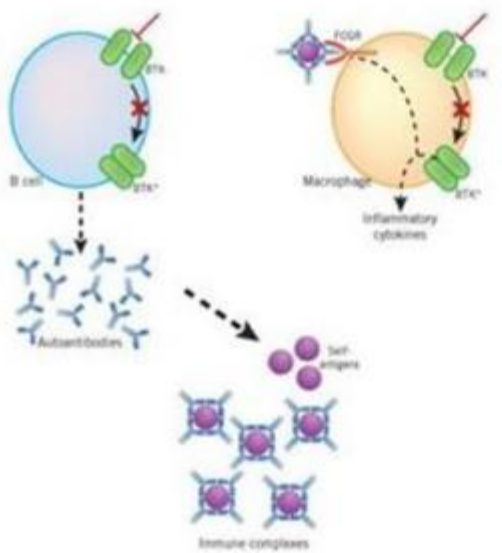
PNQ-849

Highly differentiated, Reversible BTK inhibitors for Auto-immune diseases

Bruton's Tyrosine Kinase (BTK): A Key Modulator of the B-cell Receptor (BCR) Pathway

BTK is a critical target for B-cell differentiation, activation and signaling and targeting BTK can be a compelling therapeutic modality for autoimmune diseases



- BTK is expressed in B lymphocytes, myeloid and mast cells
- BTK is essential for B-cell differentiation, activation and proliferation following engagement of B-cell antigen receptor (BCR)
- BTK plays a critical role in regulating the activity of macrophages, myeloid cell populations, mast cells, platelets, and osteoclasts.
- BTK Inhibition reduces autoantibody levels in collagen-induced arthritis.
- BTK inhibition reduces inflammatory cytokines like TNFα, IL-1β and IL-6.

BTK-inhibitors: Potential indications

B-cell Targeted Agents	Diseases Being Targeted
• Ritxuan (CD20) • Ofatumumab (CD20) • Epratuxumab (CD22) • AME-133v (CD20) • PCI-32765 (BTK) • Galiximab (CD80) • Dacetuzumab (CD40) • Afutuzumab (CD20) • R788 (SYK) • CAL-101 (PI3Kd) • Dasatinib (SYK) • Veltuzumab (CD20) • Belimumab (BLyS) • Atacicept (BLyS) • Ocrelizumab (CD20)	• Rheumatoid arthritis • Systemic lupus erythematosus • Multiple sclerosis • Non Hodgkin's lymphoma • Chronic lympocytic leukemia • Sjorgren's • Waldenstrom's macroglobulinemia • Idiopathic thromocytopenic purpura • Grave's ophthalmopathy • Myasthenia gravis • Urticaria • Biliary cirrhosis • Myositis, dermatomyositis • Vasculitis, Wegener granulomatosis • Renal transplant rejection • Diabetic nephropathies • Glomerulonephritis • Chronic focal encephalitis • Churg-Strauss syndrome • Ankylosing spondylitis

B-cell Targets and Therapies: Multiple Diseases for Potential Intervention

Recommendations for Rare Diseases:

- Explore orphan autoimmune indication as a fast-to-approval strategy
  - ➢ Anti-Neutrophil Cytoplasmic Antibodies (ANCA) -Associated Vasculitis
    - Granulomatosis with polyangiitis
    - Microscopic polyangiitis
  - ➢ Polymyositis and Dermatomyositis
- Type 1 Diabetes
  - Characterized by antibodies to islet autoantigens, GAD65, insulin etc
  - Suppression of immune activation by BTKi may delay onset or better

In blue: autoimmune diseases

PNQ-849: Summary

- A **potent, selective and reversible** BTK inhibitor with a potential "First-in-Class" opportunity for auto-immune diseases
- PNQ-617 is a potential back-up for the lead candidate
  - Composition of Matter Patent covering PNQ-849 and PNQ-617 has been granted in US (US9,233,983) and EU
- **Superior potency** (whole blood) and efficacy in multiple RA models with favorable **target engagement duration (24h)**
  - Potential for monotherapy and opportunity for once-daily dosing
- **Excellent selectivity** over other BTK and diverse non-BTK family kinases vs. irreversible inhibitors and other drugs that affect T-cell functions directly e.g. Tofacitinib
  - Potential for superior **long term safety** profile in RA and other chronic indications due to lower risk from immunosupression [general and opportunistic infections (e.g., PML, TB) and cancer] as it spares T cells & plasma memory cells while retaining efficacy
  - Unlike irreversible inhibitors, no potential for 1) covalent protein conjugate adduct formation leading to immunogenicity, 2) drug resistance due to mutation of Cys-481 residue of BTK that forms covalent bonding with the irreversible inhibitors

PNQ-849: Summary, Contd.

- A general safety profile that that could provide a **wide therapeutic window**
  - Selectivity vs. diverse 100 kinase and 121 DrugMatrix targets and hERG
  - Non-mutagenic in mini-Ames test
  - Well tolerated with dose-related TK profile in 28-day safety study in rat with a NOEL of 180 mg/kg/day
- Potential **utility in multiple auto-immune diseases** (see indications slide)
  - Explore **orphan autoimmune indication** as a fast-to-approval strategy and then repurpose it for larger autoimmune indications
  - Position as an **alternative to Rituximab** (anti-CD20 Ab), a B-cell targeted approved therapy for a number of autoimmune diseases (adverse effects and loss-of-response are common with Rituximab)
- Ready for IND filing

PNQ-849 : a Best-in-class BTKi

Comparable Potency

- **Nanomolar potency for BTK**
- Demonstrated effect on signaling & relevant cell based assays

Reversible Inhibition
No Covalent bond formation

- **No covalent bond formation** – no reactive moieties which can form covalent bond; binding to a different hydrophobic pocket – no interaction with Cys481
- **Reversibility** of enzymatic activity on dilution after pre-incubation
- **Similar potency vs wild type in C481S mutant** - Minimal potential for drug résistance

Comparable Efficacy

- **Robust efficacy in animal models of Rheumatoid arthritis**
  - Collagen Induced Arthritis & Adjuvant Induced Arthritis
- **Translational assay established**

Increased compound safety
Selectivity over other kinases

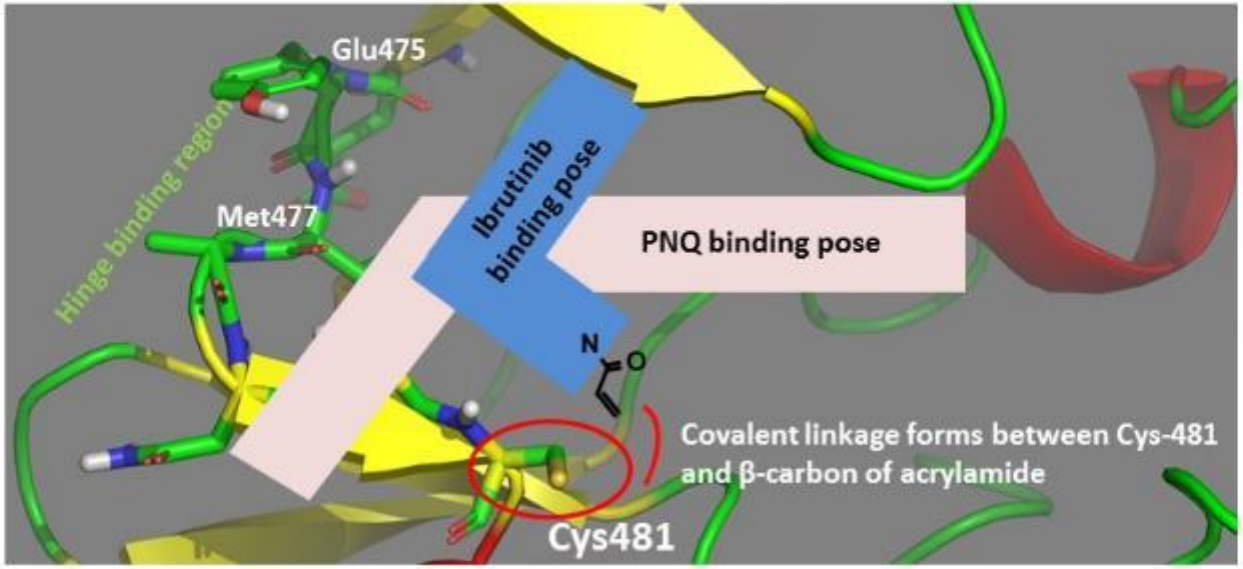
- **Superior selectivity against family kinases & diverse non-family kinases**

Reversible Inhibition and superior kinase selectivity vs covalent inhibitors such as Ibrutinib may offer **superior long term safety** in chronic treatment with equivalent/**improved efficacy**

PNQ-840: Reversible Non Covalent BTK Inhibition

- No covalent bond formation – no reactive moieties which can form covalent bond
- Proposed binding pose different from Ibrutinib and Acalabrutinib

Proposed binding pose of PNQ compounds compared to Ibrutinib



- Ibrutinib's acrylamide moiety approaches Cys481, a key residue responsible for making covalent bond
- Docking pose of PNQ-849 indicates binding to a different hydrophobic pocket – no interaction with Cys481

Reversibility vs. Irreversibility of Inhibition

Brief Protocol

Preincubate 40x IC_{80} inhibitor with 40x enzyme

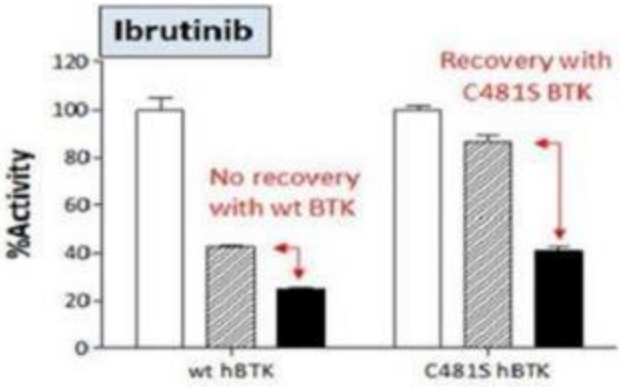
↓

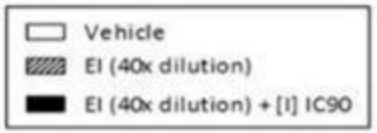
Add buffer to dilute to 1x, allow dissociation

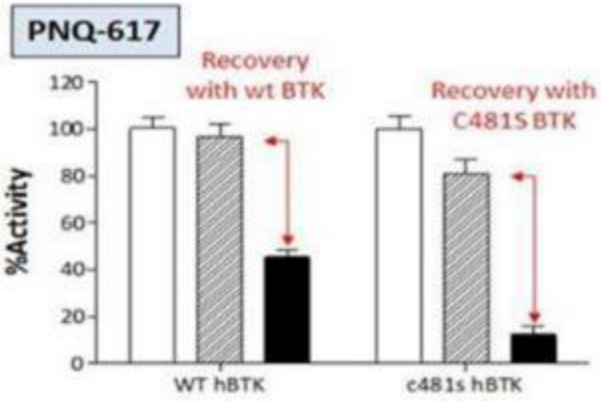
↓

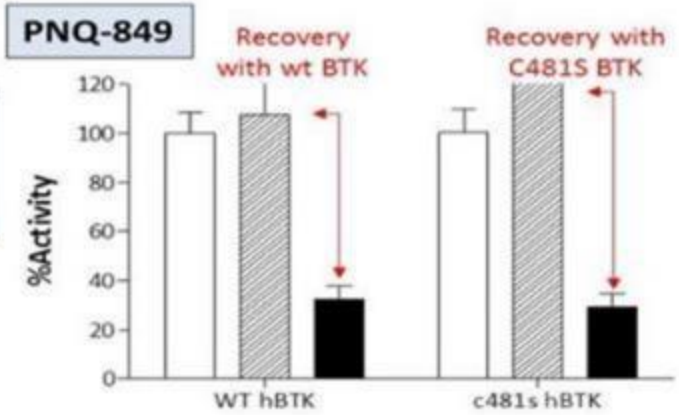
Add substrate, measure enzyme activity

Recovery of activity = Reversible
No recovery of activity = Irreversible
Inhibition control: Inhibitor concentration maintained at IC_{80} through entire experiment









- PNQ compounds are reversible potent Inhibitors of both *wt* and C481S mutant BTK

Inhibition of C481S-BTK Mutant Better than Ibrutinib

Compound	IC_{50} (nM)		
	Wt BTK	C481S BTK	Fold Shift
PNQ-849	2.7	22.5	8
PNQ-617	1.5	8.4	6
Ibrutinib	0.4	31	80

- Inconsequential shift in potency for PNQ compounds with the C481S mutant of BTK as compared to Ibrutinib

Superior Kinase Profile of PNQ-849 vs. Competitors

Kinase	Ibrutinib, IC_{50} (nM)	ACP-196, IC_{50} (nM)	CC-292, IC_{50} (nM)	PNQ-849, IC_{50} (nM)	PNQ-617, IC_{50} (nM)
BTK	1.6 (IH); 0.46[lit-1]	5.1[Lit-5]	4 (IH); <0.5[lit-2]	2.6 (IH)	0.9 (IH)
TEC	77[lit-1]	93[Lit-5]	6.2[lit-2]	~3,000*	51% @ 1 µM*
BMX	0.76[lit-1]	46[Lit-5]	0.7[lit-2]	~1,000**	80% @ 1 µM**
ITK	10.7 (IH)	>1000[Lit-5]	36 (IH)	>10000 (IH)	>10,000 (IH)
JAK3	16.1 (IH)	>1000[Lit-5]	31 (IH)	>10000 (IH)	>10,000 (IH)
SYK	>10,000 (IH)		976 (IH); 1,134[lit-3]	>10000 (IH)	>10,000 (IH)
LYN	200[lit-1]	>1000[Lit-5]	4401[lit-3]	>10000**	>10000**
c-SRC	170[lit-1]	>1000[Lit-5]	1729[lit-3]	46% @ 1 µM**	60% @ 1 µM**
LCK	33[lit-1]	>1000[Lit-5]	9079[lit-3]	~10,000**	26% @ 1 µM**
BLK	0.5[lit-4]	>1000[Lit-5]		>10,000**	>10,000**
EGFR	5.5[lit-4]	>1000[Lit-5]		>10,000**	>10,000**
ABL	86[lit-4]			~10,000**	28% @ 1 µM**
CSK	2.2[lit-4]			>10,000**	>10,000**
YES	6.5[lit-4]	>1000[Lit-5]		>10,000**	~10,000**
FLT3	72.9 [Lit-4]			>10,000**	~10,000**
FGR	2.31 [Lit-4]	>1000[Lit-5]		ND	ND
HCK	3.67[Lit-4]	>1000[Lit-5]		ND	ND
Brk	3.34			ND	ND

Poor selectivity

Moderately selective

Highly selective

Lit-1: *Proc Natl. Acad Sci*, **2010**, 107, 13075-13080

Lit-2: *J. Pharmacol. Exp. Ther.* **2013**, 346, 219-28

Lit-3: 16th congress of EHA Meeting, **2011**

Lit-4: NDA # 205552

Lit-5: ACP-196 (Acalabrutinib)/ASH2015: Abstract#831

IH: Advinus in-house data

**Binding or ** activity* based kinase panel screening at 1&10 µM*

ND: Not done

Profile of PNQ-849: *In Vitro* Pharmacology

Parameter	Potency, IC_{50} (nM)		
	Ibrutinib Approved: MCL, CLL (Irreversible)	CC-292 P-I/II (CLL/RA) (Irreversible)	PNQ-849 (Reversible)
hBTK IC_{50} (nM)	1.6 ± 0.4 (Literature: 0.5)	4 ± 0.7 (Literature: <0.5)	2.6±0.3 (n=15)
Mouse splenocyte IC_{50} (nM) (BCR mediated; ↑CD69)	2.6 ± 1.2	15.7 ± 2.5	3.9±0.7
Rat splenocyte IC_{50} (nM) (BCR mediated; ↑CD86)	1.24 ± 0.49	ND	6.9
Human whole blood IC_{50} (nM) (BCR mediated; ↑CD69)	16 ± 3	731 (65% inh. at 10 µM)	89±37
Mouse whole blood IC_{50} (nM) (BCR mediated; ↑CD69)	136.5±27.6	ND	261.3±1.8
BTK phosphorylation in mouse splenocyte IC_{50} (nM)	ND	ND	214±138

Potency comparable to Ibrutinib and superior to CC-292 in human whole blood assay

Profile of PNQ-849: *In Vitro* Pharmacology

Parameter	Selectivity		
	Ibrutinib Approved: MCL, CLL (Irreversible)	**CC-292 P-I/II (CLL/RA) (Irreversible)**	**PNQ-849 (Reversible)**
ITK, JAK3, SYK, BMX, TEC IC_{50} (µM)	0.011, 0.016, >10, Lit: 0.0008, 0.08	0.036, 0.031, 0.98 Lit: 0.0007 (Bmx); 0.006 (Tec)	>10, >10, >10 ~1, ~1*
Mouse splenocyte, TCR mediated cell based selectivity, IC_{50} (nM)	1700±900	4150	>30,000
Drug matrix screen: list of hits with >50% inhibition (% inhibition at 10 µM)	ND	ND	iNOS (76), Adrenergic α1D (53), Angiotensin AT2 (90), Sigma σ2 (52), Sodium Channel, Site 2 (85), Transporter, Adenosine (95)
100 Kinase Panel screen at 1 and 10 µM (selection based on relevance)	Literature: hits 14 kinases at 50nM	Lit: hits 4 kinases with IC_{50}<50 nM (out of 61 kinases)	Selective in 100-kinase panel (Approx. >500X over AurA, Bmx, Src, Tec; >5000X over the rest)

PNQ-849: Summary of Efficacy & PD Studies

	Study	Doses	Outcomes
PK-PD	Collagen Induced Arthritis (DBA/1J Mouse)	PNQ 849 -10 mg/kg, PO, BID	• PNQ-849 inhibited anti-IgD stimulated CD69 up-regulation on B cells *ex vivo* in whole blood, post-dosing; effect observed up to 24 h • > 50% inhibition 14 h post dosing
Efficacy	Collagen Induced Arthritis (DBA/1J Mouse)	Therapeutic treatment (PO) PNQ-849 1, 3, 10, and 30 mg/kg, BID & QD	Robust efficacy of PNQ-849 on BID & QD dosing • Dose-dependent efficacy • Supported by reduction in joint histopathology scores and loss of proteoglycan • Decrease in serum amyloid A (SAA), IL-6 and anti-collagen IgG levels
Efficacy	Adjuvant Induced Arthritis (Lewis Rat)	Therapeutic treatment (PO) PNQ-849 3,10,30 and 60 mg/kg, QD; CC-292 30mg/kg,QD	Superior effcacy of PNQ-849 compared to comparator CC-292 • Dose-dependent efficacy • Supported by reduction in joint histopathology scores and loss of proteoglycan • At 30 mg/kg, plasma concentrations were above its whole blood IC_{50} for the length of the study

PNQ-849: Summary

- PNQ-849 is a **reversible**, highly differentiated and efficacious BTK inhibitor
- PNQ-849 has demonstrated excellent preclinical PoC in multiple models with a likely **best-in-class profile**

Safety pharmacology studies

Pulmonary (Rats)
Functional Observational Battery (Rats)
Telemetry (Dogs)

All IND regulatory tox studies completed

- 28 Day Repeat Oral Dose Toxicity in Rat with TK
- 28 Day Repeat Oral Dose Toxicity in Dog with TK
- Male fertility studies in rats
- NOAEL of 180 mpk (highest tested dose)in rats and 20 mpk in dogs

Genotoxicity

- Ames test
- MUT-HGPRT-CHO or MUT-CHAB/HPBL
- Micronucleus test (MNT) in rat

CMC

- Process optimization completed
- cGMP campaign to be initiated

PNQ-849 is an IND ready compound

Thank You