Prospective Phase 1 clinical development and optimization of the interferon-biased TLR7 agonist prodrug PRTX007

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Abstract #138

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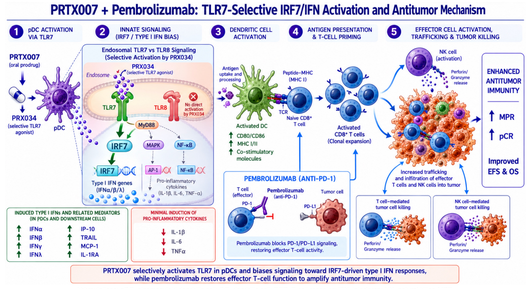
Background and prospective selection

- **Clinical challenge:** Systemic TLR7 and TLR7/8 agonism can induce inflammatory cytokine responses that constrain tolerability and the intensity of repeated immune stimulation.
- **Prospective optimization:** We hypothesized that a clinically useful systemic TLR7 agonist should preferentially engage the pDC–IRF7/interferon pathway while minimizing systemic inflammation(1).
- **Optimization strategy**: Our prospectively defined design criteria included:
  - **Controlled immune induction:** Graded, dose-dependent immune activation rather than an abrupt response threshold, with selective TLR7 agonism and minimal TLR8 activity..
  - **Differentiated functional pharmacology:** Preferential interferon-associated responses with limited proinflammatory cytokine induction in primary human PBMCs.
  - **Systemic delivery and PK/PD:** An intrinsically inactive oral prodrug enabling efficient systemic delivery, appropriate distribution, and pharmacodynamic activity persisting beyond exposure to the active agonist.
- **Clinical development:** The resulting agonist, PRX034, is delivered orally as PRTX007. Previously reported PRTX007-001 findings established dose-dependent systemic immune activation and PK/PD characteristics(2–4). PRTX007-002 evaluated consecutive-day administration(6), informing regimen selection for Phase 2 INFLECTION-003.

Proposed antitumor mechanism



Abbreviations: pDC, plasmacytoid dendritic cell; DC, dendritic cell; TCR, T-cell receptor; IFN, interferon; NK, natural killer; MPR, major pathologic response; pCR, pathologic complete response; EFS, event-free survival; OS, overall survival.

Steps 3–5 and the outcomes shown are INFLECTION-003 rationale, not findings of this study.

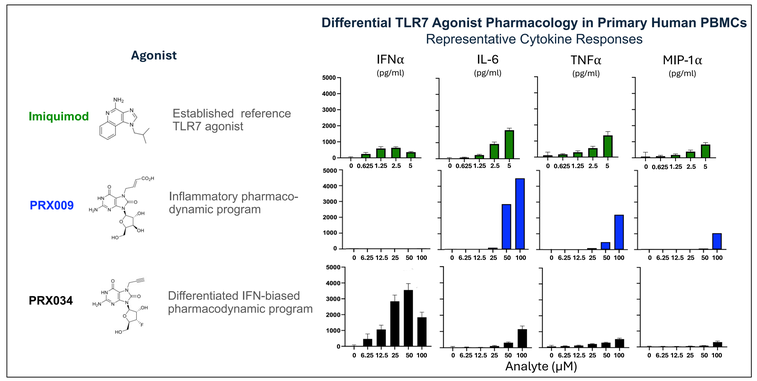
- **pDC targeting:** Human pDCs are TLR7-dominant, with little or no functional TLR8 activity; TLR8 is included in the schematic to represent other immune-cell populations.
- **Signaling bias:** TLR7 agonists can elicit interferon-associated or inflammatory responses. PRX034 was selected to favor IRF7/interferon signaling while limiting inflammatory cytokine induction.
- **TLR8 avoidance:** TLR8 activation in human myeloid cells can promote inflammatory cytokine release, supporting the additional requirement for TLR7 selectivity.

Why functional PBMC profiling?

Conventional NF-κB/AP-1 reporter assays(7) measure one downstream transcriptional readout, not interferon production. Optimizing against that readout alone can favor a profile different from the intended pharmacology.

Primary human PBMCs contain the pDCs that produce IFNα, so cytokine output resolves the responses that matter for candidate selection. Reporter assays did not distinguish the programs shown in Figure 1.

Figure 1 | PBMC studies demonstrate distinct pharmacodynamic profiles among TLR7-engaging agonists and support selection of interferon-biased PRX034



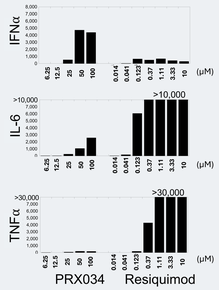
Key Findings

TLR7 agonists exhibit markedly different functional pharmacodynamic profiles in primary human PBMCs.

- **Imiquimod:** Reference TLR7 agonist exhibiting an intermediate cytokine response profile.
- **PRX009:** Structurally related TLR7 agonist with limited IFNα and pronounced inflammatory cytokine induction.
- **PRX034:** Optimized TLR7 agonist demonstrating robust IFNα induction with comparatively limited inflammatory cytokine responses.

Functional PBMC profiling distinguished responses not resolved by conventional TLR reporter assays, supporting prospective selection of PRX034.

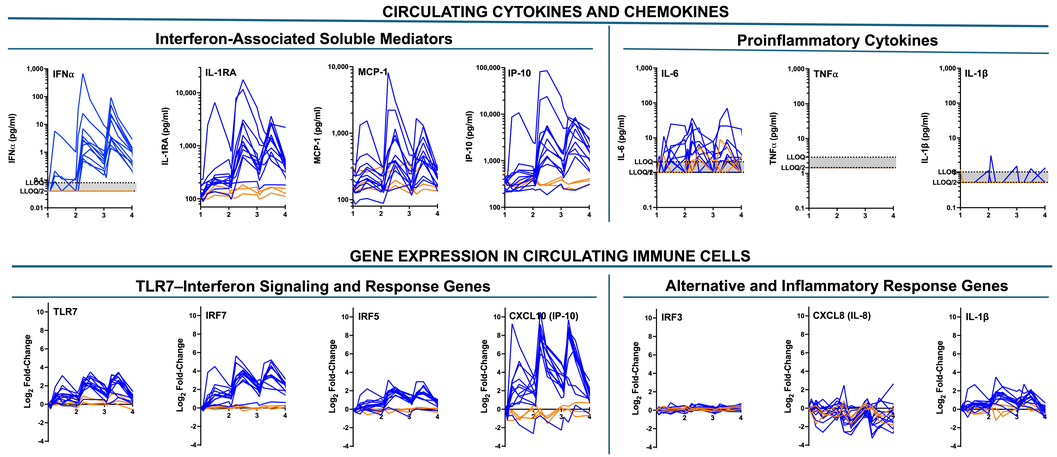
Exploratory comparator: Resiquimod (TLR7/8)



Resiquimod elicited substantially greater inflammatory cytokine responses than PRX034, despite lower maximal IFNα induction

Methods: PBMCs from healthy donors were cultured at high cell density (750,000 viable cells/100 μL) in 96-well plates. Supernatants were harvested 24 hours after agonist or vehicle exposure. Pilot studies established that high cell density was important for resolving differentiated pharmacodynamic responses. PRX034 and imiquimod were compared head-to-head in 16 independent experiments, each involving at least three donors; PRX009 was included in three of these experiments. Results were pooled at the individual-donor level across experiments and are presented as mean ± SEM. The separate exploratory PRX034–resiquimod comparison presents median responses from three donors.

Figure 2 | Interferon-biased systemic pharmacodynamic responses during three consecutive days of PRTX007 administration in healthy volunteers



Individual subject time courses
(one line per subject)

Twelve subjects received PRTX007

Three subjects received placebo

Key Findings

Robust interferon-associated activation occurred with limited inflammatory cytokine and gene induction.

- Marked IFNα, IL-1RA, MCP-1, and IP-10 responses accompanied coordinated induction of TLR7, IRF7, IRF5, and CXCL10.
- The magnitude of interferon-associated induction exceeded that observed with QOD dosing at the same 750 mg dose level in PRTX007-001.
- IL-6, TNFα, IL-1β, IRF3, CXCL8, and IL1B exhibited comparatively limited responses.

The prospectively selected interferon-biased pharmacodynamic phenotype was reproduced in humans.

Methods: PRTX007-002: Healthy volunteers received oral PRTX007 750 mg or placebo on a weekly 3-days-on/4-days-off schedule for two cycles plus one additional dose. Individual-subject soluble and transcriptional pharmacodynamic responses are shown for Days 1–4 of the first treatment cycle. **Panel Notation**: X-axis - study day, PRTX007 doses on days 1–3. Values below the lower limit of quantification (LLOQ) are plotted at LLOQ/2.

Clinical trials

PRTX007-001 - Randomized, double-blind, placebo-controlled SAD/MAD in healthy volunteers; every-other-day dosing(2-4) [ACTRN12621000468820]

PRTX007-002 (Days 1-3 PD shown) - Robust interferon-associated activation during consecutive-day dosing(6)* [ACTRN12624000981527]

INFLECTION-003 (PRTX007-003) - Phase 2: selected regimen + pembrolizumab, neoadjuvant, resectable stage III melanoma [NCT07565285]

*__PRTX007-002 tolerability:__ No serious adverse events occurred. The three participants with the greatest pharmacodynamic responses following the second dose discontinued treatment because of interferon-like adverse events. Symptoms were managed with acetaminophen, NSAIDs, or supportive care and resolved following discontinuation.

Conclusions and next step

- **Differentiated TLR7 pharmacology:** Structurally distinct TLR7 agonists elicited markedly different cytokine profiles in primary human PBMCs, demonstrating that TLR7 engagement does not dictate a single pharmacodynamic response.
- **Prospective compound selection:** Functional PBMC profiling distinguished interferon-biased and inflammatory cytokine programs not resolved by conventional TLR reporter assays, supporting selection of PRX034.
- **Preclinical-to-clinical translation:** The interferon-biased pharmacodynamic profile identified in human PBMCs was supported by previously reported NHP findings(5) and subsequently demonstrated in healthy volunteers receiving oral PRTX007, with coordinated induction of interferon-associated soluble and transcriptional biomarkers (2-4).
- **Limited inflammatory induction:** Robust systemic interferon-associated activation was accompanied by comparatively limited induction of IL-6, TNFα, IL-1β, and selected inflammatory response genes, consistent with the prospectively defined pharmacodynamic objective.
- **Next step:** The intermittent 3-days-on/4-days-off regimen, selected using the broader PRTX007-002 clinical pharmacodynamic dataset, is now being evaluated with pembrolizumab in the Phase 2 INFLECTION-003 study (NCT07565285) in patients with resectable stage III melanoma.

References and disclosures

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With respect to Clinical Study PRTX007-002:
- This work was funded by Defense Threat Reduction Agency contract HDTRA1-24-C-0014 . Approved for public release
- Funding also provided by Primmune Therapeutics, Inc.
- We thank the study participants and the Scientia Clinical Research site staff.

Nonclinical experimentation described herein was funded by Primmune Therapeutics, Inc.