; IL=interleukin; ISG15=interferon-stimulated gene 15; TNFα=tumor necrosis factor α.

- ISG15 mRNA induction increases with exposure to active agonist; accordingly, the magnitude of response is a function of dose. Shown are SAD cohorts 1 through 8, 50 mg to 600 mg
- ISG15 RNA levels are at, or exceeding, levels in the blood associated with antiviral therapeutic benefit based on reference to published ANA773 (a TLR7 agonist)[5,6,7]
- AUCs likely to be associated with therapeutic benefit for ISG15 are also indicated in the boxes in **Figure 4B**; benefit may be observed at lower exposures
  - The dashed line is the geometric mean (ISG15) + 2*SD of placebo group
- No induction of proinflammatory factors (TNFα, IL-1β, IL-6; right panel)

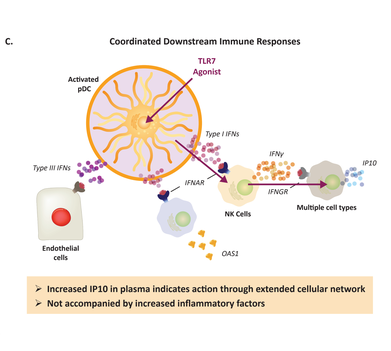
C. ISG15 time course: extended duration of PD response (SAD cohorts 1-8, 50-600 mg)



ISG15=interferon-stimulated gene 15; PD=pharmacodynamic; SAD=single-ascending dose; SEM=standard error of the mean.

- There is an extended duration of PD response over 24 hours
  - HVs were classified by anonymized ID and color-coded by ISG15 fold-change.
  - Nonresponders (clear) = ISG15 fold-change <2-fold (n=32)
  - Responders (gold) = ISG15 fold-change from ≥2- to <5-fold (n=9)
  - Robust Responders (purple) = ISG15 fold-change ≥5-fold (n=7)
- The duration of PD response is in excess of duration of exposure to active levels of PRX034 (see also **Figure 3A**, PK analysis)

IP10 Induction Demonstrates Coordinated Downstream Immune Cascade



AUC=area under the plasma drug concentration-time curve; CXCL10=C-X-C motif chemokine ligand 10; IFN=interferon; IFNAR=interferon alpha receptor; IFNGR=interferon gamma receptor; IP10=interferon gamma-induced protein 10; OAS1=2′-5′-oligoadenylate synthetase 1; pDC=plasmacytoid dendritic cell; TLR7=toll-like receptor 7.

Figure 5. IP10 (CXCL10): breadth of immune induction

- Selected circulating markers induced by PRX034 exposure in 50-600 mg SAD
  - IP10, IL-1RA, MCP1, TRAIL are expressed at high levels in plasma (data not shown for IL-1RA, MCP1, or TRAIL)
  - IP10 protein levels and mRNA increase in response to drug exposure (**Figure 5A** and **B**)
    - Panel A, n=48; Panel B, n=64 (this includes placebo-treated HVs)
    - The inset for Panel B shows that in the lower exposure range, there are some responses that occur at lower magnitudes. These responders are above the dashed line (the dashed line [n=16] represents the geometric mean of IP10 mRNA + 2*SD of placebo group; therefore, responders above this line likely represent signal above noise)
  - IP10 levels are at, or exceeding, levels in the plasma associated with antiviral therapeutic benefit based on reference to published ANA773 (a TLR7 agonist) data[5,6]
  - The AUC expected to be associated with therapeutic benefit for IP10 is indicated in the box in **Figure 5**; benefit may be observed at lower exposure
  - There is no increase in circulating IFNα (<LOQ with standard assay); however, detectable increases in IFNβ (<100 pg/mL) accompanied IP10 increase (**Figure 5A**, circles)
    - IFNβ movement was not seen except in conjunction with those IP10 points highlighted in the 5 HVs with higher AUC exposures (**Figure 5A**, circles)
- Increased IP10 in plasma indicates activation of multiple cell types downstream from activated pDCs (see cell types in **Figure 5C**)
- Inflammatory factor production is not observed even in the face of this profound immune stimulation (**Figure 4B**)

- Increased IP10 in plasma indicates action through extended cellular network
- Not accompanied by increased inflammatory factors

Conclusions

This phase 1 study demonstrates:

- Efficient systemic delivery and well-behaved PK of TLR7 agonist PRX034 by oral administration of the prodrug PRTX007
- Dose-dependent and exposure-dependent coordinated induction of TLR7-mediated immune response
  - Agonist exposure in excess of 4300 hr*ng/mL is required for pharmacologic activity
  - Degree of immune induction well managed above threshold
- Expected pattern of coordinated TLR7-mediated immune induction observed without increases in IL-6, TNFα, IL-1β
- Magnitude of immune induction expected to translate to therapeutic benefit based on benchmarking to published clinical studies by Anadys Pharmaceuticals[5,6,7]
- In sum, interim analysis of PRTX007 demonstrates a favorable safety profile with dose-dependent systemic exposure and demonstrated activation of innate immune response

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Disclosures

This study was funded by Primmune Therapeutics, Inc. James Appleman and Richard Daniels are employees and stockholders of Primmune Therapeutics, Inc. Charlotte Lemech, Christopher Argent, and Curtis Scribner are independent contractors for Primmune Therapeutics, Inc.

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