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DI PARMA

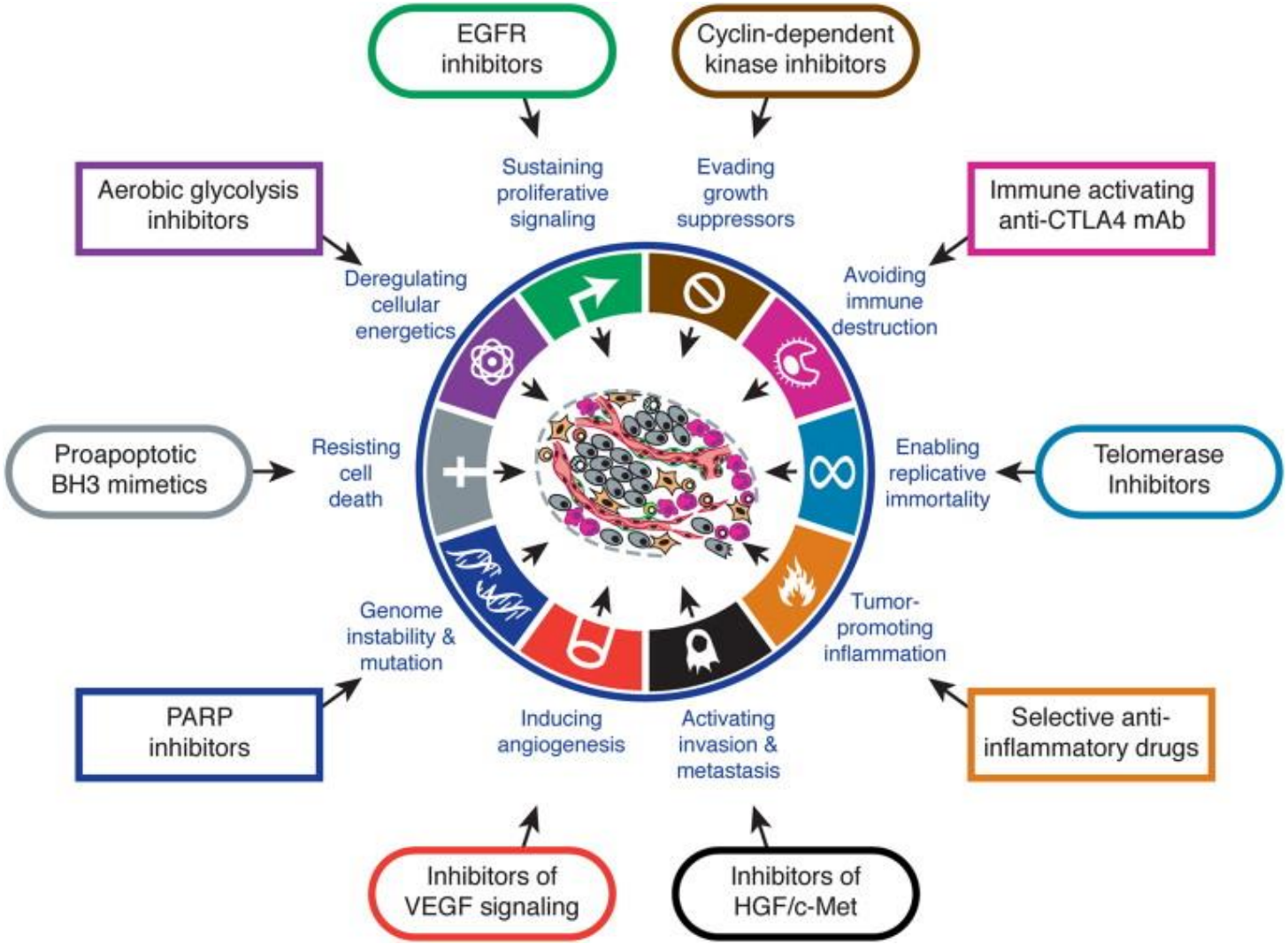
Cancer-associated alteration of glutamine metabolism

The case of hematological neoplasia

Martina Chiu, PhD

Florence, 21st January

The hallmarks of cancer



Hanahan and Weinberg, Hallmarks of cancer: Next generation, Cell (2011)

Cancer as a Genetic Disease

- Every cancer cell has mutations leading to over-expression or perturbations of oncogenes or tumor suppressor genes. First oncogene discovered in 1970.
- Oncogene: a gene that can potentially cause cancer, due to mutations or increased expression (Ras, Myc, Raf, Src, EGFR, HER2/neu, HIF-1α, Wnt, Erk, Trk, Bcr-Abl).
- Tumor suppressor gene (TSG): a normal gene that prevents tumor development (BRCA1, p53, PTEN).

How Was Cancer Viewed Prior to 1970?

- Prevailing opinion among most oncologists was that cancer was a "metabolic disease"
- Cancer cells were metabolically dysregulated (cause of the metabolic dysregulation was unknown)
- Cancer drugs were called "anti-metabolites" and cancer chemotherapy was called anti-metabolite therapy

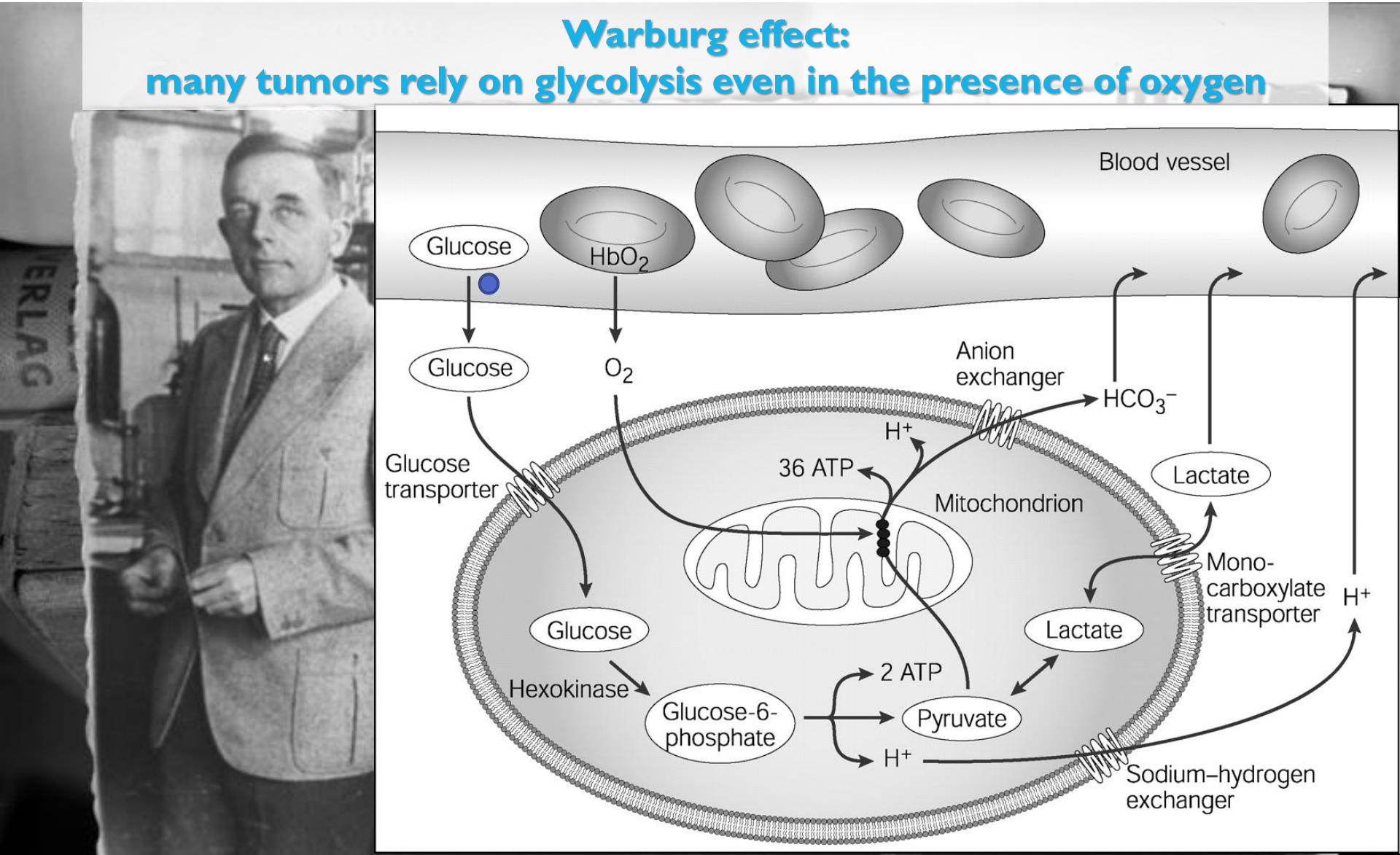
D. Wishart (2015)

Anti-Metabolite Cancer Drugs

Anti-metabolite	Metabolite equivalent
5-Fluorouracil (5-FU) - 1957	Uracil
Gemcitabine (Ara-C) - 1981	Cytosine
6-Mercaptopurine - 1951	Adenine/Guanine
Fludarapine (Ara-A) - 1968	Adenine
Methotrexate - 1956	Folate
Aminopterin - 1947	Folate
Megestrol acetate - 1956	Progesterone
Asparaginase* - 1963	Asparagine/Glutamine*

Otto Warburg: the pioneer

Warburg effect:
many tumors rely on glycolysis even in the presence of oxygen

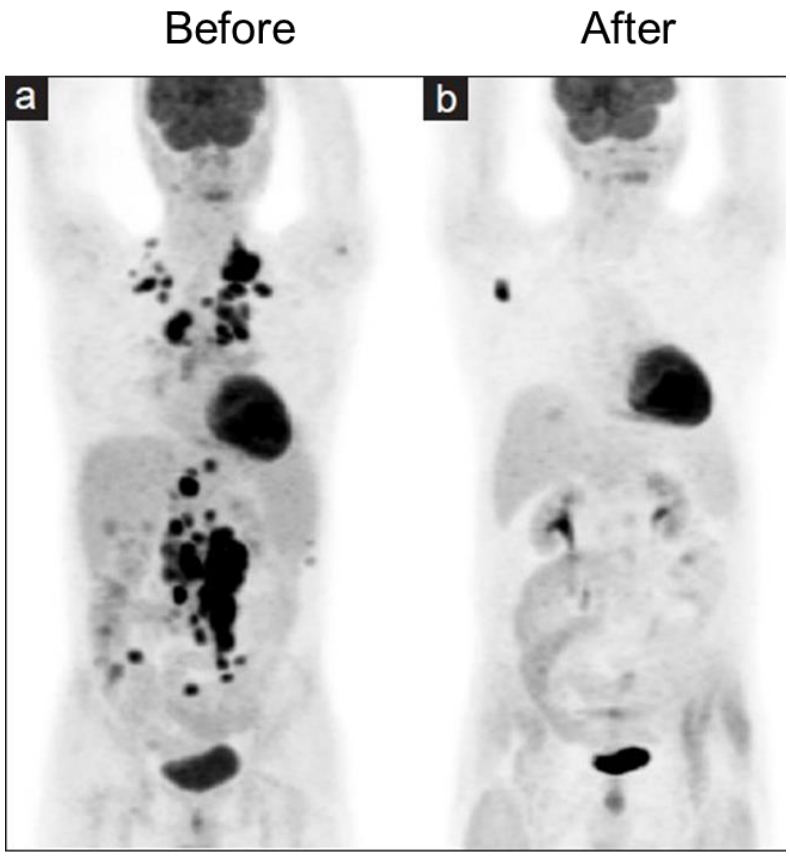


Garber K JNCI J Natl Cancer Inst 2004;96:1805-1806

Cancer is a Metabolic Disease

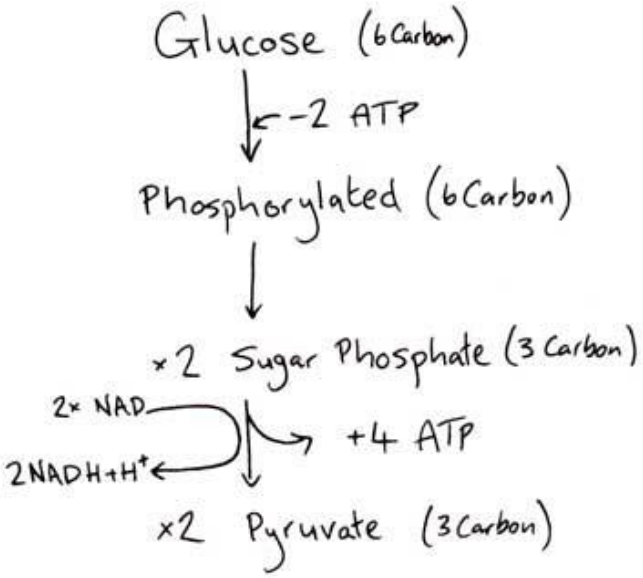
- Cancer cells consume 100-200X more glucose that other cells in the body

- This unique metabolism is the basis to PET (positron emission tomography) scans for cancer using fluorinated deoxyglucose

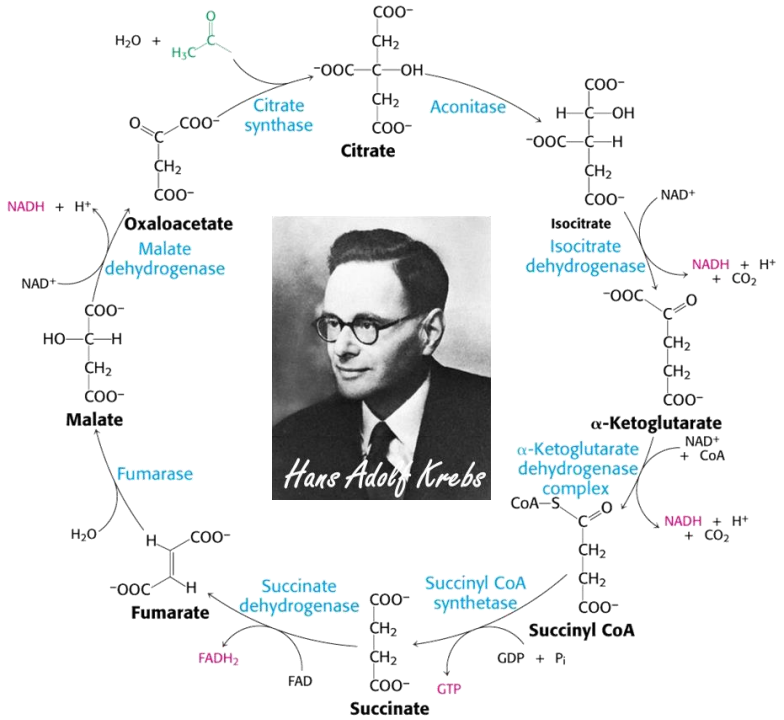


Tumors are marked in black in this PET image (lots of glucose)

Why glycolysis?



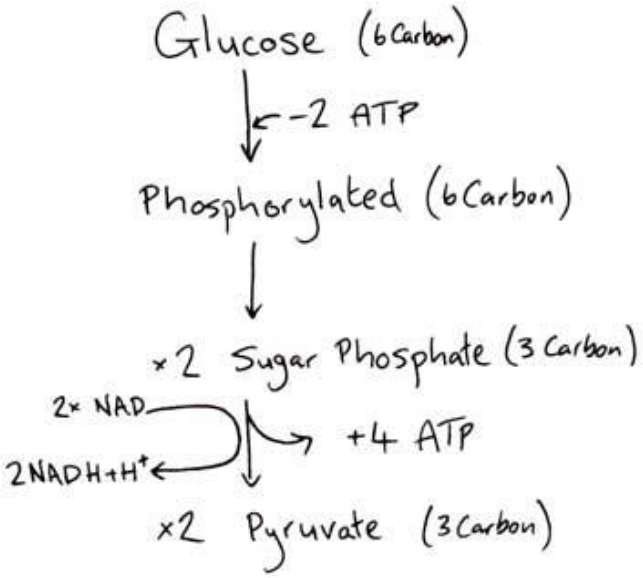
2 mol ATP/ mol glucose



36 mol ATP/ mol glucose

- **Increased resistance to hypoxia**
- **Mitochondrial defects/ Mutations in metabolic enzymes**
- **Extracellular acidification**
- **"Crabtree" effect**
- **Anaplerosis**

Why glycolysis?



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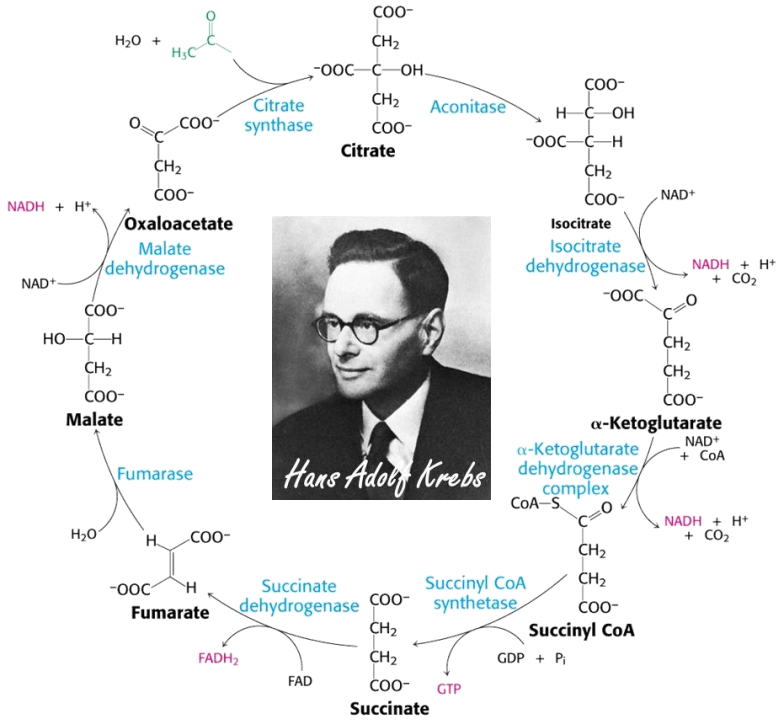

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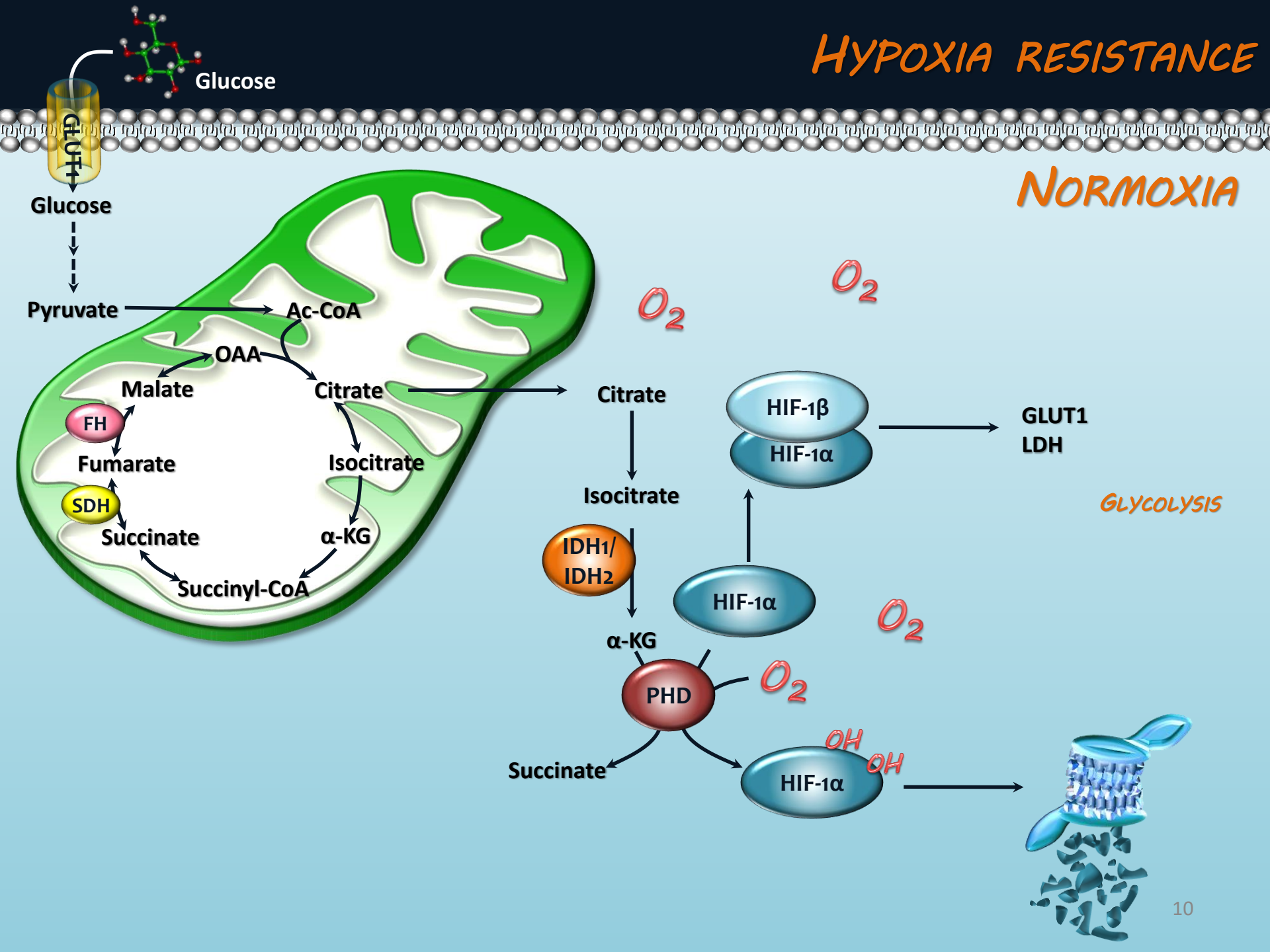
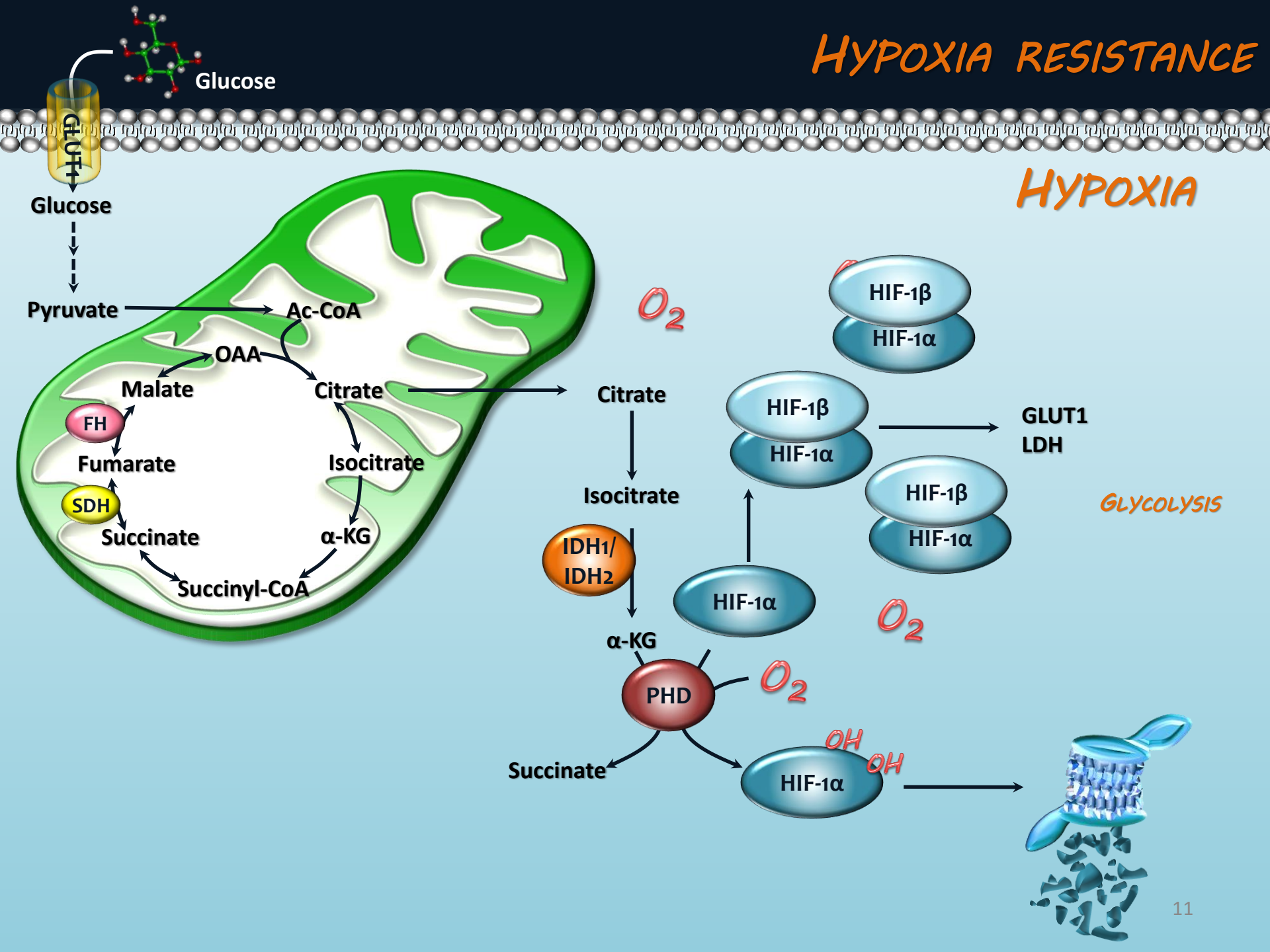
Hypoxia resistance



Hypoxia resistance



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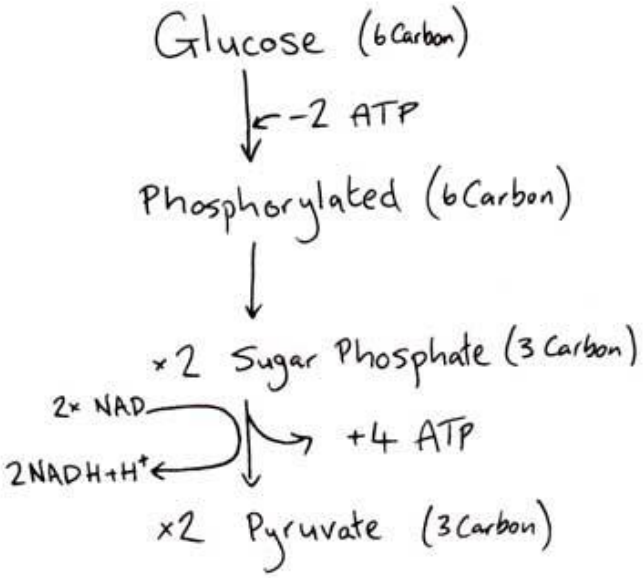

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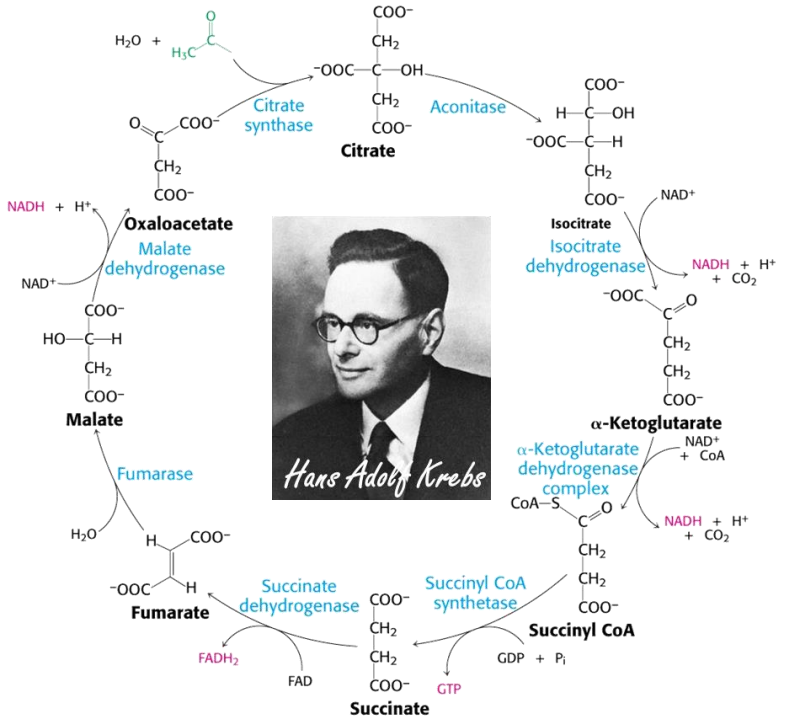

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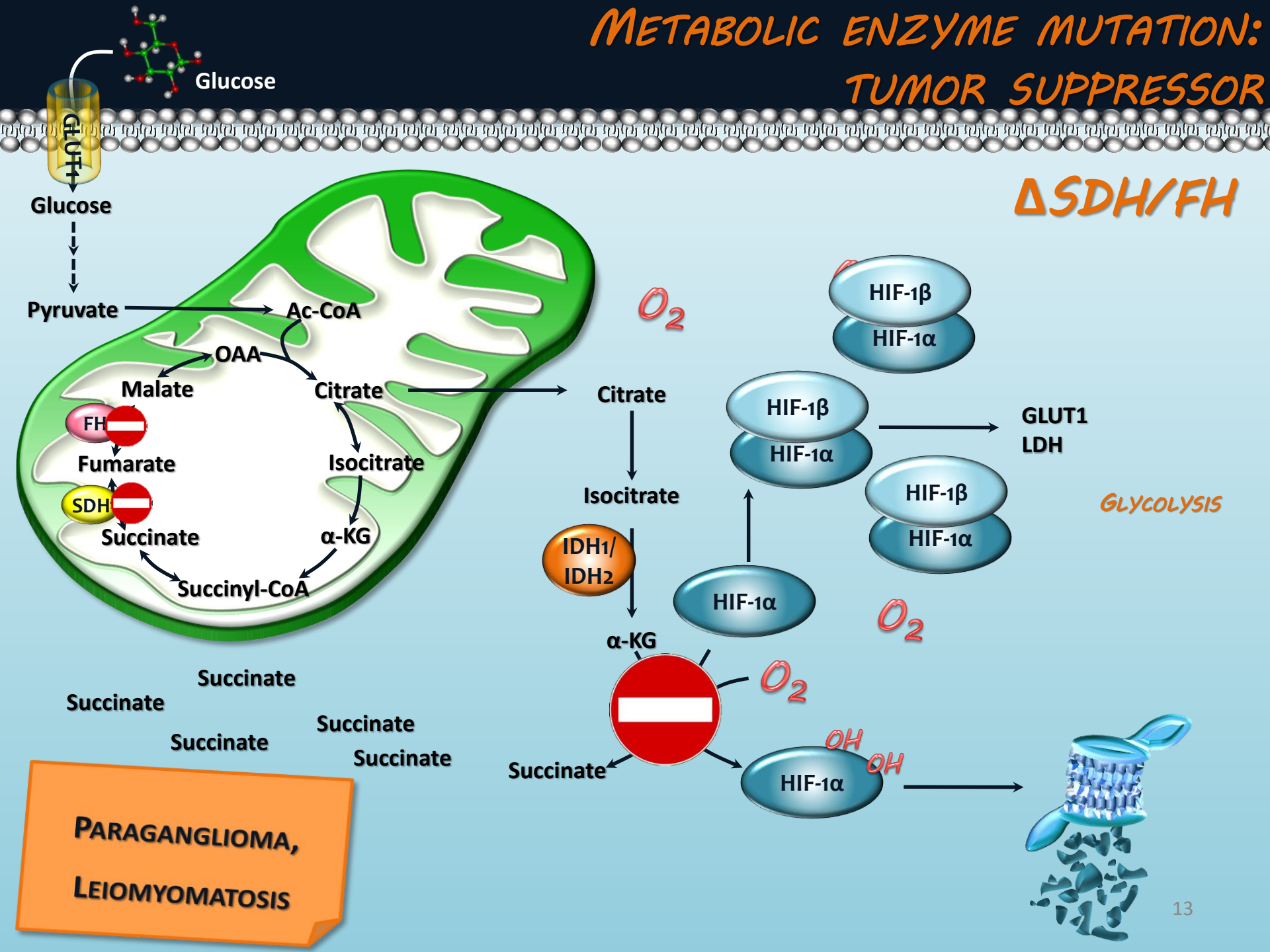
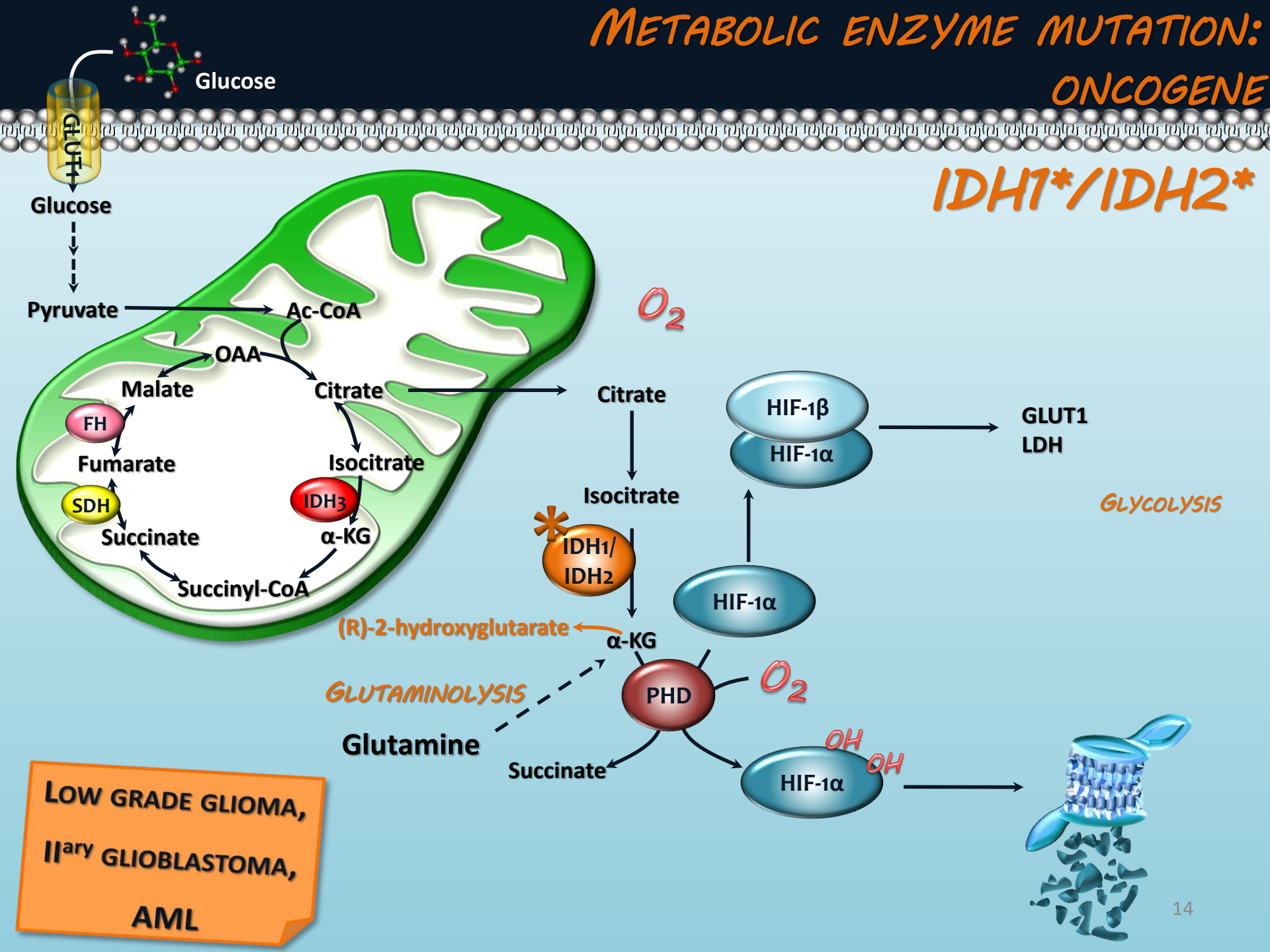
Metabolic enzyme mutation: tumor suppressor



Metabolic enzyme mutation: oncogene

IDH1*/IDH2*



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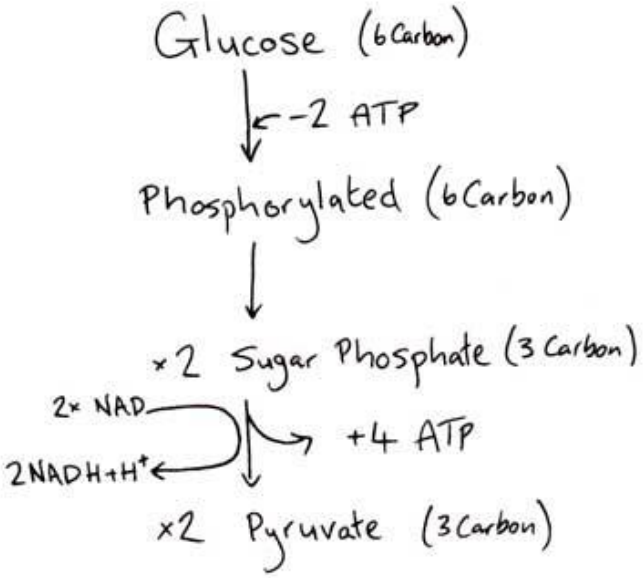

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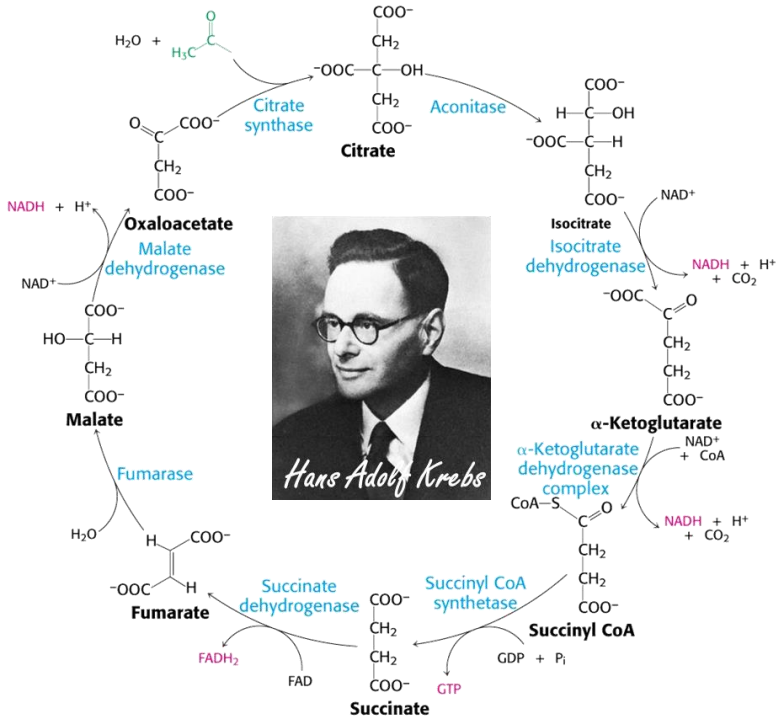

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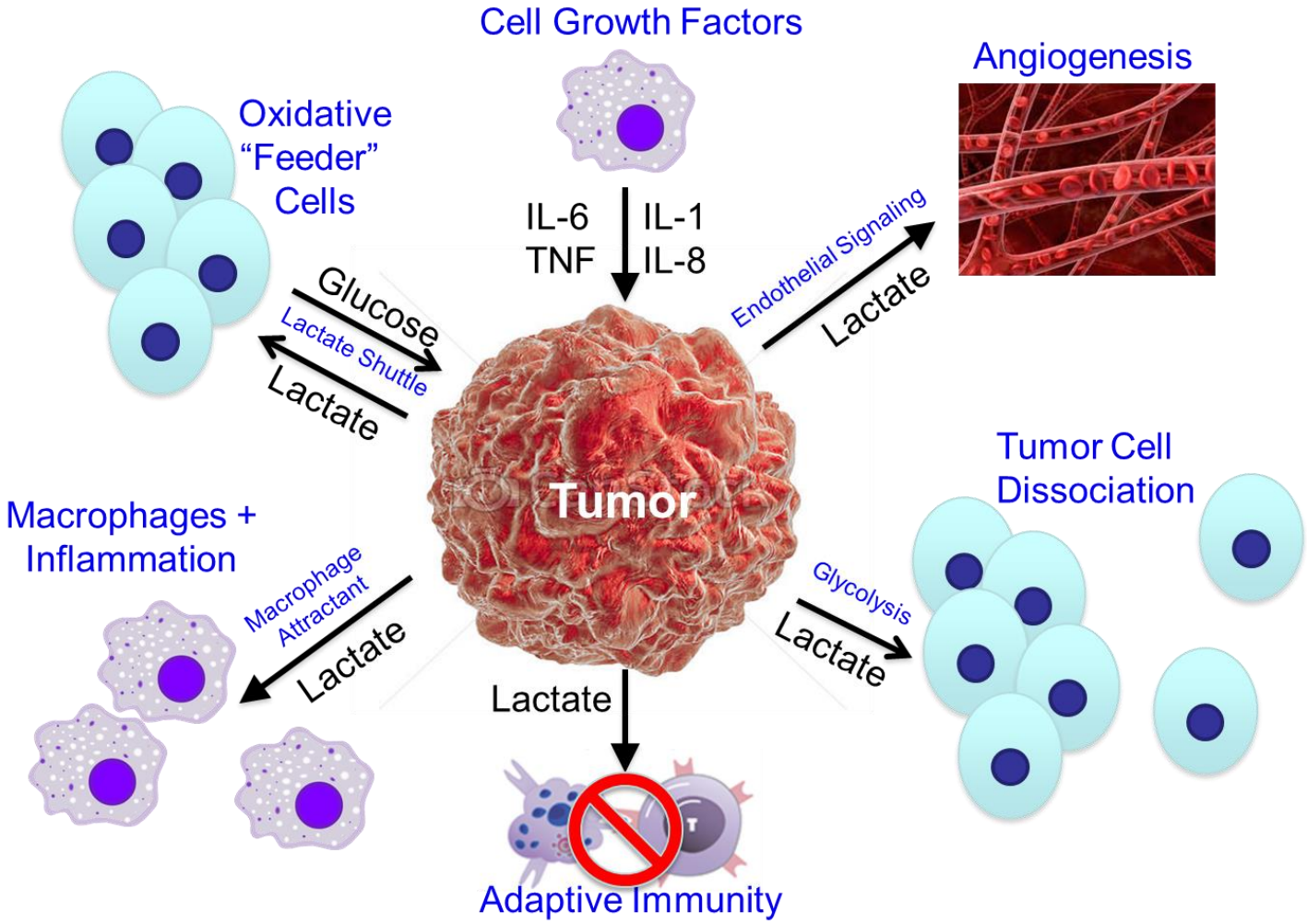
Extracellular acidification



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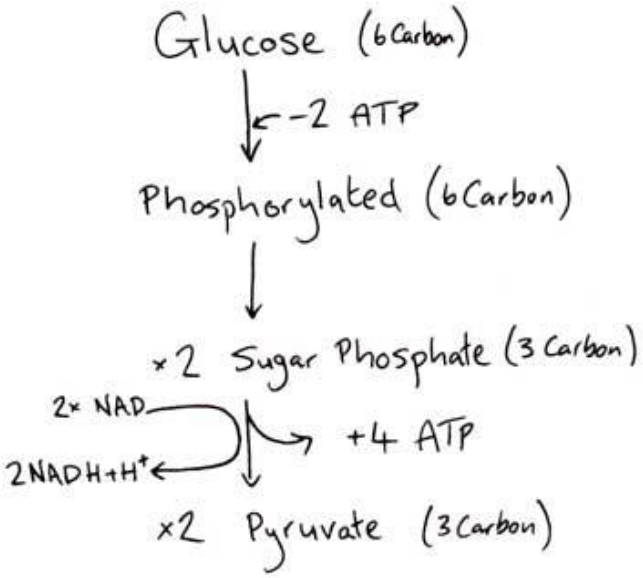

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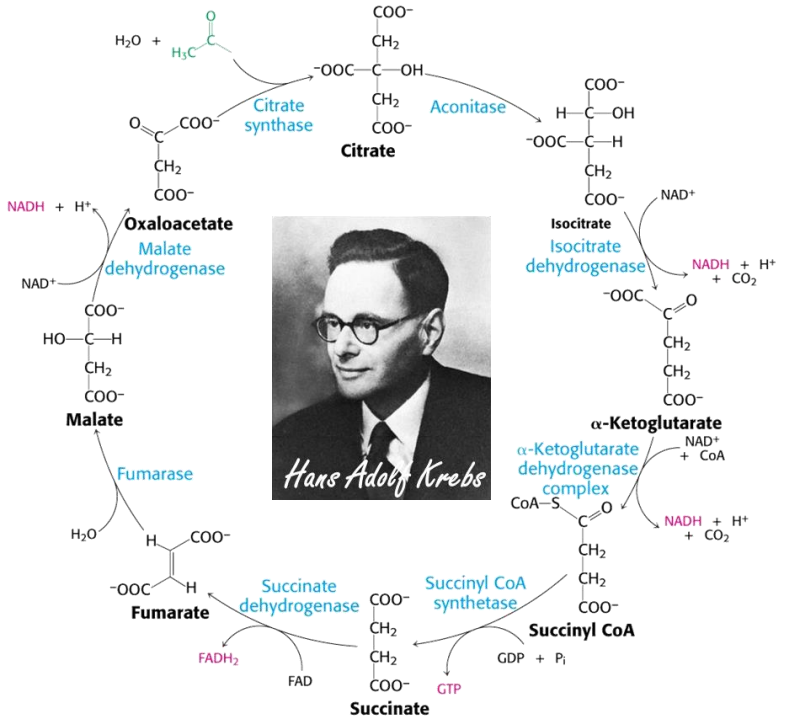

36 mol ATP/ mol glucose

- **Increased resistance to hypoxia**
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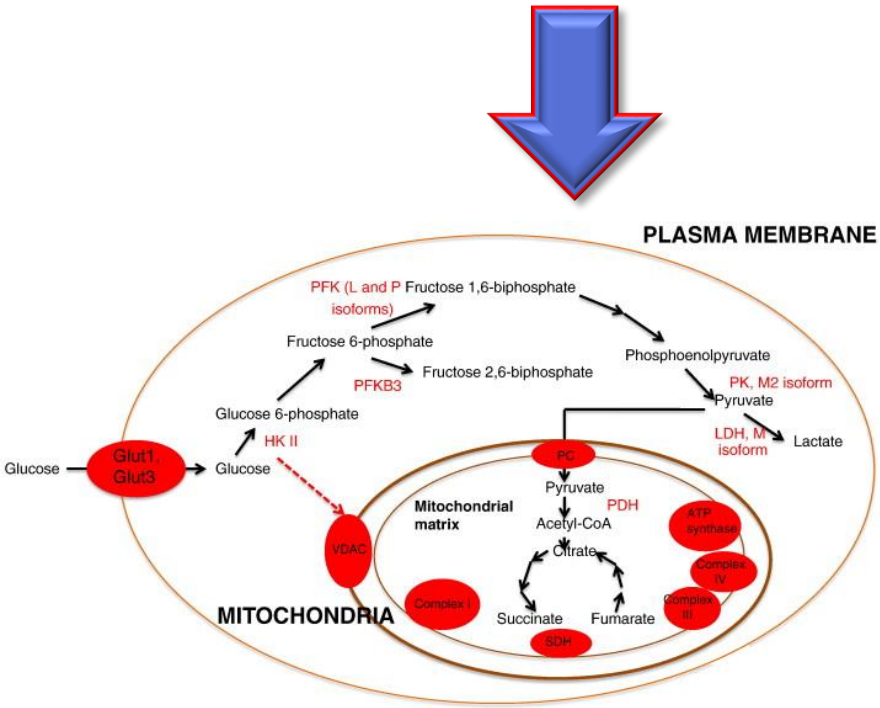
Crabtree effect

Excess of cytosolic ATP production through glycolysis inhibits mitochondrial ATP synthase, induces a chemiosmotic backpressure and hyperpolarizes the mitochondrial membrane.



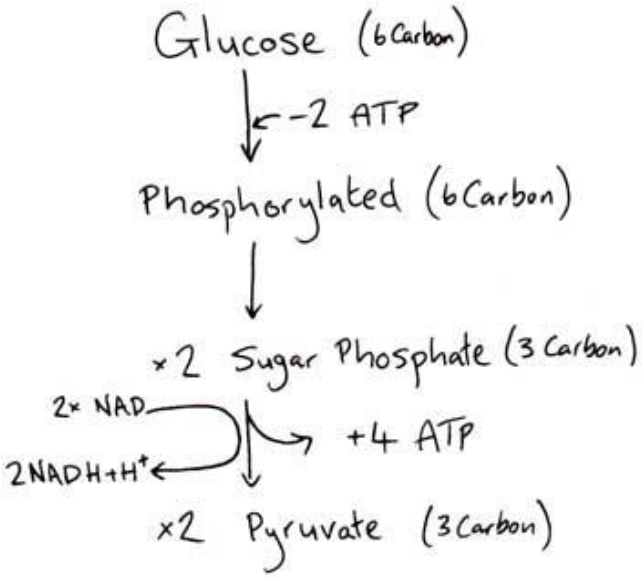
Decreasing in O_2 consumption after glucose supplementation

Cytosolic ATP production

MITOCHONDRIAL MEMBRANE HYPERPOLARIZATION (this fixes mitochondria in an **ANTI-APOPTOTIC STATE**)

Decreased ROS production

Why glycolysis?



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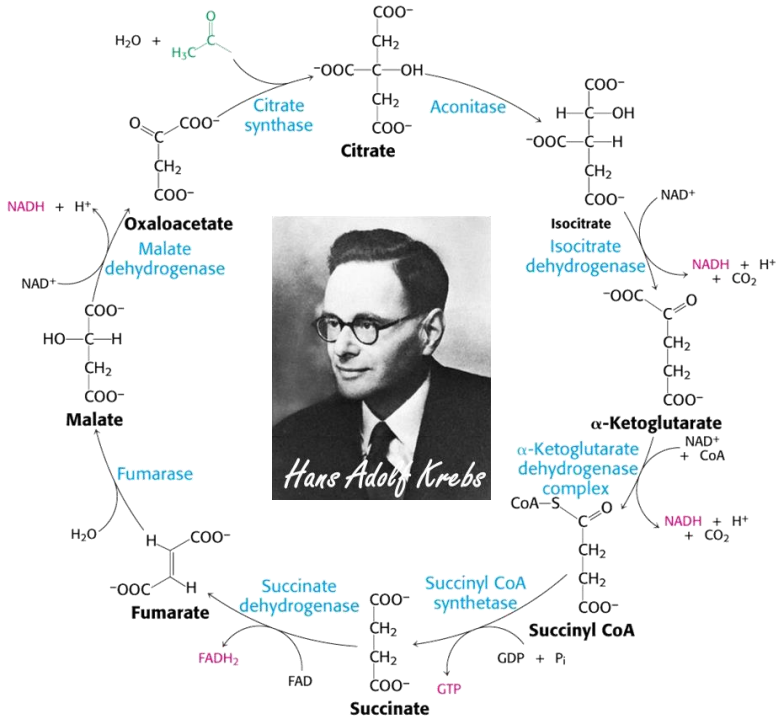

36 mol ATP/ mol glucose

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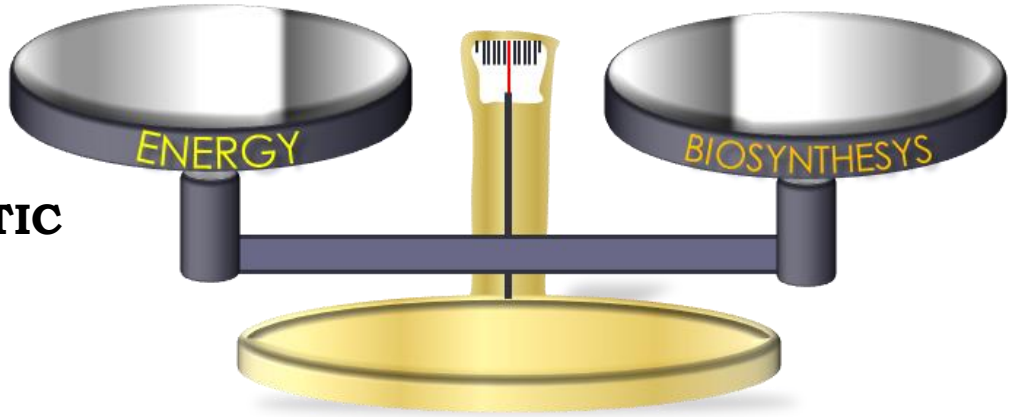
What is ANAPLEROSIS?

ANAPLEROSIS is the act of replenishing Krebs cycle intermediates that have been extracted for biosynthesis (**CATAPLEROTIC REACTIONS**)



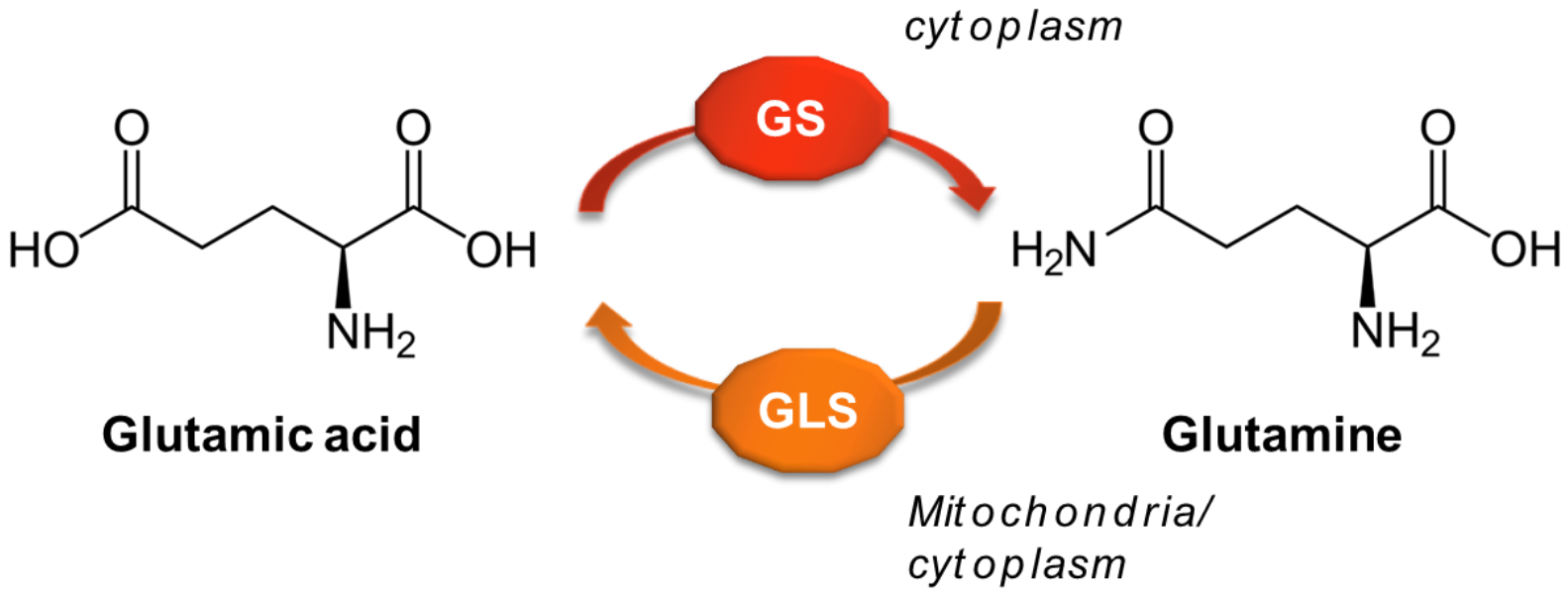
A solution for the antagonism between ENERGY PRODUCTION (bioenergetics) and CELL GROWTH (biosynthesis)

Glucose can be replaced by glutamine as an anaplerotic substrate

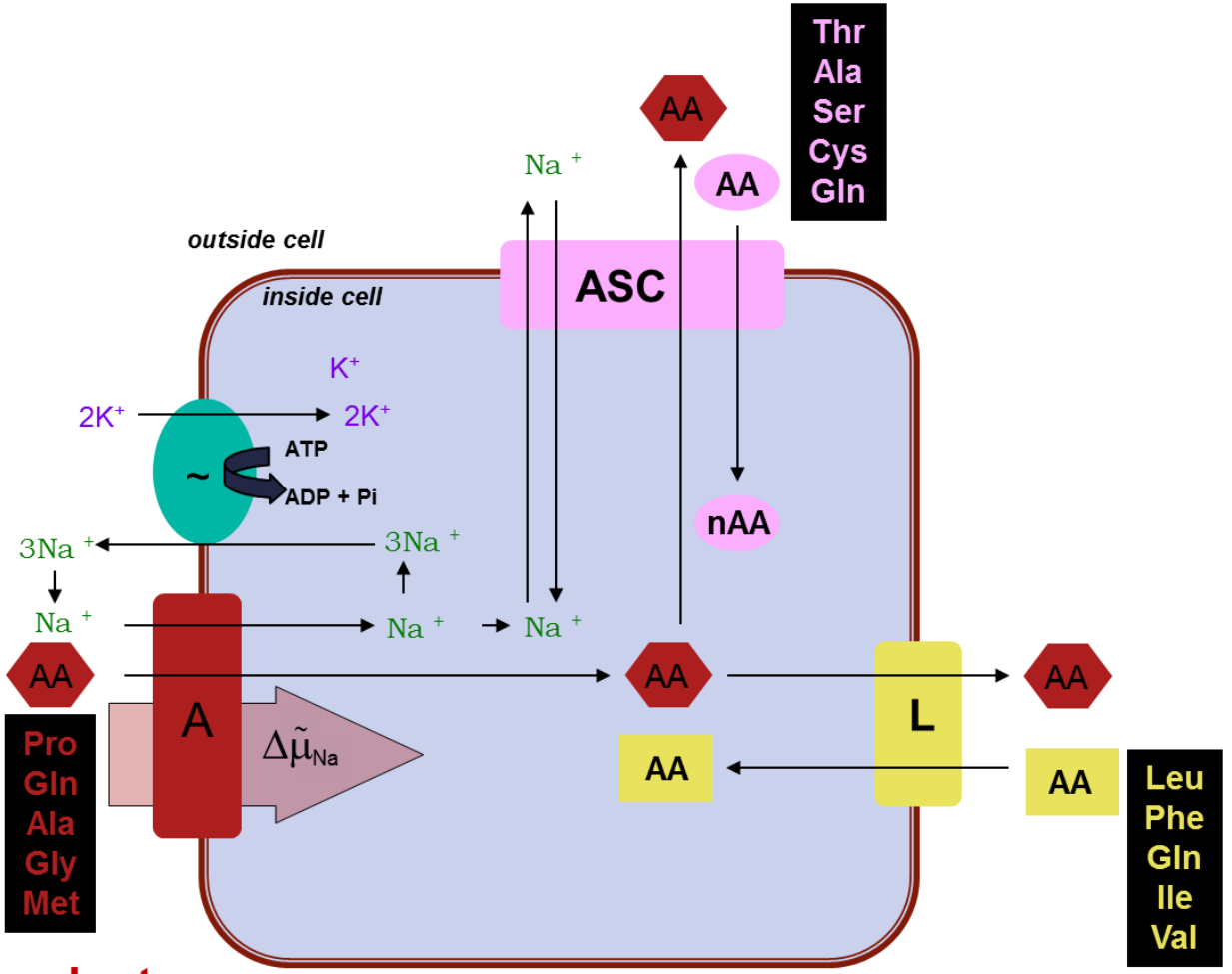
- Some tumors use glutamine when they are glucose-starved
- Some tumors become Gln-dependent because of mutations of genes for enzymes involved in glucose metabolism
- Some tumors preferentially use glutamine even in the presence of high glucose (glutamine-addicted cancers)

Glutamine, a non-essential amino acid needed for cell growth

- The most abundant amino acid in plasma (0.4-0.6 mM)
- The major nitrogen carrier among tissues
- The most abundant organic osmolyte in many tissues



Transport systems



- Na+ dependent
- Concentrative

Transport systems & Cancer

ELSEVIER

Seminars in Cancer Biology 15 (2005) 254–266

www.elsevier.com/locate/semcancer

Review

Amino acid transporters ASCT2 and LAT1 in cancer: Partners in crime?

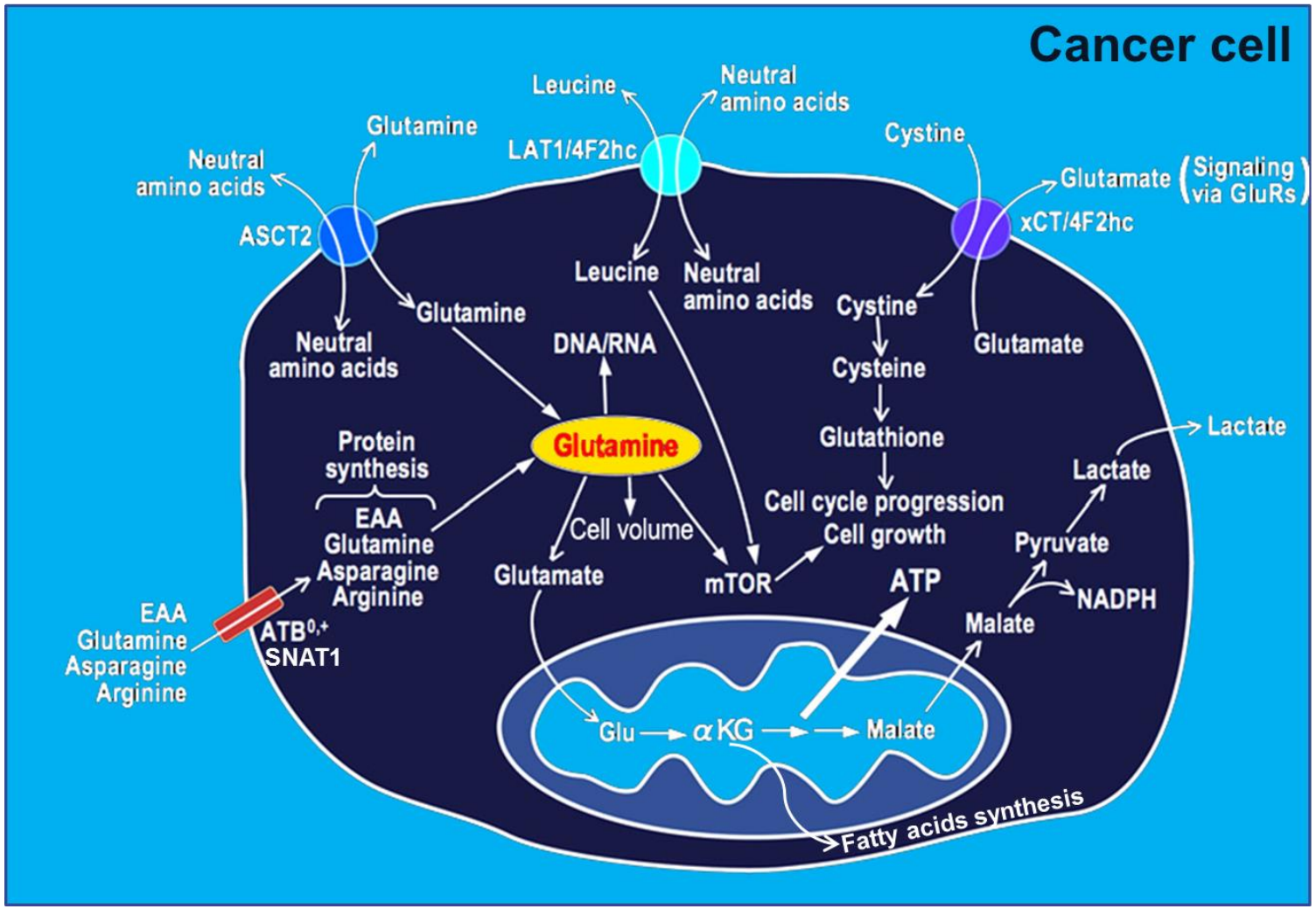
Bryan C. Fuchs, Barrie P. Bode*

Department of Biology, Saint Louis University, MW128, 3507 Laclede Avenue, St. Louis, MO 63103-2010, USA

Relative expression levels of amino acid transporter mRNA (ESTs) in normal and cancerous human tissues

	Normal ESTs (2,267,112)	Cancer ESTs (2,083,497)	Cancer:normal ratio	*P*-value
System A				
SLC38A1 (SNAT1)	433	304	0.70	<0.01
SLC38A2 (SNAT2)	398	349	0.88	0.26
SLC38A3 (SNAT4)	53	13	0.25	<0.01
System ASC				
SLC1A4 (ASCT1)	140	163	1.16	0.02
SLC1A5 (ASCT2)	175	542	**3.10**	<0.01
System L				
SLC7A5 (LAT1)	220	633	**2.88**	<0.01
SLC7A8 (LAT2)	180	95	0.53	<0.01
SLC43A1 (LAT3)	52	71	1.37	0.02
SLC2A3 (4F2hc)	535	656	**1.23**	<0.01

Why Glutamine?

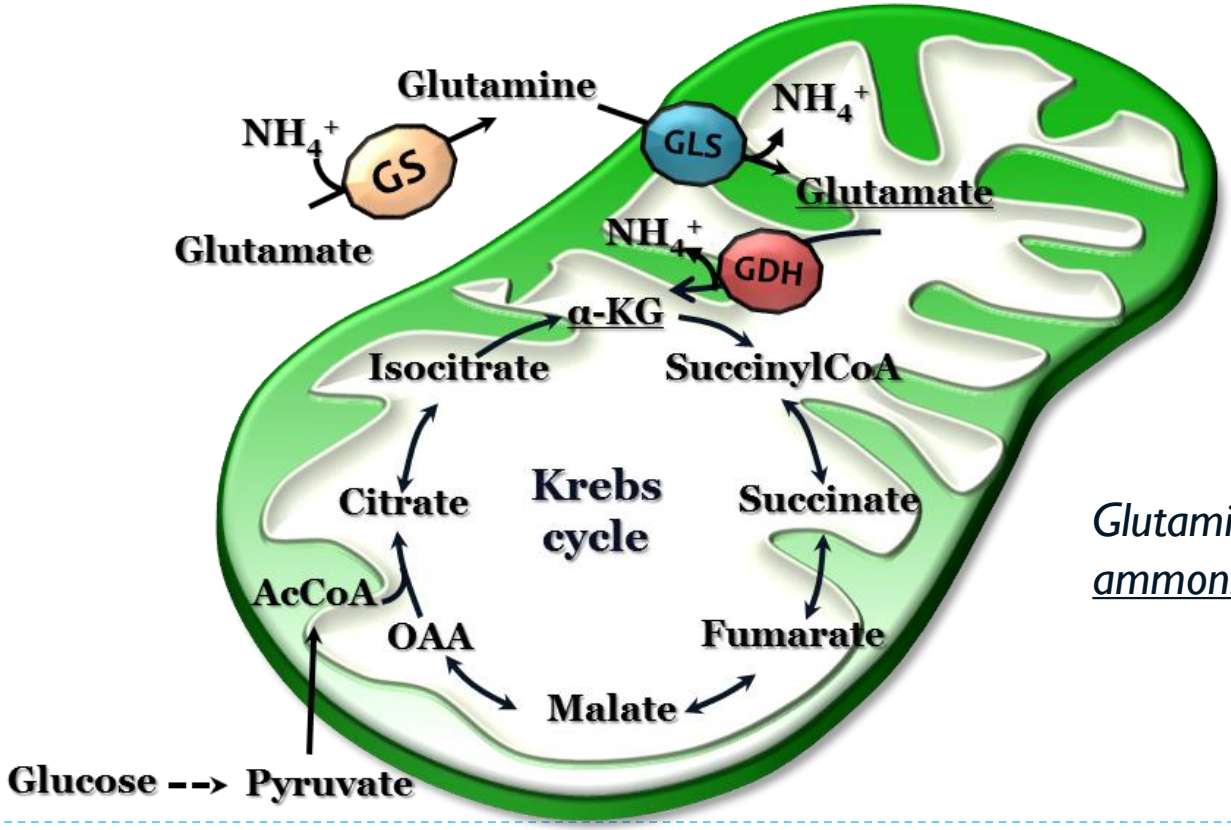


Adapted from Ganapathy et al. Pharmacology & Therapeutics 121 (2009) 29–40

Glutaminolysis → from glutamine to α-ketoglutarate

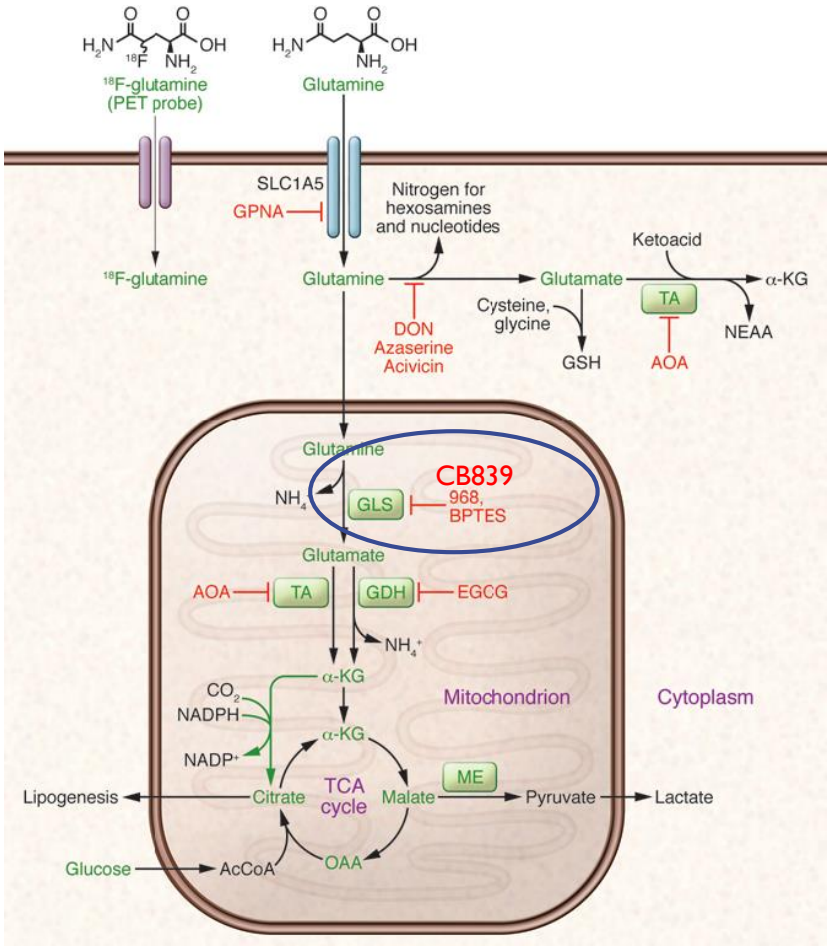
- Enzymes involved:

1. Glutaminases (GLS, GLS2) Gln → Glu
2. Glutamate Dehydrogenase (GDH) or transaminases (AAT,AST…) Glu → α-KG



Glutaminolysis produces ammonia

How to take ADVANTAGE from a BAD phenotype



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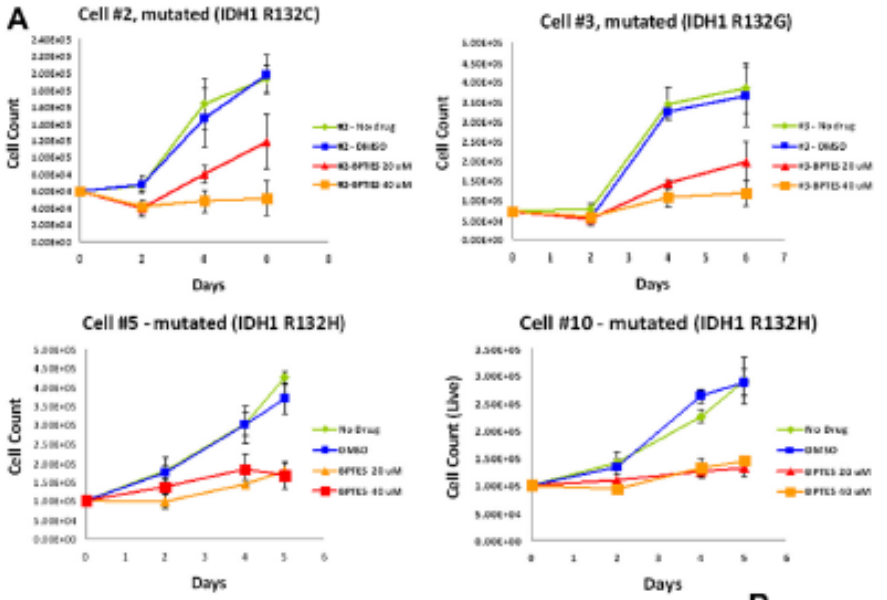
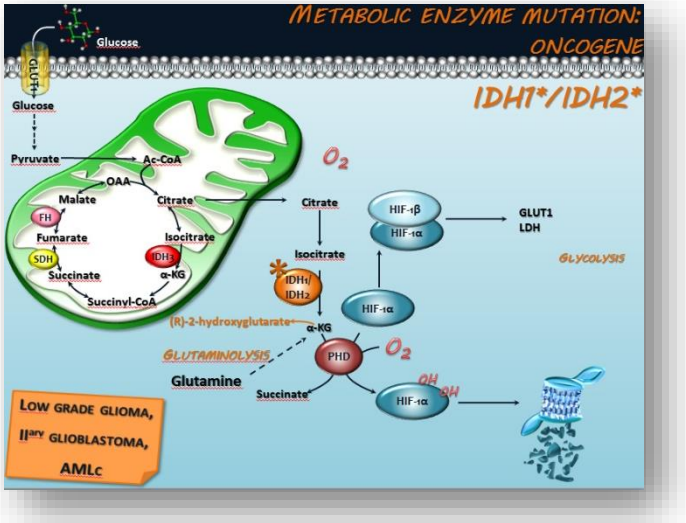
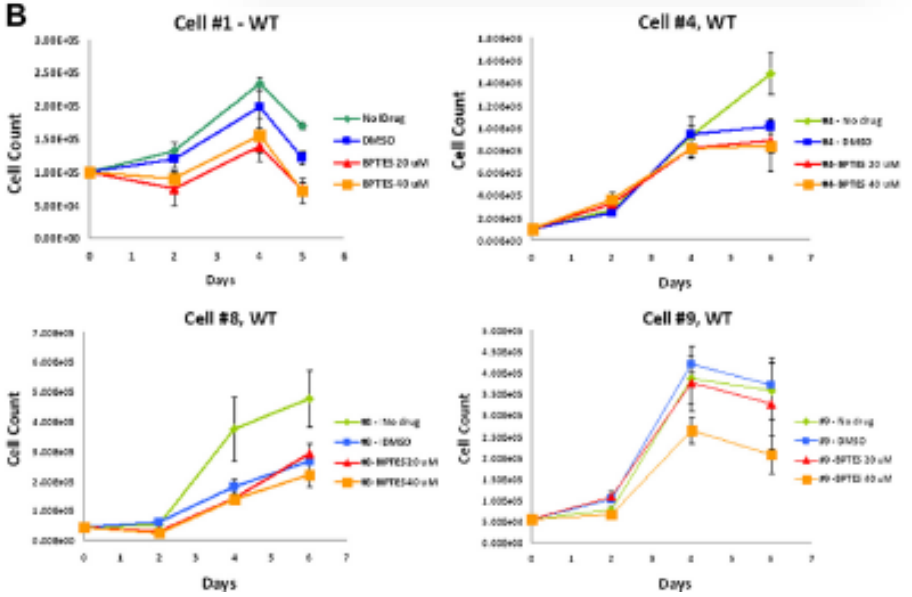
Inhibition of glutaminase selectively suppresses the growth of primary acute myeloid leukemia cells with *IDH* mutations

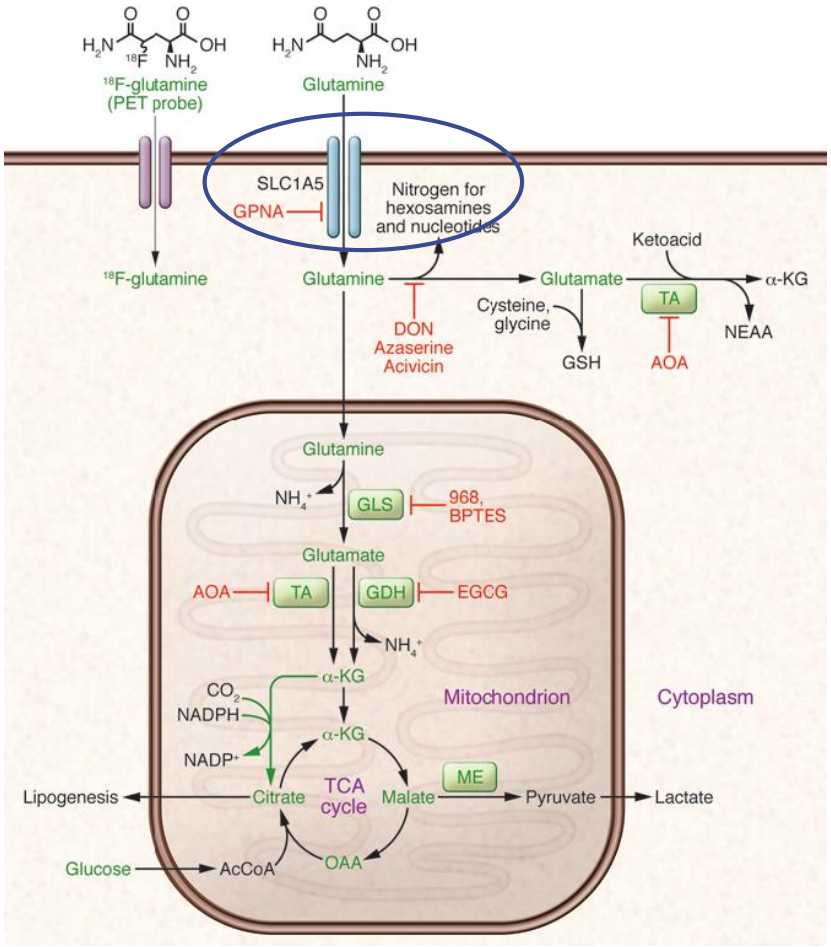






Emadi et al. Exp Hematology 2014

How to take ADVANTAGE from a BAD phenotype



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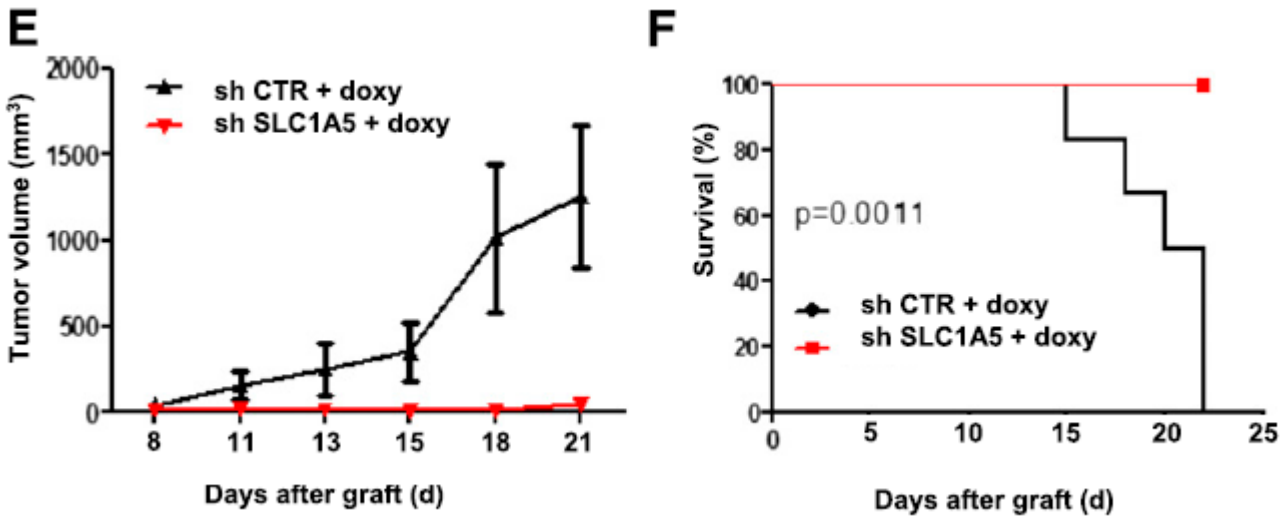
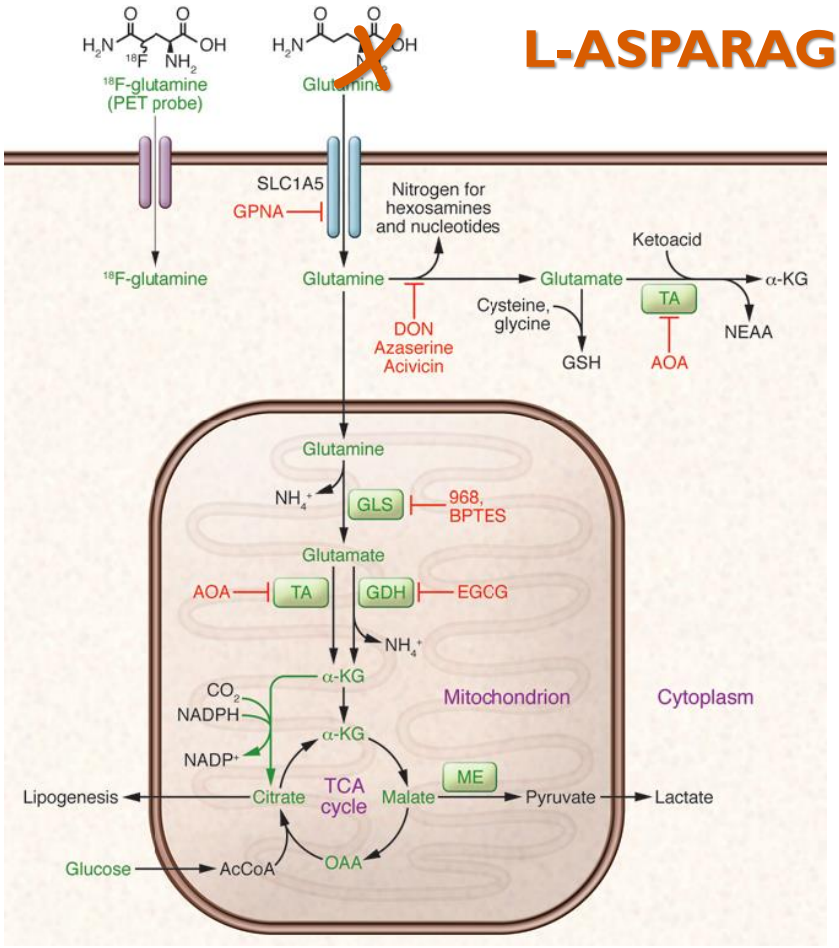
Inhibiting glutamine uptake represents an attractive new strategy for treating acute myeloid leukemia



Inhibiting Glutamine uptake by knockdown of ASCT2 transporters induces apoptosis and inhibits tumor formation in AML xenograft model

Williems et al. Blood 2013 122:3521-3532

How to take ADVANTAGE from a BAD phenotype



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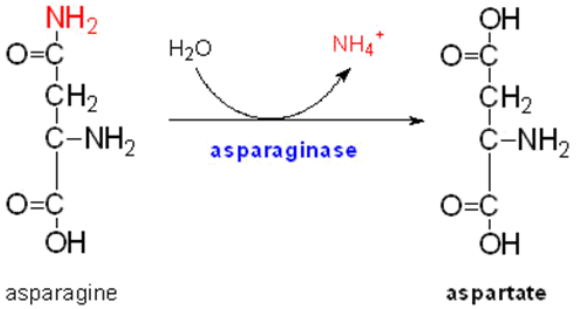
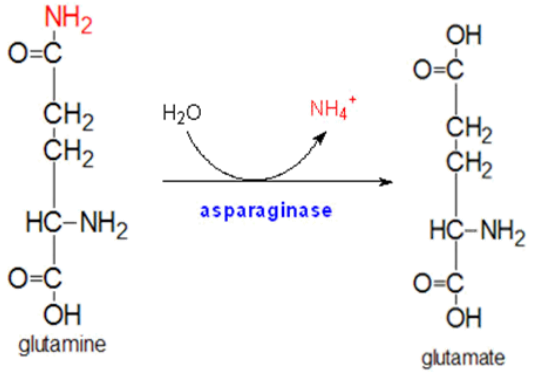
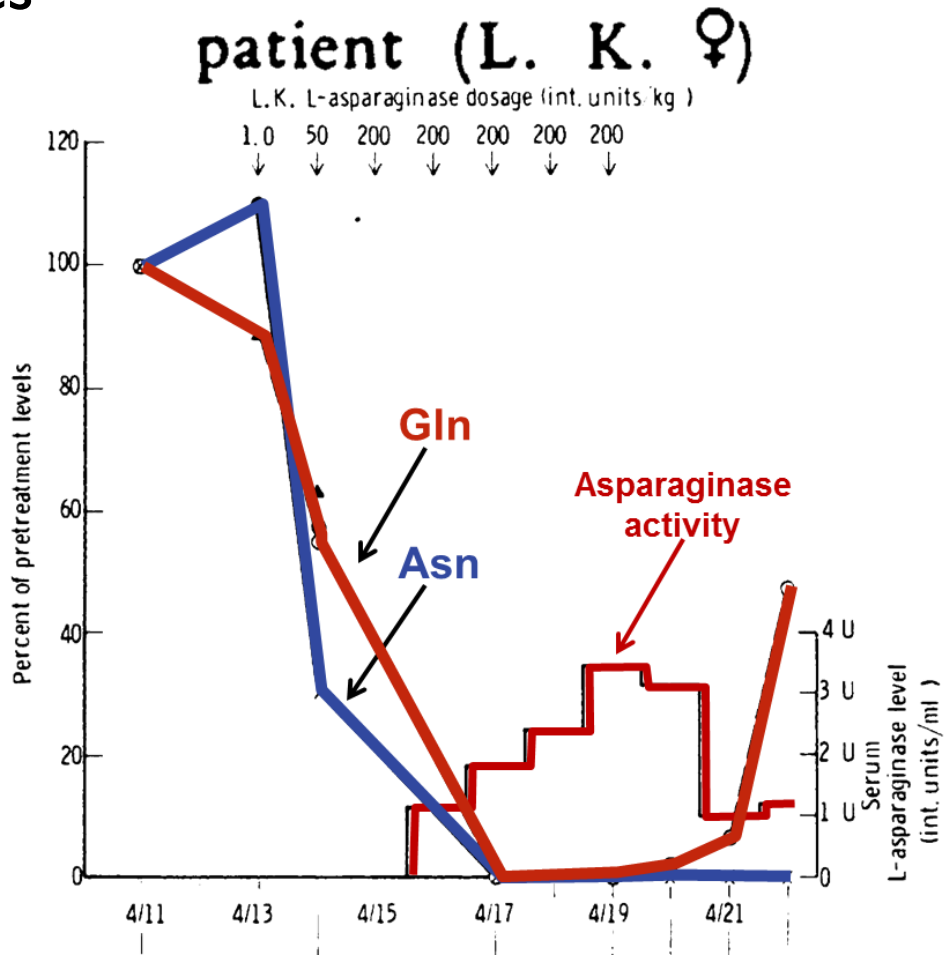
L-ASPARAGINASE: depletes blood of Asn/Gln

- Derived from two sources
  - Escherichia coli
  - Erwinia chrysanthemi







L-ASPARAGINASE: The First «metabolic» drug

Evidence that the L-Asparaginase Activity of Guinea Pig Serum is responsible for its Antilymphoma Effects

J Exp Med. 1963; 118(1) 99–120.

Experimental groups	Mouse No.	Result of implantation * Days following implantation: 11	12	13	14	15	16	17	18	19	
1. Untreated control mice.	1	N	•	•	•	•	•	•	•	•	† D 27
	2	•	•	•	•	•	•	•	•	•	† D 23
	3	•	•	•	•	•	•	•	•	•	† D 24
		0 ⌐¬ 2 cm									
2. Mice given 1.0 ml guinea pig serum, left at 23°C for 30 min. Assay of L-asparaginase activity: 100% standard (55.7 units/ml).	4	N	N	N	N	N	N	N	N	N	† D 26
		N	N	N	N	•	•	•	•	•	
	5	N	N	N	N	N	N	N	N	N	† D 34
		N	N	N	N	N	•	•	•	•	
	6	N	N	N	N	N	N	N	N	N	No tumor D 60
		N	N	N	N	N	N	N	N	N	

GLN GLN GLN

Blood vessel

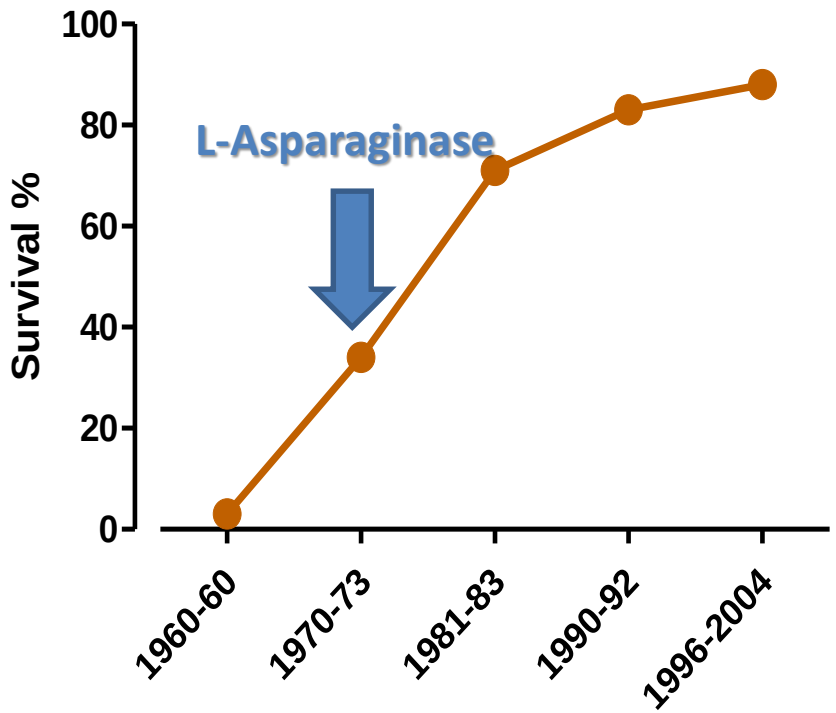


Figure 1. Improvement in survival for children with acute lymphoblastic leukemia (ALL).[1] Five-year survival rates for children less than 15 years old with ALL: 1960–2004. SEER Cancer Statistics Review 1975-2005.

Endothelial cells

GLS Glutaminase

Asparaginase

AS Asparagine synthetase

GS Glutamine synthetase

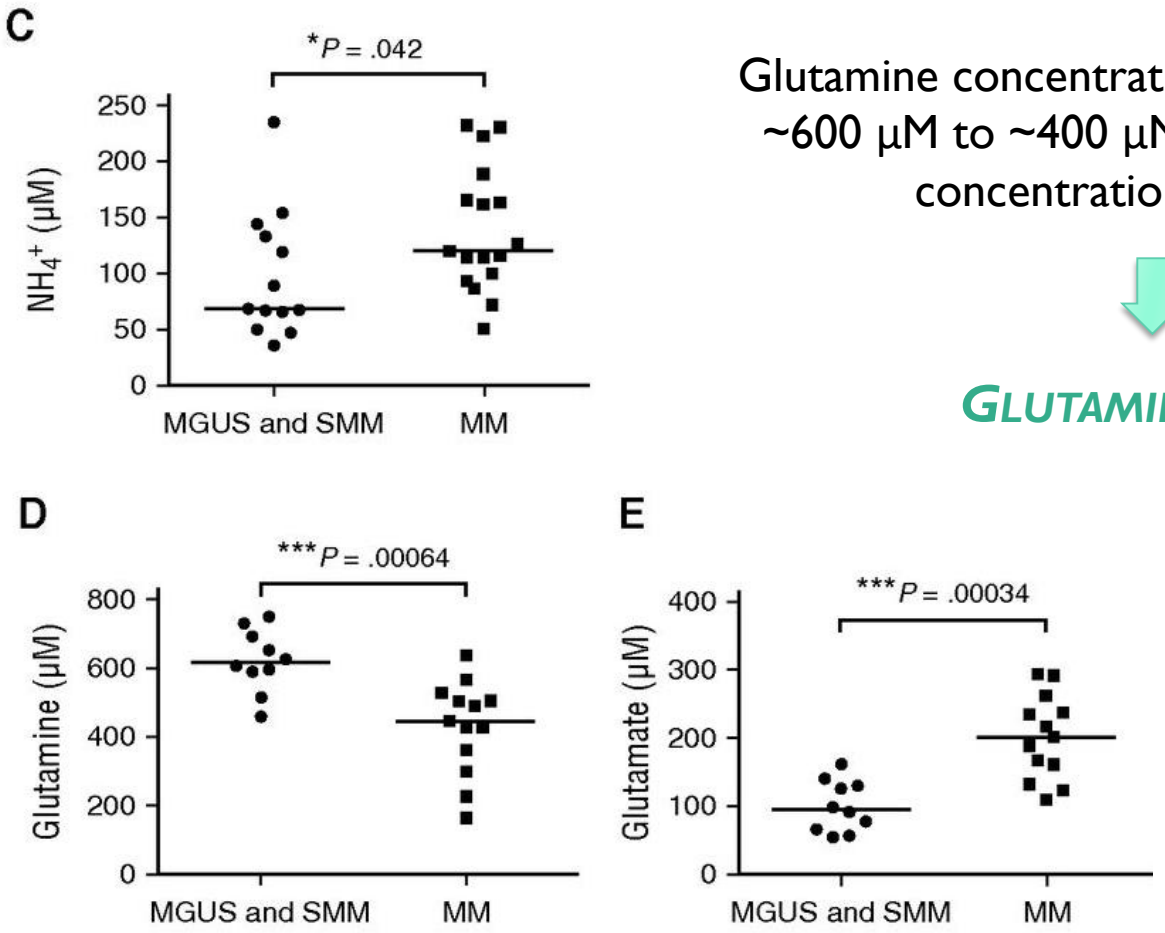
L-ASPARAGINASE & ALL

Multiple Myeloma

- Hematologic cancer characterized by clonal proliferation of malignant plasma cells in the bone marrow microenvironment
- Multiple myeloma is currently an incurable but treatable disease.
- In relapsed/refractory MM patients hyperammonemia is a rare clinical manifestation associated with high mortality rate
- Active multiple myeloma is characterized by osteolytic bone lesions

https://www.cancer.gov/types/myeloma/hp/myeloma-treatment-pdq

During MM progression bone marrow Gln decreases, while Glu and NH_4^+ increase

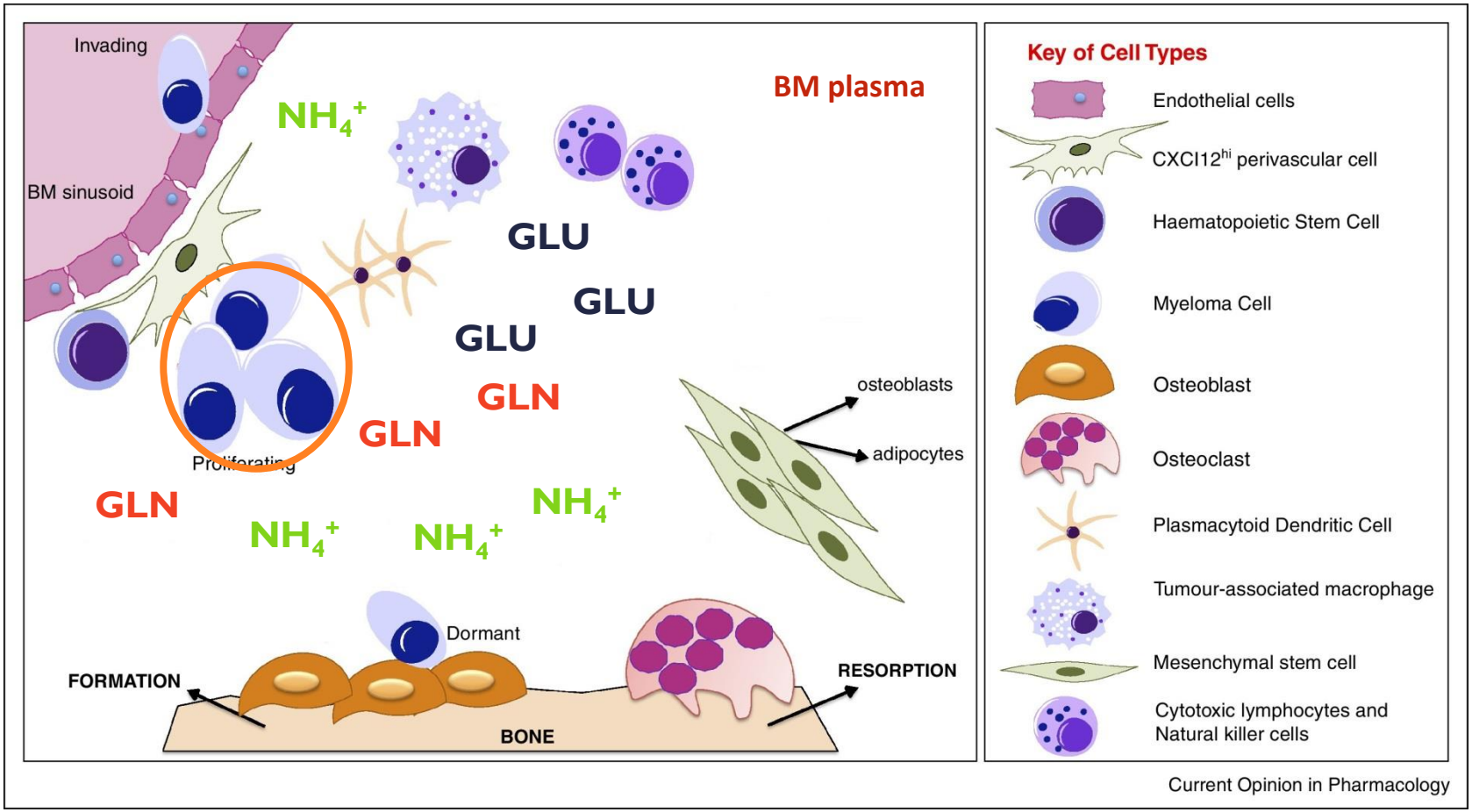


Glutamine concentration decreases from ~600 μM to ~400 μM, while glutamate concentration doubled

GLUTAMINOLYSIS?

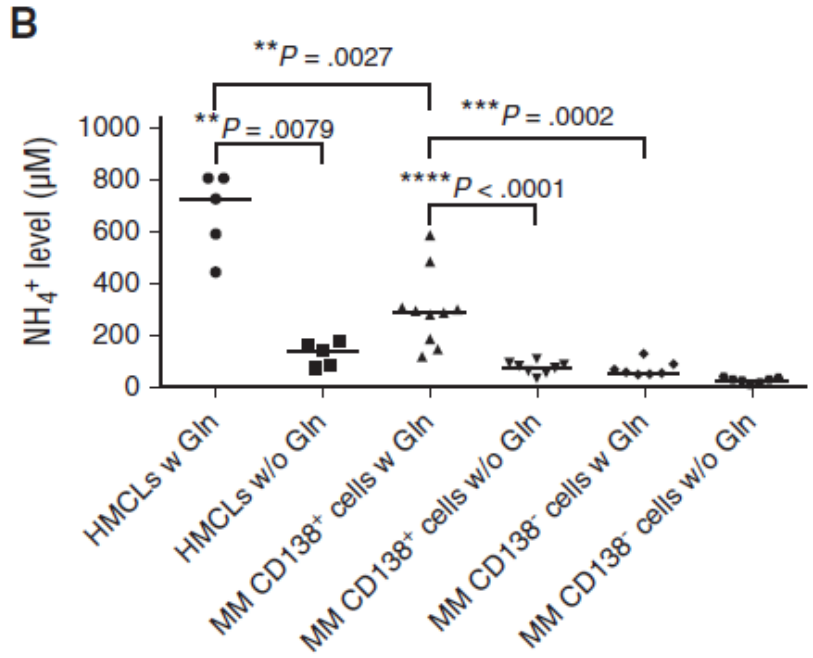
Bolzoni M, Chiu M, et al Blood 128:667-679 (2016)

Bone marrow MM niche

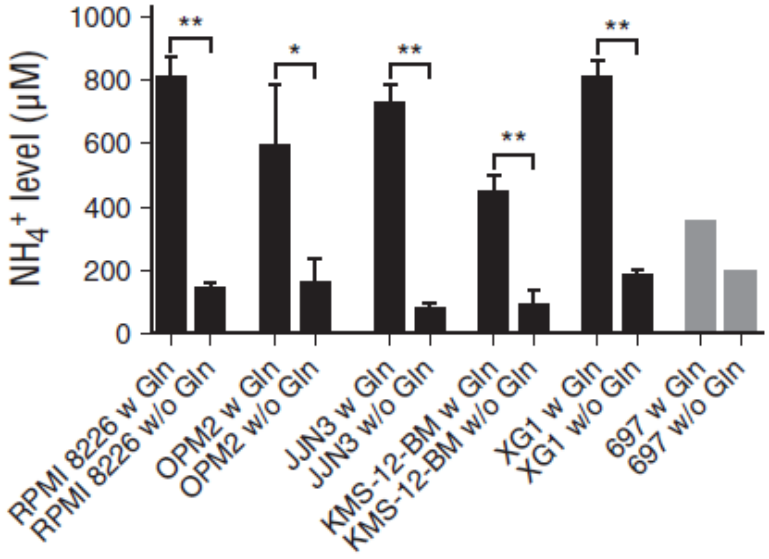


Adapted from Gooding S and Edwards CM. Current Opinion in Pharmacology (2016)

Gln is needed for NH_4^+ production by MM cells



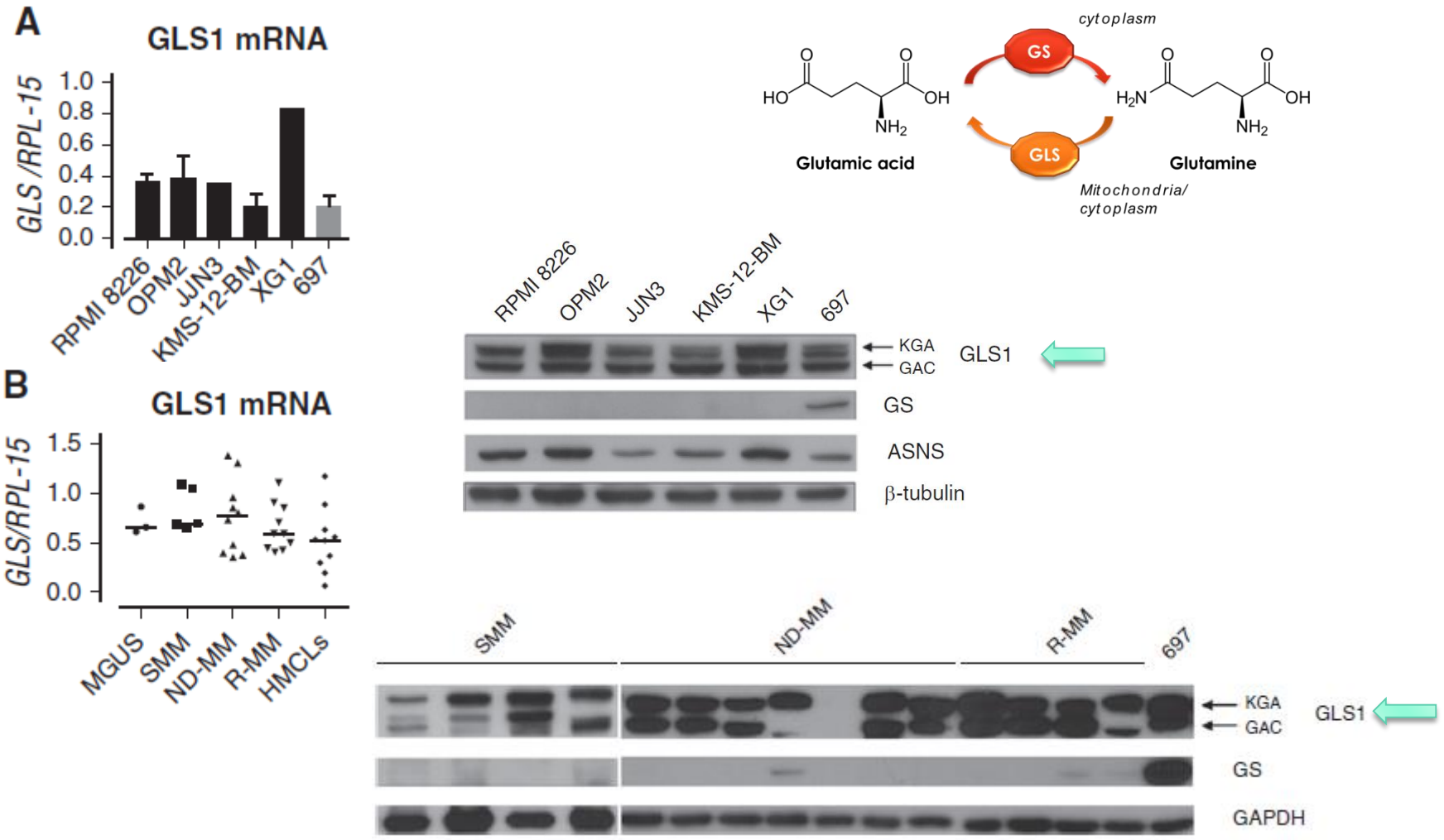
CD 138$^+$, purified from patients, produce NH_4^+ in the presence of Gln



MM cell lines produce high levels of NH_4^+ in the presence of Gln

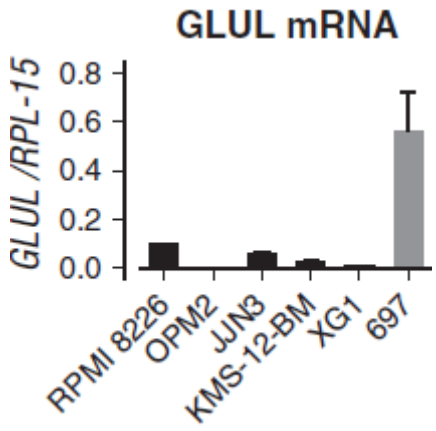
Bolzoni M, Chiu M, et al Blood 128:667-679 (2016)

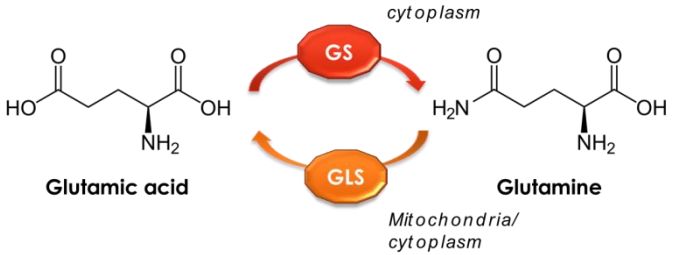
MM cells express Glutaminase

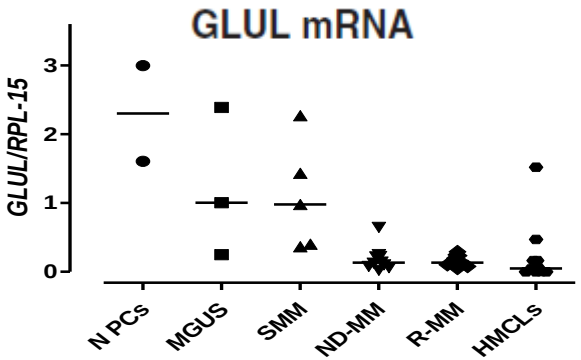


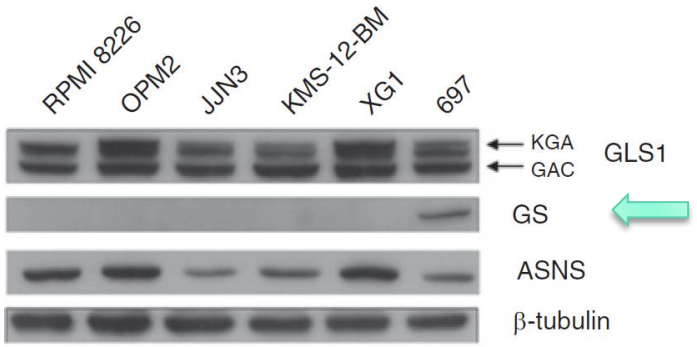
Bolzoni M, Chiu M, et al Blood 128:667-679 (2016)

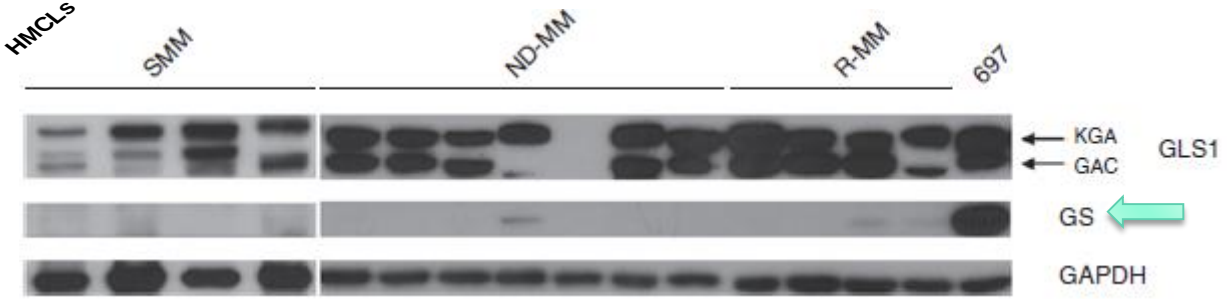
Gln Synthetase is downregulated during MM progression





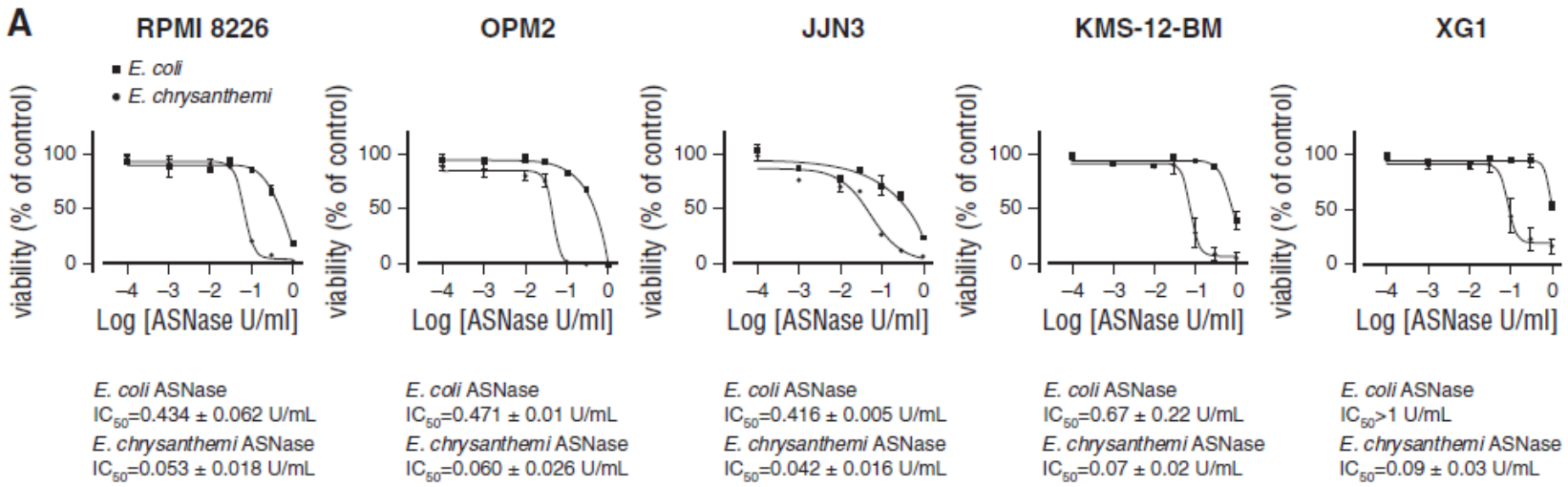






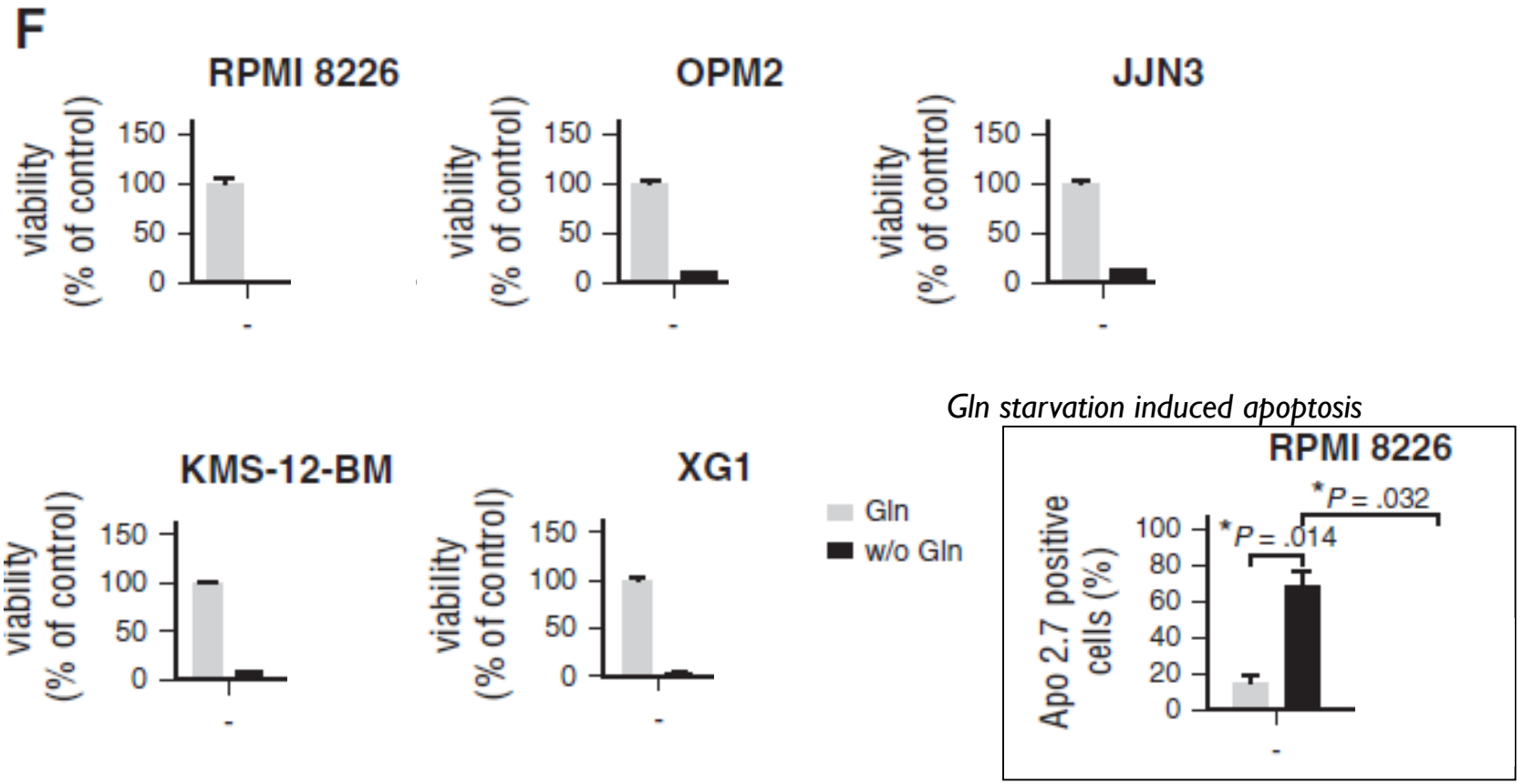
Bolzoni M, Chiu M, et al Blood 128:667-679 (2016)
Unpublished results

MM cells are extremely sensitive to ASNase



Bolzoni M, Chiu M, et al Blood 128:667-679 (2016)

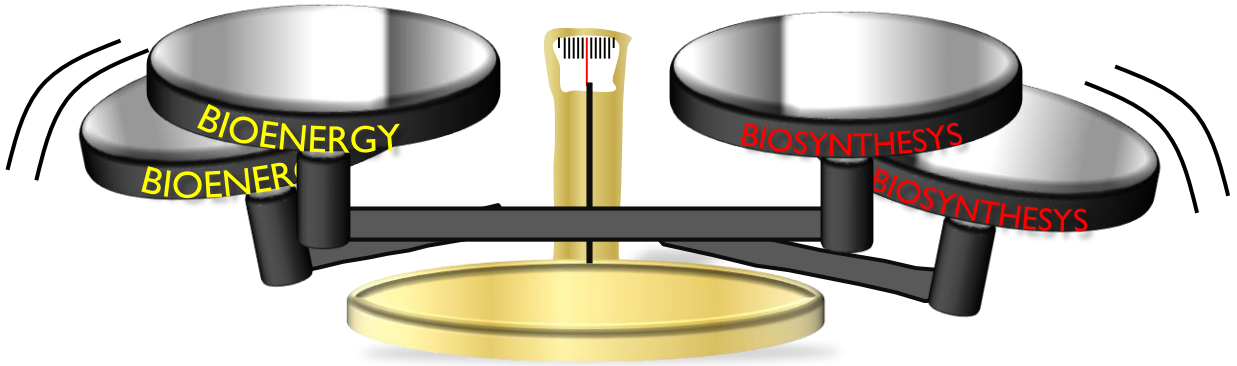
MM cells are extremely sensitive to Gln starvation



α-ketoglutarate counteracts Gln deprivation-induced apoptosis of MM cells

Bolzoni M, Chiu M, et al Blood 128:667-679 (2016)

Take Home Message:



DISRUPTING THE DELICATE BALANCE BETWEEN BIOSYNTHETIC AND BIOENERGETICS METABOLIC PATHWAYS MAY REPRESENT A POTENTIAL TOOL FOR CANCER CONTROL

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