BTK Leu528Trp - a Potential Secondary Resistance Mechanism Specific for Patients with Chronic Lymphocytic Leukemia Treated with the Next Generation BTK Inhibitor Zanubrutinib

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BTK inhibitors in CLL

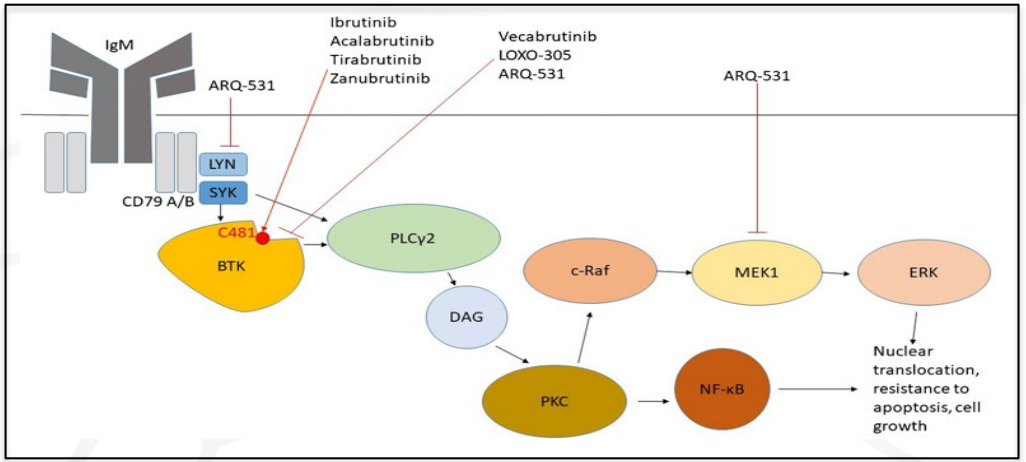
- Highly efficacious class of agent in frontline and relapsed/refractory CLL
  (Byrd *et al*, NEJM 2014; Shanafelt *et al*, NEJM 2019; Woyach *et al*, NEJM 2018)

- BTKi covalently bind to Cys481 residue in BTK resulting in blocking of enzymatic activity

- "First generation" BTKi
  - Ibrutinib

- "Second generation" BTKi
  - Acalabrutinib, tirabrutinib, zanubrutinib

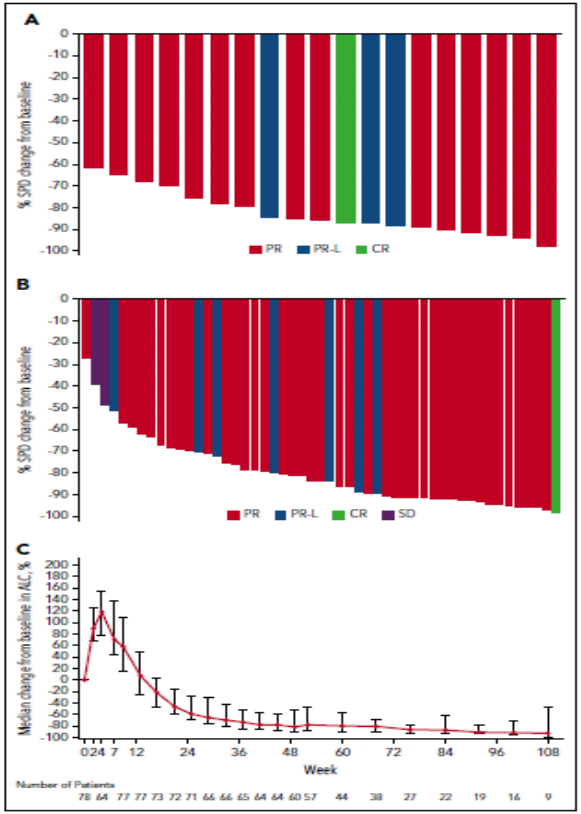
- Non-C481 dependent BTKi
  - Vecabrutinib, LOXO-305, ARQ-531



(Bond & Woyach, 2019)

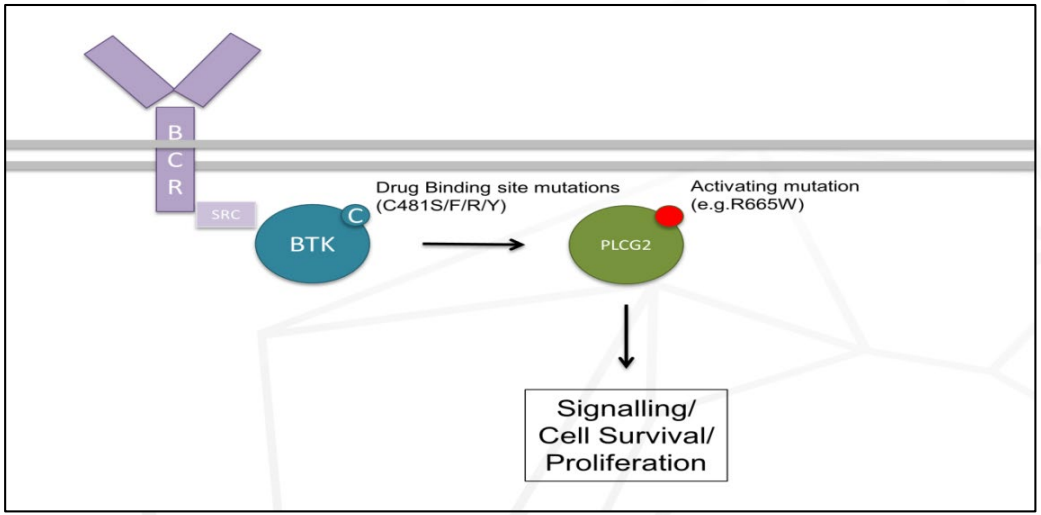
Zanubrutinib in CLL

- Second generation BTK inhibitor
- Efficacious in treatment-naïve and relapsed/refractory CLL/SLL (Tam *et al,* Blood 2019)
- Multiple clinical trials in a variety of B-cell lymphoma subtypes ongoing
- Greater selectivity for BTK (over EGFR, ITK, and TEC) than ibrutinib (Tam *et al*, Blood 2019)



Resistance to BTK inhibitors in CLL

- BTK inhibitor (ibrutinib) resistance mechanisms
  - (i) Drug-binding site Cys481 mutations (Cys481Ser, Cys481Phe/Arg/Tyr) (Woyach *et al,* NEJM 2014)
  - (ii) Downstream activating PLCG2 mutations (Liu *et al,* Blood 2015)



Aim

- To investigate possible resistance mechanisms to the second generation BTK inhibitor zanubrutinib (ZANU) in patients with CLL

Cohort

- 38 patients with relapsed/refractory CLL treated with ZANU on clinical trials (NCT02343120, NCT02569476, NCT03336333, NCT02795182) at three centres in Melbourne, Australia

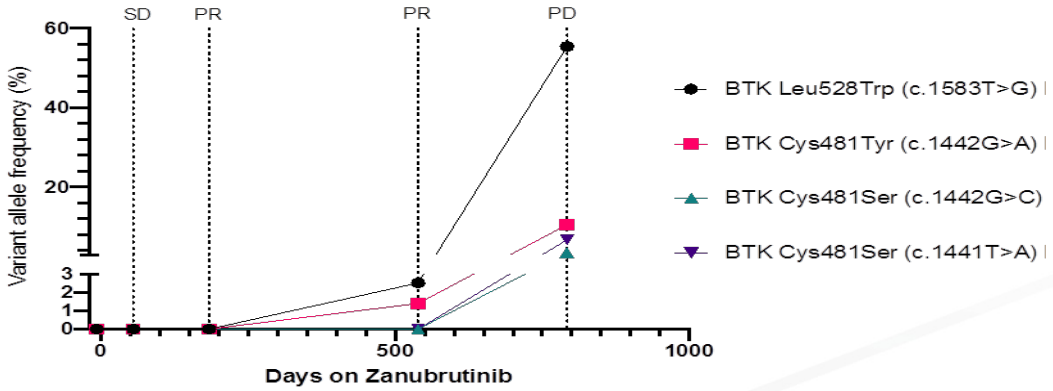
- Four of 38 patients had CLL progression on ZANU (time to progression 5, 26, 29 and 48 months)

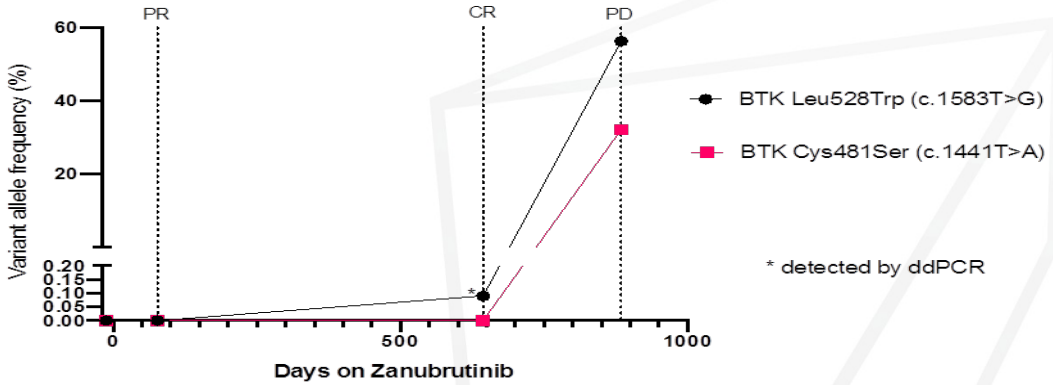
- Amplicon next generation sequencing (NGS)
  - Targeted amplicon sequencing (sensitivity approx 3-5% VAF)
    - ARAF, BCL2, BIRC3, BRAF, BTK (exon 11, 15, 16) , CARD11, CD79B, CXCR4, DNMT3A, EZH2, FOXO1, FYN, ID3, IDH1, IDH2, JAK3, KRAS, MAP2K1, MYD88, NOTCH1, NRAS, PHF6, PLCG1, PLCG2(exon 16, 19-20, 24, 27-28), RHOA, RUNX1, SF3B1, STAT3, STAT5B, STAT6, TCF3, TP53, XPO1

BTK Leu528Trp detected in 4/4 patients with progressive CLL on zanubrutinib

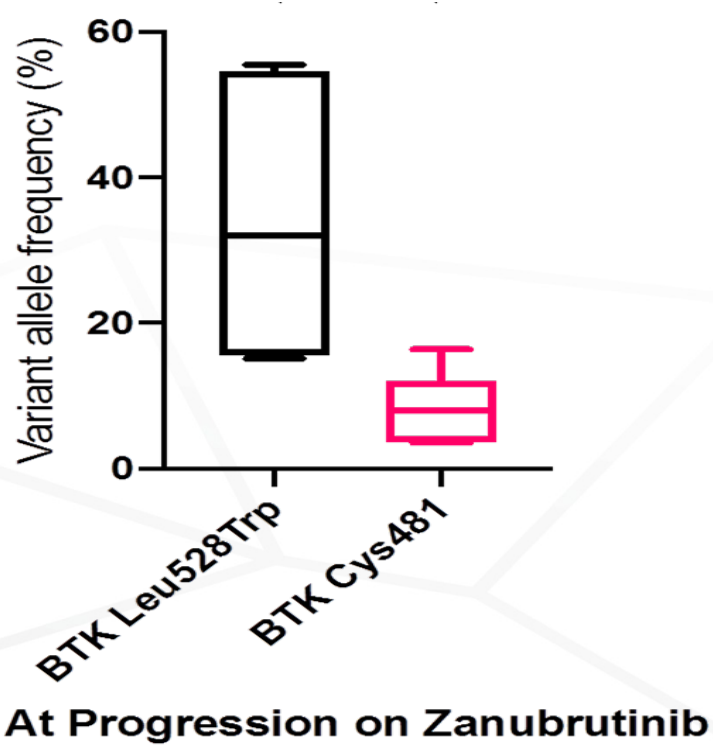
Patient ID	Pre-ZANU	Post-ZANU
CLLZ1	TP53 c.659A>G; p.(Tyr220Cys)	TP53 c.659A>G; p.(Tyr220Cys) **BTK c.1441T>A; p.Cys481Ser** **BTK c.1583T>G; p.Leu528Trp**
CLLZ2	BRAF c.1799T>A; p.(Val600Glu) NOTCH1 c.7541_7542del; p.(Pro2514Argfs*4)	BRAF c.1799T>A; p.(Val600Glu) NOTCH1 c.7541_7542del; p.(Pro2514Argfs*4) **BTK c.1441T>A; p.(Cys481Ser)** **BTK c.1442G>C; p.(Cys481Ser)** **BTK c.1583T>G; p.(Leu528Trp)**
CLLZ3	No mutations detected	TP53 c.1125_1140del; p.(Ser376Lysfs*41) **BTK c.1441T>A; p.(Cys481Ser)** **BTK c.1442G>C; p.(Cys481Ser)** **BTK c.1442G>A; p.(Cys481Tyr)** **BTK c.1583T>G; p.(Leu528Trp)**
CLLZ4	BRAF c.1406G>C; p.(Gly469Ala) XPO1 c.1711G>A; p.(Glu571Lys)	BRAF c.1406G>C; p.(Gly469Ala) XPO1 c.1711G>A; p.(Glu571Lys) **BTK c.1442G>C; p.(Cys481Ser)** **BTK c.1583T>G; p.(Leu528Trp)**

Leu528Trp is detectable in zanubrutinib treated patients before clinical CLL progression





* detected by ddPCR



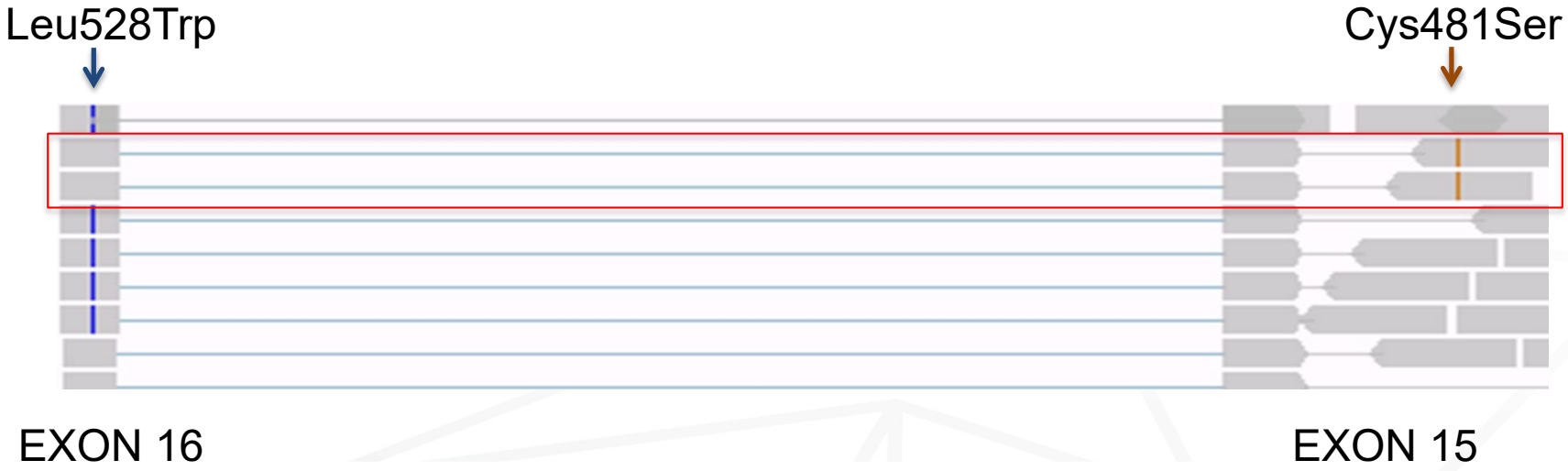
At Progression on Zanubrutinib

Leu528Trp identified in 3 out of 34 patients on zanubrutinib in steady state

- ddPCR performed on 34 patients without disease progression but persistent measurable disease on zanubrutinib

- BTK Leu528Trp detected in 3 out of 34 patients (VAF <1%)

BTK Leu528Trp and Cys481 mutations are present in different cells in zanubrutinib progressors



BTK Leu528Trp and Cys481 mutations are present in different cells in zanubrutinib progressors

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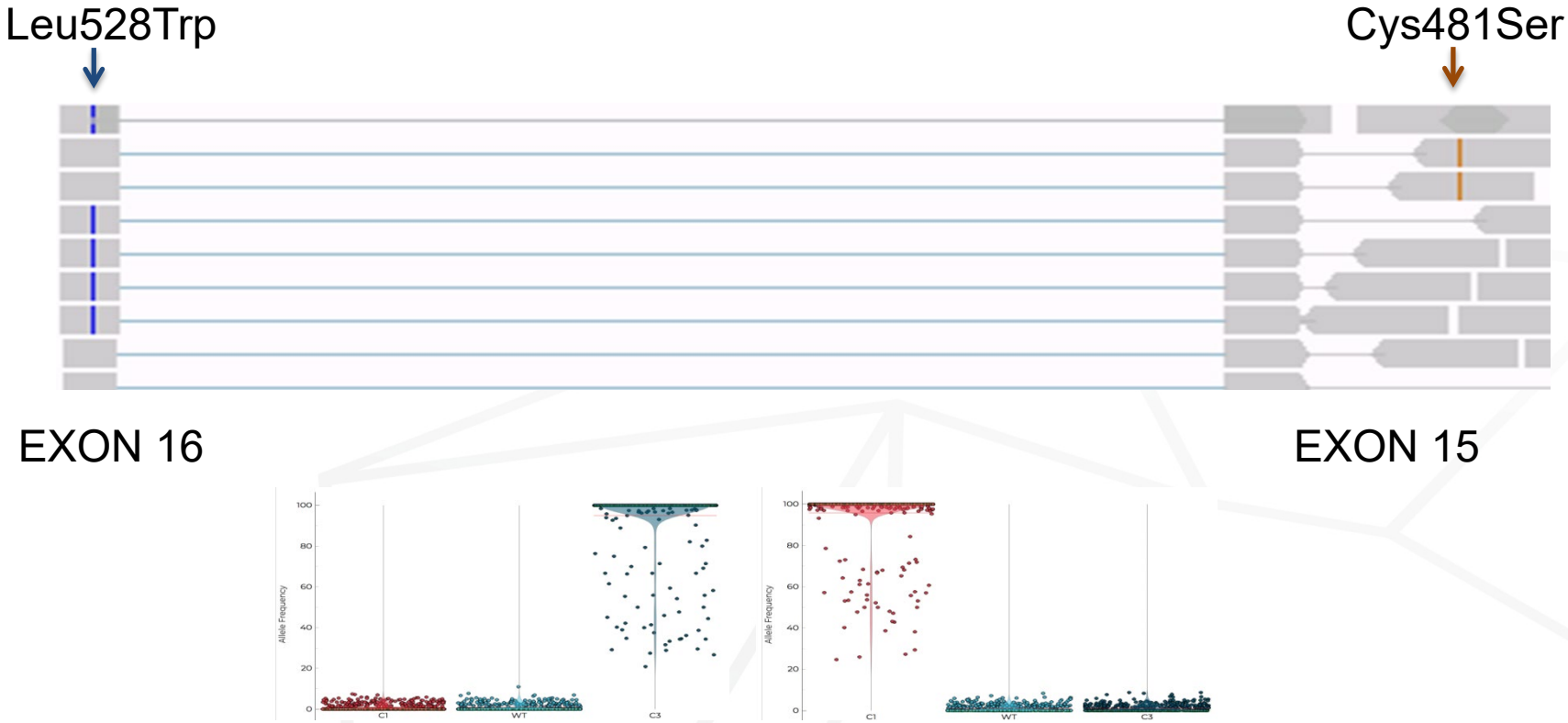


BTK Leu528Trp is rarely observed in patients treated with ibrutinib

Author	BTK Leu528 codon assessed	Patients with progressive CLL	BTK Cys481 (and non-Leu528Trp)	BTK Leu528Trp
Woyach et al, NEJM 2014	Yes	6	5	0
Maddocks et al, JAMA 2015	Yes	19 (8 RT)	13	1
Sharma et al, Oncotarget 2016	Yes	1	1	0
Woyach et al, J Clin Oncol 2017	Yes	46	37	0
Gango et al, Int J Cancer 2019	Yes	20	8	0
Kanagal-Shamanna et al, Cancer 2019	Yes	29	19	0
Total		**121**	**83 (68.5%)**	**1 (0.8%)**

49 patients with progressive CLL on ibrutinib (European Research Initiative CLL [ERIC])

- Targeted next generation sequencing (Haloplex)
- 0/49 patients found to harbor the BTK Leu528Trp

BTK Leu528Trp mutation disrupts the binding pose of ibrutinib, zanubrutinib and tirabrutinib

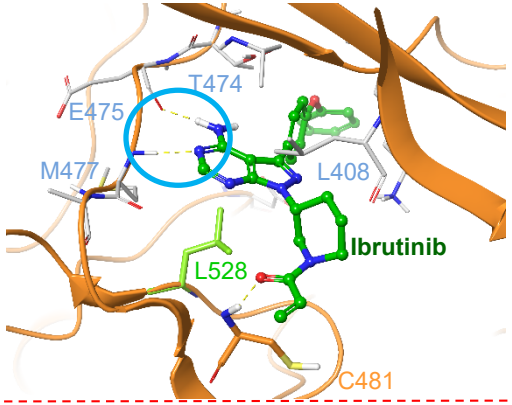
Net change in binding free energy: Trp528 vs Leu528: 9.37±0.38 kcal/mol

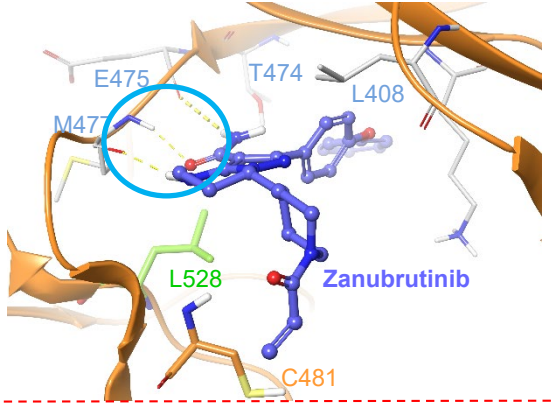
Net change in binding free energy: Trp528 vs Leu528: 10.04±0.53 kcal/mol

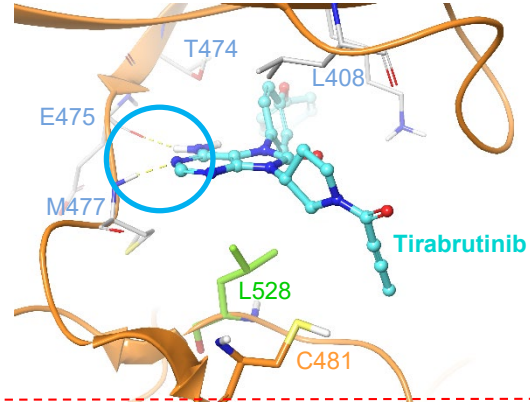
Net change in binding free energy: Trp528 vs Leu528: 7.57±0.34 kcal/mol

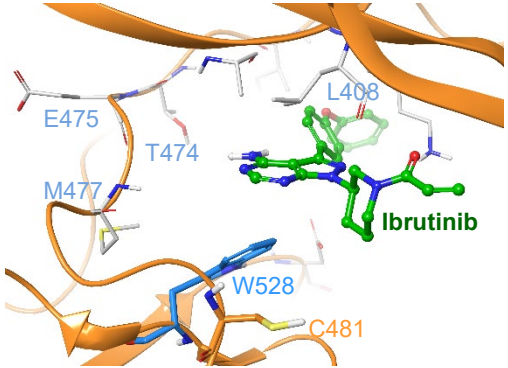
Wildtype BTK

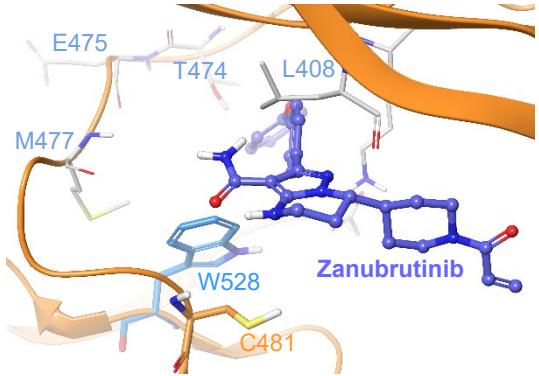
BTK Leu528Trp

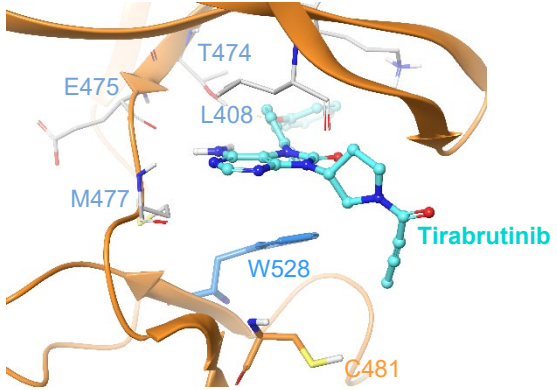










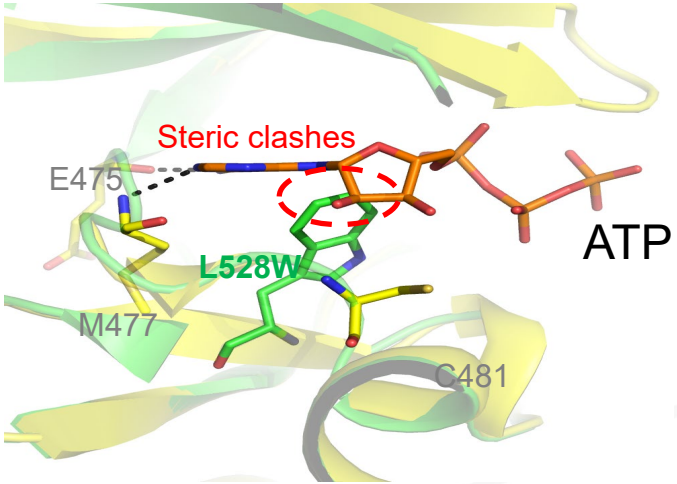


IBRUTINIB

ZANUBRUTNIB

TIRABRUTINIB

BTK Leu528Trp leads to abrogated kinase function in biochemical and cellular models



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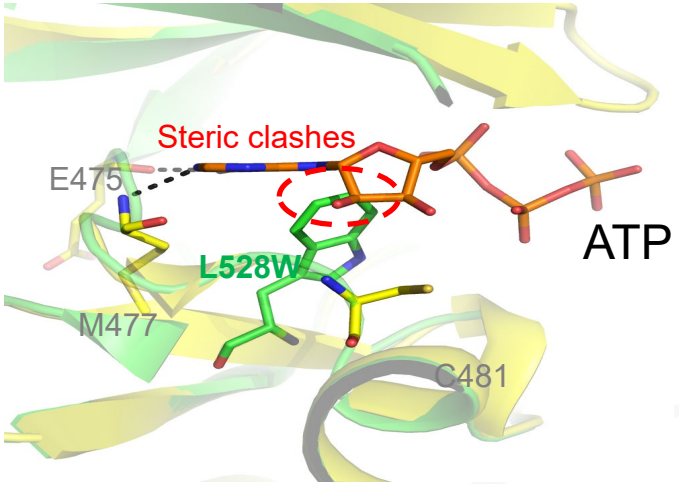

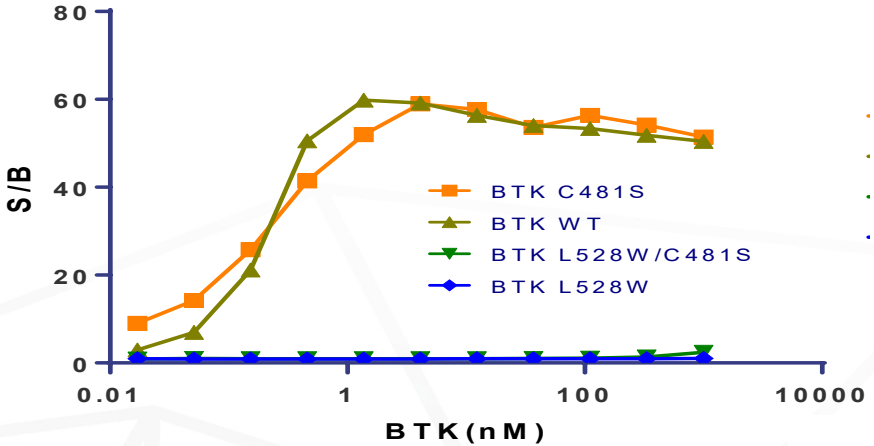

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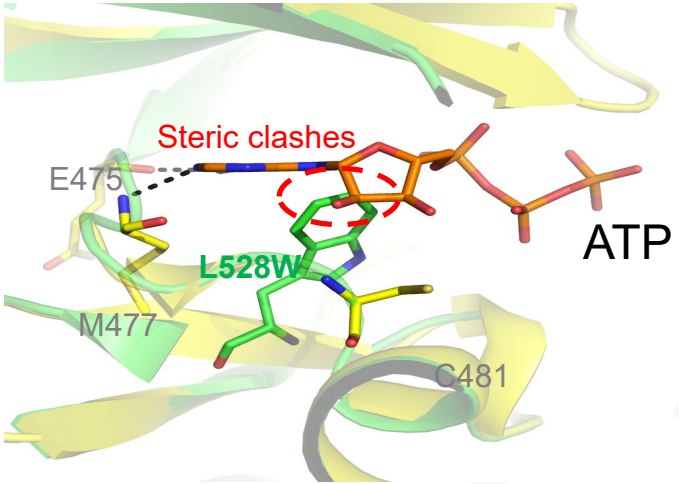
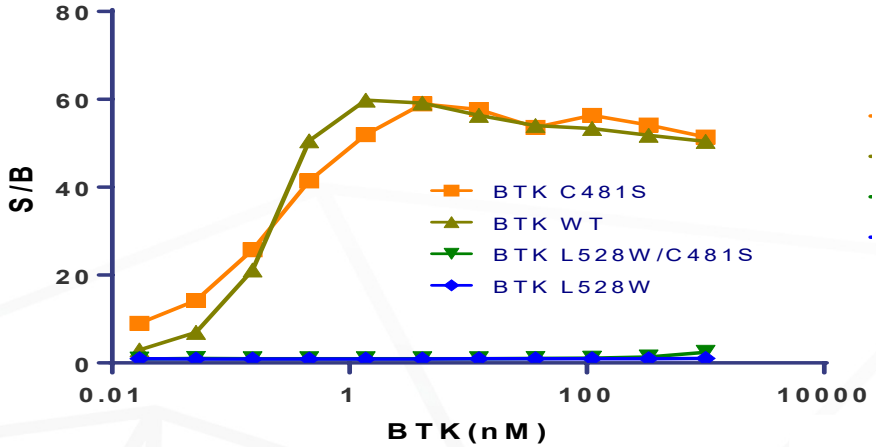
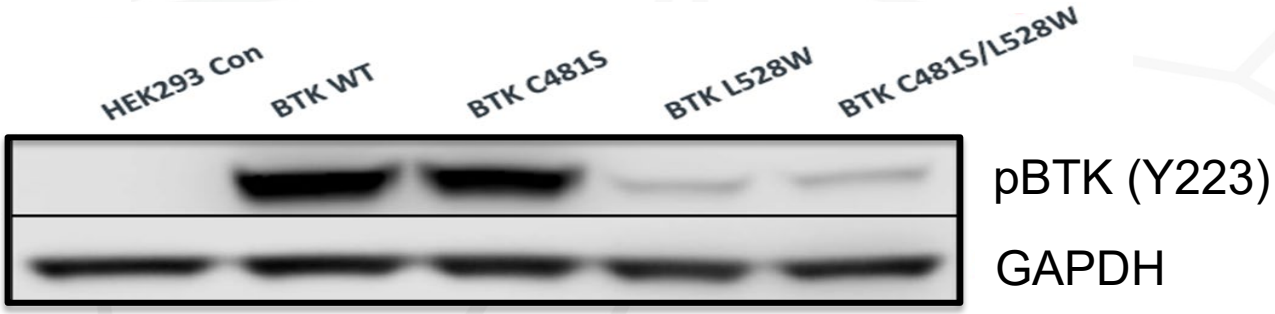
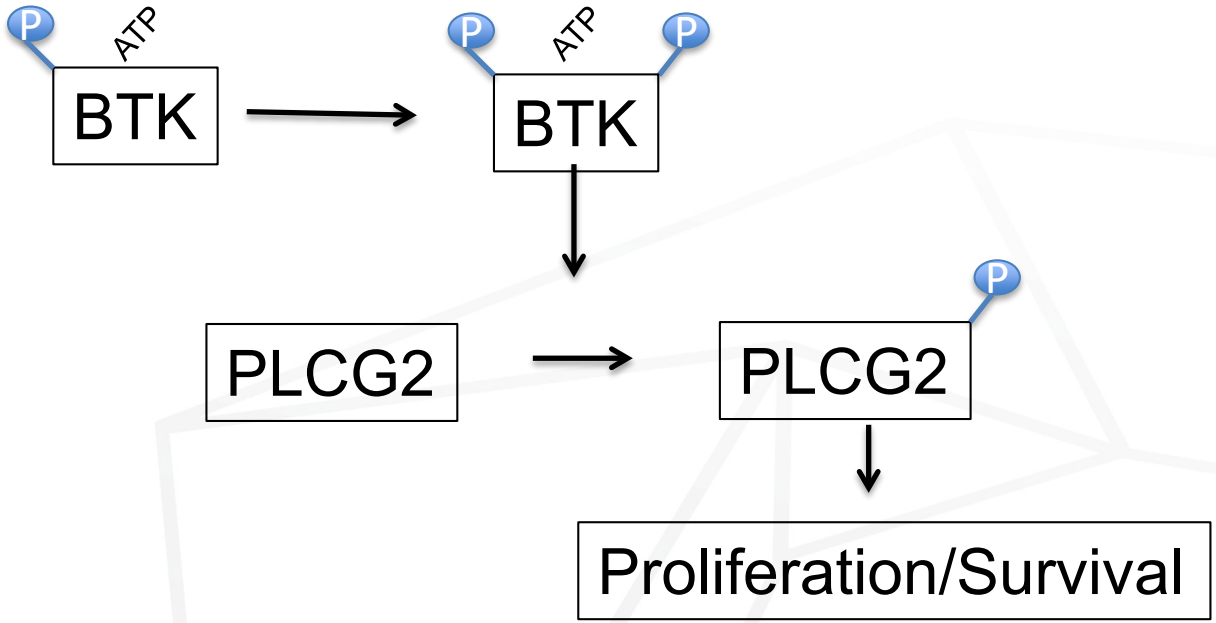






BTK Leu528Trp and downstream signalling



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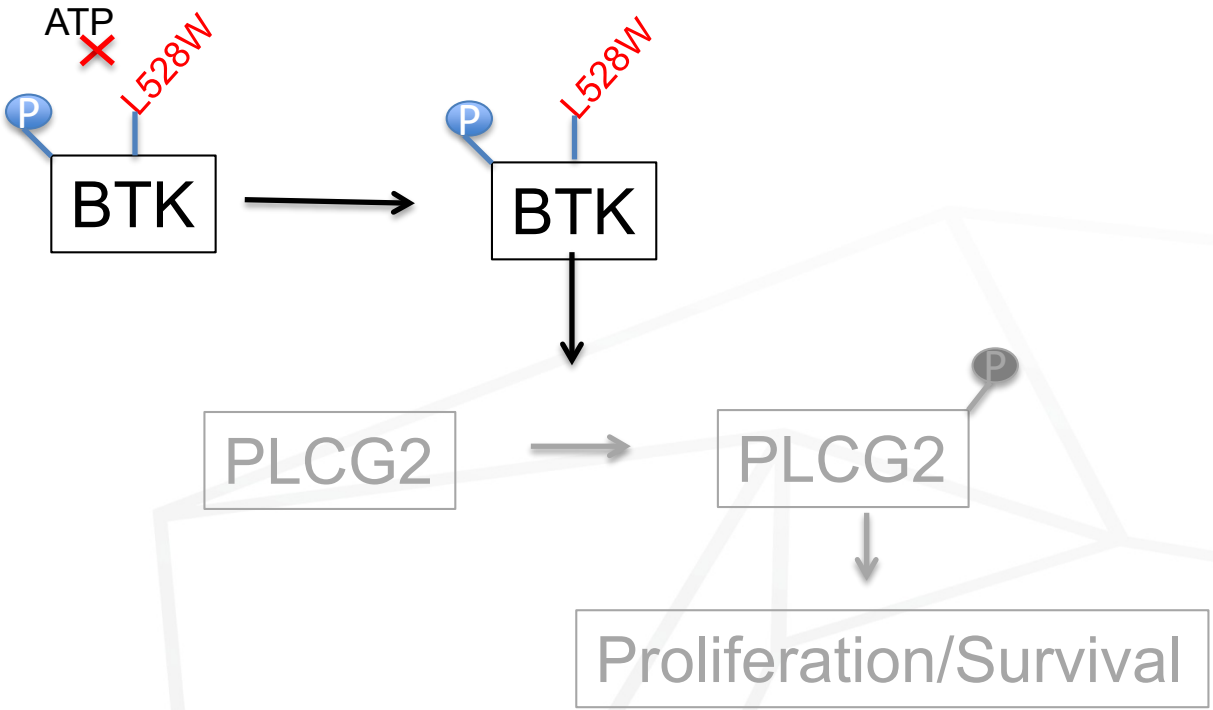

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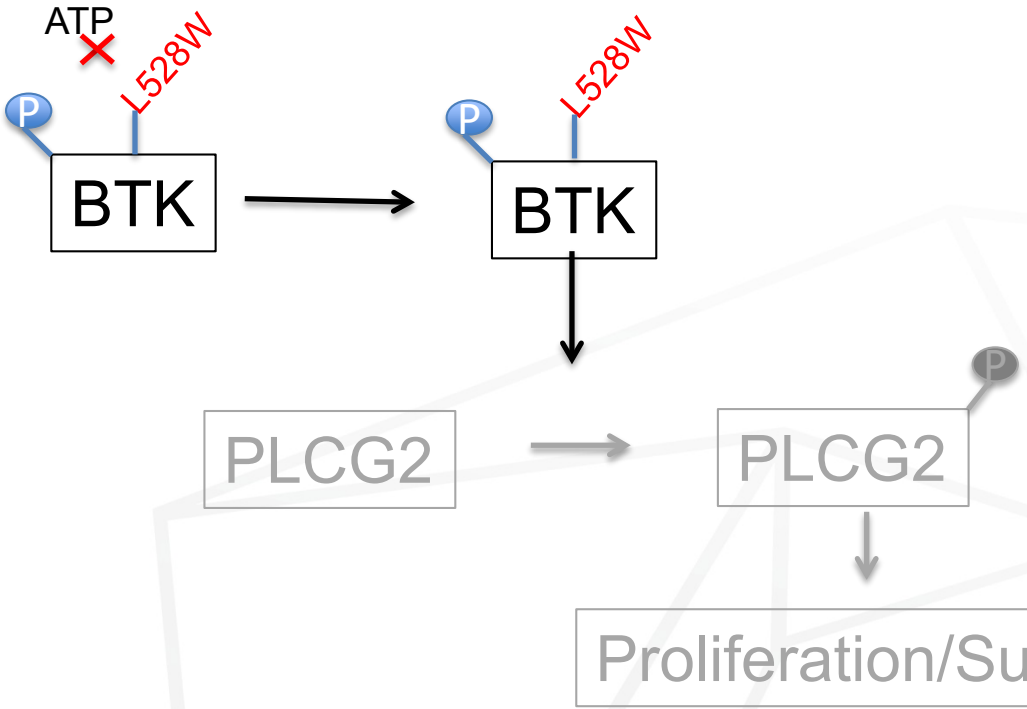
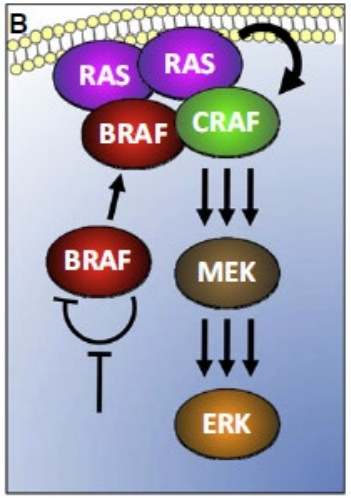
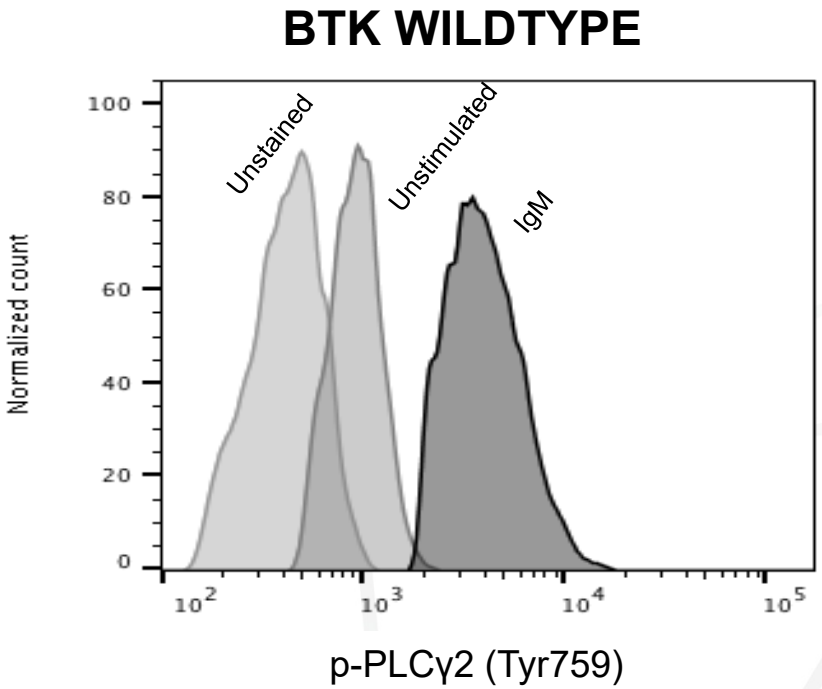




Heidorn *et al*, Cell 2010

Patient CLL cells harboring Leu528Trp show downstream activation of PLCG2



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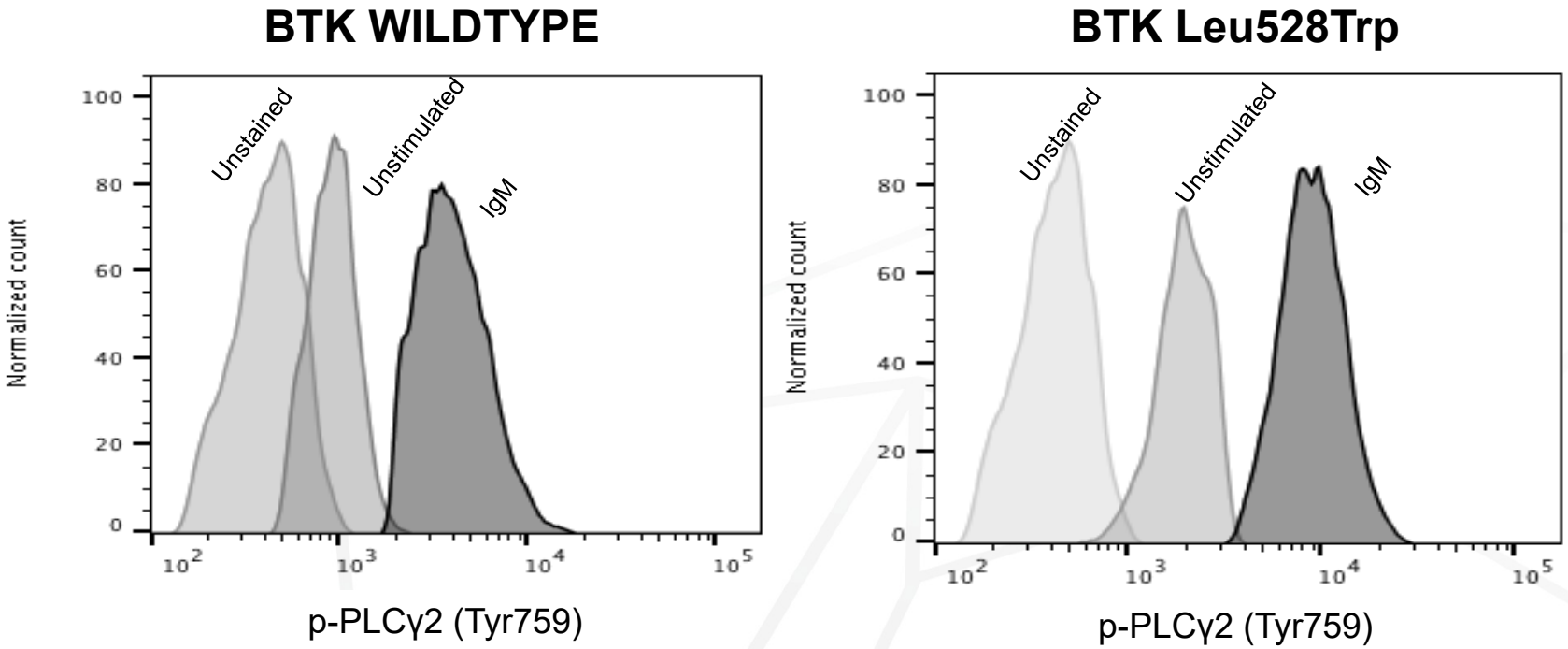

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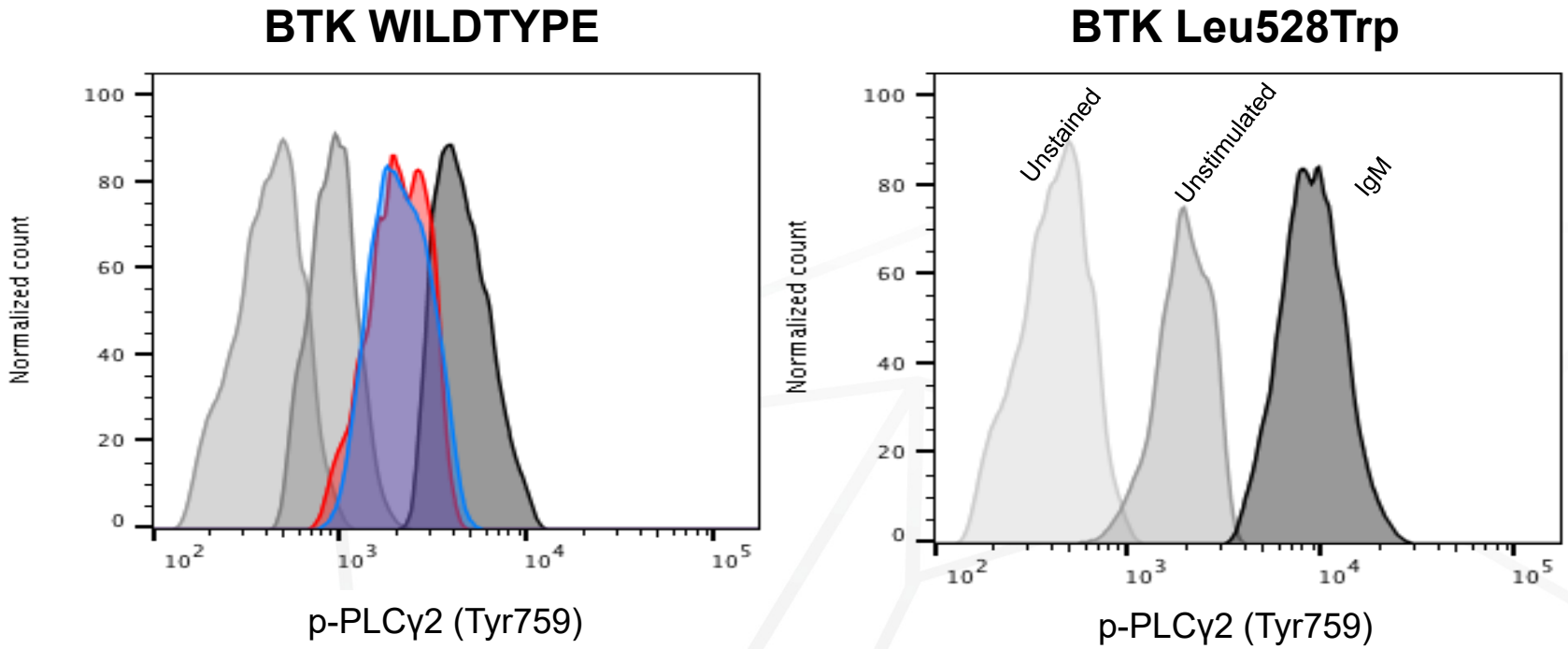

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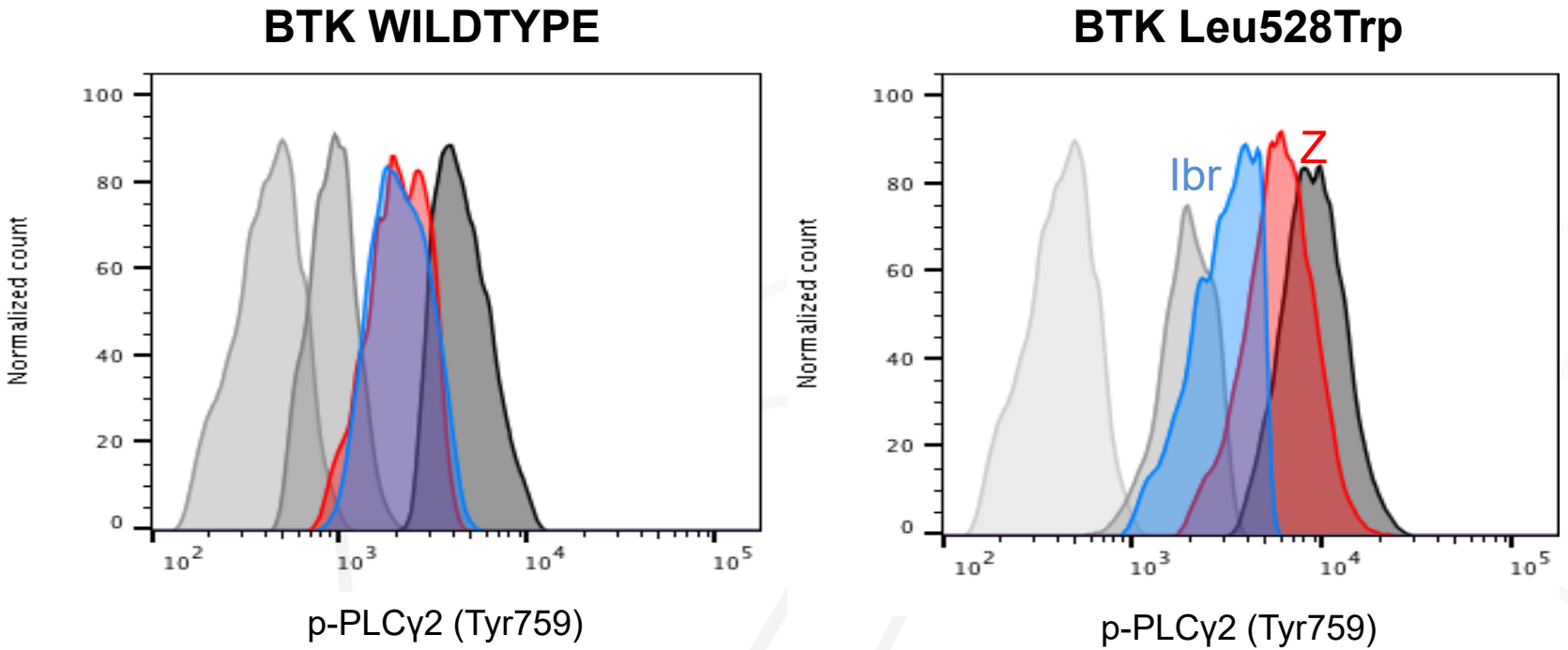

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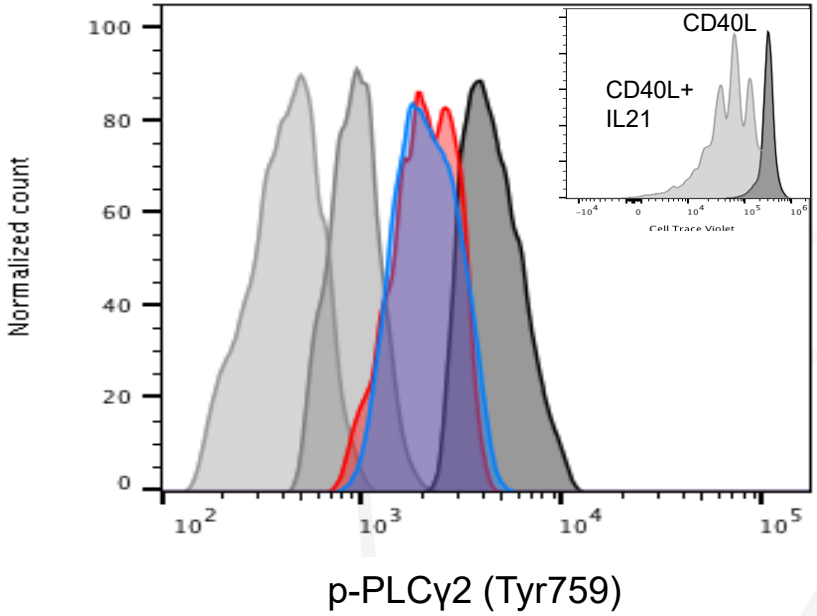
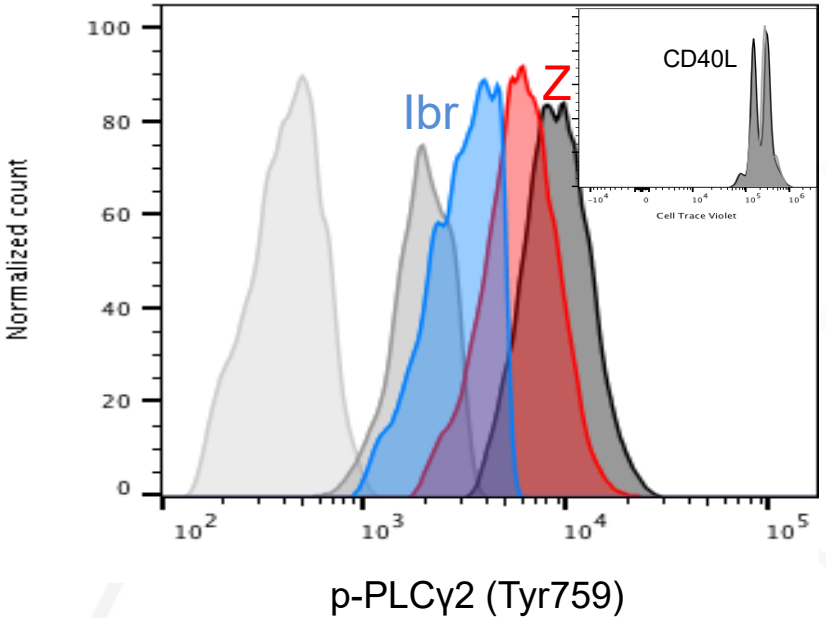
BTK WILDTYPE



p-PLCγ2 (Tyr759)

BTK Leu528Trp



p-PLCγ2 (Tyr759)

Summary

- BTK Leu528Trp mutations are enriched in CLL progression on zanubrutinib compared to ibrutinib
- BTK Leu528Trp results in a marked impairment of binding of zanubrutinib (as well as ibrutinib and tirabrutinib) to BTK
- BTK Leu528Trp occurs with Cys481 mutations but is present in different CLL cells in the tumor compartment
- BTK Leu528Trp is associated with loss of native kinase function however downstream signalling pathways appear intact in patient CLL cells suggesting an alternative mechanism of PLCG2 phosphorylation

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