



CLL Global Patient-Focused Research Symposium

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Mutations and Treatment Resistance: Finding Better Answers for CLL Patients

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Postdoctoral Fellow

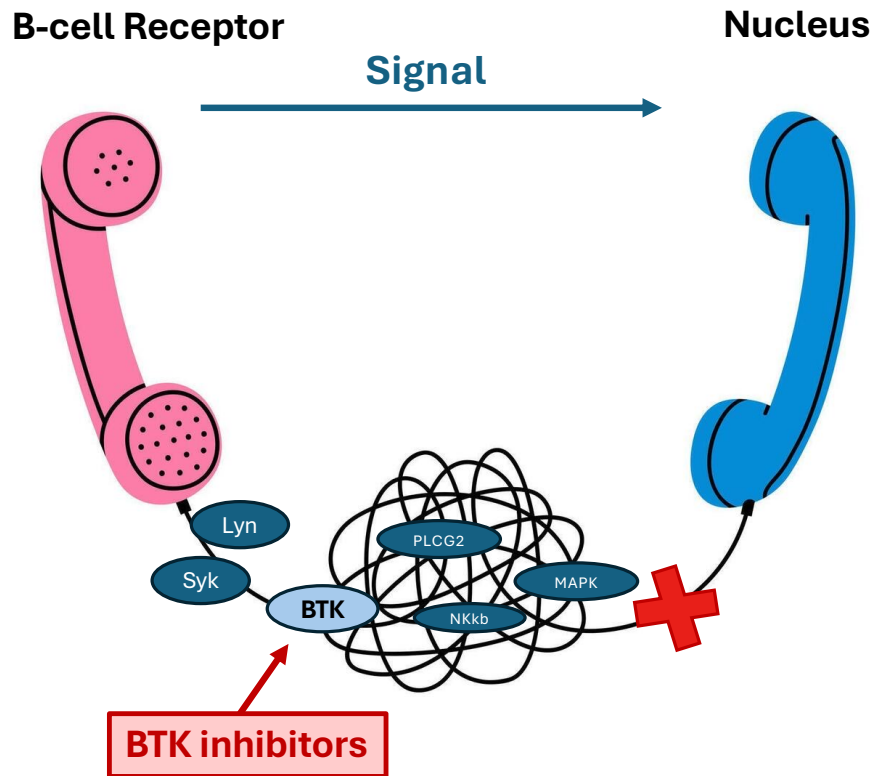
Dr. Gandhi's lab

Translational Molecular Pathology Department

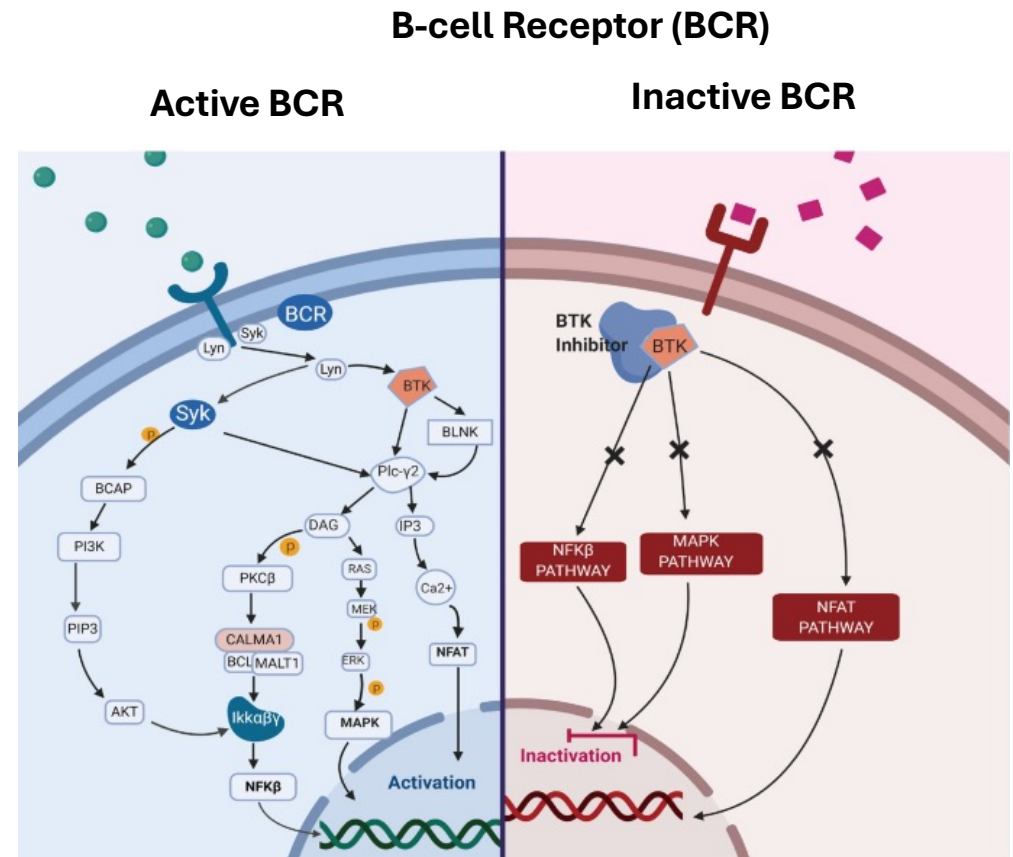
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How Targeted Treatments Work?

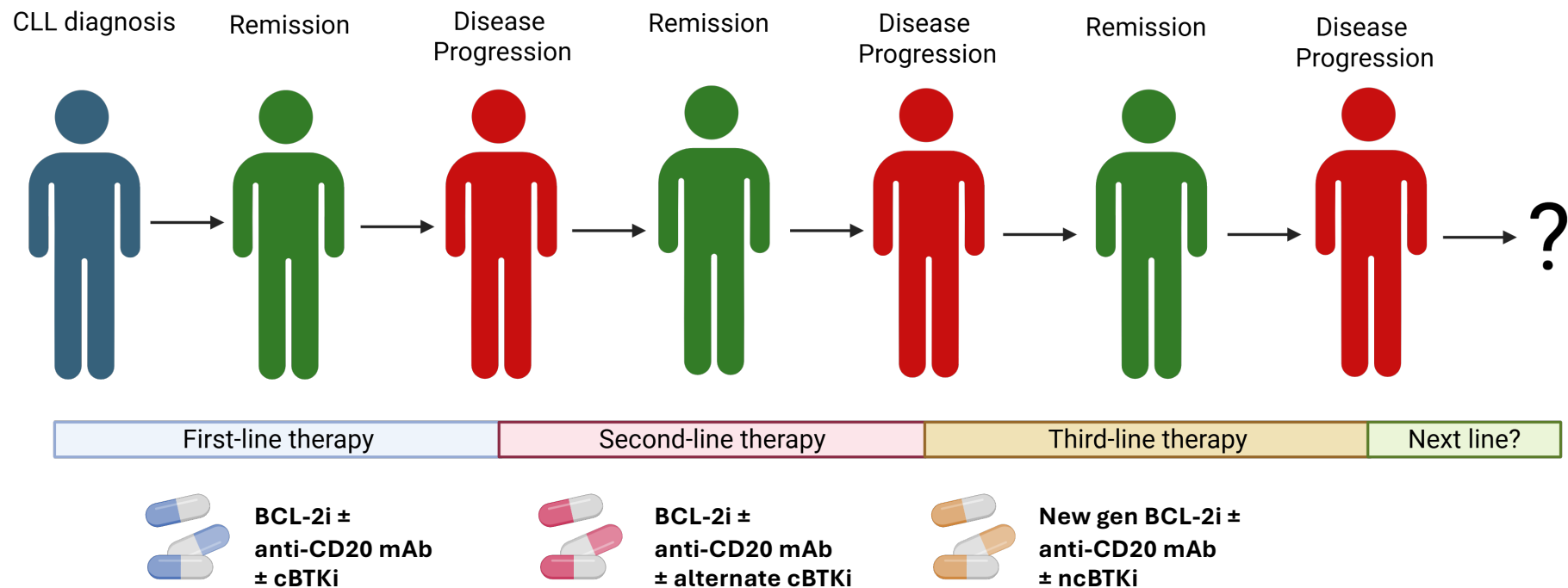


cBTKi/ncBTKi – covalent/non-covalent BTK inhibitors
 BCL-2i – BCL-2 inhibitors
 mAb – monoclonal antibodies



Chao Xue et al, Cancer Cell International, 2020

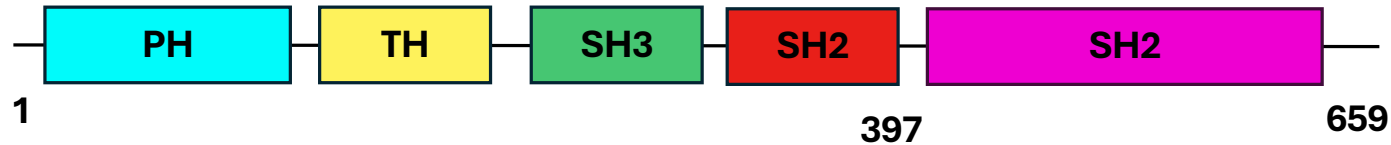
The CLL Therapy Journey: Remission and Relapse Cycle



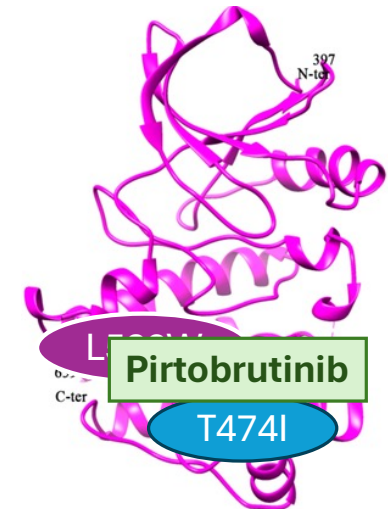
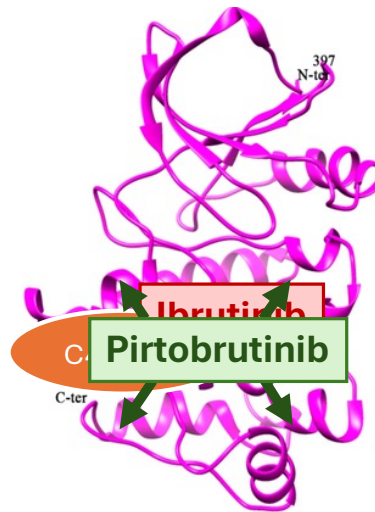
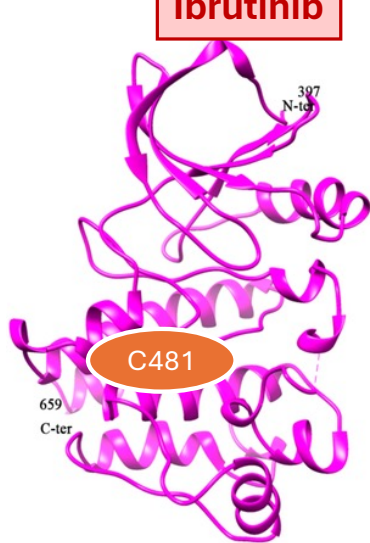
cBTKi/ncBTKi – covalent/non-covalent BTK inhibitors
 BCL-2i – BCL-2 inhibitors
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Why the Drugs Stop Working?

BTK structure

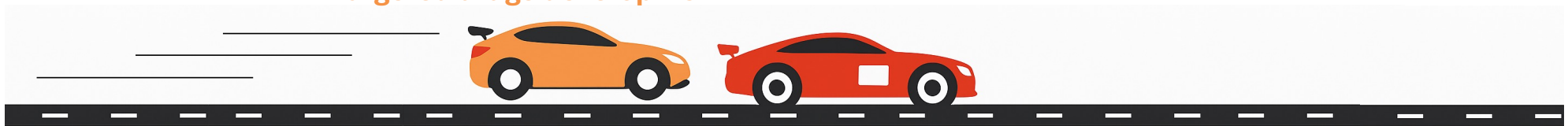


BTK kinase domain



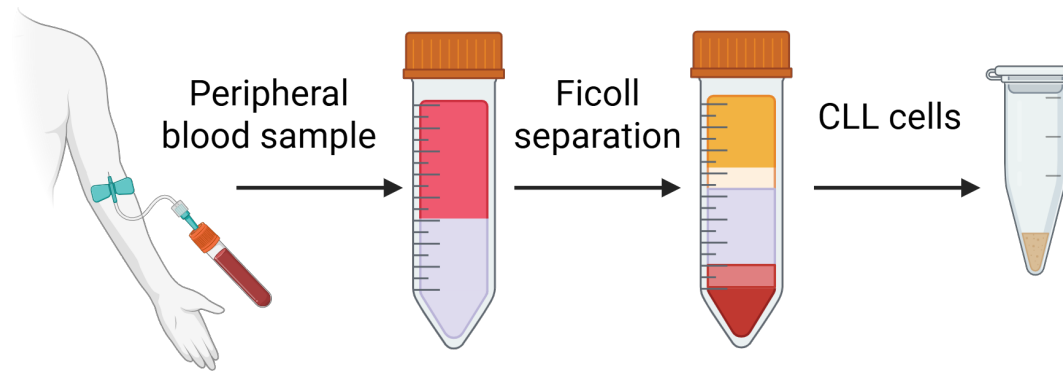
Targeted drugs development

Resistance mechanisms

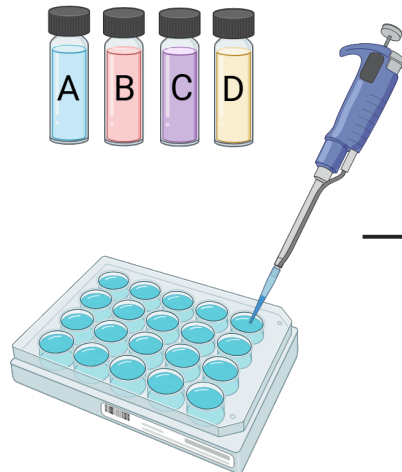


What is “Ex Vivo” drug profiling?

Ex vivo – “outside the living”



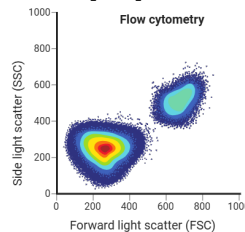
Compounds of interest



Specific Proteins

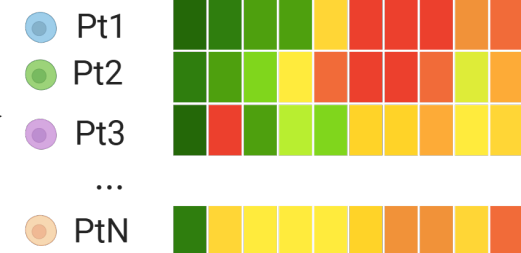


Apoptosis

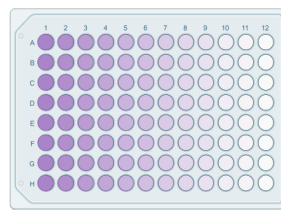


Drug Sensitivity Profile

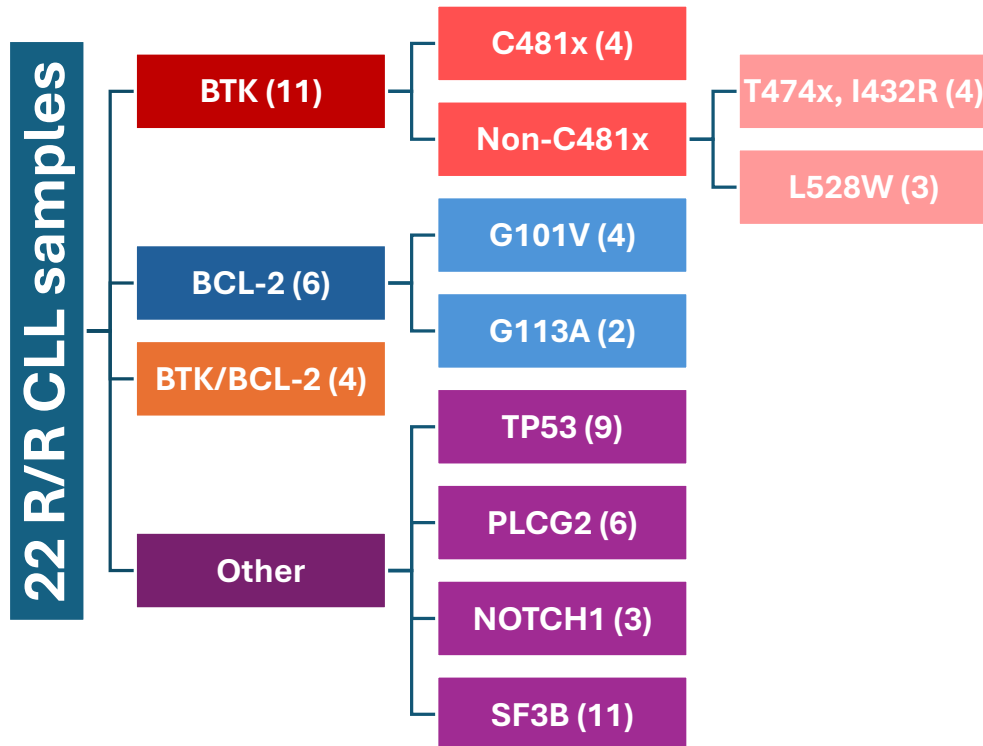
Drugs A, B, C, A+B etc.



Cell viability



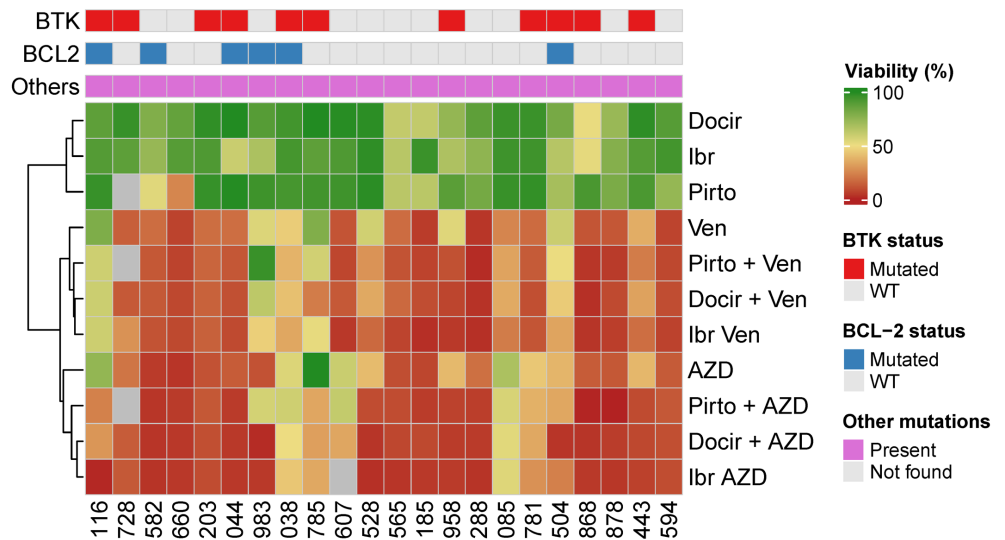
Ex vivo drug profiling of samples from relapsed CLL patients



Drugs:

- BTKi (covalent: *ibrutinib*; non-covalent: *pirtobrutinib*, *docirbrutinib*)
- BCL-2 inhibitor (*venetoclax*)
- MCL-1 inhibitor (*AZD5991*)

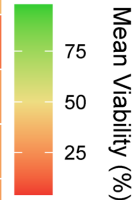
Ex vivo treatment for 72 hrs → Cell death assessment



Why One Treatment Doesn't Fit All

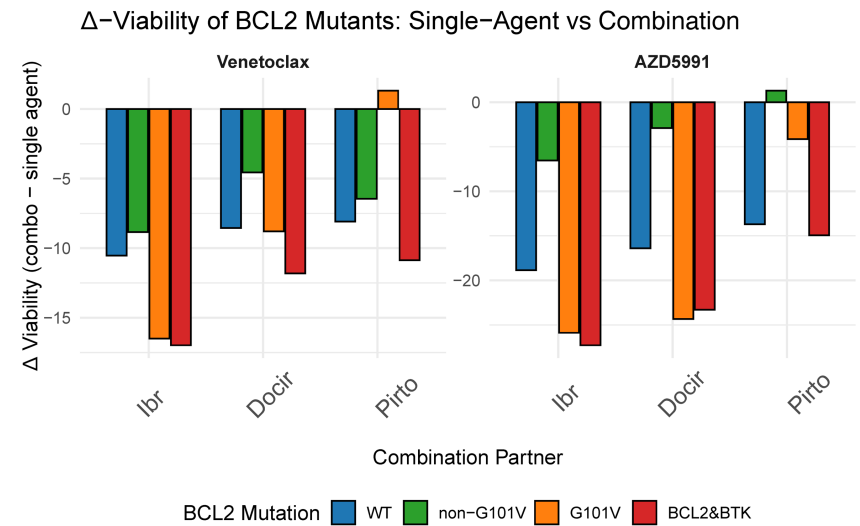
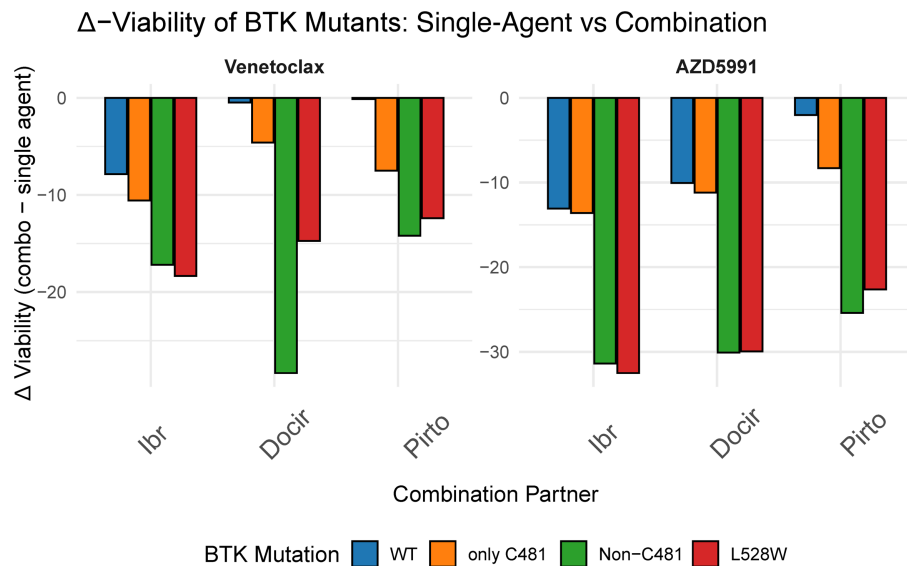
Mean Viability (\pm SEM) by Mutation Group and Treatment

	lbr	Docir	Pirto	Ven	lbr + Ven	Docir + Ven	Pirto + Ven	AZD	lbr + AZD	Docir + AZD	Pirto + AZD
all patients (n=22)	82.1 (\pm 13.9)	86.5 (\pm 14.2)	83.4 (\pm 19.1)	29.5 (\pm 24.5)	18 (\pm 17)	21.3 (\pm 18)	23.7 (\pm 24.4)	31.5 (\pm 26.8)	12.5 (\pm 15.1)	14.9 (\pm 16.1)	21.1 (\pm 21.5)
BTK WT (n=11)	84 (\pm 3.7)	83.9 (\pm 4.1)	75.3 (\pm 6.8)	19.7 (\pm 5.8)	11.9 (\pm 3.8)	19.2 (\pm 5.4)	19.6 (\pm 8.2)	22.4 (\pm 6.9)	9.3 (\pm 5)	12.3 (\pm 4.9)	20.3 (\pm 7.4)
BTK mutated (n=11)	80.2 (\pm 4.7)	89.2 (\pm 4.5)	92.2 (\pm 2.7)	39.3 (\pm 7.8)	24.2 (\pm 5.7)	23.3 (\pm 5.6)	28.1 (\pm 6.7)	40.7 (\pm 8.5)	15.5 (\pm 4.3)	17.6 (\pm 4.9)	21.9 (\pm 5.9)
only C481 (n=4)	83.8 (\pm 8)	97.9 (\pm 1.4)	95.6 (\pm 1.6)	29.3 (\pm 6.8)	18.7 (\pm 5.6)	24.7 (\pm 7.5)	21.8 (\pm 5.8)	29.2 (\pm 10.7)	15.7 (\pm 9.3)	18 (\pm 10.8)	21 (\pm 12.6)
non-C481 (n=4)	84.7 (\pm 12.1)	92 (\pm 12)	93.6 (\pm 4.2)	41.1 (\pm 31.3)	19.9 (\pm 13.8)	21.2 (\pm 14.4)	25.9 (\pm 17)	51.3 (\pm 35.3)	23.9 (\pm 21.2)	12.8 (\pm 6.4)	26.9 (\pm 26.9)
L528W (n=3)	69.4 (\pm 17.3)	73.8 (\pm 17.5)	86.3 (\pm 13.1)	50.3 (\pm 31.6)	9.5 (\pm 10.7)	12 (\pm 13.3)	19.3 (\pm 15.4)	42 (\pm 27.6)	31.9 (\pm 24.9)	35.5 (\pm 26.5)	37.9 (\pm 26.6)
BCL2 WT (n=16)	84.8 (\pm 3.3)	85.6 (\pm 4)	82.8 (\pm 5.1)	23.4 (\pm 5.5)	12.8 (\pm 3.2)	14.8 (\pm 2.7)	15.3 (\pm 3.8)	31.1 (\pm 6.8)	12.3 (\pm 3.9)	14.7 (\pm 3.8)	17.4 (\pm 5.2)
BCL2 G101V (n=4)	70.8 (\pm 6.7)	90.2 (\pm 4)	90.2 (\pm 7)	53.1 (\pm 12.9)	36.6 (\pm 10.8)	44.3 (\pm 12.1)	54.5 (\pm 17.4)	34 (\pm 14.9)	8.2 (\pm 5)	9.7 (\pm 6.5)	29.9 (\pm 10.9)
BCL2 non-G101 (n=2)	83.6 (\pm 10.2)	86.8 (\pm 7.7)	73.7 (\pm 19.9)	31.4 (\pm 13.8)	22.5 (\pm 11.8)	26.8 (\pm 14.7)	24.9 (\pm 13)	29.9 (\pm 25)	23.3 (\pm 20)	27 (\pm 23.4)	31.1 (\pm 27.4)
BTK&BCL2 (n=4)	77.3 (\pm 8.6)	91.3 (\pm 4.1)	89.8 (\pm 6.9)	50.7 (\pm 13)	33.8 (\pm 10.4)	38.9 (\pm 10.4)	39.9 (\pm 10.3)	45.2 (\pm 13)	17.9 (\pm 9.8)	21.9 (\pm 11.2)	30.2 (\pm 11.2)
TP53 (n=9)	79.5 (\pm 4.9)	80.4 (\pm 5.5)	79.2 (\pm 8.6)	30.6 (\pm 8.3)	18.6 (\pm 6.3)	20.9 (\pm 6.4)	19.3 (\pm 6.9)	29.5 (\pm 8)	9.3 (\pm 4.4)	14 (\pm 5.3)	13.8 (\pm 6.9)
NOTCH1 (n=3)	74.1 (\pm 8)	80.9 (\pm 3.6)	61 (\pm 19.1)	40.2 (\pm 16.9)	15.2 (\pm 9.1)	20 (\pm 12.5)	22.5 (\pm 14.2)	27.2 (\pm 11.8)	10.7 (\pm 6.2)	4.2 (\pm 0.5)	15.1 (\pm 9.6)
PLCG2 (n=6)	82.1 (\pm 5.5)	89.8 (\pm 4)	79.5 (\pm 11.4)	18.2 (\pm 6.2)	13.4 (\pm 5)	17.6 (\pm 6.7)	16 (\pm 6.3)	28.2 (\pm 10.8)	18.9 (\pm 9.6)	20.4 (\pm 10)	21.6 (\pm 11.4)
SF3B1 (n=11)	81.7 (\pm 4.6)	80.7 (\pm 5.2)	86.7 (\pm 3.8)	28.6 (\pm 7.8)	13.9 (\pm 5.3)	19 (\pm 5.5)	16.9 (\pm 5.4)	30.8 (\pm 7.2)	8 (\pm 4)	14.6 (\pm 4.8)	17.3 (\pm 6.7)

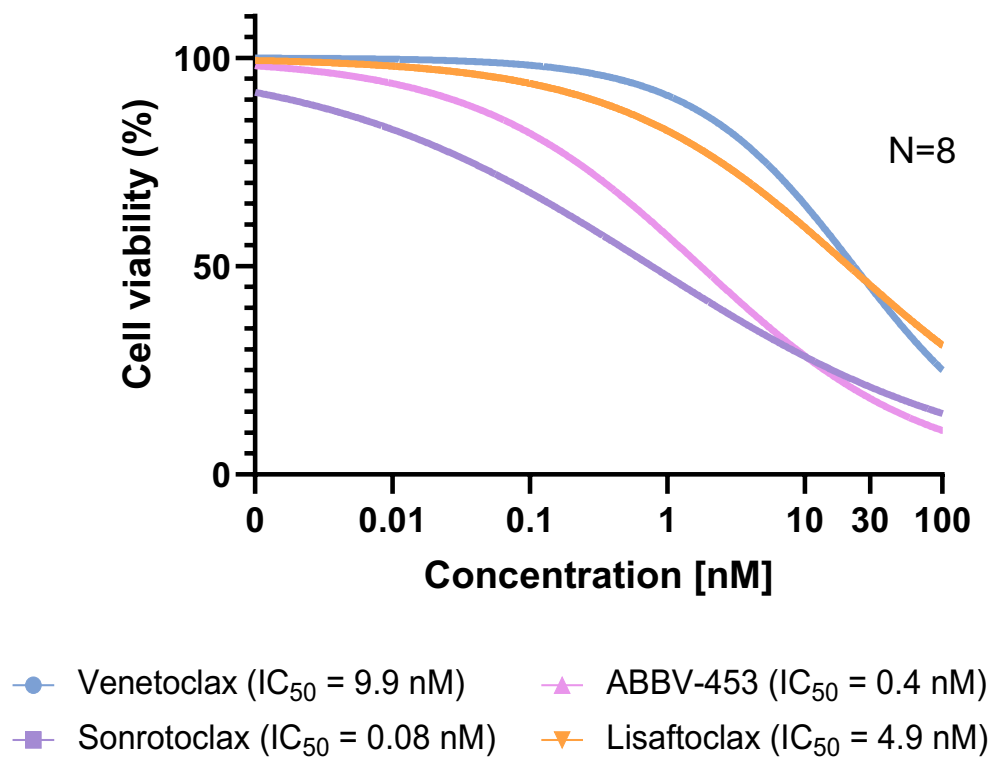


Combination Of Drugs With Different Mechanism Of Actions Helps To Increase Efficacy

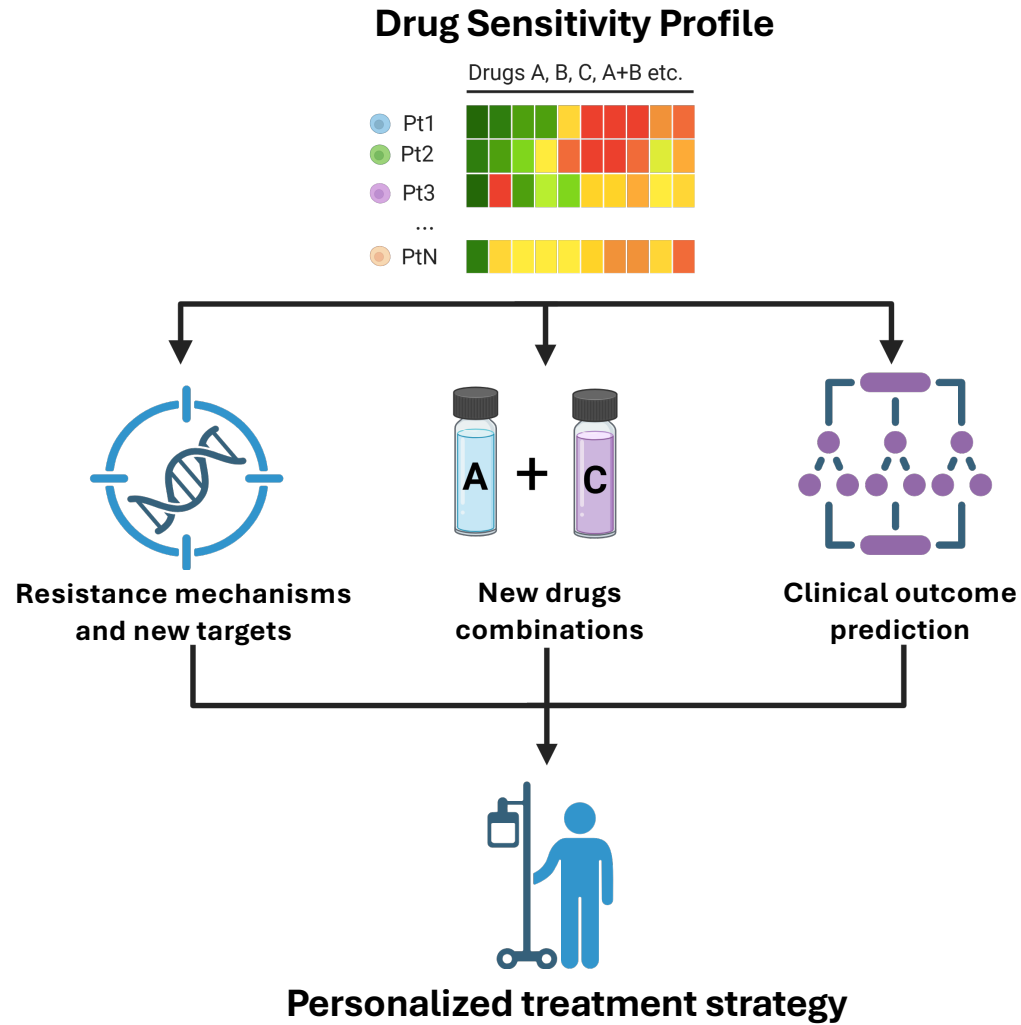
Combining drugs can be powerful when chosen correctly



Newer BCL-2 inhibitors are more potent than first-generation venetoclax in relapsed CLL samples



How *ex vivo* drug profiling can help CLL patients?



Conclusions & Acknowledgements

- CLL cells from different patients show very different sensitivities to the same drugs.
- Genetic mutations (like those in **BTK** or **BCL-2**) influence how well treatments work.
- Some resistant samples still respond to new or combination therapies.
- Studying these differences *ex vivo* helps predict which treatments are most effective for each patient.
- Understanding resistance patterns guides the design of smarter, more durable therapies.

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