A Role for ABC Transporters and Porphyrin Metabolism Leukemia ?

John D. Schuetz

St. Jude Childern's Research Hospital
Memphis, TN 38105

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Solvo Biotechnology Transporter Conference

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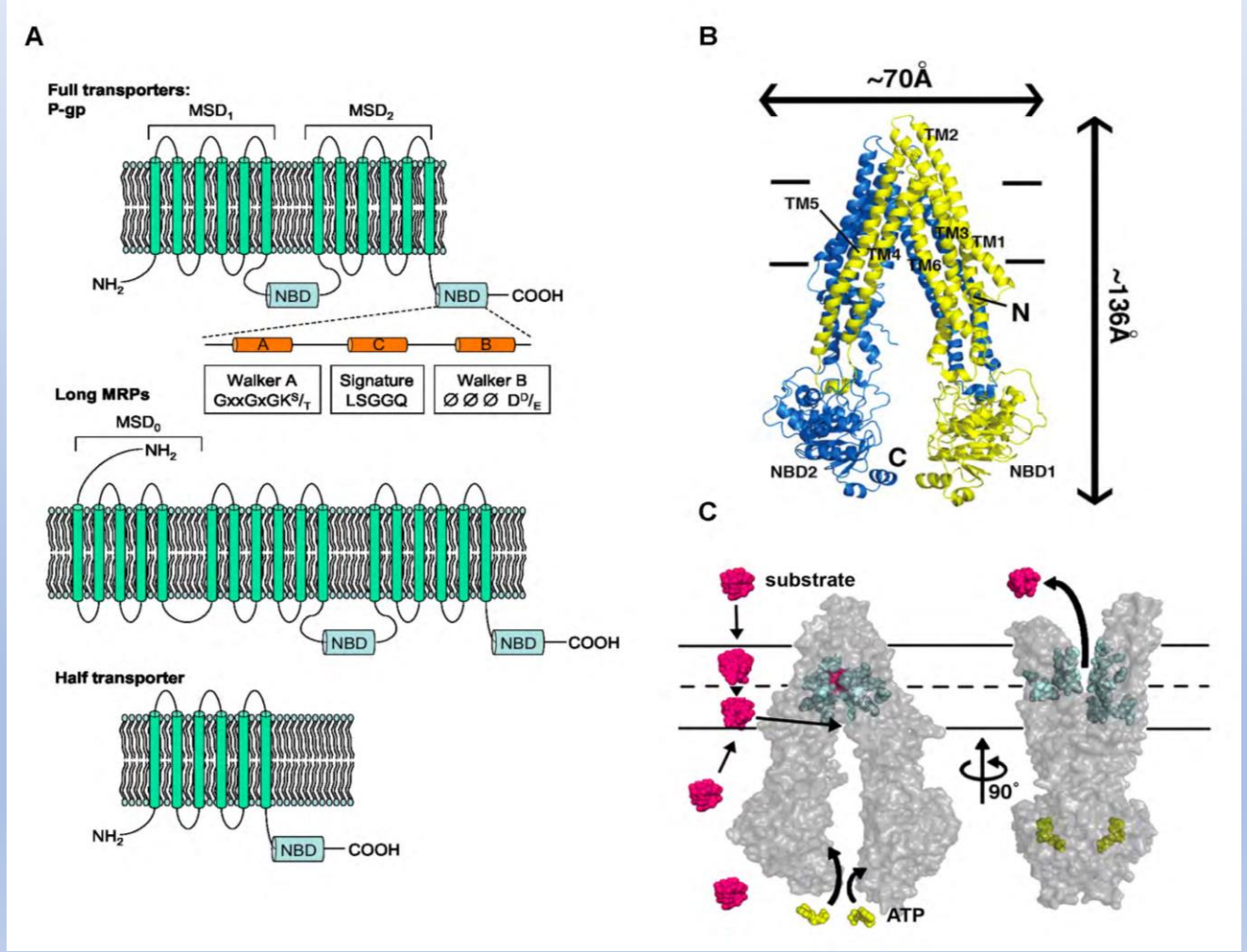
St. Jude Children's Research Hospital
ALSAC • Danny Thomas, Founder
Finding cures. Saving children.

Outline

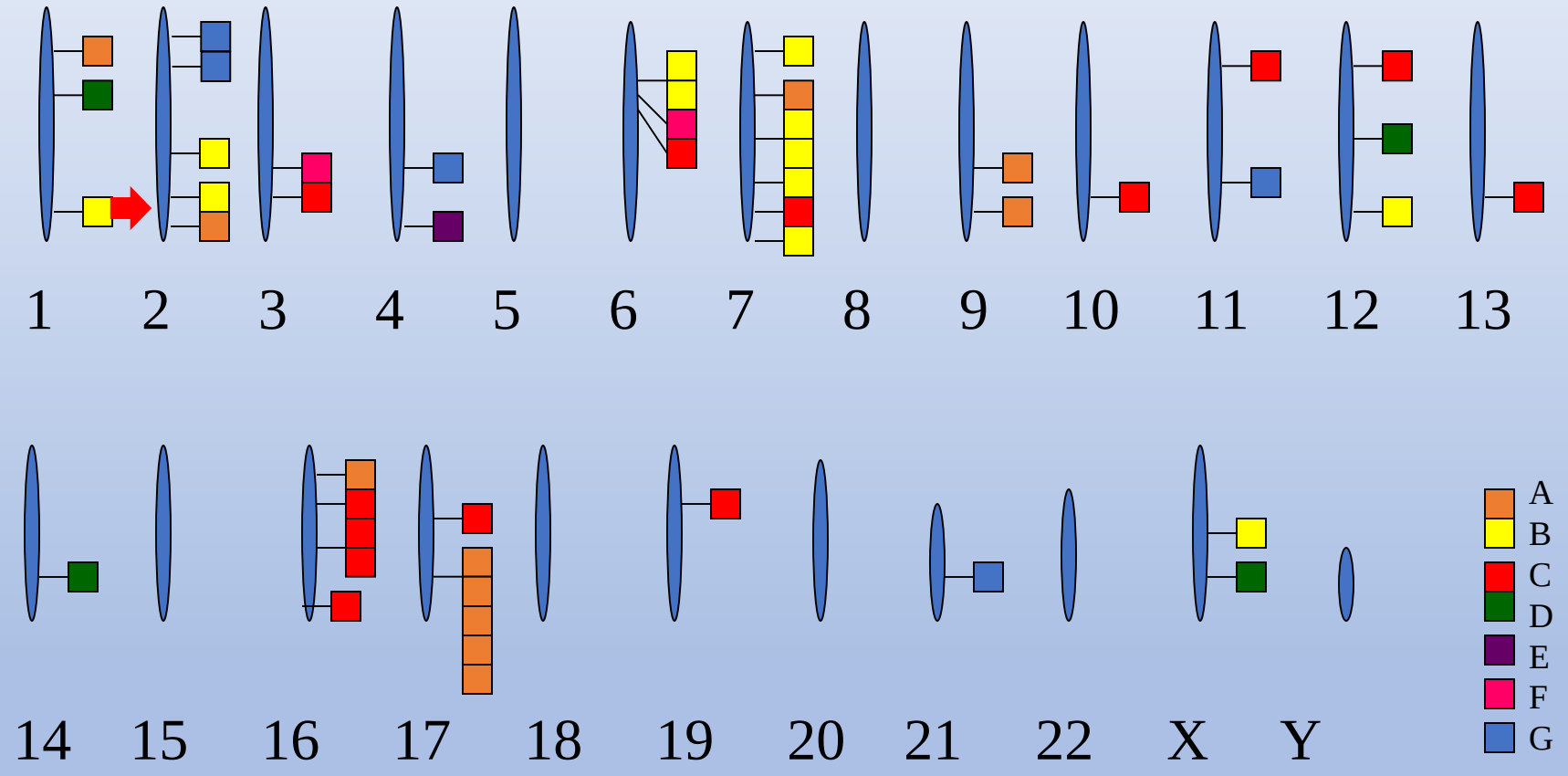
- Intro-ABC transporters
- ABCG2 in acute myeloid leukemia (AML)
- Heme/porphyrin synthesis
- PPIX mechanism of death
- Identification of dual function ABCG2 inhibitors

ABC transporters have two primary functional domains



Fukuda & Schuetz Bioch Pharm 2012

48 Human ABC Genes



Acute myeloid leukemia (AML) is a disease of altered hematopoiesis in myeloid lineage

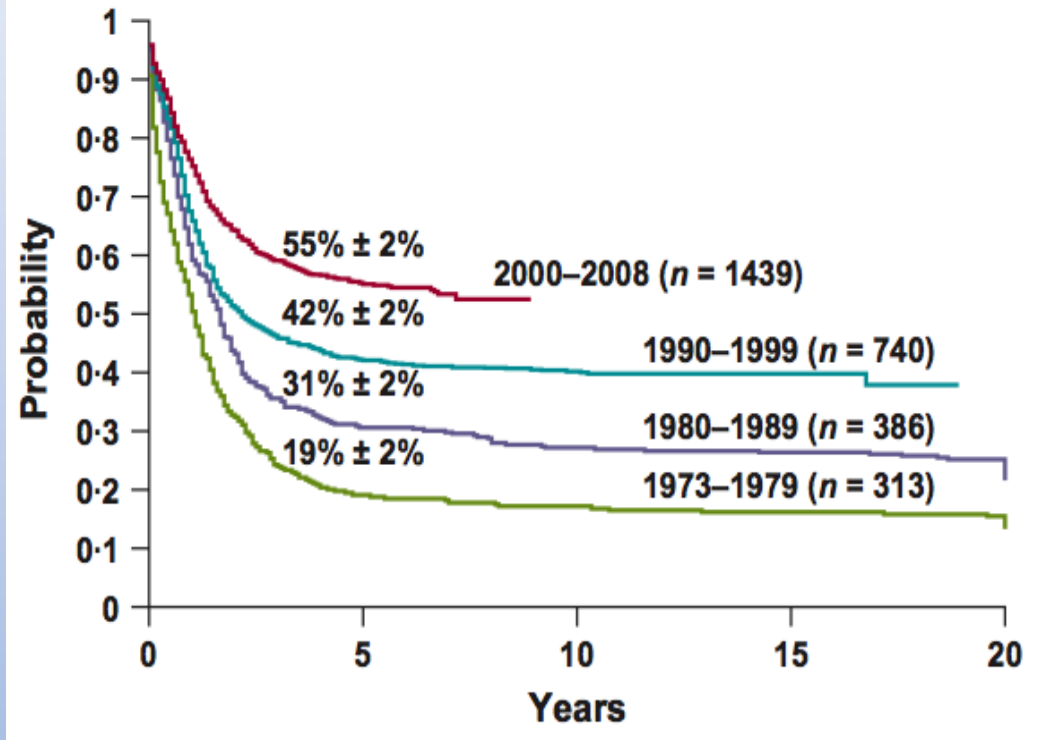
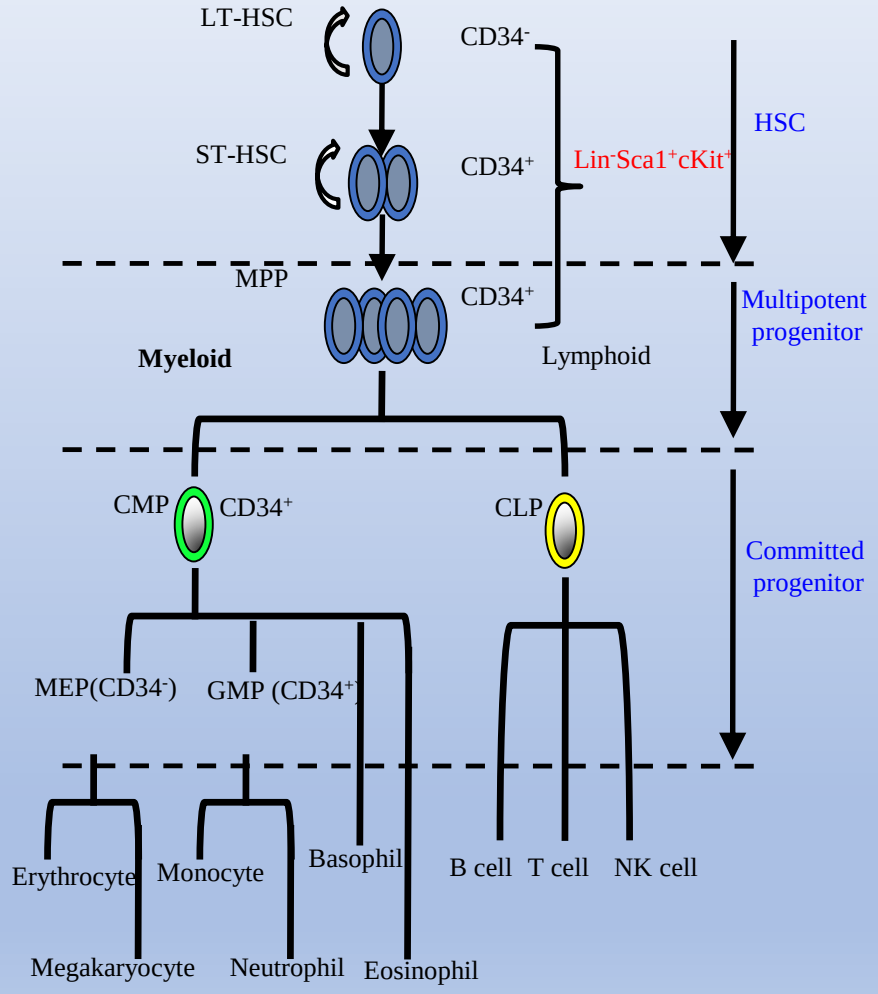
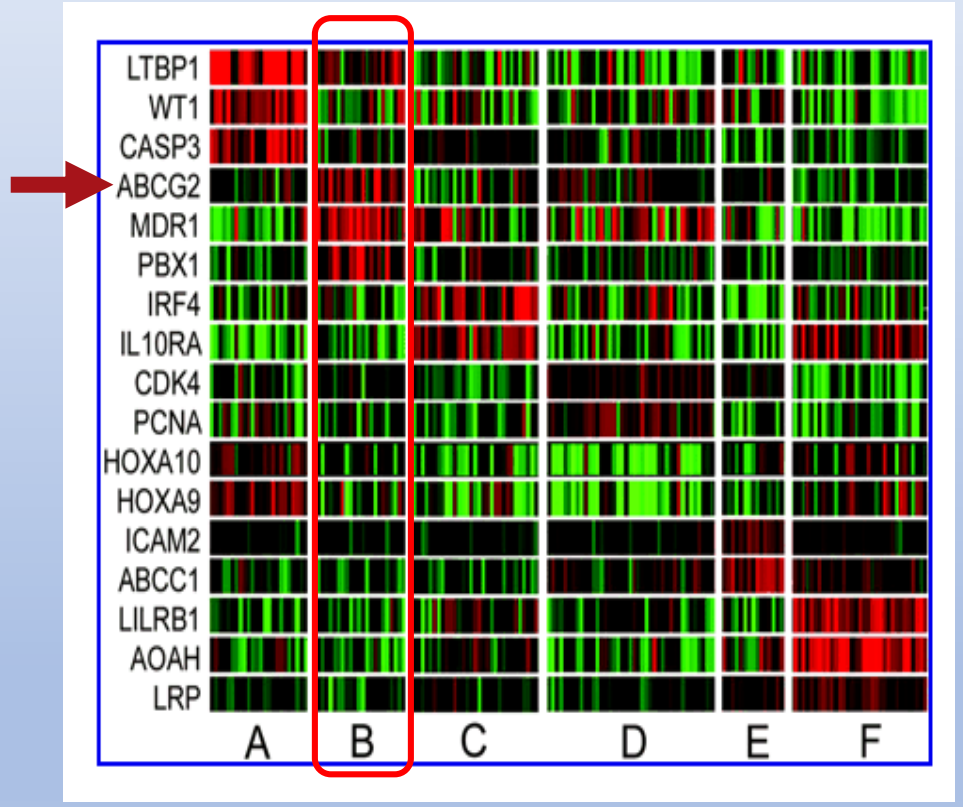


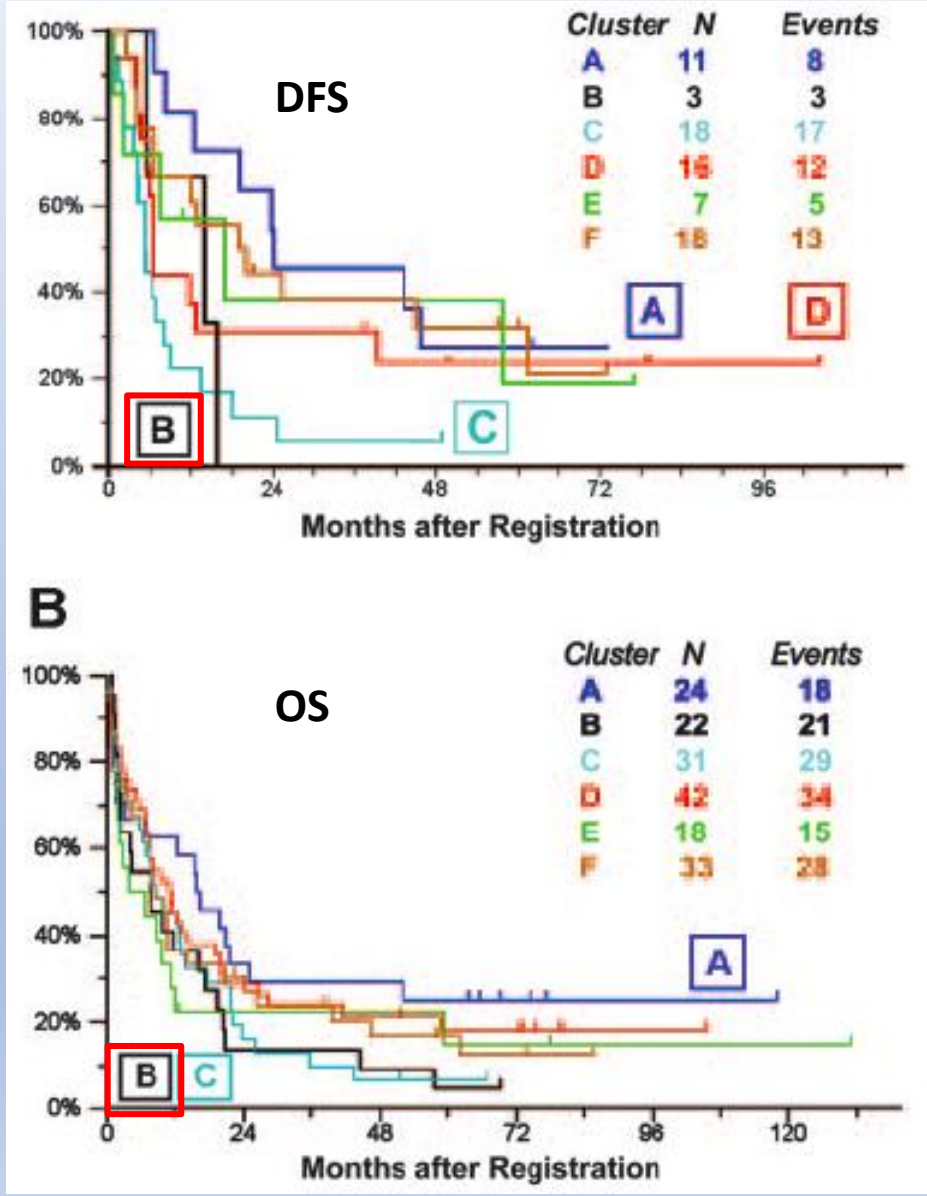
Fig 1. Overall survival of patients who were <20 years of age and diagnosed with AML during the time periods indicated. The data were obtained from www.seer.cancer.gov/popdata.



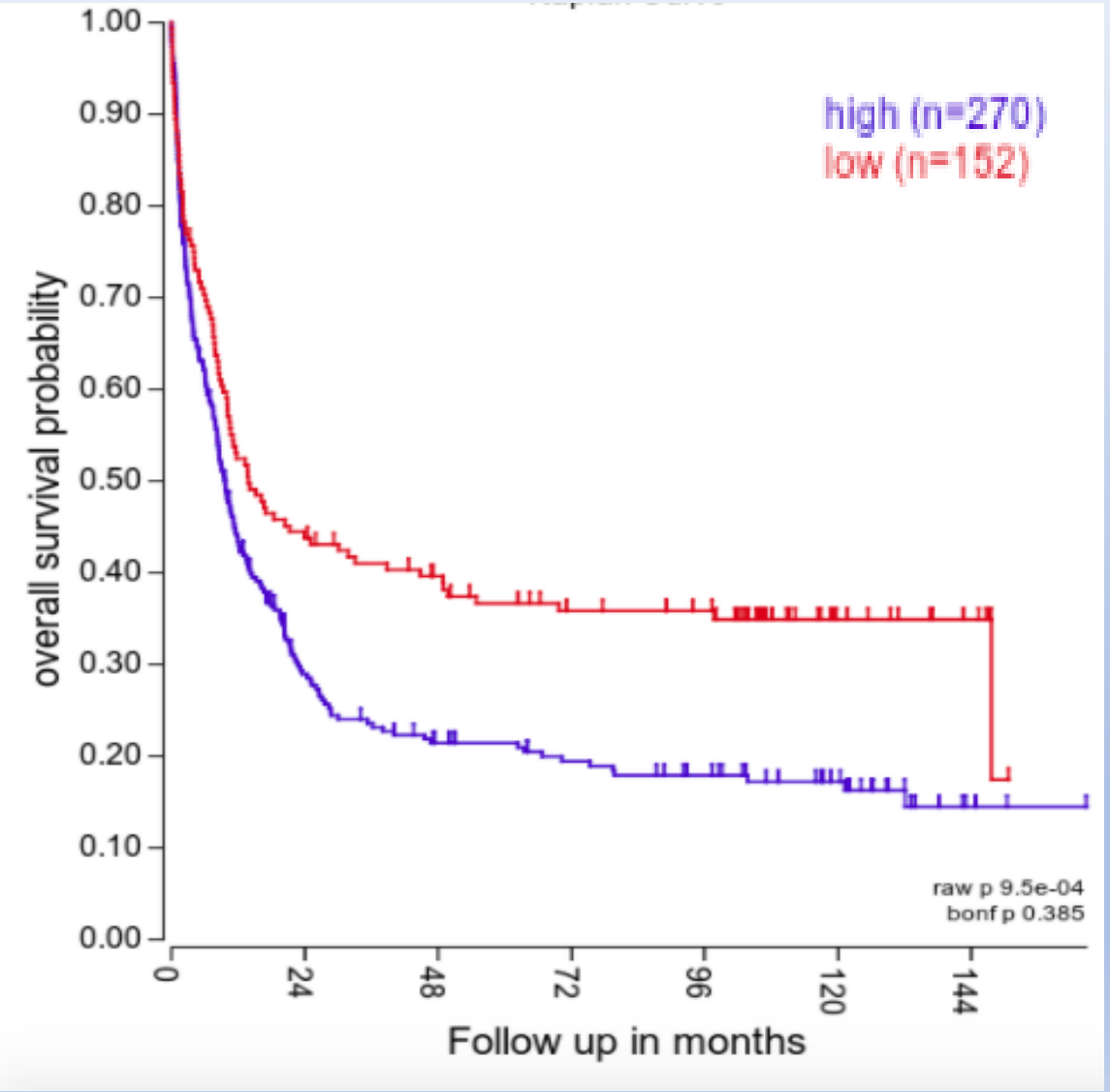
ABCG2 is highly expressed among AML patients with poor disease-free and overall survival



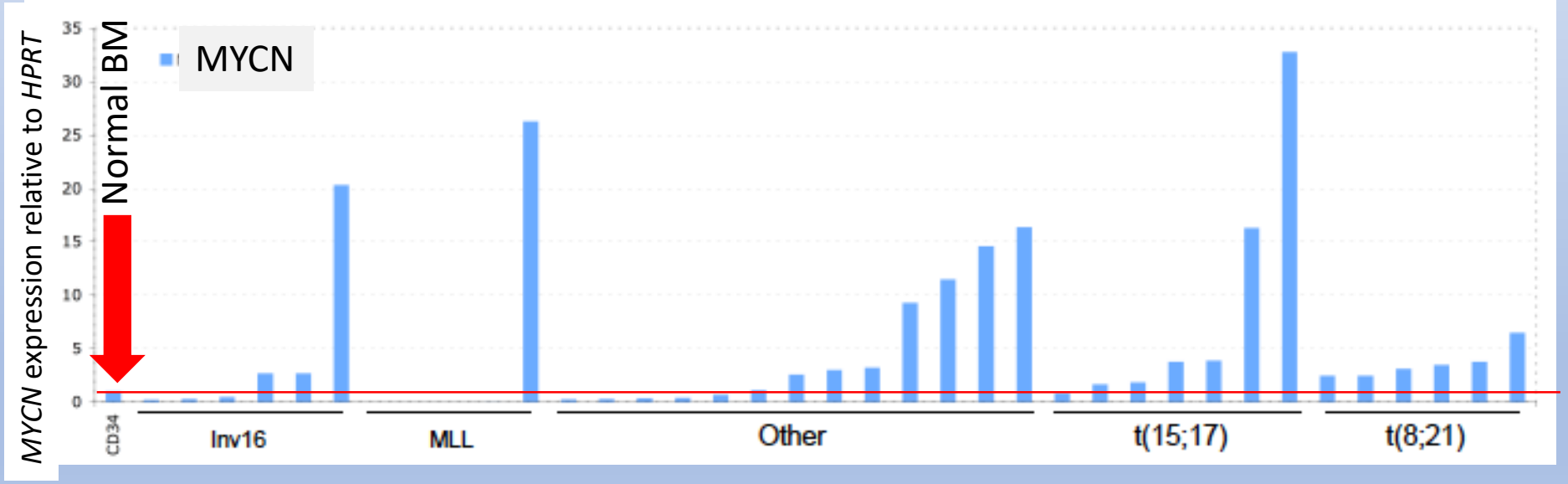
Blood 2006



ABCG2 alone is a poor prognostic factor in AML

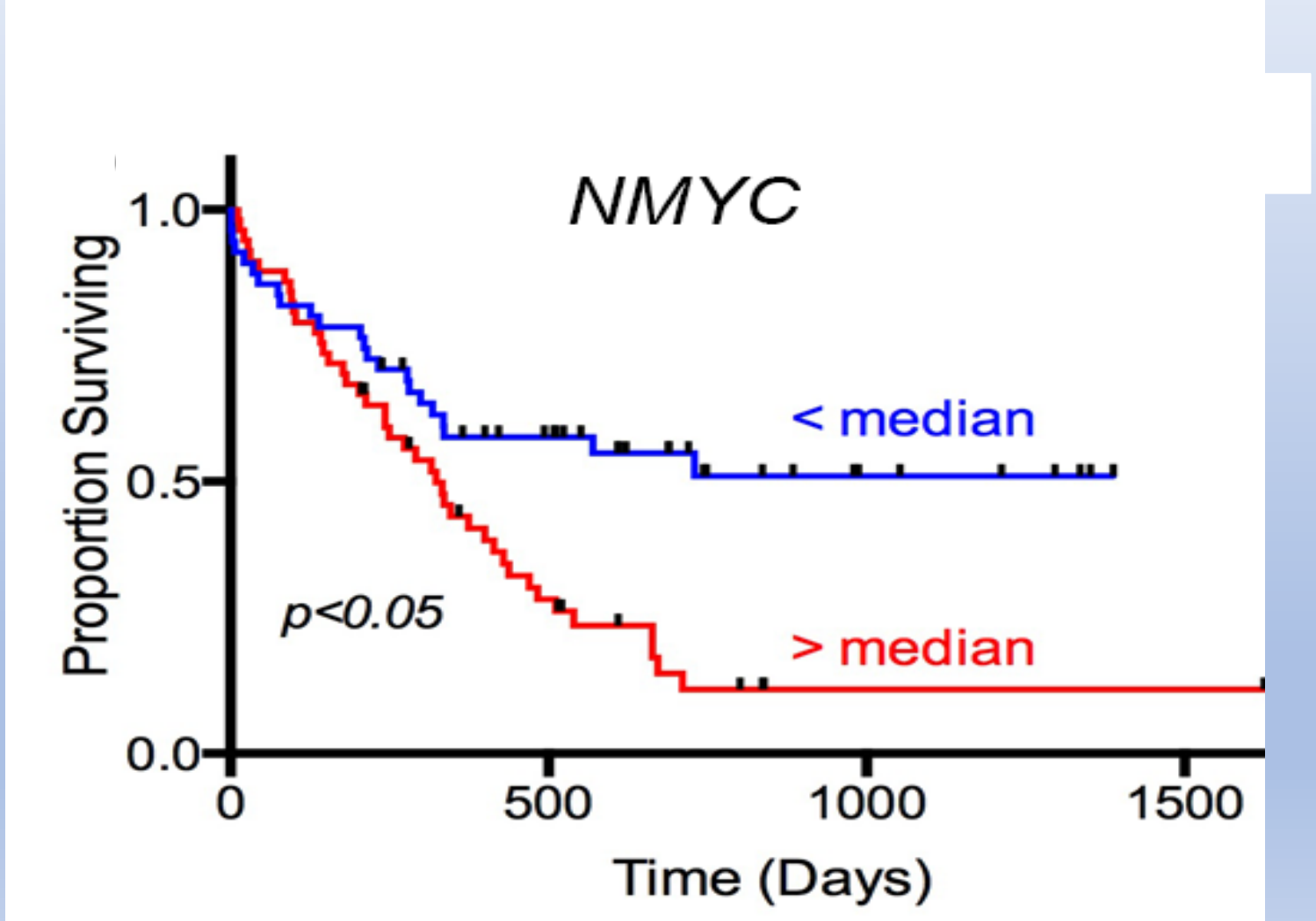


MYCN is a transcription factor highly expressed in pediatric AML



Kawagoe et al., Cancer Res. 2007

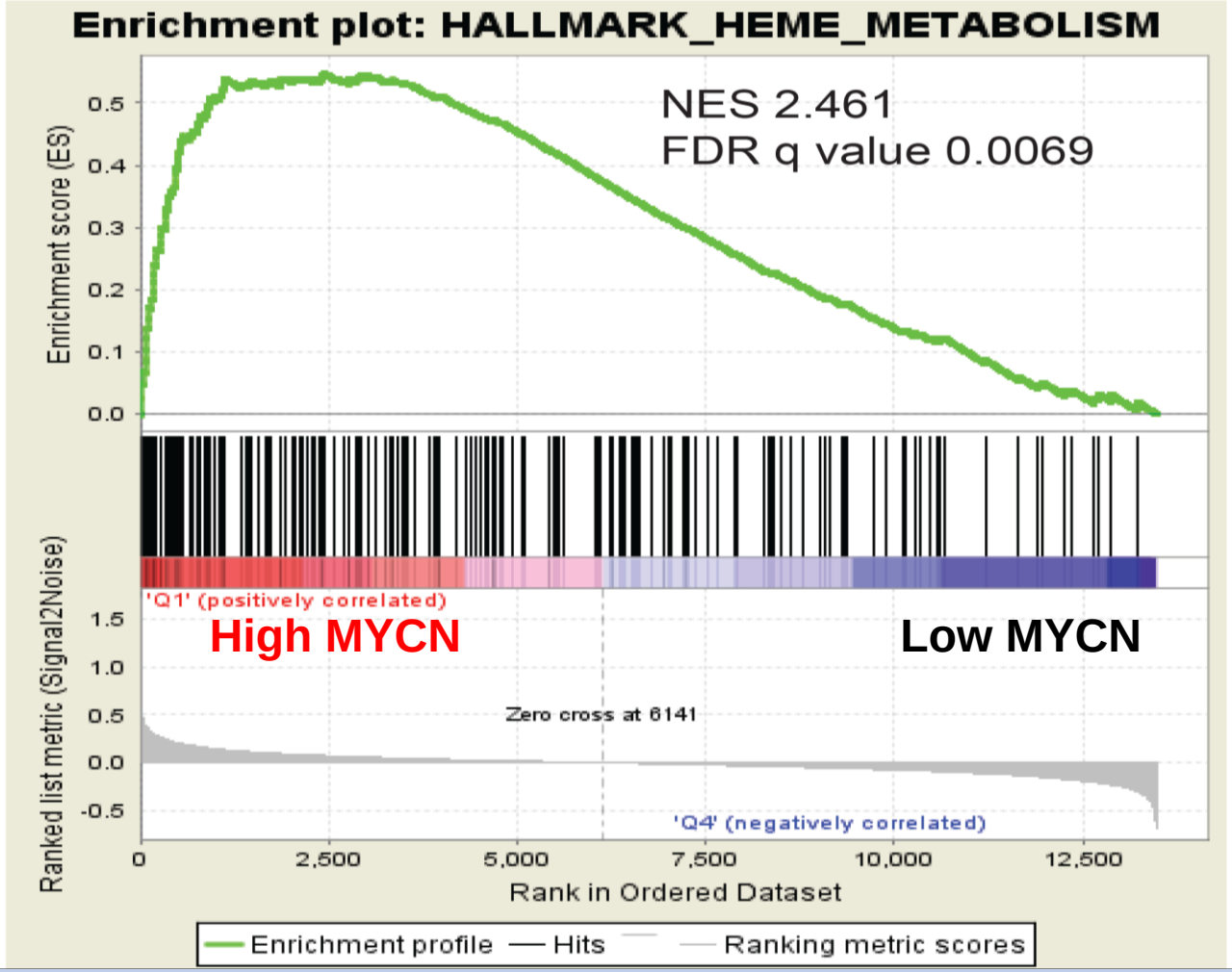
MYCN is a poor prognostic factor in AML



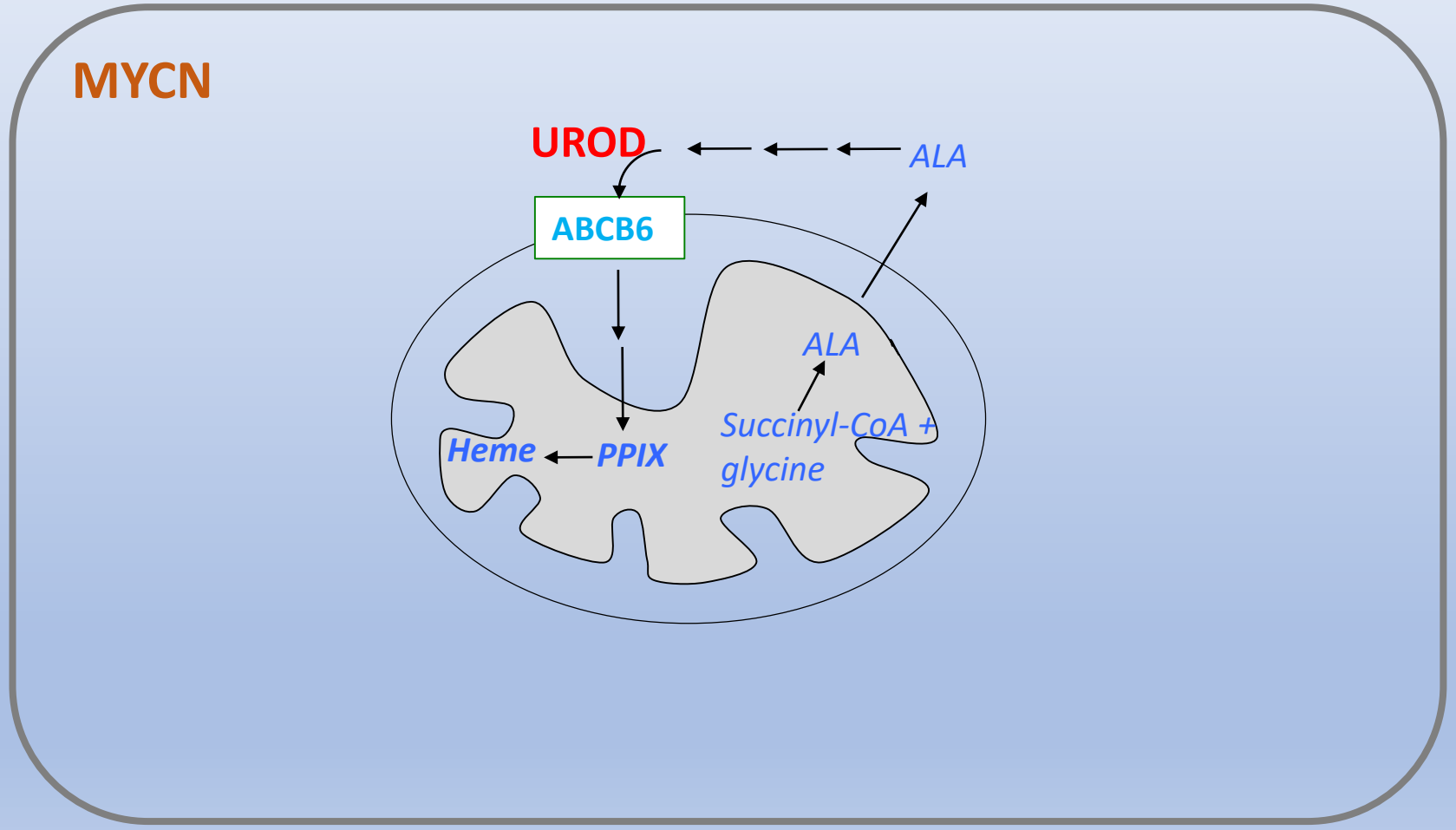
www.oncomine.org

Does MYCN produce metabolic effects?

GSEA reveals upregulated heme metabolism in pediatric AML with high MYCN expression

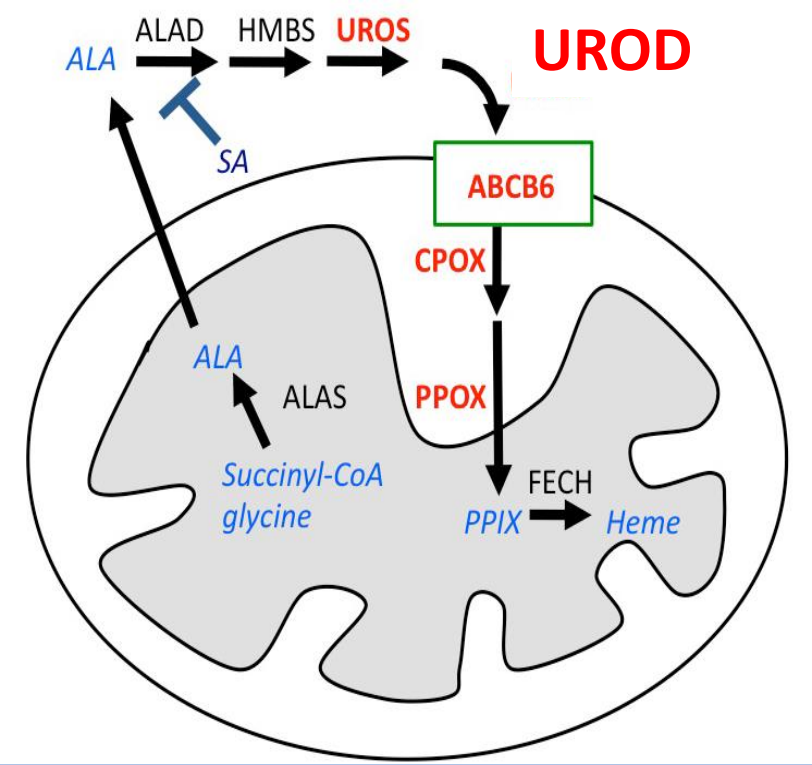


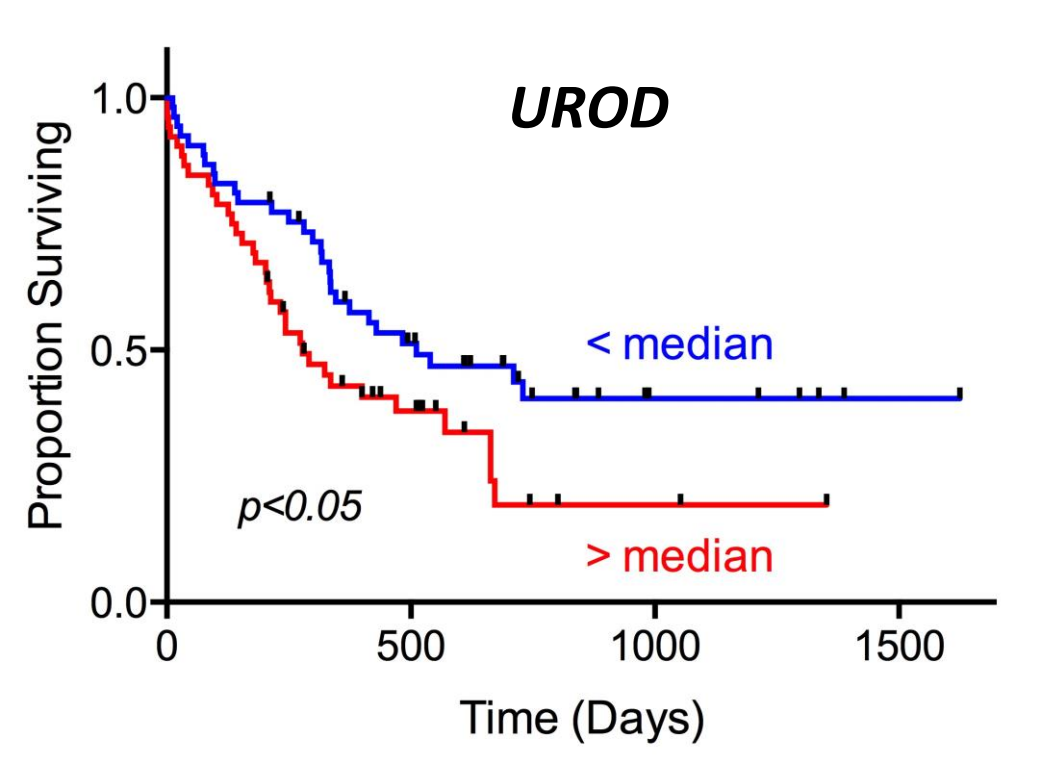
UROD was the most elevated <u>heme pathway</u> gene in High MYCN adult and pediatric AML



Fukuda et al JCI-Insight, 2017

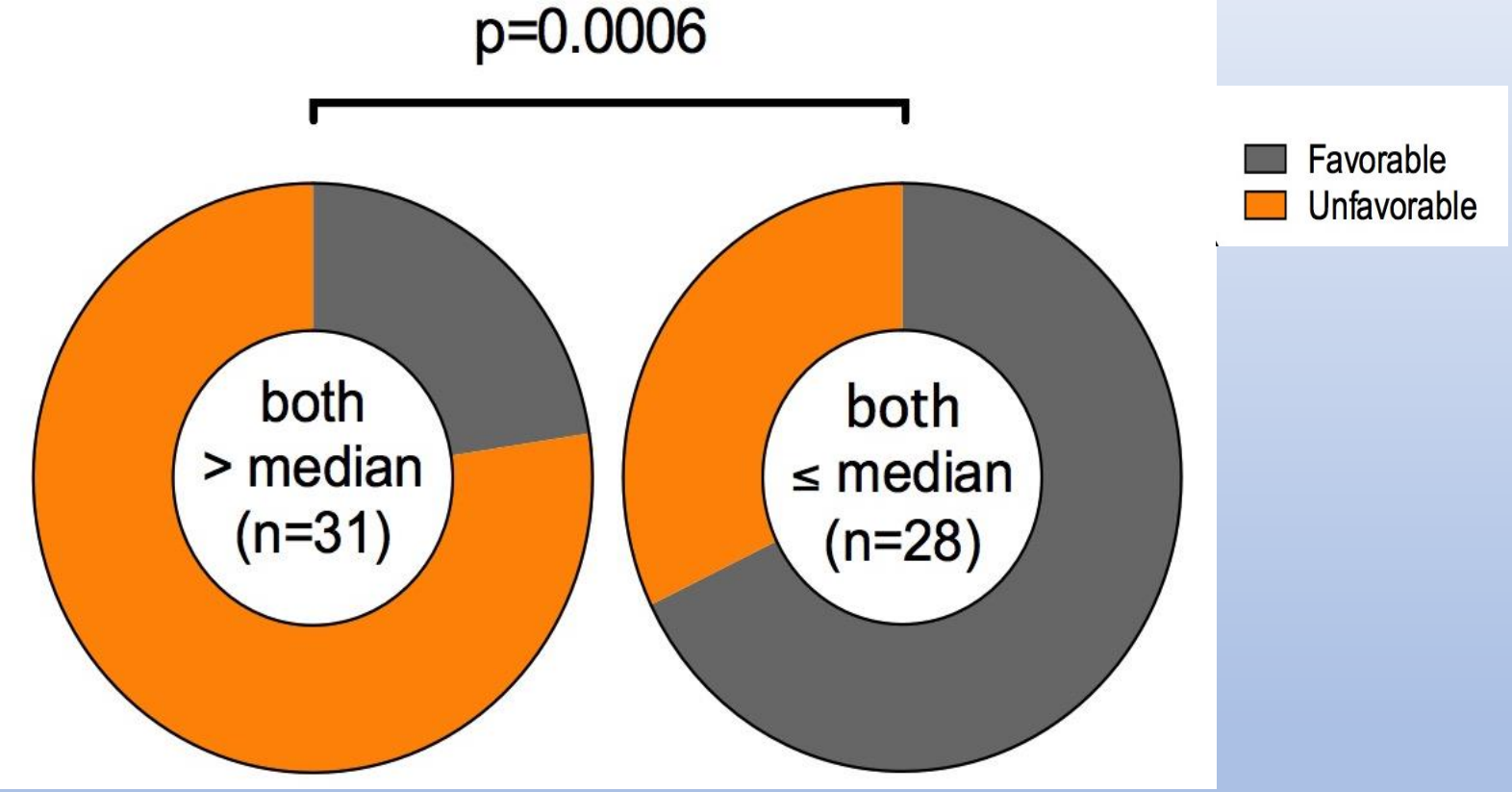
Porphyrin biosynthesis is a poor prognostic factor in adult AML





www.oncomine.org

High *MYCN* and *UROD* expression increases unfavorable outcome in adult AML

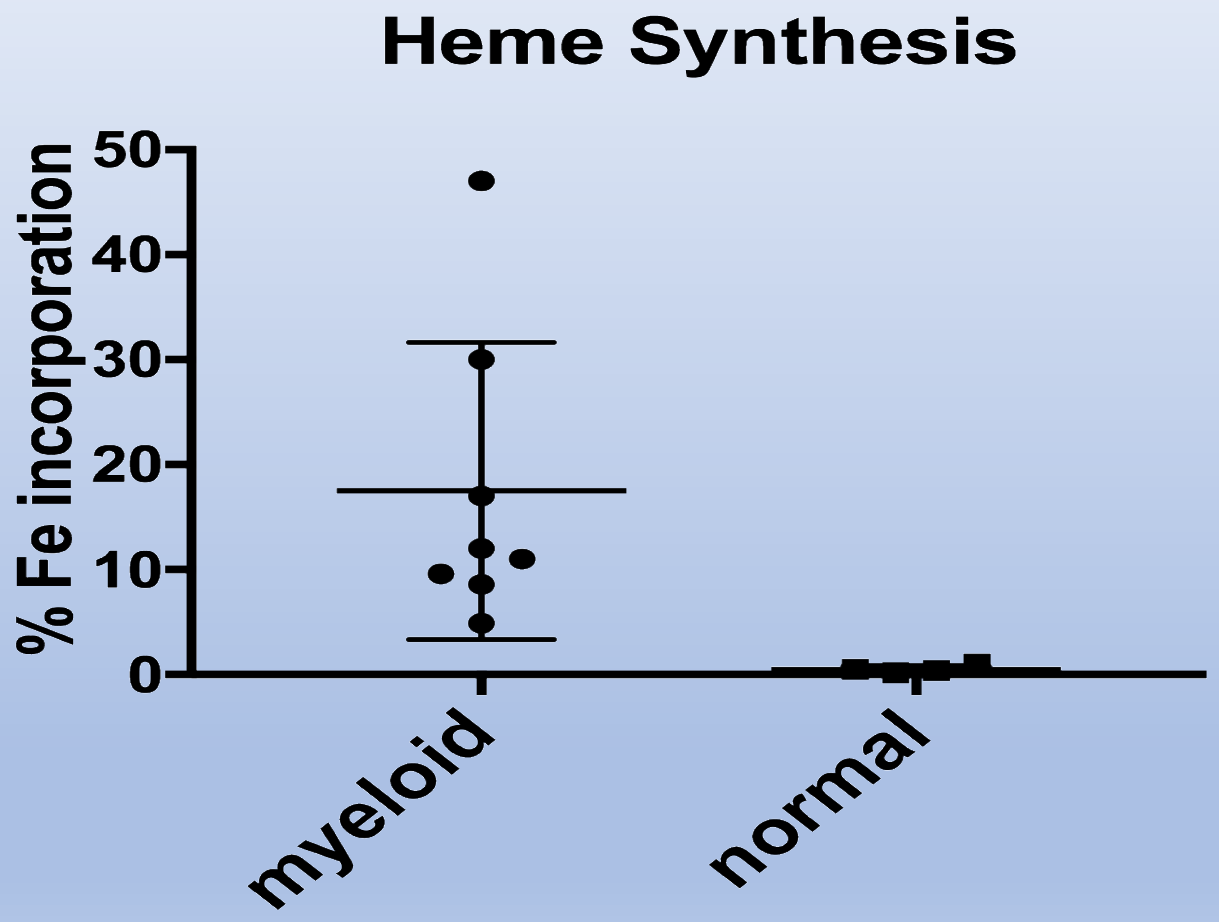


Favorable: complete remission
Unfavorable: relapse and refractory disease

Oncomine.org

Fukuda et al JCI-Insight, 2017

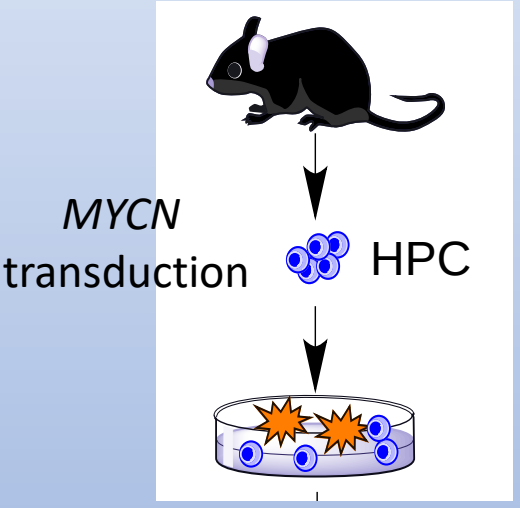
AML patients have increased heme synthesis

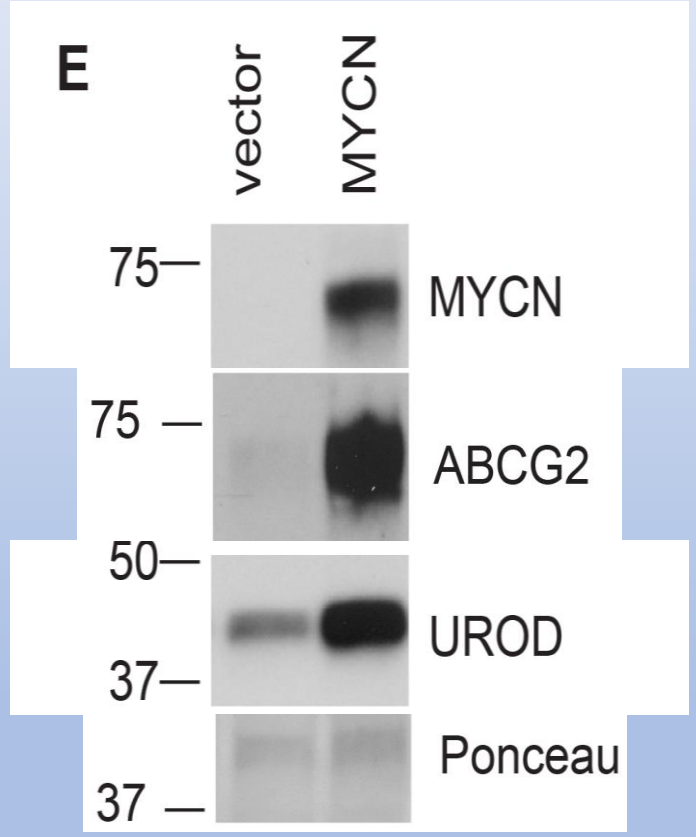


1967 *Cancer* TR Walters et al pg 1117

What is the purpose of increased heme biosynthesis ?

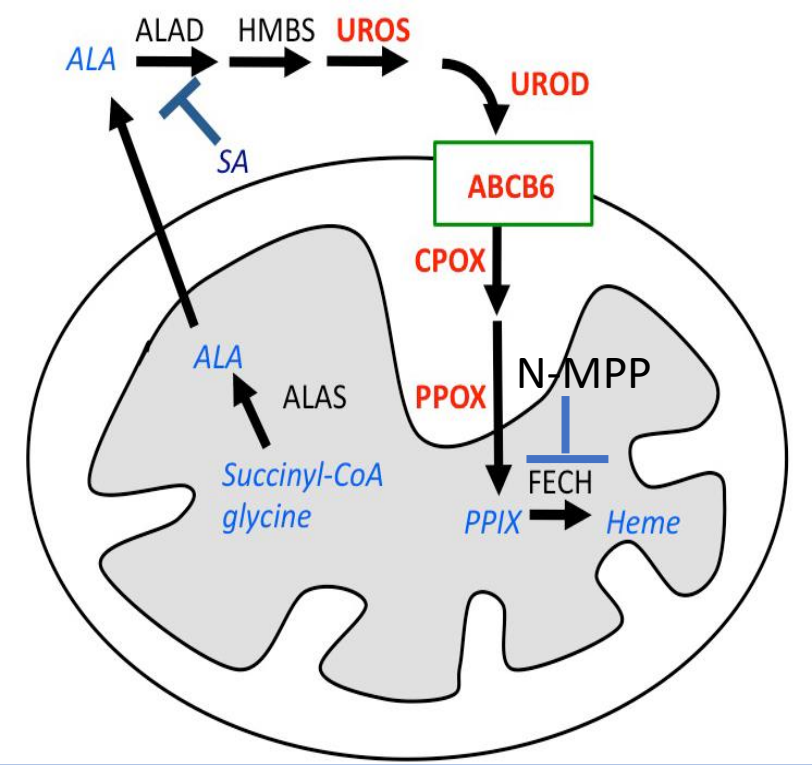
In Leukemic Progenitors MYCN upregulates heme/porphyrin synthesis, UROD and ABCG2 expression

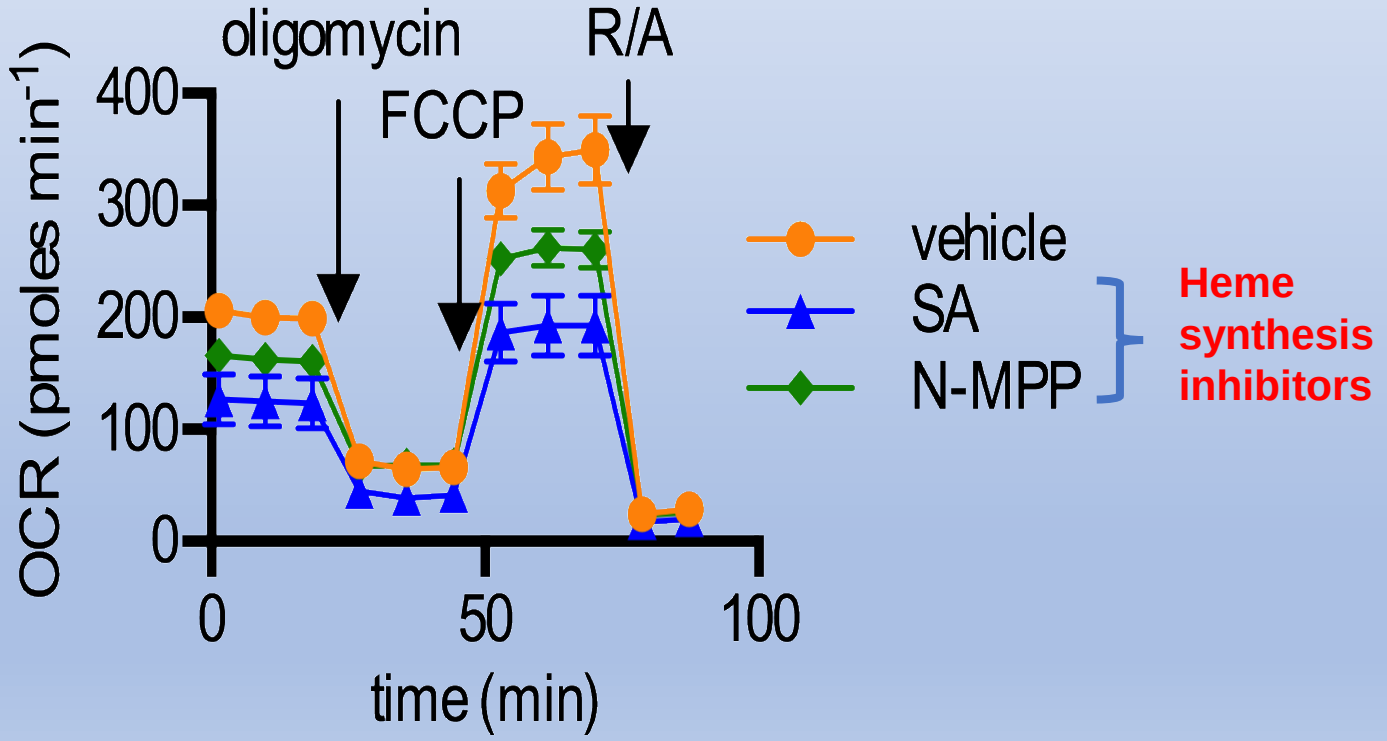




Fukuda et al JCI-Insight, 2017

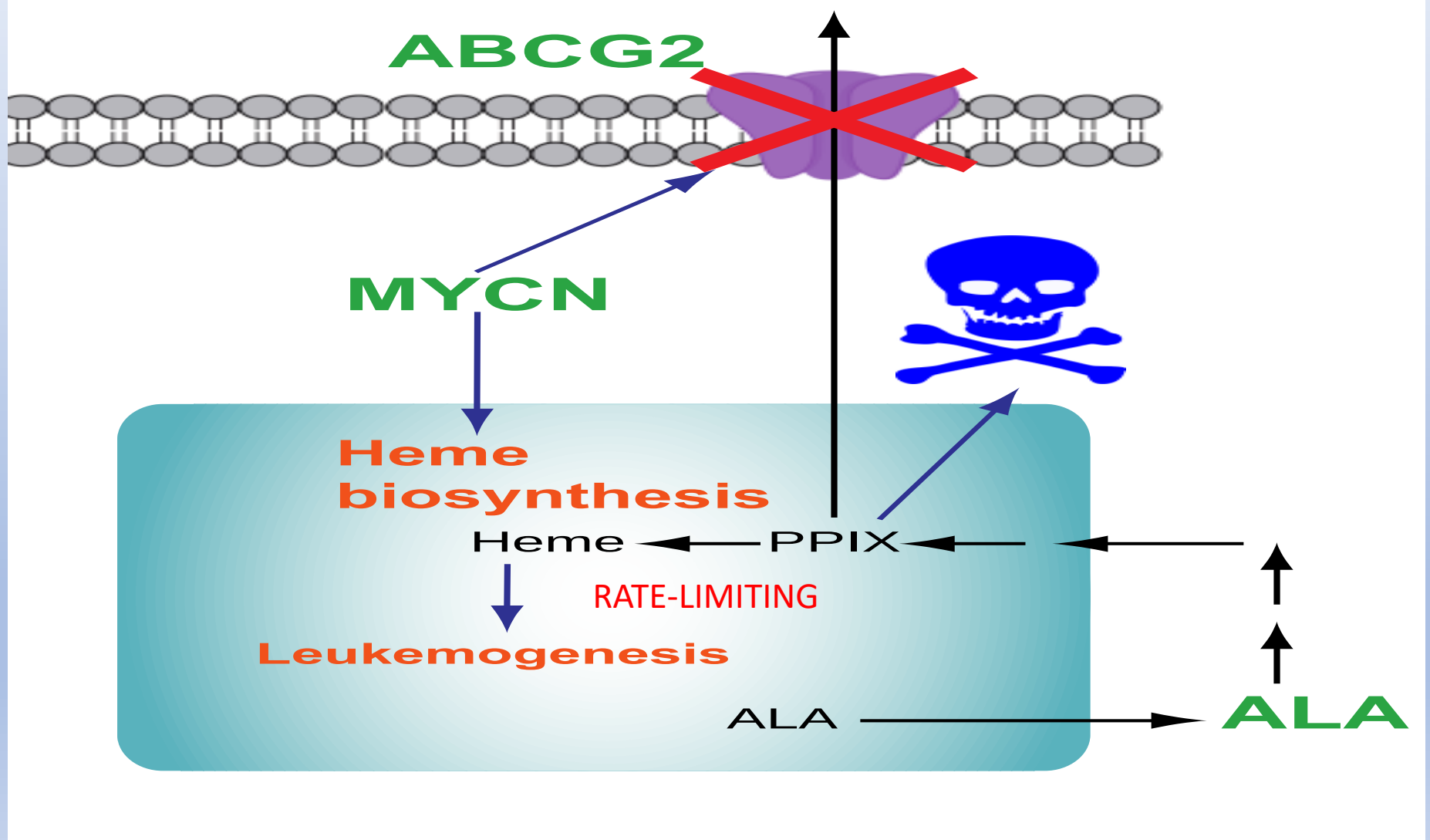
Heme synthesis is required for maximal mitochondrial oxygen consumption (OCR) in MYCN-Leukemic Progenitors





Fukuda et al JCI-Insight, 2017

Hypothesized that upregulated heme biosynthetic pathway would be a metabolic vulnerability in MYCN driven AML



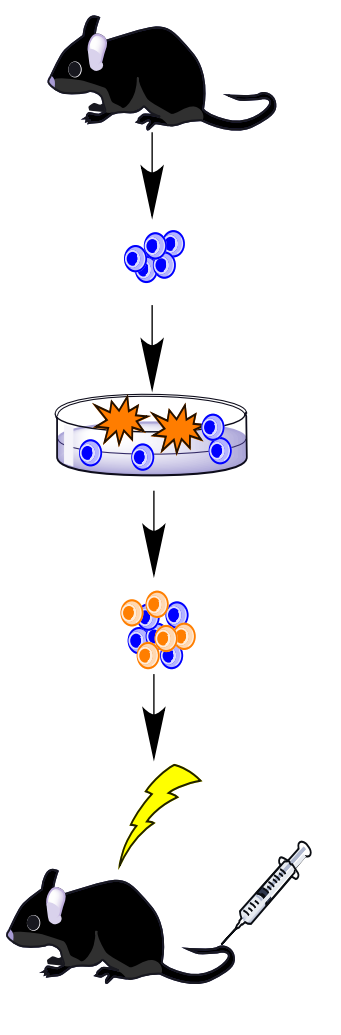
A mouse model shows that loss of *Abcg2* increases MYCN driven myeloid leukemia survival

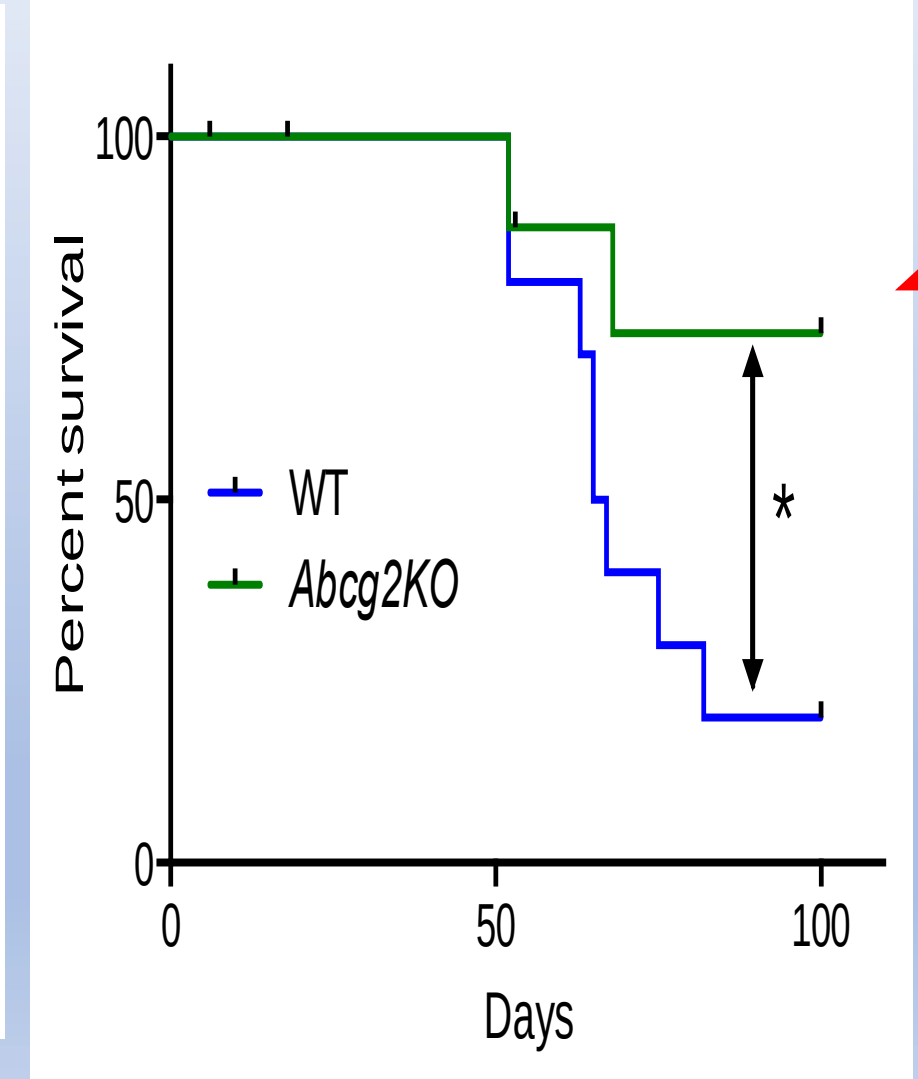
WT or Abcg2KO donor mouse

HPCs

MYCN transduction (Leukemic progenitors)

Transplant into lethally irradiated congenic recipient mice

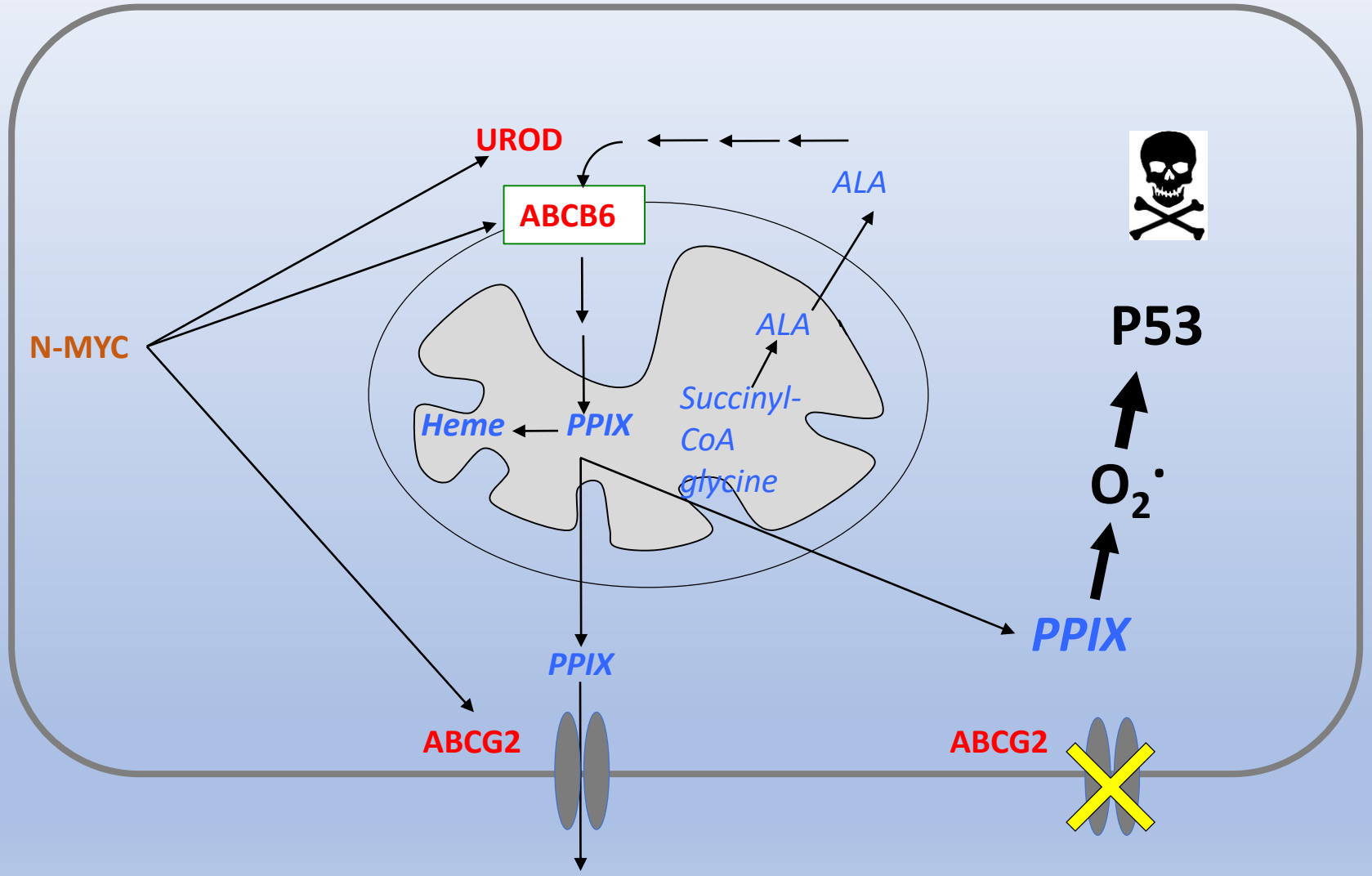




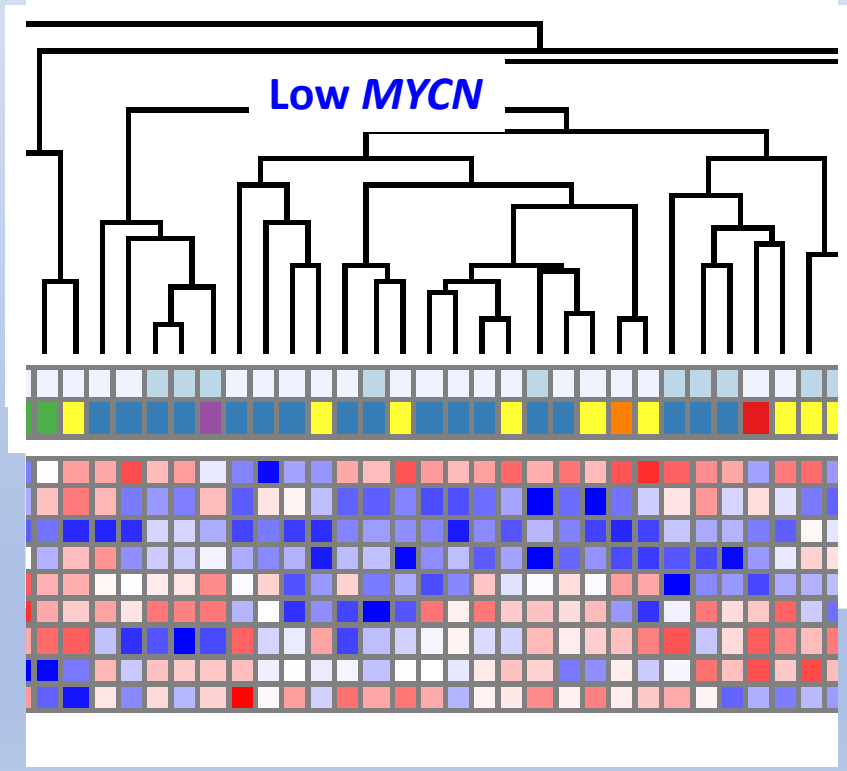
PPIX

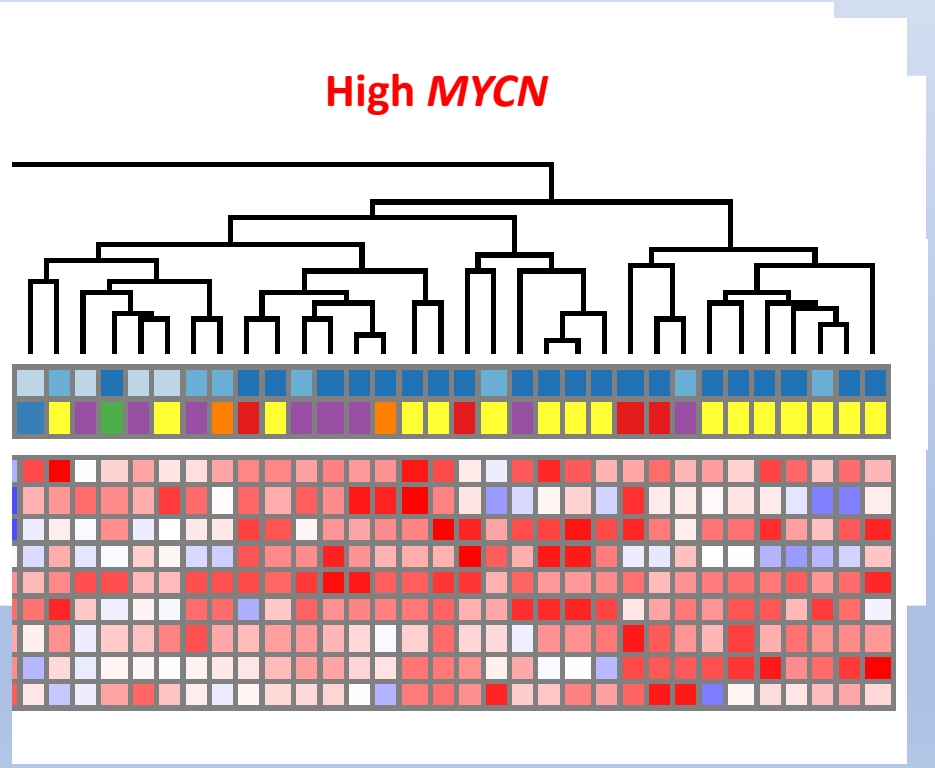
Fukuda et al JCI-Insight, 2017

Does PPIX activate P53?

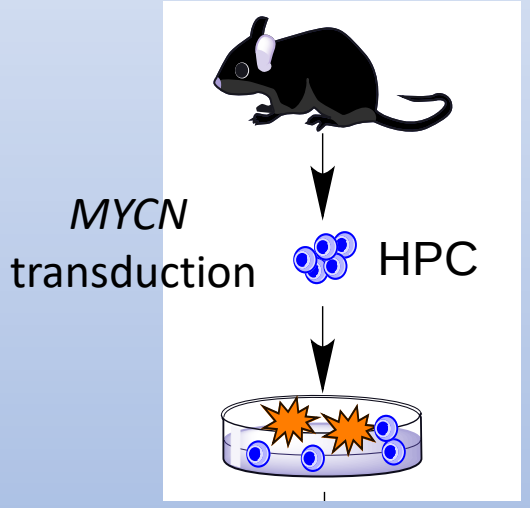


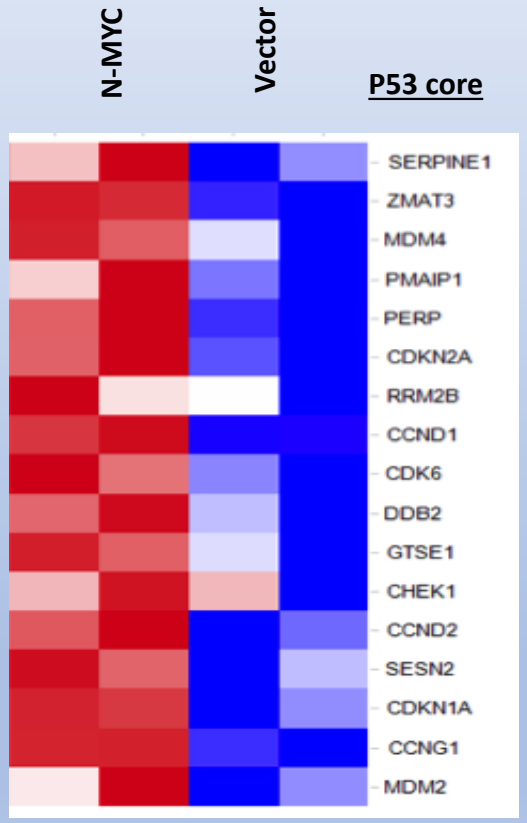
Higher *MYCN* correlates with p53 activation in SJ AML patients



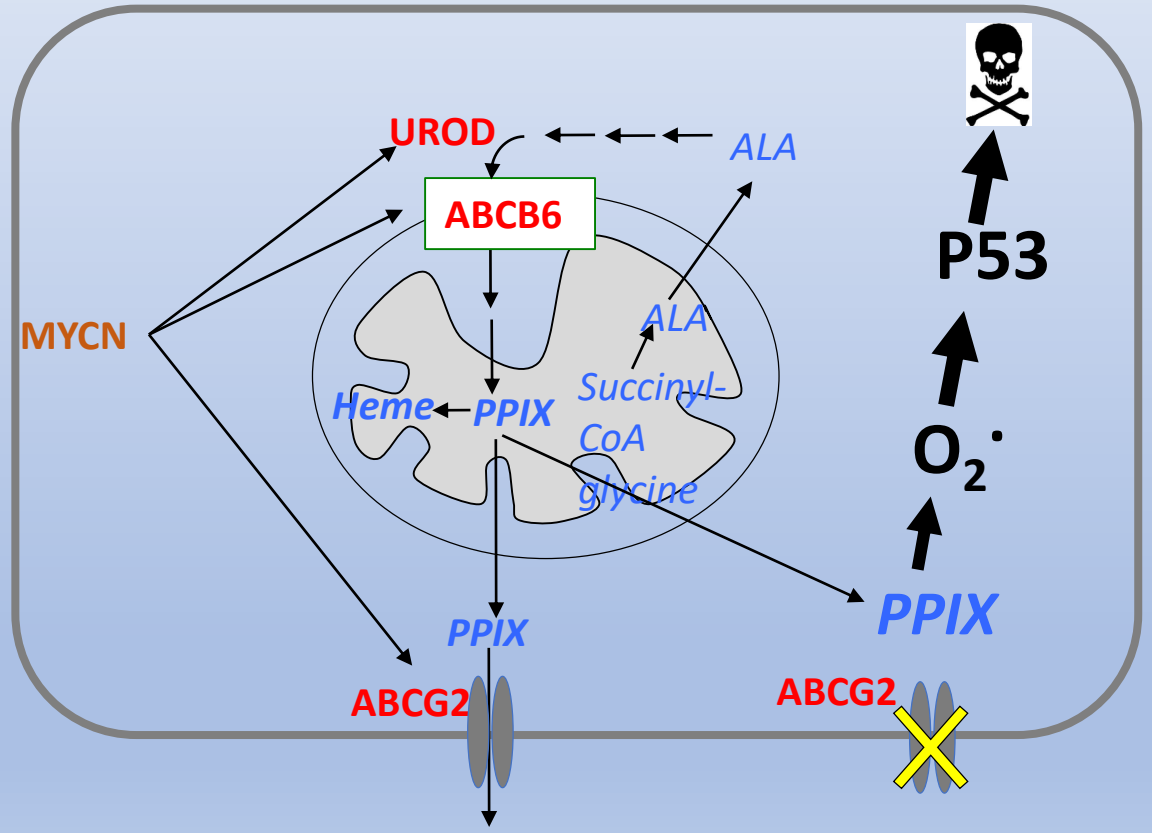


P53 is activated in MYCN leukemic progenitors

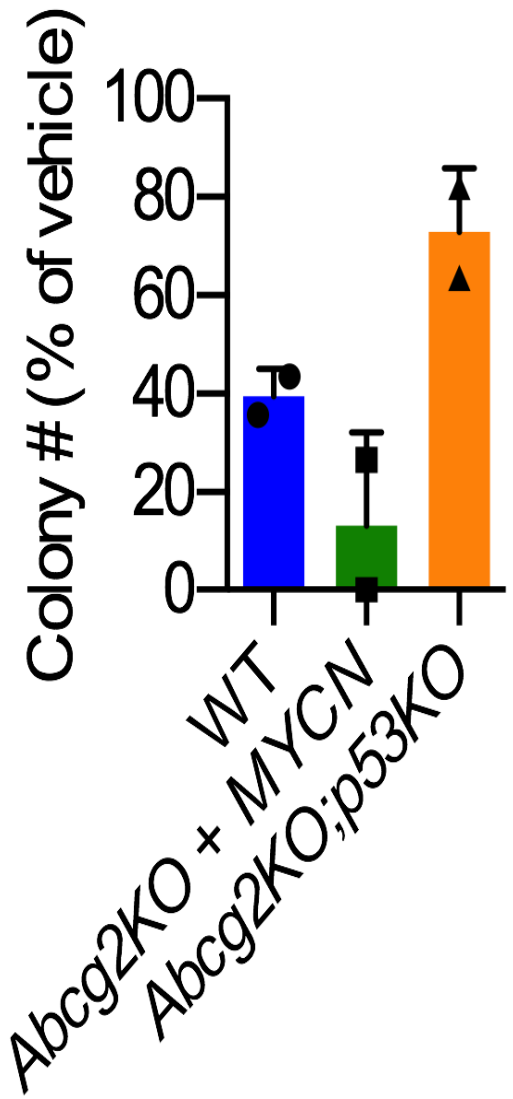




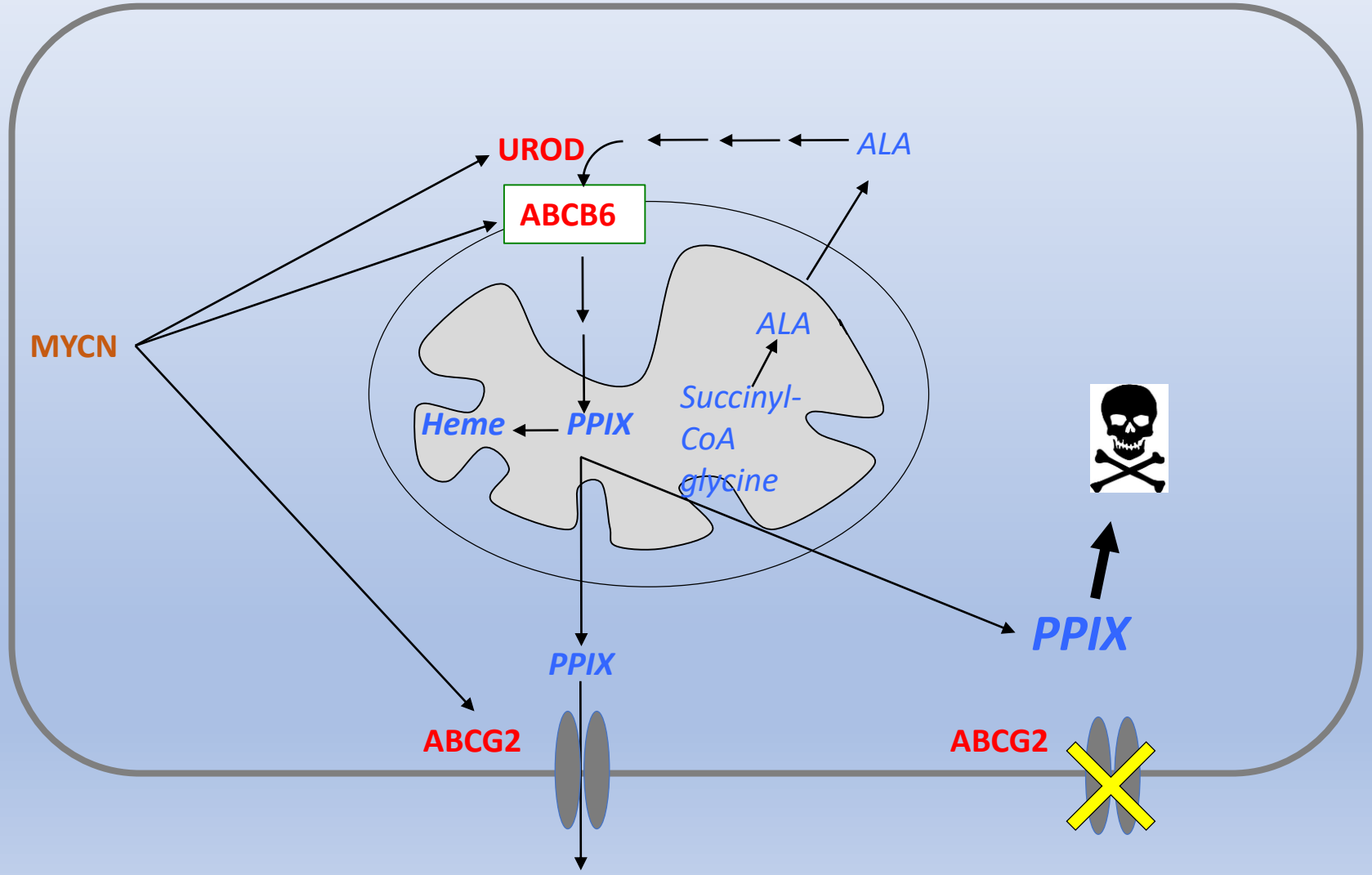
P53 deletion mostly rescues self-renewal of porphyrin treated Abcg2KO MYCN cells



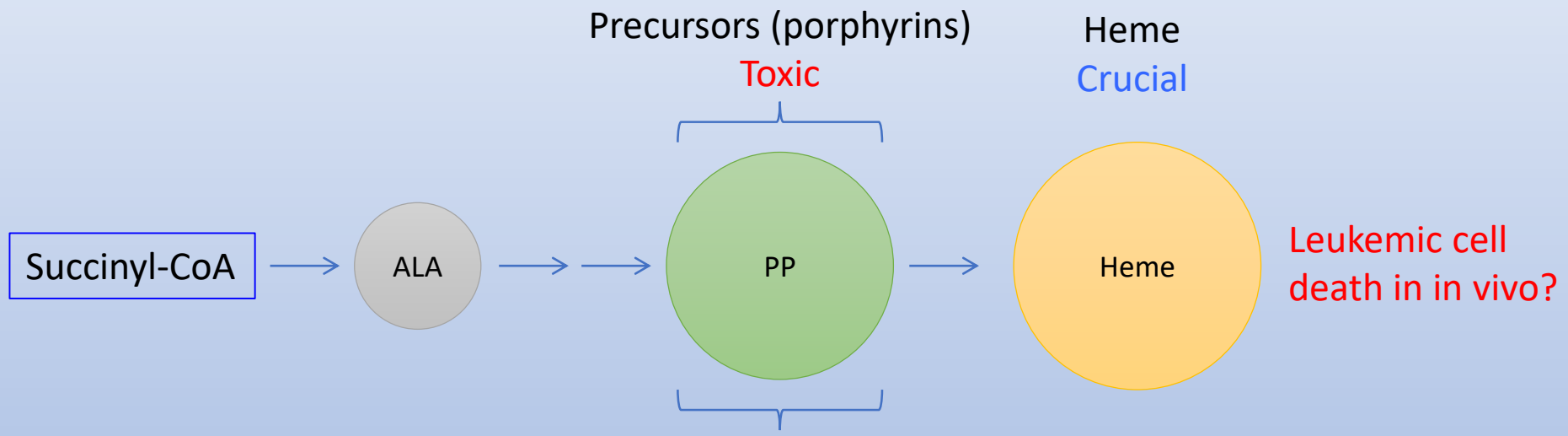
p53 null mice- Martine Roussel and Frederique Zindy



Do additional PPIX-protein interactions produce cytotoxicity?



Targeting the heme biosynthetic pathway in leukemic progenitors

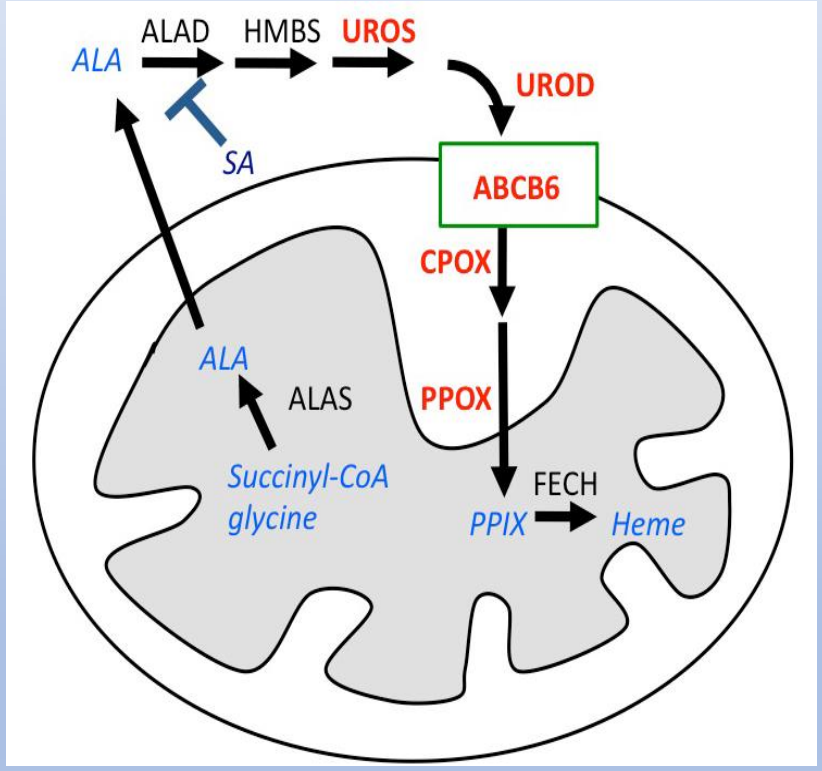


Loss of Abcg2

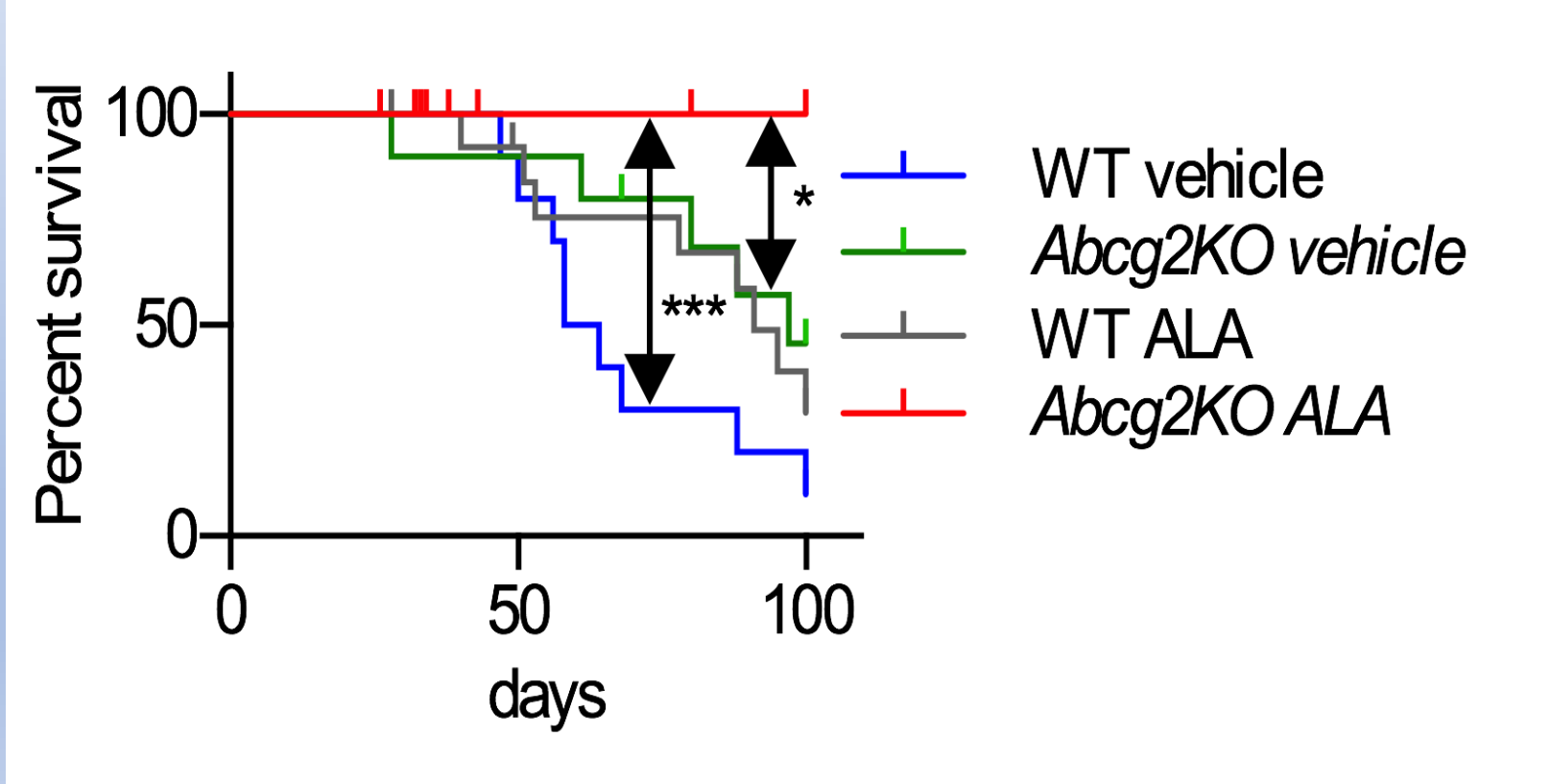
P53 activation

Other targets?

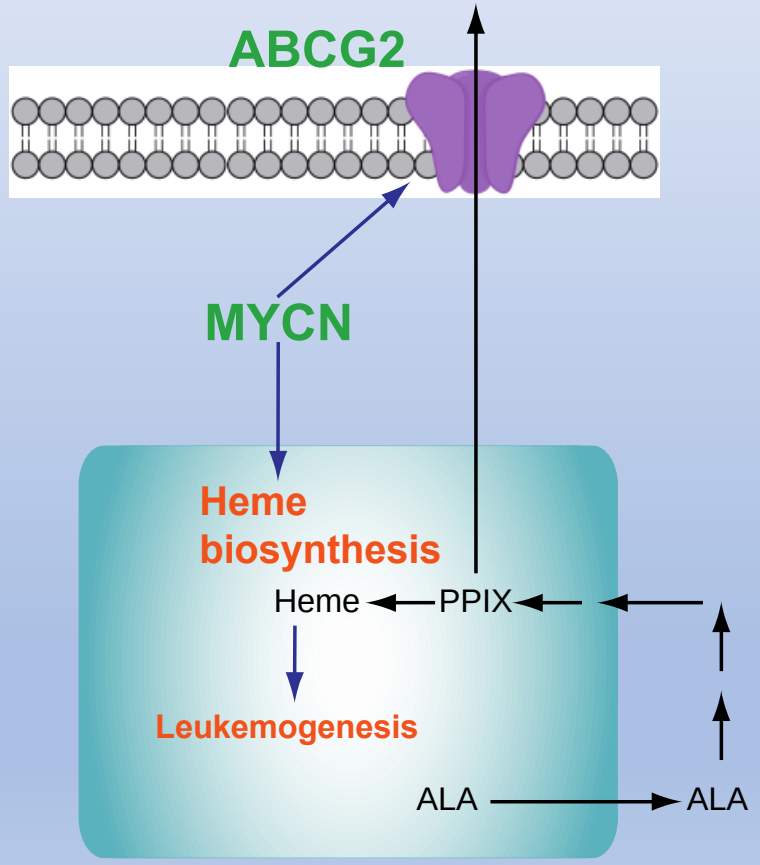
Does increasing PPIX cure AML?

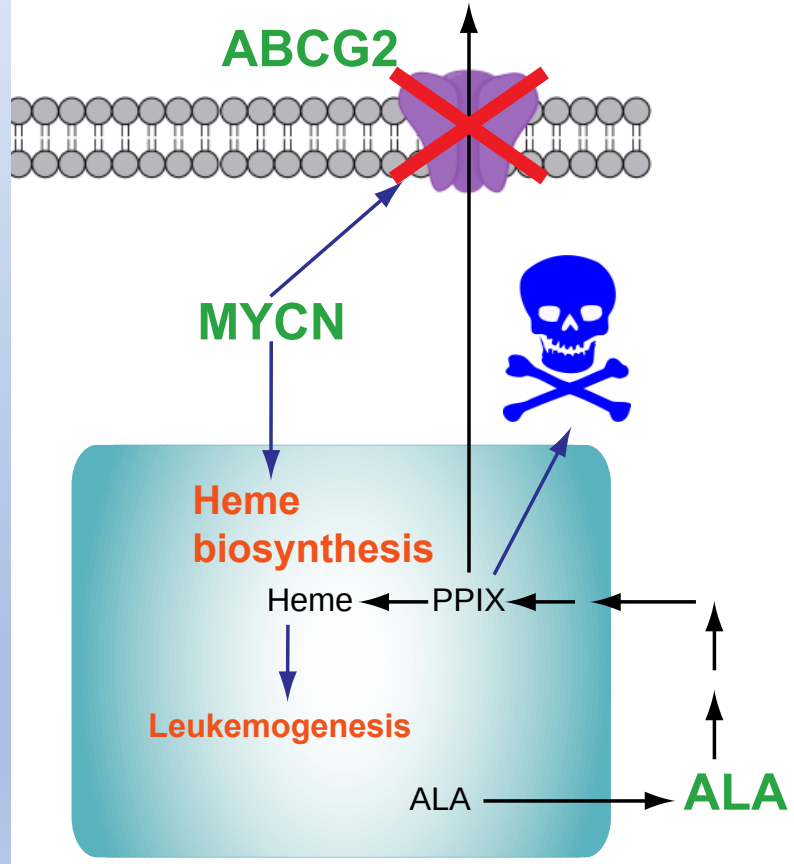


ALA-treatment of MYCN Leukemic Progenitors cures ABCG2 KO AML



Summary

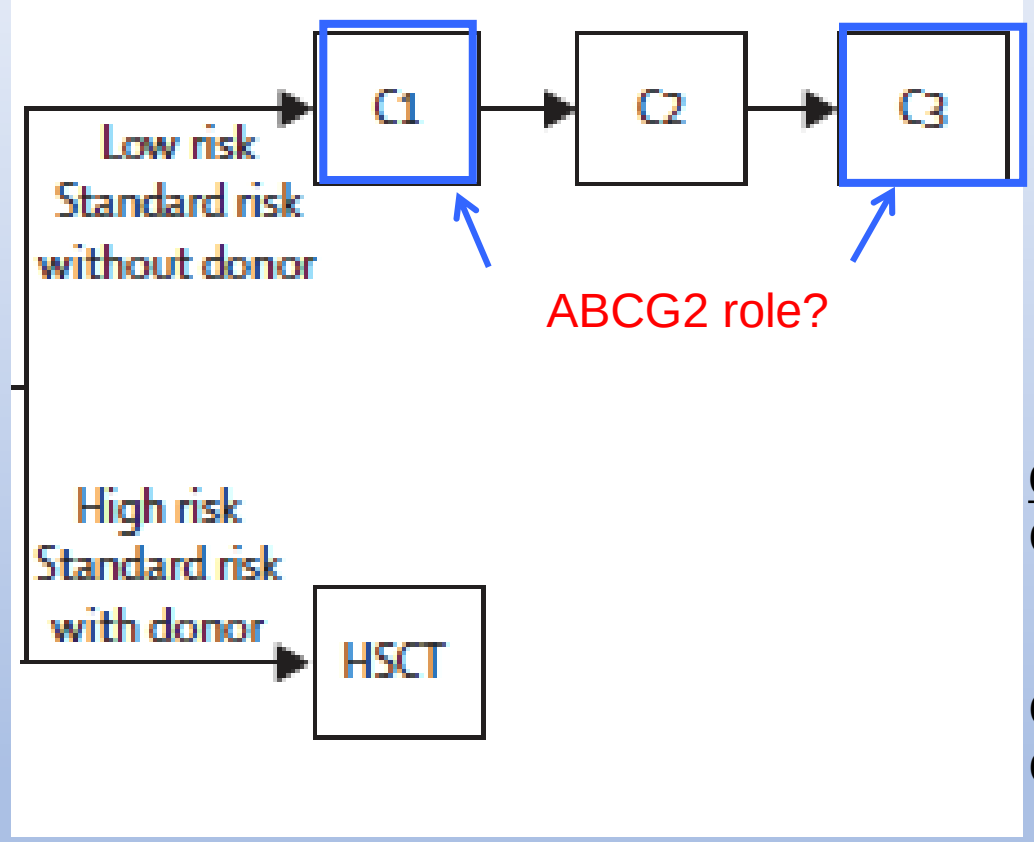

ABCG2
MYCN
Heme
biosynthesis
Heme
PPIX
Leukemogenesis
ALA
ALA


ABCG2
MYCN
Heme
biosynthesis
Heme
PPIX
Leukemogenesis
ALA
ALA

ABCG2 substrates are used in conventional AML therapy

AML02 treatment scheme

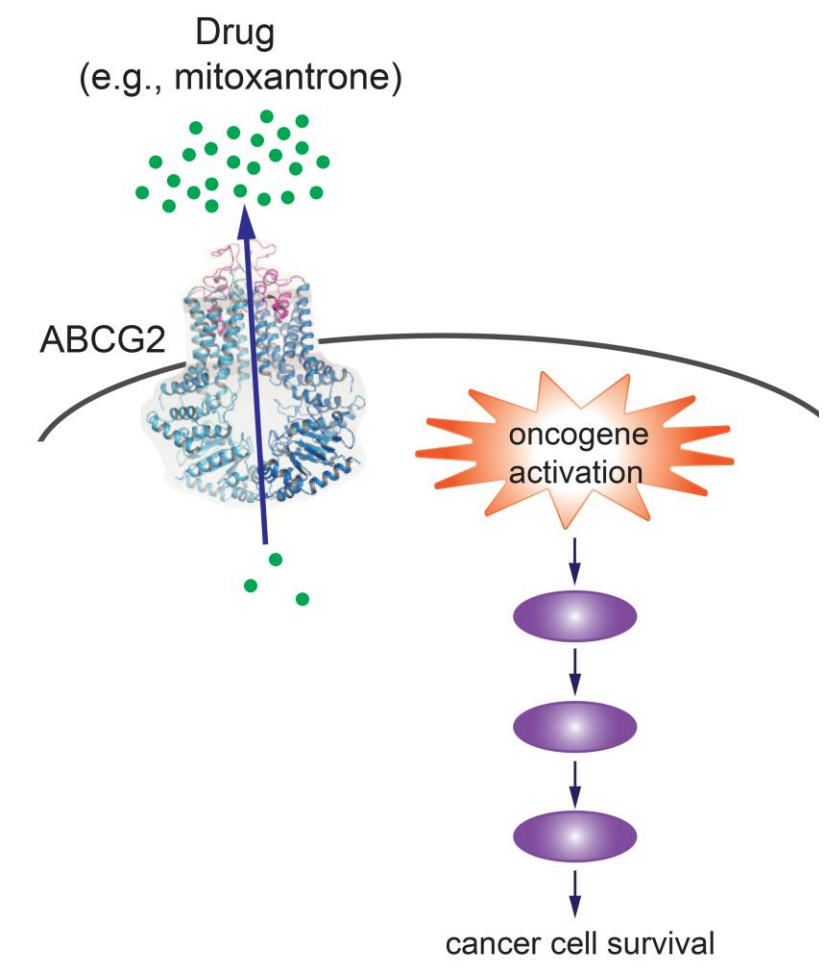
Induction
Daunorubicin
etoposide
cytarabine



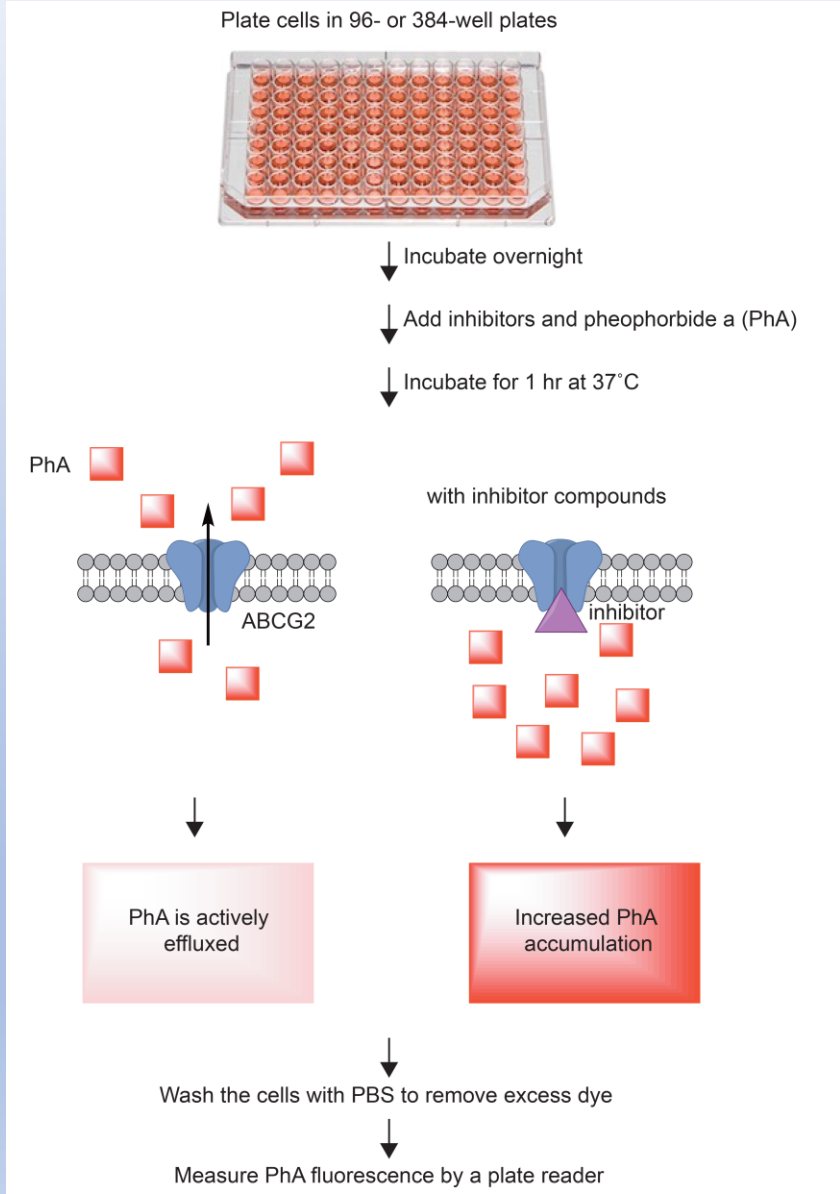
Consolidation
C1: cytarabine & **cladribine**
cytarabine & etoposide
cytarabine & **mitoxantrone**
C2: cytarabine & L-asparaginase
C3: cytarabine & **mitoxantrone**

Rubnitiz et al. 2010 Lancet Oncol

Screening for ABCG2 and Cancer liability inhibitors



High throughput screening to identify ABCG2 inhibitors



mM) FDA-approved bioactive
orbide a ef flux from WT MEFs.
positive control.

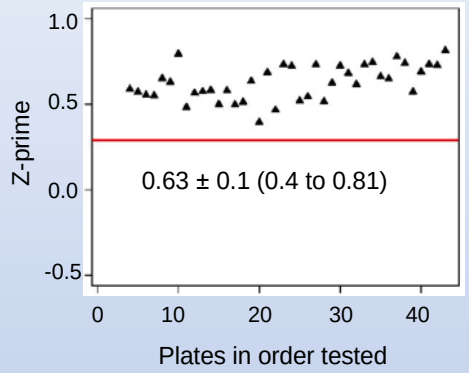
80% of lapatinib

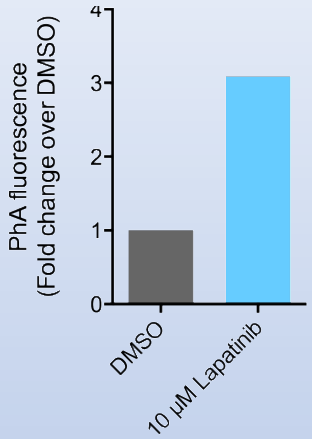
NO

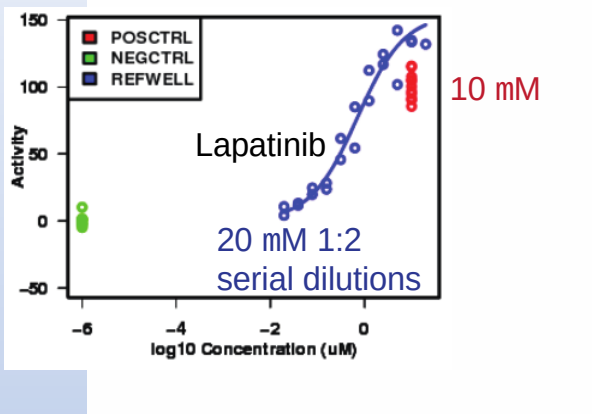
Not inhibitors

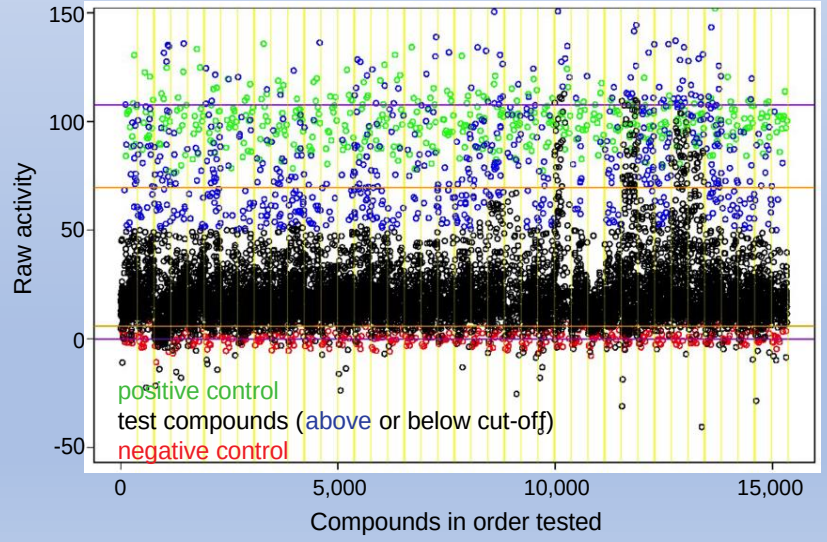
Validate hit compounds for ABCG2 inhibition and test whether they also act as subs

High throughput screening for ABCG2 inhibitors identified novel inhibitors









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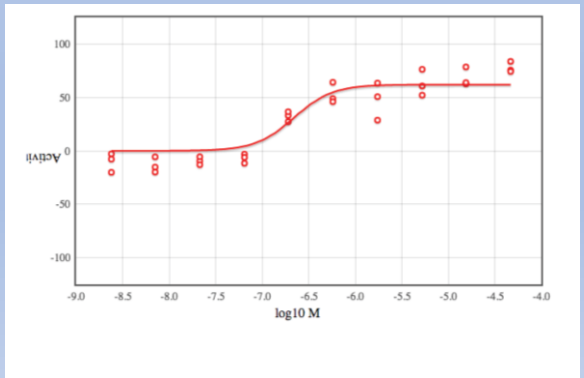
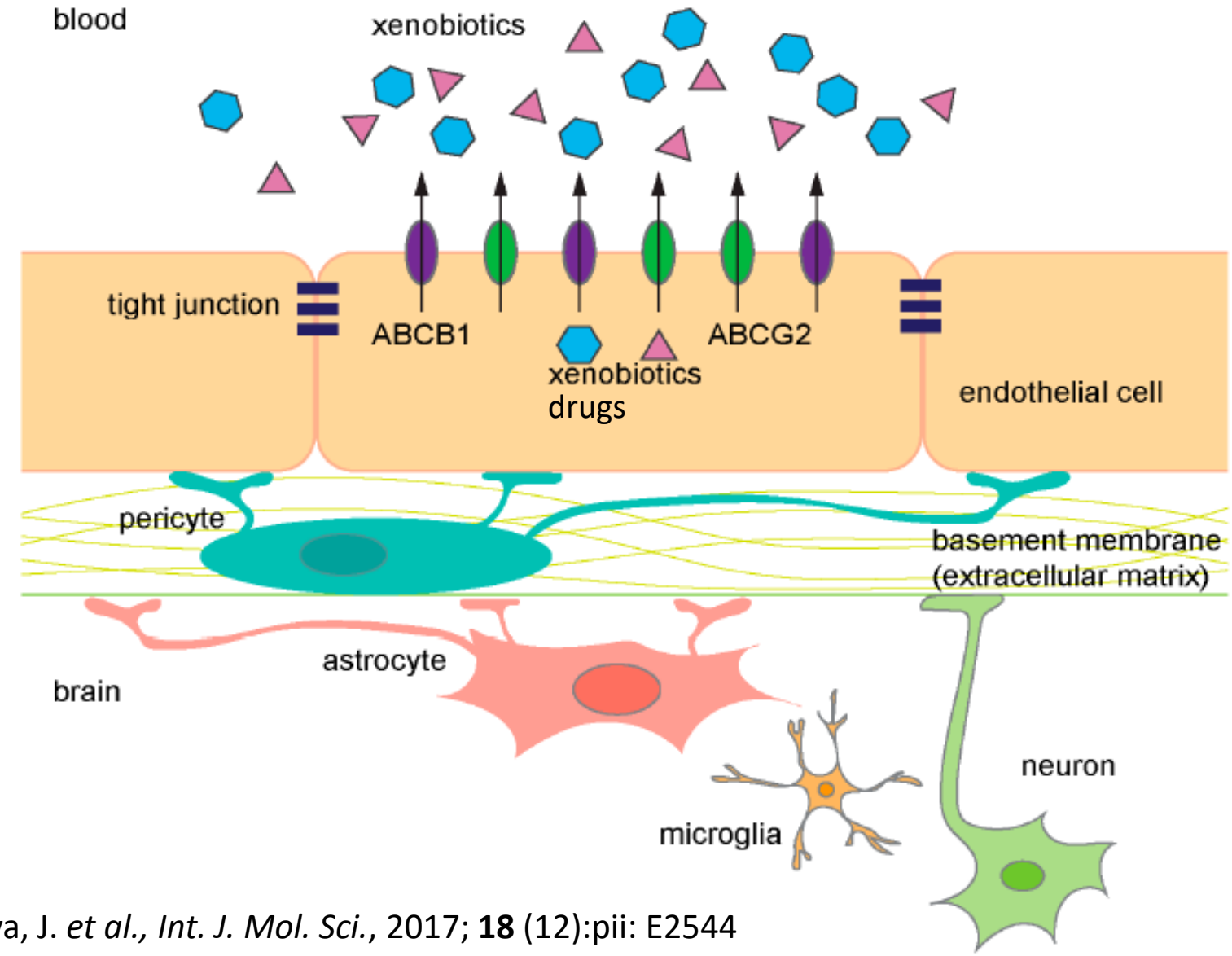


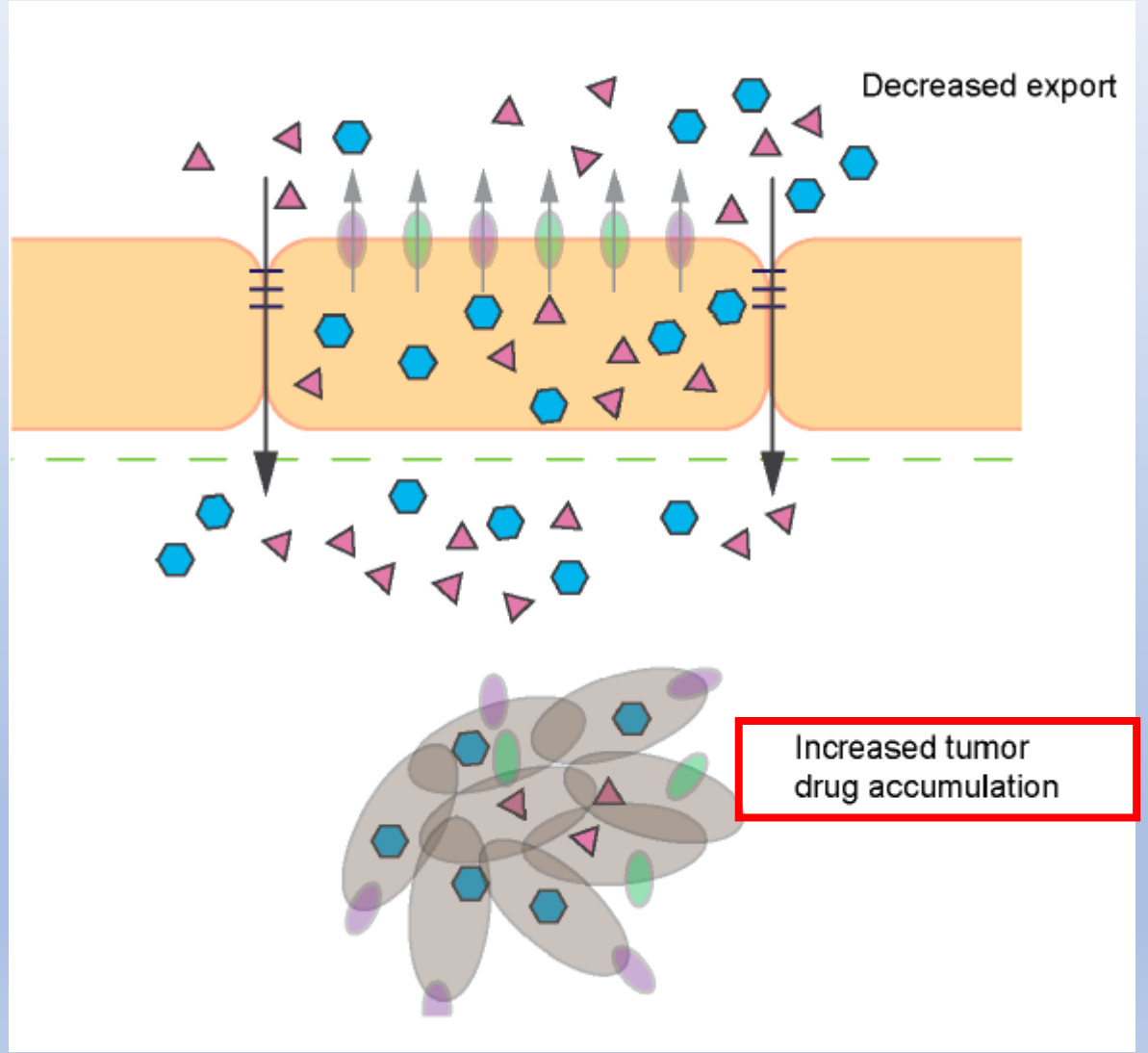
Unpublished 2019

Inhibition of ABCG2 at the blood-brain barrier (BBB)



Wijaya, J. *et al., Int. J. Mol. Sci.*, 2017; **18** (12):pii: E2544

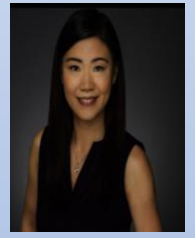
ABCG2 inhibition may allow the drugs accumulation in the tumor



Wijaya, J. *et al., Int. J. Mol. Sci.*, 2017; **18** (12):pii: E2544

“Teamwork to make the Dream work”

AP
Naren
CCHMC



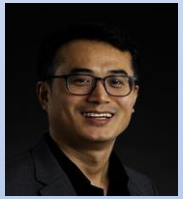
Jin
Zhang
UCSD

Yu
Fukuda

J Schuetz Lab-2018

St Jude Collaborators

Martine
Roussel
Tumor Cell Biol



Jiyang
Yu
Comp. Bio

Taosheng
Chen
Chem. Bio.

Junmin
Peng
Struc.
Biol

Funding
- P30 Cancer Center Support Grant
- ALSAC
- NIH R01s