Activation of innate and adaptive immune response with a clinical stage TLR7 agonist prodrug PRTX007

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CT180

primmune Therapeutics

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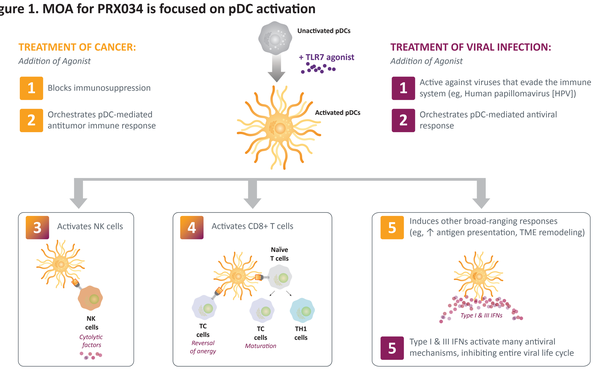
Background

- Use of the toll-like receptor 7 (TLR7) pathway to stimulate innate and adaptive immunity for treatment of cancer and multiple viral diseases has been well characterized
- Historically, systemically administered TLR7 agonists present a variety of challenges in providing therapeutic benefit that have been successfully addressed with PRTX007
- PRX034, an orally administered TLR7 agonist comprised of the prodrug PRTX007, elicits a robust, well-tolerated immune response involving activation of plasmacytoid dendritic cells (pDCs), CD8+ T cells, and natural killer (NK) cells throughout the body with no evidence of nuclear factor κB (NF-κB)–mediated inflammation[1,2]; it is expected to also activate these cells in cancer patient tumor microenvironments
- We performed a first-in-human, phase 1, prospective, randomized, double-blind, placebo-controlled, single-ascending dose (SAD) and multiple-ascending dose (MAD) study of PRTX007, and reported interim results on the following[2]:
  - PRTX007 demonstrated a favorable safety profile with no serious adverse events (SAEs) and a lack of the adverse events (AEs) historically associated with TLR7 agonists
  - Robust systemic immune induction was achieved, without counter-regulation or evidence of systemic inflammation, in healthy volunteers (HVs) receiving PRTX007 in the 300- to 500-mg MAD cohorts
- Here we report clinical pooled safety for the phase 1 study and new data on the immune correlate endpoints for the 750-mg cohort
- Data from preclinical studies (including primates) and this phase 1 clinical study suggest a novel mechanism of action (MOA) for PRX034. Like other classical TLR7 agonists, PRX034 stimulates systemic immune induction via activation of pDCs; however, unlike the classical TLR7 agonists, downstream activation is restricted to NK cells (innate immunity) and naïve and cytotoxic T cells (adaptive immunity), with minimal activation of B cells (**Figure 1**). The activation and downstream cascade elicit effective antitumor and antiviral responses

Mechanism of Action (MOA)

Activation of pDCs by TLR7 Agonist PRX034 Elicits Effective Antitumor and Antiviral Responses

Figure 1. MOA for PRX034 is focused on pDC activation



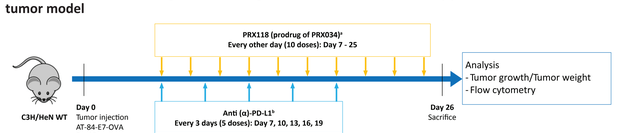
IFN=interferon; NK=natural killer; TC=cytotoxic T cell; TH1= T helper type 1; TME=tumor and its surrounding microenvironment; TLR7=toll-like receptor 7.

- **PRTX007** is an oral prodrug that delivers the TLR7 agonist **PRX034** throughout the body
- PRX034 pharmacologic activities are more restricted than most TLR7 agonists
  - Avoids induction of NFκB-mediated biosynthesis of proinflammatory factors
  - Minimizes activation of B cells
- **PRX034** pharmacologic activity engages CD8+ T cells and NK cells, the two most important immune cells for killing cancer cells
- **Avoids systemic inflammation while delivering therapeutic benefit**

PRX118 and the Mouse Model

Experimental Design

Figure 2. Experimental design for assessing mono and combination therapy of PRX118 in a mouse tumor model



Tumor cell
AT-84-E7-OVA
5×10^5 cells in right flank

Mouse
C3H/HeN, female, 12 weeks old

Analysis
- Tumor growth
- Flow cytometry

Groups (n=7 or 8 per group)
1. Control (PBS) (n=8)
2. α-PD-L1 (n=8)
3. PRX118 (n=7)
4. PRX118 + α-PD-L1 (n=7)

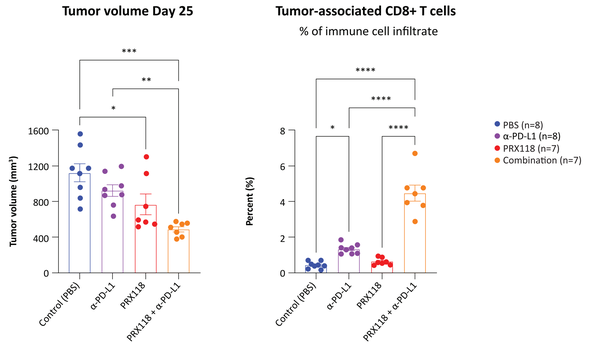
PRX118: 100 mg/kg, every 2 days, total 10 doses, i.p.
α-PD-L1: 200 μg/mouse, every 3 days, total 5 doses, i.p.

i.p.=intraperitoneal; PBS=phosphate buffered saline.
[a]PRX118, a mouse-compatible prodrug surrogate for PRTX007 that converts to PRX034.
[b]Anti (α)-PD-L1, an antibody directed against programmed cell death ligand 1 (PD-L1) and used an immune checkpoint inhibitor.

- Preclinical testing of the MOA was carried out in a mouse tumor model (**Figure 2**)
- Mono and combination immunotherapy of PRX118, the mouse-compatible prodrug surrogate for PRTX007, was assessed in a murine tumor model expressing the E7 oncogene from the human papillomavirus (HPV)[3,4]

PRX118 Mono and Combination Immunotherapy in Murine Tumor Model Expressing the E7 Oncogene From the HPV Demonstrate Importance of TLR7 Agonist

Figure 3. Tumor volume reduction and enhanced infiltration of tumor-associated CD8+ T-cells observed after mono and combination therapy

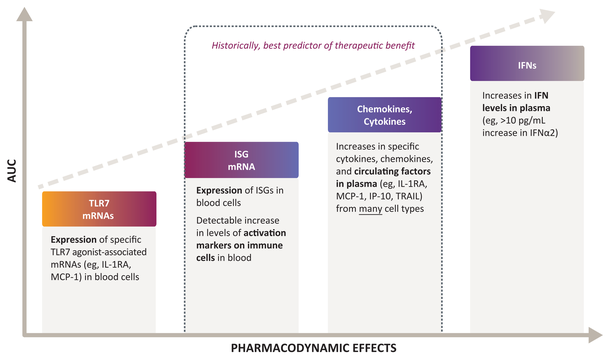


*P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001; ****P ≤ 0.0001

- Significant antitumor activity of TLR7 agonist monotherapy was observed (exceeding that of PD-L1 inhibitor alone) (**Figure 3, left panel**)
  - Increased antitumor activity was obtained when combining these 2 mechanisms
- The percent of tumor growth inhibition observed at Day 25 compared to placebo was 18% for α-PD-L1, 31% for PRX118, and 56% for combination therapy (PRX118 + α-PD-L1)
- Orthogonal antitumor mechanisms were induced (**Figure 3, right panel**)
- An increase in CD8+ T-cell tumor infiltration was observed with combination therapy compared with monotherapy
  - Systemic activation of CD8+ T cells resulted in increased CD8+ T-cell infiltration in the presence of the CPI, resulting in increased killing of tumor cells
- PRX034 and closely related compounds have also demonstrated activity in CT26 colon carcinoma and B16F10 melanoma rodent tumor models (data not shown)

Overview of Pharmacodynamic (PD) Markers and Relative Sensitivity to PRTX007 Dose in the Phase 1a Clinical Study

Figure 4. PD markers and their sensitivity to optimized TLR7 agonists

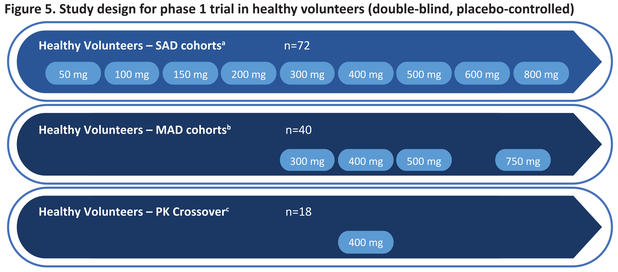


AUC=area under the plasma drug concentration-time curve; ISG=interferon-stimulated gene.

Methods

- This was 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 every other day (QOD) orally to adult healthy volunteers (HVs) in Sydney, Australia[2]
  - The 500-mg MAD cohort terminated early (after 5th dose) because of COVID-19
- Primary objectives were to assess clinical safety and tolerability of PRTX007 in HVs
- Secondary objectives were to (1) assess the pharmacokinetic (PK) characteristics of both PRTX007 and PRX034, (2) assess the PD responses of PRX034 over single and multiple doses in normal HVs, and (3) compare the PK profiles of free-base and salt forms of PRTX007
- Rationale for conducting a phase 1 study in HVs included: (1) identification of drug-specific AEs and PD markers, which is not possible to do in combination therapy in oncology patients, (2) increased clinical sampling, allowing investigators to build a comprehensive PD profile, and (3) identification of the specific active dosing range for use in cancer studies, which will help significantly reduce time, costs, and patient burden in future studies

Figure 5. Study design for phase 1 trial in healthy volunteers (double-blind, placebo-controlled)



[a]Each SAD cohort contained 6 treated and 2 placebo HVs/group; food effect study at 100 mg.
[b]Each MAD cohort contained 8 treated and 2 placebo HVs/group; administered QOD over 13 days (7 doses).
[c]Comparison of free-base and salt forms of PRTX007; single dose, crossover design.

Results

Clinical Pooled Safety Summary

AE profile for the 104 of 130 HVs receiving study drug.
The total number of AEs recorded in treated HVs was 110, with 42 attributed to PRTX007.

- There were no Grade 3 or higher AEs
- There were no clinically meaningful changes observed in creatinine, BUN, or uric acid
- There was no dose modification or discontinuation due to treatment-related AEs

Table 1. Most common AEs coded as treatment-related compared with placebo

AEs (X from N=130)	Placebo (n=26)	PRTX007 (n=104)	PRTX007 Percent (X/104=%) Grade Response		
			Grade 1	Grade 2	Grade 3
Nervous System Disorders					
Headache (40)[a]	4	17[a]	12%	5%	0
Hepatobiliary Disorders					
Elevated ALT (5)[b]	0	5[b]	5%[c]	0	0
General Disorders					
Fever (transient) (4)	1	3[d]	3%	0	0

ALT=alanine transaminase
[a]19 additional headaches in the PRTX007-treated group were coded as unrelated to drug. There was no dose dependence.
[b]No significant changes in aspartate transferase, bilirubin, or alkaline phosphatase were observed.
[c]4 of 5 HVs exhibited levels between 1.5x and 2x the upper limits of normal (ULN); 1 HV was at ~2.8x the ULN.
[d]Transient fever; resolved within 24-36 hours and did not recur upon subsequent dosing.

Individual HV Clinical Data for the 750-mg MAD Cohort

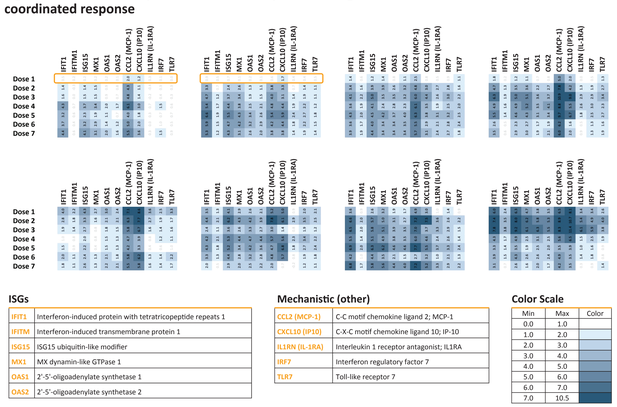
Well-behaved PK of PRX034 Following Oral Administration of 750 mg

As presented previously,[2] the PKs of PRTX007 and PRX034 were well behaved with exposure increasing proportionally to PRTX007 dose without accumulation upon repeated dosing (mean PRX034 AUC (hr*ng/ml) on D1: 13,692; D13: 13,933 for 750-mg dose cohort)

PD Responses in 750-mg Cohort

Induction of mRNAs for IFN-Stimulated Genes (ISGs) and Other TLR7-Associated Genes

Figure 6. mRNA transcript induction heat maps for 750 mg in the multi-dose cohort maintained a coordinated response



ISGs

IFIT1	Interferon-induced protein with tetratricopeptide repeats 1
IFITM	Interferon-induced transmembrane protein 1
ISG15	ISG15 ubiquitin-like modifier
MX1	MX dynamin-like GTPase 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 (IP10)	C-X-C motif chemokine ligand 10; IP-10
IL1RN (IL-1RA)	Interleukin 1 receptor antagonist; IL1RA
IRF7	Interferon regulatory factor 7
TLR7	Toll-like receptor 7

Color Scale

Min	Max	Color
0.0	1.0	
1.0	2.0	
2.0	3.0	
3.0	4.0	
4.0	5.0	
5.0	6.0	
6.0	7.0	
7.0	10.5	

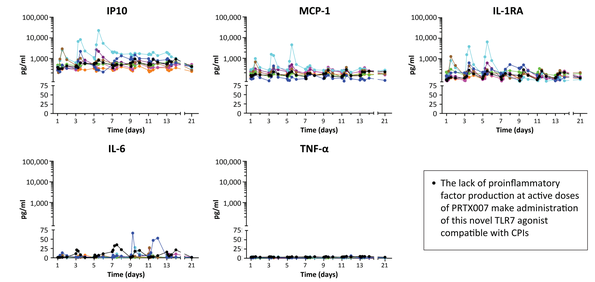
Values are Log_2 $R_{\text{Week 2 Day 1}}$ (Log_2 maximal fold-change from pretreatment baseline during 24-hr period following administration of PRTX007).
All HVs receiving PRTX007 in the 200- to 800-mg SAD cohorts, and the 300- to 750-mg MAD cohorts (n=68) are shown. Color intensity is as shown in scale.
ISG=interferon-stimulated gene.

- Coordinated expression of ISGs and genes known to be related to TLR7 agonism were observed in immune cells in blood in all HVs
  - The well-controlled expression seen with a single dose was maintained during repeated doses
- Two HVs that were poorly responsive to the first dose (first dose response highlighted in orange) established a robust response upon repeated dosing
  - The increased responsiveness in HVs 1 and 2 was not due to increased drug exposure, but rather due to increased sensitivity to drug
- There was no change in expression of NF-κB–regulated gene products

Circulating Markers in Plasma

PRTX007 Immune Cytokine Profile Demonstrates Compatibility for Combination Treatment With CPIs

Figure 7. Individual time course of cytokine profiles for all subjects in the 750-mg PRTX007 cohort (n=8)[a,b]



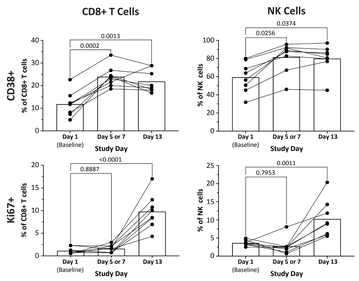
[a]PRTX007 was administered for 2 weeks to 8 HVs (n=8) at 7 doses at 750 mg/dose QOD. Each colored line represents an individual HV.
[b]Day 1 was the predose data point and serves as a baseline. Day 21 was 8-days post last dose and shows cytokine levels return to baseline.

- The lack of proinflammatory factor production at active doses of PRTX007 make administration of this novel TLR7 agonist compatible with CPIs

- A coordinated downstream immune response is suggested by the induction of selected circulating markers by the oral PRTX007 TLR7 agonist prodrug
  - Increases in IP10, monocyte chemoattractant protein 1 (MCP-1), and IL-1RA in plasma were observed
  - IL-6 and TNF-α remained essentially unchanged from pretreatment levels
  - No substantive increase in circulating Type I IFN or IL-1β were measured; these markers were less than lower limit of quantitation

Activation and Proliferation Markers Demonstrate Systemic CD8+ T Cell and NK Cell Engagement in 750-mg PRTX007 Cohort

Figure 8. Activation and proliferation in CD8+ T Cells and NK Cells in 750-mg PRTX007 cohort (n=8)



- The proportion of CD8+ T cells and NK cells, with significant expression of the activation marker CD38 and proliferation marker Ki67, increased markedly from pretreatment to end of dosing in all HVs. During this period:
  - CD38+ CD8+ T cells increased from 11.8% to 21.9% and CD38+ NK cells increased from 59.4% to 80.0%
  - Ki67+ expressing CD8+ T cells increased from 1.1% to 9.8% and Ki67+ expressing NK cells increased from 3.6% to 10.3%
- In contrast, minimal change in activation and proliferation markers was observed for CD4+ T cells and B cells (data not shown; data on file). See also **Figure 1**

Conclusions & Discussion

- PRTX007 demonstrated a favorable safety profile in all HVs receiving drug in phase 1
- In the 750-mg MAD cohort, activation of the innate and adaptive immune response, including important effector cell populations, were observed without systemic increases in proinflammatory factors
  - Induction of ISGs without significant increases in circulating IFNs was observed
  - No increase in expression or circulating levels of proinflammatory cytokines (eg, TNF-α) was noted
  - CD8+ NK and T-cell activation (CD38+ markers) increased markedly from pretreatment to end of dosing in all HVs
- Both the clinical characteristics and unique pattern of immune induction by PRTX007 support its use in combination with CPIs
  - Local antitumor activity of intratumorally administered pDC-activating agents in combination with systemic CPIs is well recognized, but overall clinical benefit has been limited
  - Use of PRTX007 in combination with CPIs in the murine tumor model expressing the HPV E7 oncogene revealed robust antitumor activity. Increased CD8+ T-cell infiltration of tumors occurred only in the presence of the CPI
  - We believe the proposed combination will increase therapeutic benefit in cancer immunotherapy
- These data justify further exploration of PRTX007 as a potential cancer therapeutic; a future study will include a combination with a CPI in solid tumors at active doses identified in this HV study

References

1. Appleman JA, et al. Abstract 582: Selection of a novel toll-like receptor 7 (TLR7) agonist PRX034 for immunotherapy of cancer. *Cancer Res*. 2020;80(16S):582. **2.** Lemech C, et al. Abstract CT189: PRTX007, an optimized TLR7 agonist for systemic immunotherapy of cancers: Interim analysis of phase I study in healthy volunteers. *Cancer Res*. 2022;82(suppl 12):CT189-CT189. **3.** Paolini F, et al. Immunotherapy in new pre-clinical models of HPV-associated oral cancers. *Hum Vaccin Immunother*. 2013;9(3):534-543. **4.** Kim SS, et al. B cells improve overall survival in HPV-associated squamous cell carcinomas and are activated by radiation and PD-1 blockade. *Clin Cancer Res*. 2020;26(13):3345-3359.

Disclosures & Acknowledgements

This study was funded by Primmune Therapeutics.
We would like to acknowledge Sharabi lab members, Department of Radiation Medicine, University of California at San Diego.
Medical writing services were provided by SCIENT Healthcare Communications and funded by Primmune Therapeutics, Inc., (Carlsbad, CA).