# Disclosure Information

*Craig B. Thompson MD*

**I have the following financial relationships to disclose:**

**Employee of: Memorial Sloan-Kettering Cancer Center**

**Grant/Research support from: NIH/NCI, Damon Runyon, Leukemia and Lymphoma Society, Hope Funds**

**MAB member: Howard Hughes Medical Institute**

**Board member: Regeneron, Charles River Laboratories**

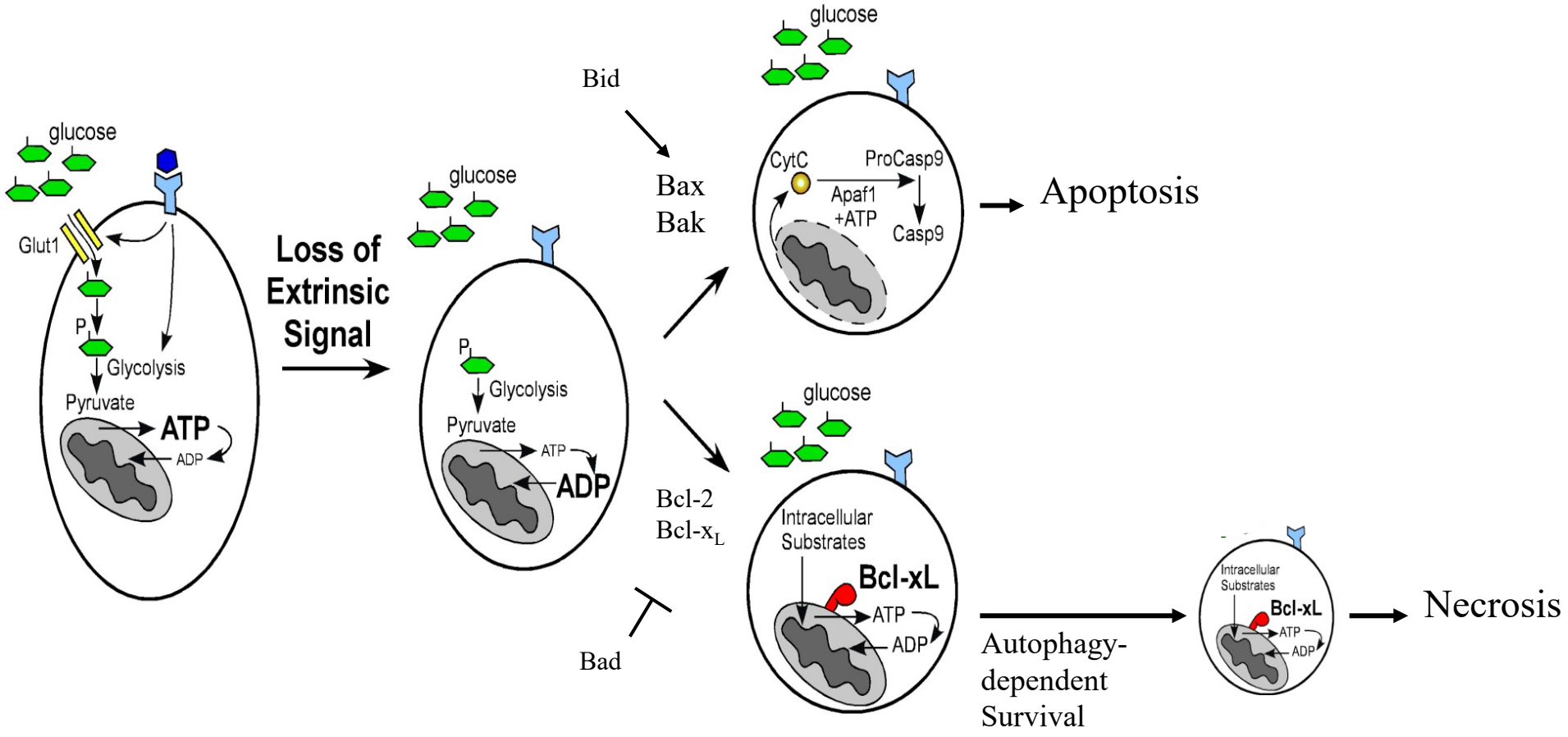
**Royalties received from: Elsevier Press, UMichigan**

**Founder: Agios**

***- and -***

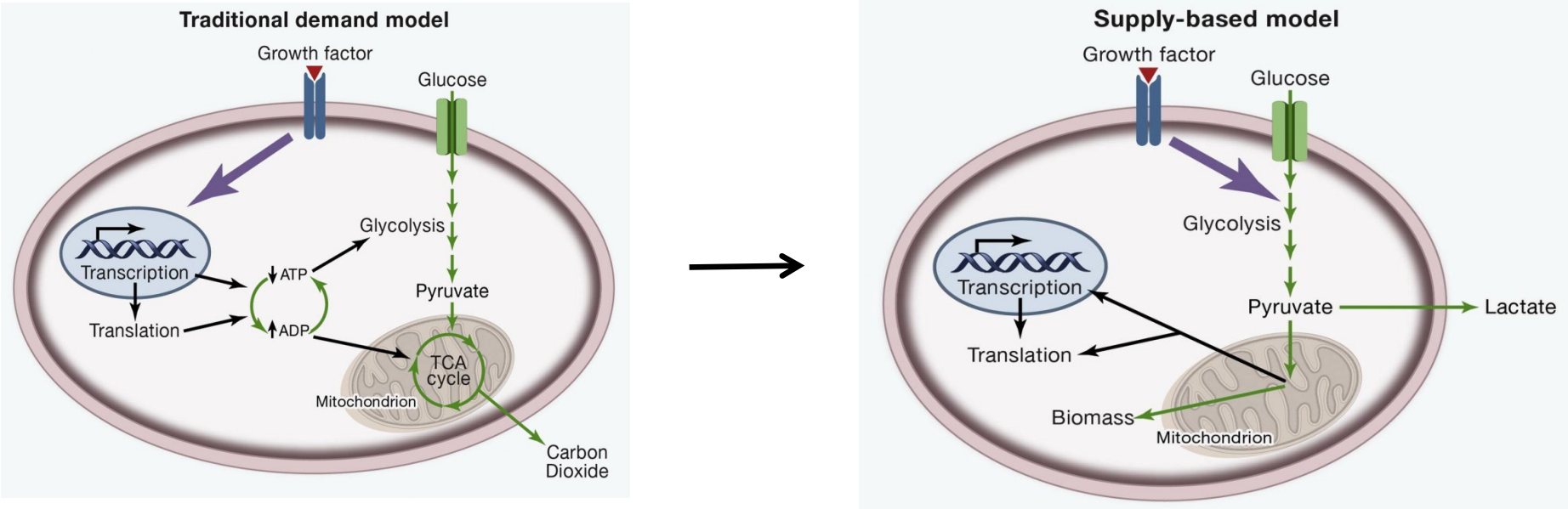
**I will discuss investigational use in my presentation: IDH inhibitors**

# Unlike single cell organisms, mammalian cells are unable take up nutrients in the absence of growth factor-initiated instruction.



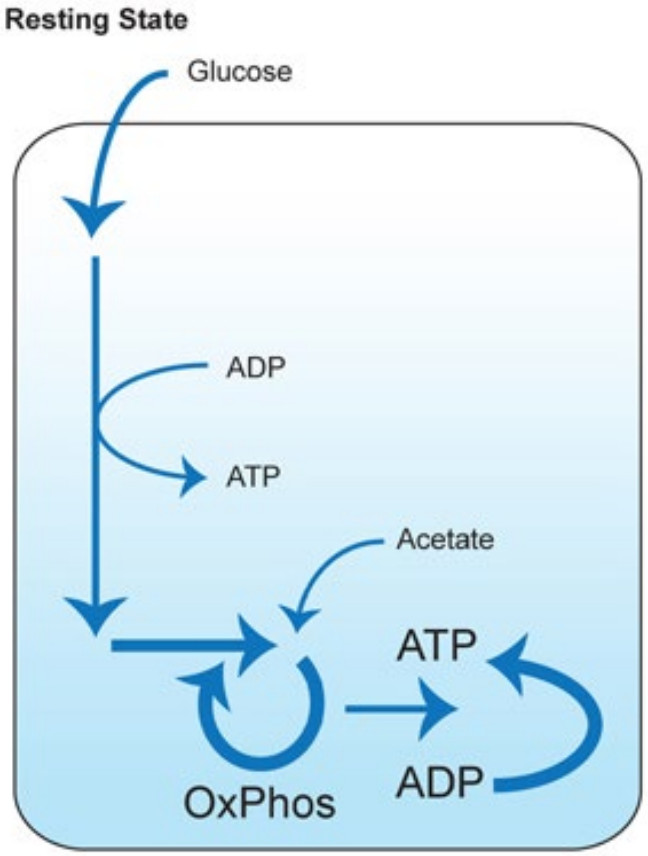
# Metazoan cells have lost the cell-autonomous ability to take up nutrients.

**To support growth, growth factor receptors evolved with the ability to direct nutrient uptake and utilization**

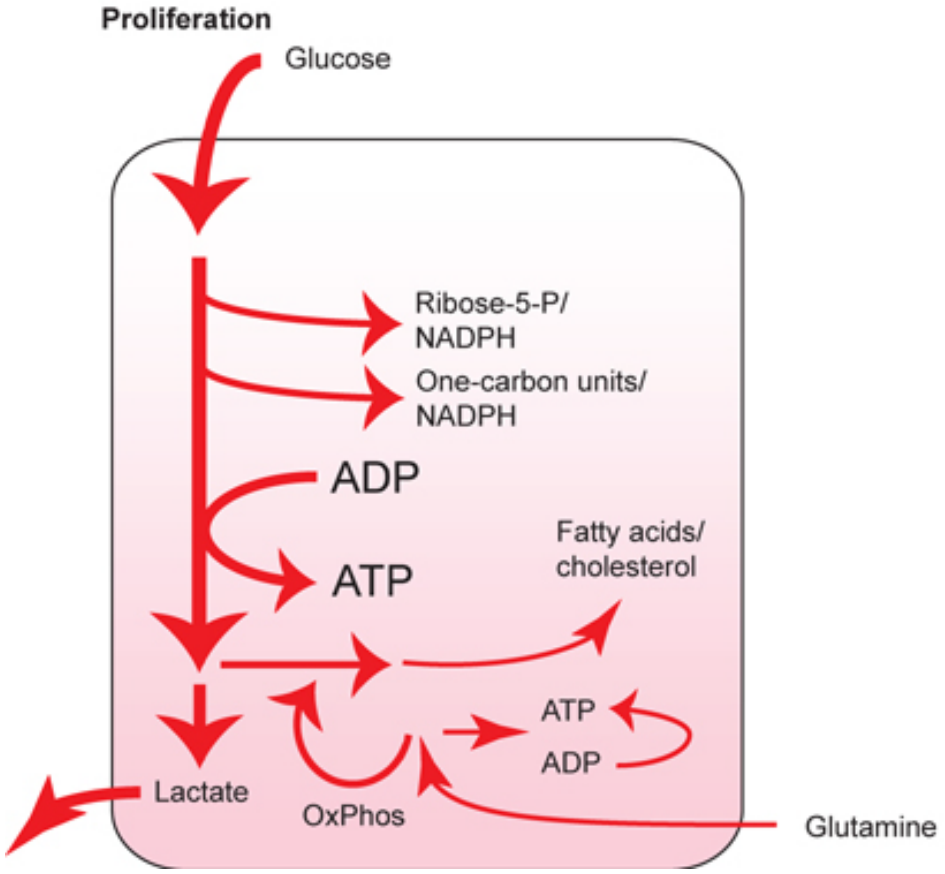


# Cellular Metabolism

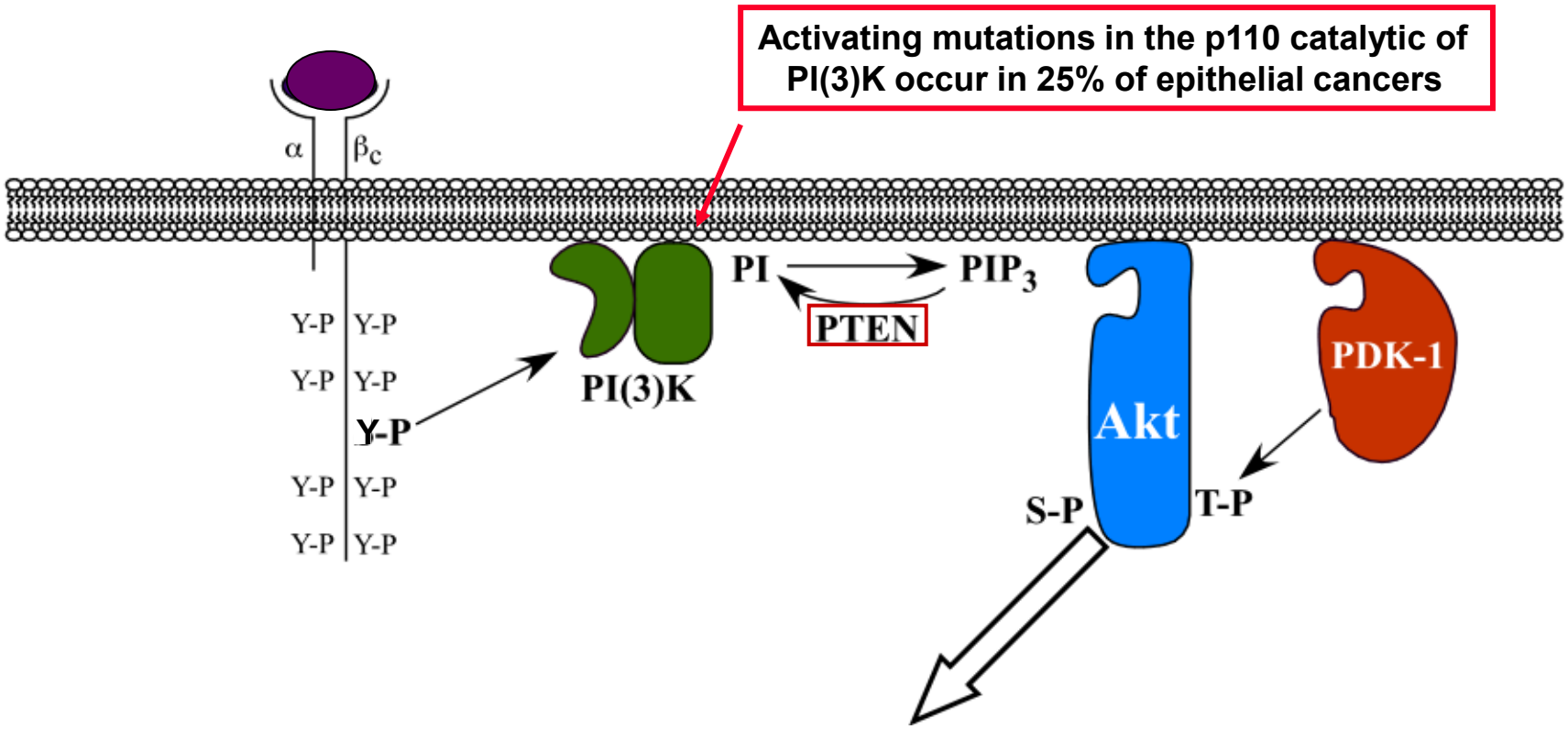
## What is understood:




## What is not understood:

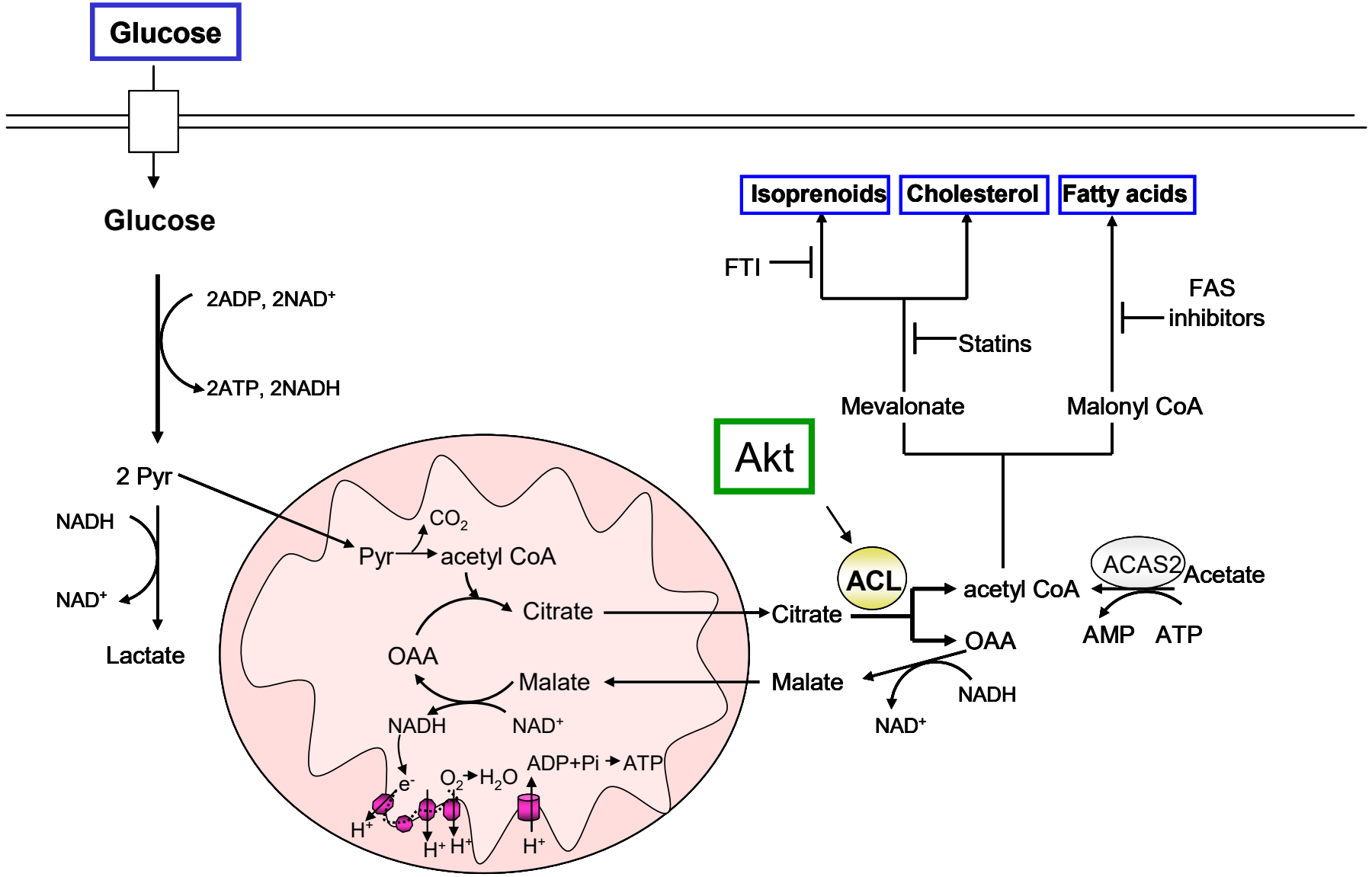


# Tyrosine kinase receptor signal transduction

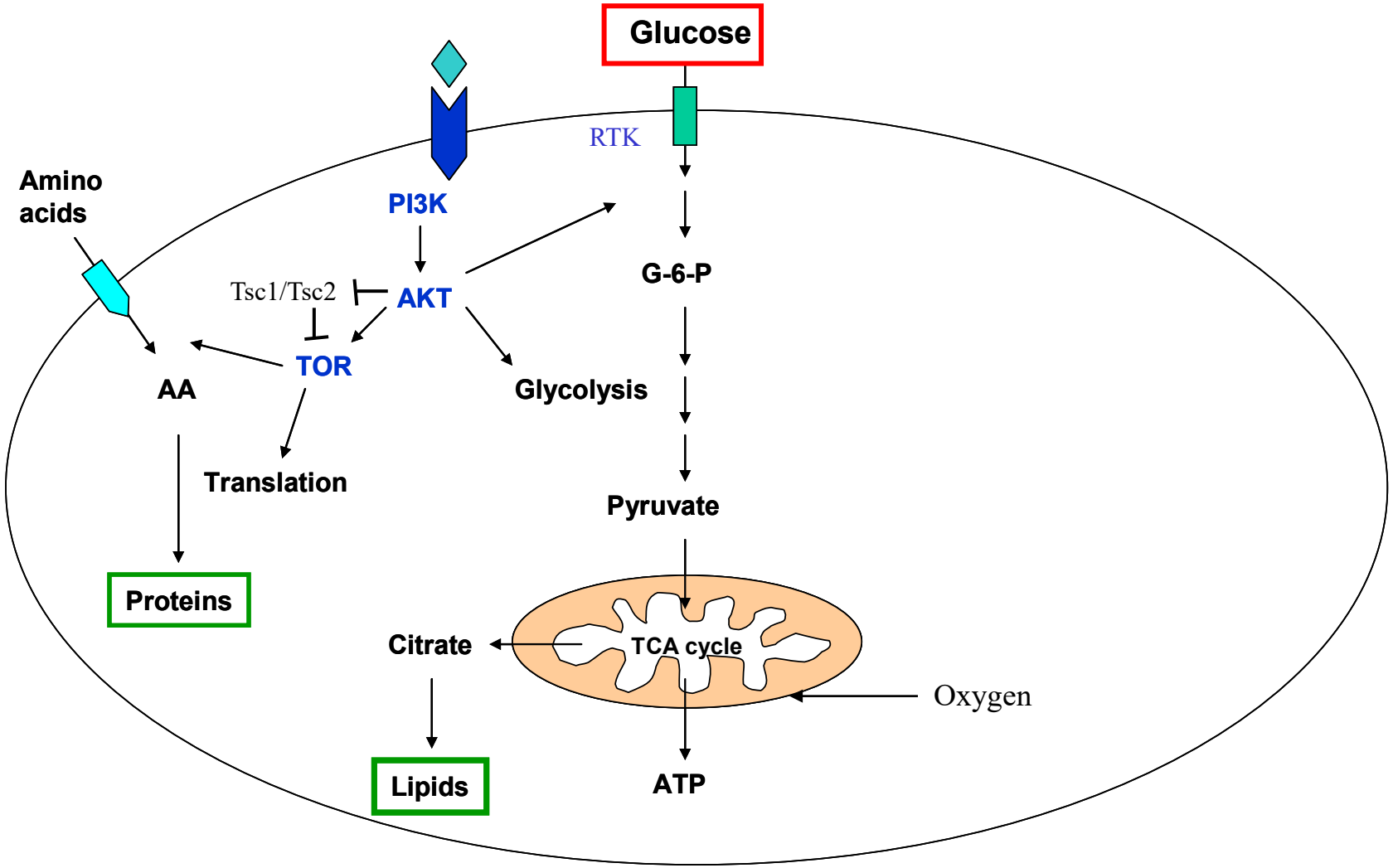



Glucose transporter surface expression
Hexokinase activity and localization
PFK-1 activation

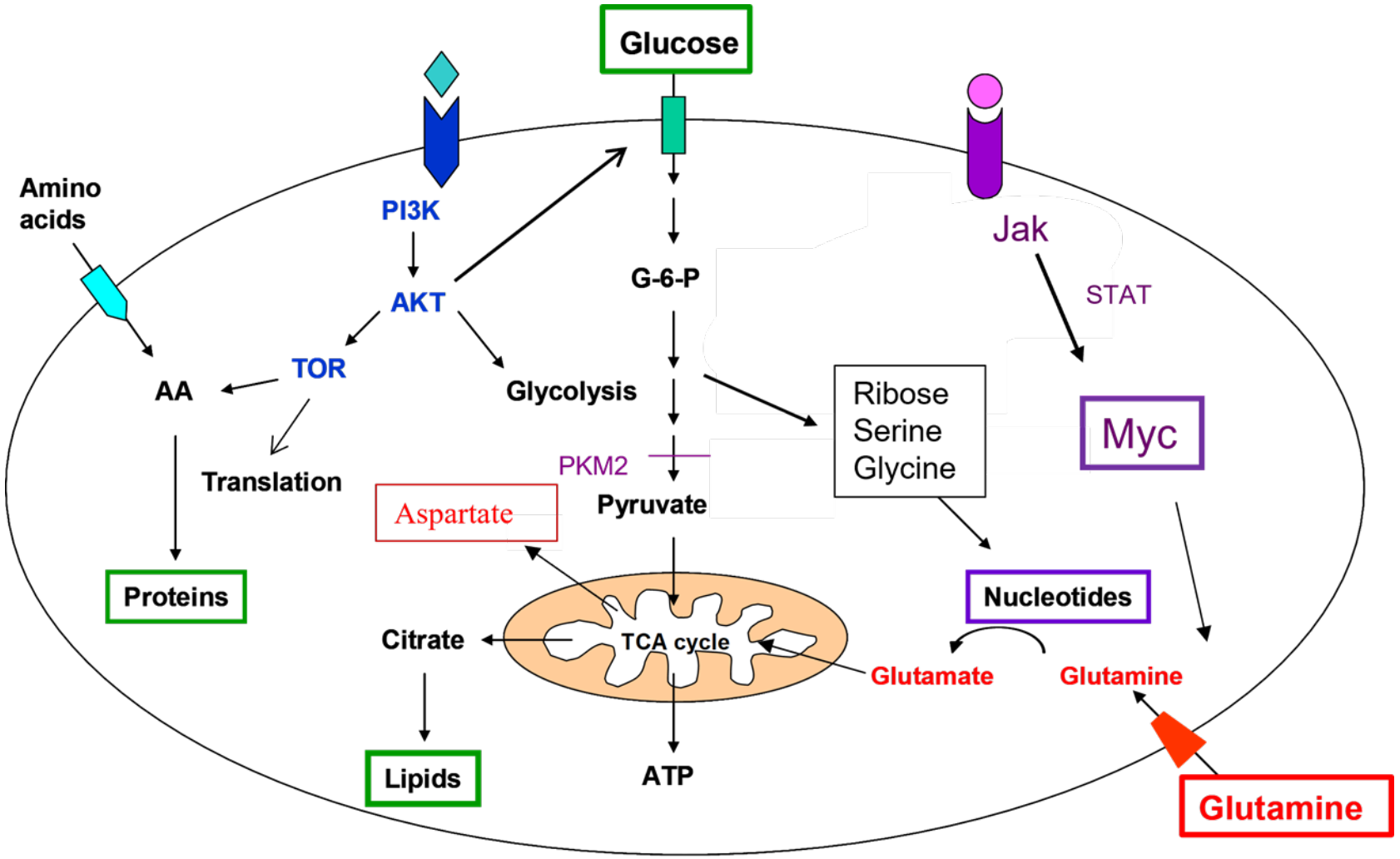
# Akt phosphorylation of ATP Citrate Lyase (ACL) results in glucose-dependent lipid synthesis



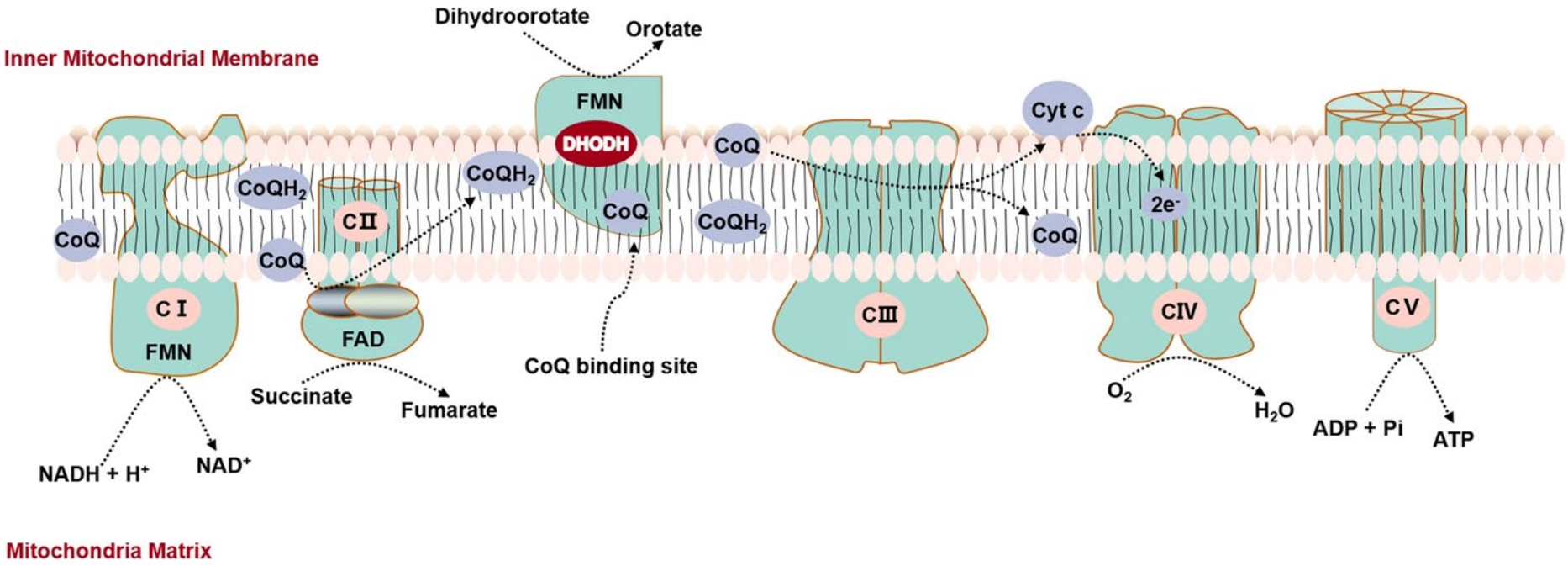
# PI3K/AKT-dependent TOR activation promotes protein synthesis



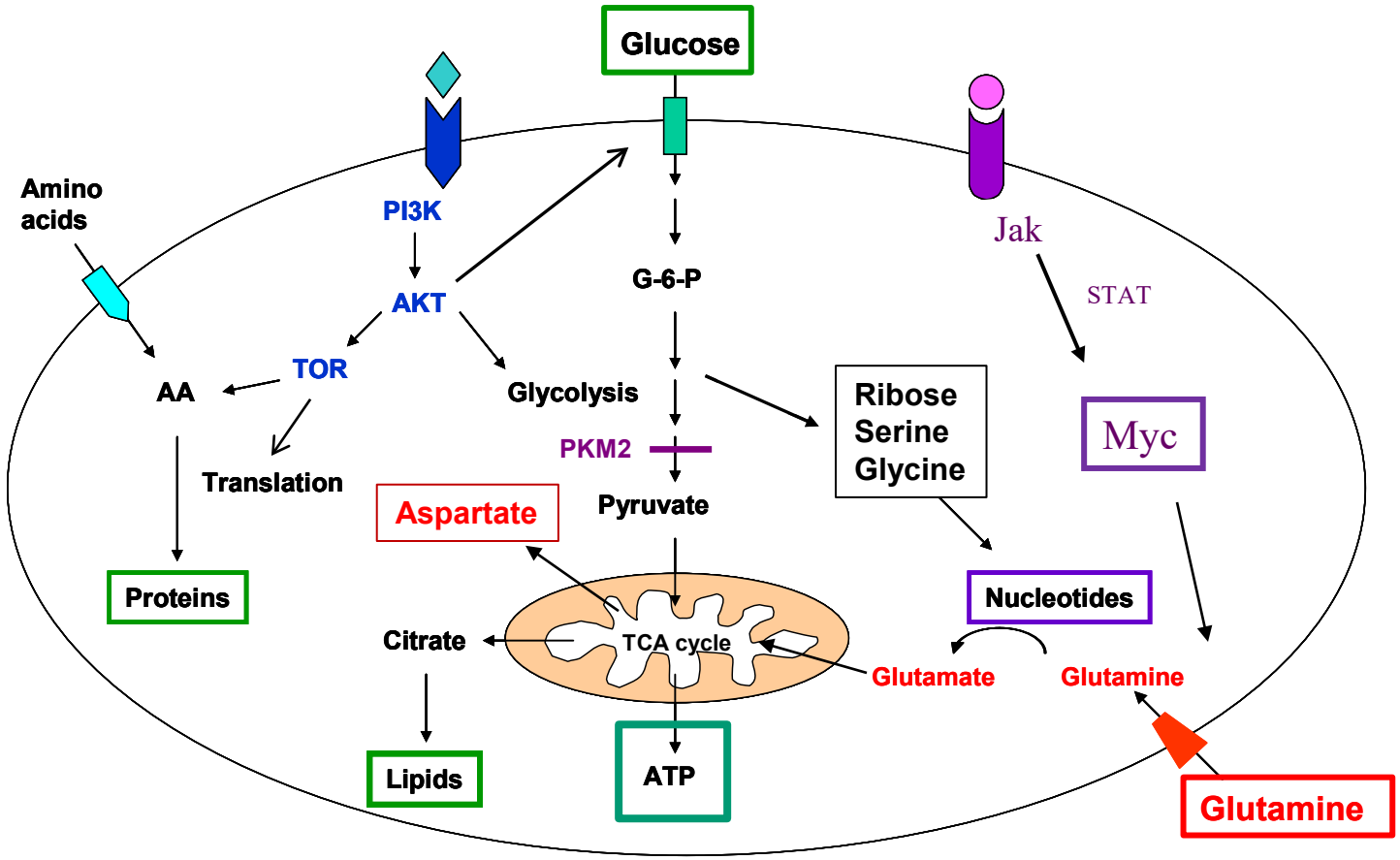
# Receptor signaling determines glucose and glutamine uptake independently



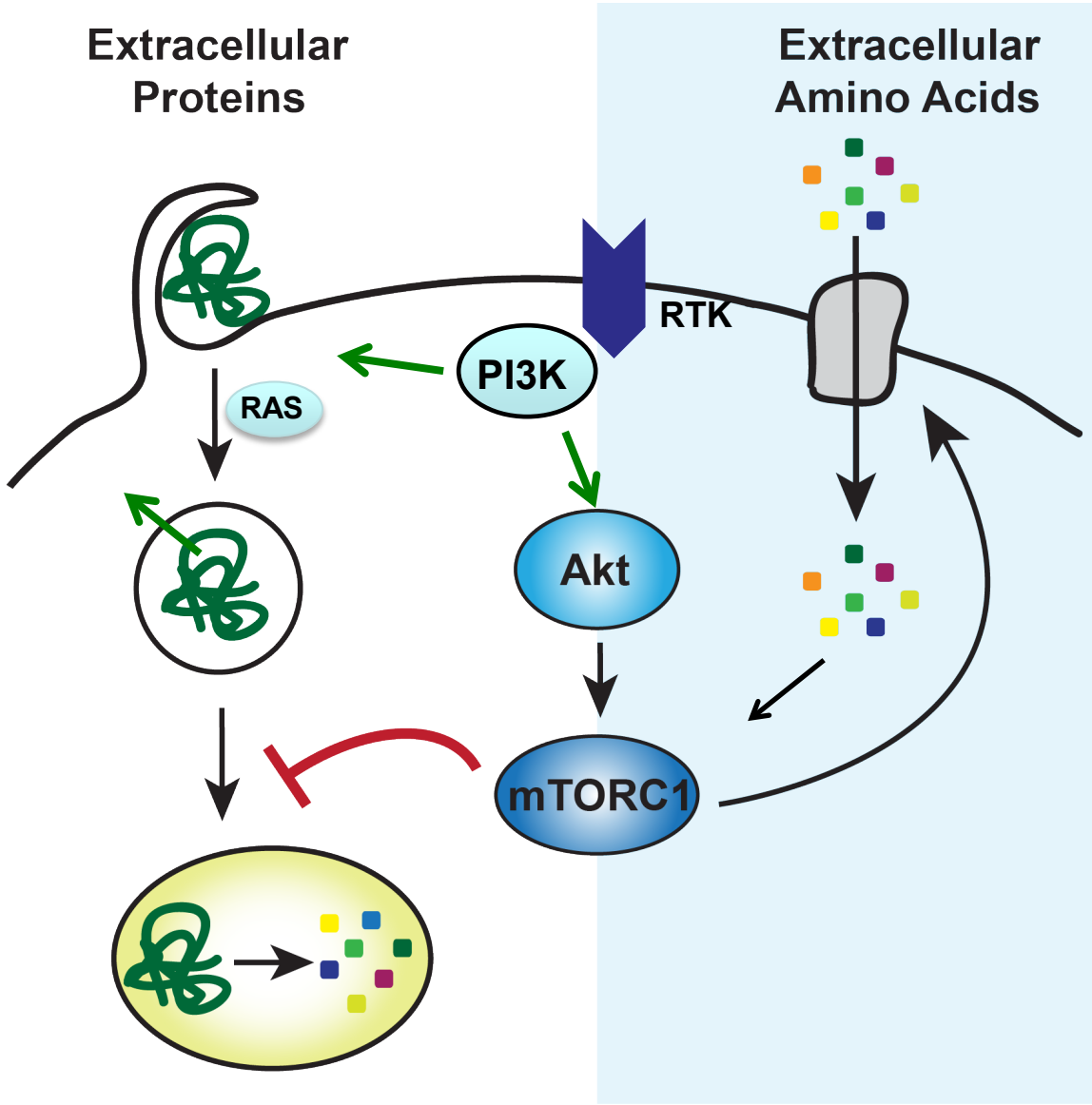
# Pyrimidine biosynthesis requires intact electron transport and ongoing oxidative phosphorylation



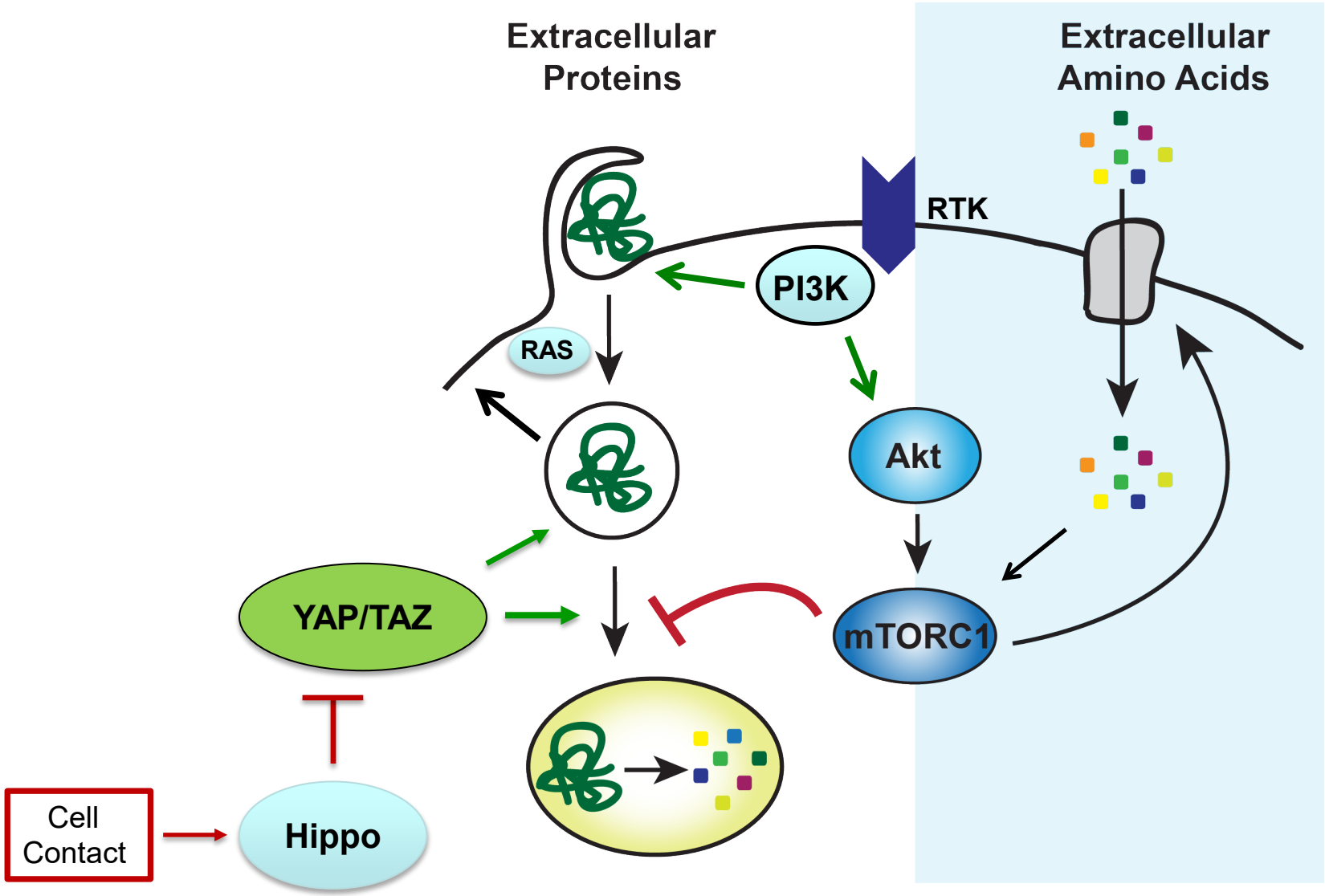
# Cell proliferation requires oxidative phosphorylation



# RTK/PI3K signaling initiates uptake of both extracellular proteins and amino acids

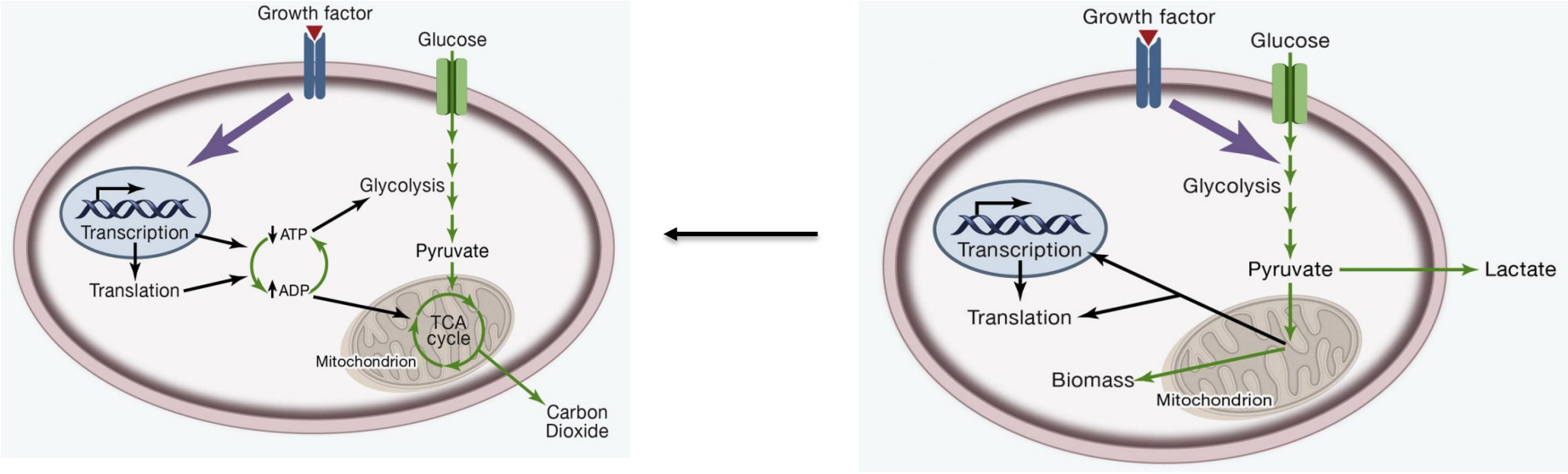


# Receptor Signaling and Cell Contact regulate the uptake of extracellular proteins and amino acids

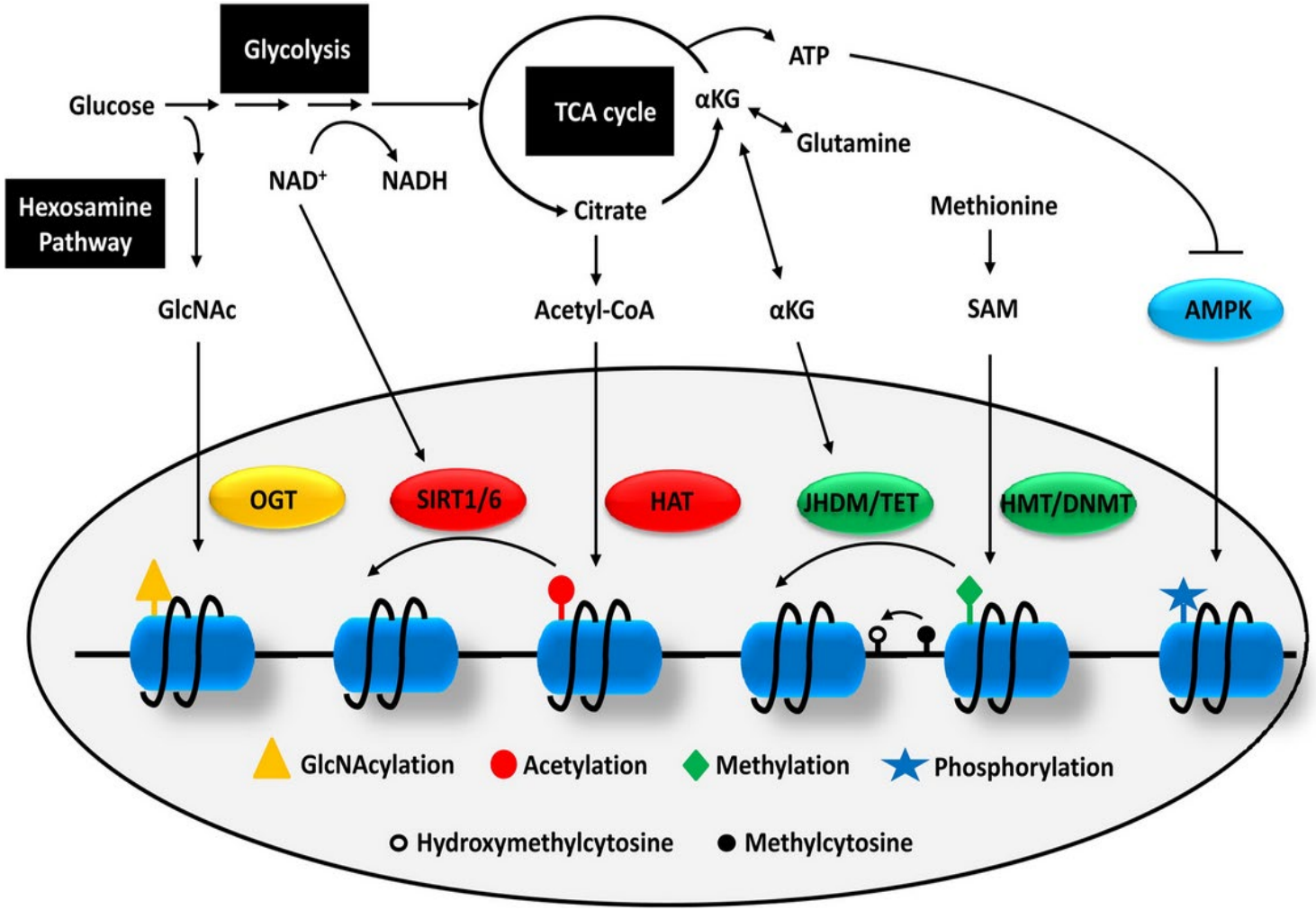


# Metazoan cells use parallel pathways to maintain their effector function

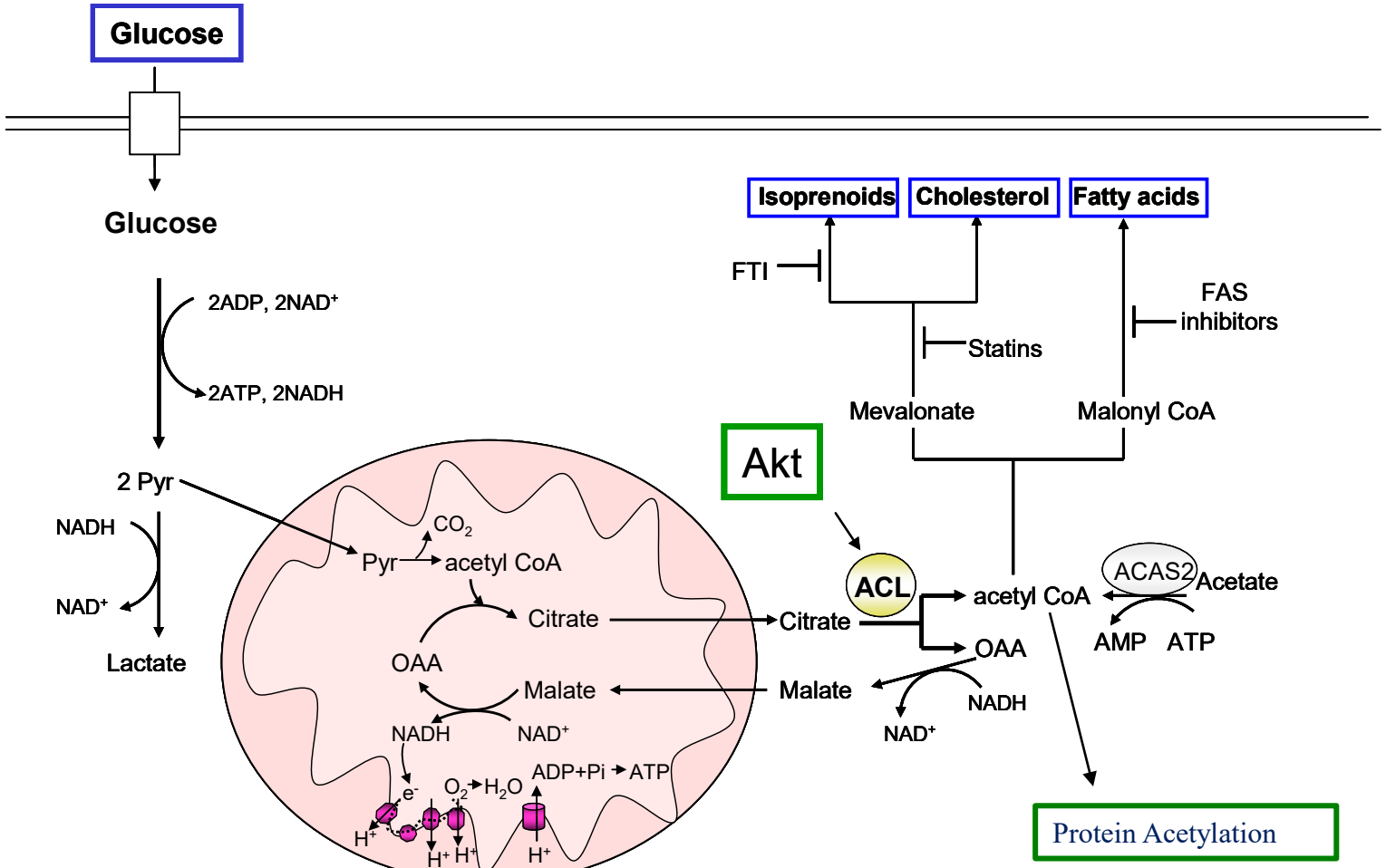
**Differentiated parenchymal cells maintain their physiologic role through lineage specific transcription and translation and mitochondrial bioenergetics.**



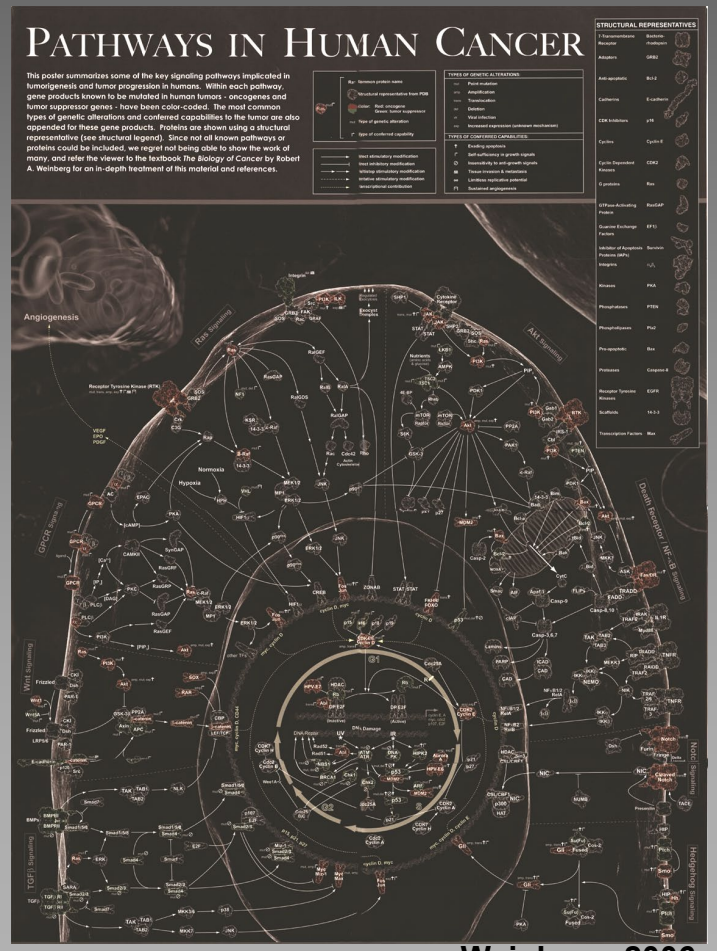
# Crosstalk between Metabolism and Epigenetics



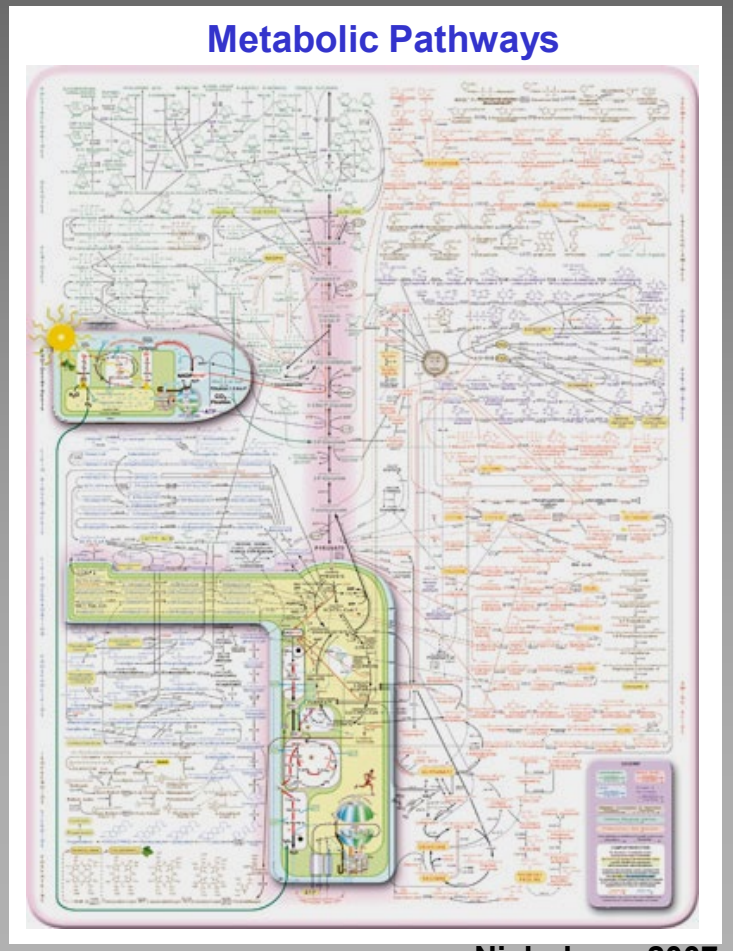
# Akt phosphorylation of ATP Citrate Lyase (ACL) results in glucose-dependent lipid synthesis and histone acetylation



# Many growth factors and oncogenes regulate cellular metabolism




Weinberg, 2006




Nicholson, 2007




The Genome Sequencing Centers (GSCs) use high-throughput Sanger/di-deoxy technology to sequence gene and genomic target regions. Putative mutations in tumor genomes are verified to have a somatic origin by comparison to DNA sequence derived from normal tissue from the same patient. The result of these analyses will be identification of tumor mutations at single nucleotide resolution.

The targets for the TCGA genomic sequencing studies will consist of genes and candidate regions selected through the combination of two different approaches. In one approach, genes of interest (e.g., tumor repressors or oncogenes) are identified from the scientific literature and by consultation with experts in the field. The second approach, genes and genomic regions are identified by analyses of the data produced by the TCGA Cancer Genome Characterization Centers (CGCCs).

View the entire gene and miRNA list.

NEW* TCGA Network Selects More than 6,000 Gene and miRNA Targets:

The TCGA network has selected more than 6,000 gene and miRNA targets for sequencing that represent both protein-coding genes and microRNAs (miRNAs). While not exhaustive, this list represents genes and sequences with a potential for being associated with human cancers based on published and unpublished research.

GBM gene lists:

Click here for the integrated GBM target list for phases one and two.
Click here for the phase one GBM gene list.
Click here for the phase two GBM target list.

Genes Being Sequenced in Glioblastoma:

Approximately 600 genes were selected for the first round of glioblastoma multiforme (GBM) tumor sequencing. To see the first GBM gene list, click here.

This list of genes was generated through a cooperative process; for details about the process, click here. The process is unique to the selection of the initial GBM targets and may or may not reflect future processes for selecting targets.

From the characterization data generated as of October 22, 2007 as well as input from the GBM disease experts, approximately 700 targets were selected for the second round of GBM tumor sequencing. To read a brief description of the selection process, click here. To see the GBM target list for phase two, click here.

To see the integrated GBM target list for phases one and two, click here.

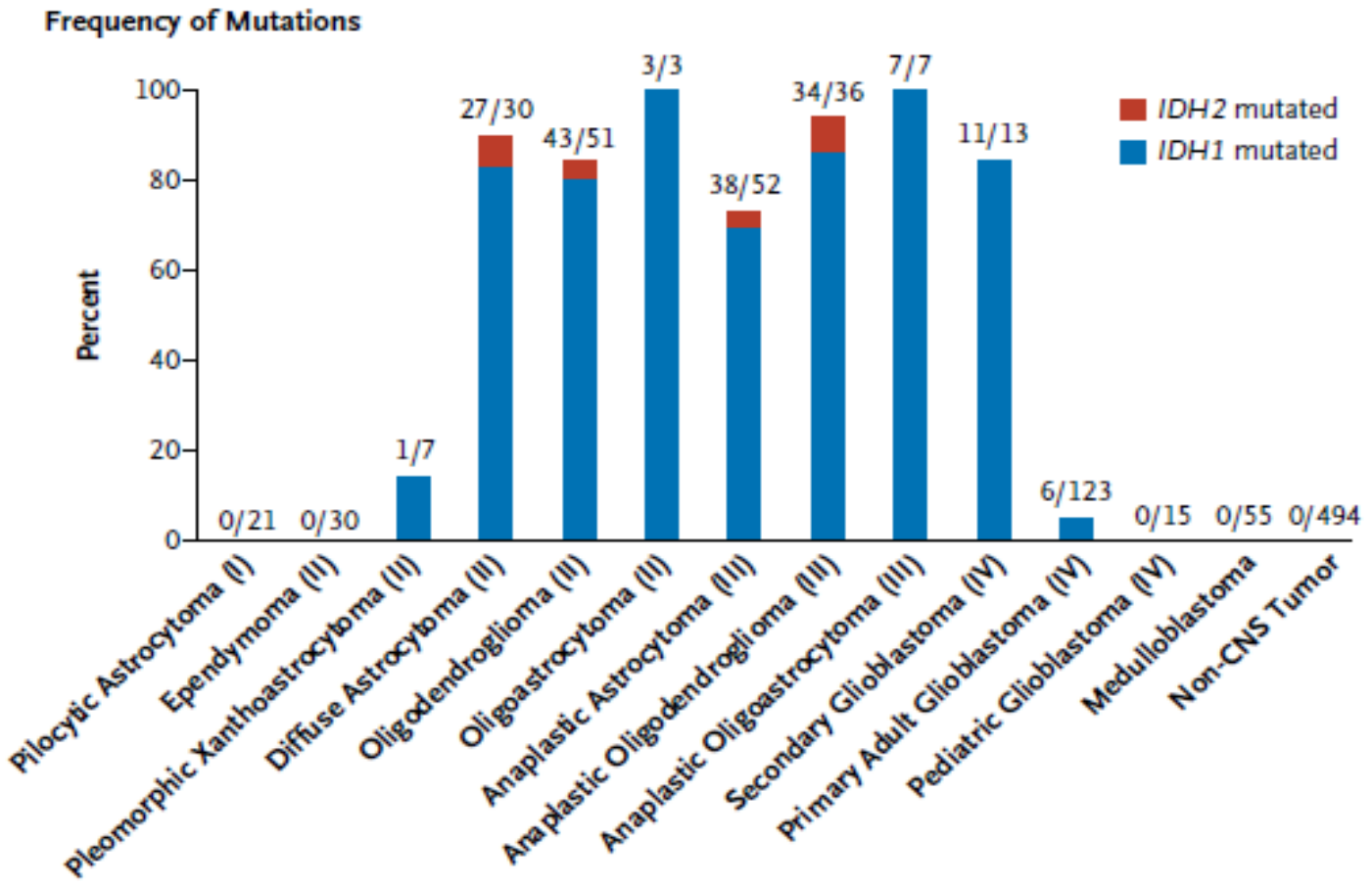
# An Integrated Genomic Analysis of Human Glioblastoma Multiforme

D. Williams Parsons,[1,2*] Siân Jones,[1*] Xiaosong Zhang,[1*] Jimmy Cheng-Ho Lin,[1*] Rebecca J. Leary,[1*] Philipp Angenendt,[1*] Parminder Mankoo,[3] Hannah Carter,[3] I-Mei Siu,[4] Gary L. Gallia,[4] Alessandro Olivi,[4] Roger McLendon,[5] B. Ahmed Rasheed,[5] Stephen Keir,[5] Tatiana Nikolskaya,[6] Yuri Nikolsky,[7] Dana A. Busam,[8] Hanna Tekleab,[8] Luis A. Diaz Jr.,[1] James Hartigan,[9] Doug R. Smith,[9] Robert L. Strausberg,[8] Suely Kazue Nagahashi Marie,[10] Sueli Mieko Oba Shinjo,[10] Hai Yan,[5] Gregory J. Riggins,[4] Darell D. Bigner,[5] Rachel Karchin,[3] Nick Papadopoulos,[1] Giovanni Parmigiani,[1] Bert Vogelstein,[1†] Victor E. Velculescu,[1†] Kenneth W. Kinzler[1†]

| Gene | Point mutations* | | Amplifications† | | Homozygous deletions† | | Fraction of tumors with any alteration (%) | Passenger probability‡ |
|---|---|---|---|---|---|---|---|---|
| | No. of tumors | Fraction of tumors (%) | No. of tumors | Fraction of tumors (%) | No. of tumors | Fraction of tumors (%) | | |
| CDKN2A | 0/22 | 0 | 0/22 | 0 | 11/22 | 50 | 50 | <0.01 |
| TP53 | 37/105 | 35 | 0/22 | 0 | 1/22 | 5 | 40 | <0.01 |
| EGFR | 15/105 | 14 | 5/22 | 23 | 0/22 | 0 | 37 | <0.01 |
| PTEN | 27/105 | 26 | 0/22 | 0 | 1/22 | 5 | 30 | <0.01 |
| NF1 | 16/105 | 15 | 0/22 | 0 | 0/22 | 0 | 15 | 0.04 |
| CDK4 | 0/22 | 0 | 3/22 | 14 | 0/22 | 0 | 14 | <0.01 |
| RB1 | 8/105 | 8 | 0/22 | 0 | 1/22 | 5 | 12 | 0.02 |
| IDH1 | 12/105 | 11 | 0/22 | 0 | 0/22 | 0 | 11 | <0.01 |
| PIK3CA | 10/105 | 10 | 0/22 | 0 | 0/22 | 0 | 10 | 0.10 |
| PIK3R1 | 8/105 | 8 | 0/22 | 0 | 0/22 | 0 | 8 | 0.10 |

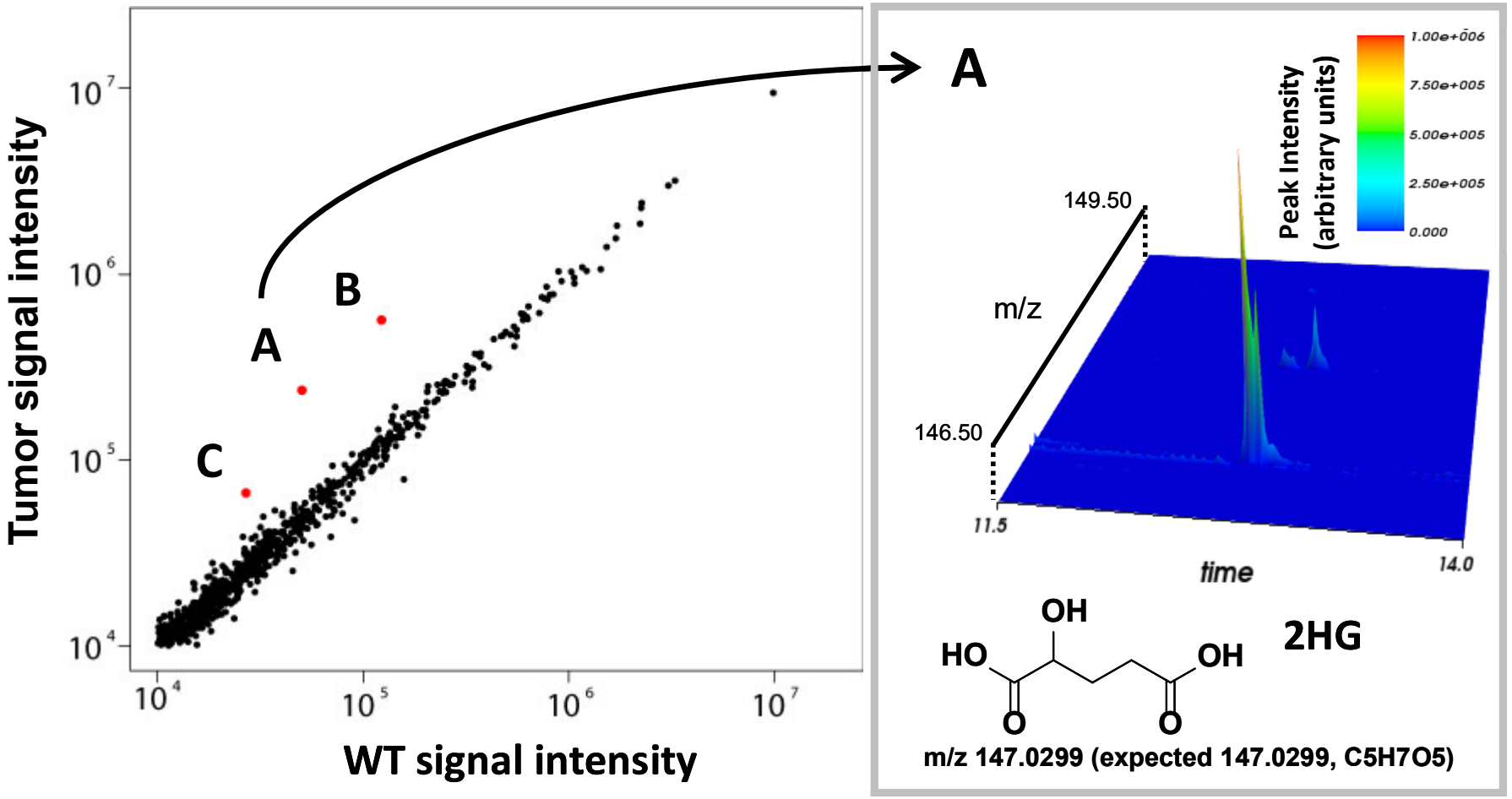
*Fraction of tumors with point mutations indicates the fraction of mutated GBMs out of the 105 samples in the Discovery and Prevalence Screens. CDKN2A and CDK4 were not analyzed for point mutations in the Prevalence Screen because no sequence alterations were detected in these genes in the Discovery Screen. †Fraction of tumors with amplifications and deletions indicates the number of tumors with these types of alterations in the 22 Discovery Screen samples. ‡Passenger probability indicates the probability obtained using the average of the lower and upper bound background mutation rates (*12*).

# IDH mutations are common in intermediate grade gliomas.

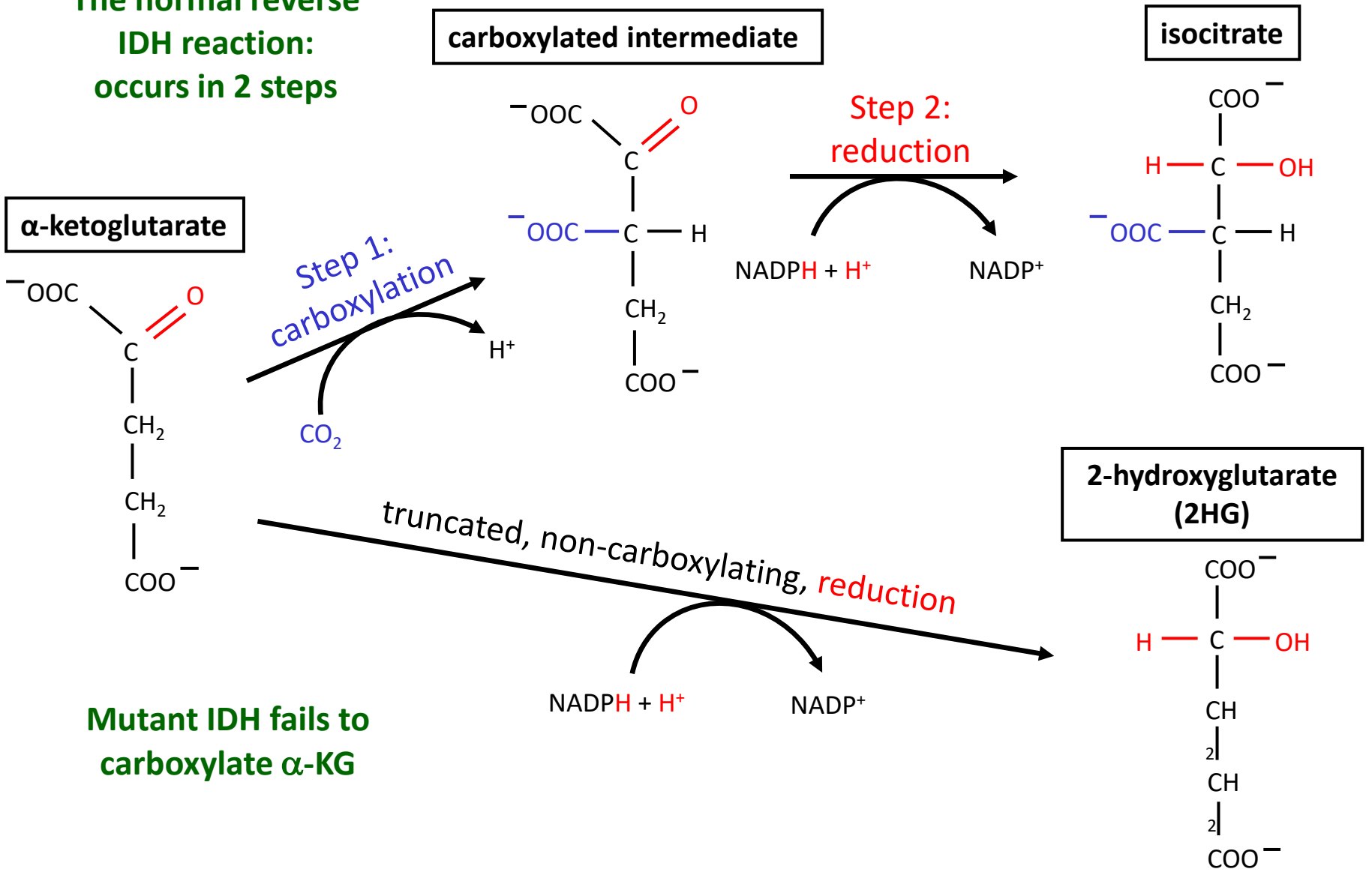



Yan, H., *et al.* NEJM 360:8, Feb 2009

# Cells transfected with R132H IDH1 accumulate an abnormal metabolite

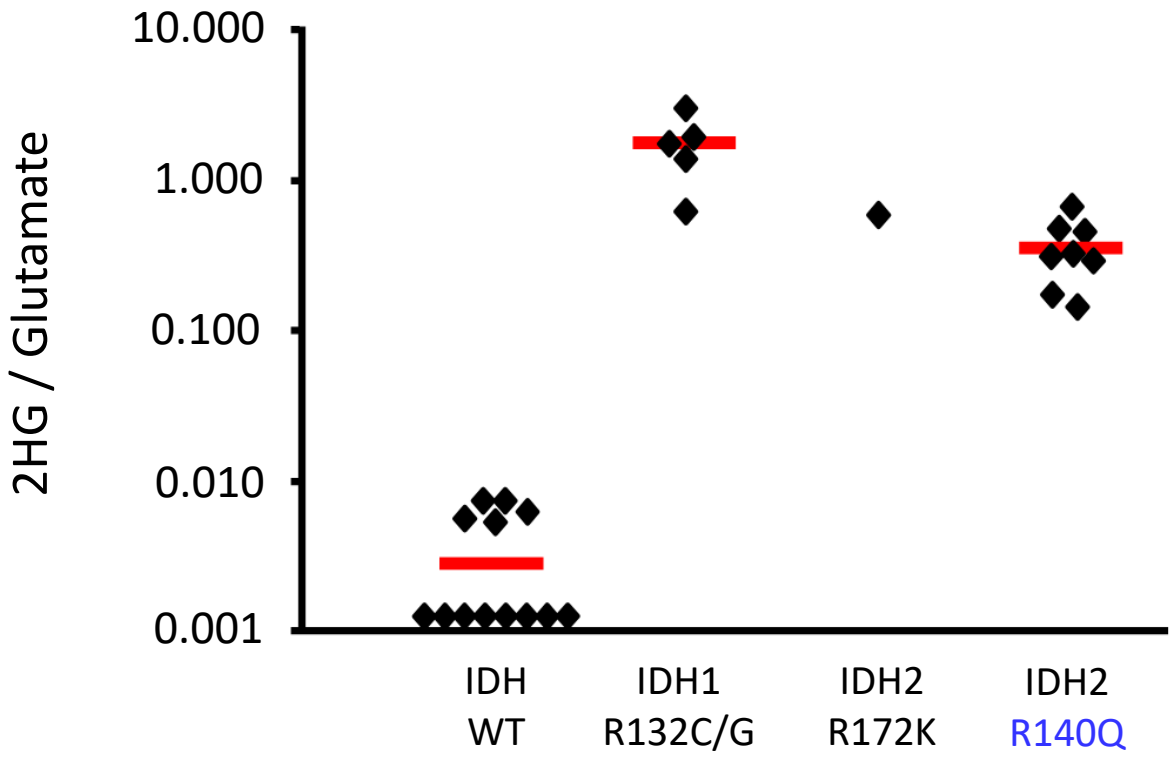


**The normal reverse IDH reaction: occurs in 2 steps**



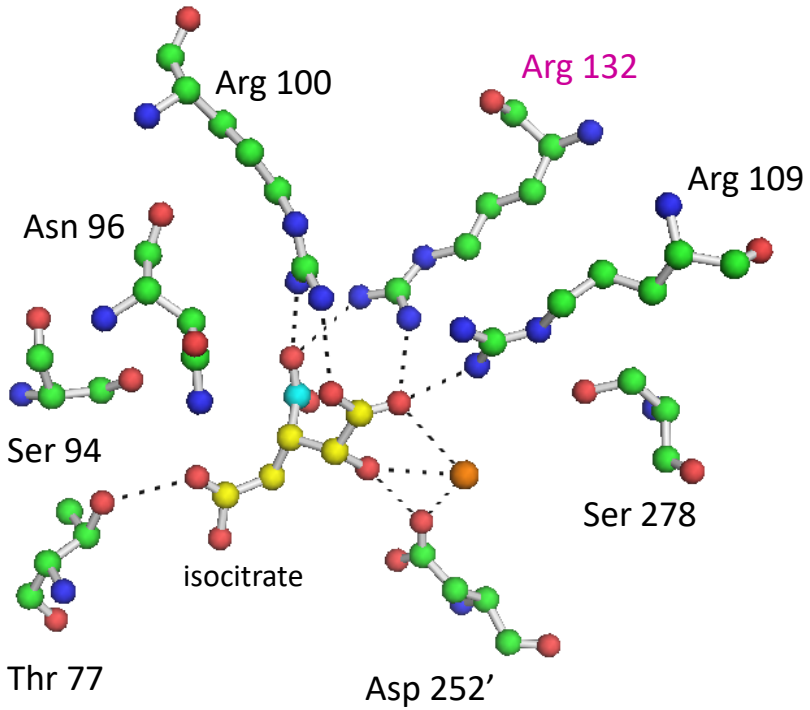

**Mutant IDH fails to carboxylate α-KG**

# Screening human AML samples for elevated 2HG uncovers yet another IDH neomorph.

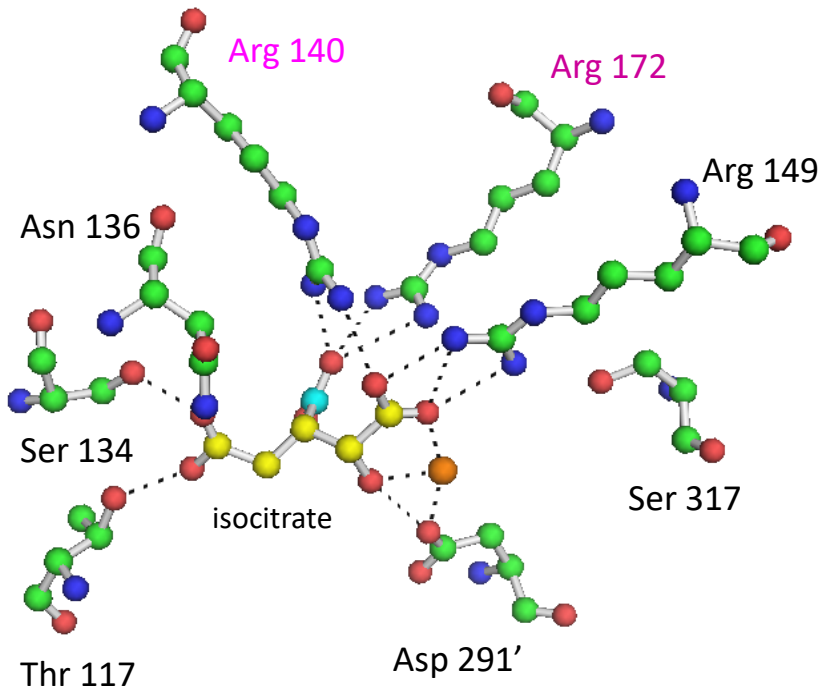


# IDH2 R140 coordinates the same isocitrate carboxyl as IDH1 R132 and IDH2 R172

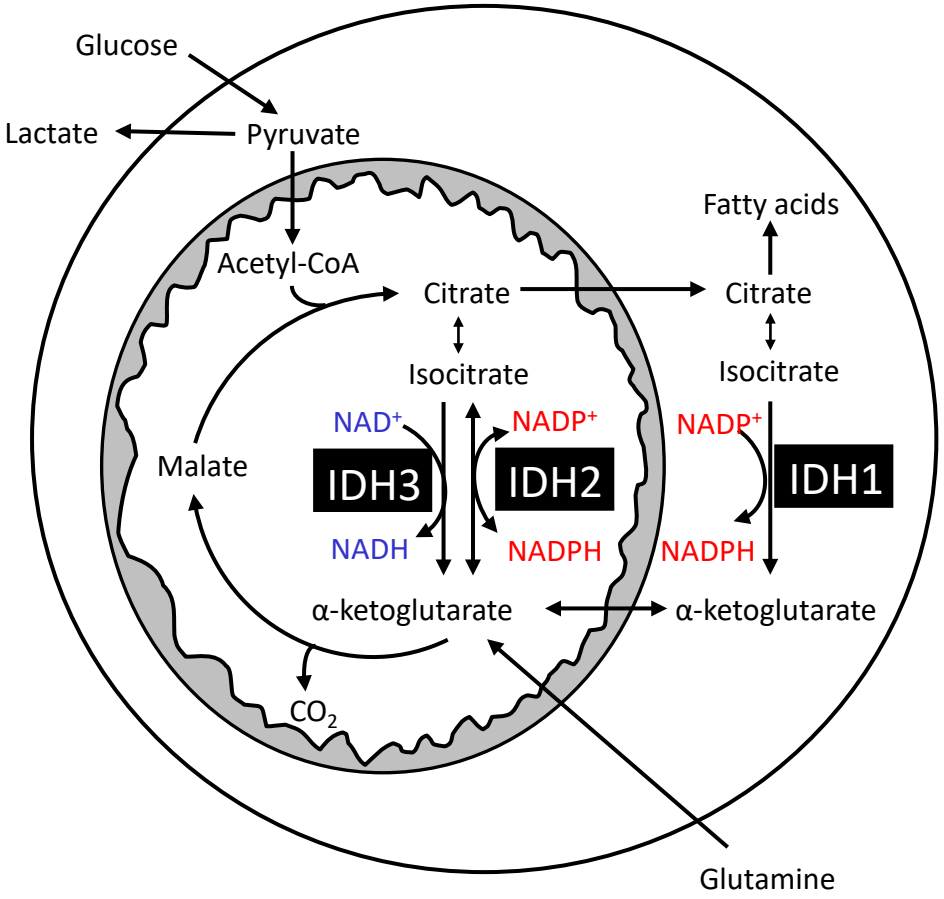
Cytosolic IDH1




Mitochondrial IDH2

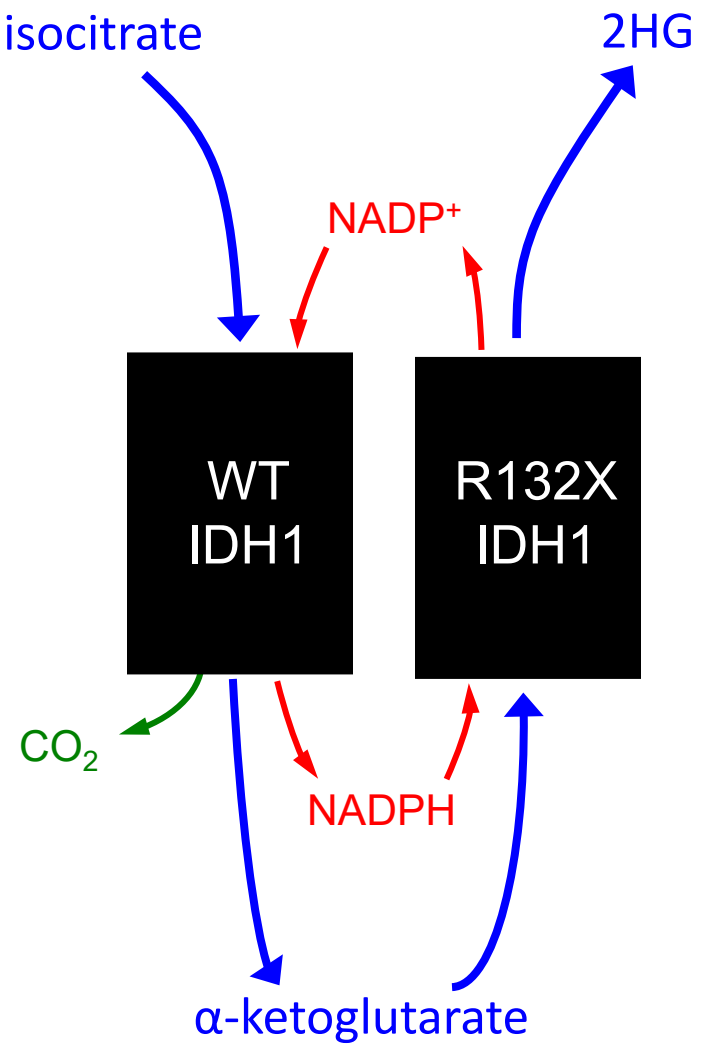


# Isocitrate dehydrogenase (IDH) mutations

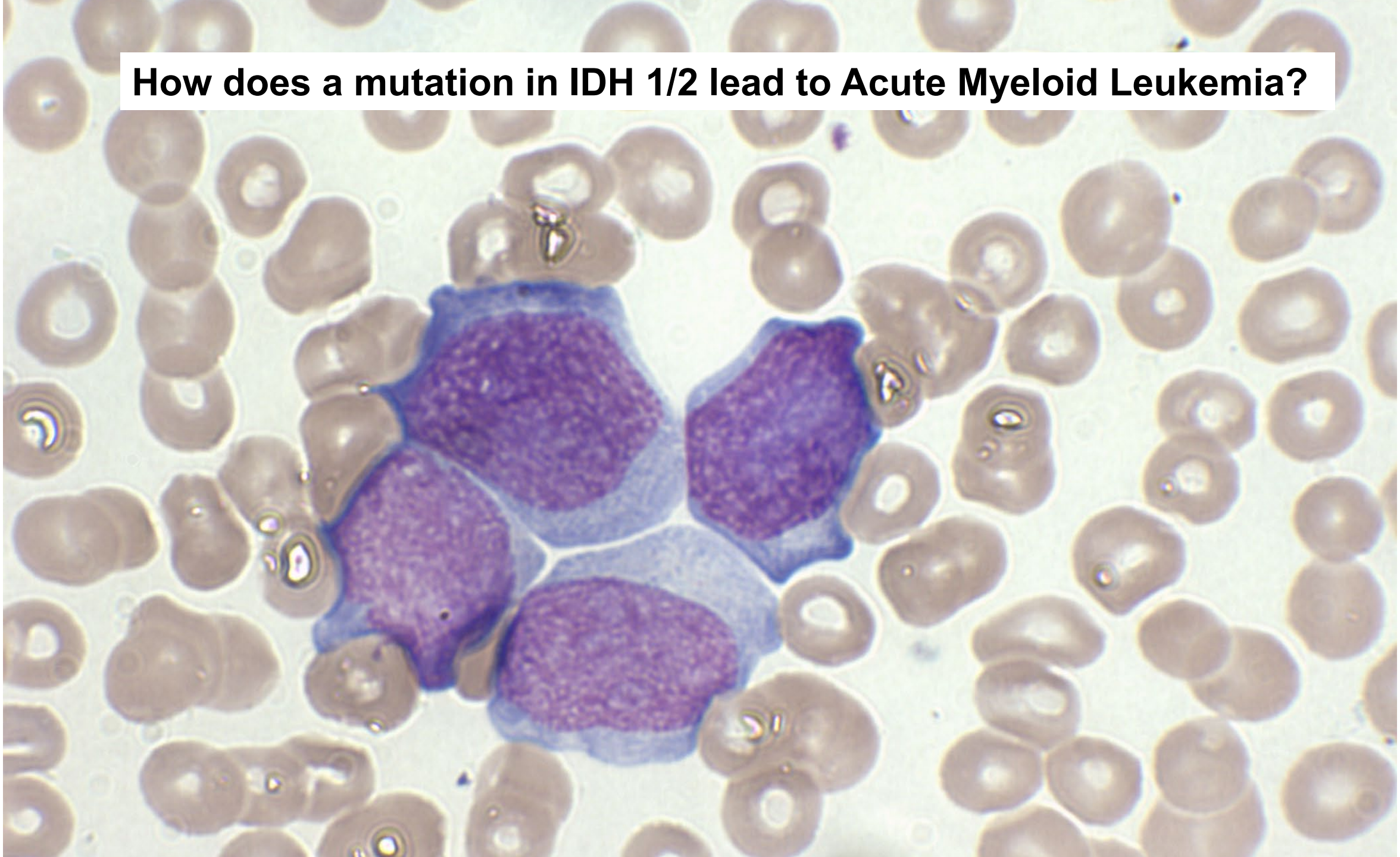



- **Early, somatic, monoallelic mutations in cytosolic IDH1/2 are found in 80% of intermediate-grade gliomas**
- **All mutations are loss-of-function for IDH1/2's normal conversion of isocitrate to α-ketoglutarate.**
- **All mutations are missense and specific to residues in the active site.**
- **One wild-type IDH1 allele is always retained in tumors.**

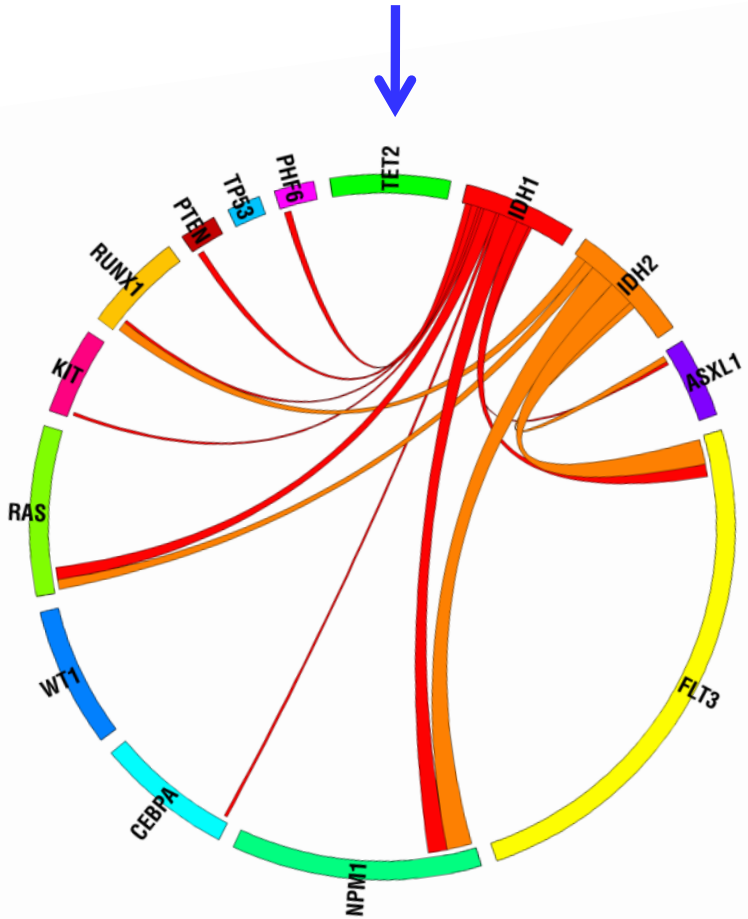
# A heterodimer between wildtype and mutant IDH1 potentiates 2HG production



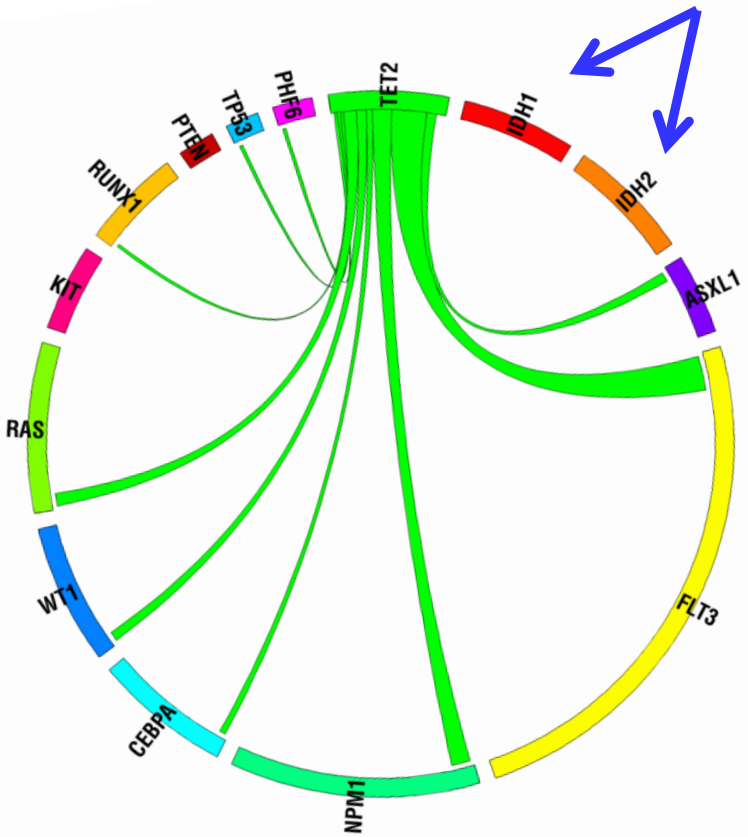
# How does a mutation in IDH 1/2 lead to Acute Myeloid Leukemia?



# IDH1/2 neomorphic mutations are mutually exclusive with loss-of-functions TET2 mutations.

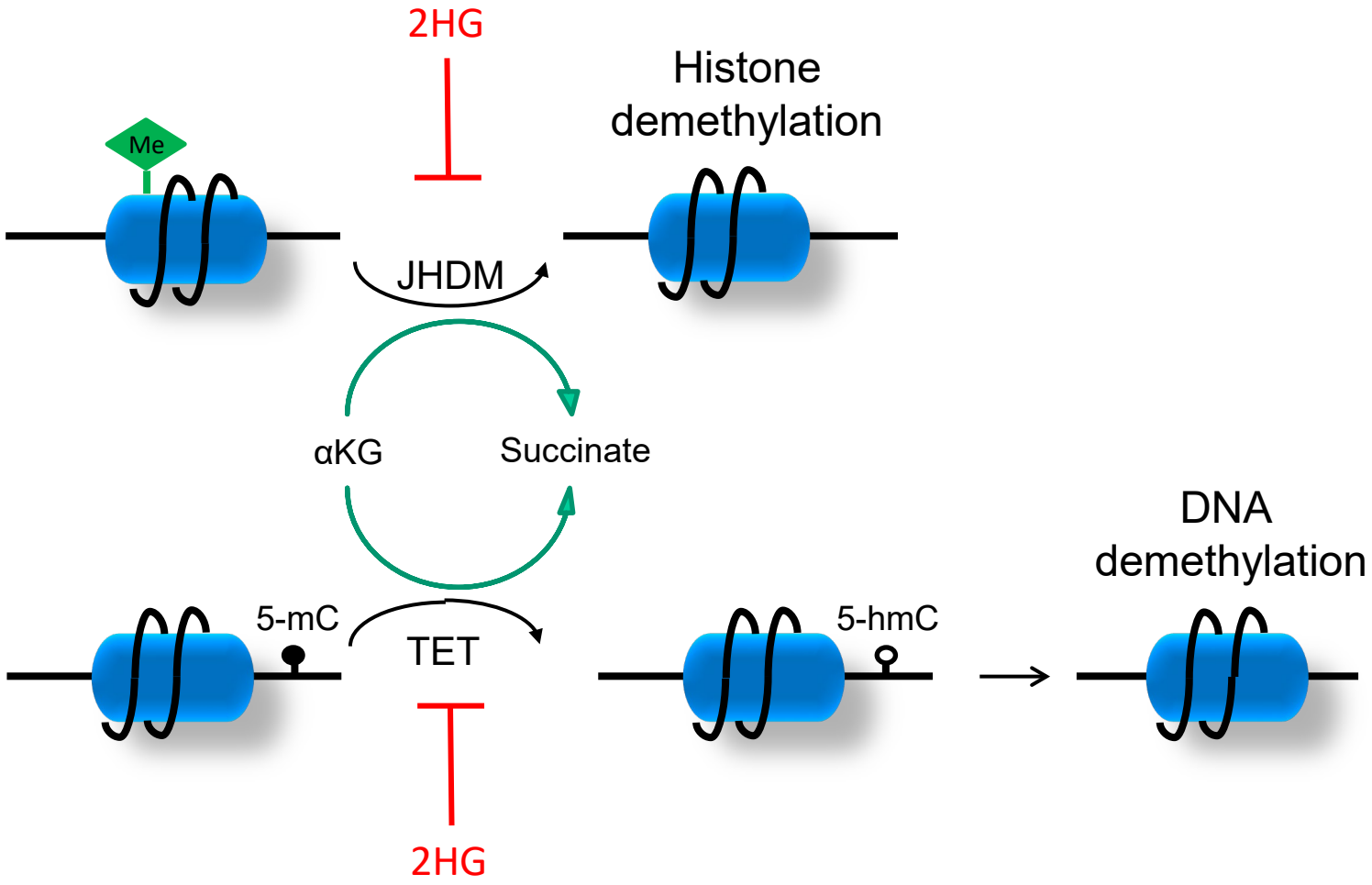



57 IDH1/2 mutant AMLs

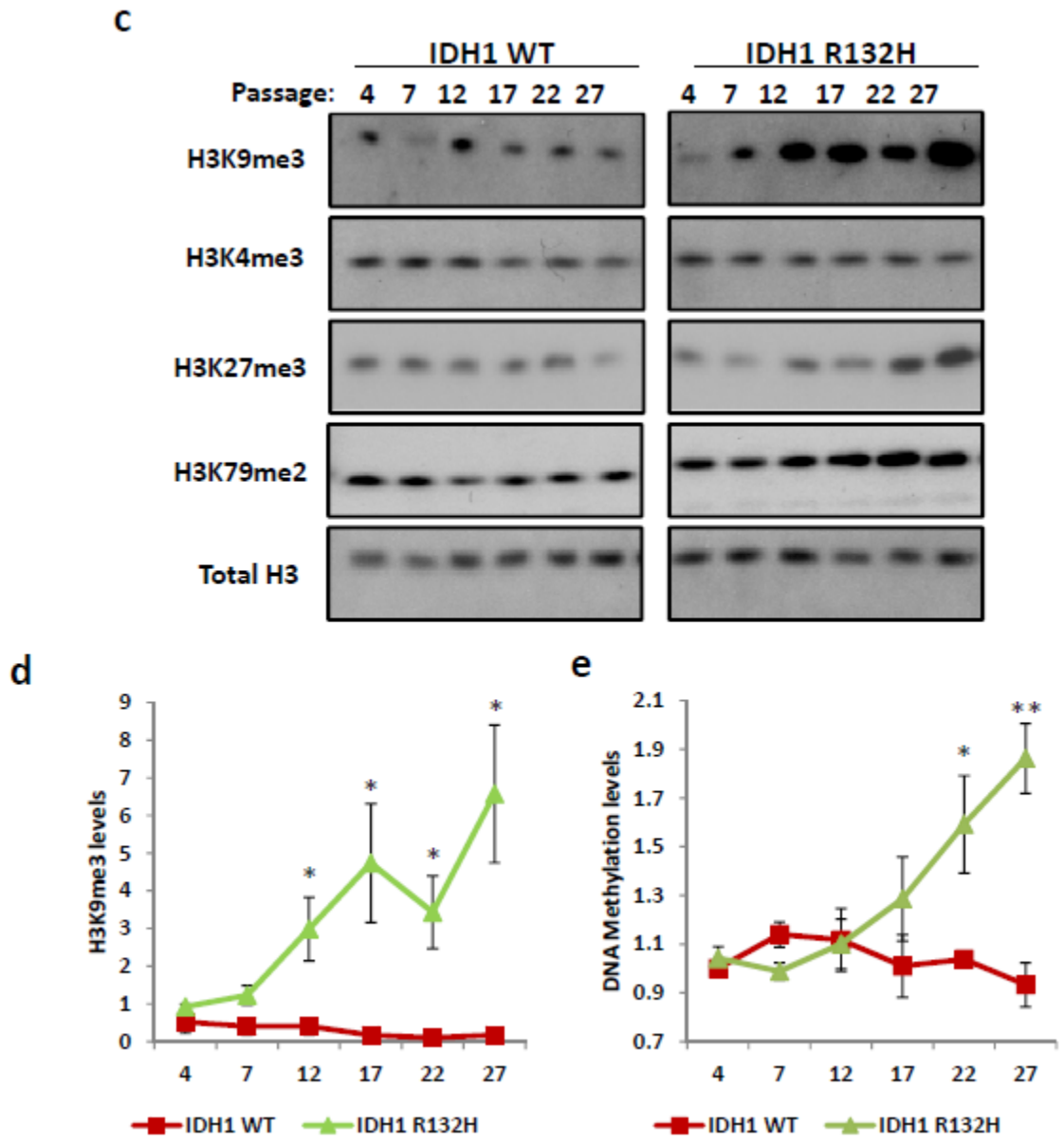



28 TET2 mutant AMLs

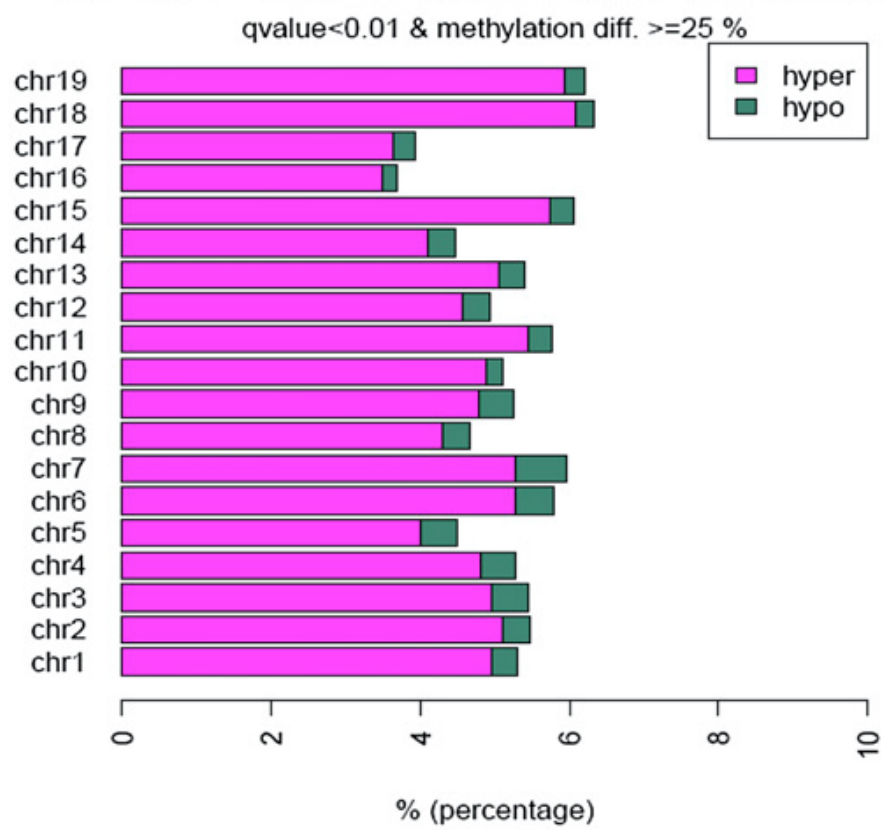
# α-KG-dependent histone and DNA demethylases



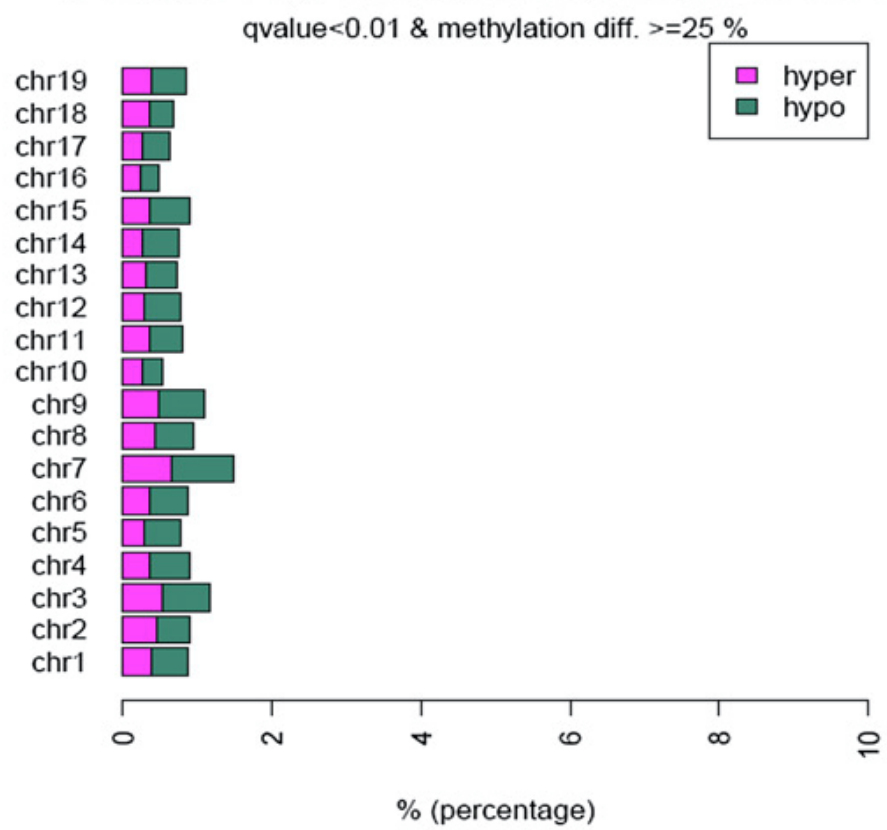
# IDH1 mutants induce progressive histone followed by DNA methylation



# Promoter DNA hypermethylation in IDH mutant cells




Mutant vs. WT

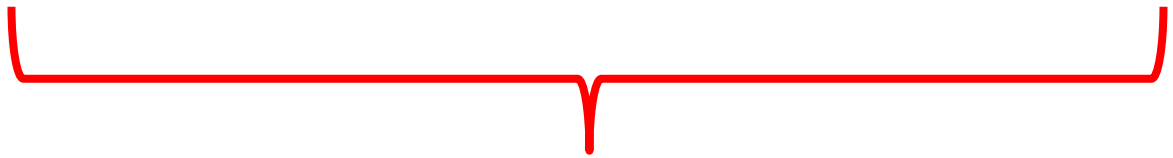



Vector vs. WT

# Preferential establishment of DNA hypermethylation at PRC2/TET1 binding promoters in ESC

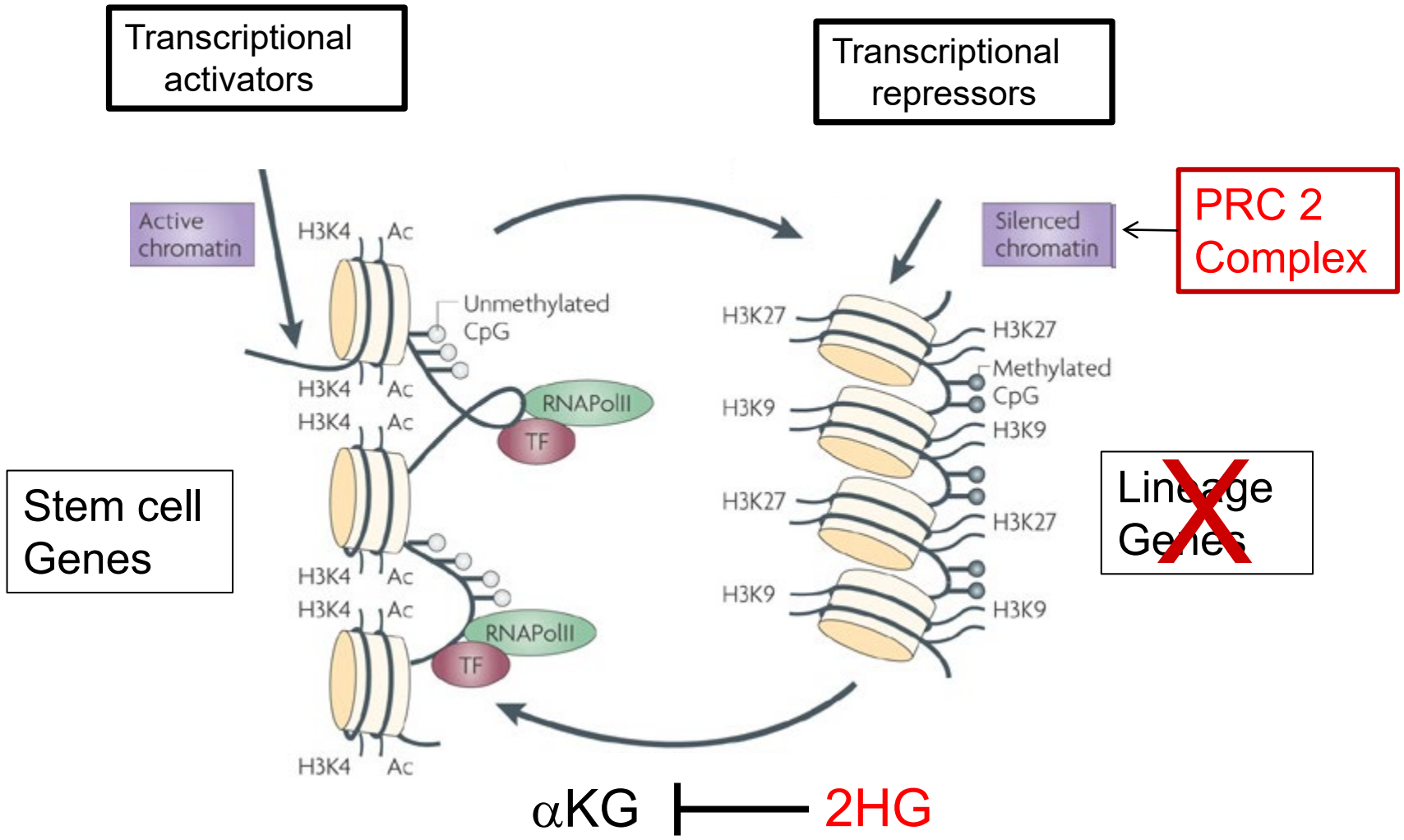
**Hypermethylated gene list -> Broad Molecular Signatures Database (3272 gene sets)**

| Gene set ID | P-value |
|---|---|
| H3K27me3 marked genes in ESC | 3.09E-49 |
| SUZ12 Targets in ESC | 9.91E-37 |
| EED Targets in ESC | 6.71E-30 |
| PRC2 Targets in ESC | 5.37E-23 |

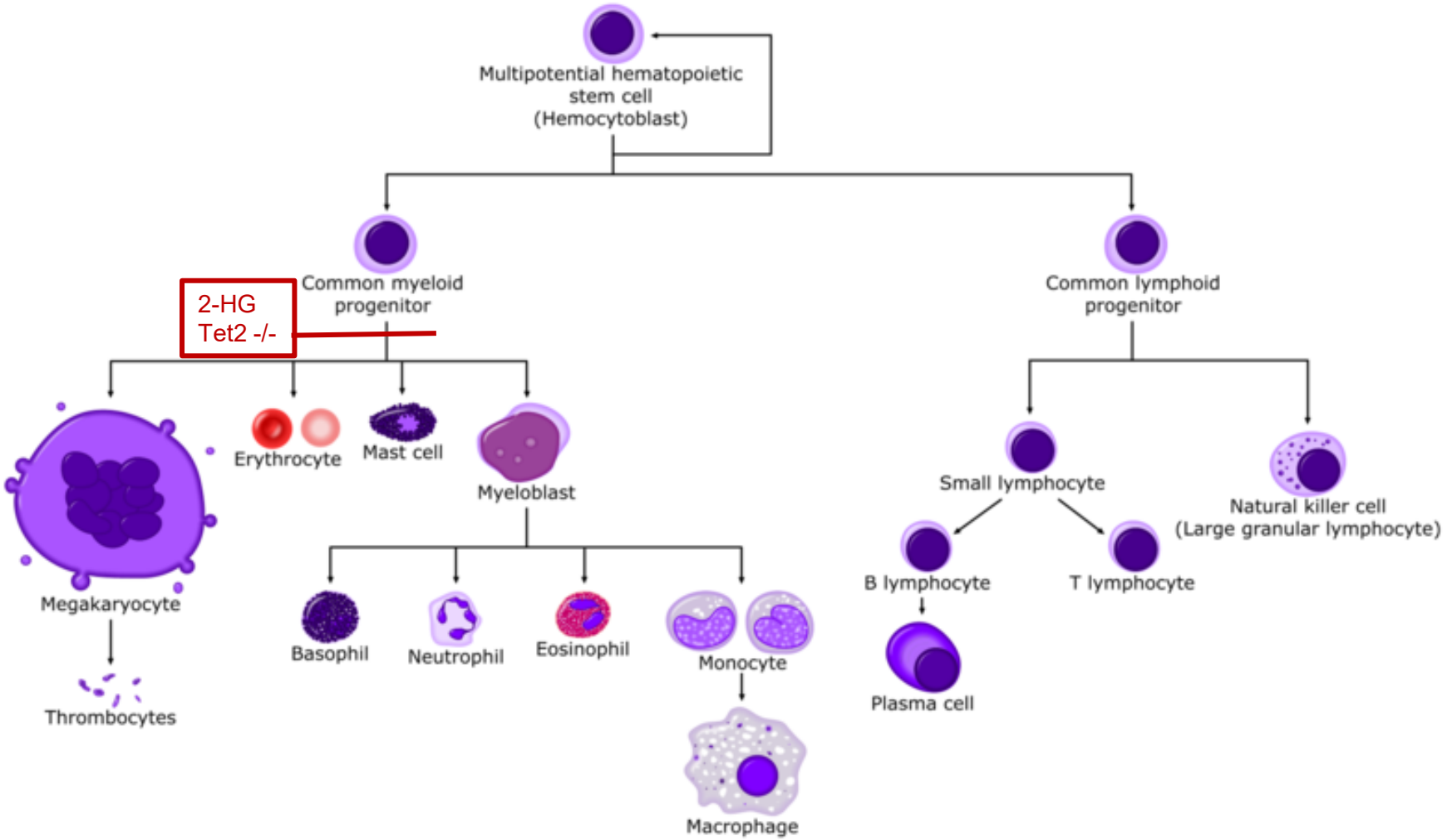


Bound by TET1

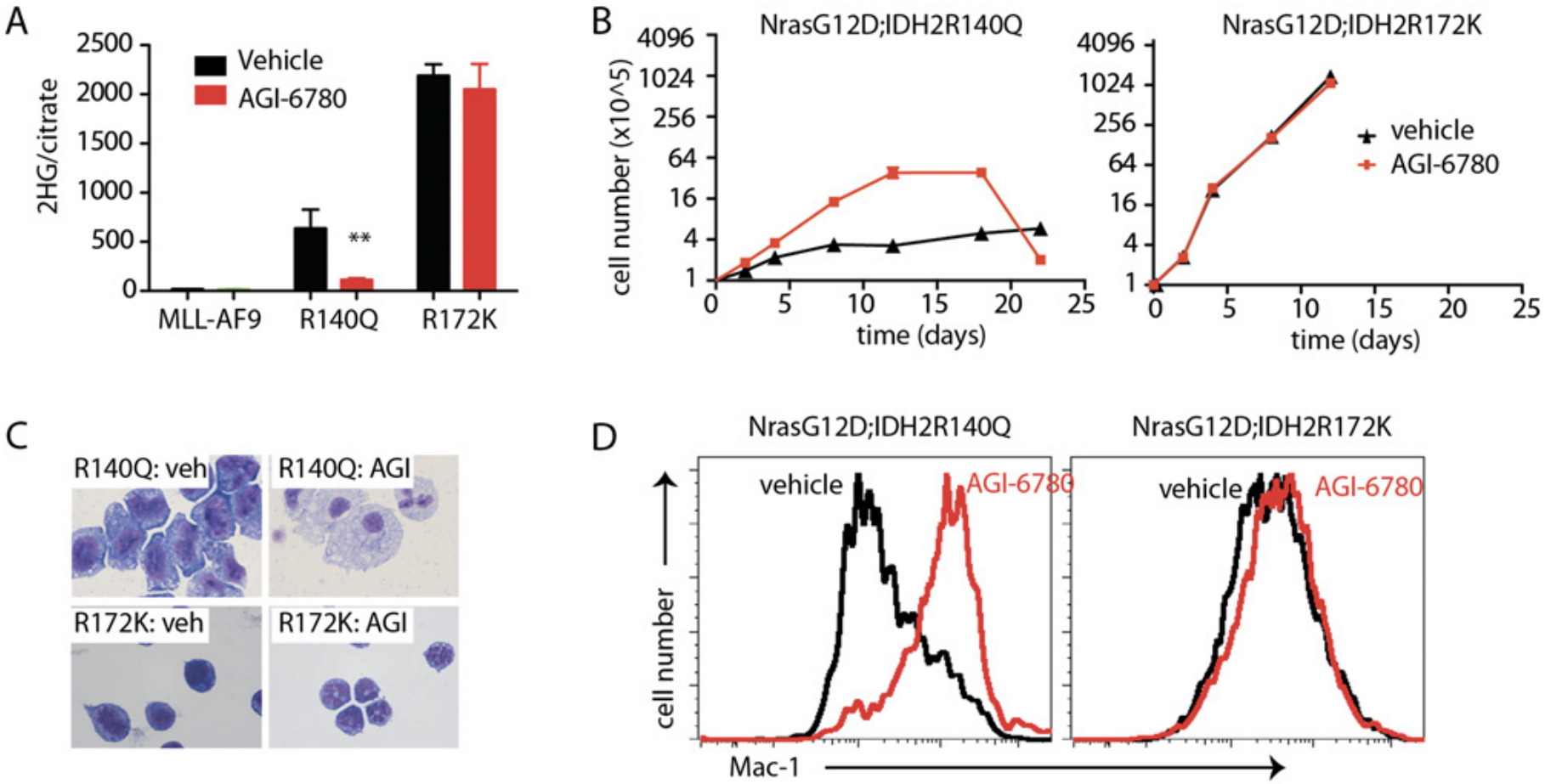
# During embryonic development silenced chromatin is established by the Polycomb Repressor Complex 2 (PRC 2)



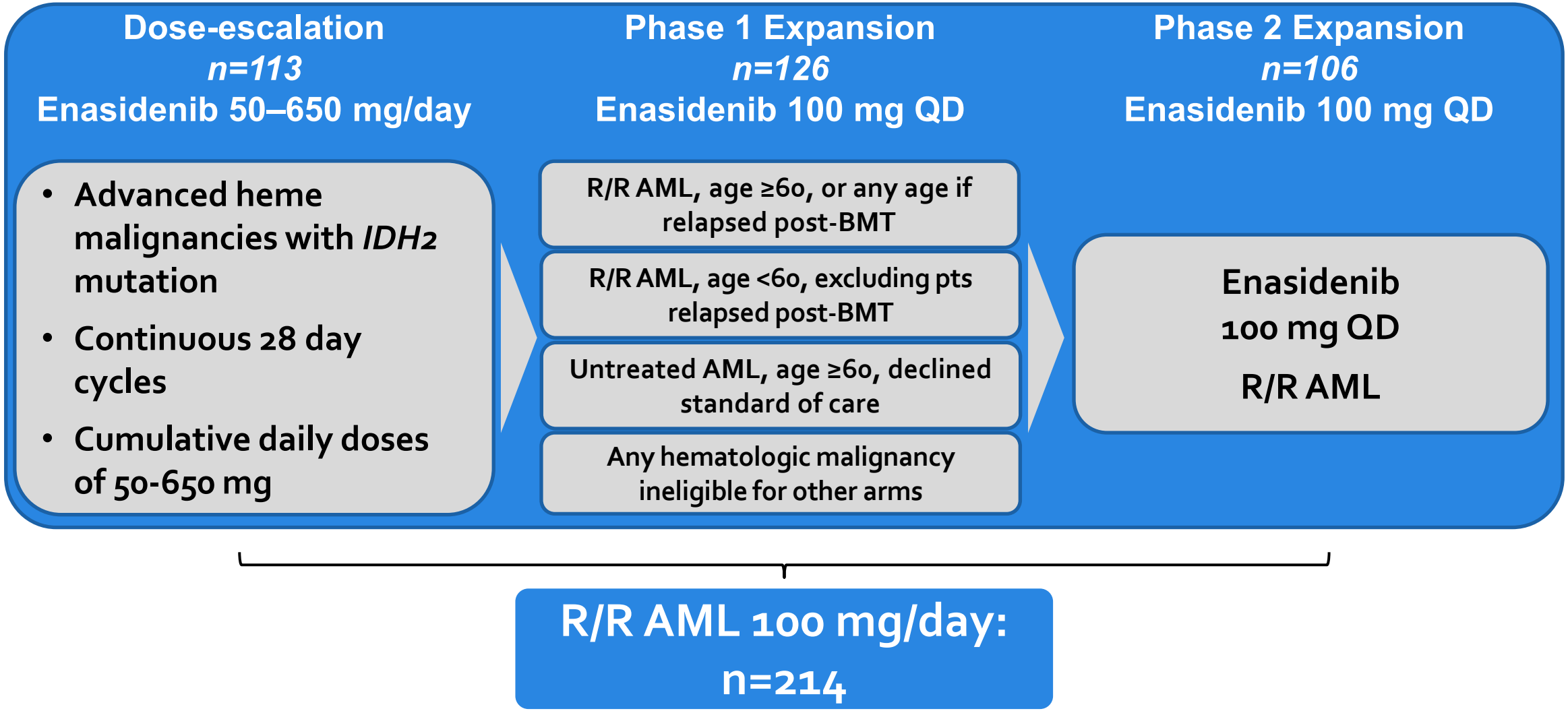
# Loss of the ability to demethylate DNA results in a block to myeloid differentiation



# Selective inhibitors of mutant IDH 2-HG production reverse leukemia by promoting differentiation

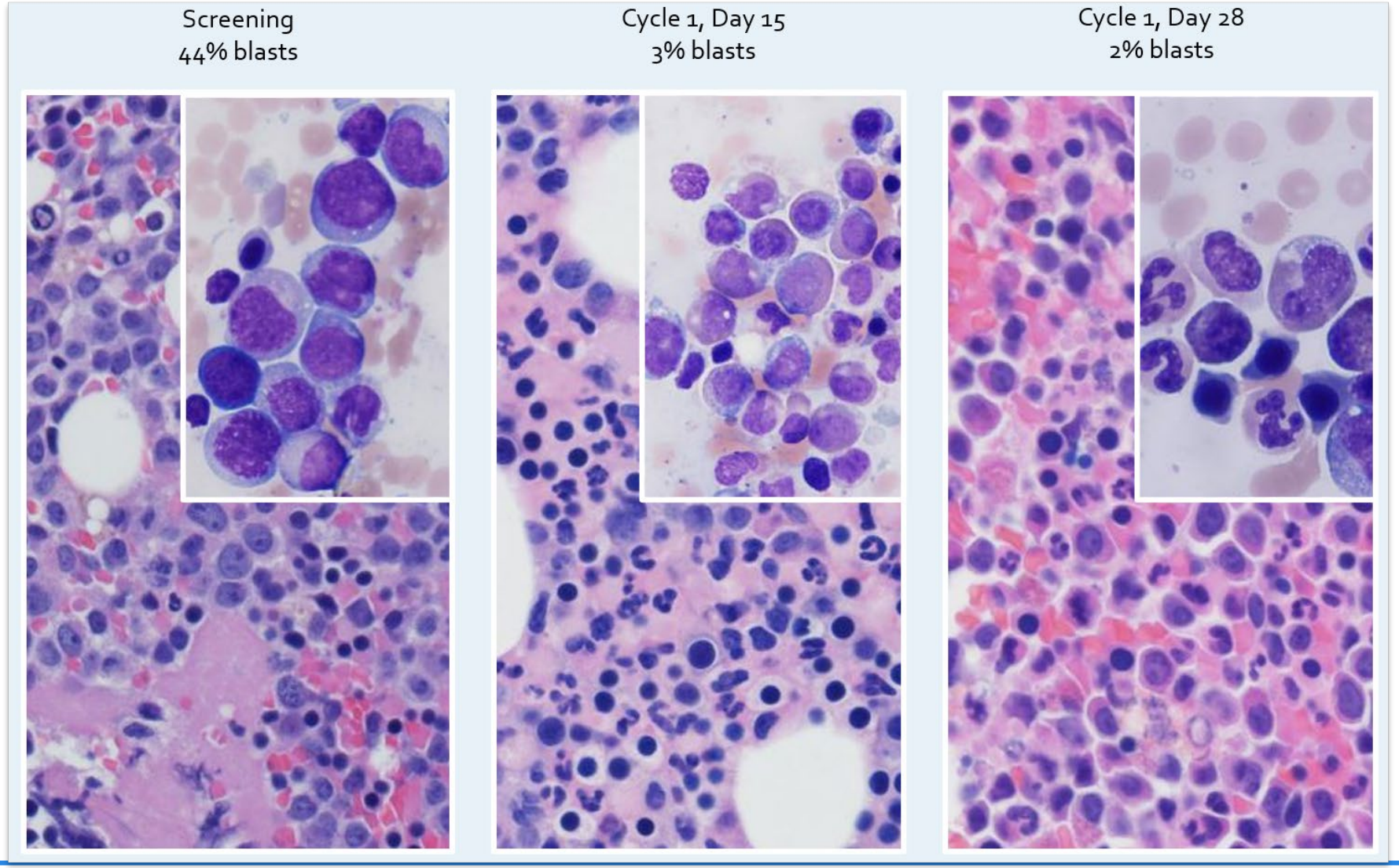


# Enasidenib - Phase 1/2 Study Design (n=345)



# Differentiation Effect in the Bone Marrow

## *Patient Achieved CR by the End of the First Cycle*

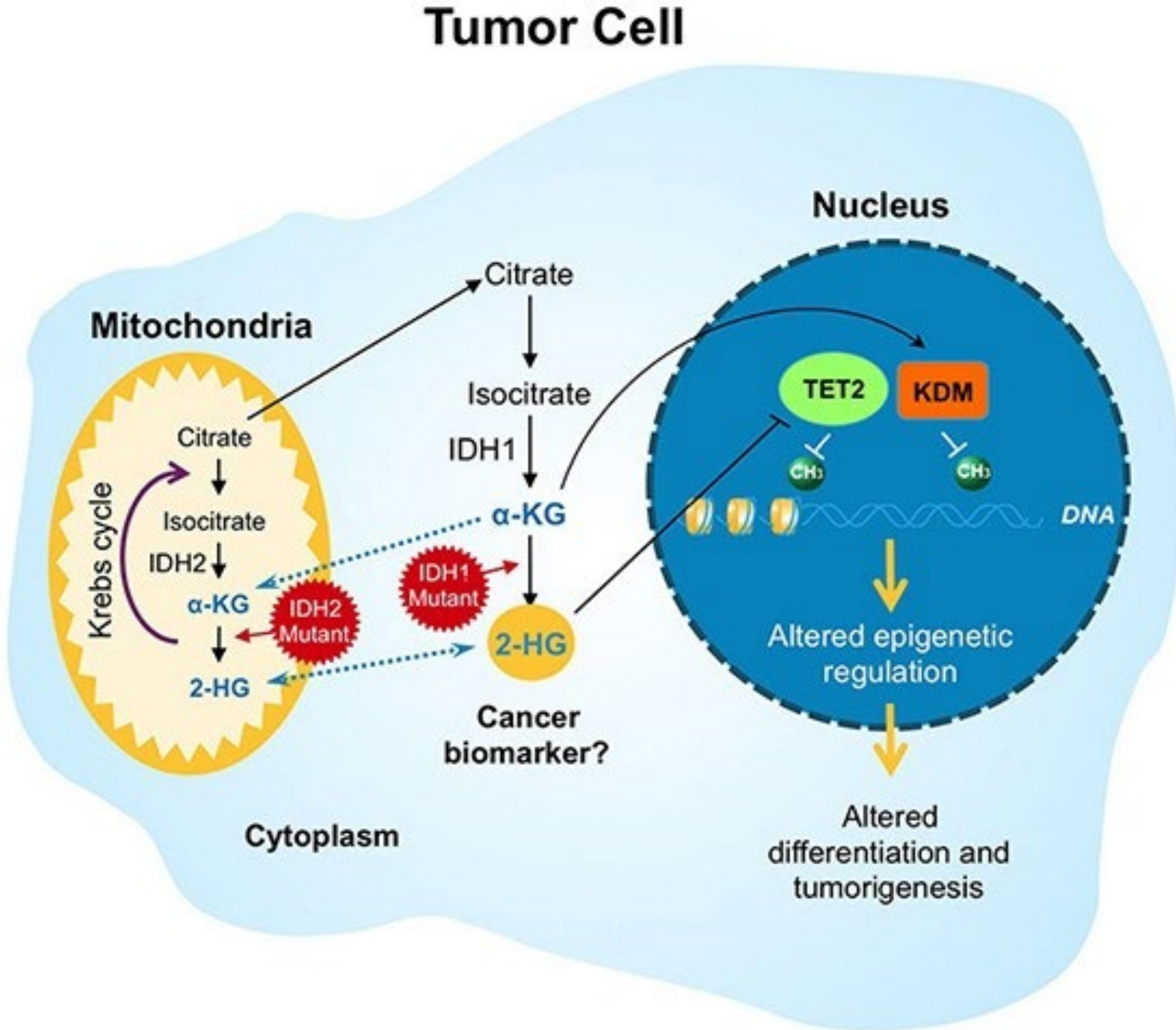


# Efficacy of Enasidenib in R/R AML

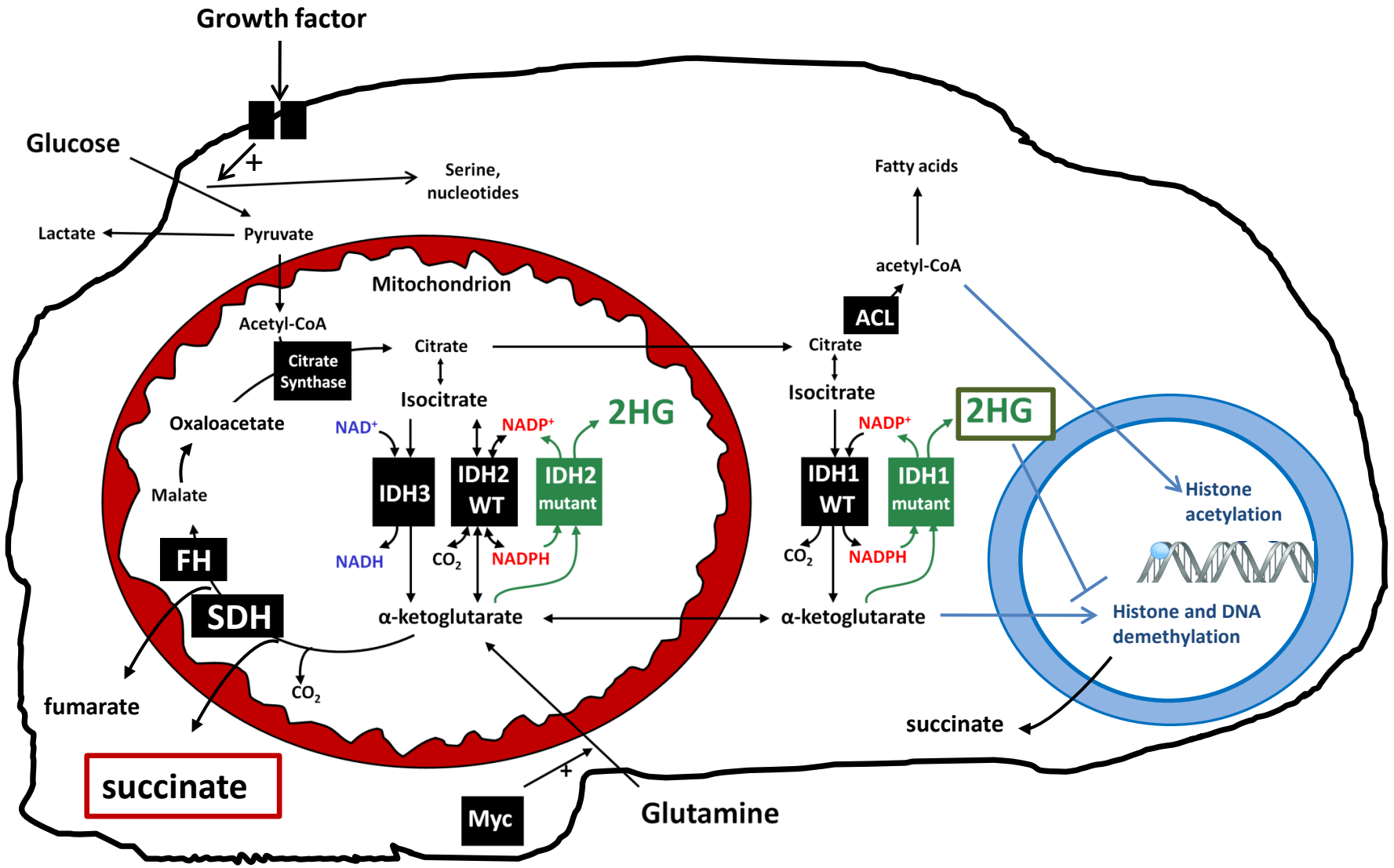
| | Relapsed/Refractory AML | |
|---|---|---|
| | **Enasidenib 100 mg/day (n=214)** | **All patients (N=280)** |
| **Overall response rate (ORR)**,* % (n/N)<br>[95%CI for ORR] | **38.8% (83/214)**<br>[32.2%, 45.7%] | **39.6% (111/280)**<br>[33.9%, 45.6%] |
| CR + CRi/CRp rate, % (n/N) | 29.0% (62/214) | 27.9% (78/280) |
| **Best response** | | |
| Complete remission (CR), n (%)<br>[CR rate 95%CI] | 42 (19.6)<br>[14.5%, 25.6%] | 53 (18.9)<br>[14.5%, 24.0%] |
| CR with incomplete count recovery (CRi/CRp), n (%) | 20 (9.3) | 25 (8.9) |
| Partial remission, n (%) | 9 (4.2) | 17 (6.1) |
| Morphologic leukemia-free state, n (%) | 12 (5.6) | 16 (5.7) |
| Stable disease,† n (%) | 98 (45.8) | 122 (43.6) |
| Progressive disease,‡ n (%) | 19 (8.9) | 26 (9.3) |
| Not evaluable, n (%) | 3 (1.4) | 4 (1.4) |
| **Time to first response**, months, median (range) | 1.9 (0.5-9.4) | 1.9 (0.5-9.4) |
| **Duration of response**, months, median [95%CI] | 5.6 [3.8, 7.4] | 5.6 [4.6, 6.5] |
| **Time to best response,** months, median (range) | 3.7 (0.6-14.7) | 3.7 (0.5-14.7) |
| **Time to CR**, months, median (range) | 3.7 (0.7-14.7) | 3.8 (0.5-14.7) |
| **Overall survival**, months, median [95%CI] | 8.8 [7.7, 9.6] | 8.8 [7.8, 9.9] |
| **Event-free survival,**§ months, median [95%CI] | 4.7 [3.7, 5.6] | 4.6 [3.7, 5.6] |



Stein EM, Dinardo CD, Blood 2017


Tumor Cell
Nucleus
Citrate
Mitochondria
Isocitrate
IDH1
TET2
KDM
Citrate
CH3
CH3
Isocitrate
α-KG
DNA
IDH2
Krebs cycle
IDH1
Mutant
α-KG
IDH2
Mutant
2-HG
2-HG
Altered epigenetic
regulation
Cancer
biomarker?
Cytoplasm
Altered
differentiation and
tumorigenesis

# SDH and Fumarate Hydratase mutations lead to elevated levels of succinate and result in chromatin hypermethylation

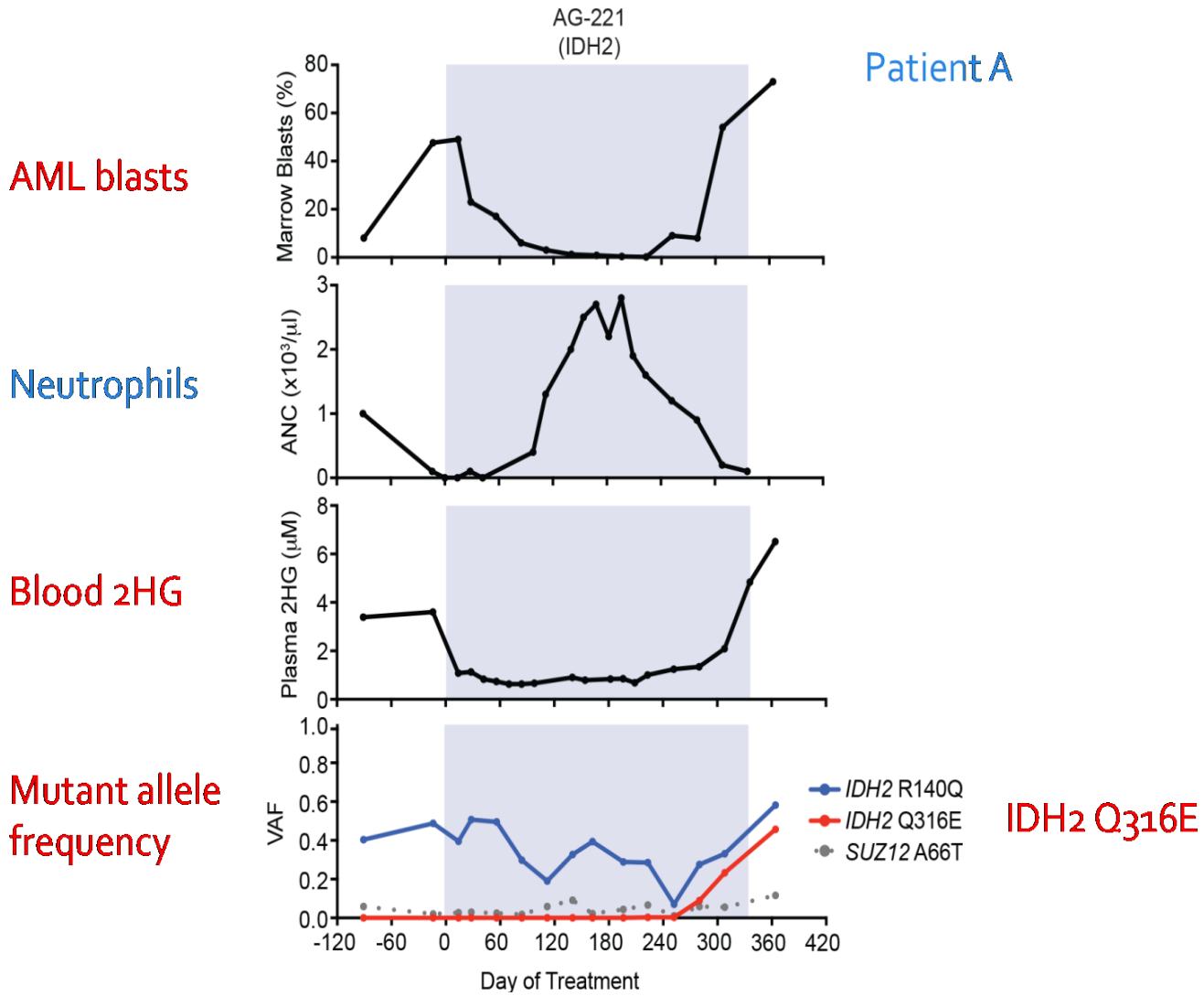


# IDH Resistance Studies

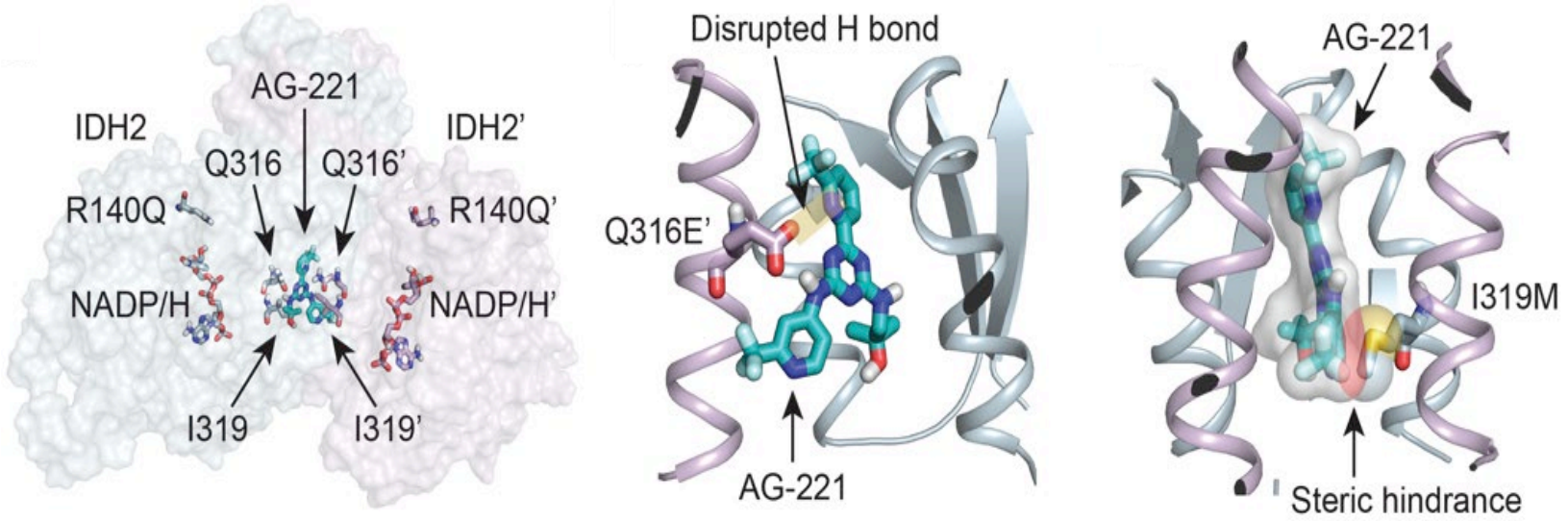
- **Collected serial samples from patients on IDH2/IDH1 inhibitor trials at MSK**
- **Detailed, serial genomic analysis at each timepoint**
- **Followed 2-HG levels including at time of clinical resistance**

**Andy Intlekofer, Alan Shih, Eytan Stein**

# Acquisition of Second Site Mutations in IDH2 at Time of Clinical Resistance



# IDH2 Resistance Mutations Occur at Inhibitor/IDH2 Dimeric Interface



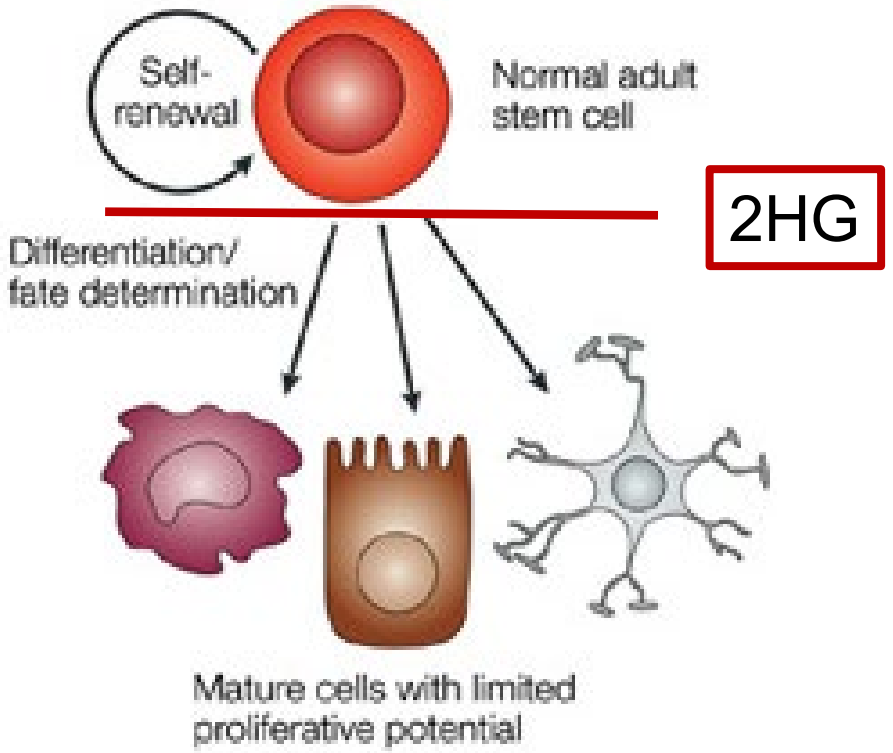

- **Mutations affect residues present in both members of IDH2 dimer**
- **Do the mutations occur in cis with the IDH2 gain-of-function allele, or in trans?**

John Chodera

# Conclusions

- A subset of patients on IDH inhibitor therapy develop acquired resistance with recurrent 2HG elevation
- This is associated with acquisition of second-site mutations which impair drug binding without affecting enzymatic function->allows 2-HG production to resume in presence of drug
- These mutations can occur in trans or in cis, and are seen with IDH2 and IDH1 inhibition
- These data further validate IDH1/2 mutant enzymes as a therapeutic target in AML

# Cancer arises in adult stem cells have the capacity to replace differentiated cells that are lost and/or damaged



RESEARCH SUMMARY

# Vorasidenib in IDH1- or IDH2-Mutant Low-Grade Glioma

Mellinghoff IK et al. DOI: 10.1056/NEJMoa2304194

## CLINICAL PROBLEM

Gliomas, the most common malignant primary brain tumor type in adults, are categorized by histologic and molecular features and by tumor grade. Almost all grade 2 gliomas have mutations in the genes encoding the metabolic enzymes isocitrate dehydrogenase 1 (IDH1) or 2 (IDH2).

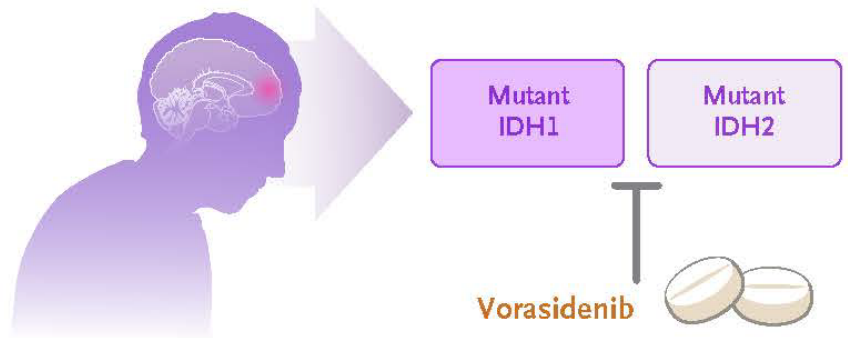
## CLINICAL TRIAL

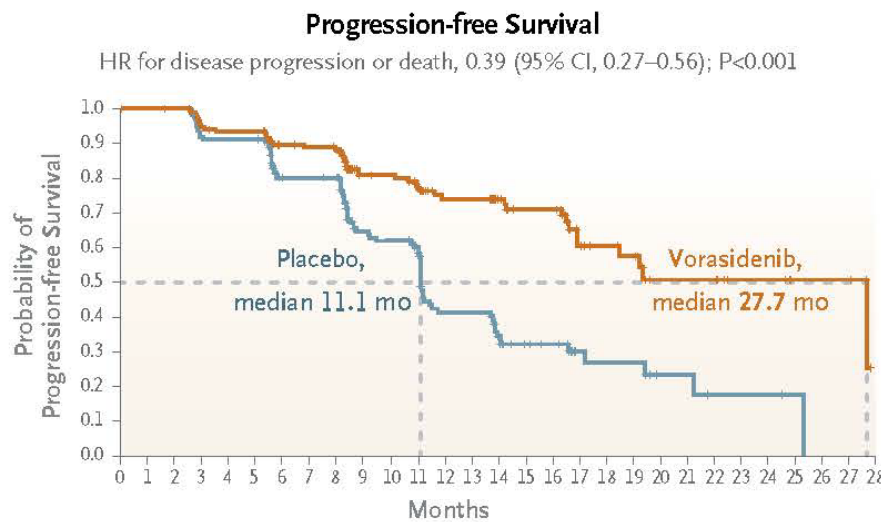
**Design:** This phase 3, double-blind, randomized, placebo-controlled trial tested the clinical effects of vorasidenib — an oral brain-penetrant inhibitor of mutant IDH1 and IDH2 enzymes — in patients with residual or recurrent grade 2 IDH-mutant glioma who had undergone surgery as their only previous treatment.

**Intervention:** 331 patients were assigned to receive oral vorasidenib (40 mg once daily) or matched placebo in 28-day cycles. The primary end point was imaging-based progression-free survival.

## RESULTS

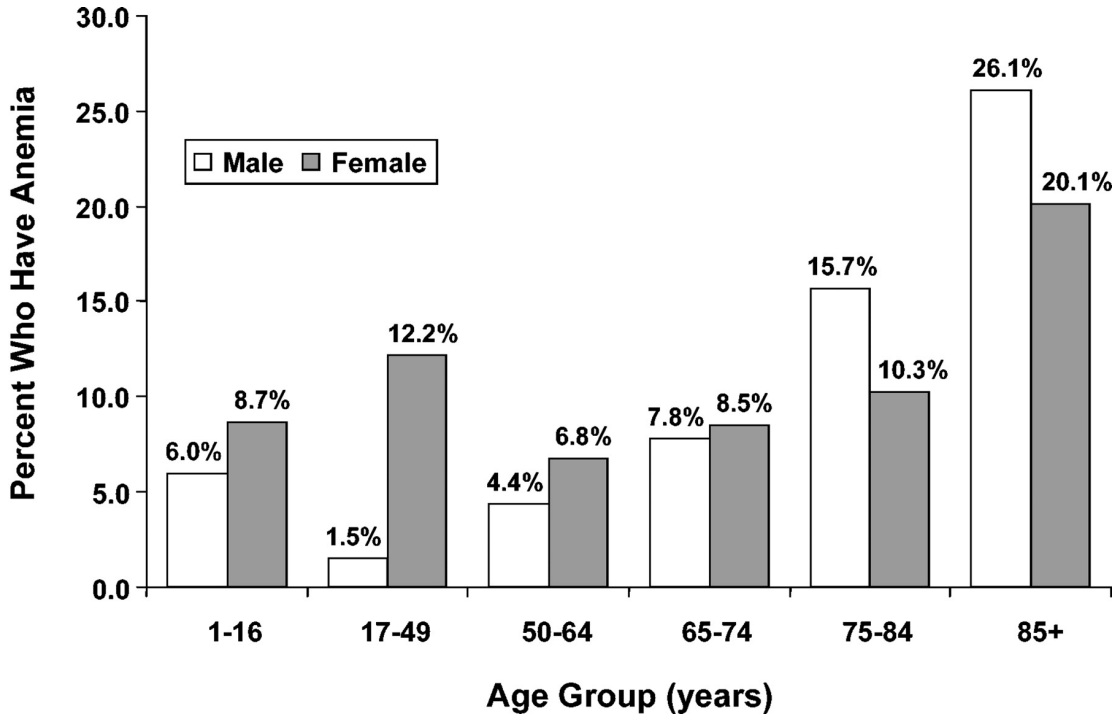
**Efficacy:** Progression-free survival was significantly longer with vorasidenib than with placebo.



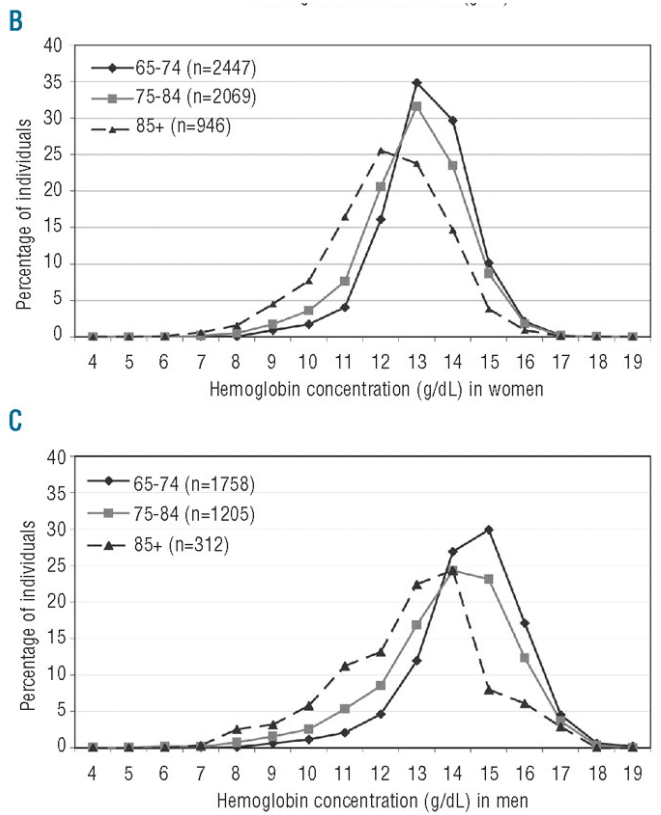



# Precision Therapy to prevent malignancy:

## Cytopenias are VERY common in the elderly




Jack et al., Blood 2004



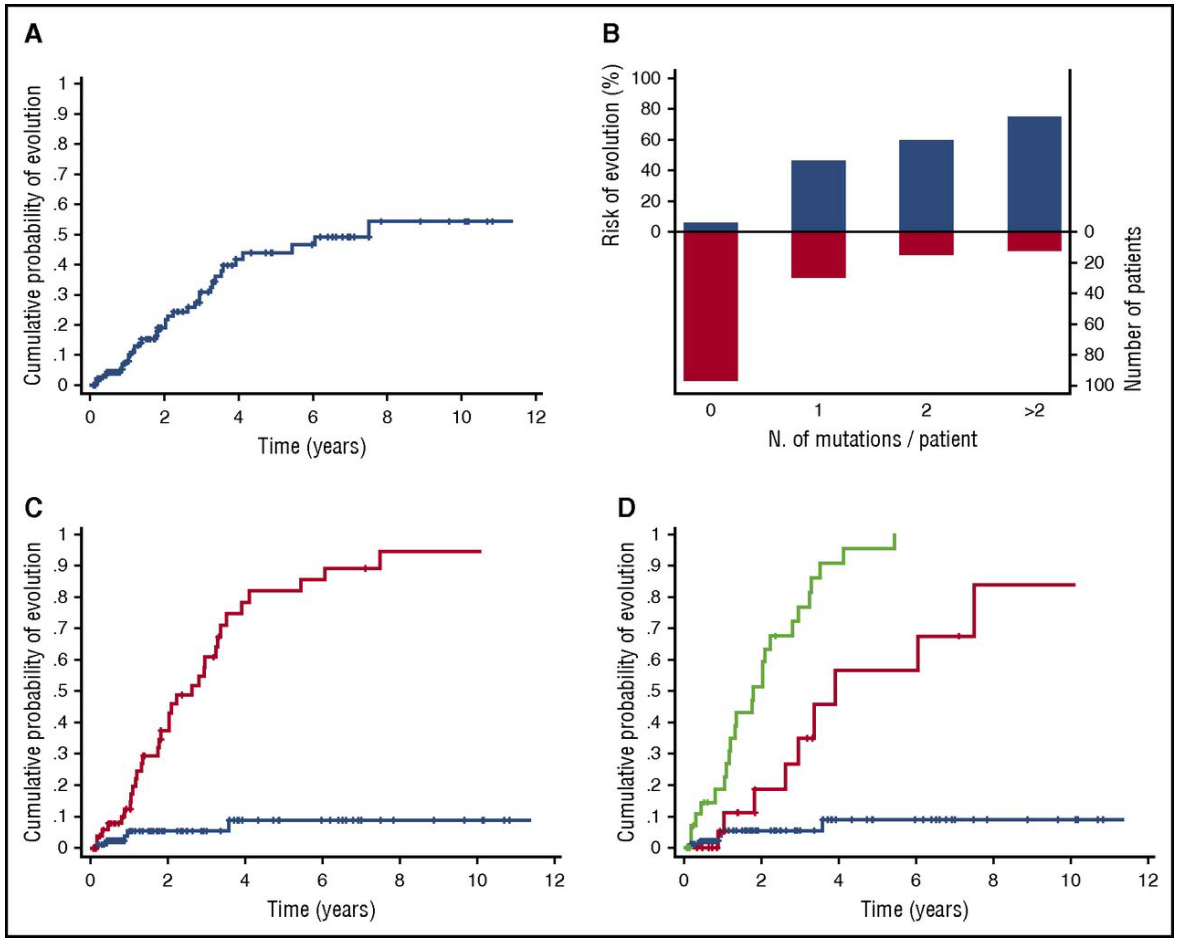

Tettamanti et al., Haematologica 2010

# Clonal Cytopenias of Uncertain Significance (CCUS) almost always progresses to malignancy

**154 patients with cytopenias of unknown origin**

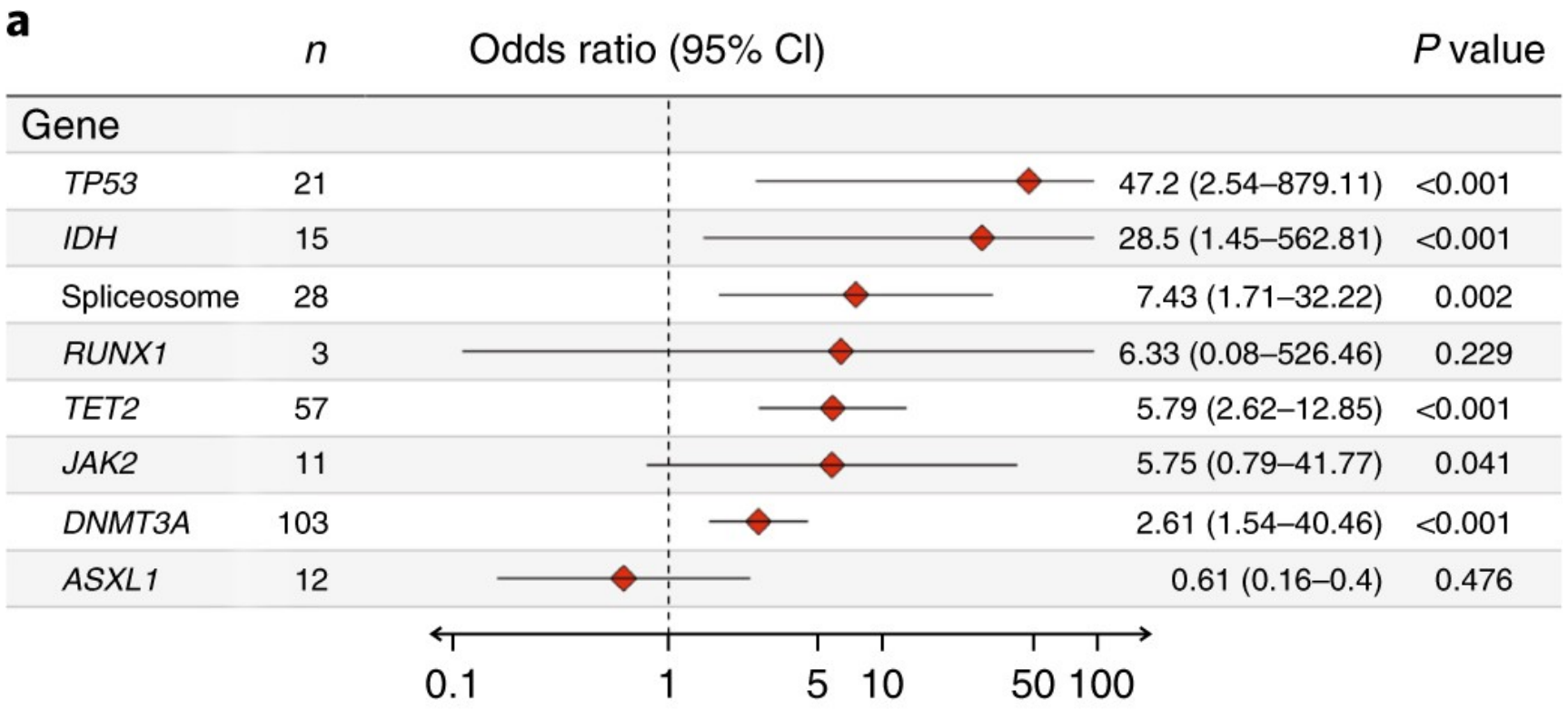
**Followed for 10 years**

**<u>95% of CCUS</u> developed a malignancy**




Malcovati et al., Blood 2017

# Cytopenic patients with IDH1/2 mutations carry a high risk leukemic progression




Desai et al., Nature Medicine 2018

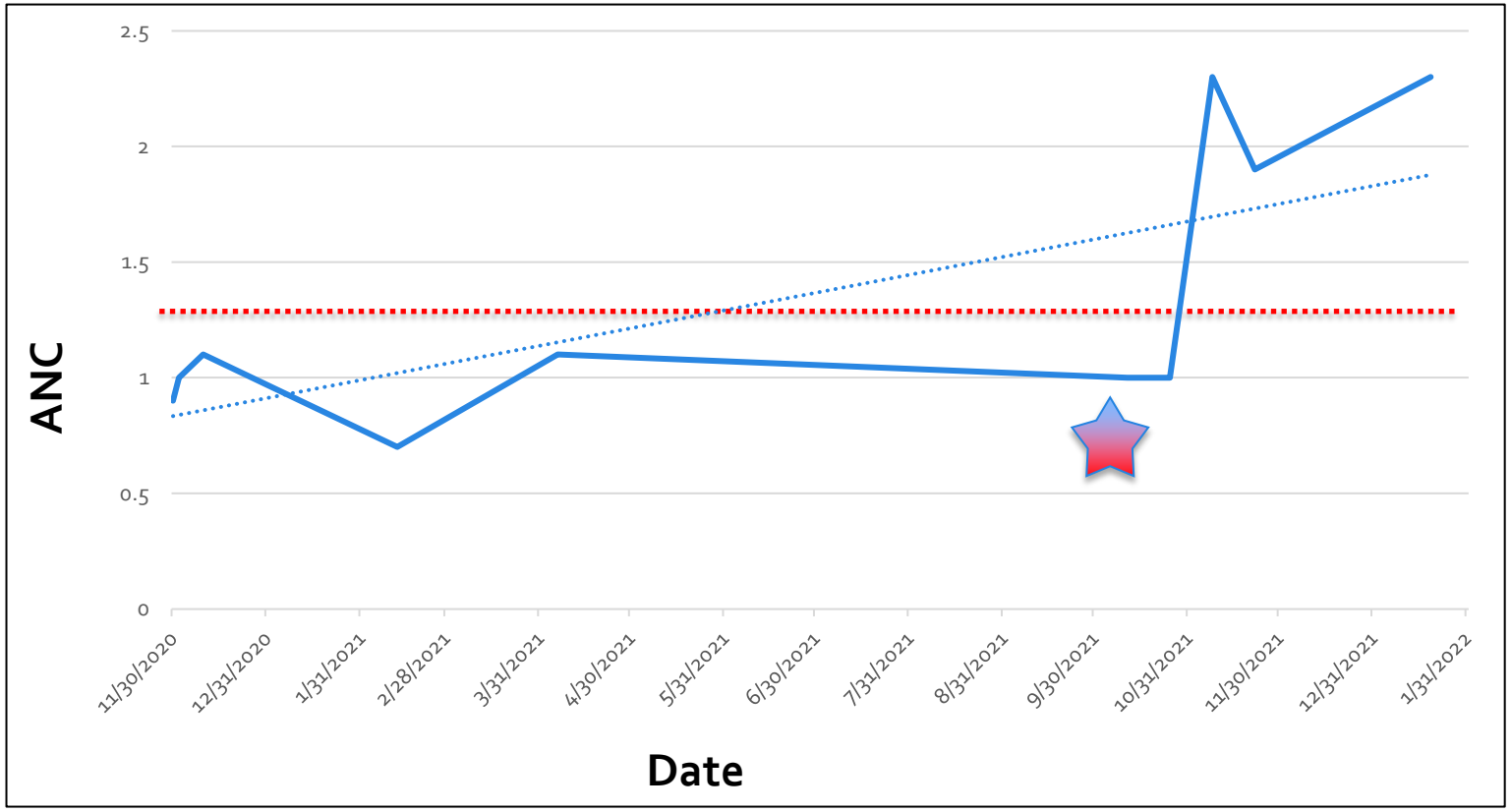
Memorial Sloan Kettering Cancer Center

# A Pilot Study of IDH2 Inhibitors for Patients with Clonal Cytopenia of Undetermined Significance and Mutations in IDH2


Eytan M. Stein, MD
Associate Attending Physician
Director, Program for Drug Development in Leukemia
Leukemia Service, Department of Medicine
Memorial Sloan Kettering Cancer Center
New York, New York

Kelly Bolton, MD, Ph.D.
Assistant Professor
Division of Biology and Biomedical Sciences
Washington University School of Medicine
Saint Louis, Missouri

# Neutropenia responding to Enasidenib



# Summary

- The IDH2 inhibitor enasidenib is well-tolerated and safe
- Patients with CCUS and IDH2 mutations almost certainly progress to AML/MDS: excellent opportunity for chemoprevention
- The first study using targeted therapy to prevent myeloid neoplasms
- Promising response observed and ongoing correlative work will hopefully provide a foundation for larger-scale, long-term studies of enasidenib in IDH2-mutant CCUS