PRTX007, a TLR7 Agonist, Demonstrates Broad-Spectrum Antiviral Activity and Is Appropriate for Pandemic Preparedness

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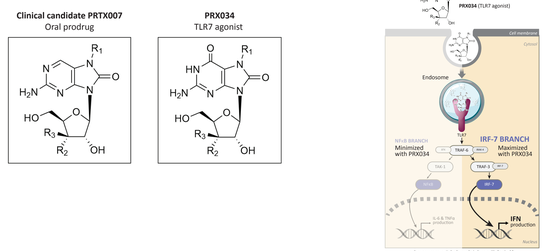
Background

We have experienced devastating viral outbreaks involving emerging viruses such as SARS coronaviruses, Zika, dengue, and Ebola. Despite the existence of some successful antivirals, small-molecule, broad-spectrum drugs against emerging viruses are limited. Toll-like receptor (TLR)7 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] Within the innate immune system, pDCs are key effector cells in combatting viral infection.[1,3] TLR7 ligand–mediated stimulation of pDCs leads to high constitutive expression of IRF-7, allowing them to respond to viruses with rapid, robust interferon (IFN) production. Moreover, pDCs form an interferogenic synapse with virally infected cells, targeting local IFN production at the infection site via paracrine transfers.[4] The challenge is that many viruses evade detection by TLR sensors.[1,3]

The oral prodrug PRTX007 converts to PRX034, its corresponding systemically acting TLR7 agonist (**Figure 1A**), which can activate pDCs by binding to the guanosine site on the TLR7 (**Figure 1B**) and overcome viral evasion of TLR7 activation. Historically, the challenge with activation of pDCs via TLR7 engagement has been that although a type I IFN response is pivotal for antiviral defense and leads to secretion of IFN I/III and IFN-stimulated genes, concurrent induction of NF-κB–mediated proinflammatory factors like IL-6, TNFα, are known to be detrimental to the host in large quantities.[1,2] In a variety of respiratory viral diseases, including COVID-19,[5] cytokine release syndrome is a major driver of inflammation, mediated partly by hyperactivation of the NF-kB pathway. IL-6 and TNFα are key mediators of hyperinflammation. Therefore, any effective systemic TLR7 agonist must avoid aggravating this pathology.

Here we report the broad-spectrum antiviral activity in vitro and in vivo (murine model) of PRTX007 the oral prodrug and PRX034 (**Figure 1A**), which can stimulate induction of IFNs without induction of the NF-κB pathway, leading to biosynthesis of proinflammatory cytokines (**Figure 1B**).



BTK=Bruton's tyrosine kinase; IL-6=interleukin 6; IRF-7=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; TNFα= tumor necrosis factor alpha; TRAF=tumor necrosis factor receptor (TNFR)-associated factor 6.

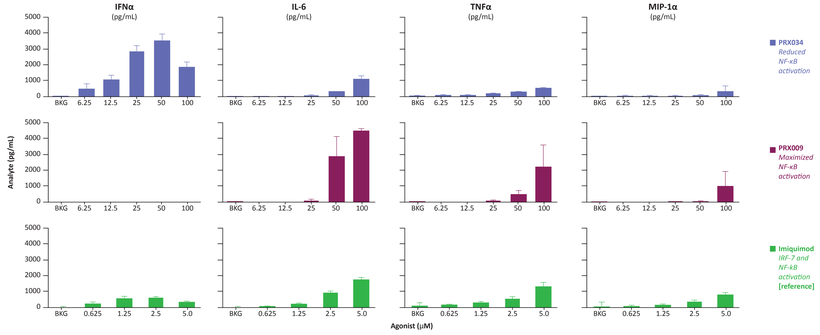
Figure 1. PRX034 induction of innate immunity without production of proinflammatory factors
A. Structures of PRTX007 and PRX034. **B.** PRX034 binds the host TLR7 receptor, triggering an intracellular signaling cascade within pDCs. PRX034 preferentially activates IRF-7 branch, leading to robust IFN production with minimal NF-κB pathway activation.

Methods

- **In vitro viral challenge with conditioned media (CM) and in vivo murine respiratory syncytial virus (RSV) viral challenge.** PRTX007's antiviral activity was assessed in a standard cellular model of viral infection using CM prepared by incubating human peripheral blood mononuclear cells (hPBMCs) with PRX034 and harvesting the supernatant. hPBMCs were isolated using standard procedure. For the RSV murine model, female BALB/c mice older than 6 weeks on arrival received PRX118 (PRX034 prodrug) administered at 60 mg/kg by intravenous (IV) bolus (n = 7/group). Intranasal infections were done with 50 µL RSV-A2 across both nares, ~$2x10^6$ pfu/mL
- **Cytokine release assay from hPBMCs.** hPBMCS were prepared by standard procedure; after 24-hour incubation with each treatment, supernatant was collected and analyzed for cytokine secretion using Millliplex kits (Merck, Cat# HCYTOMAG-60K-04; Cat# HCYTA-60K-04) according to manufacturer instruction. Cytokines analyzed were: IFNα2, IL-6, TNFα, and macrophage inflammatory protein-1α. IFNα2 is a well-known isoform of IFNα reported in the literature and its levels reflect the level IFN type in response that occurs in pDC and B cells after TLR7 stimulation[1]
- **Phase 1 study.** This first-in-human, single-center, prospective, randomized, double-blind, placebo-controlled phase 1 study of 9 single-ascending dose (SAD) cohorts and 4 multiple-ascending dose (MAD) cohorts of PRTX007 administered orally to adult healthy volunteers (HVs) is ongoing in Sydney, Australia. The primary objective is to assess the safety and tolerability of PRTX007 following SAD and MAD in normal HVs. Multiple secondary objectives include assessment of 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 and mRNA expression over 24 hours postdose for all SAD cohorts. This poster presents PD data for the first 8 SAD cohorts (50 mg-600 mg)

Results

PRX034 is a TLR7 Agonist That Induces IFN Expression With Reduced NF-κB Activation

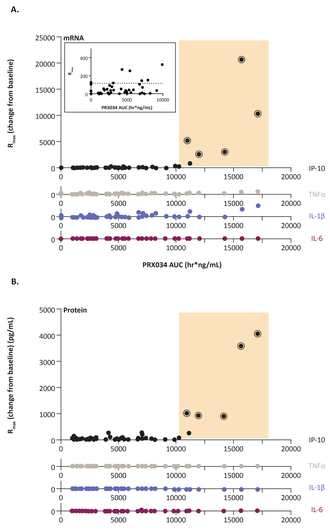


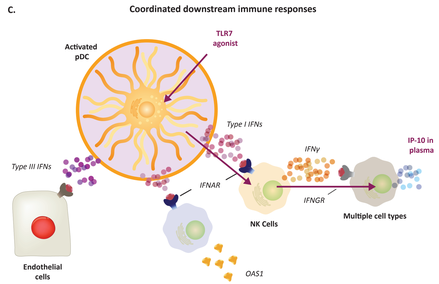
BKG=background; IFN=interferon; IL-6=interleukin 6; IRF-7=interferon regulatory factor 7; MIP-1A=macrophage inflammatory protein 1A; NF-κB=nuclear factor kappa B; PBMC=peripheral blood mononuclear cell; TLR7=toll-like receptor 7; TNFα=tumor necrosis factor alpha.

Figure 2. Two TLR7-specific agonists, PRX034 and PRX009, elicit distinct cellular pharmacology.
Human PBMC cytokine release profiles of PRX034, PRX009, and imiquimod are shown. The active metabolite of PRTX007, PRX034, was utilized for in vitro assays. IFNα2, IL-6, TNFα, and MIP-1α release (pg/mL) in media after 24 hours' treatment with agonists: PRX034 (n = 49), blue bars; PRX009 (n = 4), maroon bars; imiquimod (n = 49), green bars; in human PBMCs. Imiquimod serves as a reference, indicating a traditional TLR7 agonist that engages both arms of the TLR7 signaling pathways (IRF-7 and NF-κB). Error bars are standard error of the mean.

- We have successfully decoupled the link between NF-κB activation and induction of IFN biosynthesis, as shown in preclinical (**Figure 2**) and clinical studies of HVs (**Figure 3**)
- Preclinically, PRX034 shows reduced NF-κB activation (see **Figure 1B** for pathway); by contrast, PRX009 demonstrates a highly elevated or maximized NF-κB activation (**Figure 2**)
  - PRX009 induces <5 pg/mL of IFNα vs 3556 pg/mL induced at the same concentration of 50 µM of PRX034, a >3500-fold difference in induction
  - Conversely, at 50 µM of PRX009, 2851 pg/mL of IL-6 were induced, vs 210 pg/mL induced at the same concentration for the PRX034 agonist
  - Similarly, 100 µM PRX009 induced 2204 pg/mL of TNFα vs 535 pg/mL at the same concentration of PRX034
- The imiquimod served as a reference/control for a traditional TLR7 agonist, showing activation of both arms of the TLR7 signaling pathways (IRF-7 and NF-κB; see also **Figure 1B**)

IP-10 Induction Demonstrates Coordinated Downstream Immune Cascade in the Absence of Inflammatory Markers





AUC=area under the plasma drug concentration-time curve; CXCL10=C-X-C motif chemokine ligand 10; GM=geometric mean; HV=healthy volunteer; IFN=interferon; IFNAR=IFN alpha receptor; IFNGR=IFN gamma receptor; IL=interleukin; IP-10=interferon gamma-induced protein 10; LOQ=limit of quantitation; NF-κB=nuclear factor kappa B; NK=natural killer; OAS1=2'-5'-oligoadenylate synthetase 1; R_{max}=maximum response; SAD=single-ascending dose; SD=standard deviation; TLR7=toll-like receptor 7; TNFα=tumor necrosis factor alpha.

Figure 3. IP-10 (CXCL10): breadth of immune induction without induction of NF-κB pathway products.
A. mRNA expression of markers after a SAD of PRTX007 at 50 to 600 mg (n = 64; includes placebo-treated HVs). The inset shows that in addition to robust responses highlighted in the shaded area of the mRNA figure, some statistically significant responses (values above dashed line) of lower magnitudes also occur at lower exposures to PRX034. The threshold for significance is set from the placebo group (n = 16) as the geometric mean (GM) of IP10 mRNA + 2*SD of the GM; values above this line represent true responders. **B.** Protein expression of key immune response markers in HVs after a SAD of PRTX007 at 50 to 600 mg (n = 48; does not include placebo). **C.** Coordinated downstream immune response.

- Selected circulating markers induced by PRX034 exposure in 50 to 600 mg SAD in HVs
  - IP-10, IL-1RA, monocyte chemoattractant protein 1 (MCP1), and tumor necrosis factor–related apoptosis-inducing ligand (TRAIL) are expressed at high levels in plasma (data not shown for IL-1RA, MCP1, or TRAIL)
  - IP-10 protein levels and mRNA levels increase in response to drug exposure (**Figure 3A** and **B**)
  - IP-10 levels meet or exceed levels in plasma associated with antiviral therapeutic benefit based on reference to published ANA773 (a TLR7 agonist) data[6,7]
  - The AUC of PRX034 expected to be associated with therapeutic benefit based on correlation with increased circulating IP-10 is indicated in the box in **Figure 3**; 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 IP-10 increase in certain subjects (**Figure 3A**, circled data points)
- Increased IP-10 in plasma indicates activation of multiple cell types downstream from activated pDCs (see cell types in **Figure 3C**)
- Inflammatory factor production (IL-6, TNFα, IL-1β) is not observed even in the face of this profound immune stimulation (**Figure 3A** and **B**)

- Increased IP-10 in plasma indicates action through extended cellular network
- Not accompanied by increased inflammatory factors

Broad Spectrum Antiviral Activity Demonstrated Against SARS-CoV-2 and Other RNA Viruses In Vitro

	EC_{50} as IFNα2a Concentration in Assay (pg/mL)		
	CM IFNα2a	Exogenous IFNα2a	Exog. IFNα2a/CM
SARS-CoV-2 USA WA1/2020	41.5	4455	107
Coronavirus 229E[a]	<3	<3000	–
Influenza H1N1	19	54	2.9
RSV A2[b]	121	1950	16
Rhinovirus-14	57.3	1125	19.6
HCV 1b Replicon	<0.03	<0.03	–
Dengue Serotype 2	<3	<300	–
Zika PRVABC 59	0.5	5	10

- PRX034 CM inhibits 8 RNA viruses in vitro (**Table 1**), including SARS-CoV-2, at greater potency than what would be expected by treatment with IFNα2a (see ratio of potencies expressed as EC_{50}s for exogenously added IFNα2a and CM based on its IFNα2a content; Exog. IFNα2a/CM in **Table**)

CM=conditioned media; Exog.=exogenous; EC_{50}=half maximal effective concentration; IFNα2=human interferon alpha-2; RSV A2=human respiratory syncytial virus strain A2; SARS-CoV-2 USA WA1/2020=severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolates USA-WA1/2020.
[a]This is a 6-day study.
[b]This is a prophylaxis.

Table 1. PRX034 in vitro viral challenge studies: inhibition of viral replication following incubation with PRX034 conditioned media with human peripheral blood mononuclear cells

Confirmation of Antiviral Activity in an Animal Model (Mouse Model of RSV Infection)

A. **Viral titer (plaque-forming units from lung)**

Group	Treatment	Read time postinf	Geometric mean (GM)	Log_{10} GM	Log_{10} SD	Log_{10} decrease
1	Vehicle (control)	48h	1.40×10^3	3.15	0.55	
2	PRX118 q24h	48h	6.06×10^1	1.78	1.70	**1.36**
3	PRX118 q12h	48h	6.29×10^0	0.8	1.36	**2.35**
4	Vehicle (control)	96h	2.00×10^4	4.30	0.76	
5	PRX118 q24h	96h	2.54×10^2	2.41	1.71	**1.90**
6	PRX118 q12h	96h	9.76×10^2	2.99	1.42	**1.31**

GM=geometric mean; h=hour; LOD=limit of detection; RSV=respiratory syncytial virus; SD=standard deviation.

B.

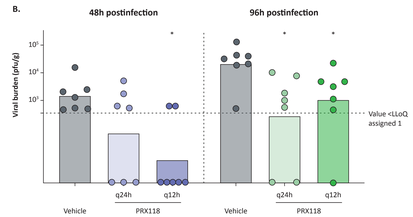


Figure 4. Time course of antiviral activity of PRX034 in mouse model of RSV infection.
Treatment was initiated 12 hours prior to infection with either vehicle (control), or PRX118 at q24h and q12h (n = 7 mice/group). Viral titers (plaque-forming units [PFU] from lung) were collected at 48 hours and 96 hours postinfection. PRX034 was delivered by IV administration of the prodrug PRX118, because PRTX007 conversion to PRX034 is inefficient in rodents. **A.** Table of viral titer results, including geometric mean and standard deviation. **B.** Individual viral titers from RSV-infected mice shown as colored dots. GMs for each treatment are shown as bars moving from left to right and correspond to treatment groups in table. Asterisks indicate statistical significance with a $P = .05$ by Kruskal-Wallis test. Dashed line indicates LoQ, values <LLoQ were assigned a 1.

- This is the second study evaluating the impact of our drug in a mouse model of RSV infection and evaluates the time course of antiviral activity
  - Significant reduction in amount of infectious virus in lung (measured as plaque-forming units) demonstrated at 48 and 96 hours postinfection (**Figure 4**)
    - Most relevant study endpoint indicating antiviral response
    - Decrease in viral RNA also observed, albeit of lower magnitude
  - In a previous study, viral load reduction with systemic PRX034 was comparable with intranasal IFNα and imiquimod
- No evidence of toxicity or adverse effects (AEs) with treatment
  - Slight increase in circulating levels of TNFα observed with q12h but not q24h dosing (no increase in IL-6 with either treatment schedule)

Key Attributes to Support Stockpiling for Pandemic Preparedness

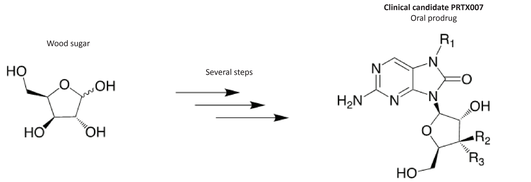


Figure 5. Manufacturing scheme for PRTX007

- PRTX007 can be synthesized on a large scale (**Figure 5**), with suitable stability (**Table 2**) to support stockpiling for pandemic preparedness
  - Crystalline, pharmaceutically acceptable salt forms identified for active pharmaceutical ingredient
  - Starting materials
    - Naturally occurring sugar feedstock
    - Accessible purine base
- As a stable, orally administered therapeutic, it could be readily distributed to the population in need (**Table 2**)
- The low water activity (<0.44) shown by PRTX007 in capsules is demonstrated to not support spore germination or microbial growth (USP <1112>), making the drug self-preserved

Conditions (temperature/%RH)	Study duration	% Purity change from T = 0
40°C/75% RH (accelerated)	6 months	<0.1%
25°C/60% RH (standard RT)	12 months	<0.1%
Projected retest	24 months	

%RH = percent relative humidity; RT = room temperature; T = 0 = time of release

Table 2. PRTX007 stability: room-temperature stable; driven by inherent chemical stability

Conclusions

- Broad-spectrum antiviral activity is demonstrated in vitro (against SARS-CoV-2 and 7 other RNA viruses) by the TLR7 agonist, PRX034; antiviral activity of PRX034 is confirmed in vivo (mouse model of RSV infection), with no evidence of toxicity or AE with treatment
  - Significant reduction in amount of infectious virus in lung (measured as PFUs) is demonstrated at 48 and 96 hours postinfection ($P = .05$)
  - No evidence of AE with treatment in the RSV murine model
- Expected pattern of coordinated TLR7-mediated immune induction is observed without increases in IL-6, TNFα, or IL-1β
- Magnitude of immune induction is expected to translate to therapeutic benefit based on benchmarking to published clinical studies by Anadys Pharmaceuticals[6,7]
  - PRX034's mechanism of action makes it uniquely suitable as a clinical candidate to successfully treat acute viral infections without exacerbating inflammatory pathology or being subject to development of antiviral resistance
- PRTX007, as the prodrug of PRX034, can be synthesized on a large scale, with suitable stability to support stockpiling for pandemic preparedness
  - As a stable, orally administered therapeutic, it could be readily distributed to the population in need

References

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