Preclinical Mechanisms of Action of PRN1008, a Reversible Covalent Bruton's Tyrosine Kinase Inhibitor in Clinical Development for Pemphigus

#011

Claire L. Langrish, Michelle R. Francesco, Yan Xing, J. Michael Bradshaw, Timothy D. Owens, and Philip A. Nunn

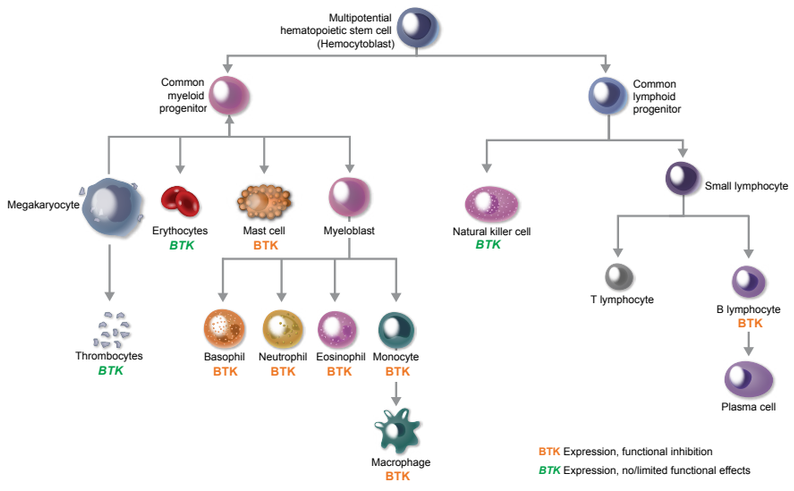
Principia Biopharma, South San Francisco, CA, USA

INTRODUCTION

Bruton's Tyrosine Kinase (BTK)

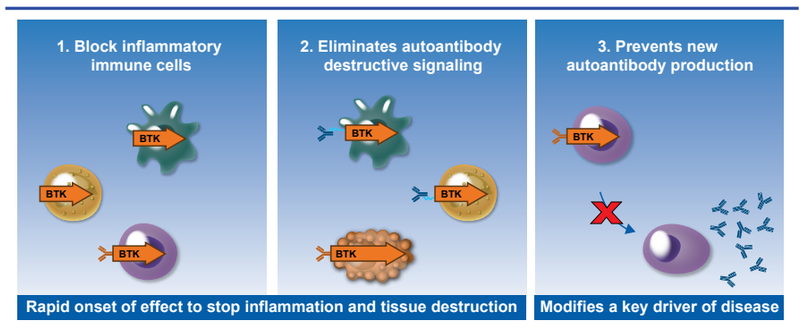
- BTK is an enzyme that plays a critical role in immune signaling pathways and is an essential signaling element downstream of the B-cell, Fcγ, and Fcε receptors[1] (**Figure 1**)

Figure 1. BTK Expression in B Cells and Innate Immune Cells[3]



- BTK has 3 key mechanisms of action in immune-mediated disease[2] (**Figure 2**)
  1. Blocks inflammatory immune cells
  2. Eliminates autoantibody destructive signaling
  3. Prevents new autoantibody production

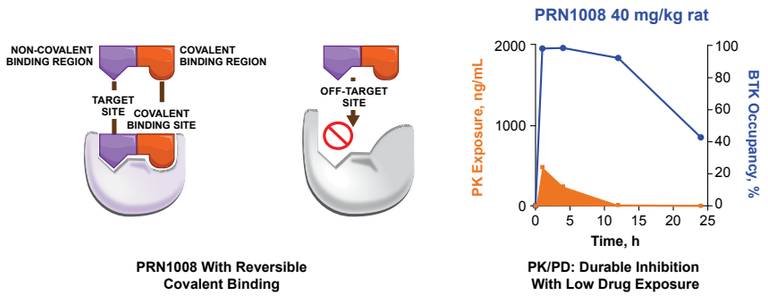
Figure 2. Three Key Mechanisms of Action of BTK for Targeting Underlying Drivers of Immune-Mediated Disease



PRN1008

- Fully reversible, oral inhibitor targeting BTK designed for immunology (**Figure 3**)
- Covalent binding achieves long BTK target engagement and durable inhibition with limited drug exposure
- Potential clinical advantage of PRN1008's rapid systemic clearance and long target residence time may **prolong efficacy, while reducing the potential for off-target toxicities**

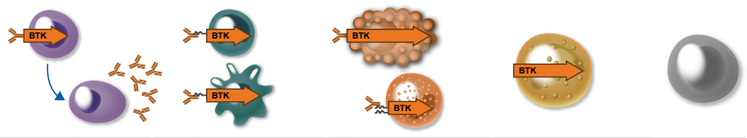
Figure 3. PRN1008 Tailored Covalency™ and Inhibition of BTK[4,5]



PD, pharmacodynamics; PK, pharmacokinetics; qd, once daily.

- PRN1008 is a fully reversible, covalent inhibitor targeting BTK, with durable inhibition at a low concentration of PRN1008 (**Figure 3**)

Figure 4. BTK Inhibition by PRN1008 Within Immune Cells



B cells, Plasma Cells	Monocyte, Macrophage	Mast cells, Basophils	Neutrophils	T Cells
Blocks B cell receptor Inhibits plasma cell differentiation and antibody production	Blocks IgG-mediated FcγR activation, phagocytosis Inflammatory mediators	Blocks IgE-mediated FcεR activation and degranulation	Inhibits activation, adhesion, recruitment, oxidative burst	No effect
BTK inhibitor PRN1008				

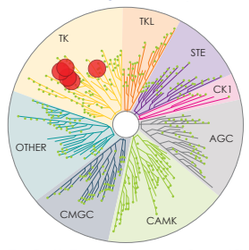
FcεR, Fc epsilon receptor; FcγR, Fc gamma receptor; Ig, immunoglobulin.

- The BTK inhibitor PRN1008 plays a critical role in neutralization of processes in immune-mediated diseases, including B and innate immune cells but not in T cells[5-7] (**Figure 4**)

RESULTS

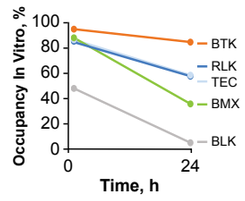
Figure 5. Preclinical Selectivity and Functional Assays for PRN1008

A Selectivity Profile (251 Kinase Panel)



Kinase Assay	IC_{50}, nM
BTK	1.3
RLK	1.2
TEC	0.8
BMX	1.0
BLK	6.3

Increased Selectivity With Time



B Cell-Based Target Specificity Assays

			On-Target Activity IC_{50} ± SD, nM
	BCR	Human B-cell proliferation	5 ± 2
	BTK	Ramos B-cell occupancy	8 ± 2
	FcγR	IgG monocyte activation	56 ± 45
	BCR	B-cell activation in HWB	123 ± 38
	FcεR	IgE basophil activation in HWB	490 ± 130
		Off-Target Activity	**IC_{50} ± SD, nM**
	TCR	Jurkat T-cell receptor signaling	> 5000
	IL-4/STAT6	Ramos B-cell IL-4-induced STAT6	> 5000
N/A	EGFR	Cellular reporter assay	> 5000
N/A	Cytotoxicity	HCT116 cells	> 16,000

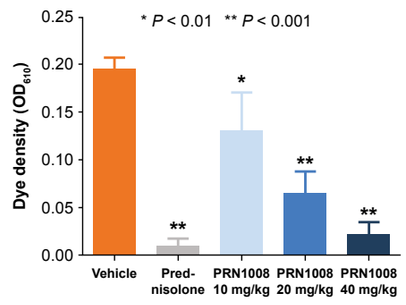
C BTK Target Engagement – Occupancy in PBMCs

Time	Mean BTK Occupancy ± SD
4 h	91% ± 2%
18 h	79% ± 2%

BCR, B-cell receptor; BLK, B-cell lymphocyte kinase; BMX, bone marrow tyrosine kinase on chromosome X; BTK, Bruton's tyrosine kinase; EGFR, epidermal growth factor receptor; HWB, human whole blood; Ig, immunoglobulin; IL, interleukin; PBMC, peripheral blood mononuclear cells; RLK, receptor-like kinase; SD, standard deviation; STAT, signal transducer and activator of transcription; TEC, tyrosine protein kinase; TCR, T-cell receptor.

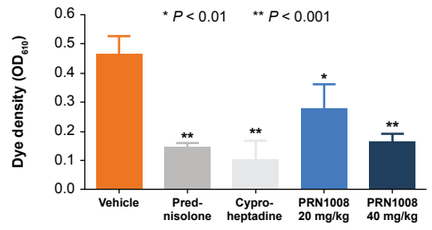
- PRN1008 has been optimized for selectivity, potency, and durability (**Figure 5A-C**)
  - Inhibits B-cell activation and Fc receptor signaling
  - Demonstrates durable BTK inhibition
  - Shows an absence of cytotoxic effects and limited functional effects on T cells and other non–BTK-dependent cellular pathways

Figure 6. Dose-Dependent Inhibition With PRN1008 in IgG Antibody-Mediated Arthus Reaction Rat Model



- In skin, PRN1008 significantly improved immune complex-mediated inflammation and injury in an IgG antibody (FcγR)-driven acute Arthus model in rats (**Figure 6**)
- PRN1008 showed significant dose-dependent inhibition of passive Arthus reaction as measured by reduction in optical density of intradermal dye extravasation
- Maximal responses similar to high-dose prednisolone (10 mg/kg) was achieved with 40 mg/kg PRN1008

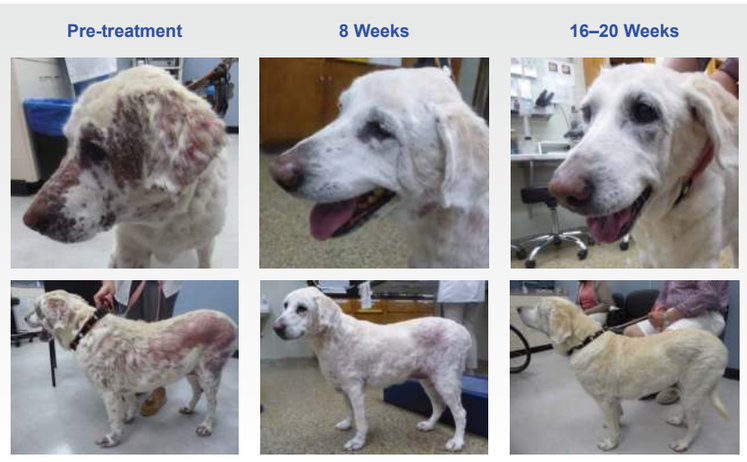
Figure 7. Dose-Dependent Inhibition With PRN1008 in IgE Antibody-Mediated Mouse Passive Cutaneous Anaphylaxis



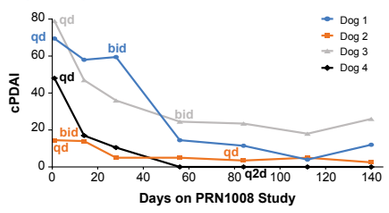
- PRN1008 inhibited mast cell activation in a passive cutaneous anaphylaxis mouse model, supporting a key role for BTK in IgE antibody (FcεR)-mediated immune responses (**Figure 7**)

Figure 8. Preclinical Proof-of-Concept for PRN1008 Monotherapy in Canine Pemphigus Foliaceus

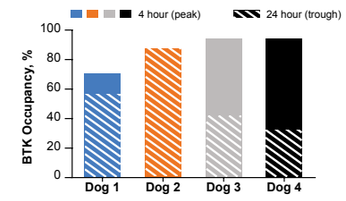
A



B Canine Pemphigus Disease Activity Index (cPDAI) Score



C BTK Occupancy (Day 1, Single Dose)



- All animals had an immediate clinical improvement, with all 4 animals achieving complete or substantial disease control as monotherapy (**Figure 8A**)
- Anti-inflammatory effects were visible within 2 weeks, and tolerability was excellent in the study
- All animals had an immediate and rapid clinical improvement by cPDAI score after treatment (**Figure 8B**)
- BTK occupancy was > 70% within 4 hours of the first day of dosing (**Figure 8C**)

MATERIALS AND METHODS

Selectivity and Functional Assays

- Biochemical studies were performed to characterize the potency, selectivity, biochemical on-rate and off-rate, and the reversibility of the interaction between PRN1008 and BTK
- Selective inhibition of BTK function and occupancy, and off-target effects were evaluated in cell-based assays in B and T cells, peripheral blood mononuclear cells (PBMC), and human whole blood (HWB)

Rat Arthus Model

- The passive Arthus reaction is an acute immunoglobulin G (IgG) antibody challenge model that is dependent on FcγR activation[8]
- Female Sprague-Dawley rats (n = 5-8) were dosed orally with vehicle; prednisolone 10 mg/kg (positive control); or PRN1008 doses of 10, 20, or 40 mg/kg once daily (qd) for 3 days before antibody challenge
- The optical dye density at OD_{610nm} of Evans blue dye extravasation in the skin was assessed 4 hours after challenge

Mouse Passive Cutaneous Anaphylaxis Model

- The passive cutaneous anaphylaxis model is an immunoglobulin E (IgE) FcεR-mediated model with mechanisms similar to those observed in human allergic disease[8]
  - Measures IgE-dependent wheal and flare response via mast cell activation, degranulation, and release of inflammatory mediators
- Female BALB/c mice (n = 5/group) were dosed orally with vehicle, PRN1008 at 20 or 40 mg/kg twice daily (bid), or positive controls prednisolone 10 mg/kg or cyproheptadine 25 mg/kg (intraperitoneal) for 3 days prior to IgE-antigen challenge
- Thirty minutes after challenge, mice were euthanized, and samples collected to assess the dye density for extravasation in the skin

Preclinical Proof-of-Concept for PRN1008 Monotherapy in Canine Pemphigus Foliaceus

- Pemphigus foliaceus is a naturally occurring autoantibody-mediated autoimmune disease in dogs that shares many similarities with human pemphigus disease
  - Most common form of pemphigus in dogs
  - Autoantibodies target epidermal antigen desmocollin in skin, necessary for cell-cell adhesion
- Four dogs with naturally-occurring, histopathologically confirmed pemphigus foliaceus received open-label treatment with PRN1008 monotherapy for 20 weeks
- Dosing was initiated at 15 mg/kg qd; 3 dogs converted to bid dosing to improve efficacy
- Response was measured using a modified canine version of the Pemphigus Disease Activity Index[8] (cPDAI)
- BTK occupancy was measured in PBMCs on day 1 at 4 and 24 hours after the first dose in a fluorescent competition assay relative to total BTK and compared with pretreatment samples

CONCLUSIONS

- PRN1008 is an oral, reversible, covalent BTK inhibitor that drives durable BTK occupancy and has a low potential for off-target effects
- Preclinically, PRN1008 showed rapid and sustained anti-inflammatory effects via 3 simultaneous mechanistic benefits
  1. Blocks inflammatory immune cells
  2. Eliminates autoantibody destructive signaling
  3. Prevents new autoantibody production
- PRN1008 significantly improved antibody-mediated inflammation and injury with responses similar to that for high-dose corticosteroids in animal models
- In autoantibody-driven, naturally occurring canine pemphigus foliaceus, PRN1008 safely and rapidly controlled disease as a monotherapy
- PRN1008 showed favorable preclinical potential for treating immune-mediated diseases, including B-cell and autoantibody-driven disorders
- PRN1008 is currently being evaluated in clinical studies (phase 3 in pemphigus [NCT03762265] and phase 2 in immune thrombocytopenia [NCT03395210])

REFERENCES

1. Crofford LJ, et al. *Exp Rev Clin Immunol.* 2016:12:763-773.
2. Rip J, et al. *Crit Rev Immunol.* 2018:38:17-62.
3. Wikimedia Commons, the free media repository. Available at and adapted from: https://commons.wikimedia.org/wiki File:Hematopoiesis_(human)_diagram.png
4. Bradshaw JM, et al. *Nat Chem Biol.* 2015;11:525-531.
5. Data on file, Principia Biopharma Inc.
6. Principia Biopharma Inc. Available at: http:/wwwprincipiabio.com/tailored-covalency/scientific-publications
7. López-Herrera G, et al. *J Leukoc Biol.* 2014;95:243-250.
8. Chang BY, et al. *Arthritis Res Ther.* 2011;13:R115.
9. Murrell DF, et al. *J Am Acad Dermatol.* 2008;58:1043-104

ACKNOWLEDGMENTS

These studies were sponsored by Principia Biopharma, South San Francisco, CA, USA

Editorial support was provided by Vaniam Group and funded by Principia Biopharma. The authors directed development of the presentation and are fully responsible for all content and editorial decisions.

DISCLOSURES

All authors report employment and stock or other ownership for Principia Biopharma

CORRESPONDING AUTHOR

claire.langrish@principiabio.com

Presented at the 49th annual meeting of the European Society for Dermatological Research (ESDR), September 18-21, 2019 in Bordeaux, France