Preclinical ITP Characterization of Pirtobrutinib: A Non-Covalent, Reversible, Bruton Tyrosine Kinase Inhibitor

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OBJECTIVES

To provide a preclinical assessment of pirtobrutinib activity in ITP using both in vitro and in vivo methods

CONCLUSIONS

- Pirtobrutinib is a highly selective, non-covalent, reversible, BTK inhibitor showing efficacy and tolerability in B-cell malignancies, leading to approvals in CLL/SLL and MCL
  - Its optimized pharmacokinetic properties ensures high levels of sustained BTK inhibition over 24 hours with once-a-day dosing
- *In vitro*, pirtobrutinib reduced B-cell activation, antibody production, and FcγR-mediated TNFα production in human monocytes
- In a mouse ITP model, pirtobrutinib concentrations increased with dose and positively correlated with platelet response. The high dose (30 mg/kg) had similar positive efficacy as control group, IVIG.
- To our knowledge, this is the first preclinical report of a non-covalent BTK inhibitor's effect on platelet response in ITP
- These data, combined with existing data demonstrating pirtobrutinib's selective and potent BTK inhibition, support further clinical investigation of pirtobrutinib for ITP treatment

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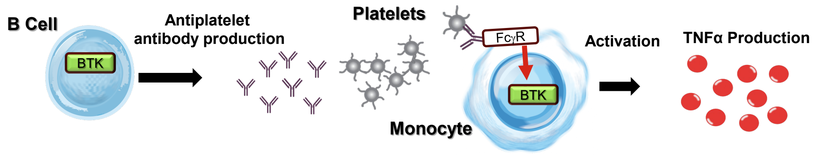
Background

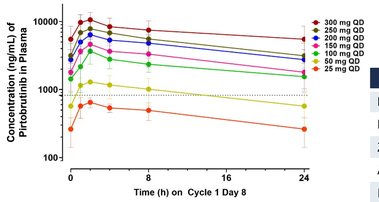
Bruton Tyrosine Kinase (BTK) Role in Key Mechanisms of ITP Pathology



- B cells play a key role in ITP through the production of antiplatelet-antibodies[1-3]
- Binding of the platelet-bound antibodies to FcγR on monocytes leads to their activation that potentiates the autoimmune response[1-3]
- Pirtobrutinib previously demonstrated dose-dependent inhibition of B cell activation[6] and could be studied for ITP therapy

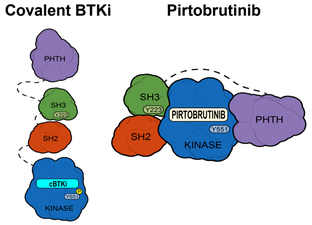
Pirtobrutinib is a Non-covalent, Reversible, BTK Inhibitor with Unique PK Properties and Binding Mechanism

Plasma exposures throughout dosing interval[5]



Half life, hours[6,8-11]	
Pirtobrutinib	19
Rilzabrutinib	3-4
Zanubrutinib	2-4
Acalabrutinib	1
Ibrutinib	4-6

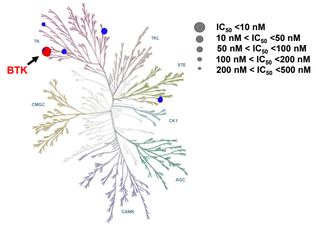
Pirtobrutinib may stabilize BTK in a closed inactive conformation[4]



- Pirtobrutinib, when dosed at 200 mg once daily, produces steady state plasma exposure corresponding to 96% BTK target inhibition[4] and a half-life of 19 hours[6]
- Pirtobrutinib is approved for treating relapsed or refractory MCL in adults in the EU (EMA Conditional Approval, Oct 2023) after prior treatment with a BTK inhibitor,[7] and in the USA (FDA Accelerated Approval, Jan 2023) after at least two lines of systemic therapy, including a BTK inhibitor, and for adult patients with CLL/SLL (FDA Accelerated Approval, Dec 2023) who have received at least two prior lines of therapy, including a BTK inhibitor and a BCL-2 inhibitor[6]
- Pirtobrutinib inhibits BTK activity by:
  - Binding to BTK via an extensive water-mediated hydrogen bond network, unlike covalent BTKi that rely on Cys481 (shared by 9 other kinases)[4]
  - Potentially stabilizing BTK in a closed, inactive conformation, blocking access to upstream kinases and phosphorylation of Y551, potentially inhibiting kinase-independent BTK signaling[4]

Pirtobrutinib Demonstrates High Selectivity for BTK

Pirtobrutinib was highly selective for BTK in >98% of the human kinome[5]

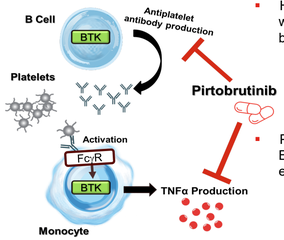


Selectivity of BTK Inhibitors in Biochemical Assays

	% Enzyme Activity[4]				Kinase Assay	
	Pirtobrutinib		Ibrutinib	Zanubrutinib	Pirtobrutinib	Rilzabrutinib[12]
	1000 nM	100 nM	100 nM	100 nM	IC_{50}, nM	IC_{50}, nM
BTK	1.8	3	1.1	2.7	3.2	1.3
ITK	103.4	106.1	2.3	85.2	>5000	440
RLK	19.6	68.4	-0.1	0.4	209	1.2
TEC	64.6	97.2	3	8.9	1234	0.8
BMX	70.2	94.6	-0.1	2.5	1155	1.00
BLK	72.8	81.7	0.6	-0.4	4100	6.3

- In follow-up cellular studies, pirtobrutinib retained >100-fold selectivity over other tested kinases, including selectivity for BTK over other TEC family member kinases (ITK, RLK, TEC, & BMX) where covalent BTKi have failed to maintain selectivity[12]
  - Pirtobrutinib only inhibits BTK (IC50) at single-digit nanomolar concentrations, whereas Rilzabrutinib inhibits 4/5 TEC family kinases at single-digit nanomolar concentrations
- Pirtobrutinib has shown favorable safety and tolerability in the oncology setting with low rates of discontinuation due to toxicity[13,14]

Methods Assessing Pirtobrutinib in ITP



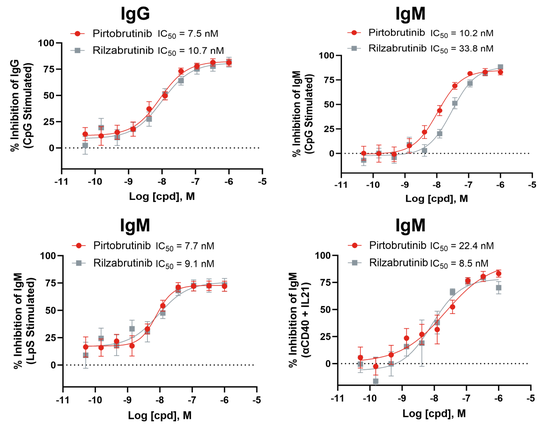
In Vitro

- Human B cells and monocytes were treated with pirtobrutinib or rilzabrutinib for one hour before stimulation to assess
  - Antibody production
  - TNFα release and gene expression

In Vivo

- Prior to antibody mediated platelet depletion, BALB/c Mice (n=7-8/group) were treated with either:
  - Pirtobrutinib (PO, 10 or 30 mg/kg)
  - Vehicle (PO, 0.5%methycelluose/0.5%tween80/water)
  - IVIG (IV, 1000mg/kg)

Pirtobrutinib Inhibits Key Mechanisms of ITP pathology: B cell antibody production



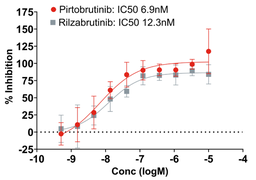
- Human B cells from 5 donors were treated with pirtobrutinib or rilzabrutinib (1μM-0.05nM) for one hour before stimulation with cytidine-guanosine dinucleotides, LpS, or αCD40 and IL21 for 7 days
- Secretion of IgM and IgG were reduced with pirtobrutinib treatment
- Secretion of IgM was reduced with pirtobrutinib treatment independent of stimulation condition

Antibody production was measured via ELISA

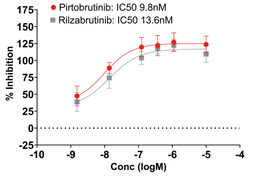
Pirtobrutinib Inhibits Key Mechanisms of ITP pathology: Antibody-dependent Monocyte Activation

Pirtobrutinib potently inhibited both TNFα release and gene expression from human monocytes stimulated with antibody immune complexes

TNFα Secretion



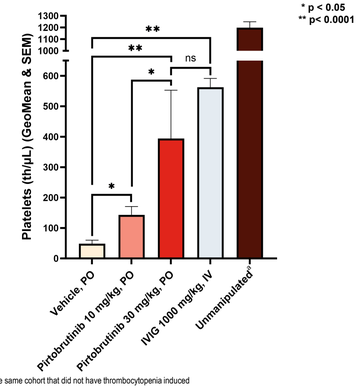
TNFα Gene Expression



- FCγR signaling was activated on human monocytes through treatment with antibody immune complexes

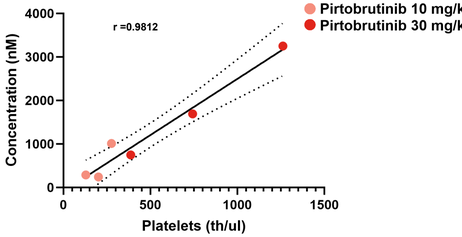
Pirtobrutinib Increased Platelet Counts in a Mouse Model of ITP

Platelet Counts in Mouse Model of ITP Treated with Pirtobrutinib



[a]Refers to the set of mice from the same cohort that did not have thrombocytopenia induced

Linear Regression of Pirtobrutinib Blood Concentrations and Platelet Counts



- Pirtobrutinib blood concentrations increased as dose increased and had a positive correlation with platelet response
- Pirtobrutinib significantly and dose-dependently increased platelet counts in mice relative to vehicle treated control mice
- Statistically, the high dose level (30 mg/kg) of pirtobrutinib was not significantly different from the positive efficacy control group, IVIG

Abbreviations

BTK, Bruton Tyrosine Kinase; CLL, chronic lymphocytic leukemia; FCγR, Fc gamma Receptor; IgG, immunoglobulin; IgM, immunoglobulin; ITP, Immune Thrombocytopenia; IVIG, Intravenous Immunoglobulin; IV, intravenous; MCL, mantle cell lymphoma; PO, by mouth; QD, once daily; SLL, small lymphocytic lymphoma; th/μL, thousands per microliter;TNF, tumor necrosis factor, th/μL.

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