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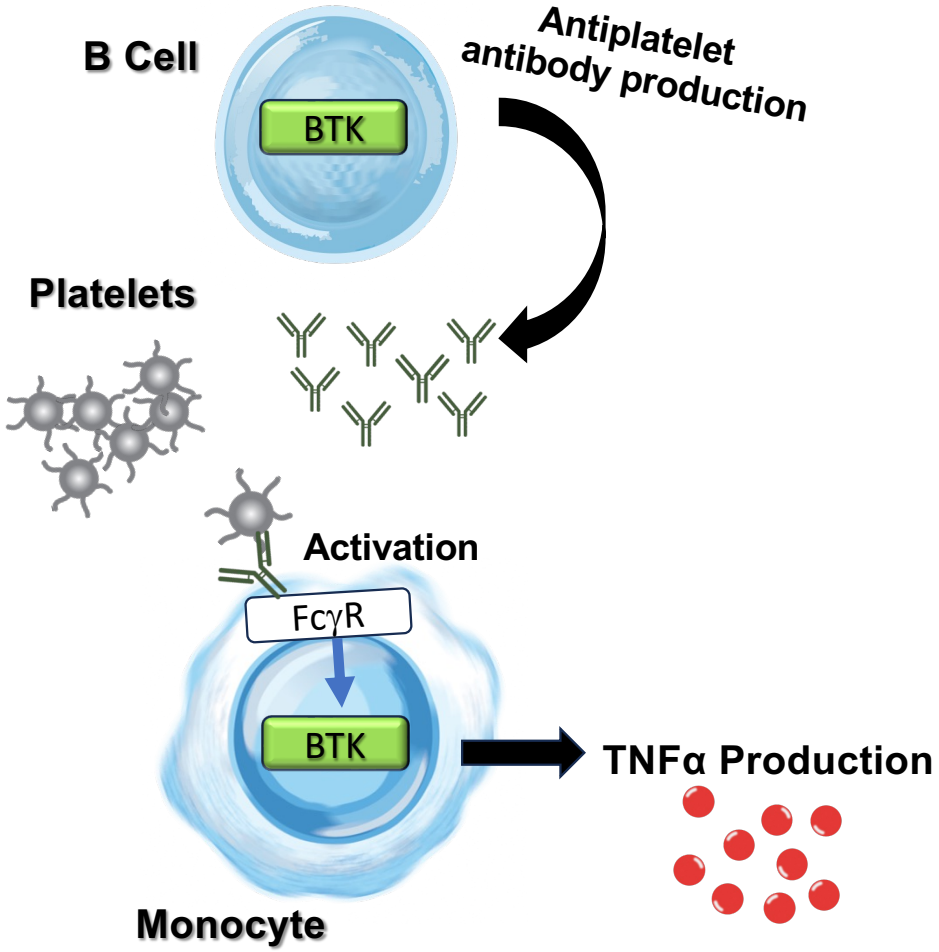
# PRECLINICAL ITP CHARACTERIZATION OF PIRTOBRUTINIB: A NON-COVALENT, REVERSIBLE, BRUTON TYROSINE KINASE INHIBITOR

# Disclosures of Tomás José González-López

| Company name | Research support | Employee | Consultant | Stockholder | Speakers bureau | Advisory board | Other |
|---|---|---|---|---|---|---|---|
| Novartis | X | | X | | X | X | |
| Amgen | X | | X | | X | X | |
| Grifols | X | | X | | X | X | |
| Sobi | X | | X | | X | X | |
| Argenx | | | X | | X | | |
| UCB | | | X | | X | | |
| Alpine | | | X | | X | | |
| Momenta | | | X | | X | X | |


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



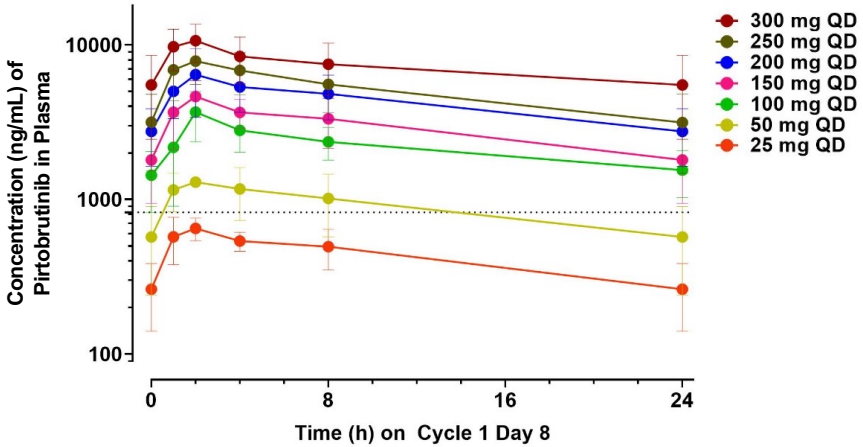

- B cells play a key role in ITP through the production of antiplatelet-antibodies[1-3]
- Binding of the platelet-bound antibodies to FcγR on monocytes leads to their activation that potentiates the autoimmune response[1-3]
- Pirtobrutinib previously demonstrated dose-dependent inhibition of B cell activation[4] and warrants further study of pirtobrutinib as a therapeutic option for ITP

**Selective BTK inhibition may impede multiple mechanisms in ITP**

[1]Cooper et al. NEJM 2019. [2]Audia et al. *J. Clin. Med.* 2021. [3]Cines et al. *NEJM* 2002. [4]Gomez et al. *Blood*.2023. Abbreviations: BTK, Bruton Tyrosine Kinase; TNF, tumor necrosis factor, FCγR, Fc gamma Receptor.

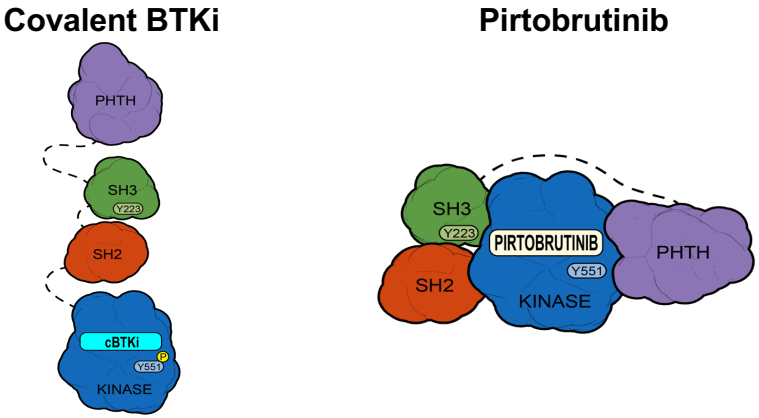
# Pirtobrutinib is a Non-covalent, Reversible, BTK Inhibitor with Unique PK Properties and Binding Mechanism

### 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]



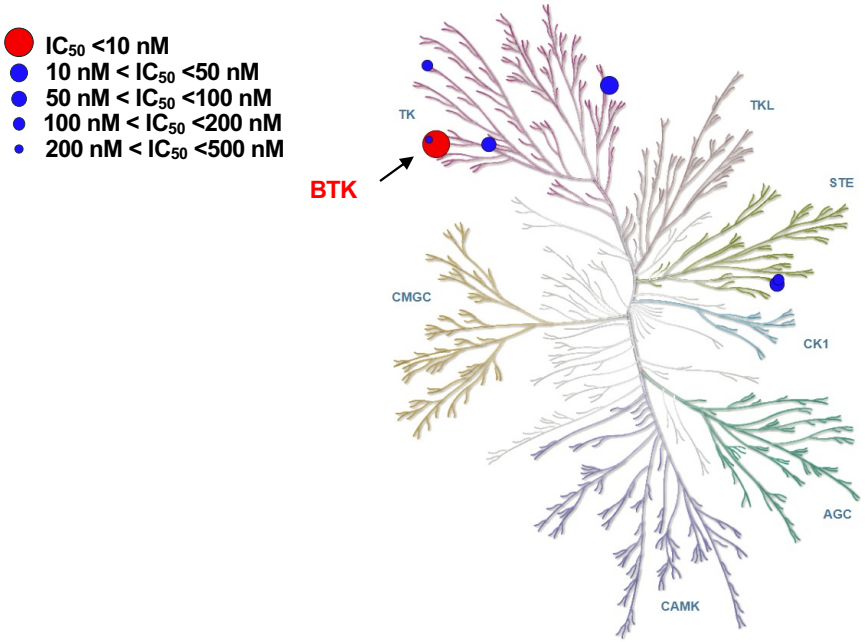

- Pirtobrutinib, when dosed at 200 mg once daily, produces steady state plasma exposure corresponding to 96% BTK target inhibition[4] and a half-life of 19 hours[6]
- Pirtobrutinib is approved for treating relapsed or refractory MCL in adults in the EU (EMA Conditional Approval, Oct 2023) after prior 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, inhibiting kinase-independent BTK signaling[4]

[4]Gomez et al. *Blood*.2023. [5]Mato et al, *Lancet* 2021. [6]Jaypirca [Prescribing Information]. Indianapolis, IN, 2023, 2024. [7]Jaypirca EPAR (EMEA/H/C/005863), European Medicines Agency, 2023. [8]Ucpinar et al. *Clin Transl Sci.* 2023. [9]Brukinsa [Prescribing Information]. San Mateo, CA: BeiGene, 2024. [10]Calquence [Prescribing Information]. Wilmington, DE: AstraZeneca, 2022. [11]Imbruvica [Prescribing Information]. South San Francisco, CA: Pharmacyclics LLC, 2024. Abbreviations: CLL, chronic lymphocytic leukemia; MCL, mantle cell lymphoma; QD, once daily; SLL, small lymphocytic lymphoma.

# Pirtobrutinib Demonstrates High Selectivity for BTK

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



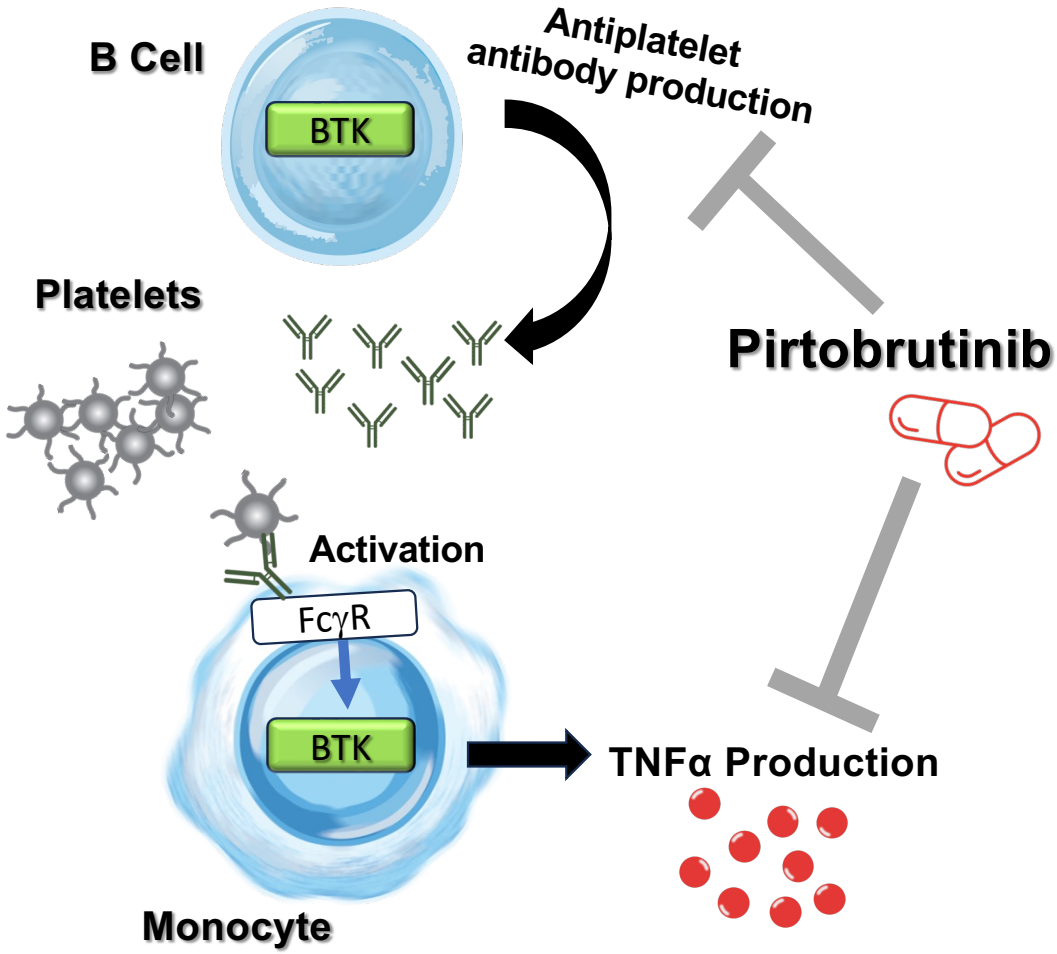

## Selectivity of BTK Inhibitors in Biochemical Assays

| | % 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 ($IC_{50}$) 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]

[12]Langrish et al, *J Immunol 2021*.[13]Coombs et al, *JCO* 2022.[14]Nirav et al, *JCO* 2023.

# Methods Assessing Pirtobrutinib in ITP




## *In Vitro*

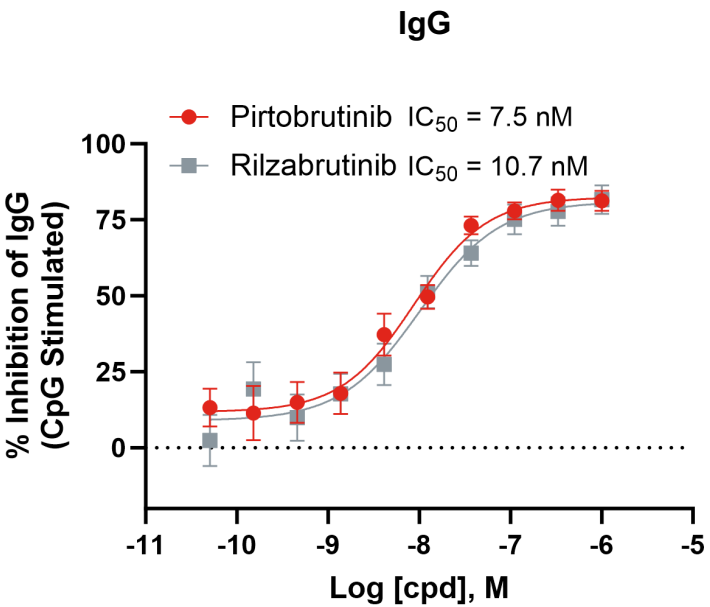
- Human B cells and monocytes were treated with pirtobrutinib or rilzabrutinib for one hour before stimulation to assess:
  - Antibody production
  - TNFα release and gene expression

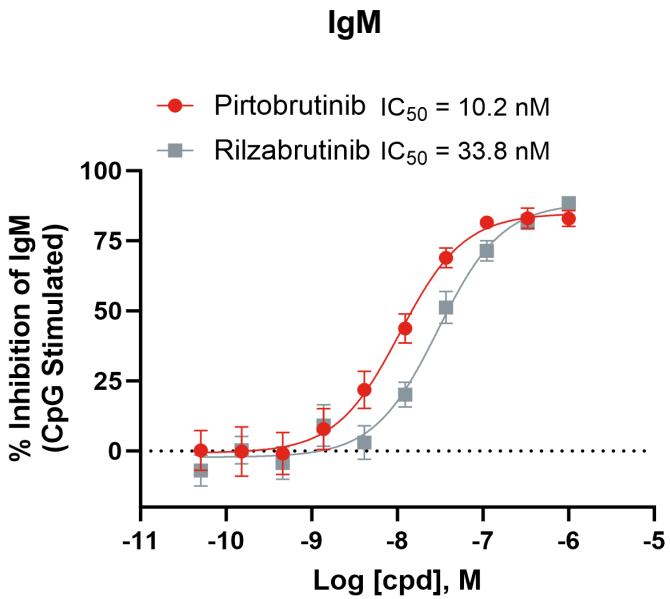
## *In Vivo*

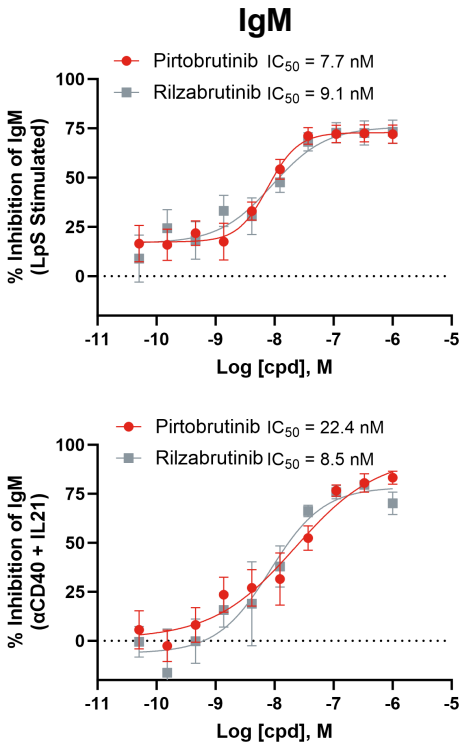
- Prior to antibody mediated platelet depletion, BALB/c Mice (n=7-8/group) were treated with either:
  - Pirtobrutinib (PO, 10 or 30 mg/kg)
  - Vehicle (PO, 0.5%methycelluose/0.5%tween80/water)
  - IVIG (IV, 1000mg/kg)

Abbreviations: BTK, Bruton Tyrosine Kinase; FCγR, Fc gamma Receptor; PO, by mouth; TNF, tumor necrosis factor.

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



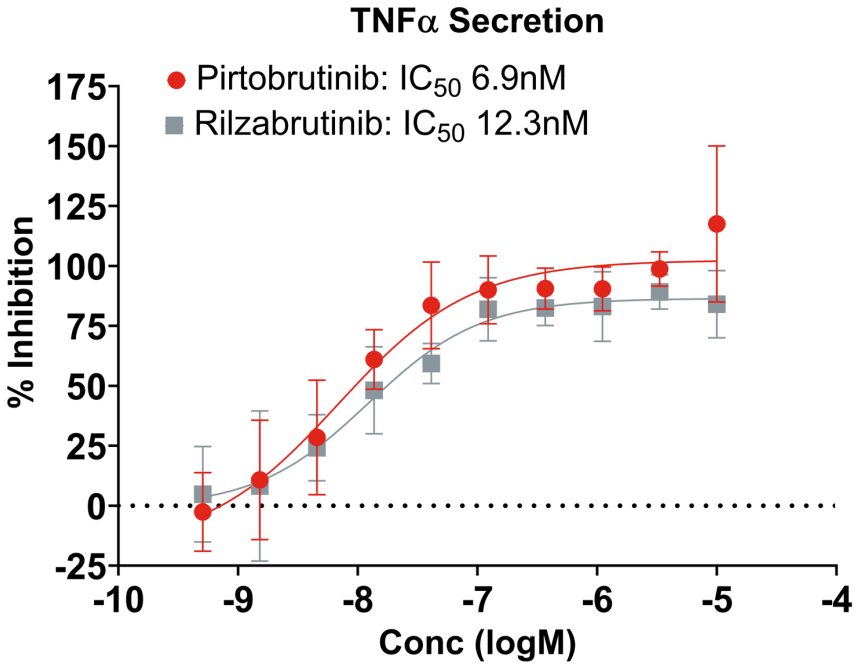



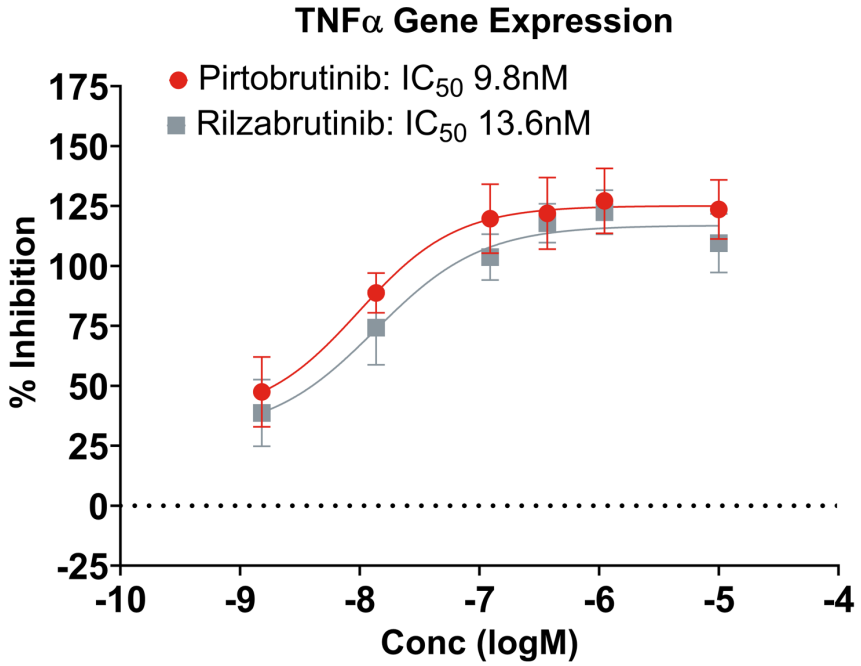

- Human B cells from 5 donors were treated with pirtobrutinib or rilzabrutinib (1μM-0.05nM) for one hour before stimulation with cytidine-guanosine dinucleotides, LpS, or αCD40 and IL21 for 7 days
- Secretion of IgM and IgG were reduced with pirtobrutinib treatment
- Secretion of IgM was reduced with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

Antibody production was measured via ELISA. Abbreviations: IgG, immunoglobulin ; IgM, immunoglobulin M.

# 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



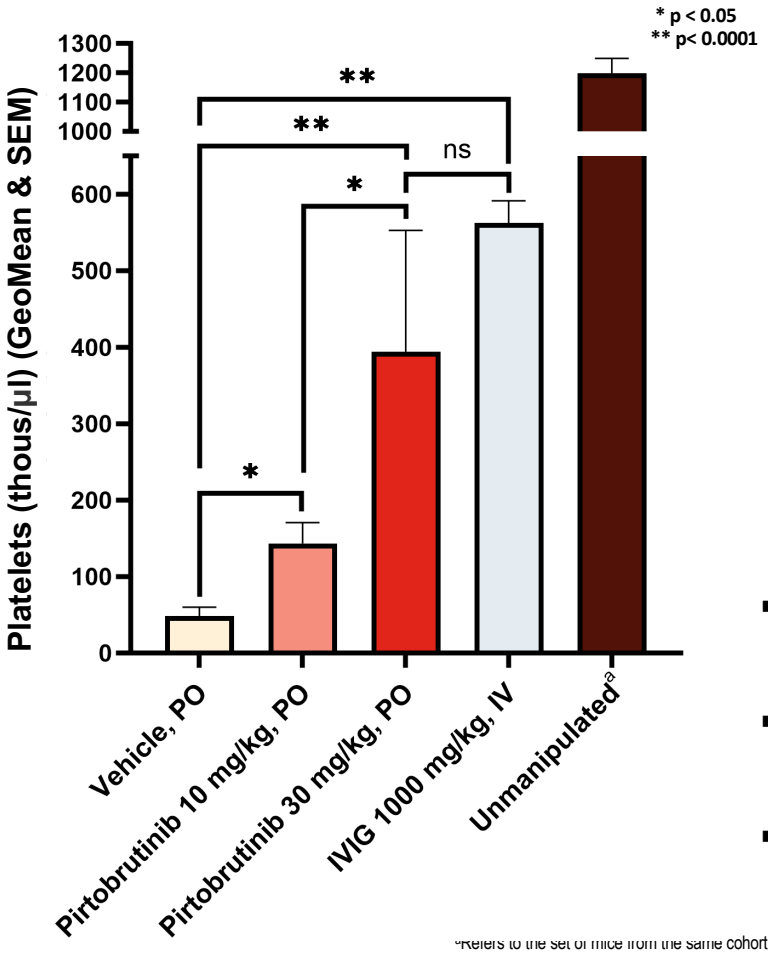




FCγR signaling was activated on human monocytes through treatment with antibody immune complexes

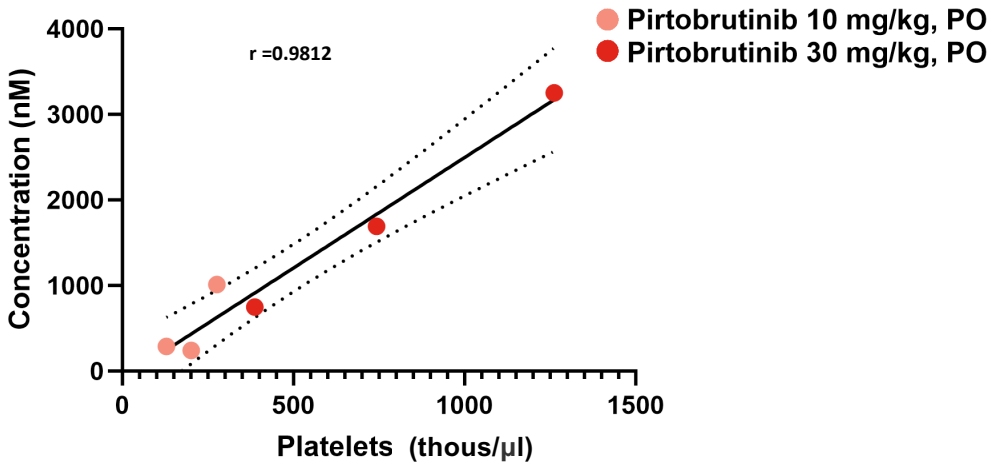
Abbreviations: ITP, Immune thrombocytopenia; FCγR, Fc gamma Receptor; TNF, tumor necrosis factor.

# Pirtobrutinib Increased Platelet Counts in a Mouse Model of ITP

**Platelet Counts in Mouse Model of ITP Treated with Pirtobrutinib**




**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

[a]Refers to the set of mice from the same cohort that did not have thrombocytopenia induced. Abbreviations: ITP, Immune thrombocytopenic; IVIG, Intravenous Immunoglobulin; IV, intravenous; PO, by mouth; thous/µL, thousands per microliter.

# Conclusion

- 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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