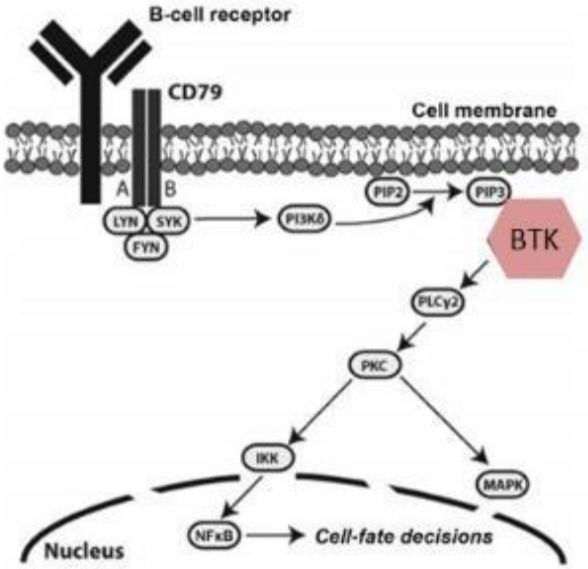
PNQ-154: Reversible Bruton's Tyrosine Kinase Inhibitors

*PNQ-154 is a novel "***Oral Reversible BTK Inhibitor ***" for the treatment of multiple B cell cancers across a wide spectrum of patients, particularly those with BTK mutations and Ibrutinib resistance*

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

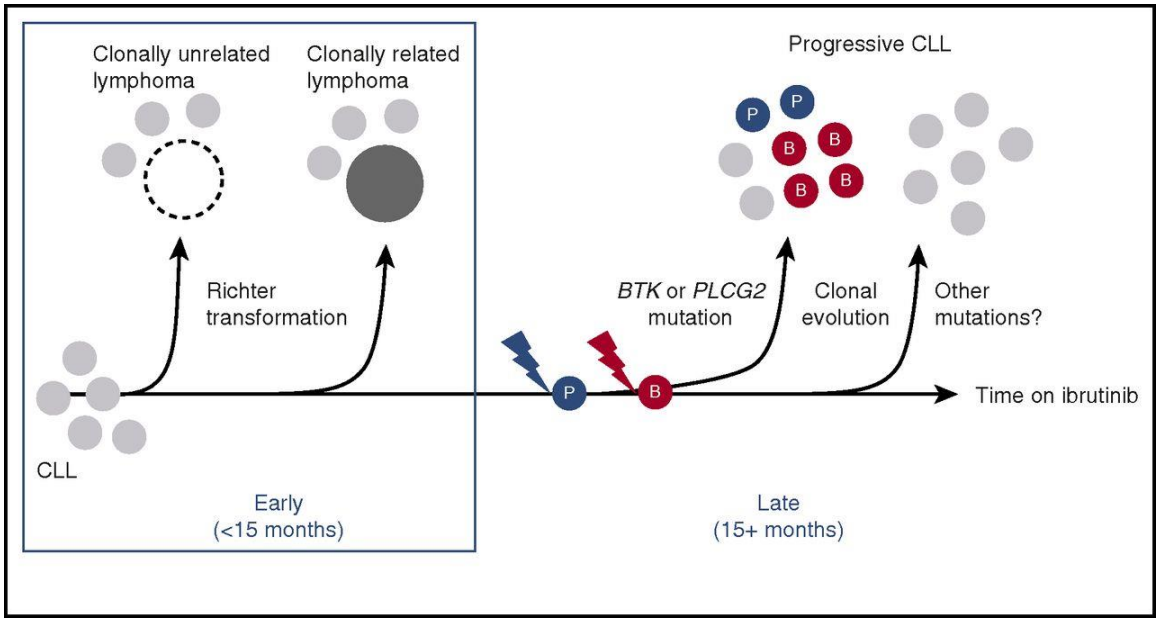
BTK is a critical target for B-cell malignancies based on its significant and exclusive role in B-cell development, fate and function by the modulation of the B-cell receptor (BCR) signaling pathway



- BTK inhibition attenuates cell migration, proliferation, and survival, disrupts integrin-mediated adhesion to fibronectin, inhibits DNA synthesis, diminishes cellular response to tissue homing chemokines and induces apoptosis
- BTK inhibition also attenuates the overactive B-Cell signaling to impact altered cell chemotaxis and adhesion and the development of a supportive tumor micro environment.

BTK inhibition is a clinically validated mechanism to treat cancers driven by dysfunctional BCR signalling

“Ibrutinib Resistance”: an emerging issue in treatment



About 30 per cent of patients of CLL develop resistance against Ibrutinib, a significant cause of which is a mutation in the BTK protein

Ibrutinib resistance:

- Development of clonally nonrelated aggressive lymphoma (Richter transformation),
- Richter transformation of a CLL subclone (both mostly occurring within 12 to 18 months of treatment initiation),
- Progressing CLL concurring with acquisition of BTK and/or PLCG2 mutations (mostly occurring after 12 to 18 months of treatment), and
- Possibly late progression in which no mutations of BTK or PLCG2 can be detected.

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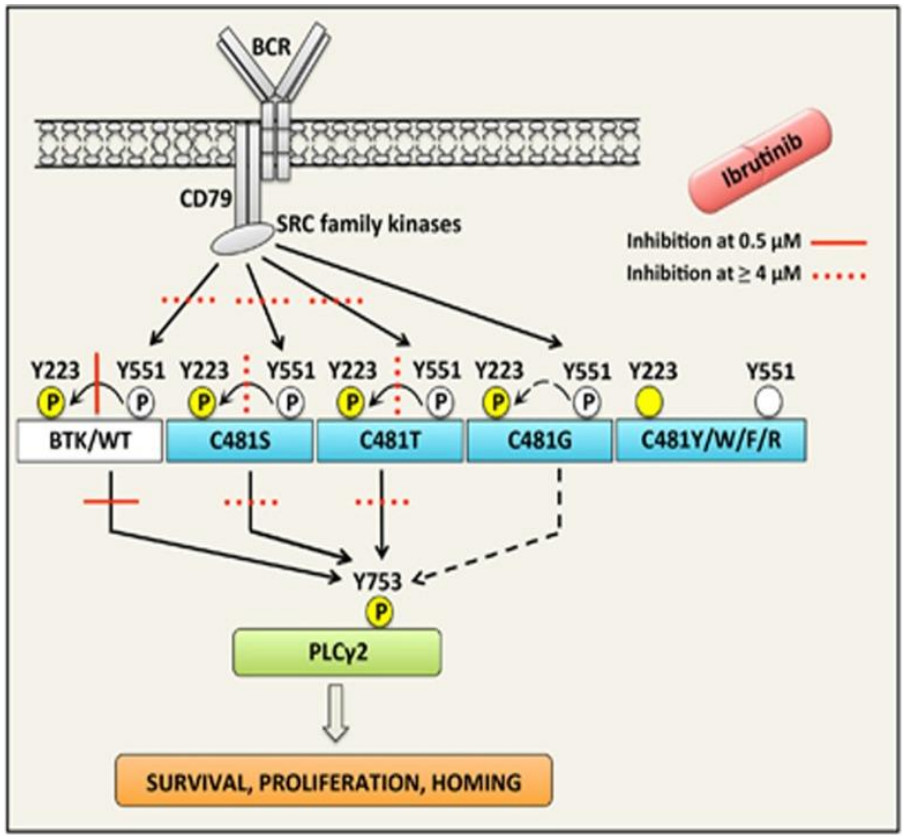
Avoiding Ibrutinib Resistance



Schematic representation of the BCR signaling pathway demonstrating the ibrutinib sensitivity of BTK C481 variants and their impact on downstream signaling

We have identified the following factors essential for a BTK inhibitor to overcome or avoid the occurrence of resistance in the treatment of CLL or other cancers driven by BCR pathway:

Nature and site of inhibition:

The C481S variant is a common mutant form caused because of bonding at the Cys-481 residue of BTK by covalent irreversible inhibition that escapes Ibrutinib action.

Potency and selectivity:

Enhanced selectivity for BTK, with low side effects would significantly block even the C481 escape variants, while being selective enough to avoid the inhibition of the T-cell kinase ITK[65, 66] and other check-point targets

PNQ-154: Reversible Bruton's Tyrosine Kinase (BTK) Inhibitor

Key Differentiators

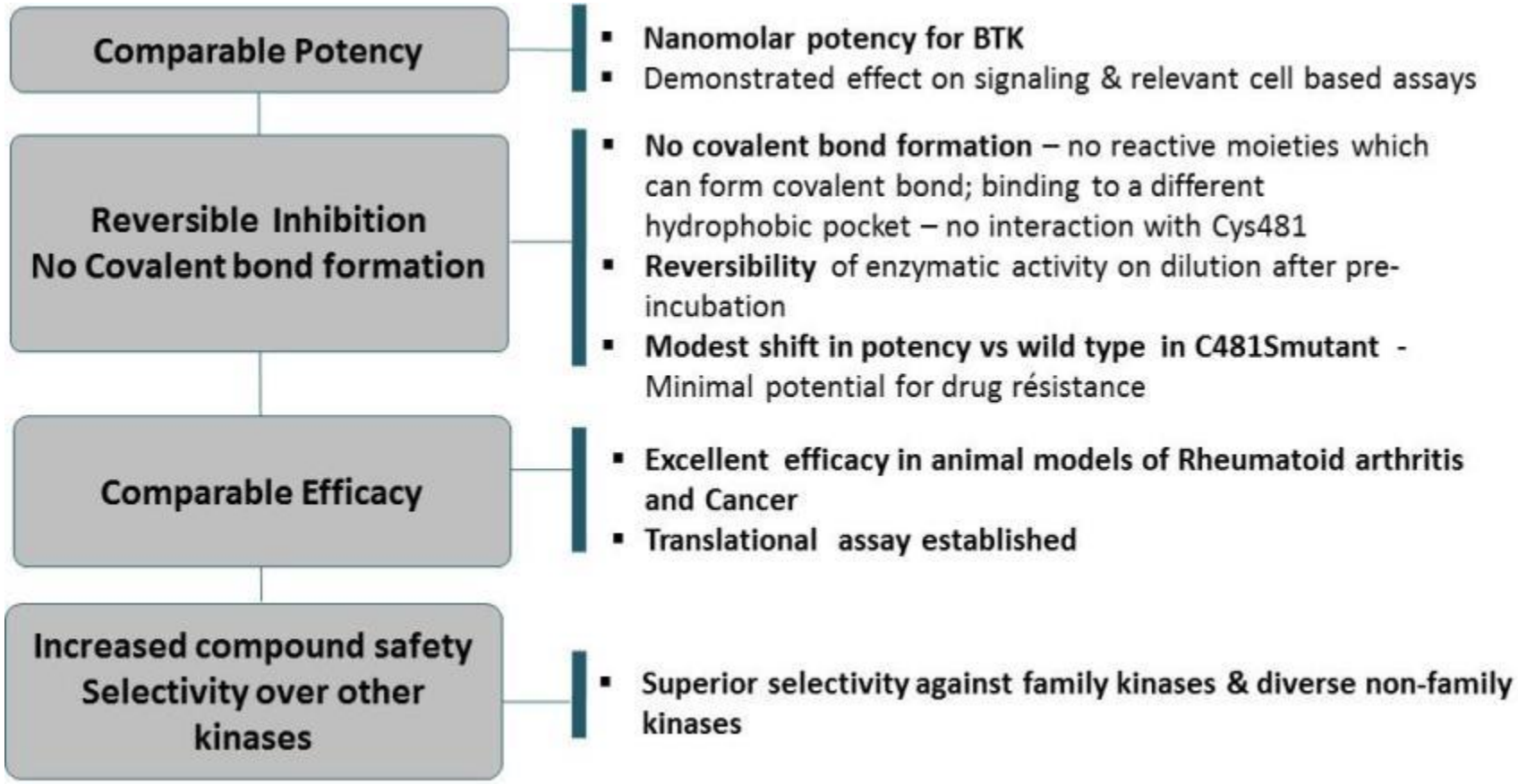
- Reversible, non-covalent BTK inhibitor
- Active against BTK Cys481 mutants

Best in Class Opportunity with demonstrated advantages over other BTK inhibitors & B cell targeted therapies and efficacy established in diffuse large B-cell lymphoma (DLBCL) Xenograft model

Status

- Patent granted.
- Long patent life till year 2034.
- 6-8 months from IND filing

PNQ-154: Advantage Over Competition



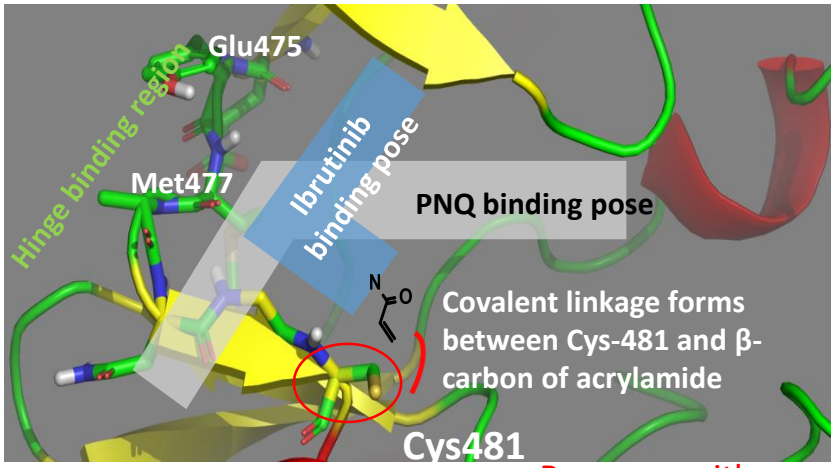
Comparable potency and efficacy in RA and cancer models model and superior kinase selectivity vs Ibrutinib may offer **superior long term safety** without compromising with efficacy in chronic treatment

PNQ-154: Differentiation based on Reversibility

Reversible inhibition

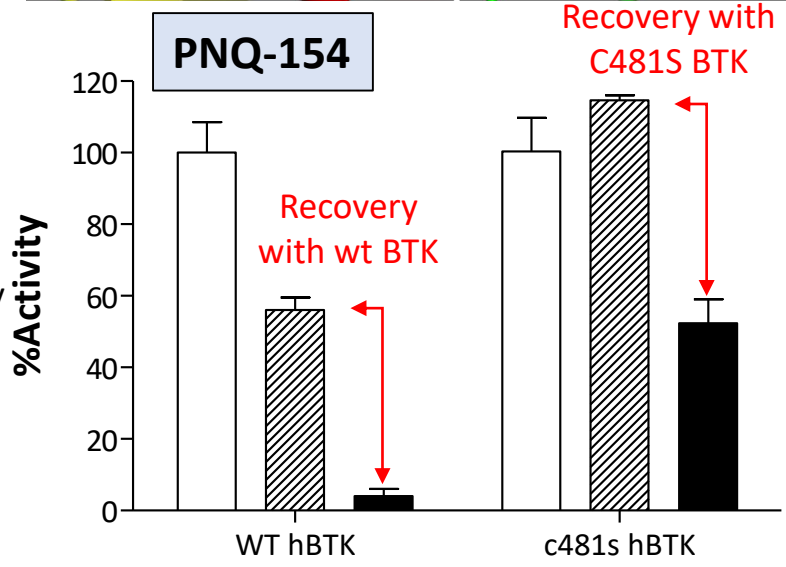
- No risk of inhibition of other cysteine-containing kinases and other enzymes by covalent modification
- No potential for drug resistance due to mutation of Cys-481 residue of BTK that forms covalent bonding with irreversible inhibitors
- No potential for covalent protein conjugate adduct formation leading to immunogenicity or loss of selectivity

Proposed binding pose of PNQ compounds



Intrinsically selectivity , with no/minimal inhibition of other kinases that may have potential safety risks

- TEC-sparing: Decreased platelet dysfunction/bleeding risk
- JAK-3-sparing: Decreased risk for profound immunosuppression
- EGFR-sparing: Decreased potential for diarrhea/GI toxicity



Small oral *bid* dose leading to full target coverage over 24 hrs, thus reducing the risk of Richter's transformation or other mutations/clones, without compromising the safety and side-effect profile

in vitro Pharmacology, selectivity and ADMET

Profile of PNQ-154: *In Vitro* Pharmacology

Parameter	Potency, IC_{50} (nM)		
	PCI-32765 Approved: MCL, CLL	**CC-292 P-I/II (CLL/RA)**	**PNQ-154**
hBTK IC_{50} (nM)	1.6 ± 0.4 (Literature: 0.5)	4 ± 0.7 (Lit: <0.5)	0.9 ± 0.08 (n=20)
Mouse splenocyte IC_{50} (nM) (BCR mediated; ↑CD69)	2.6 ± 1.2	15.7 ± 2.5	2.5 ± 0.3
Rat splenocyte IC_{50} (nM) (BCR mediated; ↑CD86)	1.24 ± 0.49	ND	5.5±1.3
Human whole blood IC_{50} (nM) (BCR mediated; ↑CD69)	16 ± 3	731 nM (max 65% inh at 10 µM)	16.5 ± 7
Mouse whole blood IC_{50} (nM) (BCR mediated; ↑CD69)	136.5±27.6	ND	91 ± 49
BTK phosphorylation in mouse splenocyte IC_{50} (nM)	ND	ND	279 ±128
Growth inhibition IC_{50} (nM) OCI-LY10 (DLBCL cell line):	2.1 ±0.2	861 ± 200	20 ± 4.5
Growth inhibition IC_{50} (nM) Mino (MCL cell line):	5350±850	1320±28	2400±1100

❖ **Potency- comparable vs Ibrutinib and superior vs CC-292 in human whole blood, cancer cell and other assays**

Profile of PNQ-154: *In Vitro* Pharmacology

Selectivity			
	PCI-32765 Approved: MCL, CLL (Irreversible)	**CC-292 P-I/II (CLL/RA) (Irreversible)**	**PNQ-154 (Reversible)**
hITK, JAK3, Syk IC_{50} (nM)	0.011, 0.016, >10	Lit: 0.036 (ITK); 0.0007 (Bmx); 0.006 (Tec)	>10,000, >10,000, 2785
Mouse splenocyte, TCR mediated cell based selectivity, IC_{50} (nM)	1170 ± 100	4150 ± 700	>30,000
Drug matrix screen: list of hits with >50% inhibition (% inhibition at 10 µM)	ND	ND	CYP2C19 (65), CYP2C9 (50), PPP3CA (52), A3 (72), Adrenergic α10 (53), Angiotensin AT2 (98), 5HT4 (56), σ2 (50), transporter adenosine (91)
100 Kinase Panel screen at 1&10 uM (Selection based on the relevance)	Literature: hits 14 kinases at 50nM	Lit: hits 4 kinases with IC_{50}<50 nM (out of 61 kinases)	More selective compared to Ibrutinib and CC-292 (Moderately selective against : Aurora, Bmx and Src (~80% inh. of at 1 µM)

❖ Superior selectivity against other BTK and 100 diverse non-BTK family kinases vs. Ibrutinib and CC-292 (see next slide)

Superior Kinase Profile of PNQ-154 vs Ibrutinib and CC-292

Kinase	Ibrutinib, IC_{50} (nM)	CC-292, IC_{50} (nM)	PNQ-154, IC_{50} (nM)
BTK	1.6 (IH); 0.46[lit-1]	4 (IH); <0.5[lit-2]	0.9 (IH)
TEC	77[lit-1]	6.2[lit-2]	~1,000*
BMX	0.76[lit-1]	0.7[lit-2]	81% @ 1 µM**
ITK	10.7 (IH)	36 (IH)	>10,000 (IH)
JAK3	16.1 (IH)	31 (IH)	>10,000 (IH)
SYK	>10,000 (IH)	976 (IH); 1,134[lit-3]	2,800 (IH)
LYN	200[lit-1]	4401[lit-3]	~10,000**
c-SRC	170[lit-1]	1729[lit-3]	78% @ 1 µM**
LCK	33[lit-1]	9079[lit-3]	~1,000**
BLK	0.5[lit-4]		>10,000**
EGFR	5.5[lit-4]		>10,000**
ABL	86[lit-4]		~1,000**
CSK	2.2[lit-4]		~10,000**
YES	6.5[lit-4]		~3,000**
FLT3	72.9 [Lit-4]		~10,000**
FGR	2.31 [Lit-4]		ND
HCK	3.67[Lit-4]		ND
Brk	3.34		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

IH: Advinus in-house data

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

ND: Not done

Profile of PNQ-154: *In Vitro* ADMET

Study	PNQ-154
Equilibrium solubility[tosylate salt] (pH: 2.1, 4, 7.4) in µM	>2000, 1147, <1 (free base); >2000, 1155, <1 (tosylate salt)
Caco-2 Permeability (nm/sec)	A-B: 54; B-A: 71
%PPB (mouse, rat, dog, human)	96.7±1.5; 95.5±2.3; 95.9±1.4; 92.6±0.5
MR (nmol/min/mg) (MLM/RLM/DLM/HLM)	<0.04, <0.04, 0.06, <0.04
Hepatocyte (% remaining at 30 min) (M, R, D, H)	83(M), 100 (R), 89 (D), 100 (H)
Stability in simulated gastric (SGF; % remaining at 1 h) and intestinal fluid (SIF; % remaining at 3 h)	97 (SGF); 100 (SIF)
CYP, IC50 (µM) (2C9, 2C19, 3A4, 1A2, 2D6, 2B6, 2C8)	>12.5 (except 2C8: 0.107)
CYP 3A4 TDI	No TDI liability
CYP phenotype assessment (1A2, 2C8, 2C9, 2C19, 2D6, 3A) CYP content: 100 pmol/mL; Incubation time: 60 min)	Predominantly metabolized by CYP3A
Interspecies metabolite fingerprinting	Metabolite finger printing was similar in the mice, rat, dog and human liver microsomes under tested in vitro conditions
hERG IC50 (Patch clamp) (µM)	6.7 µM
Cytotoxicity IC_{50} (MTT HEPG2) (µM)	>50 (100% survival)
PXR induction potential (µM)	No induction up to 50 µM
Mini Ames (5 strains±S9 fraction)	Non-mutagenic (5000 µg/plate)
Blood/plasma partitioning ratio	0.59 (mouse), 0.97 (rat)

❖ No liabilities observed for PNQ-154 from *in vitro* ADME perspective except CYP2C8 inhibition* (IC_{50}: 0.107 µM)

**Detailed studies required to understand its impact*

PK Profile of PNQ-154

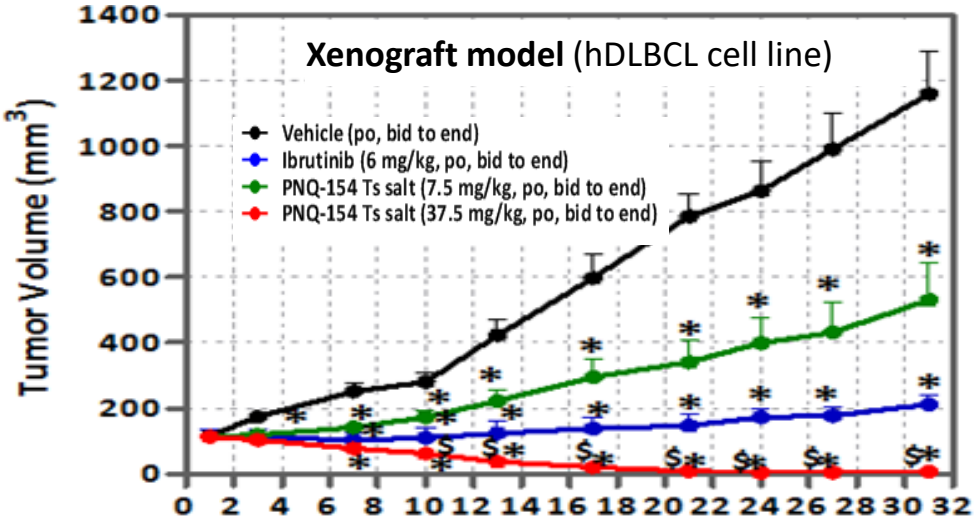
Compounds	PNQ-154	
Mouse PK profile (tosylate salt)	IV (3 mg/kg) CL: 2.7 5 mL/min/kg V_{ss}: 0.58 L/kg $T_{1/2}$: 4.45 h	PO (10 mg/kg) C_{max}: 13.8 µM AUC : 71 µM.h F(%): 82
Rat PK profile (tosylate salt)	IV (3 mg/kg) CL: 7.5 mL/min/kg V_{ss}: 2.18 L/kg $T_{1/2}$: 3.66 h	PO (10 mg/kg) C_{max}: 3.75 µM AUC : 32.6 µM.h F(%): 100
Dog PK profile (tosylate salt)	IV (1 mg/kg) CL: 5.8 mL/min/kg V_{ss}: 1.6 L/kg $T_{1/2}$: 5.2 h	PO (3 mg/kg) C_{max}: 1.4 µM AUC : 9.5 µM.h F(%): 77

Predicted human dose is in the range of 5-10 mg with elimination half life of ~15 h

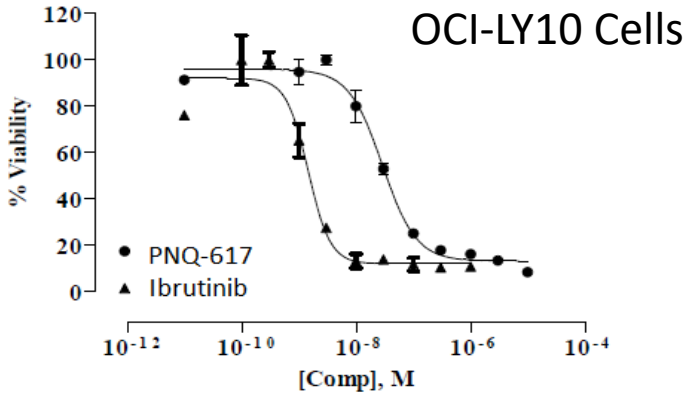
Preclinical Efficacy

PNQ-154: Efficacy in preclinical assays

Tumor Volume



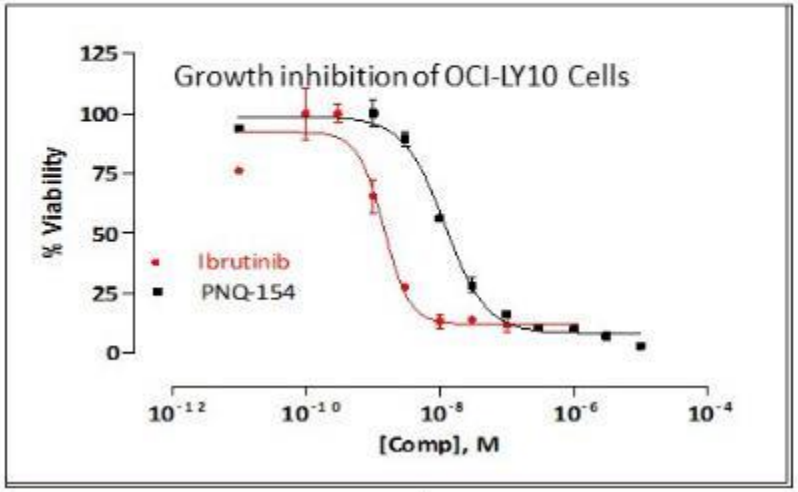
Cell Growth Suppression



- PNQ-154 has demonstrated **excellent efficacy** in cells as well in vivo
- Significant and dose dependent inhibition of OCI-LY10 (DLBCL) and Mino (MCL) cell growth
- Significant and dose-dependent tumor growth inhibition with complete regression at end of study

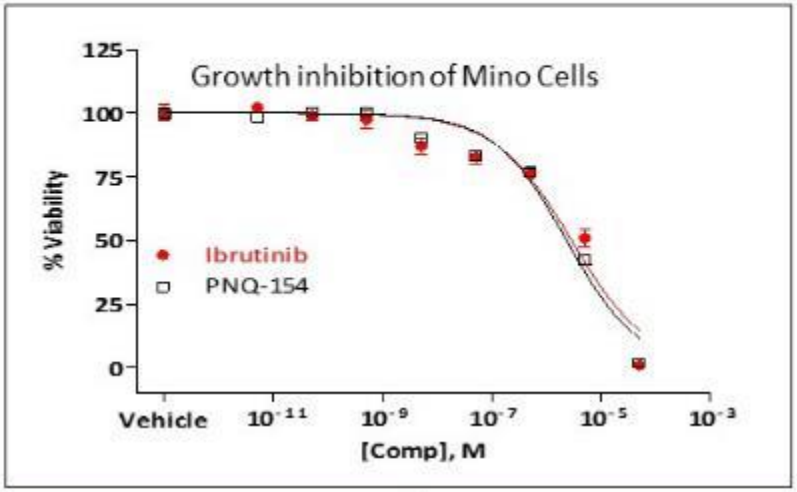
PNQ-154: Potency of Cancer Cell Growth Inhibition

OCI-LY10 (DLBCL Cell line)



Compound	Avg IC_{50} ± SD (nM)
Ibrutinib	2.1±0.02
PNQ-154	20.1± 4.5

Mino(MCL Cell line)



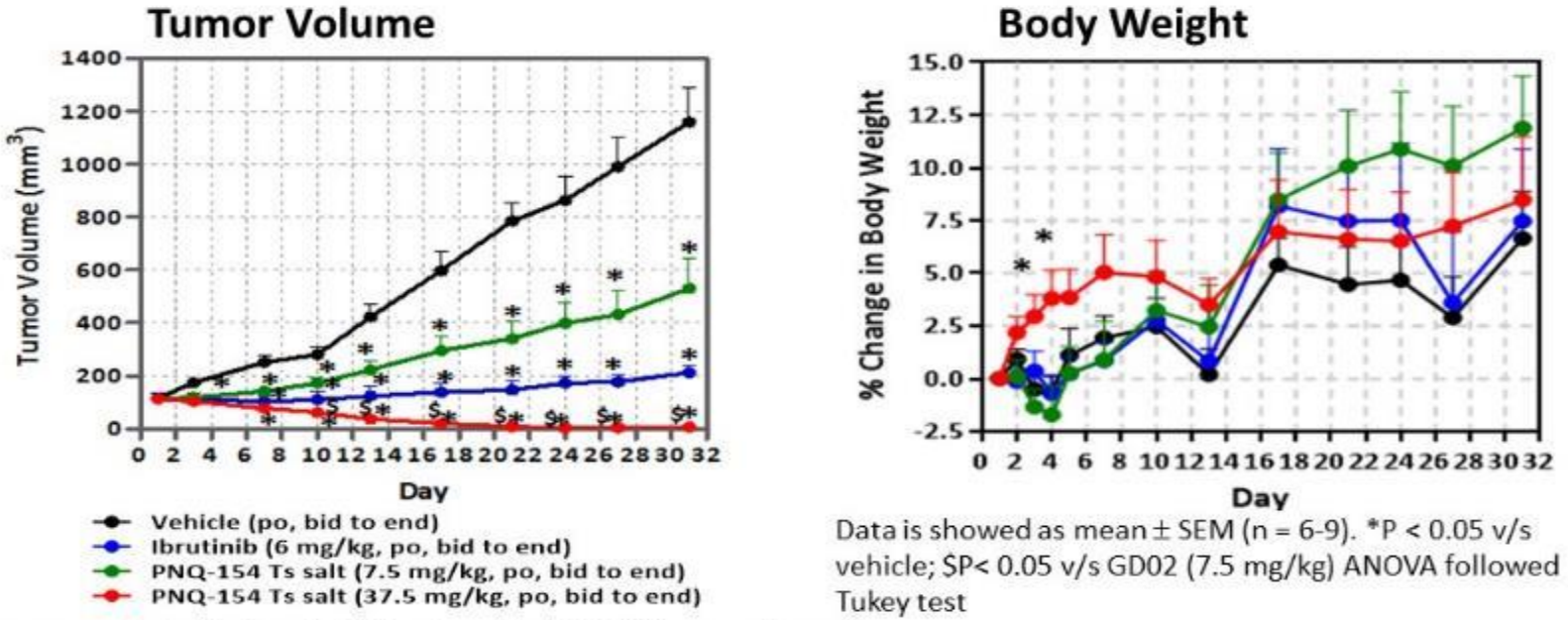
Compound	Avg IC_{50} ± SD (nM)
Ibrutinib	2670±900
PNQ-154	1940±300

OCI-LY10 is a Diffuse large B-cell lymphoma (DLBCL) cell line which exhibit aggressive & rapid growth
Mino is Mantle Cell Lymphoma Cell line (MCL)

- Dose dependent inhibition of DLBCL & MCL (Mino) cells

PNQ-154: Early Onset with Complete Tumor Regression

OCI-LY10 (human DLBCL cell line) Xenograft Model

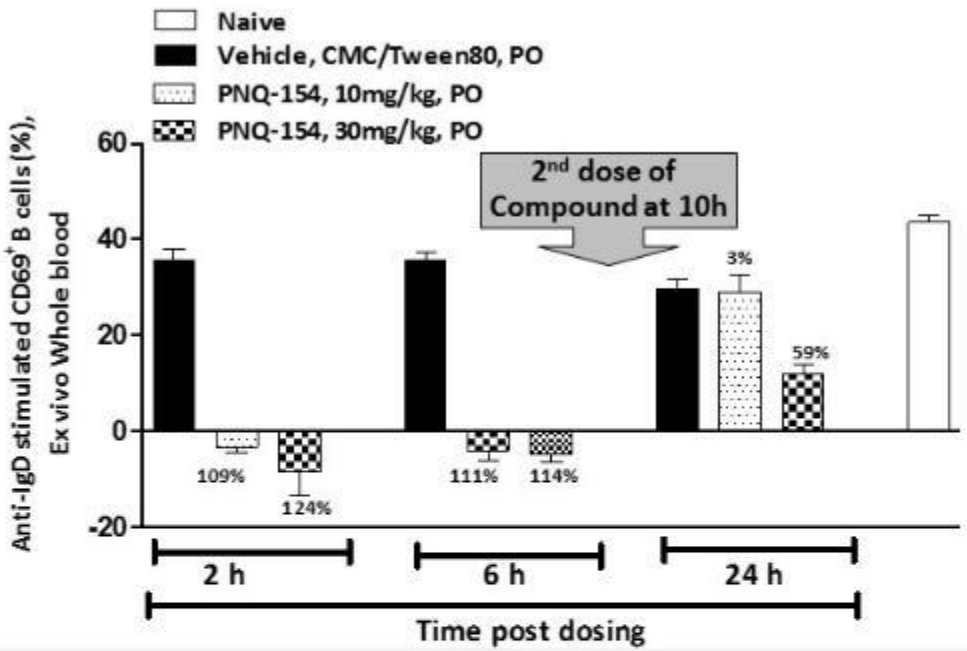


Data is showed as mean ± SEM (n = 6-9). *P < 0.05 v/s vehicle; $P< 0.05 v/s GD02 (7.5 mg/kg) ANOVA followed Tukey test

Study was conducted in female SCID mice using PNQ-154 as tosylate salt.

- PNQ-154 showed significant efficacy with early-onset and dose-dependent tumor growth inhibition; no change in body weight
- **Complete tumor regression at 30 mg/ kg by day-21**

Translational Assay for Target Engagement in FIH Established



PNQ-154	Time (h)	Plasma conc (μM)	Approx. fold above IC_{50}
10 mg/kg	2#	7.32 ± 1.28	80
	6#	3.33 ± 0.69	36
	14*	0.05 ± 0.02	0.6
30 mg/kg	2#	16.71 ± 3.24	183
	6#	13.64 ± 1.00	150
	14*	0.47 ± 0.18	5

represents post 1st dose;* post 2nd dose

- **Demonstrated target engagement:**

Inhibition of anti-IgD induced CD69 up-regulation on B cells ex vivo in whole blood from compound treated mice :

>100% inhibition up to 6 hr after first dose at 10 & 30mg/kg

It is a clinical biomarker/translational assay for BTK target engagement in FIH trials and beyond (human whole blood assay established)

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PNQ-154 : Differentiation from Competition

- Opportunity for B-cell lymphoma and other cancers
- **Comparable potency and efficacy** in a B cell lymphoma model and superior kinase selectivity vs Ibrutinib may offer **superior long term safety** without compromising with efficacy in chronic treatment
  - Unlike irreversible inhibitors:
    - **No potential for drug resistance** due to mutation of Cys-481 residue of BTK that forms covalent bonding with irreversible inhibitors (non-responder patient population is emerging in clinic with Ibrutinib due to Cys-481 mutation)
    - **No potential for covalent protein conjugate adduct formation** leading to immunogenicity or loss of selectivity
  - Ibrutinib hERG patch clamp IC_{50} ~1 µM vs. ~7 µM for PNQ-154

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Thank You