Amsterdam UMC
University Medical Centers

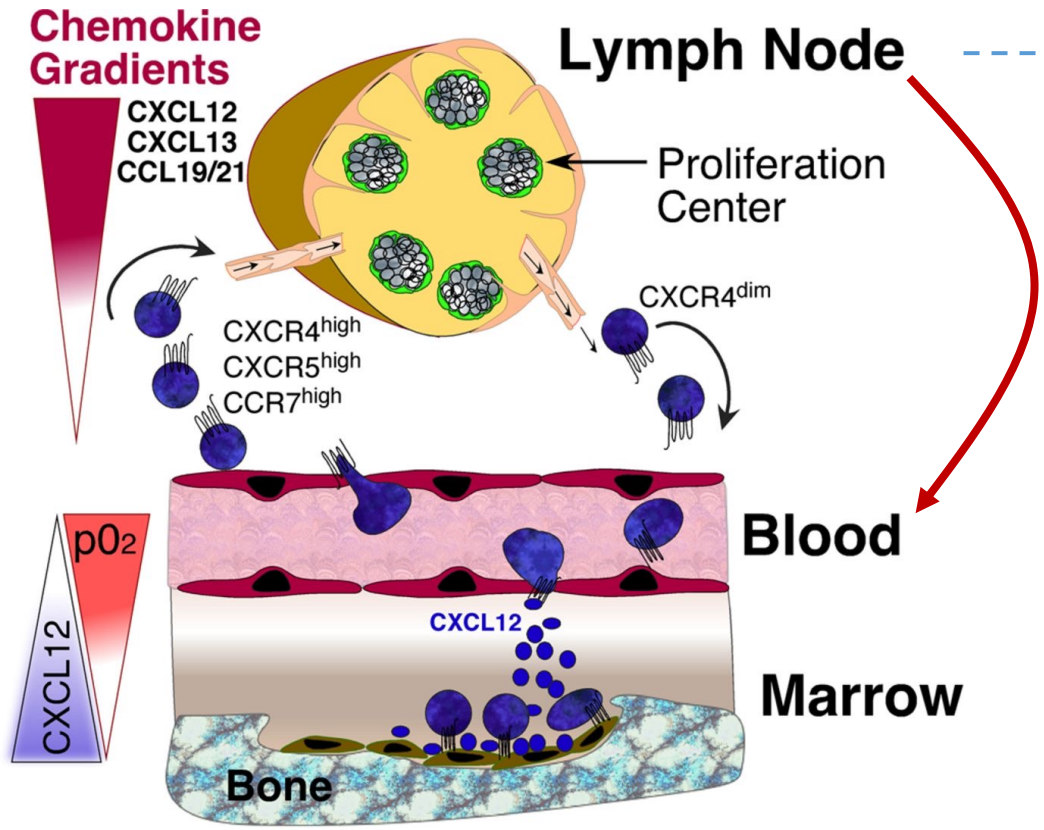
Glutamine addiction in the CLL microenvironment; towards therapeutic applications and a PET tracer as a novel diagnostic tool

Eric Eldering, Amsterdamn, The Netherlands

Brief intro on CLL & targeted drugs

- Accumulation of malignant CD5+/19+ B cells in Blood, LN, spleen and BM
- Variable clinical course, 1/3 progressive
- **B Cell Receptor dependent, cells circulate between LN and PB**
  - **Microenvironment-driven disease**
- Standard treatment immunochemotherapy (FCR) is being replaced by targeted therapies
  - **Ibrutinib (BTK inhibitor – B cell receptor signaling blocked)**
  - **Venetoclax (= ABT-199; Bcl-2 inhibitor, a BH3 mimetic)**
- Against these drugs as single agents – resistance develops with often dismal outcome
- So, there is a continued clinical need for novel therapeutic strategies

Microenvironment shapes CLL biology



Ibrutinib

Lymph node stimuli
B-cell receptor (BCR)
CD40-CD40L
and others

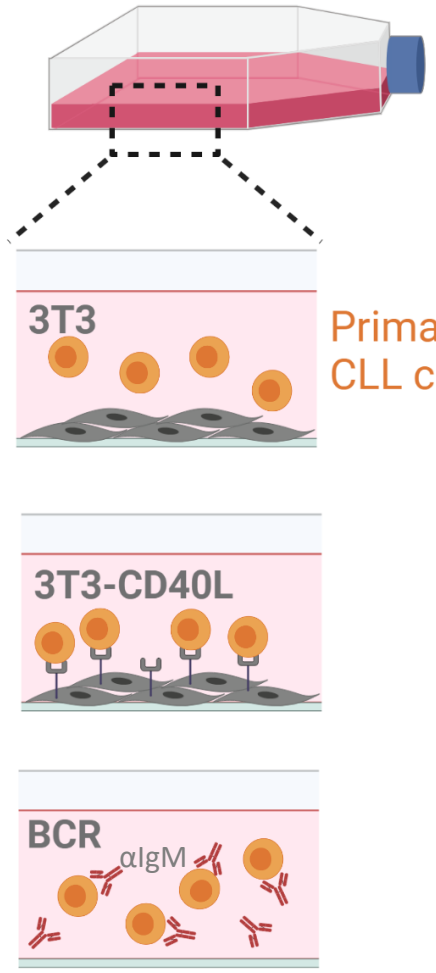
Upregulation of
anti-apoptotic proteins,
e.g. Bcl-2 — **Venetoclax**

Drug resistance

Cell death

Burger and Montserrat, Blood 2013

VEN resistance can be induced in vitro by lymph node stimuli



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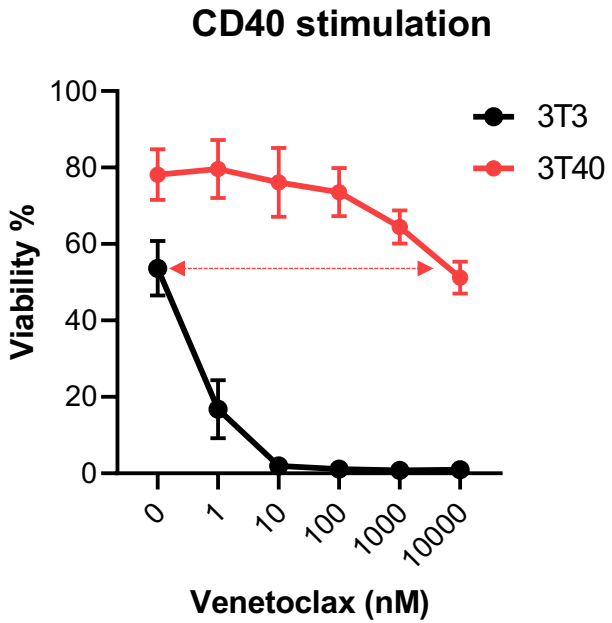
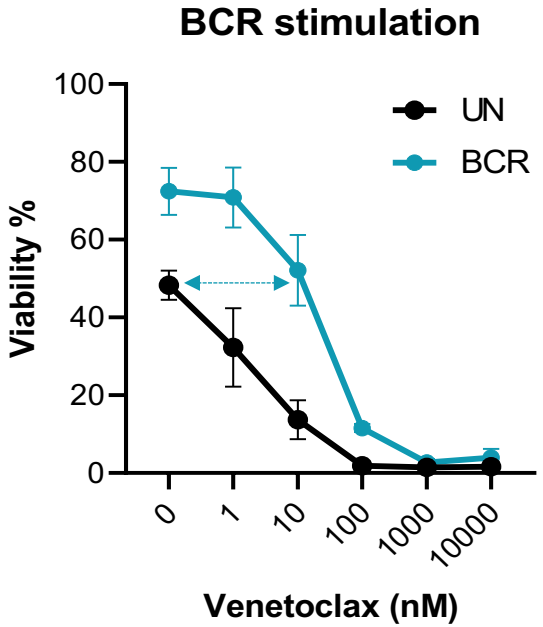
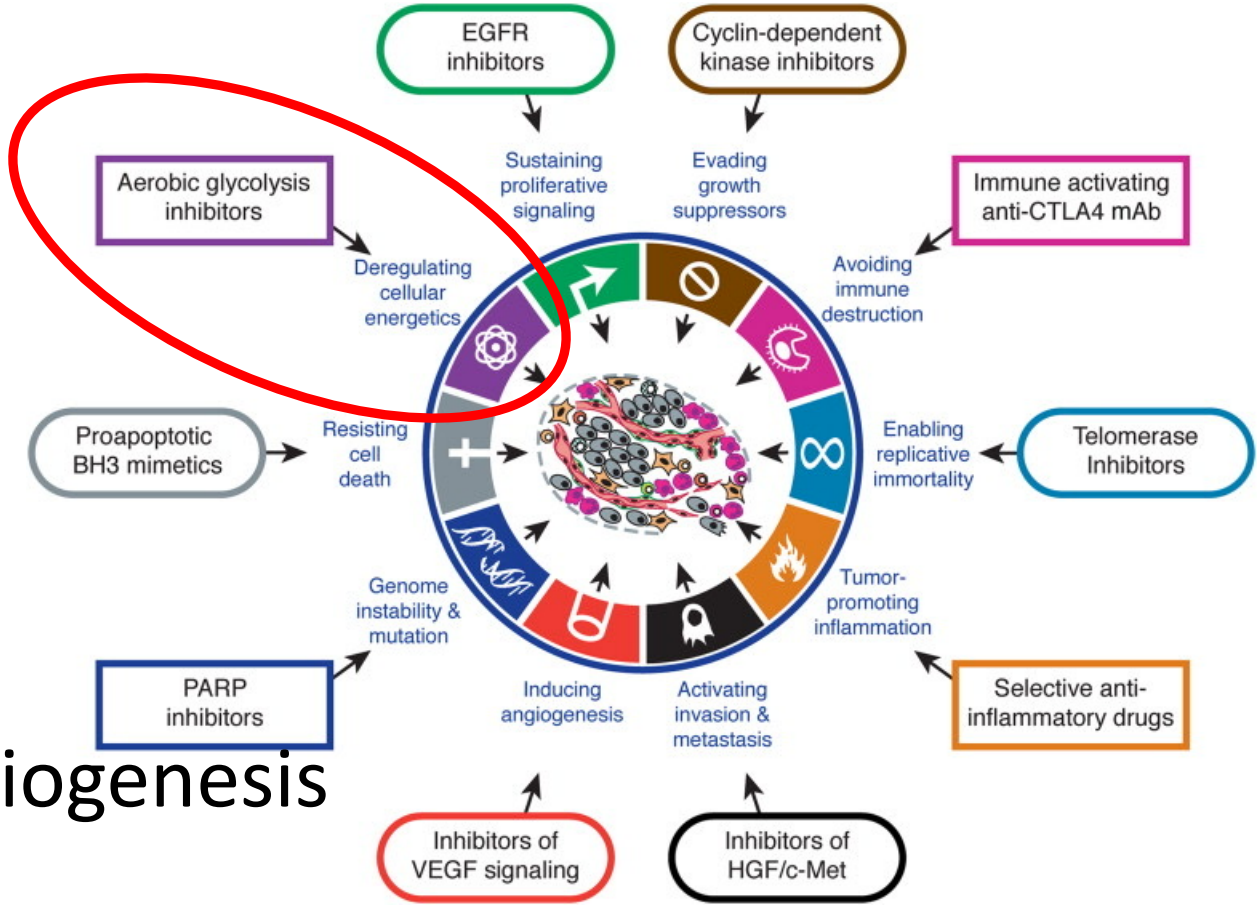




VEN resistance is due to NF-κB signalling and upregulation of Bcl-XL, Mcl-1 and Bfl-1 antiapoptotic proteins.

Cancer hallmarks*

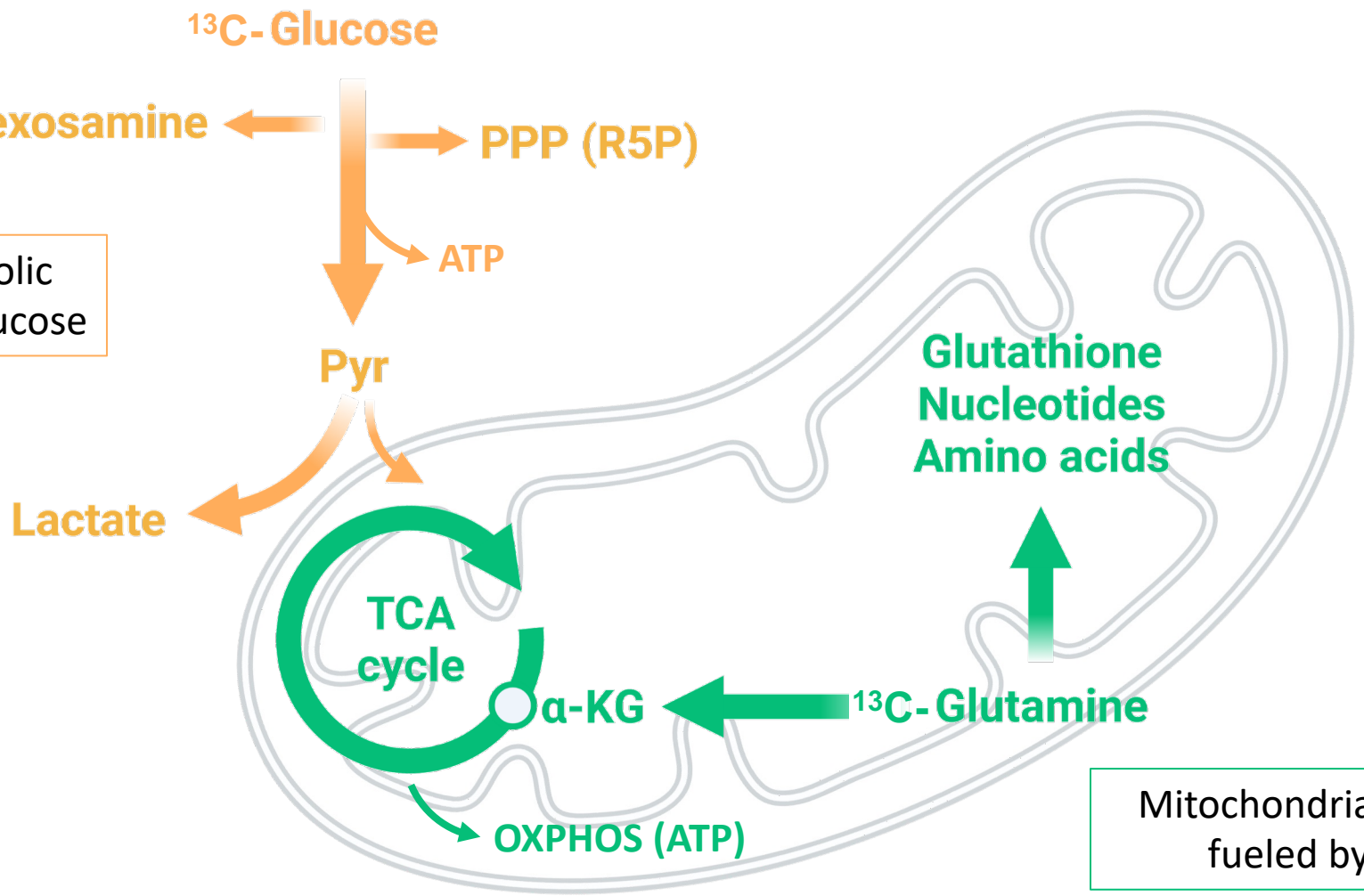
1. Unrestricted cell division
2. *Avoiding cell death*
3. *Avoiding immune attack*
4. **Changes in Metabolism**
   - **Warburg dogma under challenge**
5. Inflammation
6. Genomic instability
7. Metastasis, migration, angiogenesis



* Hanahan & Weinberg: Hallmarks of cancer, next generation, Cell 2011

The metabolism of CLL cells is reprogrammed in lymph nodes

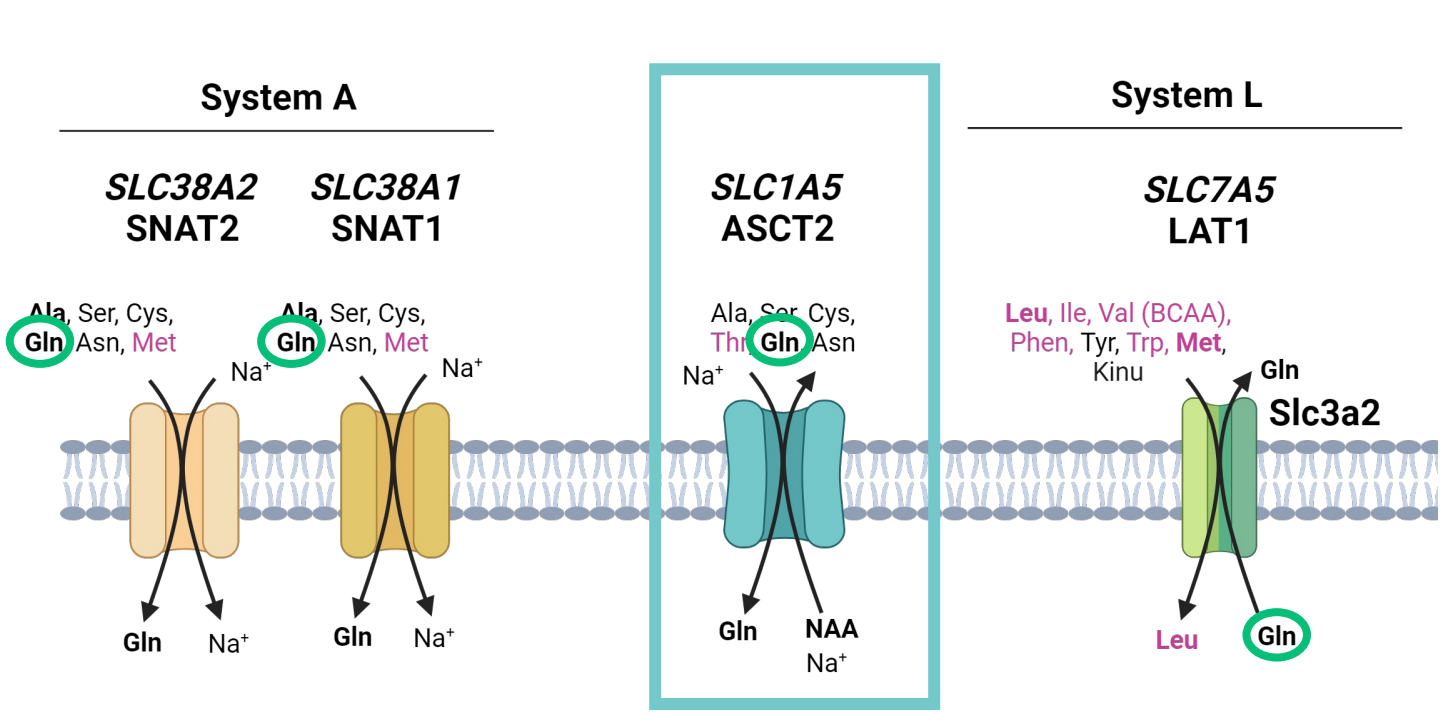
Glycolysis & other cytosolic pathways are fueled by glucose

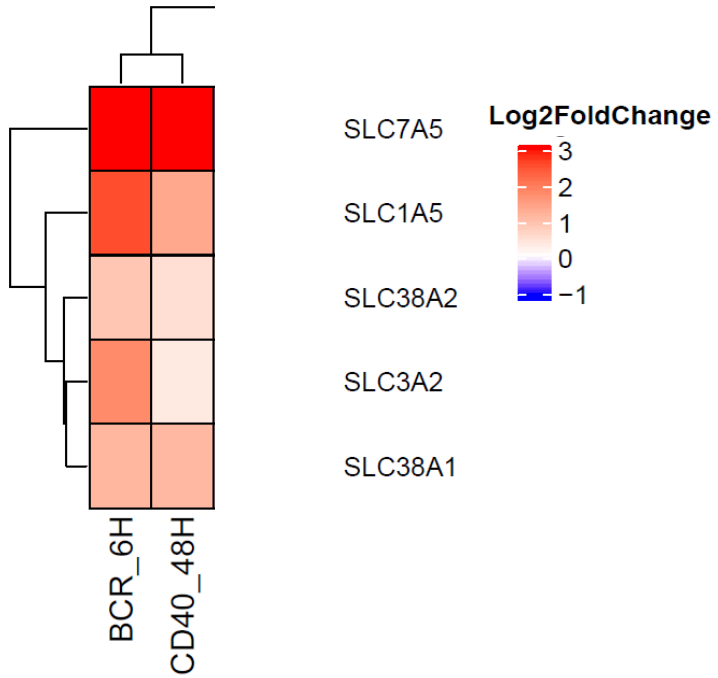


Mitochondrial pathways are fueled by glutamine

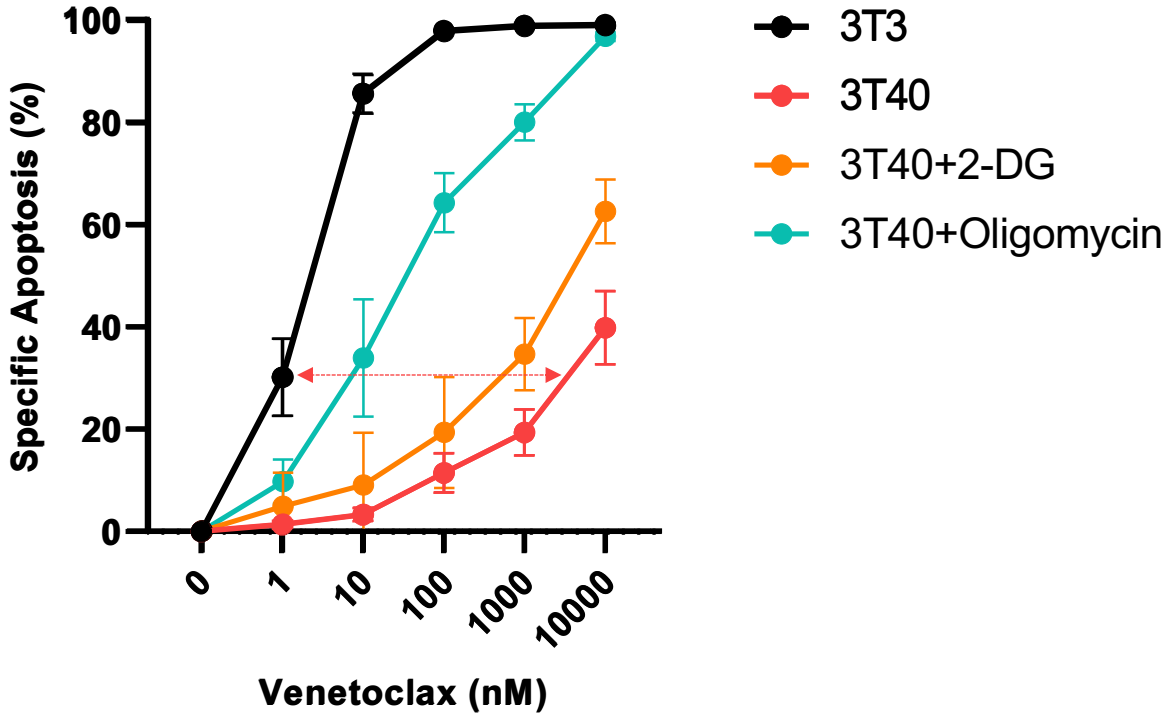
Chen, Simon-Molas, et al., Blood 2022

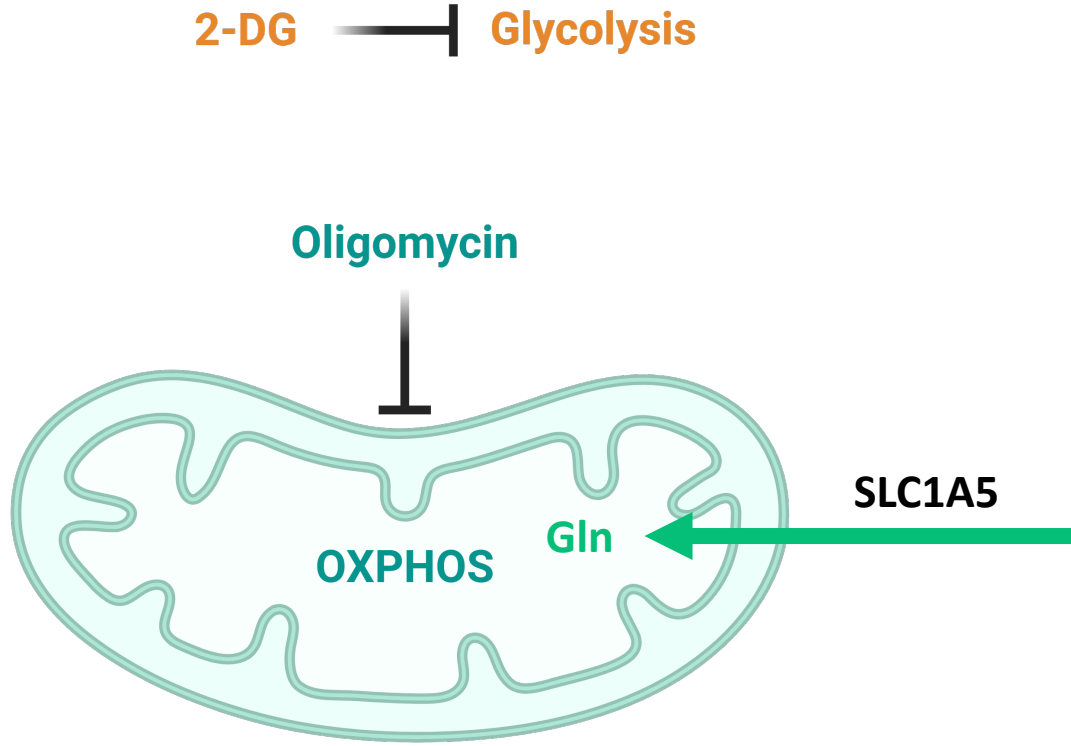
SLC1A5 and other Gln transporters are upregulated by LN stimuli and downregulated by IBR





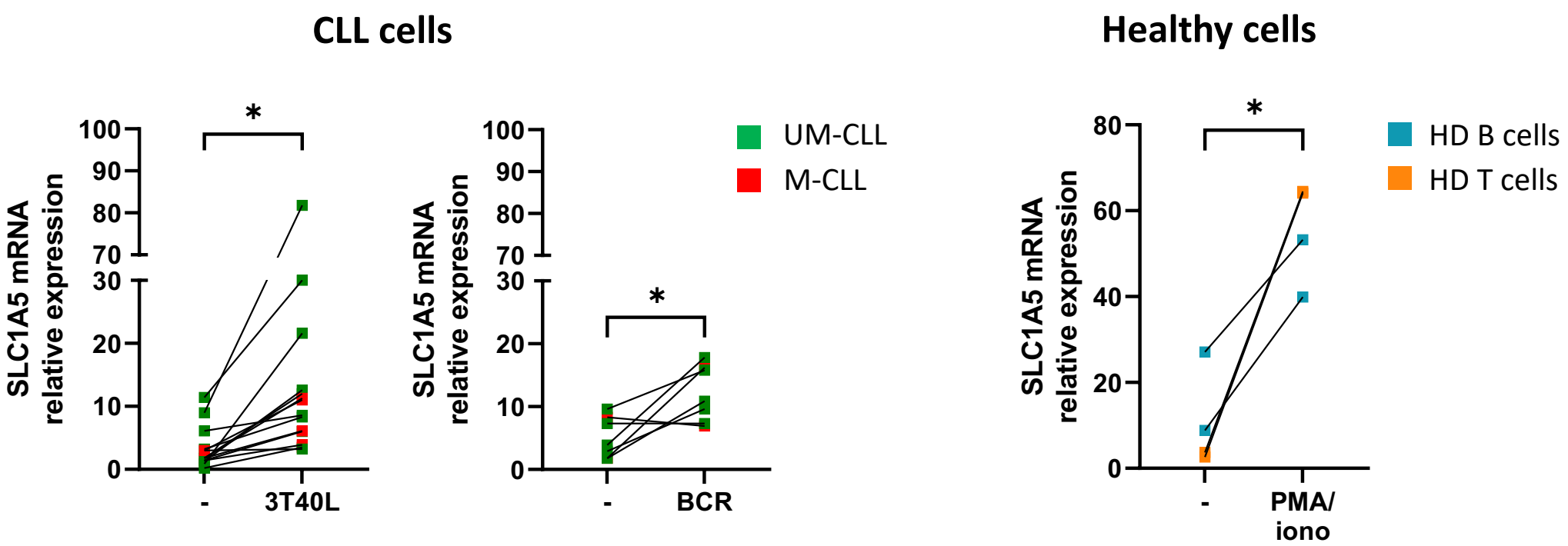
Inhibition of OXPHOS, but not glycolysis, sensitizes CLL cells to VEN



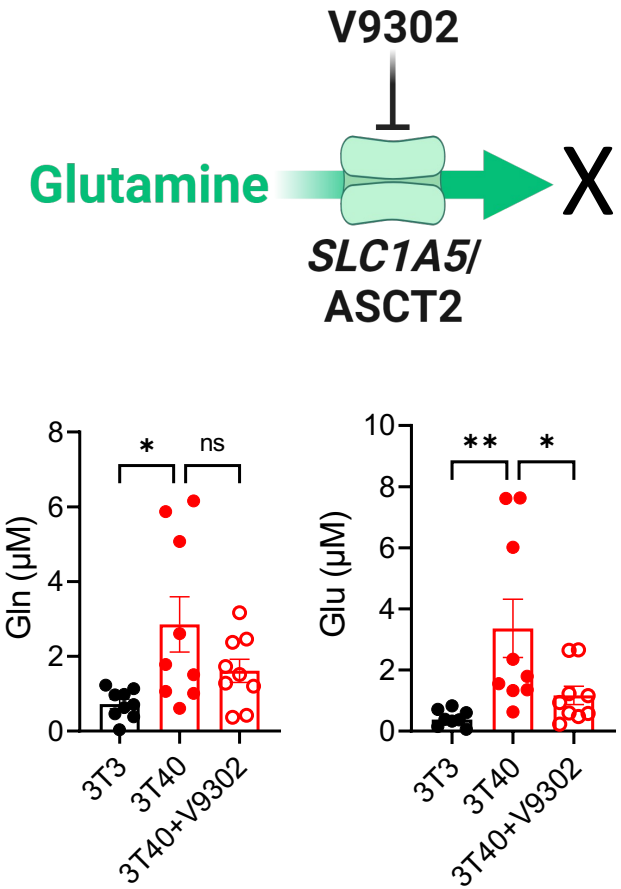


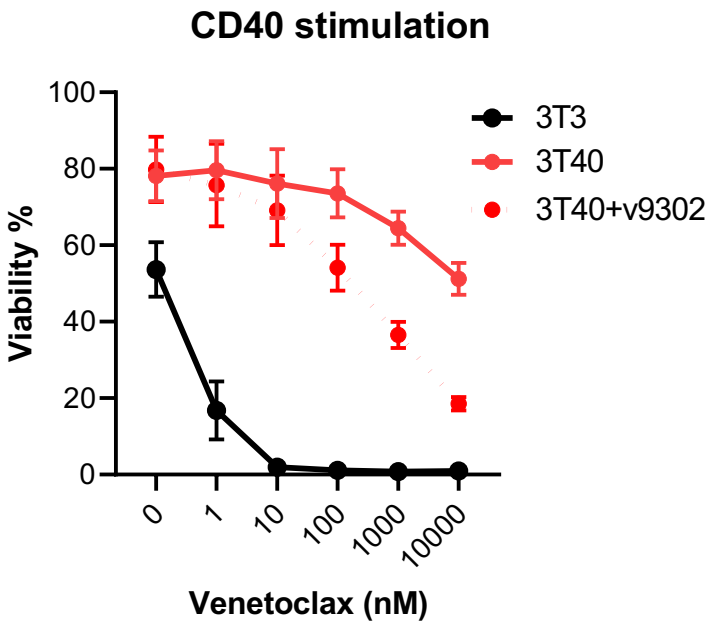
Chen, et al., Haematologica 2023

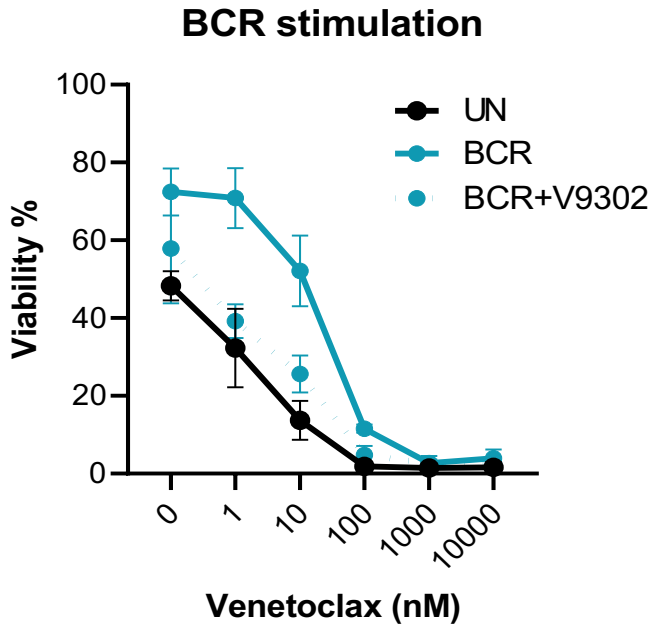
SLC1A5 is a key glutamine transporter in proliferating cells



Inhibition of glutamine uptake sensitizes CLL cells to VEN

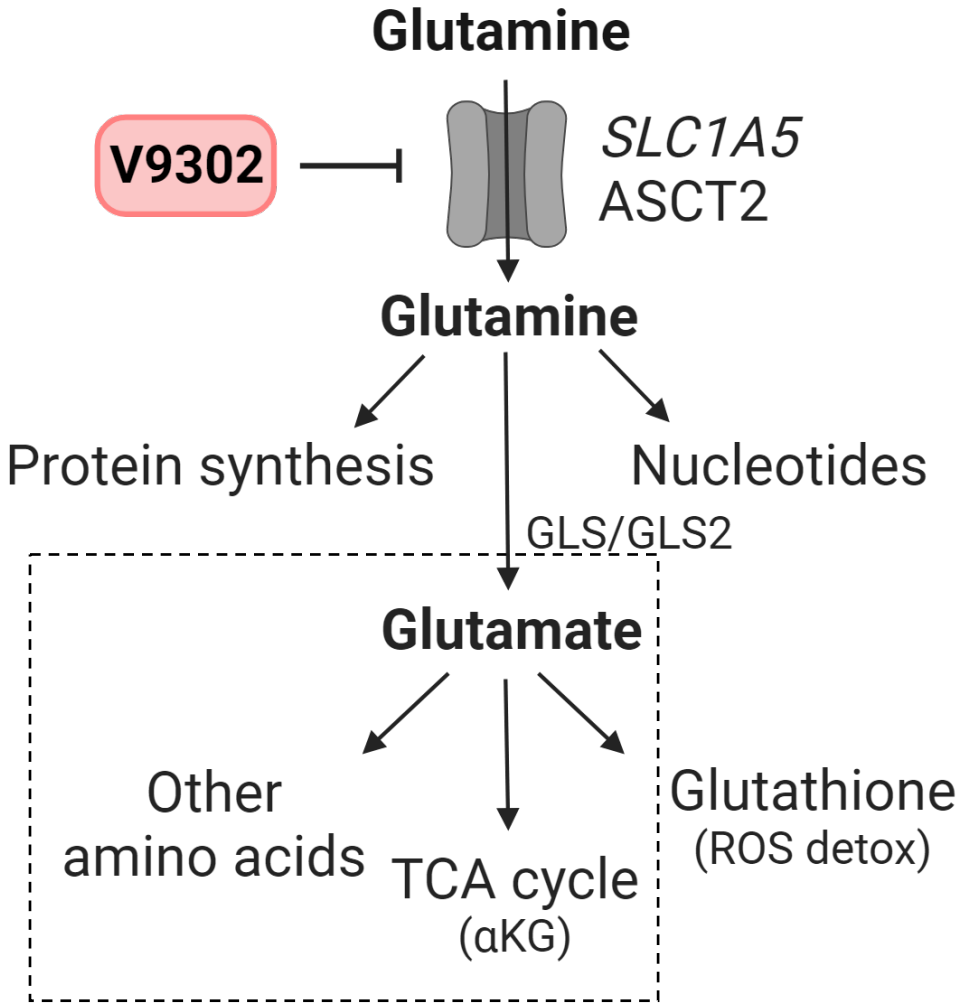


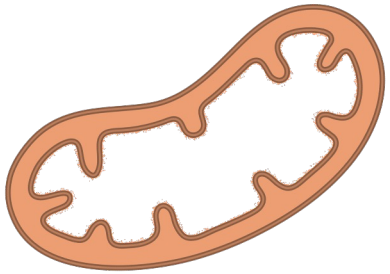




Chen, Simon-Molas, et al., Blood 2022

Roles for glutamine in proliferating cells

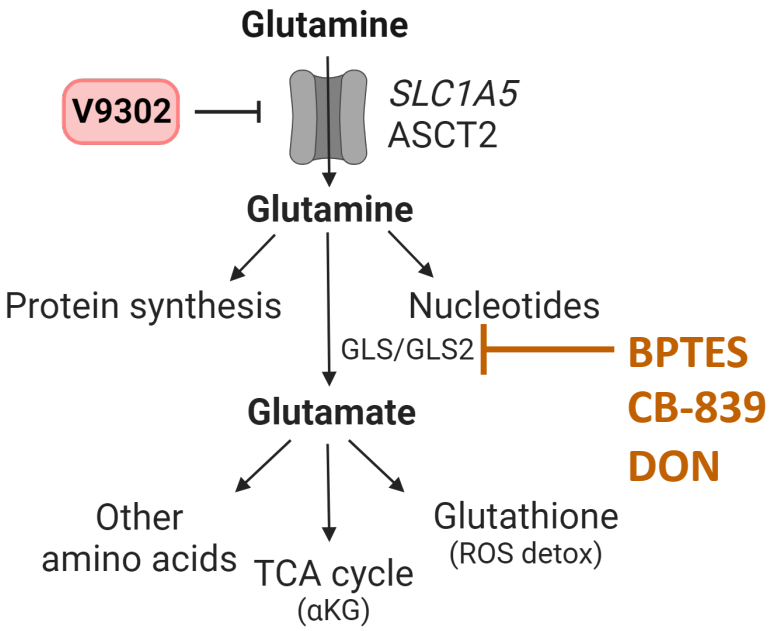


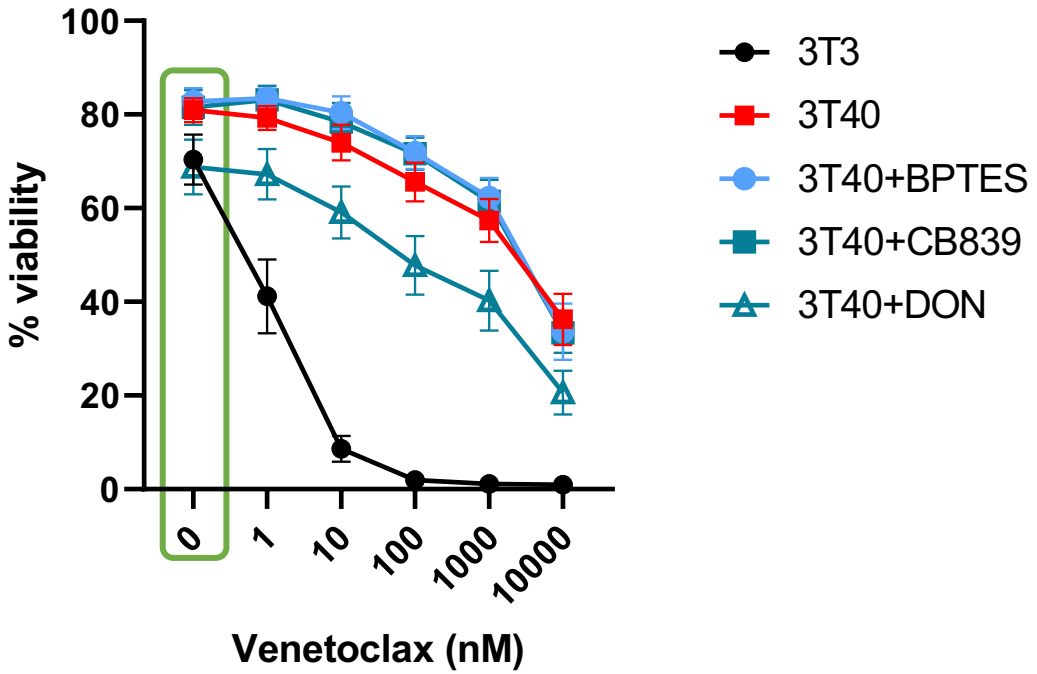


Are the effects of V9302 due to decreased Gln incorporation into the mitochondria?

V9302 does more beyond preventing Gln incorporation into the mitochondria

1. Inhibition of glutaminase does not increase sensitivity to VEN





Active site inhibitor L-DON known to be toxic and reduces the basic pro-survival effect of CD40 stimulation

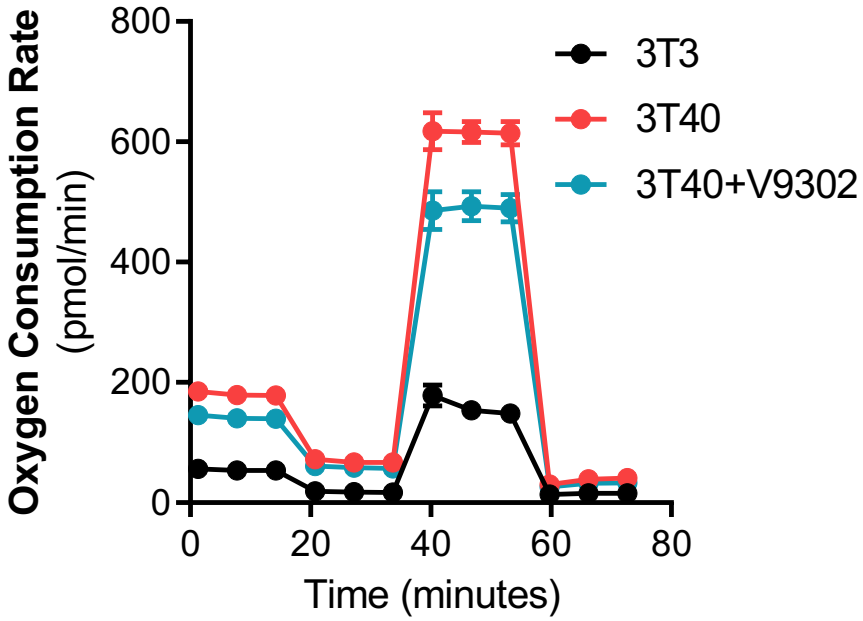
Chen, et al., Haematologica 2023

V9302 does more beyond preventing Gln incorporation into the mitochondria

1. **Inhibition of GLS does not increase sensitivity to VEN**

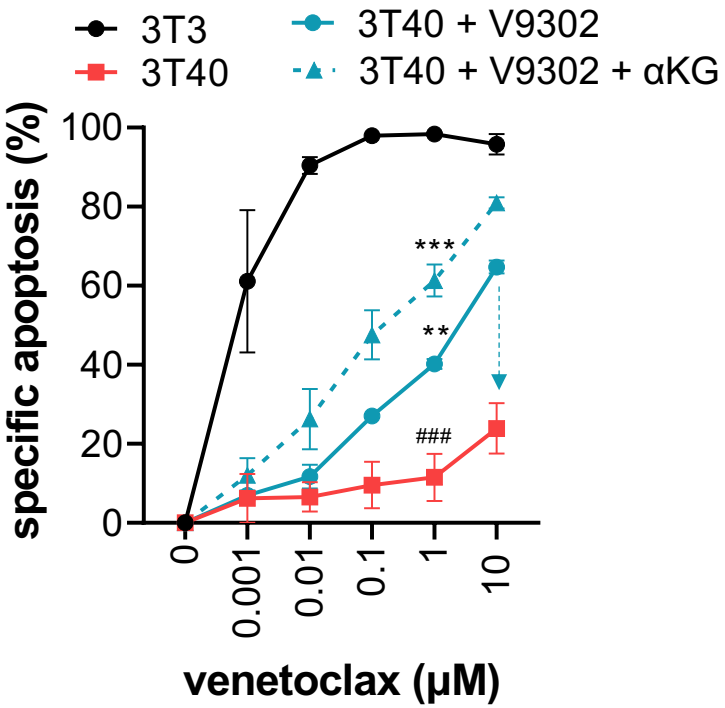
2. **V9302 only slightly decreases mitochondrial activity, as measured by extracellular flux analysis (Seahorse)**

Mitochondrial activity

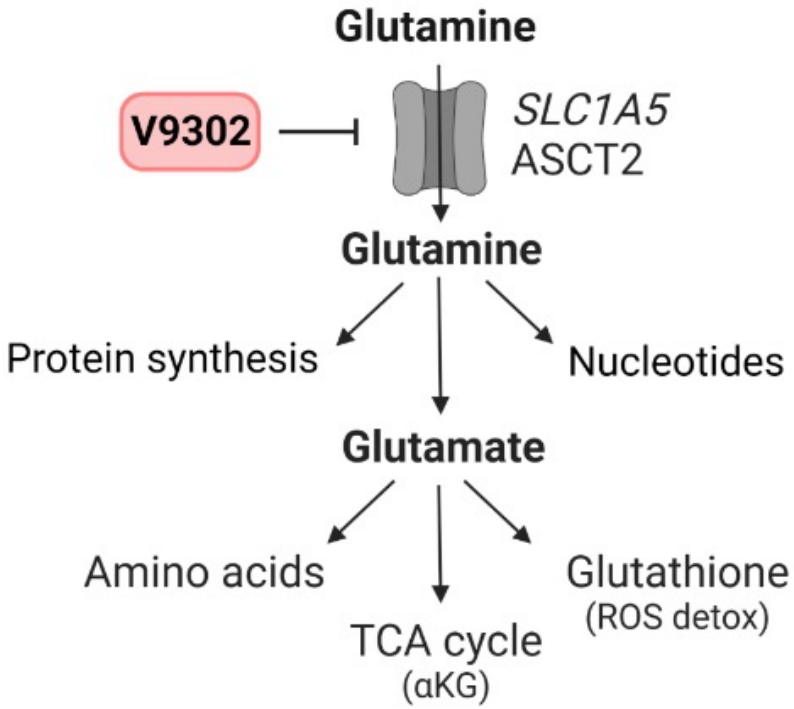


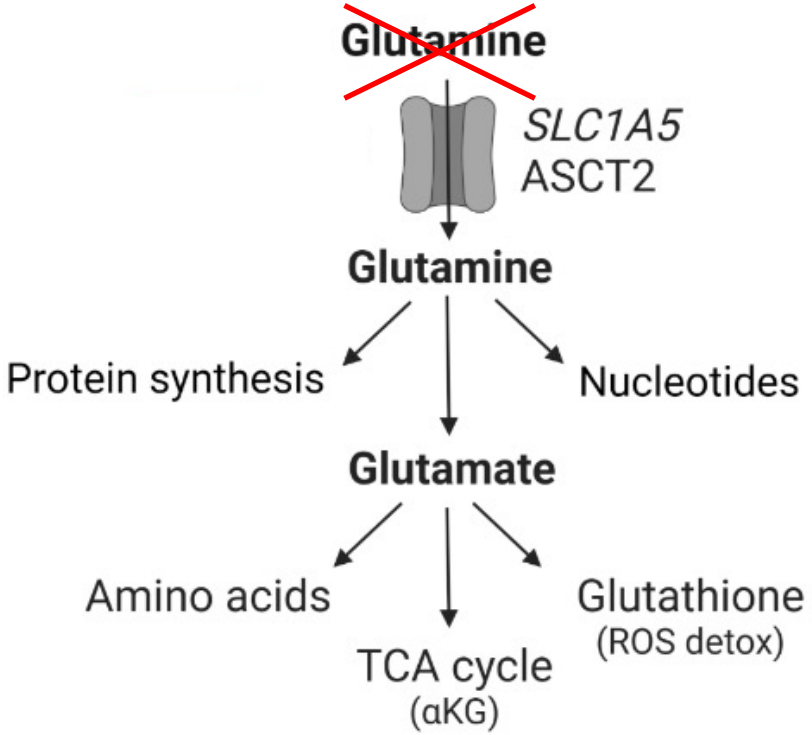
V9302 does more beyond preventing Gln incorporation into the mitochondria

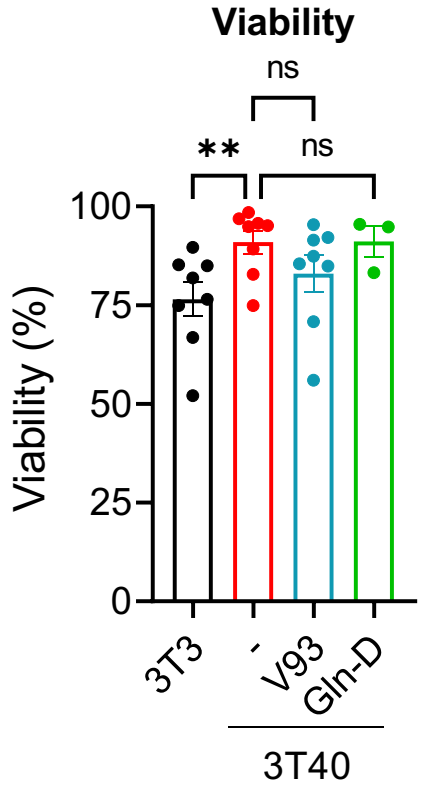
1. Inhibition of GLS does not increase sensitivity to VEN
2. V9302 only slightly decreases OXPHOS
3. TCA replenishment with α-ketoglutarate does not overcome VEN sensitivity induced by V9302



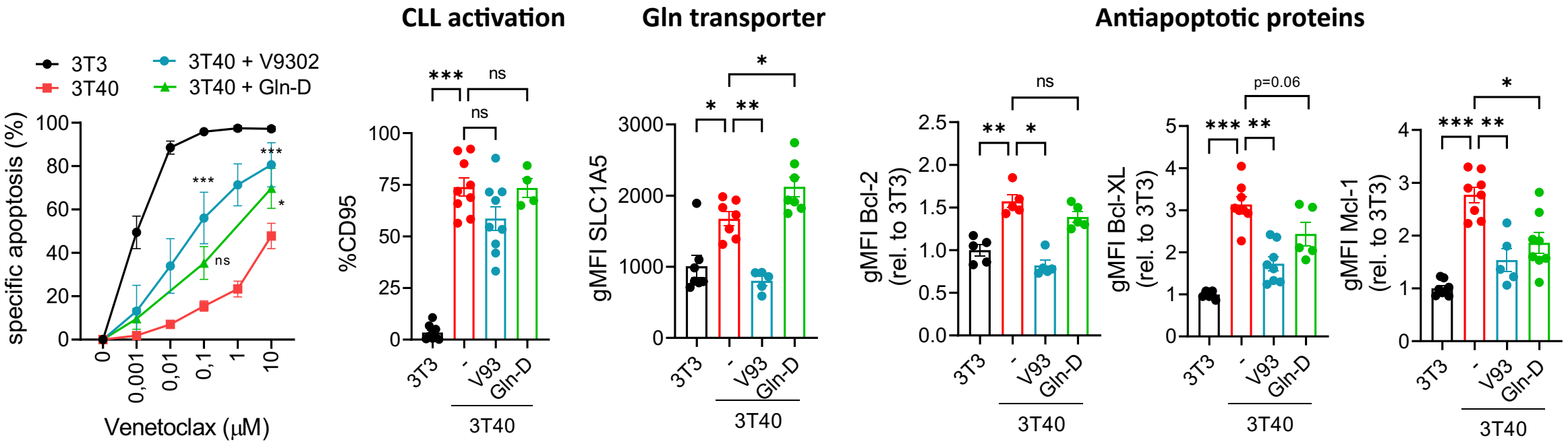
Glutamine deprivation *vs* V9302: do they do the same?





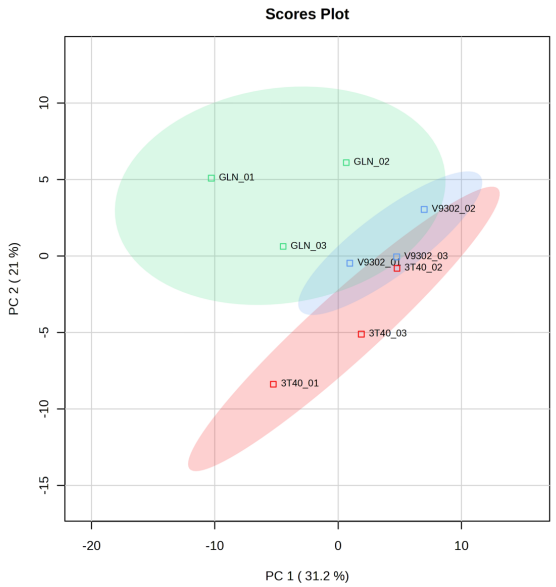


Glutamine deprivation does not recapitulate V9302 effects



V9302: 1) sensitizes CLL cells to venetoclax to a major extent compared to Gln-D
2) decreases CLL activation and ASCT2 expression, while Gln-D does not
3) prevents the upregulation of antiapoptotic proteins more than Gln-D

V9302 causes a pronounced decrease in most amino acids, while Gln-D only impacts Gln-derived pathways

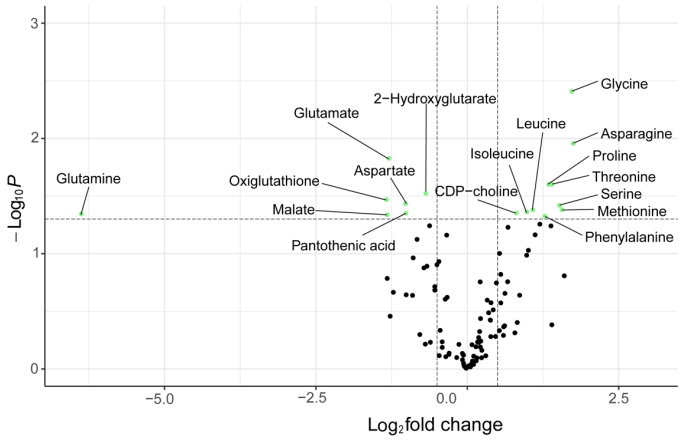


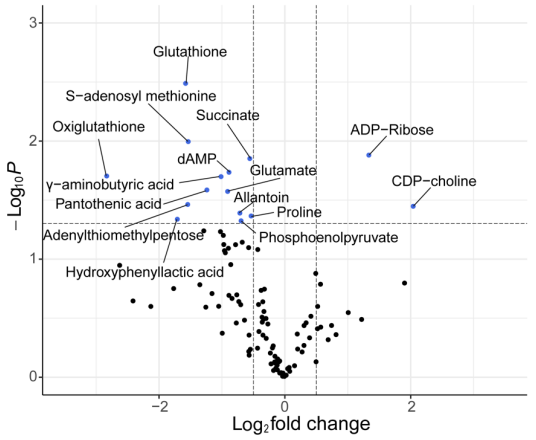
- 3T40
- Gln-
- V9302

Gln-D vs 3T40

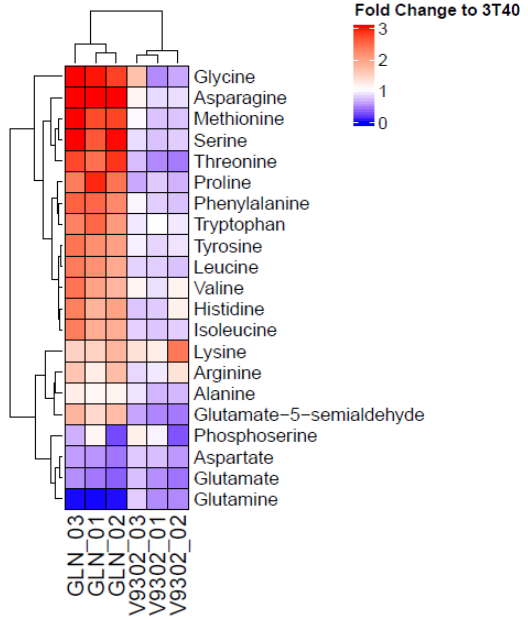
V9302 vs 3T40

$Log_2FC>1$; $p<0.05$

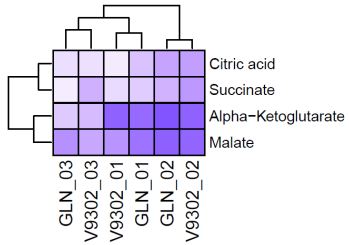




Amino acids

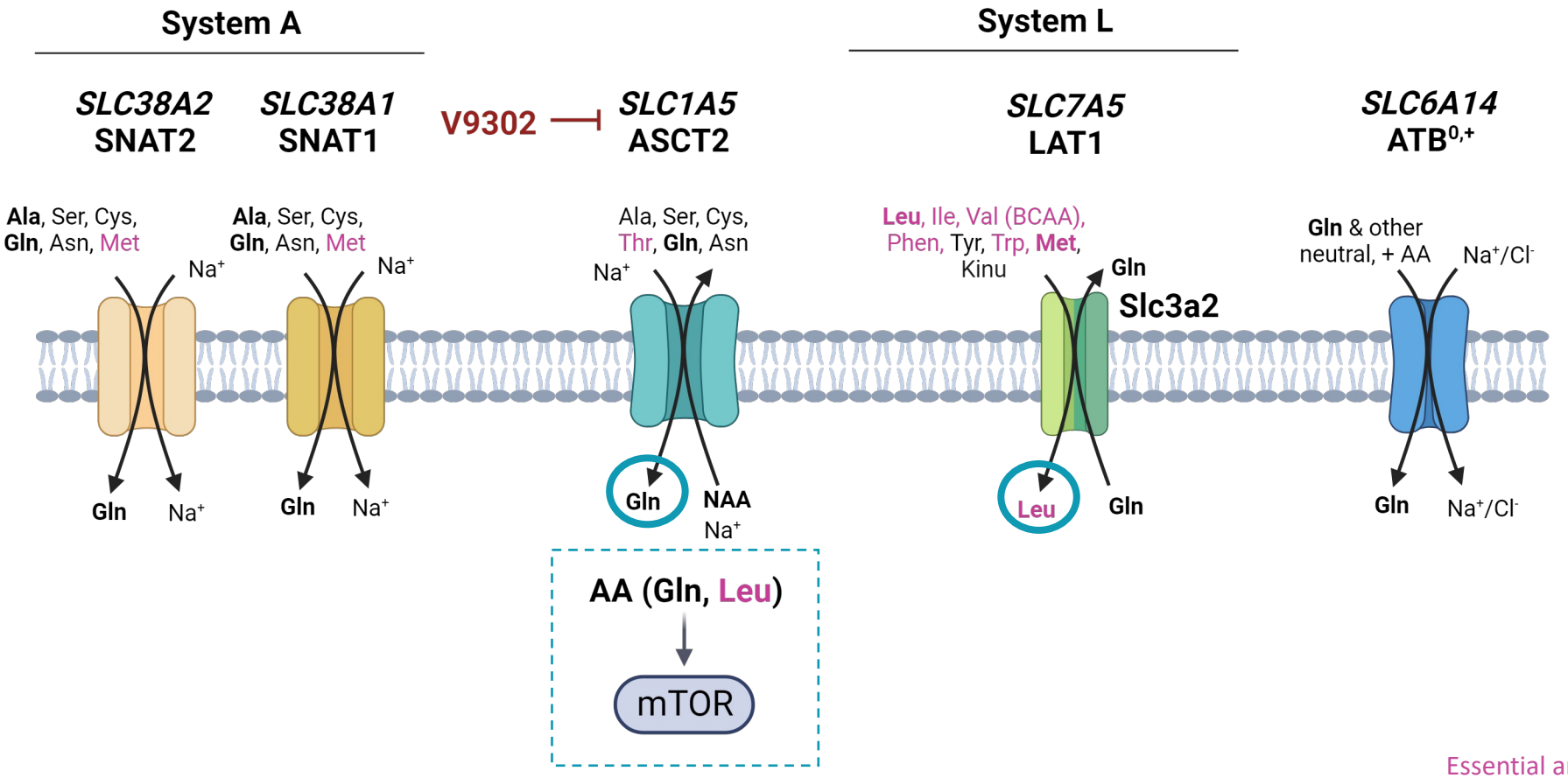


TCA cycle intermediates



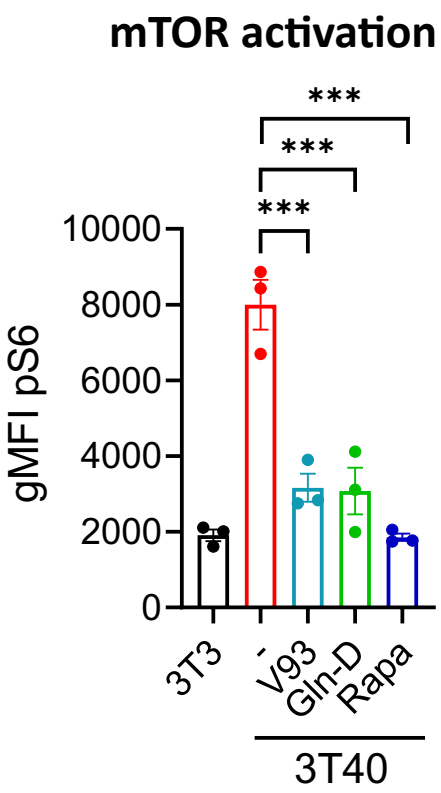
Only metabolites closely related to Gln are depleted upon Gln-D.
V9302 causes a profound decrease in all amino acids, and alters many other pathways.

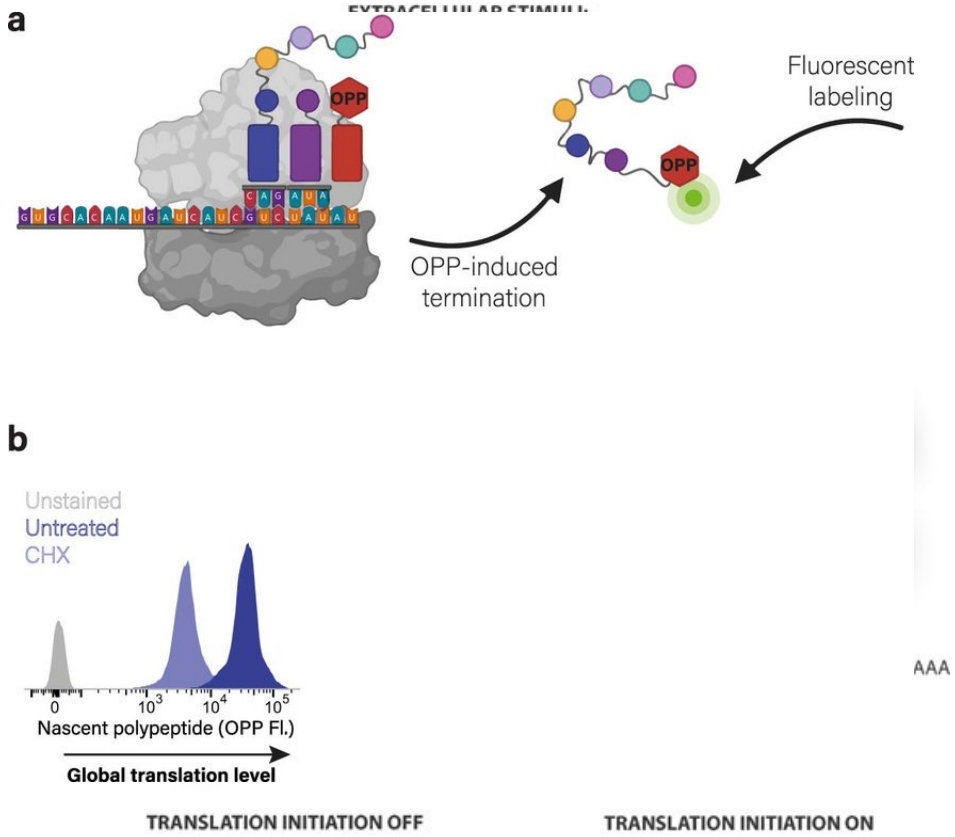
Amino acid homeostasis and mTOR signalling

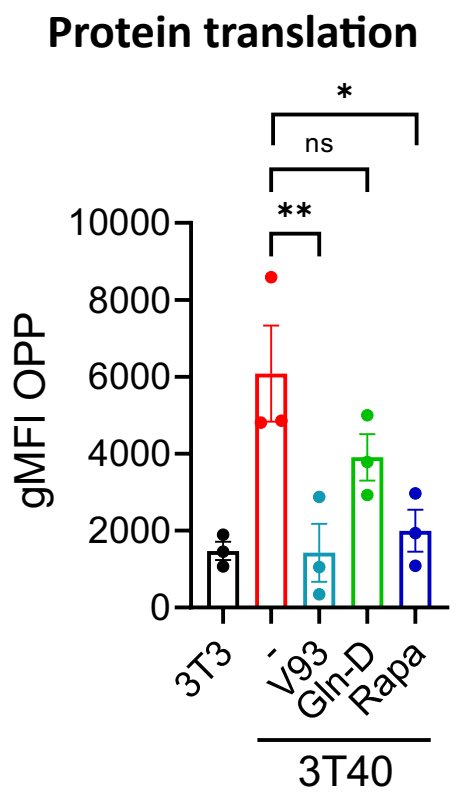


Essential amino acids

mTOR inactivation and inhibition of protein synthesis are at the basis of V9302 effect on VEN resistance



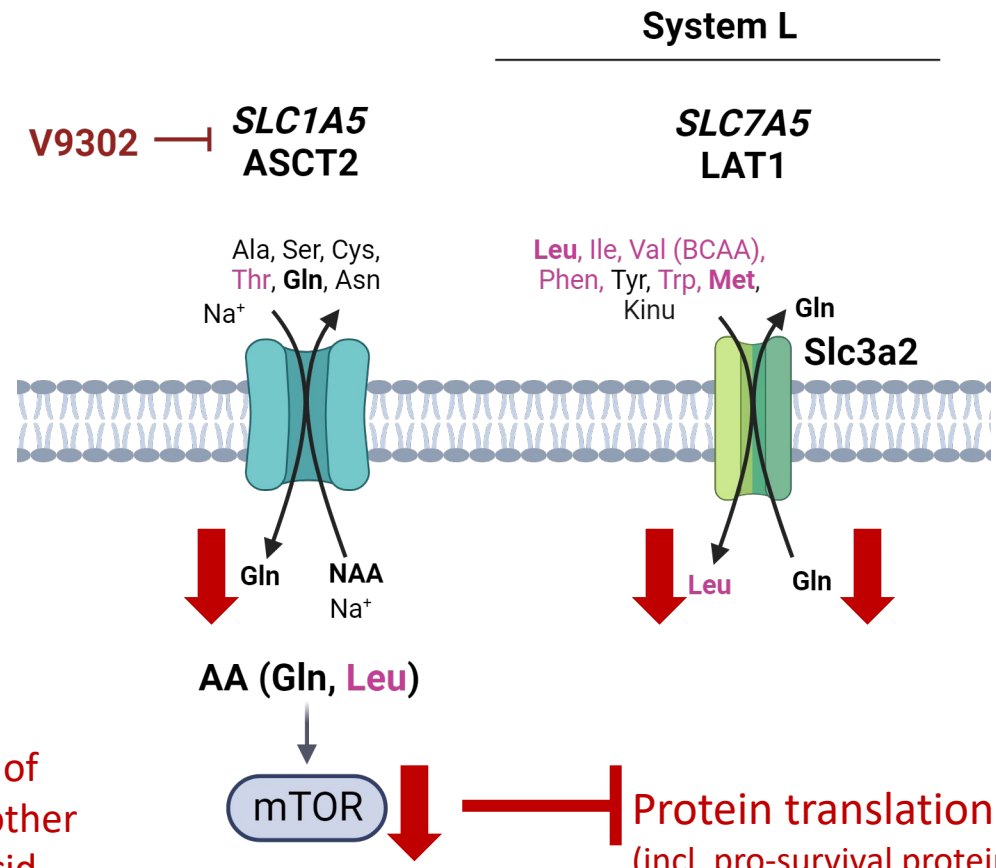




V9302, Gln-D and Rapa decrease mTOR signaling, but only V9302 and Rapa significantly block protein translation.

Finkel et al., Nature 2021
Mamane et al., Oncogene 2006

Recapitulation: Mechanism of action of V9302



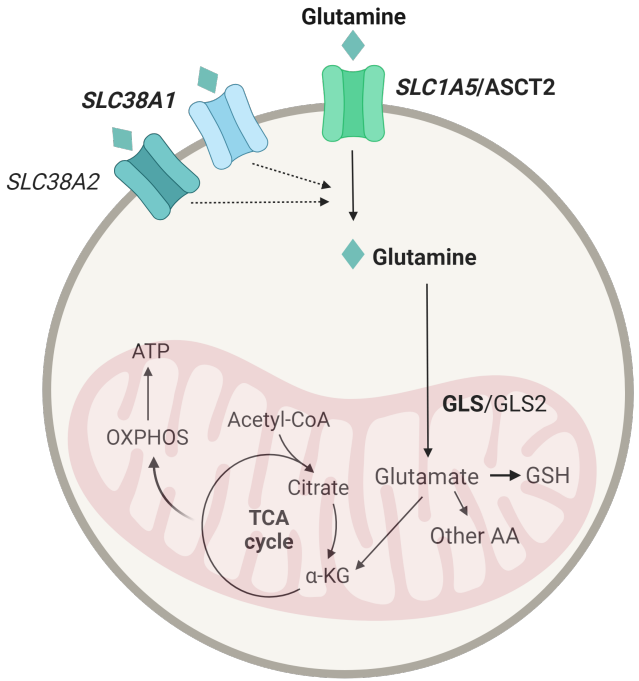
+ effects of V9302 on other amino acid transporters

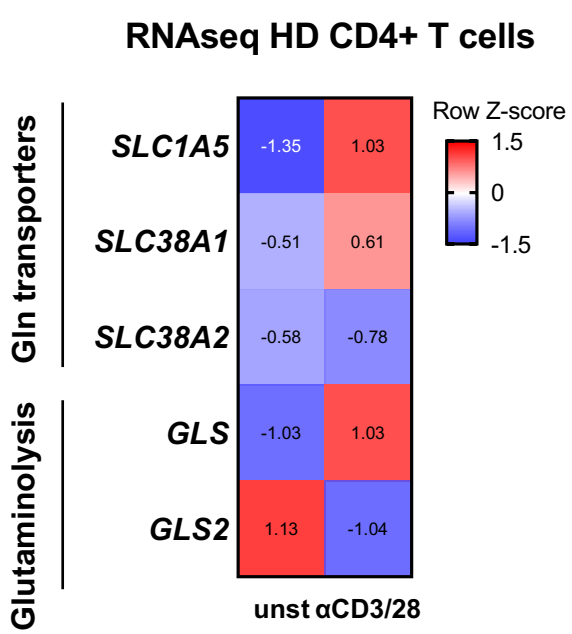

From mechanistic studies
to in vitro applications

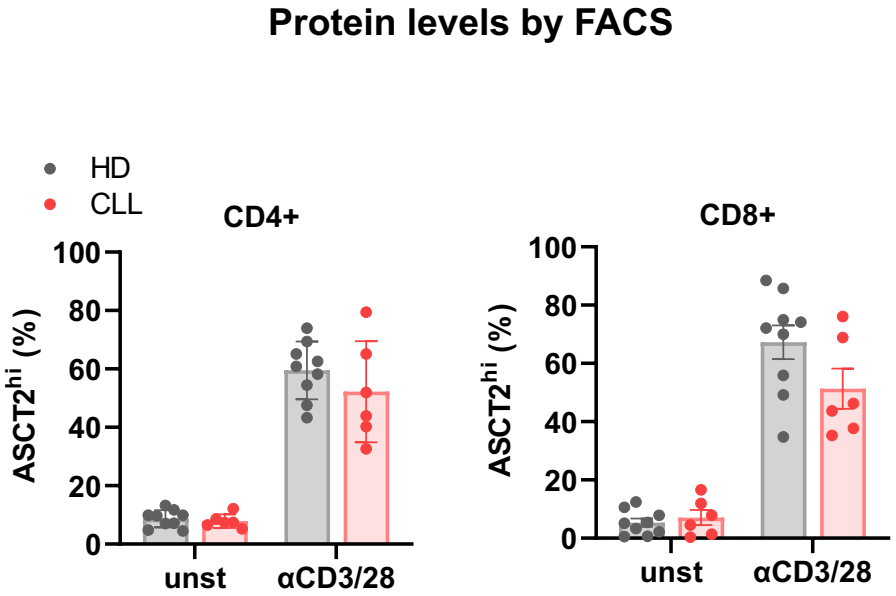
1. Glutamine addition as new therapeutic target in CLL: are the inhibitors toxic for healthy T cells?

(some of them are.. but V9302 is not..)
=> See poster Helga Simon Molas in next session

T cells from healthy controls and CLL patients also express the Gln transporter SLC1A5





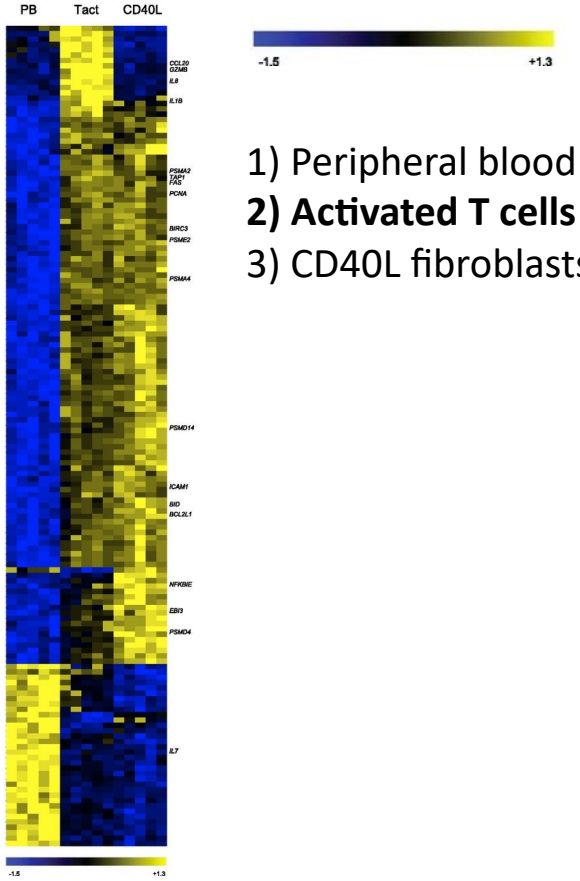


RNAseq by Chiara Montironi
RNAseq CLL T-cells: ongoing by Elena Camerini

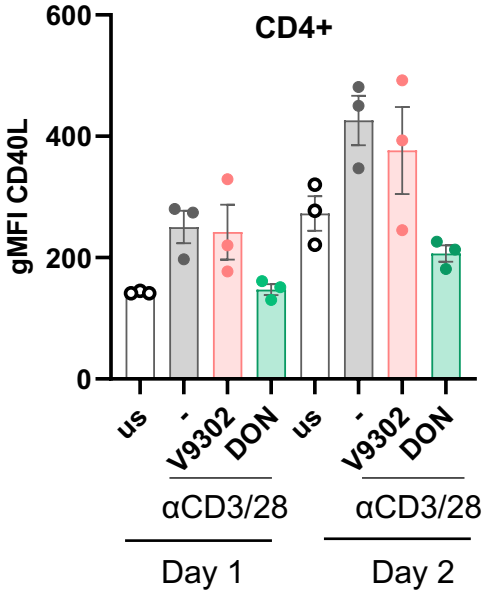
48h stimulation with soluble αCD3/αCD28

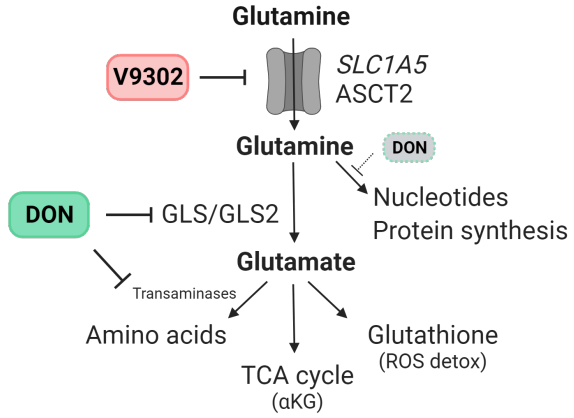
New setting: CLL stimulation through CD40L expressed by T cells

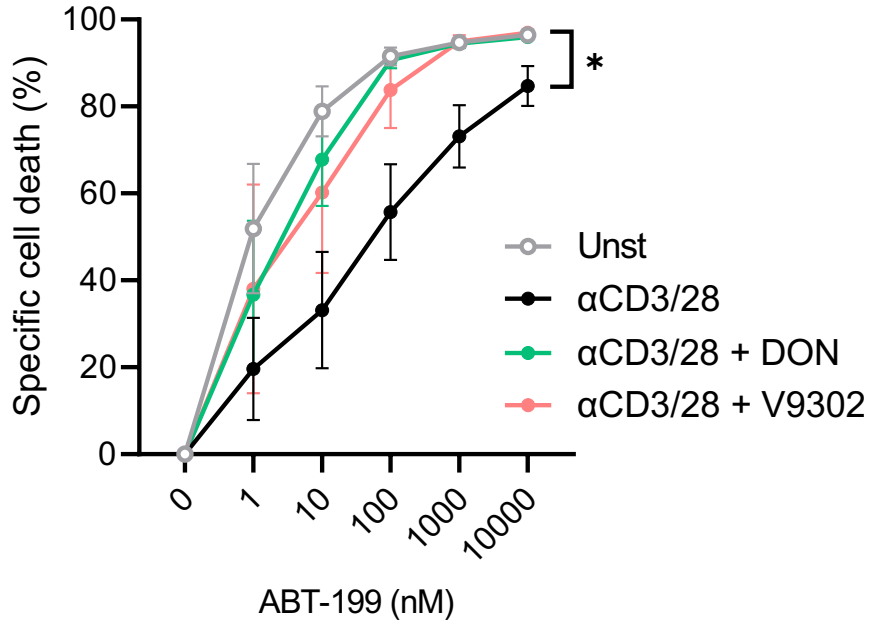
CLL transcriptomic signature upon 3T40 and T cell activation is very similar
(Pascutti Blood, 2013)



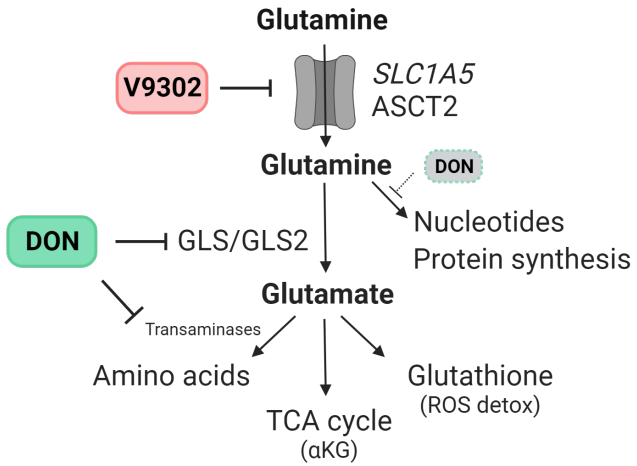
1) Peripheral blood
2) Activated T cells
3) CD40L fibroblasts

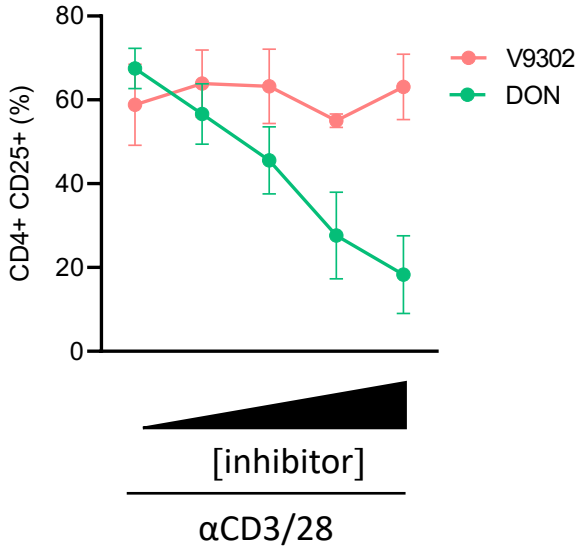


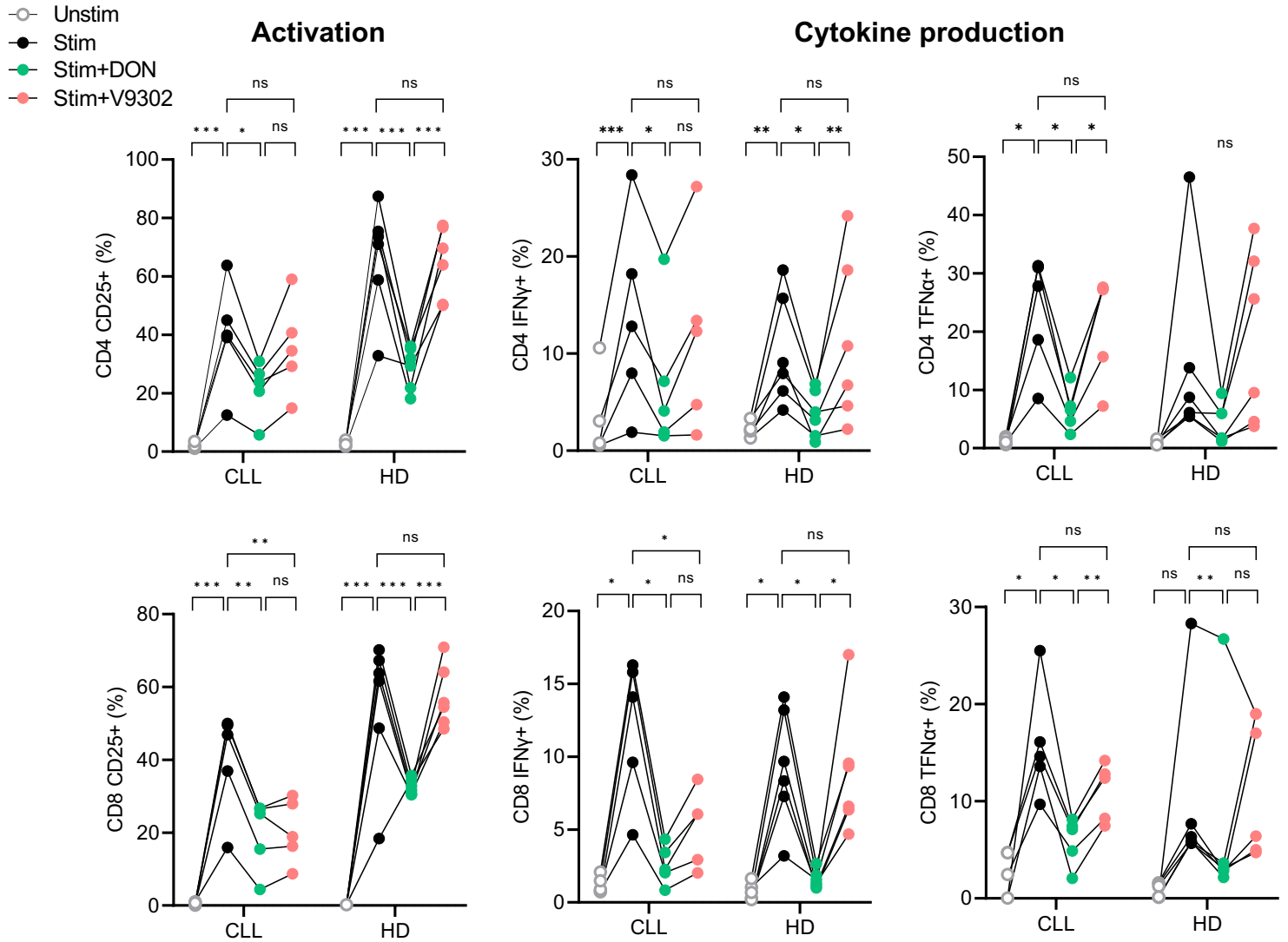




V9302 preserves T cell effector function (except CLL CD8+)



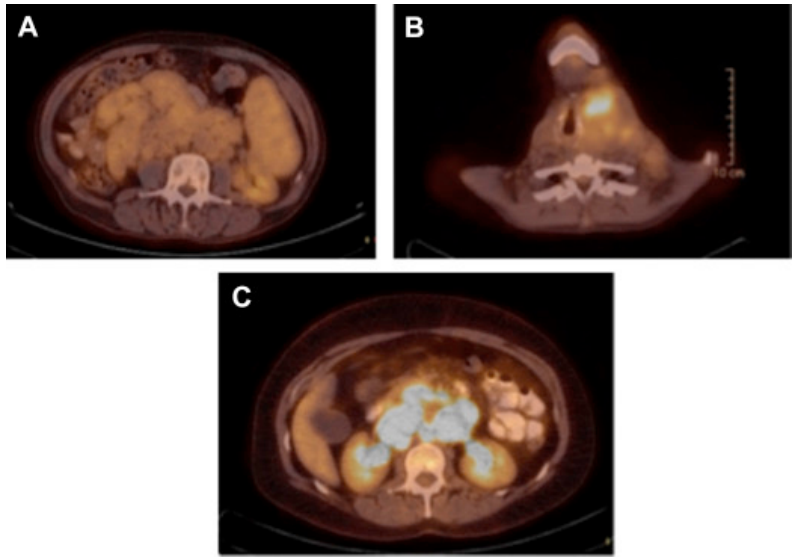




2. How can we exploit CLL glutamine addition in diagnostics?

[^{18}F]Glutamine PET as a potential new diagnostic tool in CLL

[^{18}F]FDG seems to be suboptimal to image CLL LN



[^{18}F]fluoro-2-deoxy-D-glucose PET/CT imaging:

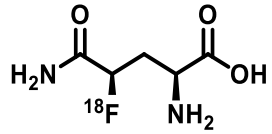
A) Indolent CLL at diagnosis
B) Biopsy-proved accelerated CLL
C) Biopsy-proved Richter Transformation

Rhodes and Mato, PET Clinics 2019

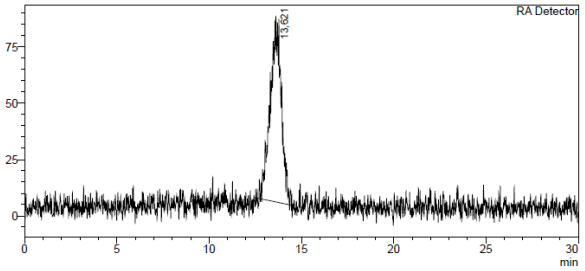
HOVON158 trial at Amsterdam UMC (A. Kater, J. Zijlstra):
^{18}F-FDG PET in CLL to investigate predictive value

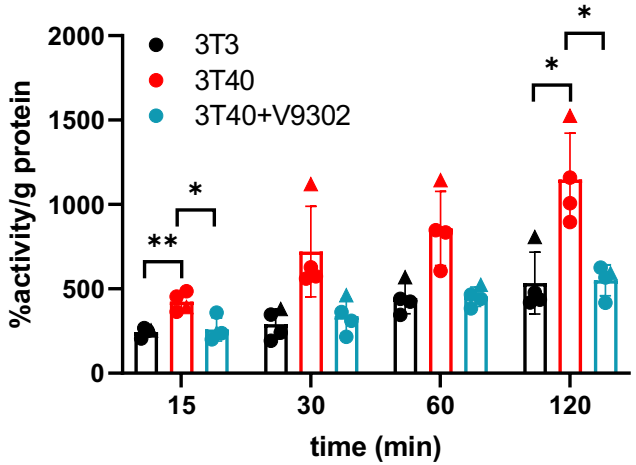
NEW: development of a ^{18}F-**Glutamine** PET for CLL

- In vitro tests – preliminary data
- In vivo tests (CLL TCL-1 mouse model) – next step!



(2*S*,4*R*)-4-[^{18}F]F-glutamine





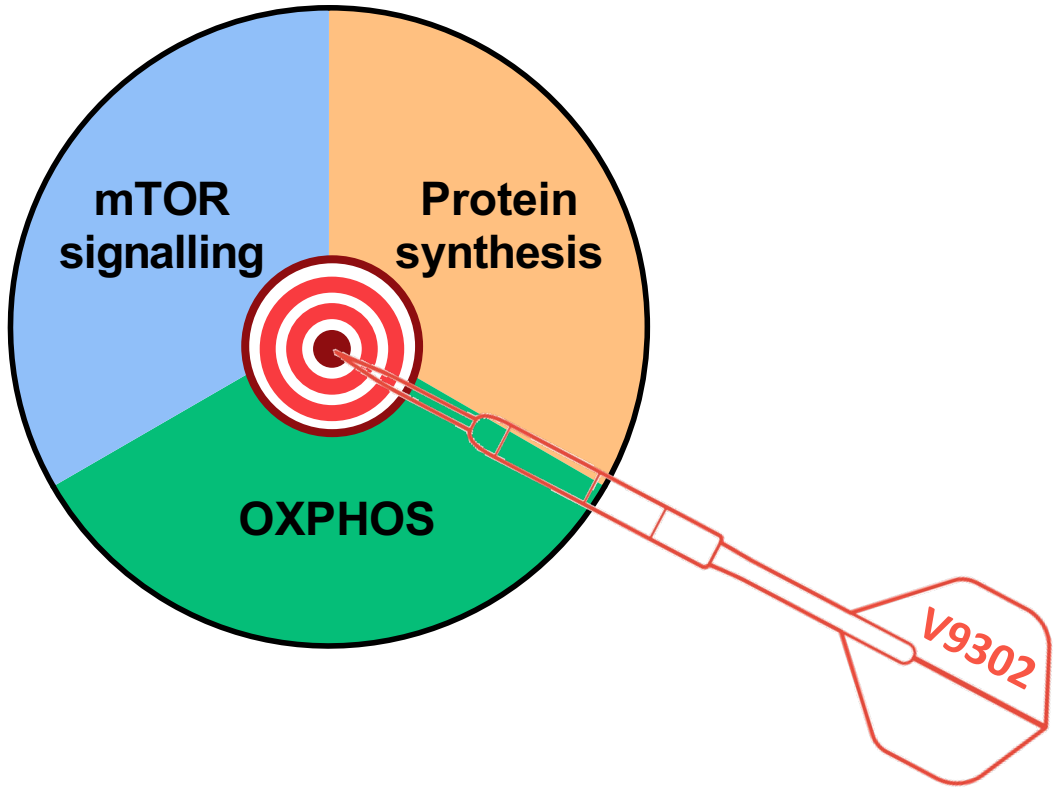
n=4; 3 unmutated CLL, 1 mutated CLL (triangles)

In collaboration with group of José Zijlstra and Bert Windhorst, VUMC

Conclusions

- Combined inhibition of mTOR signaling, protein translation and mitochondrial activity are at the basis of increased venetoclax sensitivity by V9302.
- Other amino acid transporters apart from SLC1A5 are likely to be involved in the effect of V9302. We are currently investigating this aspect.
- V9302 preserves the function of healthy CD4+ and CD8+ T cells. In CLL, CD4+ function is preserved, CD8+ function is dampened.
- Glutamine addiction of CLL cells has potential to be exploited in the clinics by i) targeting Gln import for therapy and ii) developing a ^{18}F-Gln PET tracer for diagnostics.

Glutamine metabolism in CLL cells upon LN stimuli



ImmunoHematology group & collaborators on this project

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EXPERIMENTAL IMMUNOLOGY

LYMMCARE
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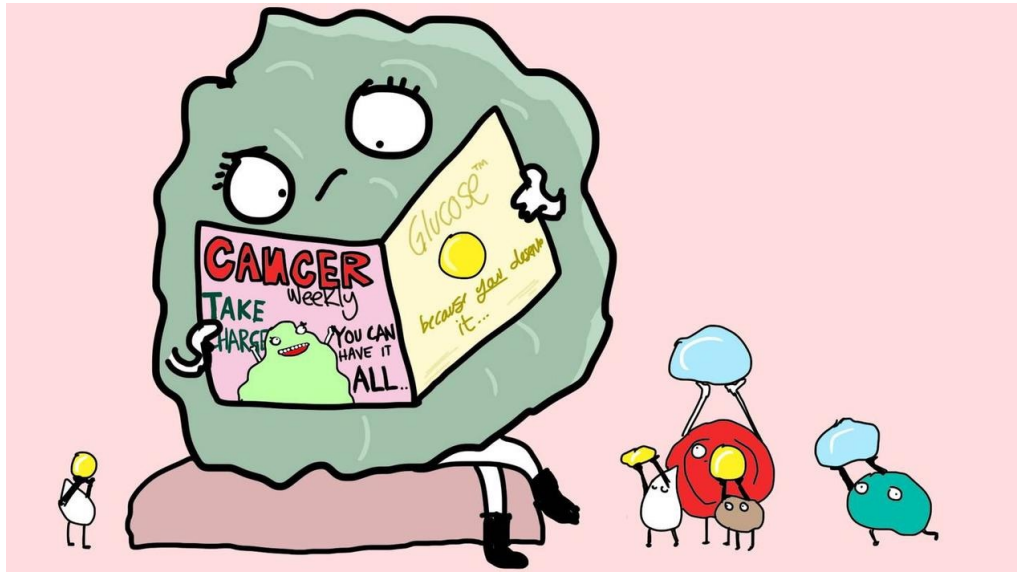
Questions?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Glutamine

Some open questions for discussion…

- Is Gln the key amino acid or is it for example Leu (or branched-chain AA)?
- Is Slc1a5 the key transporter, or others are more relevant?
- What sets the threshold for CLL cells to become sensitive to VEN? E.g. Gln-D vs V9302, mTORC1 vs mTORC1+2 inhibition.
- Which is the mechanism by which V9302 downregulates the levels of pro-apoptotic proteins? Is the general decrease in translation that we have observed, or is it more specific?
- VEN vs IBR resistance: different branches of cell metabolism?

Thanks for your attention!

Targeting Glutamine Import Counteracts Microenvironmental-Driven Venetoclax Resistance in Chronic Lymphocytic Leukemia

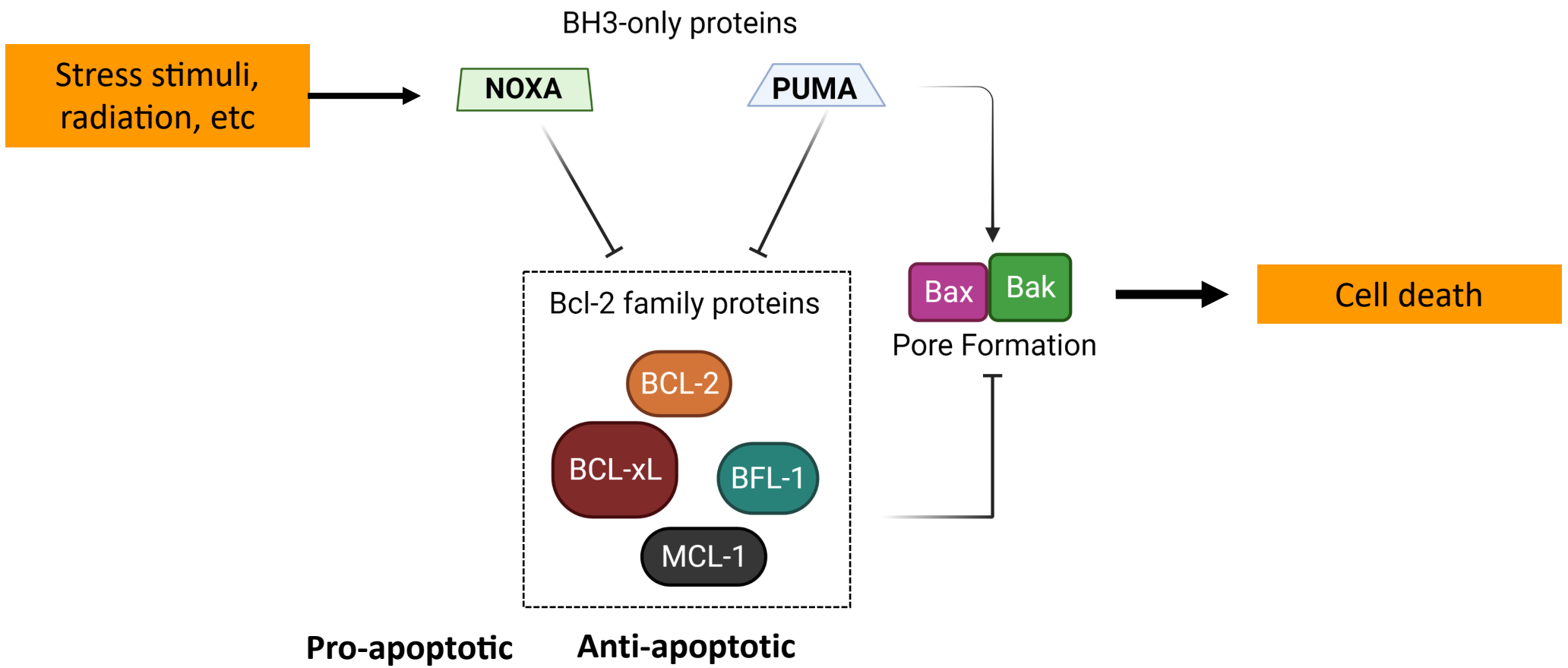
Helga Simon-Molas

ImmunoHematology Group (E. Eldering, A. Kater)

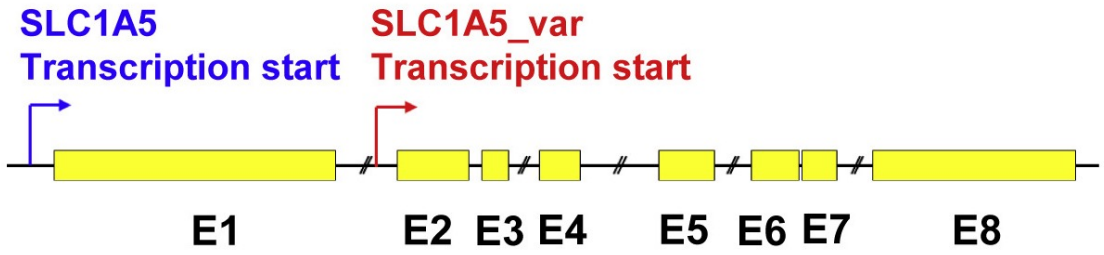
Depts. of Experimental Immunology & Hematology

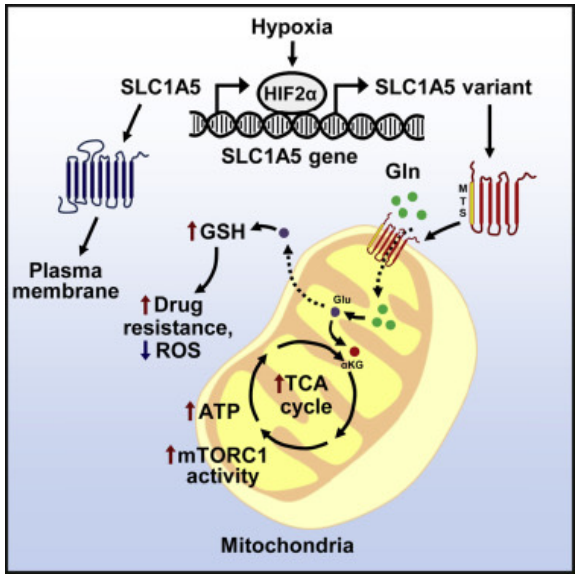
Lymmcare Work Discussion 09-10-2023

BH3 mimetics are effective targeted drugs in CLL

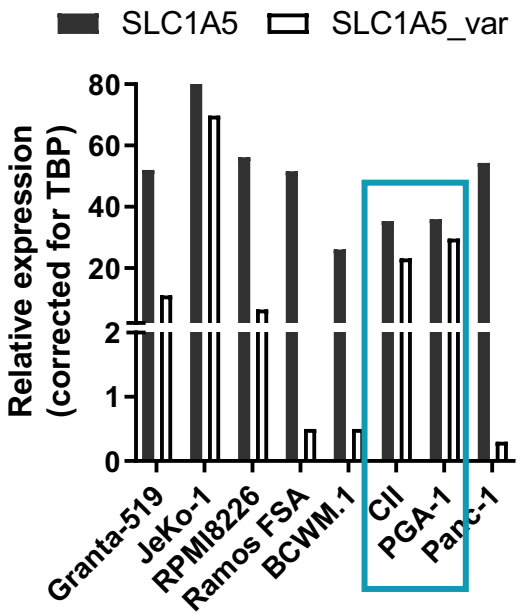


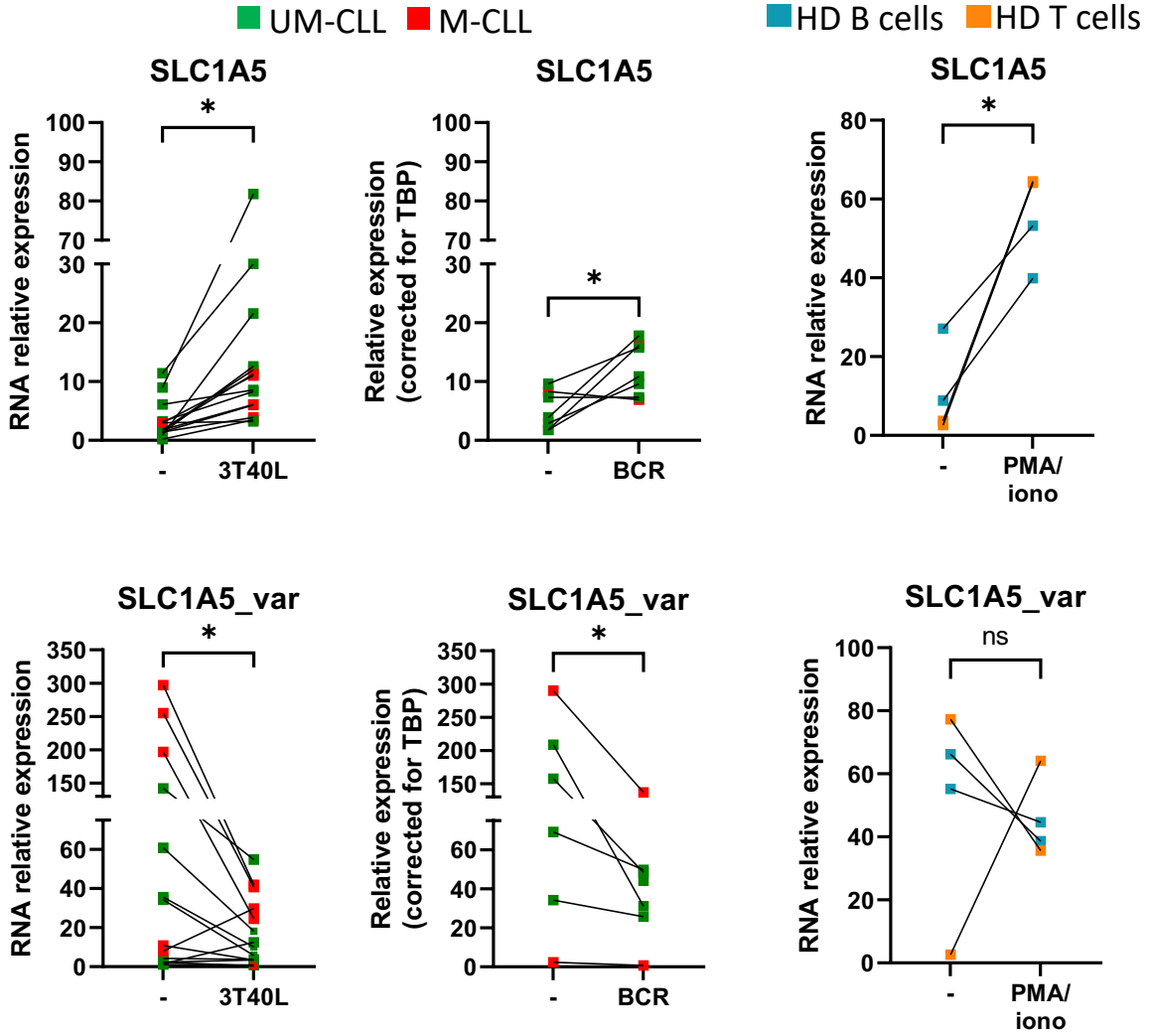
SLC1A5: plasma membrane *vs* mitochondrial form



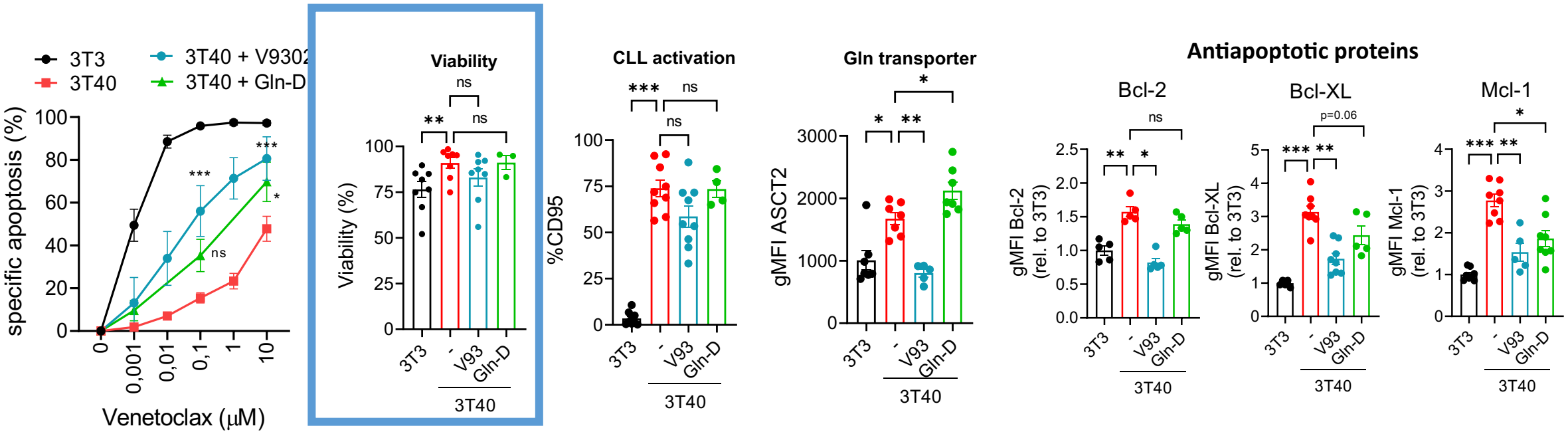


Yoo, et al., Cell Metabolism 2020



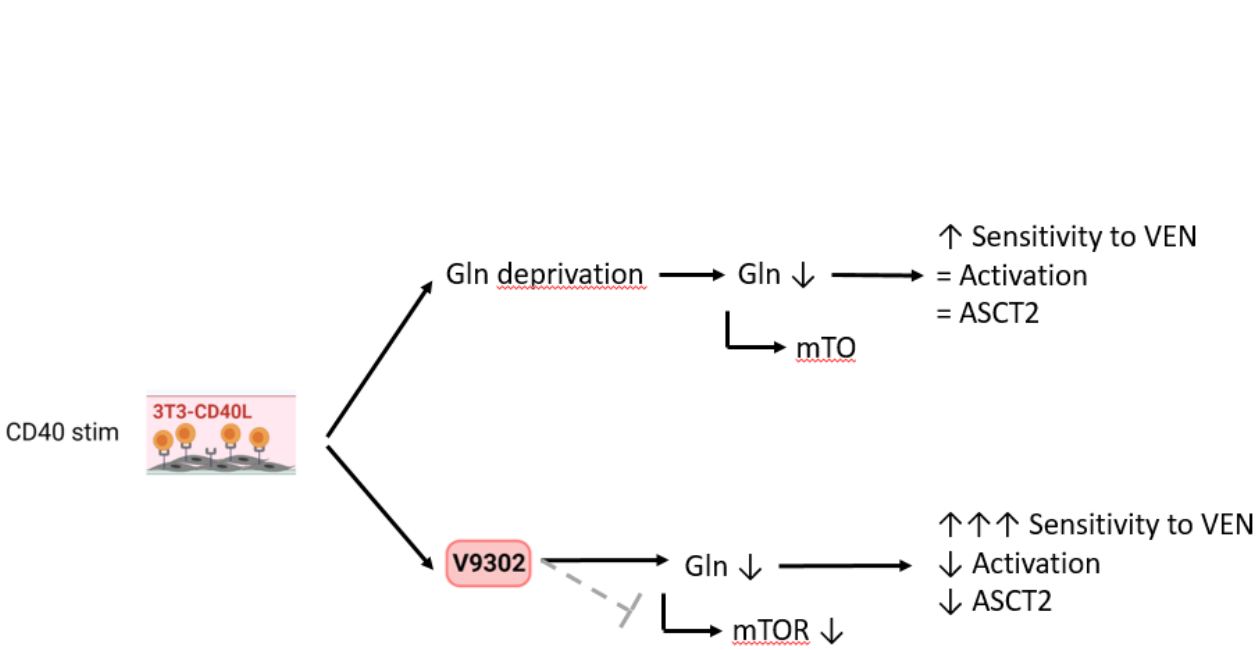


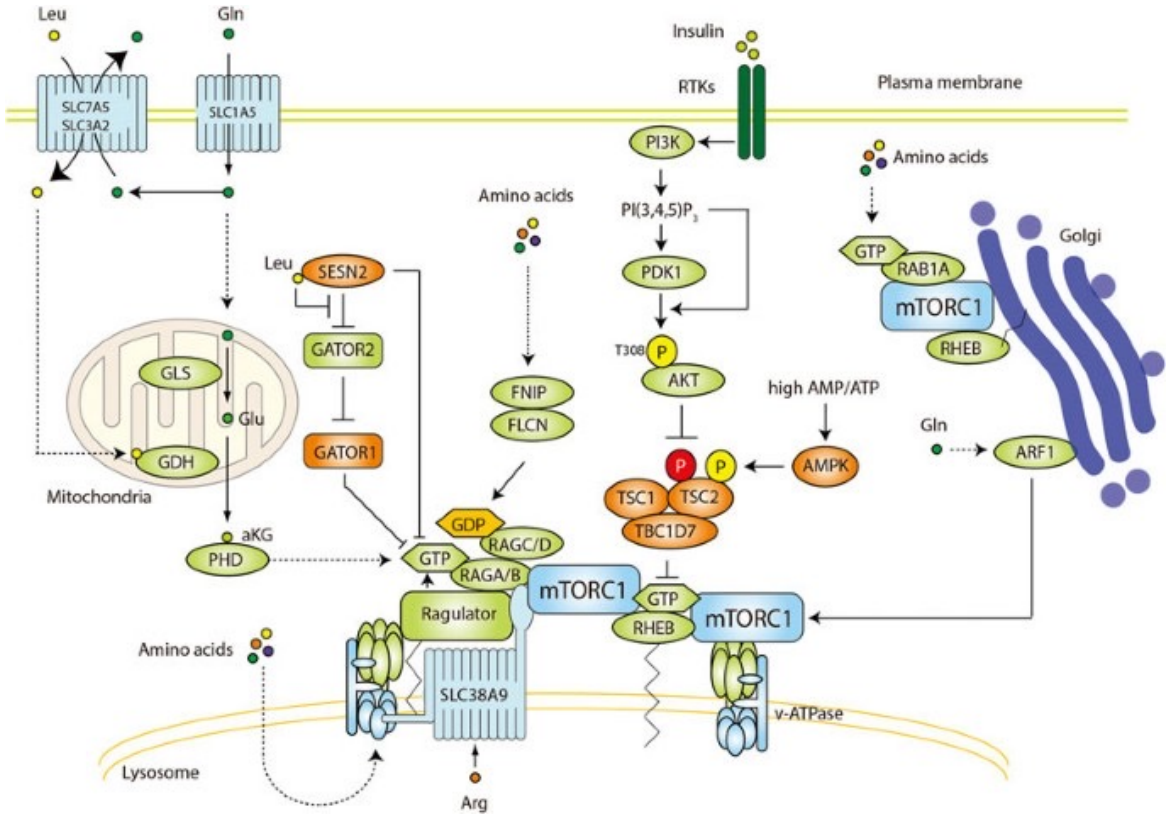
Glutamine deprivation does not recapitulate V9302 effects



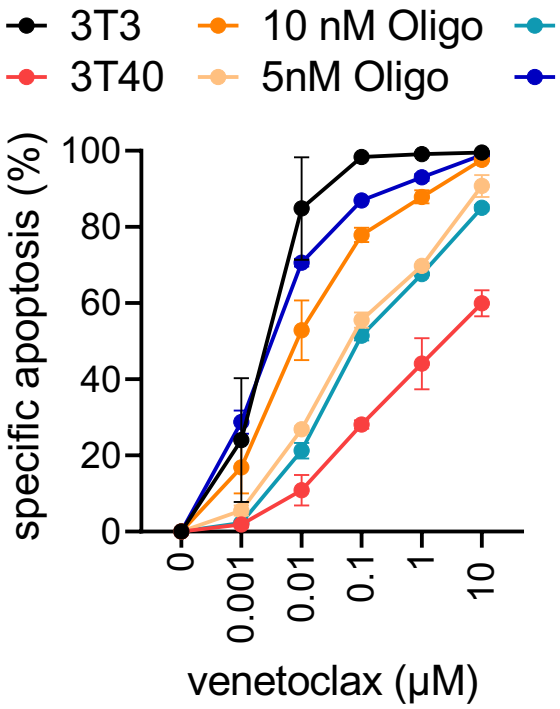
V9302: 1) sensitizes CLL cells to venetoclax to a major extent compared to Gln-D
2) decreases CLL activation and ASCT2 expression, while Gln-D does not
3) prevents the upregulation of antiapoptotic proteins more than Gln-D

Gln- *vs* V9302 scheme

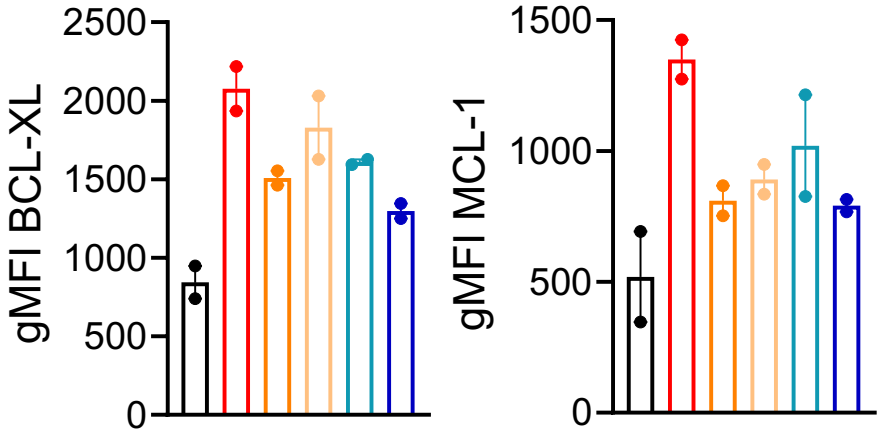




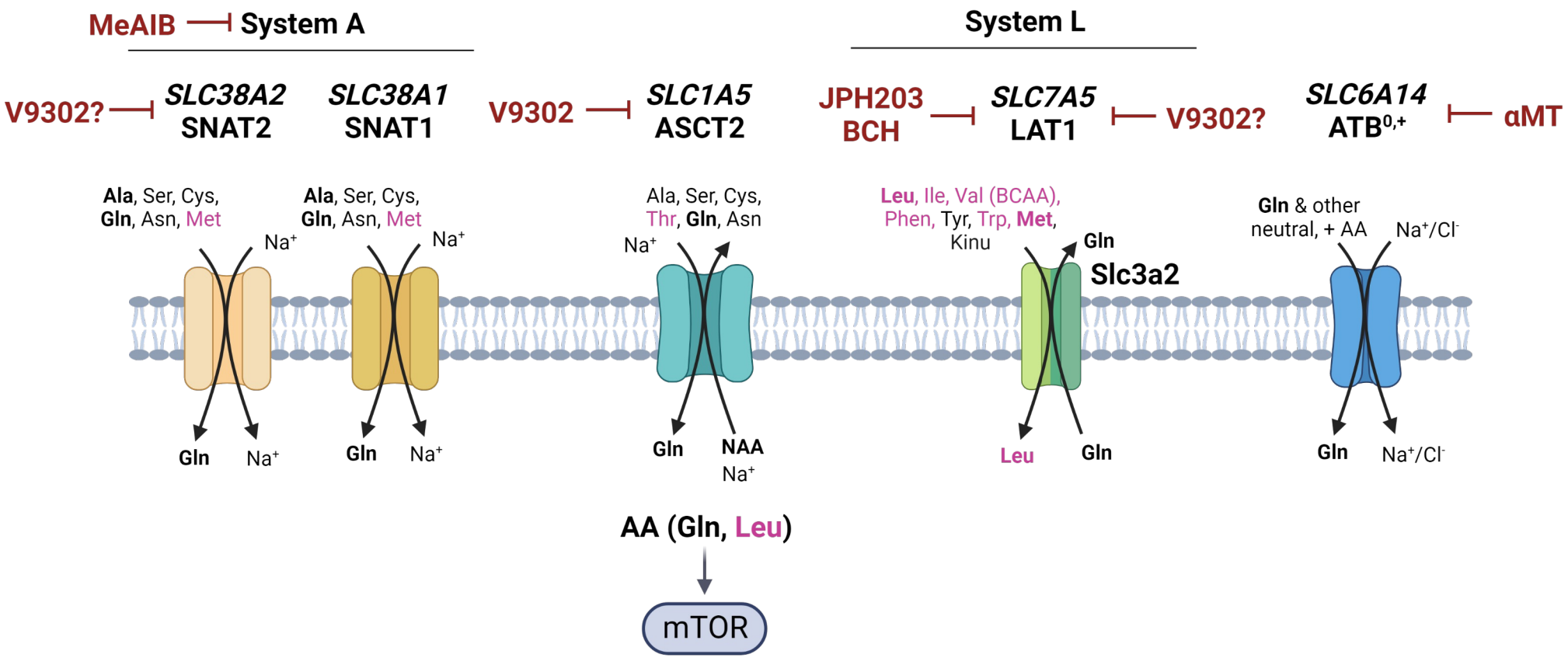
Synergy between V9302 and OXPHOS inhibition



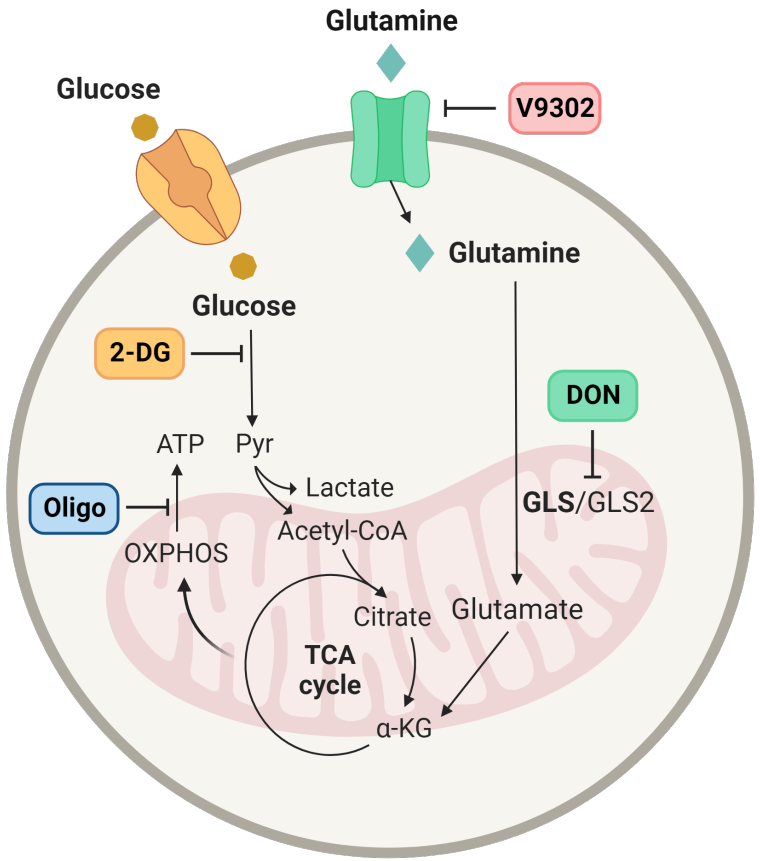
Venetoclax concentration (nM)	Synergy Score V9302+Oligomycin
0	1,08
1	0,82
10	0,48
100	0,41
1000	0,35
10000	0,17

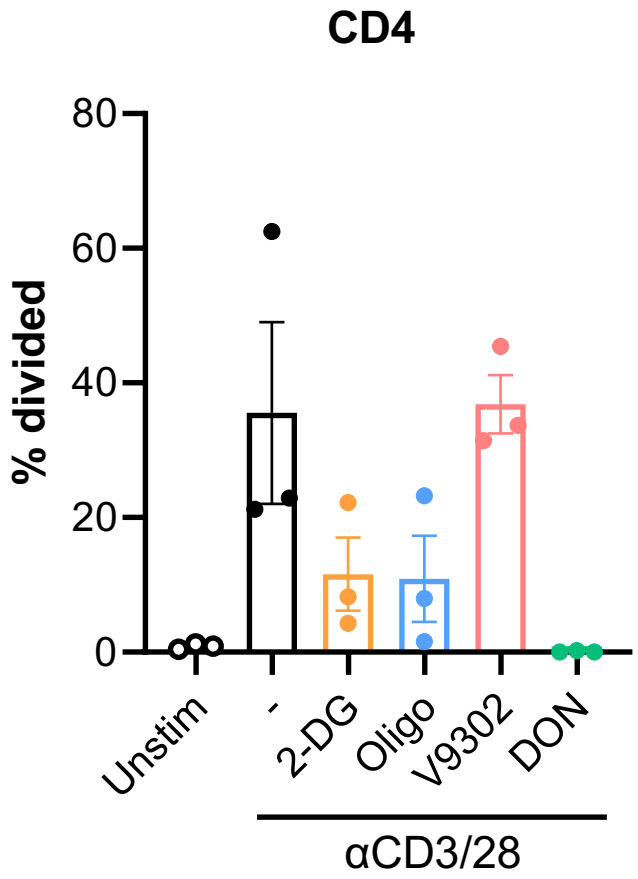


Amino acid transporters



Unlike 2-DG, Oligomycin and DON, V9302 preserves T cell proliferation

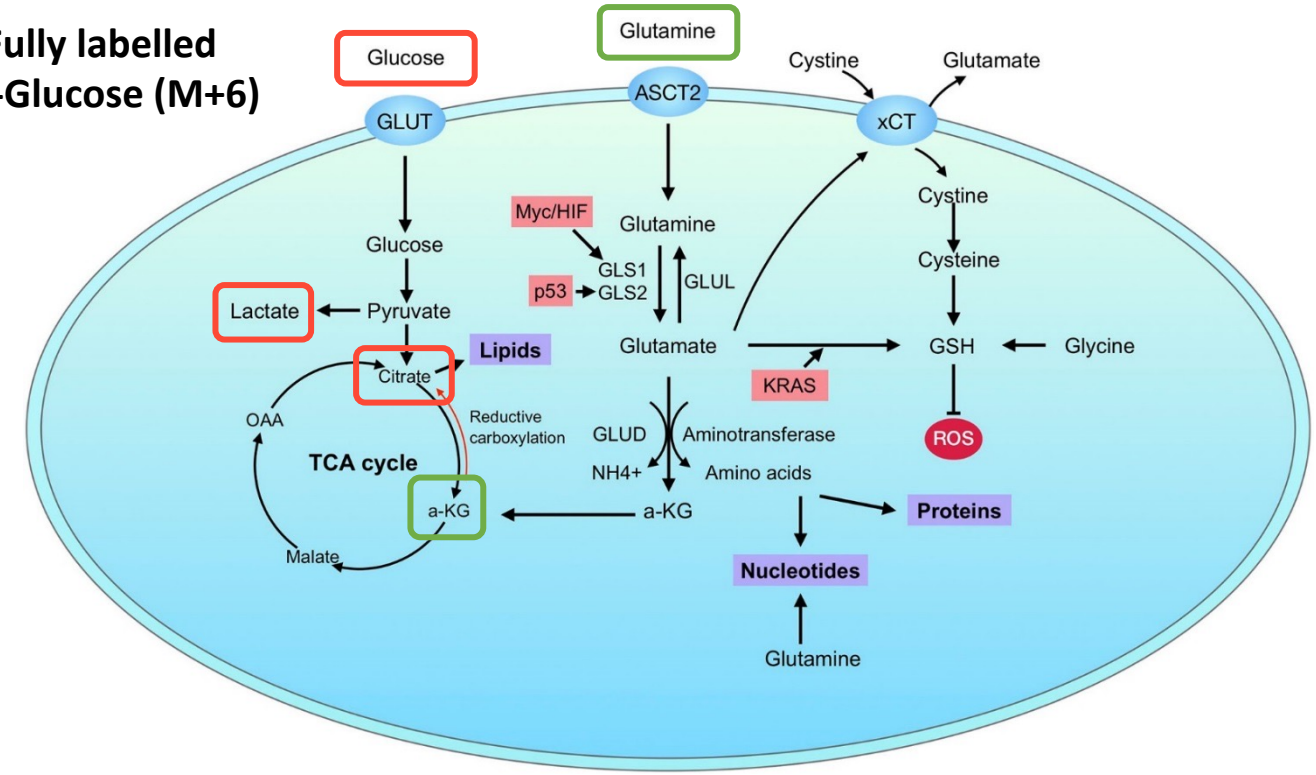




Glucose and glutamine labelling

Fully labelled
^{13}C-Glucose (M+6)

Fully labelled
^{13}C-Glutamine (M+5)



Targeting Glutaminolysis: New Perspectives to Understand Cancer Development and Novel Strategies for Potential Target Therapies, Wang, Frontiers in Oncology 2020.