CONTENTS

WELCOME

LEUKEMIA & LYMPHOMA SOCIETY®
fighting blood cancers

On behalf of The Leukemia & Lymphoma Society (LLS), thank you for joining us for ***Cellular Metabolism in Hematologic Malignancies: From Evolving Science to Therapeutic Potential***, a continuing medical education activity originally presented in Atlanta, Georgia. LLS would also like to thank our esteemed speakers for sharing their time and expertise. Through this activity, our presenters will describe how metabolic reprogramming contributes to tumor transformation and sustains progression in blood cancer cells; assess the therapeutic potential of targeting metabolic changes as they relate specifically to hematologic malignancies; apply new strategies for incorporating therapies that target metabolism in blood cancers; utilize the information presented from current and future clinical trials that evaluate cancer metabolism in order to better manage patients with hematologic malignancies; and explain the basis for therapies that affect cancer cell metabolism.

This workbook includes the presenters' slides to help guide you through the activity. If you would like to receive 3.25 *AMA PRA Category 1 Credit(s)*™, please complete the online learning assessment and evaluation.

We hope that you will find this activity rewarding and informative.

Thank you,

Richard C. Winneker, PhD
Senior Vice President, Research
The Leukemia & Lymphoma Society

PROGRAM AGENDA

Welcome
Richard C. Winneker, PhD
The Leukemia & Lymphoma Society

Introduction and Overview: Metabolic Targets in Leukemia and Lymphoma
Chi V. Dang, MD, PhD

Metabolism and Apoptotic Pathways in Leukemia
Jeffrey C. Rathmell, PhD

mTOR Links Cell Signaling and Metabolism
Mariusz A. Wasik, MD

Genetic and Metabolic Alterations in Myeloproliferative Neoplasms and AML
Ayalew Tefferi, MD

Targeting Autophagy in Hematologic Malignancies
John L. Cleveland, PhD

Question-and-Answer Session

OVERVIEW

TARGET AUDIENCE

This activity is designed for hematologists, oncologists, and other healthcare professionals who wish to enhance their knowledge of advances in cellular metabolism and implications in treating patients with hematologic malignancies.

STATEMENT OF NEED

Over 80 years ago, world-renowned biochemist Otto Heinrich Warburg observed that cancer cells exhibited high glycolysis (glucose conversion to lactic acid) even in the presence of oxygen (Warburg effect). However, not until the last decade did the reprogramming of cellular metabolism regain deserved attention for its role in cancer.

A number of common oncogenes and tumor suppressor genes have been discovered that directly control cell metabolism. In addition, a number of metabolic enzymes causally mutated in a variety of human cancers including hematological malignancies have now been identified as human tumor suppressors or oncogenes. The reprogramming of metabolic pathways is essential for tumors to survive and proliferate in their microenvironment. As a result of these more recent developments, several pharmacologic therapies have been developed and are currently under evaluation in clinical trials for their safety and efficacy in the treatment of both solid and hematologic cancers.

Cancer cell metabolism and the Warburg effect are promising targets for cancer treatment. However, physicians may lack the clinical and biochemical knowledge of the basic science behind the Warburg effect. In addition, physicians may not be up-to-date on the agents that are currently under evaluation in clinical trials. Some of these agents are in late-stage clinical trials and have demonstrated promising results. Therefore, it is important that physicians understand the basic science of the Warburg effect and the emerging pharmacologic agents that may reach the clinic, which may provide important additional options for the treatment of cancer. This knowledge will allow physicians to effectively implement these new therapies in their clinical practice, potentially improving patient outcomes and quality of life.

EDUCATIONAL OBJECTIVES

After completing this activity, the participant should be better able to:

- Describe how metabolic reprogramming contributes to tumor transformation and sustains progression in blood cancer cells
- Assess the therapeutic potential of targeting metabolic changes as they relate specifically to hematologic malignancies
- Apply new strategies for incorporating therapies that target metabolism in blood cancers
- Utilize the information presented from current and future clinical trials that evaluate cancer metabolism in order to better manage patients with hematologic malignancies
- Explain the basis for therapies that affect cancer cell metabolism

STATEMENT OF SUPPORT

This activity is jointly sponsored by The Leukemia & Lymphoma Society, RMEI, LLC and Postgraduate Institute for Medicine and supported by an educational grant from Celgene Corporation.

FACULTY BIOGRAPHIES

John L. Cleveland, PhD

Professor and Chair
The Department of Cancer Biology
The Scripps Research Institute, Scripps Florida
Jupiter, FL

John L. Cleveland, PhD, is professor and chair of the department of cancer biology for The Scripps Research Institute (TSRI), Scripps Florida in Jupiter, Florida. As head of the Cancer Biology Department, Dr. Cleveland directs and coordinates the oncology research efforts of TSRI. Dr. Cleveland completed undergraduate studies at the University of Maine and doctoral training in immunology and microbiology at Wayne State University School of Medicine. As a graduate student, Dr. Cleveland studied DNA tumor viruses and in 1984 he joined the laboratory of Dr. Ulf R. Rapp at the National Cancer Institute, where he began his studies on the roles of oncogenes in cancer. In 1989 he was appointed as assistant professor in the department of biochemistry at St. Jude Children's Research Hospital, a renowned center for pediatric cancer. There he rapidly advanced through the ranks to become a professor in 2000.

The Cleveland lab is most recognized for its discovery that oncogenes can provoke apoptosis (cell death) pathways and that these pathways are disabled during cancer progression. His work over the past decade has identified many new targets that can be exploited in cancer prevention and treatment, and at Scripps Florida, new drugs are being developed that will exploit these targets. The laboratory is currently funded by three large R01 grants as well as funding from a pharmaceutical partner.

Dr. Cleveland is editor of *Molecular Cancer Research* and *Molecular and Cellular Biology*. He also serves on the extramural scientific advisory boards for three institutions. Finally, Dr. Cleveland has served as a member of study sections for 20 years, first for the American Cancer Society, then for the National Institute of General Medicine, and finally for the National Cancer Institute. He was recruited to lead Cancer Biology at TRSI on November 1, 2006.

FACULTY BIOGRAPHIES

Chi V. Dang, MD, PhD

John H. Glick, MD, Professor of Medicine
Director, Abramson Cancer Center
University of Pennsylvania
Perelman School of Medicine
Philadelphia, PA

Chi Van Dang, MD, PhD, is Director of the Abramson Cancer Center and Director of the Abramson Family Cancer Research Institute, as well as a professor of medicine and The John H. Glick Professor at the Perelman School of Medicine, University of Pennsylvania, in Philadelphia.

After completing his doctorate in chemistry from Georgetown University, Washington, DC, Dr. Dang received a medical degree from Johns Hopkins University School of Medicine in Baltimore, Maryland, where he also did his internship and residency in medicine. Dr. Dang did a fellowship in hematology-oncology at the Cancer Research Institute at the University of California, San Francisco.

Dr. Dang currently has grants funding his studies of leukemia, lymphoma, pancreatic cancer, and MYC transcription and apoptosis. The Dang laboratory has contributed to the understanding of the function of the MYC cancer gene (www.myccancergene.org), which has emerged as a central transcription factor or gene switch in many different human cancers. His laboratory established the first mechanistic link between the MYC cancer gene and cellular energy metabolism, contributing to the concept that genetic alterations in cancers re-program fuel utilization by tumors and render cancers addicted to certain fuel sources. Dr. Dang's laboratory is now exploiting these concepts for therapeutic targeting of cancer cell metabolism as a new way to treat cancer.

He has authored more than 200 scientific and medical articles, book chapters and a book, and serves on the editorial boards of numerous journals. Dr. Dang is also Editor-in-Chief of *Cancer & Metabolism*, and a scientific editor of *Cancer Discovery*.

Dr. Dang has received numerous honors and awards, including The Tsung Hsien and Shu Yung Wu Lectureship in Cancer Research for 2011 from the University of Michigan, the Vietnamese-American Medical Research Foundation Achievement Award in 2005, the Johns Hopkins University School of Medicine Dean's Lectureship in 2001, and the MD Anderson Cancer Center Odyssey Program Distinguished Lecturer in 2000. He is a member of the Institute of Medicine and a Fellow of the American Academy of Arts & Sciences.

FACULTY BIOGRAPHIES

Jeffrey C. Rathmell, PhD

Associate Professor
Department of Pharmacology and Cancer Biology
Department of Immunology
Sarah W. Stedman Nutrition and Metabolism Center
Duke University
Durham, NC

Jeffrey C. Rathmell, PhD, is associate professor in the departments of pharmacology and cancer biology and immunology and Director of Graduate Studies in the pharmacology doctoral program at Duke University in Durham, North Carolina. He completed undergraduate study in biology at the University of Northern Iowa, doctoral training in immunology at Stanford University and post-doctoral training at the University of Pennsylvania.

Dr. Rathmell started his lab at Duke University in 2003. He is the Principal Investigator on several NIH, NCI, and foundation-supported research projects and his research has been published extensively in peer-reviewed journals. He serves on the editorial boards of *Cancer and Metabolism* and the *Journal of Biological Chemistry* and is associate editor for the *Journal of Clinical Investigation* and *BMC Cell Biology*. He currently serves as chair of the Tumor Cell Biology Study Section of the NIH Center for Scientific Review. His work at Duke has focused on regulation of cell death pathways in immunity and more recently on how metabolic pathways influence lymphocyte fate in inflammatory diseases and leukemia.

FACULTY BIOGRAPHIES

Ayalew Tefferi, MD

Professor of Medicine
Division of Hematology
Mayo Clinic
Rochester, MN

Ayalew Tefferi, MD, was born in Addis Ababa, Ethiopia and migrated to the United States in 1982 after completing his medical school education at the University of Athens in Athens, Greece. Dr. Tefferi received his hematology training at the Mayo Clinic in Rochester, Minnesota before joining the staff at the Mayo Clinic College of Medicine, division of hematology in the department of medicine. He is currently a full professor in hematology and internal medicine.

Dr. Tefferi spends the majority of his time in direct patient care and his clinical as well as laboratory interests focus on chronic myeloproliferative disorders, myelodysplastic syndromes, chronic myeloid leukemia, hypereosinophilic syndrome, and systemic mast cell disease. His academic and research achievements have been copious and include over 600 publications including books, book chapters, original articles, reviews, editorials, letters, and abstracts. In addition, Dr. Tefferi serves as an associate editor for the *Mayo Clinic Proceedings* and the *European Journal of Hematology*, and is on the editorial board for *Blood* and several other hematology journals. Dr. Tefferi has given more than 300 national and international invited lectureships and serves as faculty for the annual Hematology and Oncology Board review courses at George Washington University in Washington, DC, Harvard University in Boston, Massachusetts, and MD Anderson Cancer Center in Houston, Texas.

FACULTY BIOGRAPHIES

Mariusz A. Wasik, MD

Professor
Department of Pathology
University of Pennsylvania
Perelman School of Medicine
Philadelphia, PA

Mariusz A. Wasik, MD, is professor in the department of pathology at the University of Pennsylvania, Perelman School of Medicine, in Philadelphia.

Dr. Wasik graduated from Wroclaw Medical University in Poland, followed by fellowships in immunology at Harvard Medical School and Boston University, residency in pathology at Mallory Institute of Pathology/Boston University and fellowship in hematopathology at Beth Israel Hospital/Harvard. The key areas of his research are:

1. Mechanisms of malignant cell transformation by the chimeric ALK kinase. He and his colleagues found that pathways involving STAT3, PI3K/AKT, MEK/ERK, mTORC1 and STAT5b are constitutively activated by the chimeric form of ALK, NPM/ALK. This aberrant signaling affects expression of proteins involved in the tumor immune evasion and promotes epigenetic gene silencing of tumor suppressor genes.

2. mTOR signaling in cancer. Studies by his team indicate that rapamycin-related mTORC1 inhibitors have strong inhibitory effect on the whole spectrum of B- and T-cell lymphomas. The mechanism of mTORC1 activation is lymphoma-type dependent and involves the key oncogenic signals as well as PI3K/AKT, MEK/ERK, and other signaling pathways upstream of mTORC1.

3. Signaling of IL-2R-type receptors in malignant transformation of T lymphocytes. The studies indicate that cutaneous T-cell lymphoma displays activation of IL-2R-associated Jak/STAT signal transduction pathway due, at least in part, to the epigenetic silencing of the SHP-1 gene, directly and indirectly controlled by the transcription factor STAT3.

He has authored more than 125 peer-reviewed articles for such journals as *Nature Medicine*, *Proceedings of the National Academy of Science*, *Blood*, *American Journal of Pathology*, and *Journal of Immunology*, among others. Dr. Wasik frequently serves as the member and chair of grant review committees for the National Institute of Health and other funding organizations.

ACCREDITATION & CREDIT

CONTINUING EDUCATION INFORMATION

Physician Continuing Education
Accreditation Statement

This activity has been planned and implemented in accordance with the Essential Areas and policies of the Accreditation Council for Continuing Medical Education through the joint sponsorship of The Leukemia & Lymphoma Society, RMEI, LLC and Postgraduate Institute for Medicine. The Postgraduate Institute for Medicine is accredited by the ACCME to provide continuing medical education for physicians.

Credit Designation

The Postgraduate Institute for Medicine designates this activity for a maximum of 3.25 *AMA PRA Category 1 Credit(s)*™. Physicians should only claim credit commensurate with the extent of their participation in the activity.

METHOD OF PARTICIPATION AND REQUEST FOR CREDIT

There are no fees for participating and receiving CME credit for this activity. During the period February 28, 2013 through February 28, 2014, participants must read the learning objectives and faculty disclosures and study the educational activity.

PIM supports Green CME by offering your Request for Credit online. If you wish to receive acknowledgment for completing this activity, please complete the post-test and evaluation on www.cmeuniversity.com. On the navigation menu, click on "Find Post-Test/Evaluation by Course" and search by course ID 8934. Upon registering and successfully completing the post-test with a score of 70% or better and the activity evaluation, your certificate will be made available immediately. Processing credit requests online will reduce the amount of paper used by nearly 100,000 sheets per year.

CONTINUING EDUCATION INFORMATION FOR NURSES AND SOCIAL WORKERS

Approval for nurses has been obtained by the National Office of the Leukemia & Lymphoma Society under provider number CEP 5832 to award 3.25 continuing education contact hours through the California Board of Registered Nursing.

The Leukemia & Lymphoma Society (LLS), provider number 1105, is approved as a provider for social work continuing education by the Association of Social Work Boards (ASWB) www.aswb.org Approved Continuing Education Program (ACE). Approval Period: December 2011 – December 2014. LLS maintains responsibility for the program. Social workers should contact their regulatory board to determine course approval. Social workers will receive 3.25 CE clinical clock hours.

Upon successful completion of the entire program, post-test (grade of 70% or higher) and submission of the activity evaluation, a certificate of completion will be issued to you within 30 days, via email or US mail based on your designation on the evaluation.

FEE INFORMATION

There is no fee for this educational activity.

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DISCLOSURES & DISCLAIMER

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The ***faculty*** reported the following financial relationships or relationships to products or devices they or their spouse/life partner have with commercial interests related to the content of this CME activity:

- **John L. Cleveland, PhD**, has no affiliations with commercial interests to disclose.
- **Chi V. Dang, MD, PhD**, has no affiliations with commercial interests to disclose.
- **Jeffrey C. Rathmell, PhD**, has affiliations with Lycera *(Consulting Fees)*.
- **Ayalew Tefferi, MD**, has no affiliations with commercial interests to disclose.
- **Mariusz A. Wasik, MD**, has no affiliations with commercial interests to disclose.

The ***planners and managers*** reported the following financial relationships or relationships to products or devices they or their spouse/life partner have with commercial interests related to the content of this CME activity:

THE LEUKEMIA & LYMPHOMA SOCIETY

- **Richard C. Winneker, PhD**, has no affiliations with commercial interests to disclose.

RMEI, LLC

- **Cynthia M. Kunzer** has no affiliations with commercial interests to disclose.
- **Emma Hitt, PhD**, has no affiliations with commercial interests to disclose.

POSTGRADUATE INSTITUTE FOR MEDICINE

- **Laura Excell, ND, NP, MS, MA, LPC, NCC**, has no affiliations with commercial interests to disclose.
- **Trace Hutchison, PharmD**, has no affiliations with commercial interests to disclose.
- **Samantha Mattiucci, PharmD**, has no affiliations with commercial interests to disclose.
- **Jan Schultz, RN, MSN, CCMEP**, has no affiliations with commercial interests to disclose.
- **Patricia Staples, MSN, NP-C, CCRN**, has no affiliations with commercial interests to disclose.

DISCLOSURE OF UNLABELED USE

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The opinions expressed in the educational activity are those of the faculty and do not necessarily represent the views of the planners. Please refer to the official prescribing information for each product for discussion of approved indications, contraindications, and warnings.

DISCLAIMER

Participants have an implied responsibility to use the newly acquired information to enhance patient outcomes and their own professional development. The information presented in this activity is not meant to serve as a guideline for patient management. Any procedures, medications, or other courses of diagnosis or treatment discussed or suggested in this activity should not be used by clinicians without evaluation of their patient's conditions and possible contraindications on dangers in use, review of any applicable manufacturer's product information, and comparison with recommendations of other authorities.

Abramson Cancer Center
Penn Medicine

Metabolic Targets in Leukemia and Lymphoma

Chi Van Dang

Leukemia & Lymphoma Society
ASH
2012

1

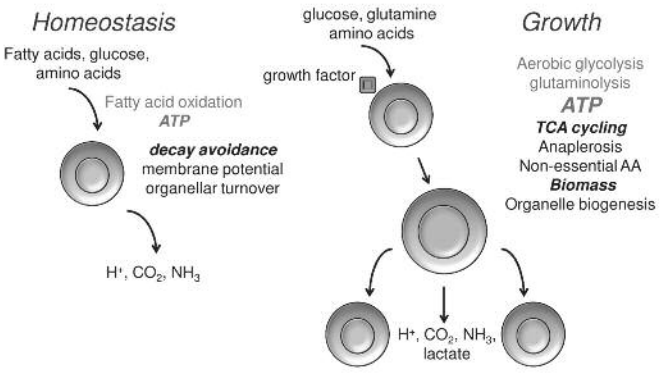
Regulation of Cell Growth and Metabolism

What regulates cell growth?

What is required metabolically for cell replication?

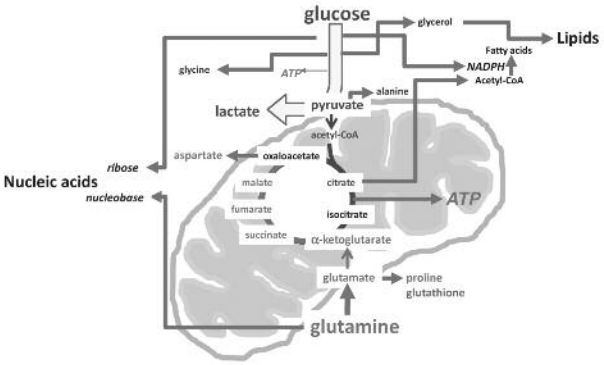
2

Energetics of Cell Homeostasis vs Growth



3

Metabolism of cell growth and proliferation



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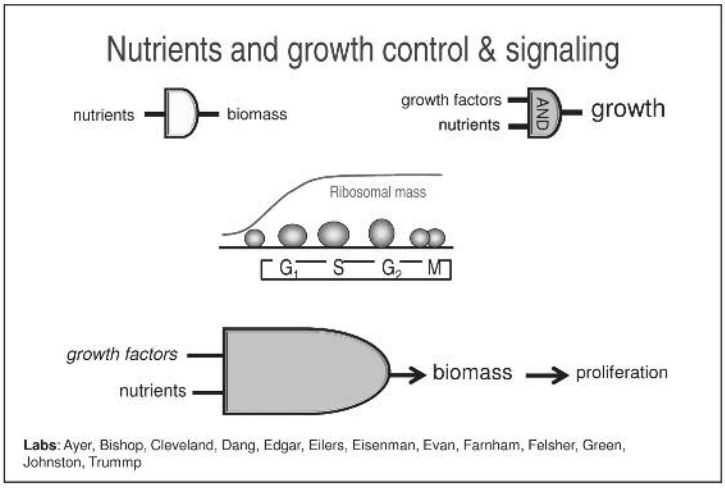
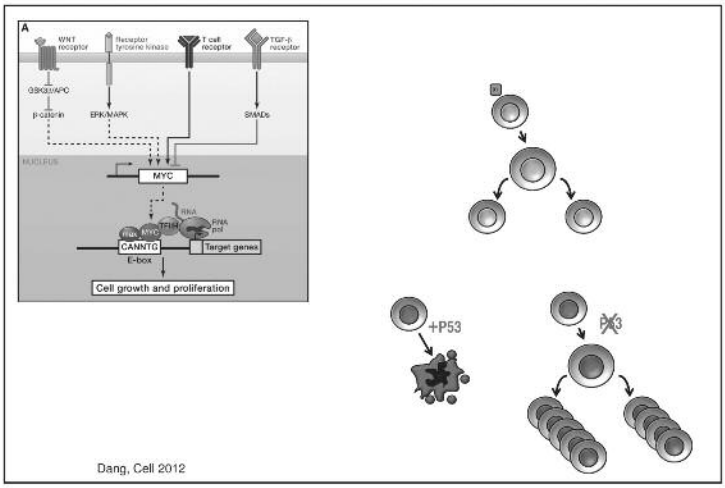
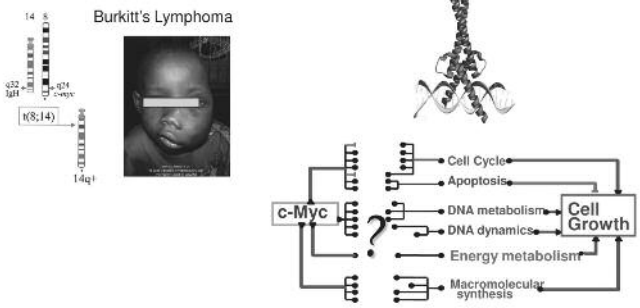

Nutrients and growth control & signaling
nutrients
biomass
growth factors
nutrients
AND
growth
Ribosomal mass
G1 S G2 M
growth factors
nutrients
biomass
proliferation
Labs: Ayer, Bishop, Cleveland, Dang, Edgar, Eilers, Eisenman, Evan, Farnham, Felsher, Green, Johnston, Trummp

5


A
WNT receptor
Receptor tyrosine kinase
T cell receptor
TGF-β receptor
GSK3β/APC
β-catenin
ERK/MAPK
SMADs
MYC
RNA
TFIIH
RNA pol
CANNTG
Target genes
E-box
Cell growth and proliferation
+P53
P53
Dang, Cell 2012

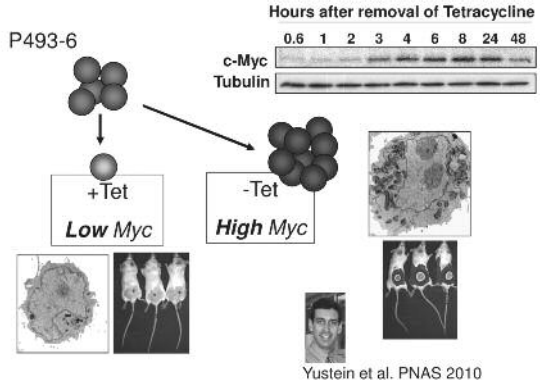
6

Cancer & Myc target genes



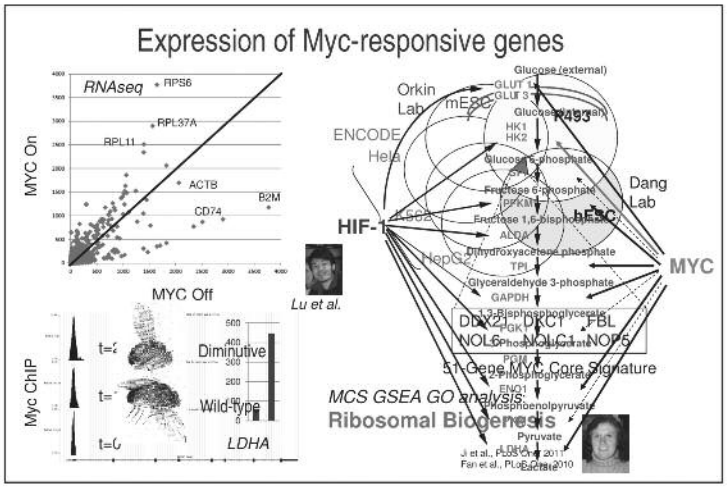
7

P493: Human Burkitt Lymphoma *model*



Yustein et al. PNAS 2010

8


Expression of Myc-responsive genes
RNAseq
RPS6
RPL37A
RPL11
ACTB
B2M
CD74
MYC On
MYC Off
Myc ChIP
Diminutive
Wild-type
LDHA
Lu et al.
Orkin Lab
mESC
ENCODE
HeLa
HepG2
Dang Lab
hESC
HIF-1
MYC
Glucose (external)
GLUT 1
GLUT 3
HK1
HK2
Glucose 6-phosphate
Fructose 6-phosphate
PFKM
Fructose 1,6-bisphosphate
ALDA
Dihydroxyacetone phosphate
TPI
Glyceraldehyde 3-phosphate
GAPDH
1,3-Bisphosphoglycerate
PGK
2-Phosphoglycerate
ENO1
Phosphoenolpyruvate
Pyruvate
LDHA
Lactate
DDX21
DKC1
FBL
NOL6
NOLC1
NOP5
51-Gene MYC Core Signature
MCS GSEA GO analysis:
Ribosomal Biogenesis
Ji et al., PLoS One 2011
Fan et al., PLoS One 2010

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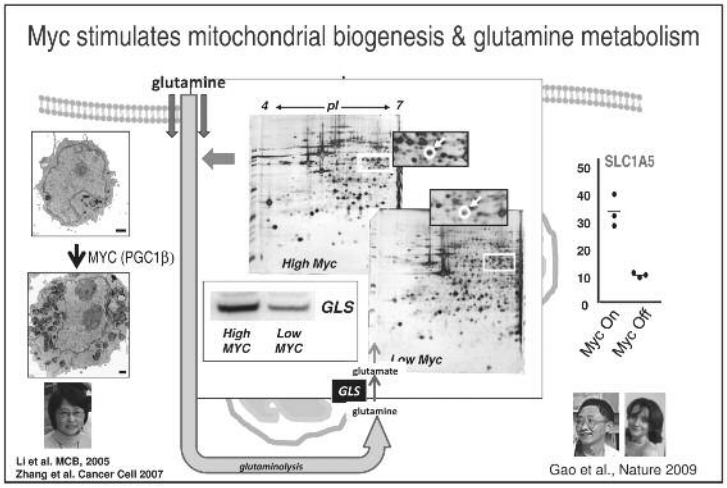
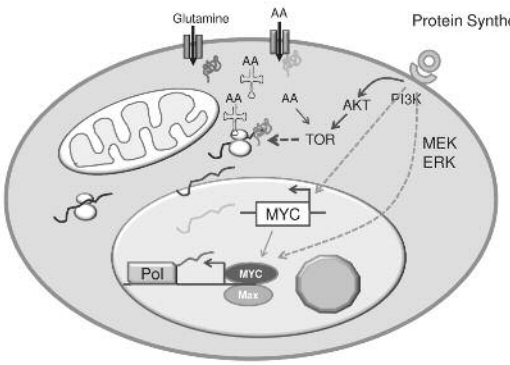

Myc stimulates mitochondrial biogenesis & glutamine metabolism
glutamine
MYC (PGC1β)
4
pI
7
High Myc
Low Myc
GLS
High MYC
Low MYC
glutamate
glutamine
glutaminolysis
SLC1A5
50
40
30
20
10
0
Myc On
Myc Off
Li et al. MCB, 2005
Zhang et al. Cancer Cell 2007
Gao et al., Nature 2009

10

Myc: Biomass accumulation and metabolism



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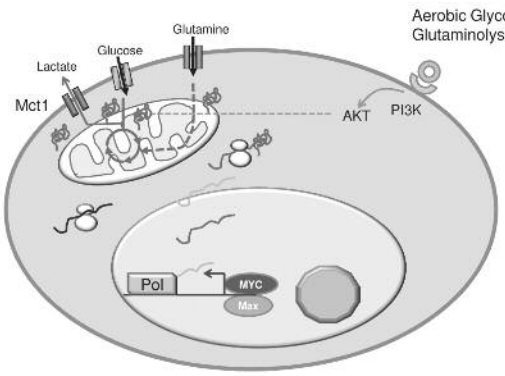
Myc: Biomass accumulation and metabolism



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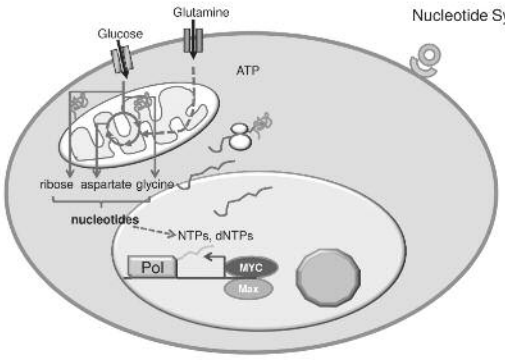
Myc: Biomass accumulation and metabolism



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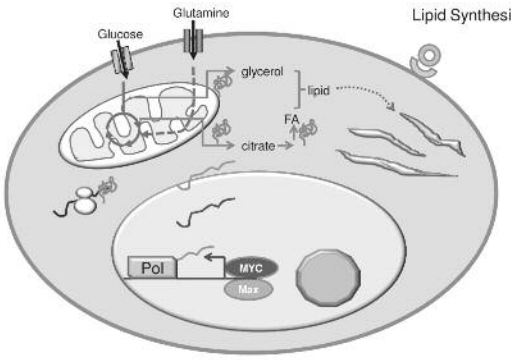
Myc: Biomass accumulation and metabolism



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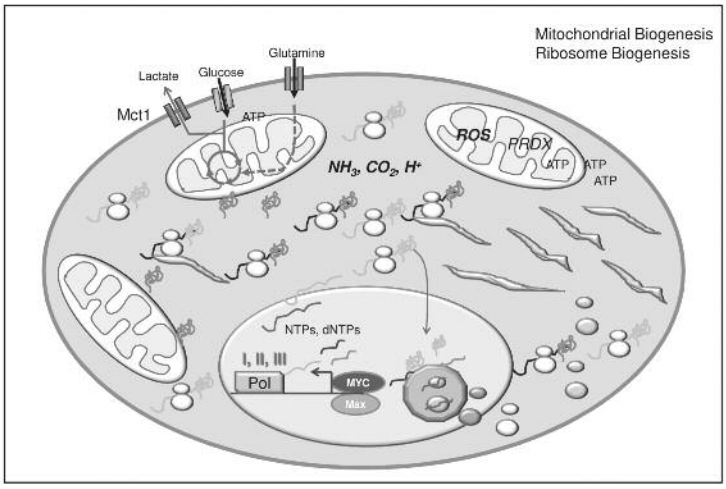
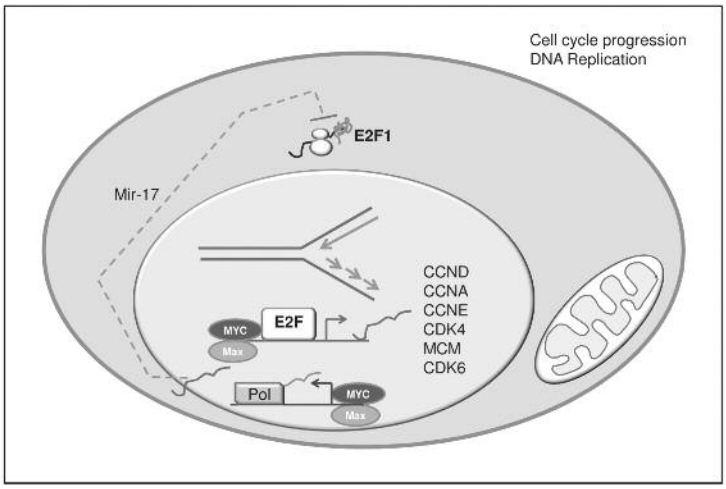
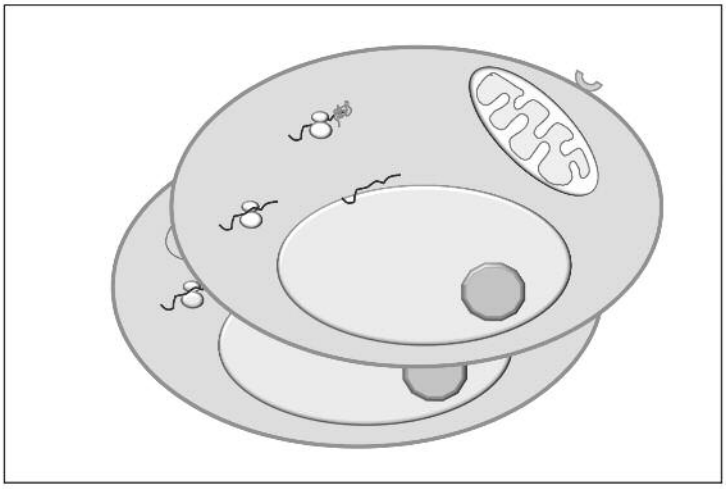

Mitochondrial Biogenesis
Ribosome Biogenesis
Glutamine
Lactate
Glucose
Mct1
ATP
ROS
PRDX
ATP
ATP
ATP
NH_3, CO_2, H^+
NTPs, dNTPs
I, II, III
Pol
MYC
Max

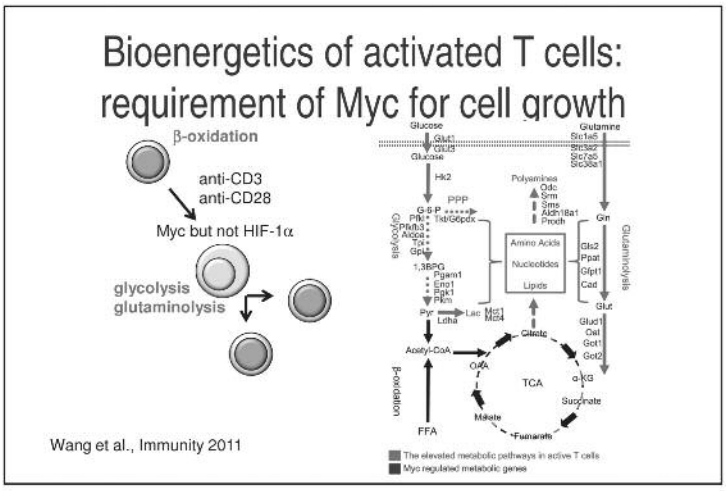
15


Cell cycle progression
DNA Replication
E2F1
Mir-17
CCND
CCNA
CCNE
CDK4
MCM
CDK6
MYC
E2F
Max
Pol
MYC
Max

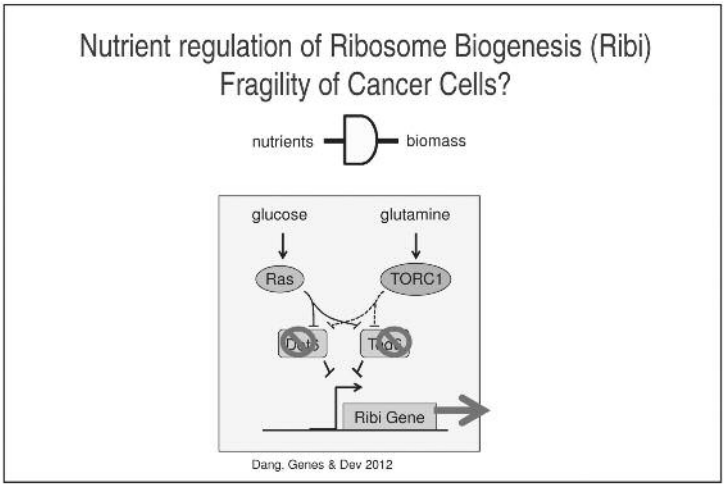
16



17


Bioenergetics of activated T cells:
requirement of Myc for cell growth
β-oxidation
anti-CD3
anti-CD28
Myc but not HIF-1α
glycolysis
glutaminolysis
Wang et al., Immunity 2011
Glucose
Glutamine
Hk2
PPP
Polyamines
Amino Acids
Nucleotides
Lipids
Pyr
Lac
Citrate
Acetyl-CoA
OAA
TCA
α-KG
Succinate
Malate
Fumarate
FFA
β-oxidation
Glutaminolysis
The elevated metabolic pathways in active T cells
Myc regulated metabolic genes

18


Nutrient regulation of Ribosome Biogenesis (Ribi)
Fragility of Cancer Cells?
nutrients
biomass
glucose
glutamine
Ras
TORC1
Ribi Gene
Dang, Genes & Dev 2012

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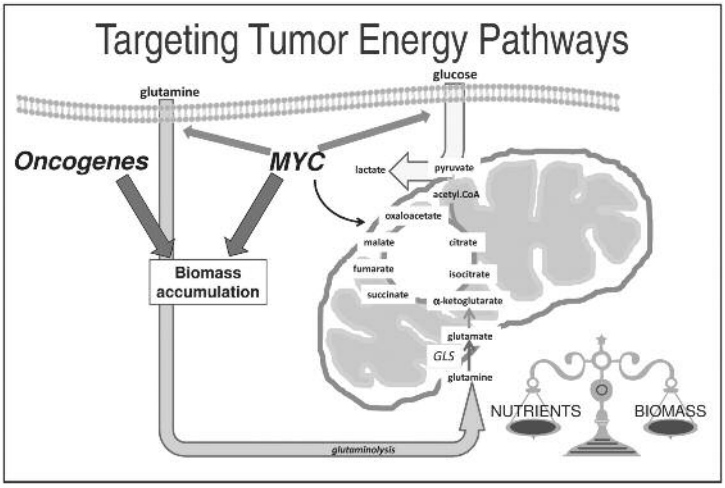
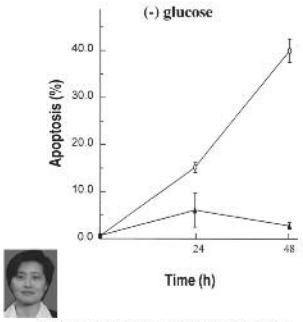

Targeting Tumor Energy Pathways
glutamine
glucose
Oncogenes
MYC
lactate
pyruvate
acetyl.CoA
oxaloacetate
malate
citrate
fumarate
isocitrate
succinate
α-ketoglutarate
Biomass accumulation
glutamate
GLS
glutamine
NUTRIENTS
BIOMASS
glutaminolysis

20

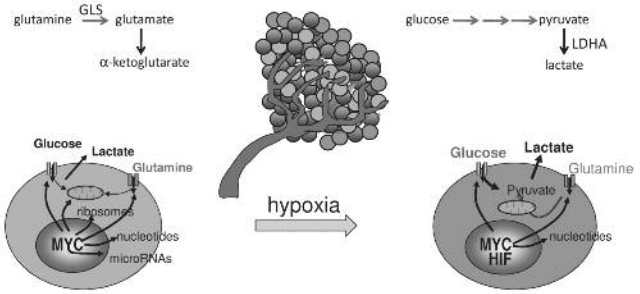
Cancer Cell Fragility: Myc-induced addiction to glucose or glutamine



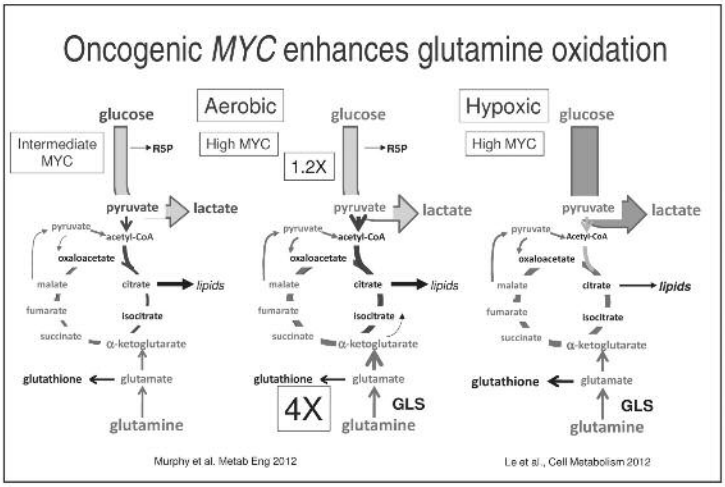
Shim H et al. PNAS 1998;95:1511-1516

21

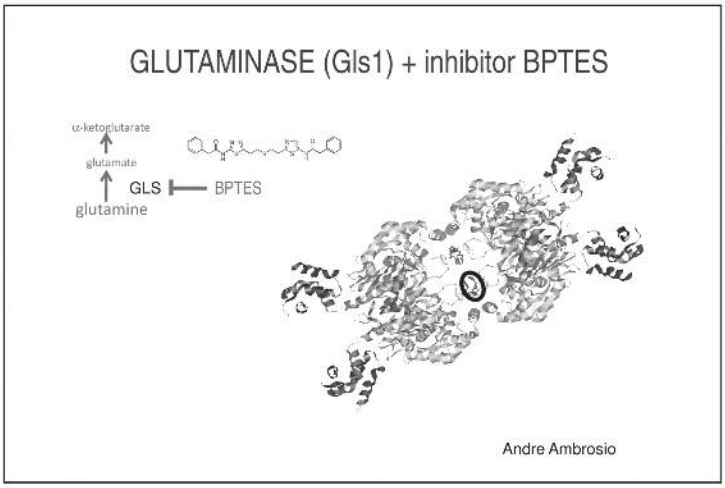
Glutamine & Tumor Tissue Metabolism



22


Oncogenic MYC enhances glutamine oxidation
glucose
Aerobic
Intermediate MYC
High MYC
R5P
pyruvate
lactate
acetyl-CoA
oxaloacetate
citrate
lipids
malate
fumarate
isocitrate
succinate
α-ketoglutarate
glutathione
glutamate
glutamine
1.2X
4X
GLS
Hypoxic
Acetyl-CoA
Murphy et al. Metab Eng 2012
Le et al , Cell Metabolism 2012

23


GLUTAMINASE (Gls1) + inhibitor BPTES
α-ketoglutarate
glutamate
GLS
BPTES
glutamine
Andre Ambrosio

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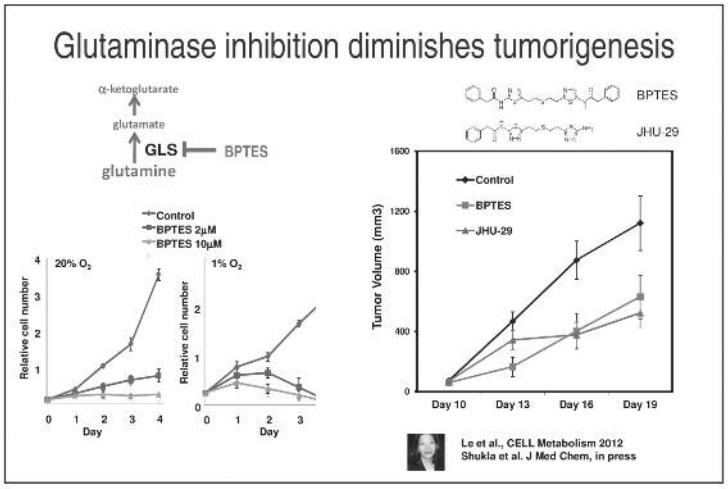
Glutaminase inhibition diminishes tumorigenesis
α-ketoglutarate
glutamate
GLS
BPTES
glutamine
BPTES
JHU-29
Control
BPTES 2μM
BPTES 10μM
20% O2
1% O2
Relative cell number
Day
Control
BPTES
JHU-29
Tumor Volume (mm3)
Day 10
Day 13
Day 16
Day 19
Le et al., CELL Metabolism 2012
Shukla et al. J Med Chem, in press

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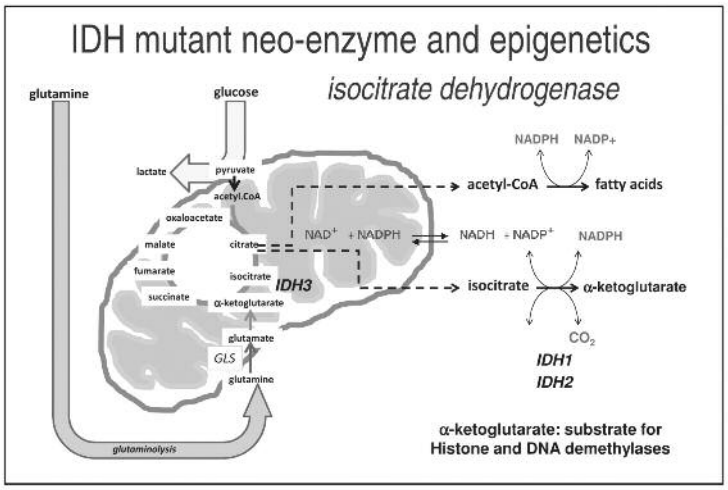
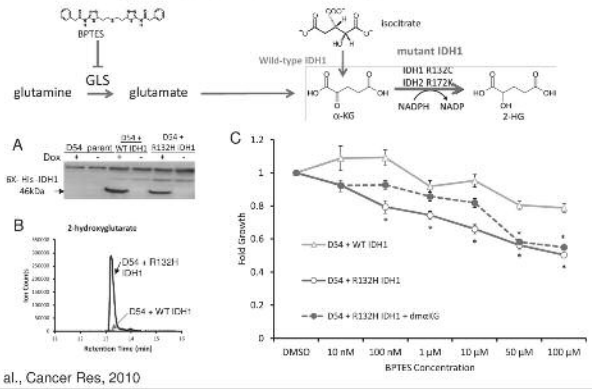

IDH mutant neo-enzyme and epigenetics
isocitrate dehydrogenase
glutamine
glucose
lactate
pyruvate
acetyl.CoA
oxaloacetate
malate
citrate
fumarate
isocitrate
succinate
α-ketoglutarate
IDH3
glutamate
GLS
glutamine
glutaminolysis
NADPH NADP+
acetyl-CoA
fatty acids
NAD+ + NADPH
NADH + NADP+
NADPH
isocitrate
α-ketoglutarate
CO2
IDH1
IDH2
α-ketoglutarate: substrate for
Histone and DNA demethylases

26

Glutaminase inhibition diminishes growth of glioma cells with mutant IDH1

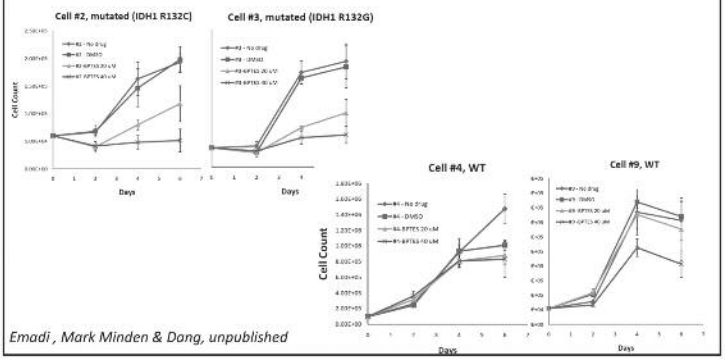


Seltzer et al., Cancer Res, 2010

27

BPTES inhibits AML cells with mutant IDH1

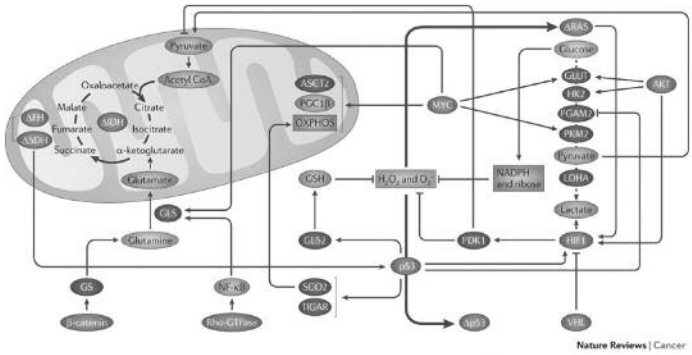
- IDH mutation found in 30% acute myelogenous leukemias
- IDH mutation found in myelofibrosis and transforming MPD ~ 20%
- Blinded study: 2/2 wildtype IDH AMLs relative resistant; 4/4 mutant IDH AMLs sensitive to BPTES



Emadi , Mark Minden & Dang, unpublished

28

Network of cancer genes and metabolism



Koppenol, Bounds and Dang, 2011

29

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ACKNOWLEDGEMENTS

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Peng Sun
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Karen Zeller

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S. Rayport
R. Kumar/A. Maitra
J Mendell
Kamphorst/Rabinowitz
G Semenza
Murphy/Young
C Thompson
A Thomas-Tikhonenko
T Tsukamoto/Shukla
B Slusher
D Vander Jagt
D Liebler/R Slebbos
P Datta, R Gillies

Metabolism and Apoptotic Pathways in Leukemia

Jeff Rathmell

Department of Pharmacology and Cancer Biology
Department of Immunology
Stedman Nutrition and Metabolism Center

Duke University

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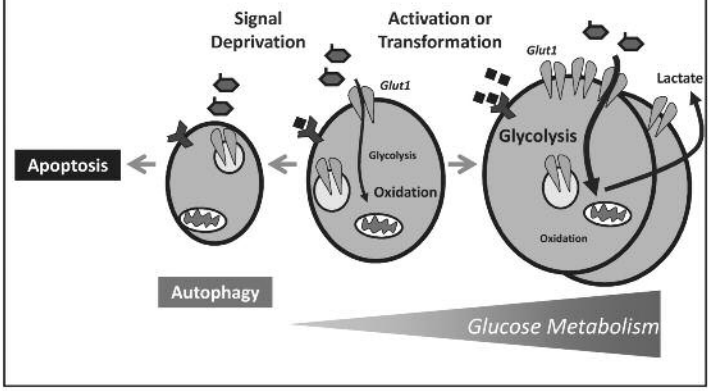
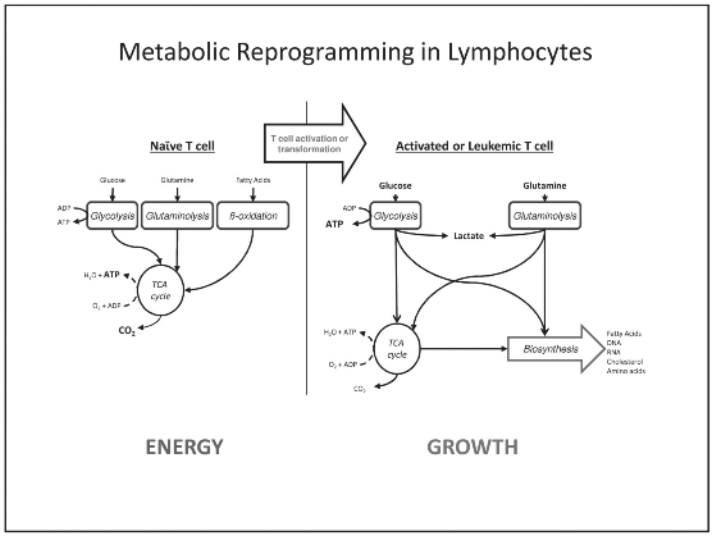
- *Glucose is a primary fuel and glucose metabolism is highly regulated*
- *Resting lymphocytes are oxidative, activated & leukemic T cells are glycolytic*
- *Cell metabolism may be a checkpoint for cell fate in immunity and cancer*



32


Metabolic Reprogramming in Lymphocytes
Naïve T cell
T cell activation or transformation
Activated or Leukemic T cell
Glucose
Glutamine
Fatty Acids
ADP
ATP
Glycolysis
Glutaminolysis
β-oxidation
TCA cycle
H_2O + ATP
O_2 + ADP
CO_2
Lactate
Biosynthesis
Fatty Acids
DNA
RNA
Cholesterol
Amino acids
ENERGY
GROWTH

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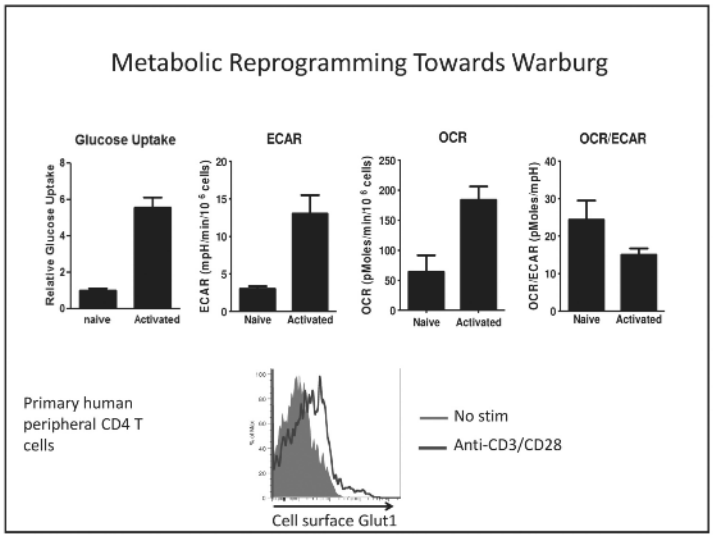
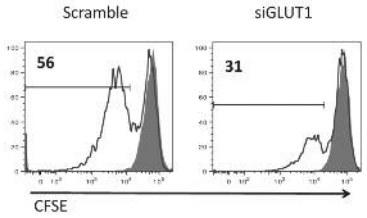

Metabolic Reprogramming Towards Warburg
Glucose Uptake
ECAR
OCR
OCR/ECAR
Relative Glucose Uptake
ECAR (mpH/min/10 6 cells)
OCR (pMoles/min/10 6 cells)
OCR/ECAR (pMoles/mpH)
naive
Activated
Naive
Activated
Primary human peripheral CD4 T cells
No stim
Anti-CD3/CD28
Cell surface Glut1

34

Glut1 is Required for T Cell Activation and Proliferation



Primary human peripheral CD4 T cells

No stim
Anti-CD3/CD28

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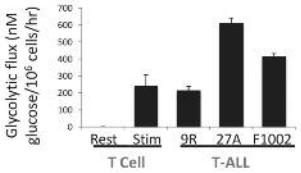
Leukemic T Cells Are Highly Glycolytic

Otto Warburg

Showed in solid tumors that cancer cells reprogram to aerobic glycolysis

Now known to be due to activation of oncogenic signaling pathways and hypoxia



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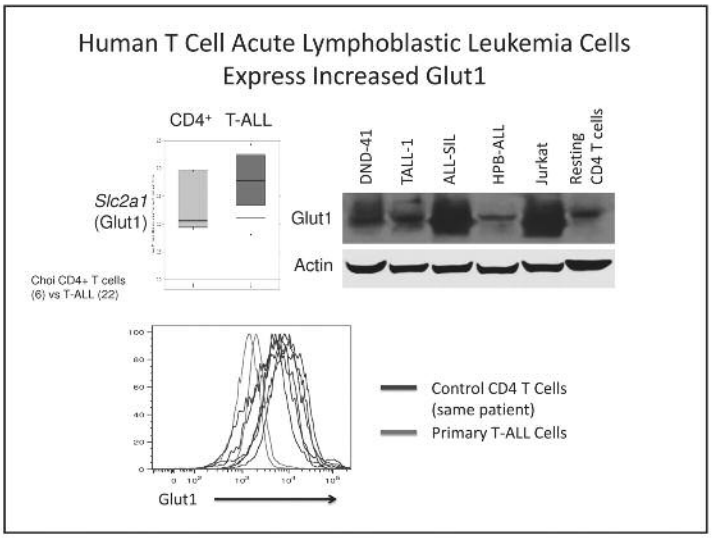
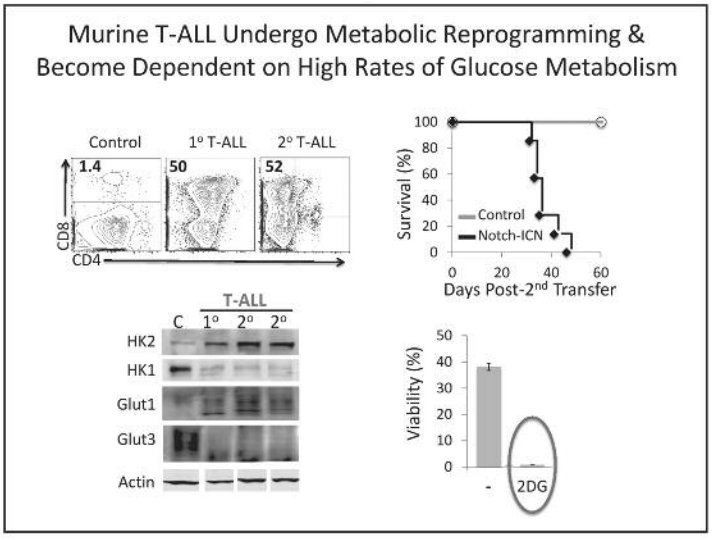

Human T Cell Acute Lymphoblastic Leukemia Cells Express Increased Glut1
CD4+ T-ALL
Slc2a1 (Glut1)
Choi CD4+ T cells (6) vs T-ALL (22)
DND-41
TALL-1
ALL-SIL
HPB-ALL
Jurkat
Resting CD4 T cells
Glut1
Actin
Control CD4 T Cells (same patient)
Primary T-ALL Cells
Glut1

37


Murine T-ALL Undergo Metabolic Reprogramming & Become Dependent on High Rates of Glucose Metabolism
Control
1° T-ALL
2° T-ALL
1.4
50
52
CD8
CD4
T-ALL
C
1°
2°
2°
HK2
HK1
Glut1
Glut3
Actin
Survival (%)
Control
Notch-ICN
Days Post-2nd Transfer
Viability (%)
-
2DG

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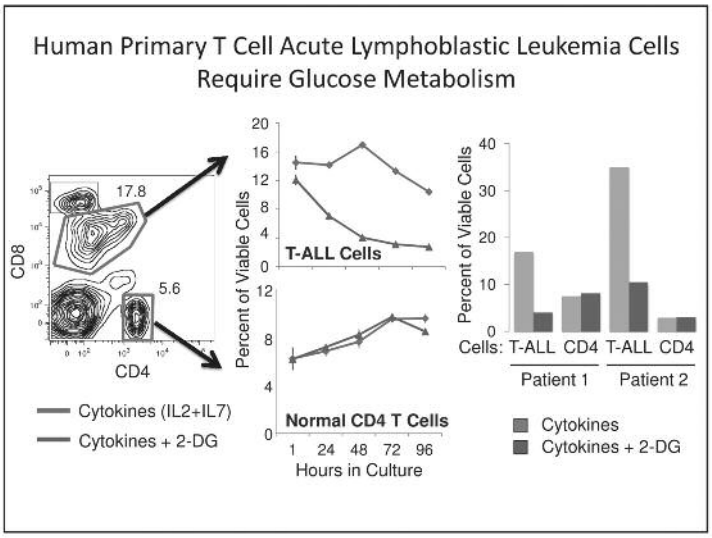
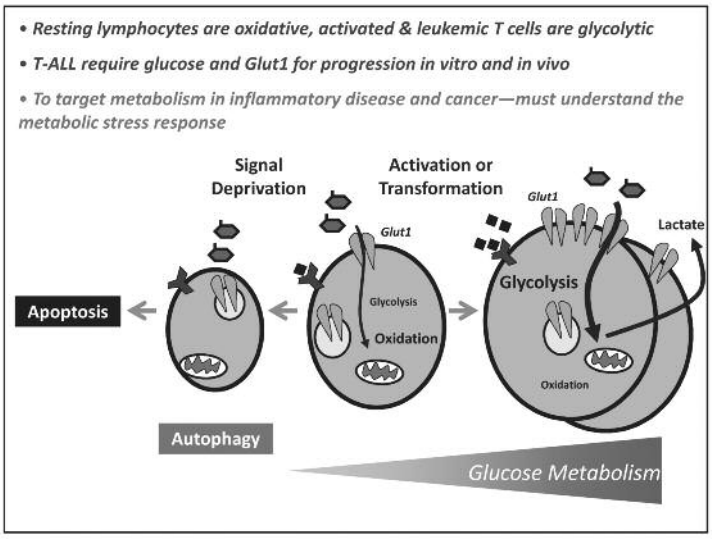

Human Primary T Cell Acute Lymphoblastic Leukemia Cells Require Glucose Metabolism
CD8
CD4
17.8
5.6
Cytokines (IL2+IL7)
Cytokines + 2-DG
Percent of Viable Cells
T-ALL Cells
Normal CD4 T Cells
1 24 48 72 96
Hours in Culture
Percent of Viable Cells
Cells: T-ALL CD4 T-ALL CD4
Patient 1
Patient 2
Cytokines
Cytokines + 2-DG

39


• Resting lymphocytes are oxidative, activated & leukemic T cells are glycolytic
• T-ALL require glucose and Glut1 for progression in vitro and in vivo
• To target metabolism in inflammatory disease and cancer—must understand the metabolic stress response
Signal Deprivation
Activation or Transformation
Glut1
Glut1
Lactate
Apoptosis
Glycolysis
Oxidation
Glycolysis
Oxidation
Autophagy
Glucose Metabolism

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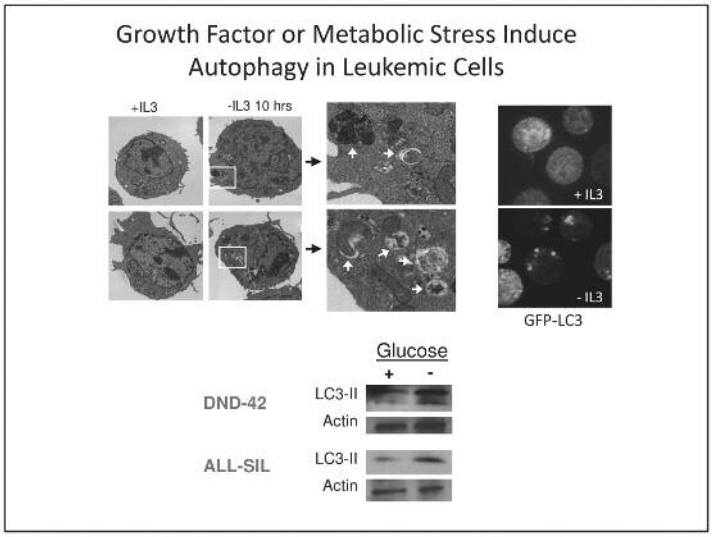
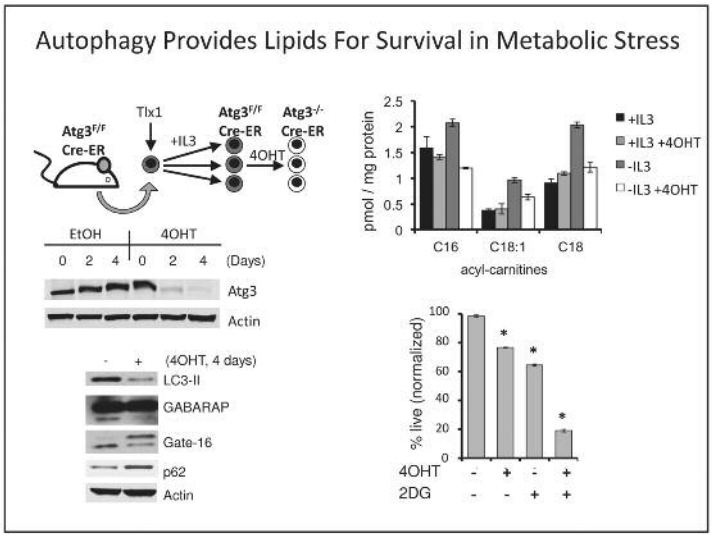
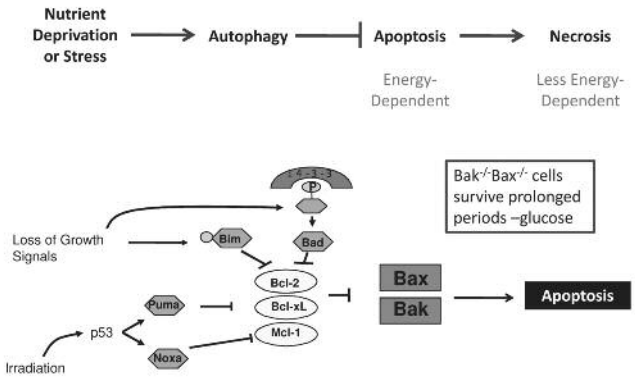

Growth Factor or Metabolic Stress Induce Autophagy in Leukemic Cells
+IL3
-IL3 10 hrs
+ IL3
- IL3
GFP-LC3
Glucose
+ -
DND-42
LC3-II
Actin
ALL-SIL
LC3-II
Actin

41


Autophagy Provides Lipids For Survival in Metabolic Stress
Atg3F/F Cre-ER
Tlx1
+IL3
Atg3F/F Cre-ER
Atg3-/- Cre-ER
4OHT
EtOH
4OHT
0 2 4 0 2 4 (Days)
Atg3
Actin
- + (4OHT, 4 days)
LC3-II
GABARAP
Gate-16
p62
Actin
pmol / mg protein
+IL3
+IL3 +4OHT
-IL3
-IL3 +4OHT
C16
C18:1
C18
acyl-carnitines
% live (normalized)
4OHT
2DG

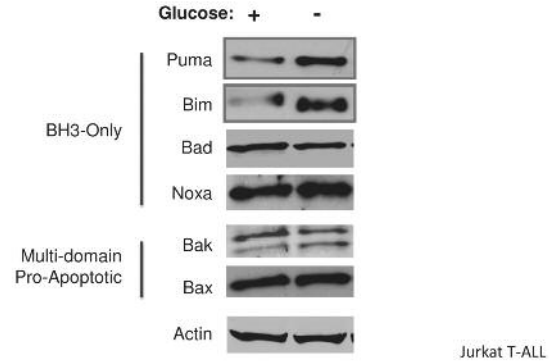
42

What is the Role of the Bcl-2 Family When Aerobic Glycolysis is Disrupted?



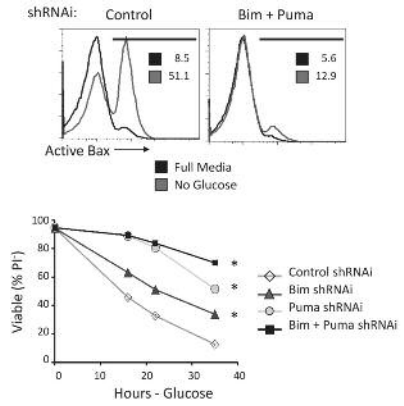
43

Glucose Deprivation Leads to Changes in Expression of Select Pro-Apoptotic Bcl-2 Family Members



44

A Bcl-2 Family Regulated Metabolic Checkpoint is ESSENTIAL for Rapid Cell Death



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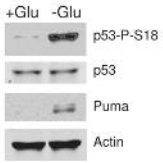
How is Puma Regulated?

Glucose Deprivation Leads to p53 Phosphorylation and Puma Induction



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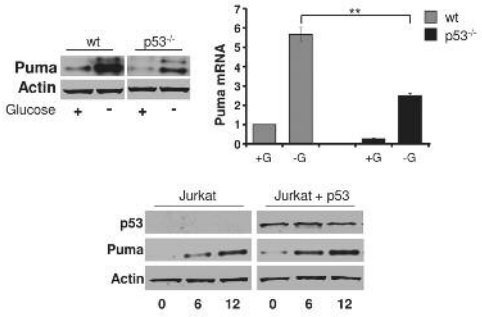
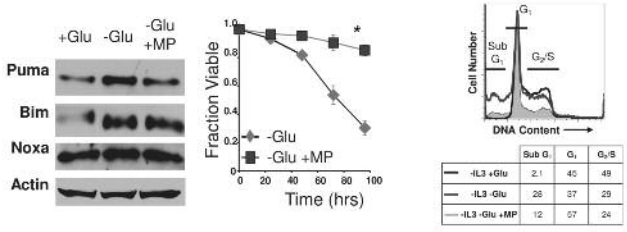
p53 Contributes But is Not Necessary for Puma Transcriptional Induction



47

Pyruvate Can Replace Glucose To Rescue Puma and Cell Survival



	Sub G1	G1	G2/S
-IL3 +Glu	2.1	45	49
-IL3 -Glu	28	37	29
-IL3 -Glu +MP	12	57	24

Bim is not affected by Pyruvate

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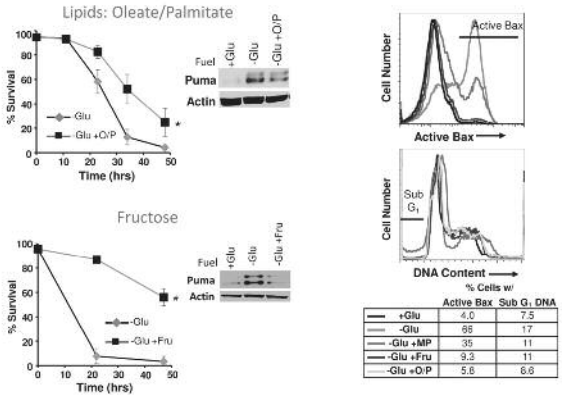
Alternative Fuels Also Rescue Puma and Cell Viability



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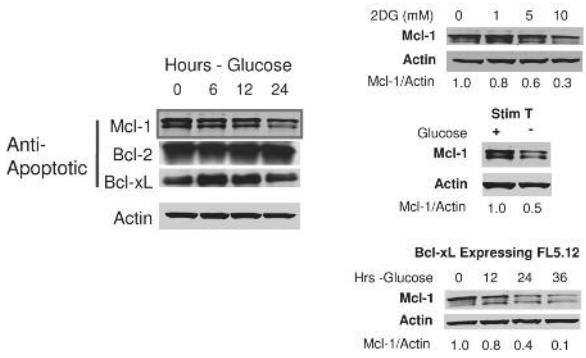
Mcl-1 is Dependent on Continued Metabolism While Bcl-2 and Bcl-xL Are Not

Glucose:	+	-
Mcl1/Actin	1	0.6
Bcl-2/Actin	1	1.1
Bcl-xL/Actin	1	1.1

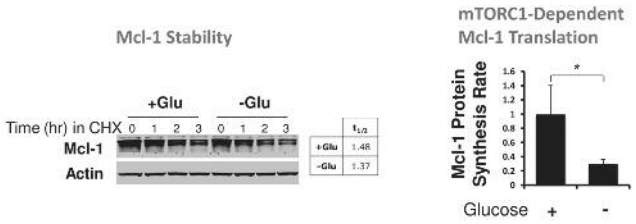
50

Mcl-1 is Dependent on Continued Metabolism While Bcl-2 and Bcl-xL Are Not

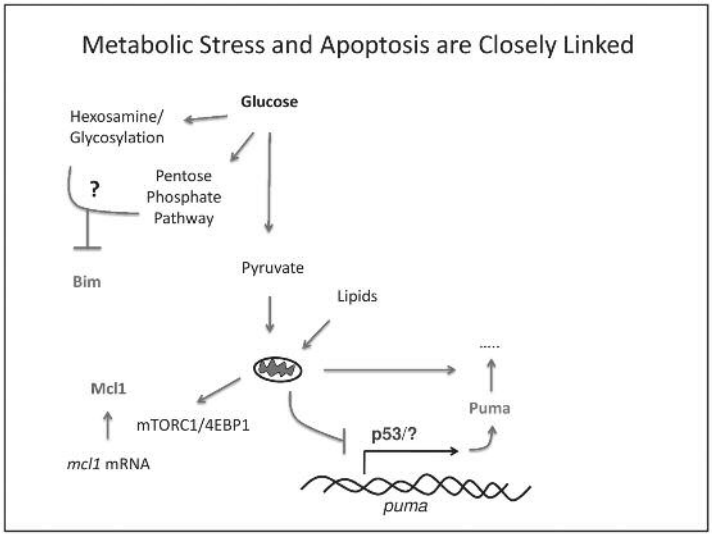


51

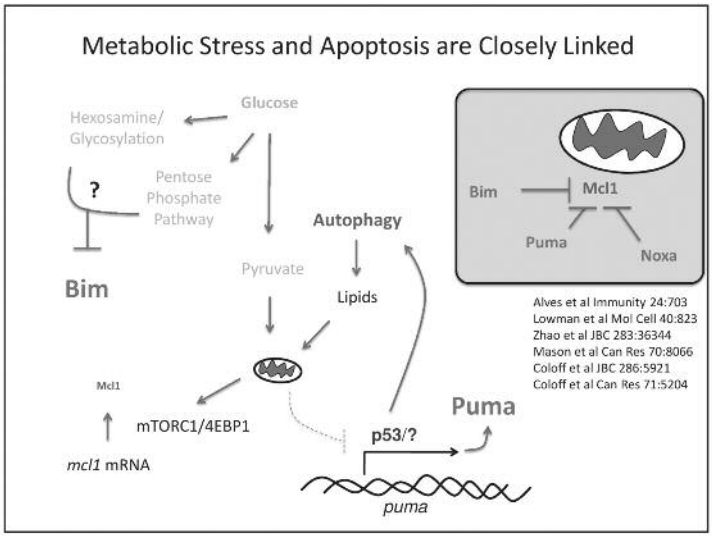
Mcl-1 Expression Decreases Due to Reduced Protein Translation



52


Metabolic Stress and Apoptosis are Closely Linked
Glucose
Hexosamine/
Glycosylation
?
Pentose
Phosphate
Pathway
Bim
Pyruvate
Lipids
.....
Mcl1
Puma
mTORC1/4EBP1
p53/?
mcl1 mRNA
puma

53


Metabolic Stress and Apoptosis are Closely Linked
Glucose
Hexosamine/
Glycosylation
?
Pentose
Phosphate
Pathway
Autophagy
Bim
Pyruvate
Lipids
Mcl1
mTORC1/4EBP1
p53/?
Puma
mcl1 mRNA
puma
Bim
Mcl1
Puma
Noxa
Alves et al Immunity 24:703
Lowman et al Mol Cell 40:823
Zhao et al JBC 283:36344
Mason et al Can Res 70:8066
Coloff et al JBC 286:5921
Coloff et al Can Res 71:5204

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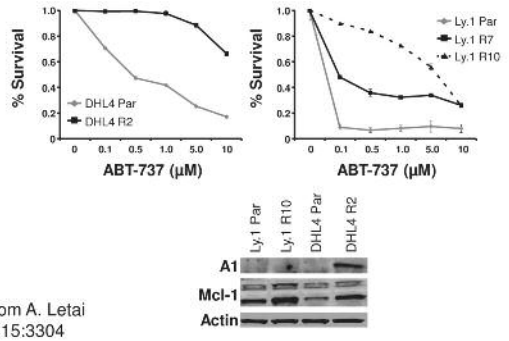
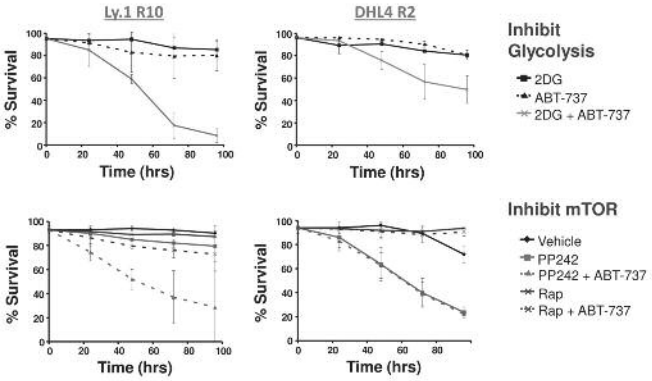
Lymphoma Cells Develop Resistance to ABT-737 by Mcl-1 and/or A1 Upregulation



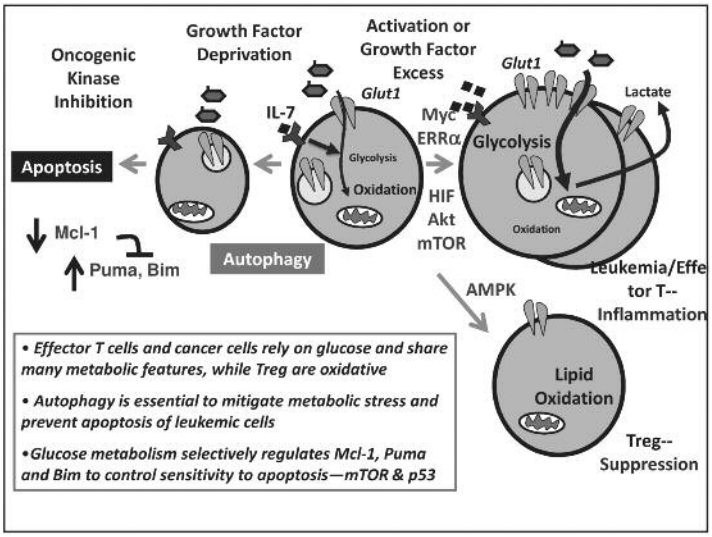
Cells from A. Letai
Blood 115:3304

55

Metabolic Interference of Mcl-1 Synergizes With ABT-737



56


Oncogenic Kinase Inhibition
Growth Factor Deprivation
Activation or Growth Factor Excess
Glut1
IL-7
Myc
ERRα
Glycolysis
Lactate
Apoptosis
Oxidation
HIF
Akt
mTOR
Mcl-1
Puma, Bim
Autophagy
AMPK
Leukemia/Effector T--Inflammation
Lipid Oxidation
Treg--Suppression
• Effector T cells and cancer cells rely on glucose and share many metabolic features, while Treg are oxidative
• Autophagy is essential to mitigate metabolic stress and prevent apoptosis of leukemic cells
• Glucose metabolism selectively regulates Mcl-1, Puma and Bim to control sensitivity to apoptosis—mTOR & p53

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Tingyu Liu
Andrew MacIntyre
Nancie MacIver
Donte Moore
Amanda Nichols
Duke University
Chris Newgard
Julia Walker
You-Wen He
Donald McDonnell
Past Lab Member Contributions
Brian Altman
Emily Mason
Jon Coloff
Ryan Michalek
McGill University
Vincent Giguere
Russell Jones
U. of Utah
E Dale Abel
U. Of Colorado
Mike Rudolph
Steve Anderson
LEUKEMIA & LYMPHOMA SOCIETY
fighting blood cancers
American Asthma Foundation, Gabrielle's Angel Foundation, Alex's Lemonade Stand, NIAID, NCI, NHLBI
Lupus Research Institute

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mTOR links cell signaling and metabolism

Mariusz A. Wasik, MD

LLS-ASH
Dec. 7, 2012

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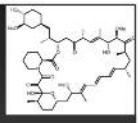
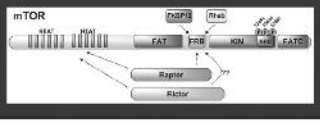
mTOR



(mammalian/mechanistic Target Of Rapamycin):

- ubiquitously expressed serine/treonine kinase

- forms complexes with several proteins including raptor (complex 1: mTORC1) or rictor (complex 2: mTORC2)

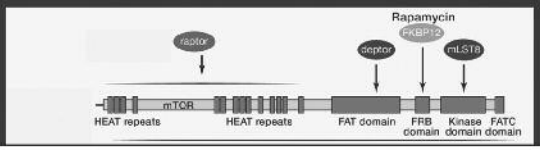


60

Inhibitors of mTOR kinase activity- type I

Rapamycin and its derivatives (Rapalogs)

- inhibit protein-protein interaction (raptor binding)
- inhibit mTOR complex 1 (mTORC1)
- are highly specific and very potent (cell IC50: pM- low nM)

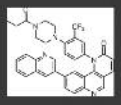


61

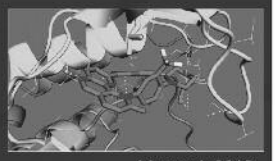
Inhibitors of mTOR kinase activity- type II

Direct kinase domain inhibitors/ATP binding competitors

- inhibit both mTORC1 and mTORC2 (some also PI3K)
- are less specific than rapalogs

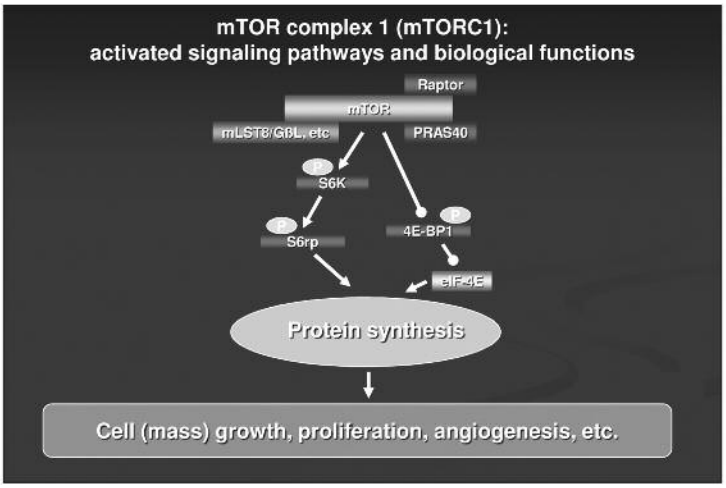


Torin

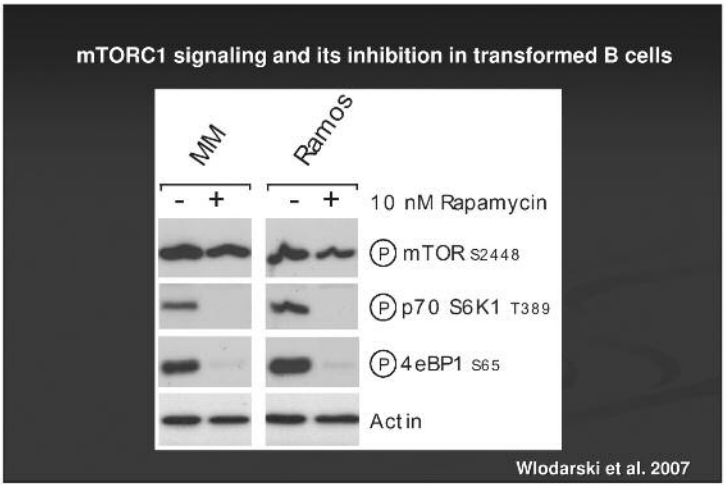


Liu et al. 2012

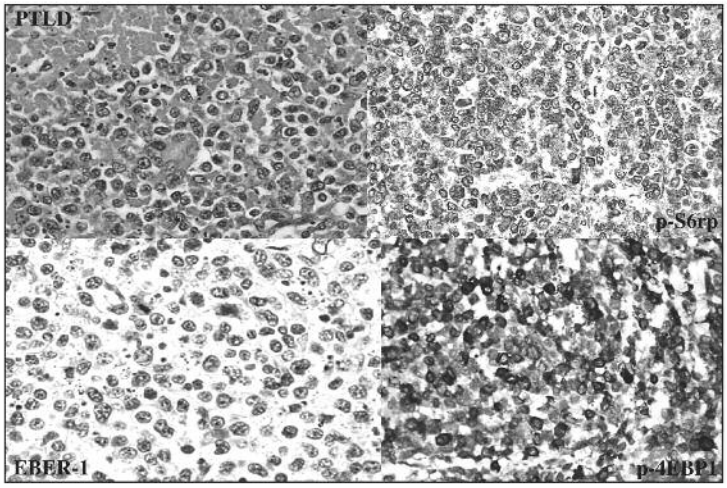
62


mTOR complex 1 (mTORC1):
activated signaling pathways and biological functions
Raptor
mTOR
mLST8/GβL, etc
PRAS40
P
S6K
P
S6rp
P
4E-BP1
eIF-4E
Protein synthesis
Cell (mass) growth, proliferation, angiogenesis, etc.

63


mTORC1 signaling and its inhibition in transformed B cells
MM
Ramos
- + - +
10 nM Rapamycin
Ⓟ mTOR S2448
Ⓟ p70 S6K1 T389
Ⓟ 4eBP1 S65
Actin
Wlodarski et al. 2007

64



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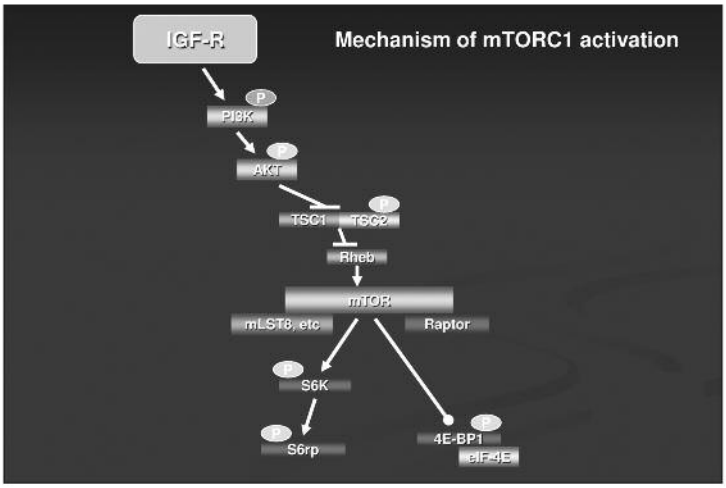
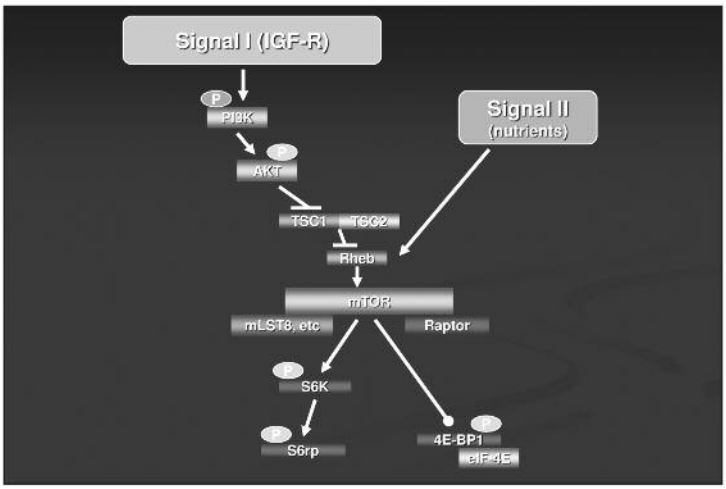
mTORC1 activation in lymphomas

- PTLD (various types including hyperplasia)
- diffuse large B-cell lymphoma
- ALK+ anaplastic large cell lymphoma
- cutaneous T-cell lymphoma
- AIDS-related lymphomas (various types)

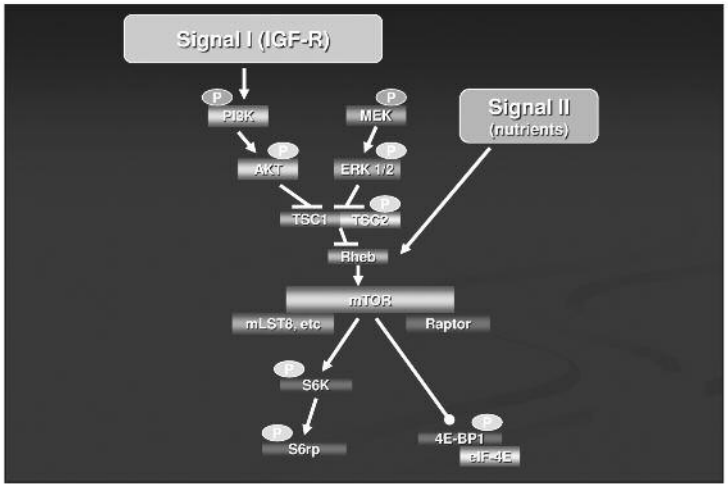
Majewski et al. 2000&2003, Wlodarski et al. 2005,
Marzec et al. 2007&2008, El-Salem et al. 2007&2009


IGF-R
Mechanism of mTORC1 activation
P
PI3K
P
AKT
P
TSC1
TSC2
Rheb
mTOR
mLST8, etc
Raptor
P
S6K
P
S6rp
P
4E-BP1
eIF-4E

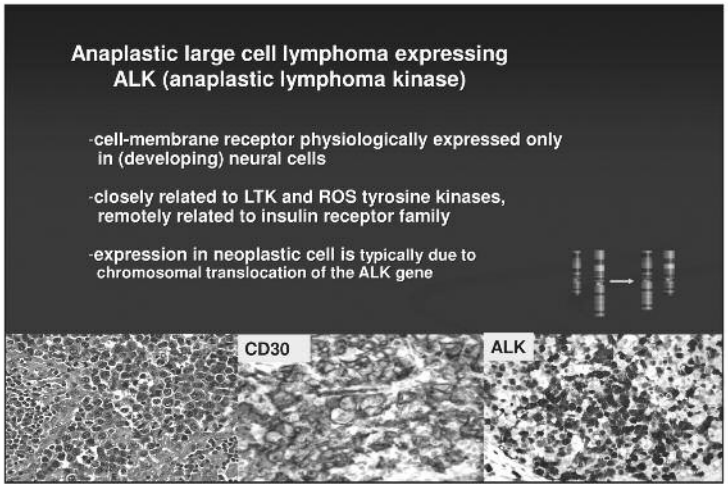
67


Signal I (IGF-R)
P
PI3K
Signal II
(nutrients)
P
AKT
P
TSC1
TSC2
Rheb
mTOR
mLST8, etc
Raptor
P
S6K
P
S6rp
P
4E-BP1
eIF-4E

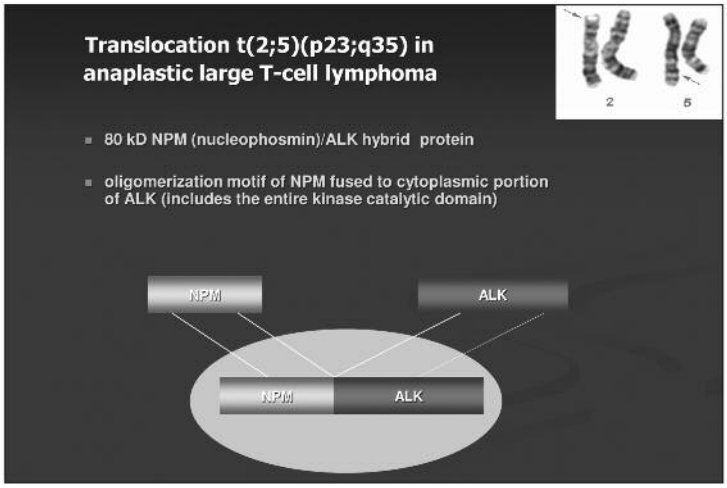
68


Signal I (IGF-R)
P
PI3K
P
MEK
Signal II
(nutrients)
P
AKT
P
ERK 1/2
TSC1
TSC2
P
Rheb
mTOR
mLST8, etc
Raptor
P
S6K
P
S6rp
P
4E-BP1
eIF-4E

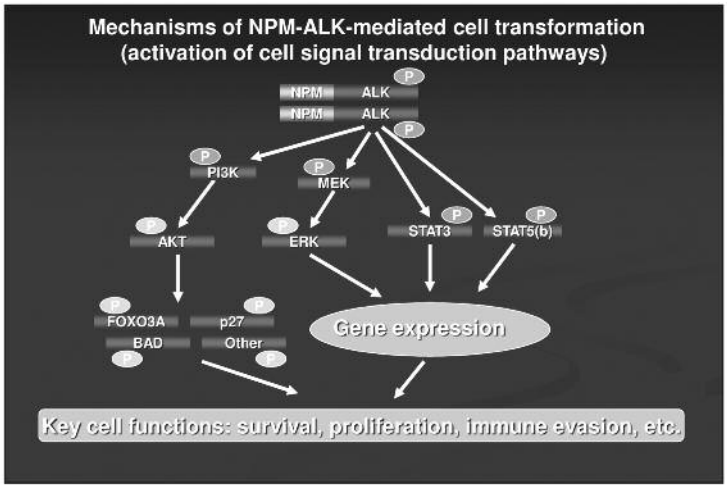
69


Anaplastic large cell lymphoma expressing
ALK (anaplastic lymphoma kinase)
-cell-membrane receptor physiologically expressed only in (developing) neural cells
-closely related to LTK and ROS tyrosine kinases, remotely related to insulin receptor family
-expression in neoplastic cell is typically due to chromosomal translocation of the ALK gene
CD30
ALK

70


Translocation t(2;5)(p23;q35) in anaplastic large T-cell lymphoma
2
5
80 kD NPM (nucleophosmin)/ALK hybrid protein
oligomerization motif of NPM fused to cytoplasmic portion of ALK (includes the entire kinase catalytic domain)
NPM
ALK
NPM
ALK

71


Mechanisms of NPM-ALK-mediated cell transformation (activation of cell signal transduction pathways)
NPM
ALK
NPM
ALK
P
PI3K
MEK
AKT
ERK
STAT3
STAT5(b)
FOXO3A
BAD
p27
Other
Gene expression
Key cell functions: survival, proliferation, immune evasion, etc.

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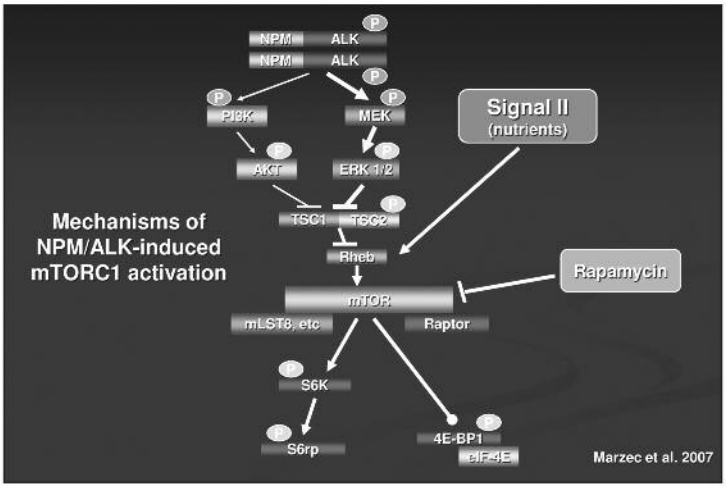
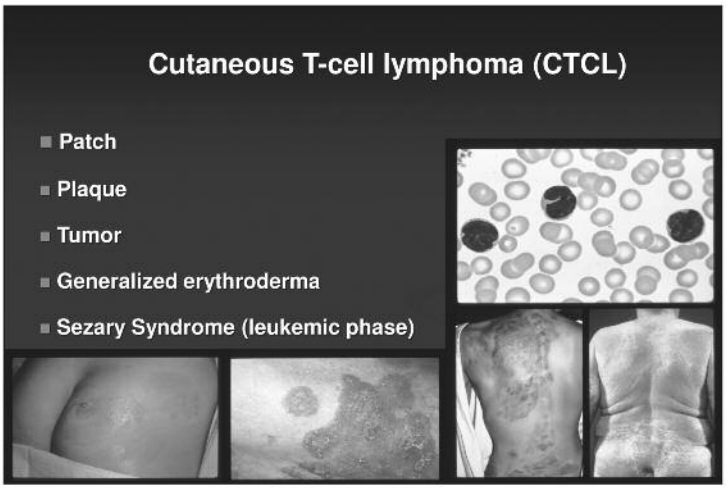
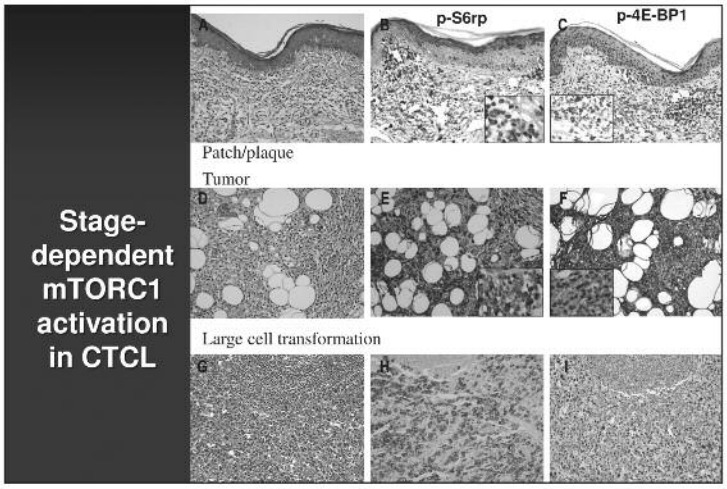


Mechanisms of NPM/ALK-induced mTORC1 activation

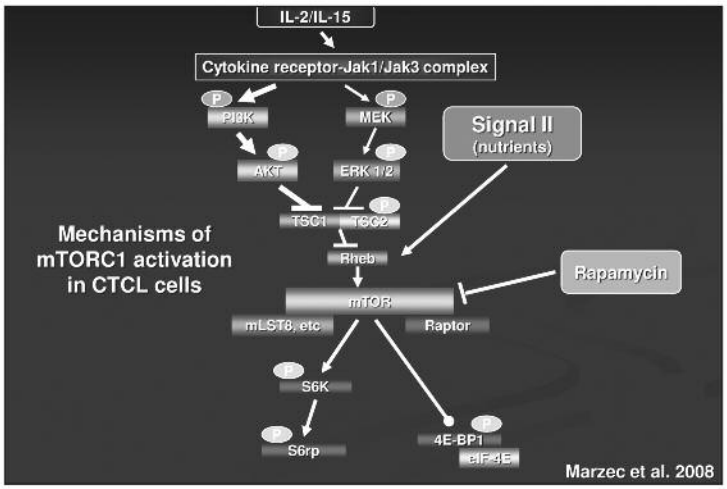
73



74


Stage-dependent mTORC1 activation in CTCL
p-S6rp
p-4E-BP1
Patch/plaque
Tumor
Large cell transformation

75


IL-2/IL-15
Cytokine receptor-Jak1/Jak3 complex
PI3K
MEK
Signal II
(nutrients)
AKT
ERK 1/2
TSC1
TSC2
Rheb
Rapamycin
mTOR
mLST8, etc
Raptor
S6K
S6rp
4E-BP1
eIF-4E
Mechanisms of mTORC1 activation in CTCL cells
Marzec et al. 2008

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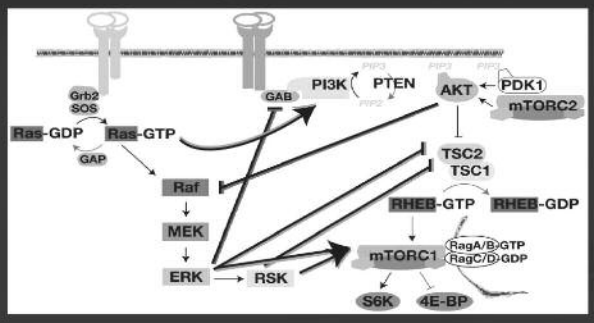
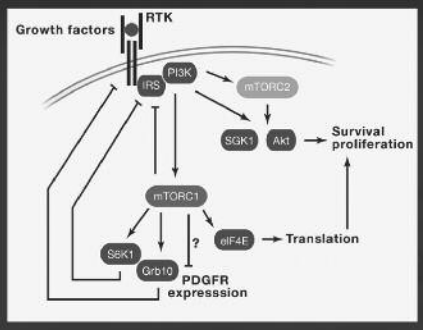
MEK-ERK and PI3K-AKT pathways: cross-talk and impact on mTORC1 activation



Mendoza et al. 2011

77

mTORC1 generates negative feedback to suppress (?fine-tunes) cytokine/growth factor signal



Laplante & Sabatini 2012

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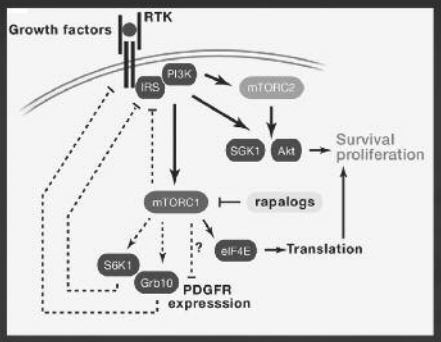
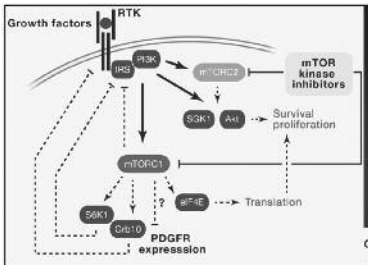
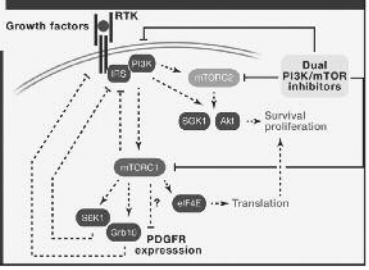
mTORC1-generated negative feedback: impact of mTORC1 inhibition



79

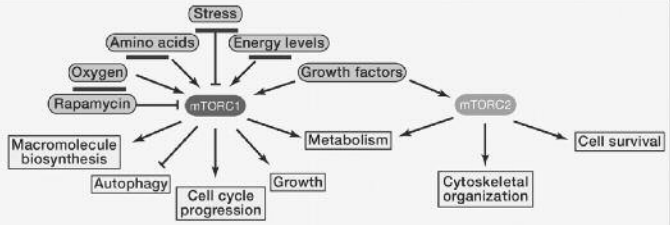


mTORC1 generated negative feedback: impact of direct mTOR (and PI3K) inhibition



80

mTORC1 activation by diverse metabolic signals



Laplante & Sabatini 2012

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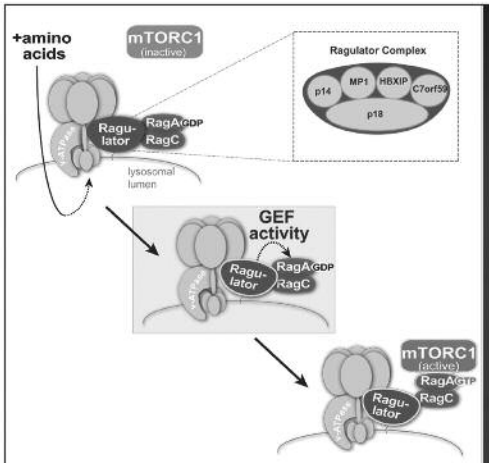
Amino acids-mediated mTORC1 activation and translocation



Efeyan et al. 2012
Bar-Peled et al. 2012

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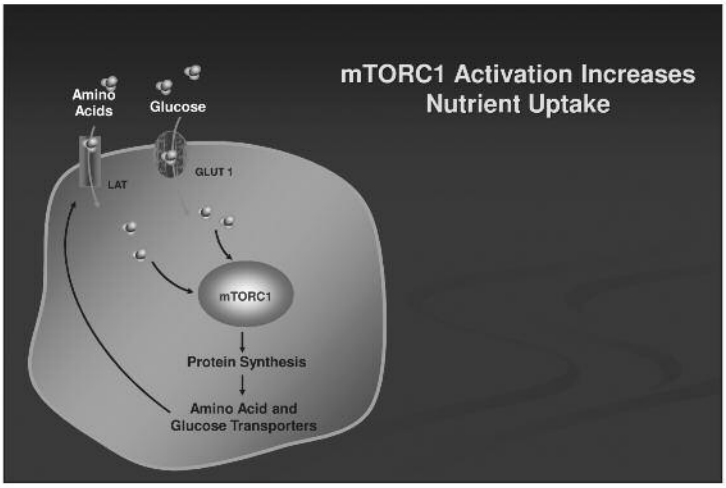
mTORC1 Activation Increases Nutrient Uptake
Amino Acids
Glucose
LAT
GLUT 1
mTORC1
Protein Synthesis
Amino Acid and Glucose Transporters

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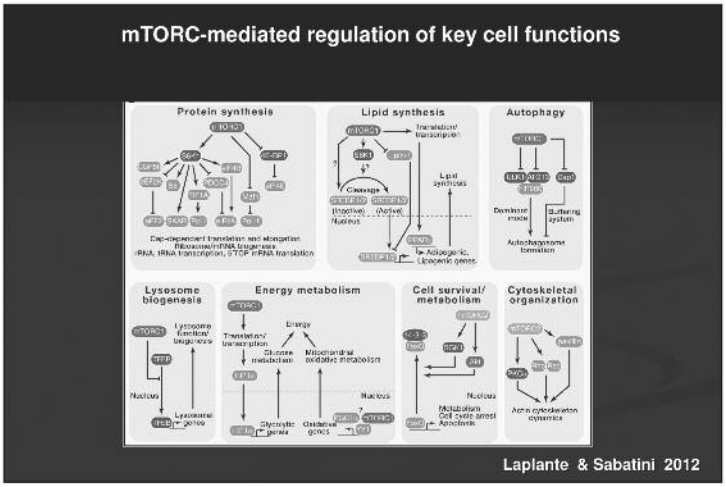
mTORC-mediated regulation of key cell functions
Protein synthesis
Lipid synthesis
Autophagy
Lysosome biogenesis
Energy metabolism
Cell survival/ metabolism
Cytoskeletal organization
Laplante & Sabatini 2012

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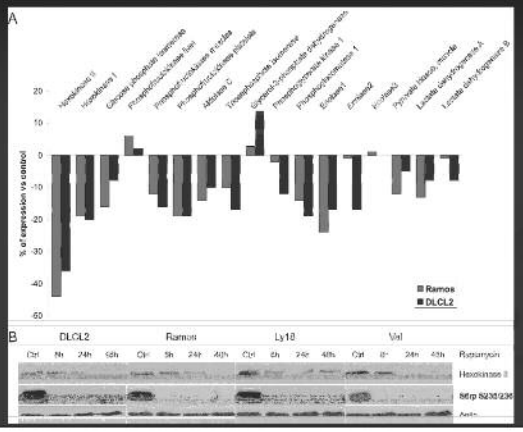
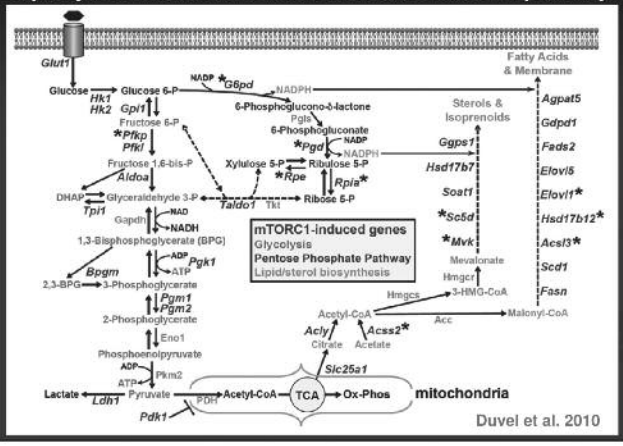
Rapamycin-induced inhibition of hexokinase II expression



Lee et al. 2012

85

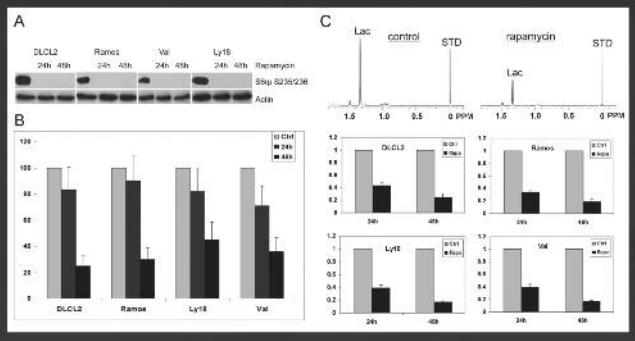
Rapamycin-induced inhibition of several metabolic pathways



Duvel et al. 2010

86

Rapamycin-induced inhibition of mTORC1 signaling (A), cell proliferation (B) and glycolysis (C)



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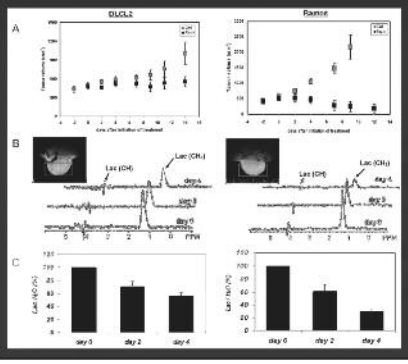
Rapamycin-induced inhibition of tumor growth and glucose metabolism in vivo



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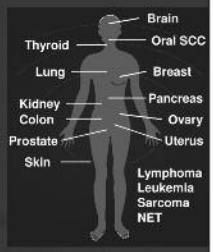
Effect of mTORC1 inhibitors as single agents in cancer

Responses seen in a whole spectrum of malignancies:
- lymphomas
- leukemias (myeloid and lymphoid)
- neuroendocrine tumors
- sarcomas
- various carcinomas



FDA approval in five non-hematopoietic tumors

There is a clear need for the "right" combination therapy

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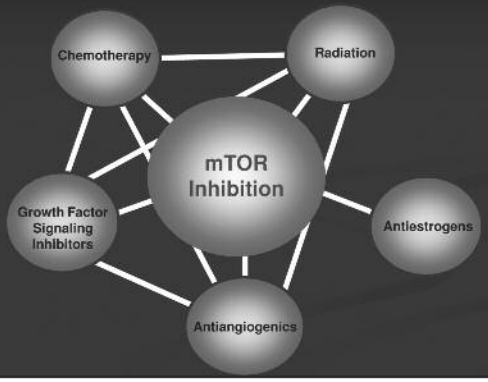
mTOR Inhibition May Enhance the Antitumor Effects of Other Therapies



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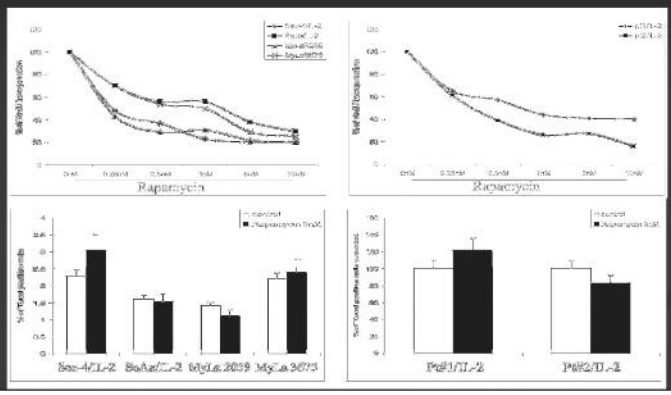
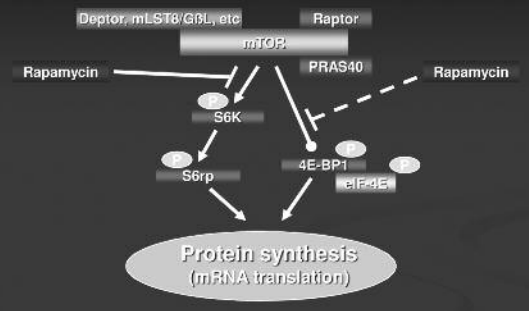
Biological effects of mTORC1 inhibition in CTCL cells



91

Rapalogs efficiently inhibit S6K but not 4E-BP1 phosphorylation



Mendoza et al. 2011

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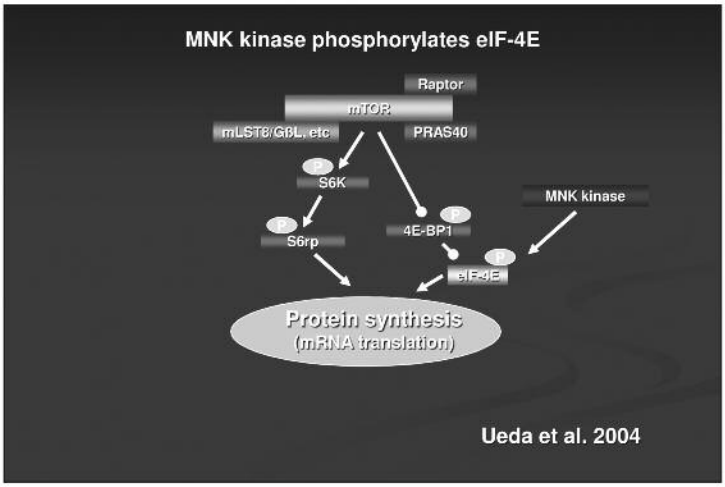
MNK kinase phosphorylates eIF-4E
Raptor
mTOR
mLST8/GβL, etc
PRAS40
P
S6K
MNK kinase
P
S6rp
4E-BP1
P
eIF-4E
Protein synthesis
(mRNA translation)
Ueda et al. 2004

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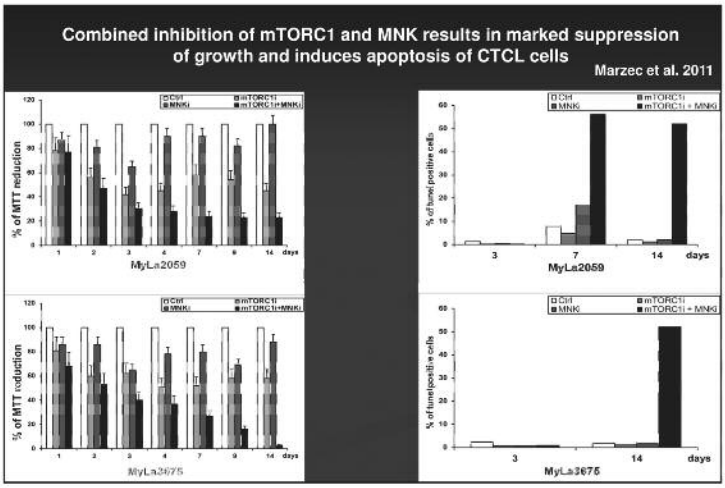
Combined inhibition of mTORC1 and MNK results in marked suppression of growth and induces apoptosis of CTCL cells
Marzec et al. 2011
% of MTT reduction
MyLa2059
% of tunel positive cells
MyLa2059
MyLa3675
MyLa3675

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Presentation summary- part 1

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- mTORC1 pathway is ubiquitously activated in lymphomas and other malignancies
- many cytokines and growth factors activate mTORC1 (signal I)- response to cell interaction cues
- mTORC1 generates negative feedback affecting cell signaling components (growth factor receptor and IRS)
- mTORC1 is highly sensitive to nutrients and other metabolic stimuli (signal II)- response to metabolic cues
- mTORC1 actively regulates nutrient uptake and cell metabolism

Presentation summary- part 2

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- mTORC1 activation can be monitored in vitro and in vivo by functional (cell metabolism-based) imaging methods
- inhibition of mTORC1 alone universally leads to inhibition of cell proliferation (but typically does not induce substantial apoptosis)
- combination of mTORC1 and MNK inhibitors induced apoptotic cell death of cutaneous T-cell lymphoma cells


Contributors- 1
RAD as anti- PTLD drug
• Miroslaw Majewski
• Magda Korecka
• Liona Fields
• Leslie Shaw
• Joanne Joergensen
• Walter Schuler
mTORC1 signaling in transformed B cells
• Pawel Wlodarski
• Michal Marzec
• Monika Kasprzycka
• Xiaobin Liu
• Erle Robertson
mTORC1 activation in reactive, PTLD and ARL tissues
• Mouna El-Salem
• P. Ragunath
• Michal Marzec

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Contributors- 2
mTORC1 activation in ALK+TCL
• Michal Marzec
• Monika Kasprzycka
• Xiaobin Liu
• Pawel Wlodarski
• Mouna El-Salem
• P. Ragunath
mTORC1&MNK combination
• Michal Marzec
• Xiaobin Liu
mTORC1 and Jak1/3 signaling in CTCL
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• Xiaobin Liu
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• John Tobias
• Don Baldwin
• Monika Kasprzycka
• Agnieszka Witkiewicz
• P. Ragunath
• Alain Rook
• Niels Odum
• Erle Robertson
Imaging of mTORC1 activation
• Michal Marzec
• Xiaobin Liu
• Kanchan Kantekure
• P. Ragunath
• Seung Cheol Lee
• Jerry Glickson

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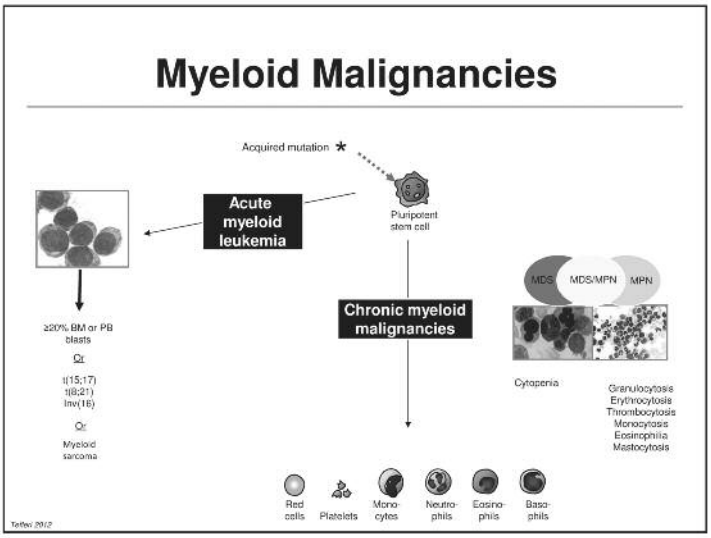
Genetic and Metabolic Alterations in Myeloproliferative Neoplasms and Acute Myeloid Leukemia

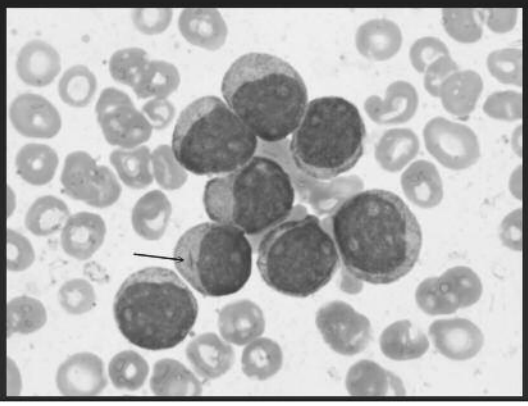
Ayalew Tefferi
Mayo Clinic, Rochester, MN

100

Myeloid Malignancies



Acute Myeloid Leukemia



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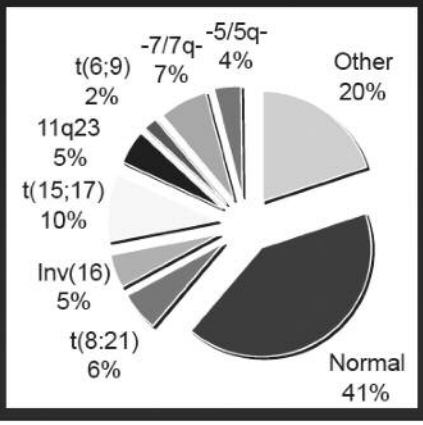
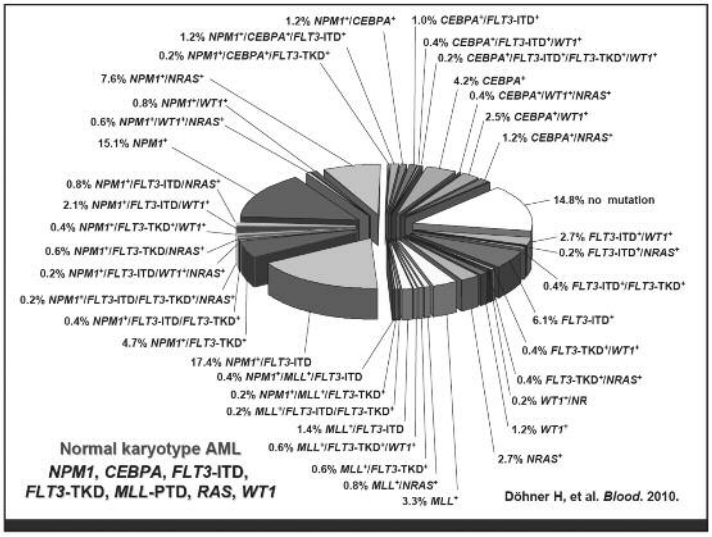
Cytogenetic abnormalities in AML



102


1.2% NPM1+/CEBPA+
1.0% CEBPA+/FLT3-ITD+
1.2% NPM1+/CEBPA+/FLT3-ITD+
0.4% CEBPA+/FLT3-ITD+/WT1+
0.2% NPM1+/CEBPA+/FLT3-TKD+
0.2% CEBPA+/FLT3-ITD+/FLT3-TKD+/WT1+
7.6% NPM1+/NRAS+
4.2% CEBPA+
0.8% NPM1+/WT1+
0.4% CEBPA+/WT1+/NRAS+
0.6% NPM1+/WT1+/NRAS+
2.5% CEBPA+/WT1+
15.1% NPM1+
1.2% CEBPA+/NRAS+
0.8% NPM1+/FLT3-ITD/NRAS+
14.8% no mutation
2.1% NPM1+/FLT3-ITD/WT1+
2.7% FLT3-ITD+/WT1+
0.4% NPM1+/FLT3-TKD+/WT1+
0.2% FLT3-ITD+/NRAS+
0.6% NPM1+/FLT3-TKD/NRAS+
0.4% FLT3-ITD+/FLT3-TKD+
0.2% NPM1+/FLT3-ITD/WT1+/NRAS+
0.2% NPM1+/FLT3-ITD/FLT3-TKD+/NRAS+
6.1% FLT3-ITD+
0.4% NPM1+/FLT3-ITD/FLT3-TKD+
0.4% FLT3-TKD+/WT1+
4.7% NPM1+/FLT3-TKD+
17.4% NPM1+/FLT3-ITD
0.4% FLT3-TKD+/NRAS+
0.4% NPM1+/MLL+/FLT3-ITD
0.2% WT1+/NR
0.2% NPM1+/MLL+/FLT3-TKD+
0.2% MLL+/FLT3-ITD/FLT3-TKD+
1.2% WT1+
1.4% MLL+/FLT3-ITD
0.6% MLL+/FLT3-TKD+/WT1+
2.7% NRAS+
0.6% MLL+/FLT3-TKD+
0.8% MLL+/NRAS+
3.3% MLL+
Normal karyotype AML
NPM1, CEBPA, FLT3-ITD,
FLT3-TKD, MLL-PTD, RAS, WT1
Döhner H, et al. Blood. 2010.

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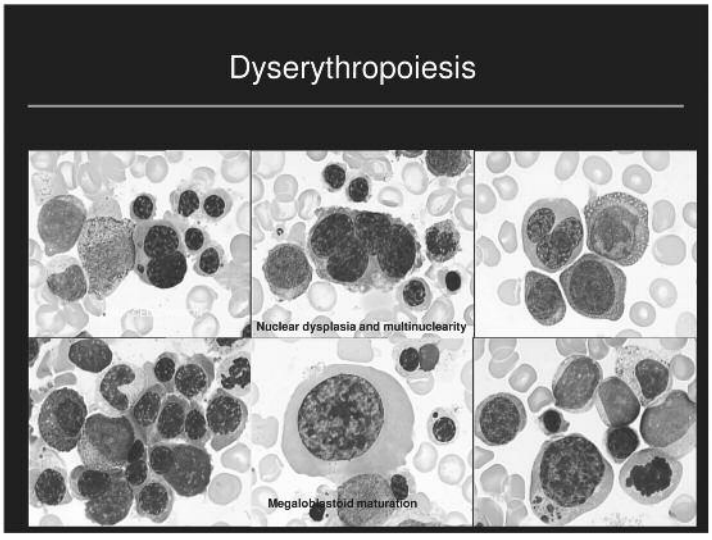
Dyserythropoiesis
Nuclear dysplasia and multinuclearity
Megaloblastoid maturation

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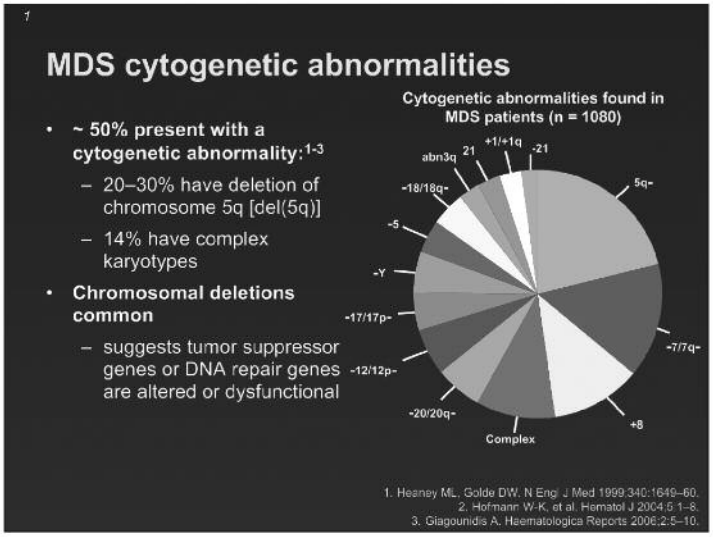
1
MDS cytogenetic abnormalities
~ 50% present with a cytogenetic abnormality:1-3
– 20–30% have deletion of chromosome 5q [del(5q)]
– 14% have complex karyotypes
Chromosomal deletions common
– suggests tumor suppressor genes or DNA repair genes are altered or dysfunctional
Cytogenetic abnormalities found in MDS patients (n = 1080)
abn3q
21
+1/+1q
-21
5q-
-18/18q-
-5
-Y
-17/17p-
-12/12p-
-20/20q-
Complex
+8
-7/7q-
1. Heaney ML, Golde DW. N Engl J Med 1999;340:1649–60.
2. Hofmann W-K, et al. Hematol J 2004;5:1–8.
3. Giagounidis A. Haematologica Reports 2006;2:5–10.

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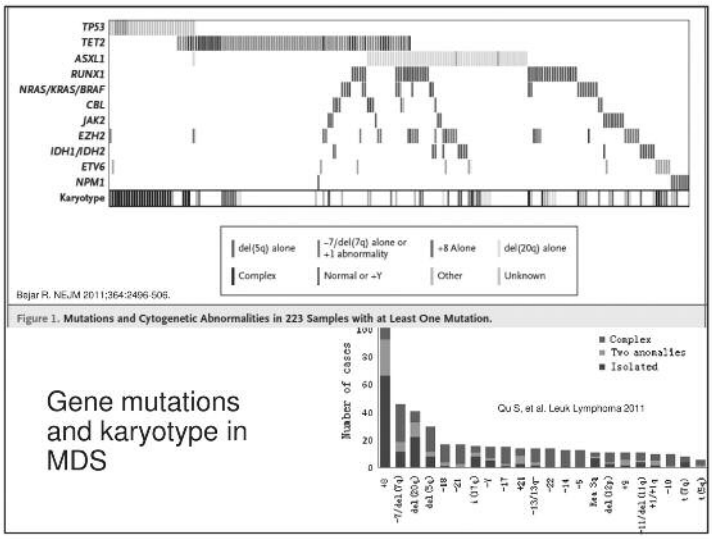
TP53
TET2
ASXL1
RUNX1
NRAS/KRAS/BRAF
CBL
JAK2
EZH2
IDH1/IDH2
ETV6
NPM1
Karyotype
del(5q) alone
-7/del(7q) alone or +1 abnormality
+8 Alone
del(20q) alone
Complex
Normal or -Y
Other
Unknown
Bejar R. NEJM 2011;364:2496-506.
Figure 1. Mutations and Cytogenetic Abnormalities in 223 Samples with at Least One Mutation.
Gene mutations and karyotype in MDS
Complex
Two anomalies
Isolated
Number of cases
Qu S, et al. Leuk Lymphoma 2011

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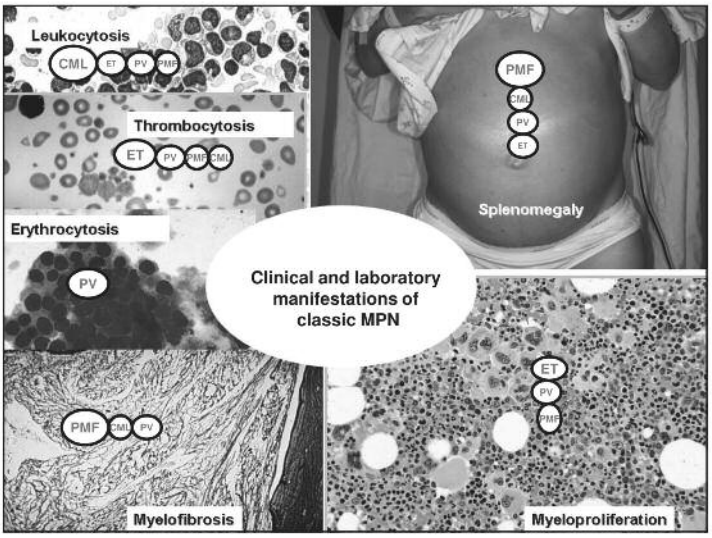

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1,000 Patients with Primary Myelofibrosis
The Mayo Clinic Experience

	All Patients (n=1,000)	Patients seen at diagnosis (n=340)
Age (years); median (range)	65 (14-92)	**66 (14-92)**
Males; n (%)	621 (62%)	**206 (61%)**
DIPSS-plus† risk group; %		
Low	10%	**16%**
Intermediate- 1	15%	**25%**
Intermediate- 2	37%	**34%**
High	37%	**25%**
Constitutional symptoms; n (%)	336 (34%)	**99 (29%)**
Circulating blasts ≥1%; n (%)	555 (56%)	**154 (45%)**
Hemoglobin <10 g/dl; n (%)	535 (54%)	**130 (38%)**
Transfusion requiring; n (%)	383 (38%)	**83 (24%)**
Leucocytes >25 x 10^9/L; n (%)	159 (16%)	**43 (13%)**
Platelets <100 x 10^9/L; n (%)	256 (26%)	**61 (18%)**
*JAK2*V617F; n (%)	358 (61%)	**115 (62%)**
Palpable spleen >10 cm; n (%)	307 (31%)	**70(21%)**
Cytogenetic categories		
Normal	568 (59%)	**218 (69%)**
Favorable††	261 (27%)	**66 (25%)**
Unfavorable††	138 (14%)	**33 (10%)**
Deaths; n (%)	590 (59%)	**172 (51%)**
Leukemic transformations, n (%)	67 (7%)	**24 (7%)**

+9, 13q-, 20q-

Complex, +8, -7/7q-, 5q-, inv(3), iso(17q), 12p-

Tefferi et al. Mayo Clinic Proceedings 2012

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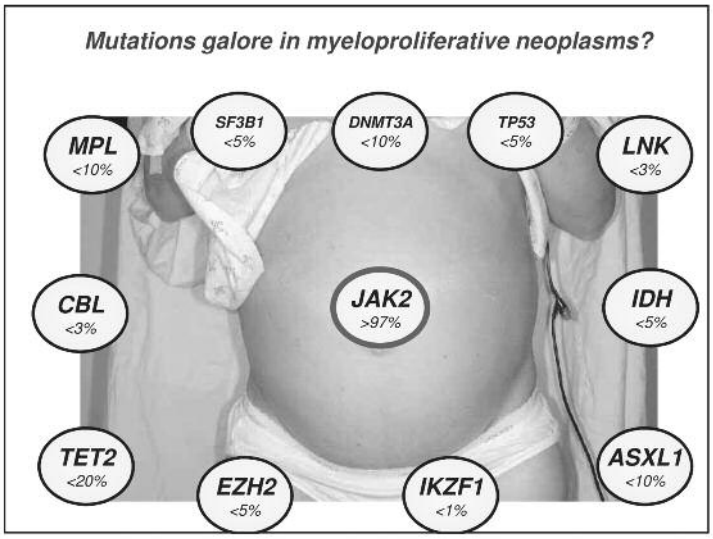

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Mutational frequency and distribution for *ASXL1*, *EZH2*, *SRSF2*, *IDH1* and *IDH2* mutations in primary myelofibrosis

ASXL1 tested 279: mutated 85 (30.5%)

SRSF2 tested 358: mutated 52 (14.5%)

EZH2 tested 270: mutated 15 (5.6%)

IDH2 tested 374: mutated 10 (2.7%)

IDH1 tested 374: mutated 7 (1.9%)

ASXL1 & SRSF2 mutated n = 11; p = 0.43
ASXL1 & EZH2 mutated n = 6; p = 0.45
ASXL1 & IDH1 mutated n = 2; p = 0.62
ASXL1 & IDH2 mutated n = 2; p = 0.70

SRSF2 & IDH1 mutated n = 4; p = 0.0002
SRSF2 & IDH2 mutated n = 5; p = 0.0009
SRSF2 & EZH2 mutated n = 2; p = 0.71

EZH2 & IDH1 mutated n = 1; p = 0.21
EZH2 & IDH2 mutated n = 1; p = 0.21

One patient was ASXL1, SRSF2, EZH2 and IDH2 mutated
Two patients were ASXL1, SRSF2 and EZH2 mutated
Two patients were ASXL1, IDH1 and SRSF2 mutated

ACUTE MYELOID LEUKAEMIA (AML) AND RELATED PRECURSOR NEOPLASMS

- **AML with recurrent genetic abnormalities**
- AML with t(8;21)(q22;q22); *RUNX1-RUNX1T1*
- AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
- Acute promyelocytic leukaemia with t(15;17)(q22;q12); *PML-RARA*
- AML with t(9;11)(p22;q23); *MLLT3-MLL*
- AML with t(6;9)(p23;q34); *DEK-NUP214*
- AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *RPN1-EVI1*
- AML (megakaryoblastic) with t(1;22)(p13;q13); *RBM15-MKL1*
- AML *with mutated NPM1*
- AML *with mutated CEBPA*
- **AML with myelodysplasia-related changes**
- **Therapy-related myeloid neoplasms**
- **Acute myeloid leukaemia, NOS**
- AML with minimal differentiation
- AML without maturation
- AML with maturation
- Acute myelomonocytic leukaemia
- Acute monoblastic and monocytic leukaemia
- Acute erythroid leukaemia
- Acute megakaryoblastic leukaemia
- Acute basophilic leukaemia
- Acute panmyelosis with myelofibrosis
- **Myeloid sarcoma**
- **Myeloid proliferations related to Down syndrome**
- Transient abnormal myelopoiesis
- Myeloid leukaemia associated with Down syndrome
- **Blastic plasmacytoid dendritic cell neoplasm**

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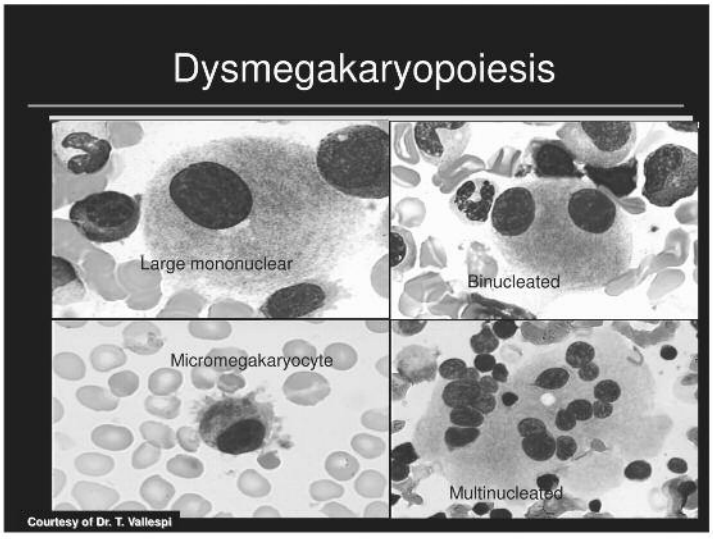

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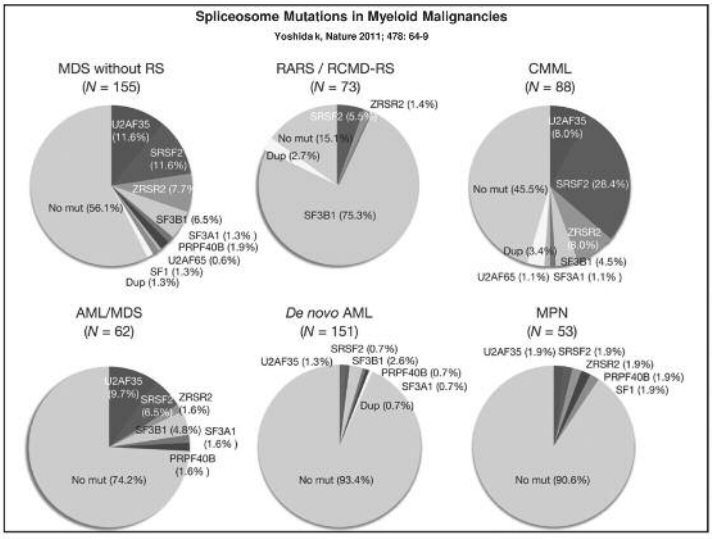
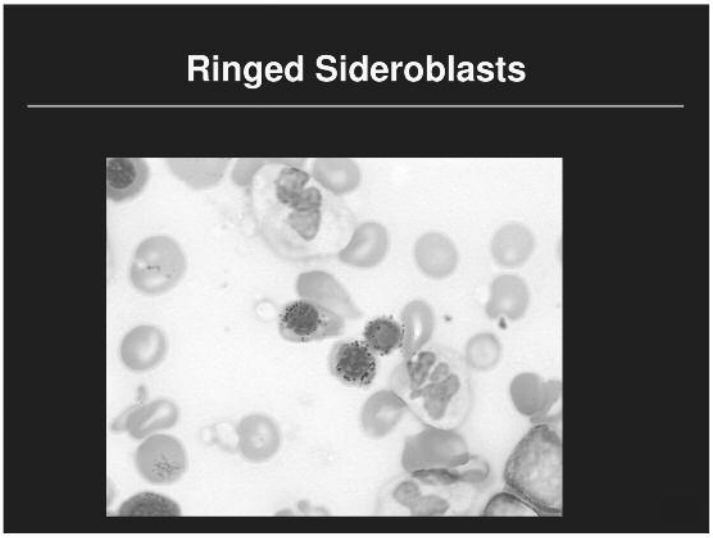
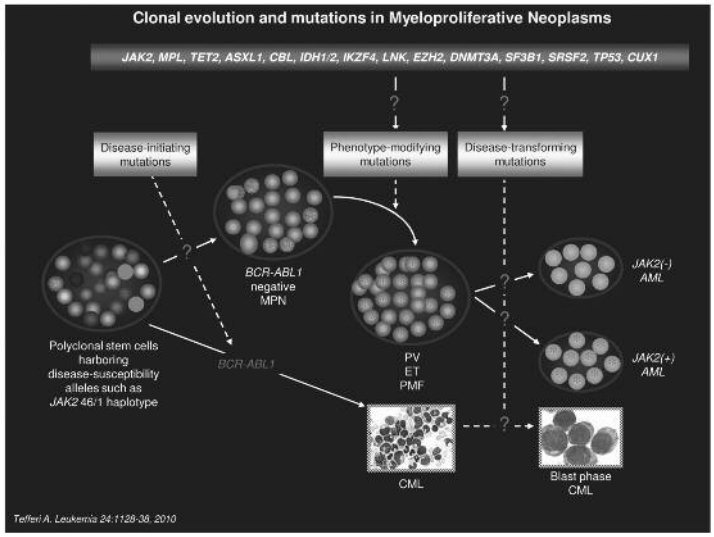

Spliceosome Mutations in Myeloid Malignancies
Yoshida k, Nature 2011; 478: 64-9
MDS without RS
(N = 155)
U2AF35 (11.6%)
SRSF2 (11.6%)
ZRSR2 (7.7%)
No mut (56.1%)
SF3B1 (6.5%)
SF3A1 (1.3%)
PRPF40B (1.9%)
U2AF65 (0.6%)
SF1 (1.3%)
Dup (1.3%)
RARS / RCMD-RS
(N = 73)
ZRSR2 (1.4%)
SRSF2 (5.5%)
No mut (15.1%)
Dup (2.7%)
SF3B1 (75.3%)
CMML
(N = 88)
U2AF35 (8.0%)
SRSF2 (28.4%)
No mut (45.5%)
ZRSR2 (8.0%)
Dup (3.4%)
SF3B1 (4.5%)
U2AF65 (1.1%)
SF3A1 (1.1%)
AML/MDS
(N = 62)
U2AF35 (9.7%)
SRSF2 (6.5%)
ZRSR2 (1.6%)
SF3B1 (4.8%)
SF3A1 (1.6%)
PRPF40B (1.6%)
No mut (74.2%)
De novo AML
(N = 151)
SRSF2 (0.7%)
U2AF35 (1.3%)
SF3B1 (2.6%)
PRPF40B (0.7%)
SF3A1 (0.7%)
Dup (0.7%)
No mut (93.4%)
MPN
(N = 53)
U2AF35 (1.9%)
SRSF2 (1.9%)
ZRSR2 (1.9%)
PRPF40B (1.9%)
SF1 (1.9%)
No mut (90.6%)

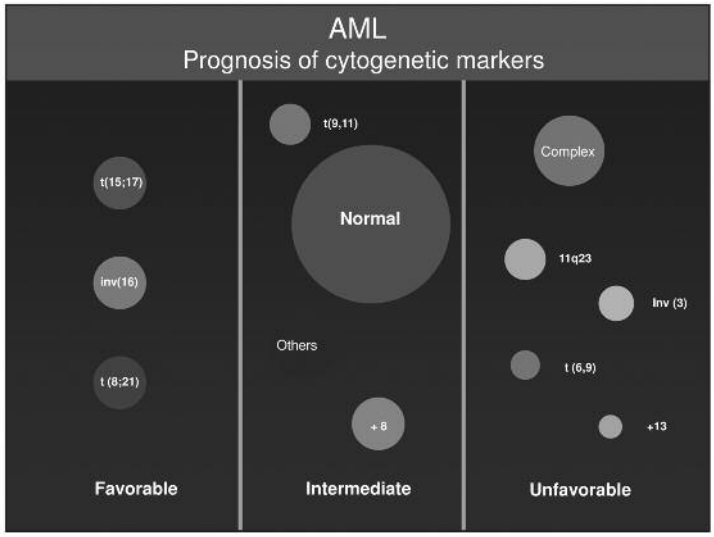
113


Ringed Sideroblasts

114


Clonal evolution and mutations in Myeloproliferative Neoplasms
JAK2, MPL, TET2, ASXL1, CBL, IDH1/2, IKZF4, LNK, EZH2, DNMT3A, SF3B1, SRSF2, TP53, CUX1
Disease-initiating mutations
Phenotype-modifying mutations
Disease-transforming mutations
BCR-ABL1 negative MPN
JAK2(-) AML
JAK2(+) AML
Polyclonal stem cells harboring disease-susceptibility alleles such as JAK2 46/1 haplotype
BCR-ABL1
PV
ET
PMF
CML
Blast phase CML
Tefferi A. Leukemia 24:1128-38, 2010

115


AML
Prognosis of cytogenetic markers
t(15;17)
inv(16)
t (8;21)
t(9,11)
Normal
Others
+ 8
Complex
11q23
Inv (3)
t (6,9)
+13
Favorable
Intermediate
Unfavorable

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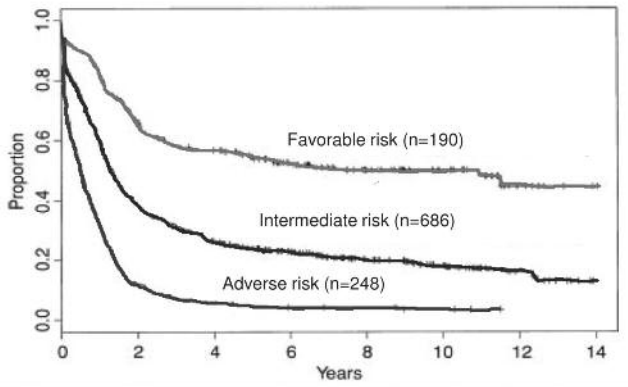
Outcomes according to cytogenetic risk group



Byrd JC et al (CALGB 8641). Blood 2002; 100:4325.

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Molecular Studies in AML

Marker	%	Prognosis
•FLT3 ITD/mutation	30	Worse
•MLL PTD	7	Worse
•↑ BAALC	6	Worse
•↑ BCL2 and WT1 mRNA	10-20	Worse
•↑ EVI1 expression	10	Worse
•C-kit mutation in CBF	15	worse
•NPM1 mutation	50	Better
•CEBPA mutation	8	Better
•IDH1-2 mutations	20-30	Worse if NPM1 +, FLT3WT
•DNMT3A	22	worse

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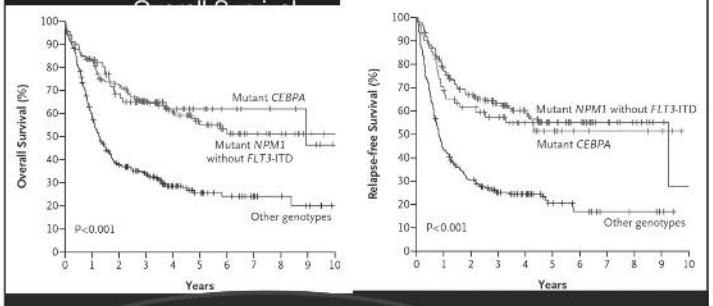
CEBPα and NPM1 mutations in AML



NPM1 and CEBPα mutations should be evaluated in all new AML with normal karyotype

Schlenk et al NEJM 2008

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Prognostic relevance of gene mutations in MDS

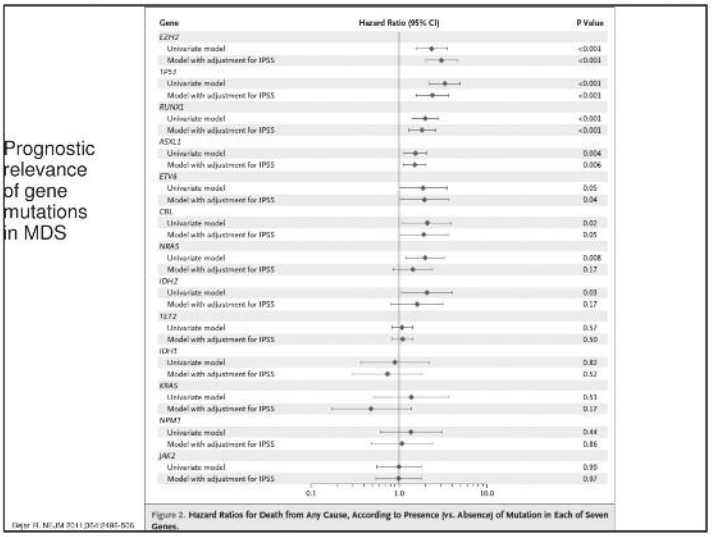
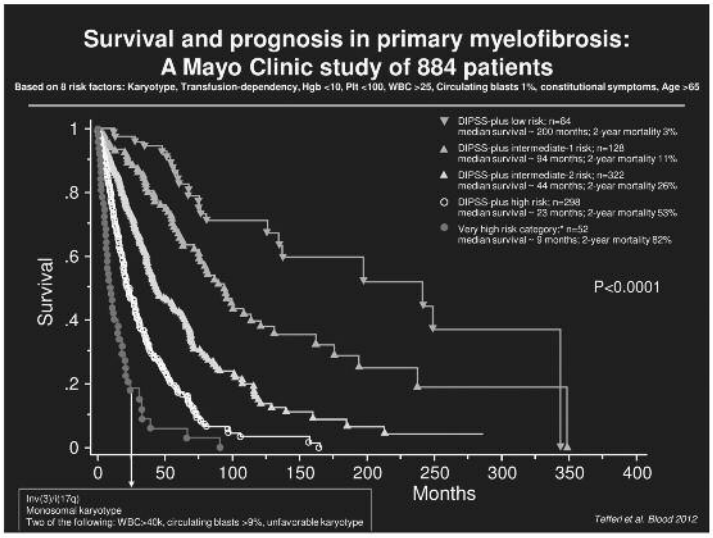


Figure 2. Hazard Ratios for Death from Any Cause, According to Presence (vs. Absence) of Mutation in Each of Seven Genes.

Bejar R. NEJM 2011;364:2496-506

120


Survival and prognosis in primary myelofibrosis:
A Mayo Clinic study of 884 patients
Based on 8 risk factors: Karyotype, Transfusion-dependency, Hgb <10, Plt <100, WBC >25, Circulating blasts 1%, constitutional symptoms, Age >65
DIPSS-plus low risk; n=64
median survival = 200 months; 2-year mortality 3%
DIPSS-plus intermediate-1 risk; n=128
median survival = 94 months; 2-year mortality 11%
DIPSS-plus intermediate-2 risk; n=322
median survival = 44 months; 2-year mortality 26%
DIPSS-plus high risk; n=268
median survival = 23 months; 2-year mortality 53%
Very high risk category:* n=52
median survival = 9 months; 2-year mortality 82%
P<0.0001
Survival
Months
Inv(3)/t(17q)
Monosomal karyotype
Two of the following: WBC>40k, circulating blasts >9%, unfavorable karyotype
Tefferi et al. Blood 2012

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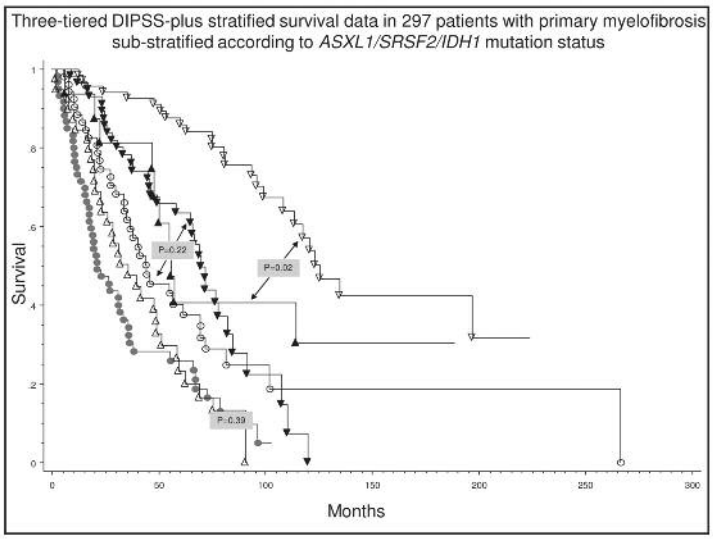
Three-tiered DIPSS-plus stratified survival data in 297 patients with primary myelofibrosis
sub-stratified according to ASXL1/SRSF2/IDH1 mutation status
P=0.22
P=0.02
P=0.39
Survival
Months

122

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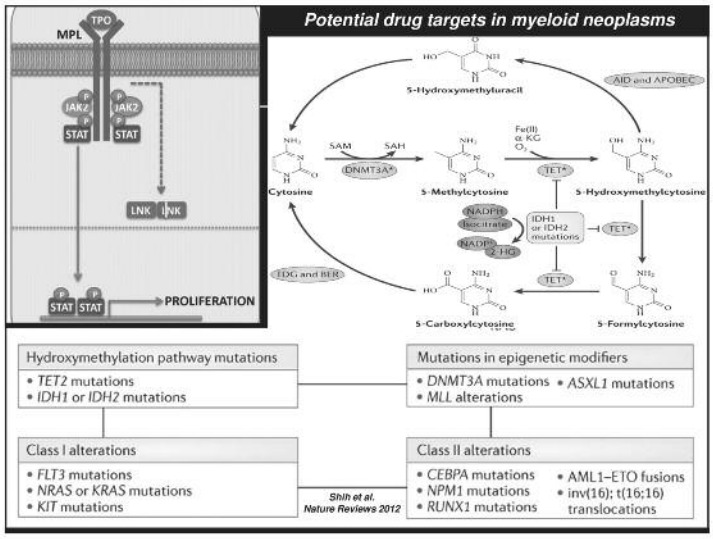

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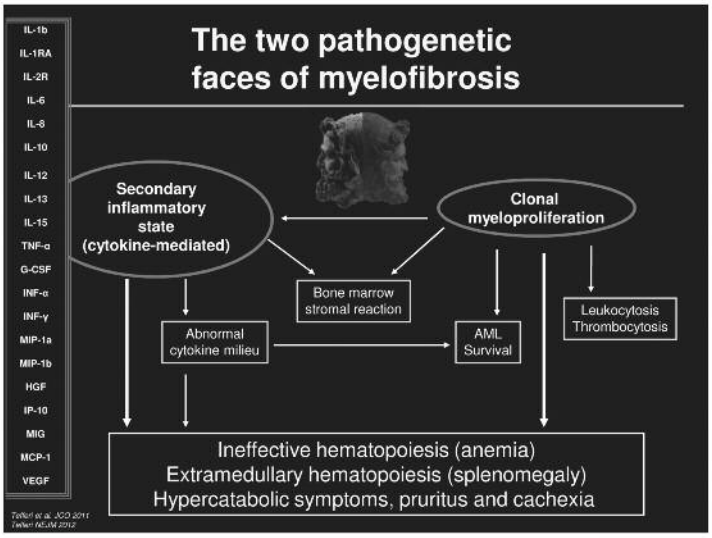
	JAK2 IC50 (nM)	JAK1 IC50 (nM)	JAK3 IC50 (nM)	TYK2 IC50 (nM)	Other targets	Current stage	Symp Resp	Spleen Resp	Anem Resp	Side effects
Ruxolitinib	2.8	3.3	428	19	None reported	FDA approved	>50%	29% (MRI)	14%	Anemia Thrombocytopenia *ruxolitinib withdrawal syndrome**
SAR302503	3	105	1040	405	FLT3, RET	Phase-3	>50%	39%	0%	Nausea/Diarrhea Anemia Thrombocytopenia Transaminasemia Hyper-lipase/amylasemia
Lestaurtinib	<1	–	3	–	FLT3, TRKA VEGFR2, RET	Phase-2	NR	>18%	25%†	Nausea/Diarrhea Anemia Thrombocytopenia
CYT387	18	11	155	17	PKD3, PKCμ CDK2, ROCK2 JNK1, TBK1	Phase-2	>50%	45%	50%	Thrombocytopenia Headaches 1st dose effect** Peripheral neuropathy Transaminasemia Hyper-lipase/amylasemia
SB1518	23	1280	520	50	FLT3	Phase-2	>50%	32% (MRI)	††	Nausea/Diarrhea
LY2784544	–	–	–	–	–	Phase-1/2	NR	>22%	NR	Nausea/Diarrhea Anemia Electrolyte abnormalities/TLS? Increases in serum creatinine
XL019	2	134	195	344	–	Halted	>50%	33%	NR	Peripheral neuropathy
AZD1480	<0.5	1.3	3.9	–	Aurora-A, TRKA FGFR1, FLT4	Phase-1/2				
BMS911543	1.1	356	73	66	None reported	Phase-1/2				
NS-018	<1	33	39	22	SRC, FYN ABL, FLT3	Phase-1/2				

Tefferi. NEJM 2012


IL-1b
IL-1RA
IL-2R
IL-6
IL-8
IL-10
IL-12
IL-13
IL-15
TNF-α
G-CSF
INF-α
INF-γ
MIP-1a
MIP-1b
HGF
IP-10
MIG
MCP-1
VEGF
The two pathogenetic faces of myelofibrosis
Secondary inflammatory state (cytokine-mediated)
Clonal myeloproliferation
Bone marrow stromal reaction
Abnormal cytokine milieu
AML Survival
Leukocytosis Thrombocytosis
Ineffective hematopoiesis (anemia)
Extramedullary hematopoiesis (splenomegaly)
Hypercatabolic symptoms, pruritus and cachexia

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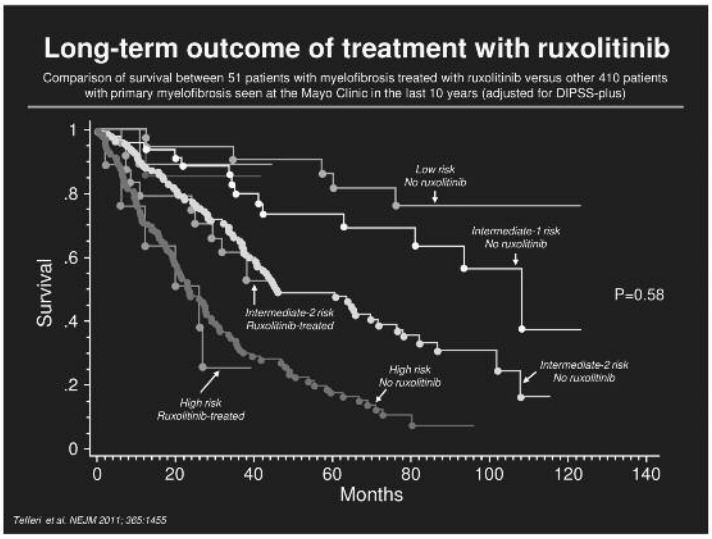
Long-term outcome of treatment with ruxolitinib
Comparison of survival between 51 patients with myelofibrosis treated with ruxolitinib versus other 410 patients with primary myelofibrosis seen at the Mayo Clinic in the last 10 years (adjusted for DIPSS-plus)
Survival
Months
Low risk
No ruxolitinib
Intermediate-1 risk
No ruxolitinib
Intermediate-2 risk
Ruxolitinib-treated
P=0.58
High risk
No ruxolitinib
Intermediate-2 risk
No ruxolitinib
High risk
Ruxolitinib-treated
Tefferi et al. NEJM 2011; 365:1455

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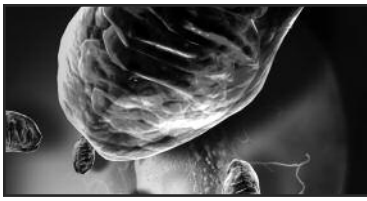

Concluding remarks

- Most myeloid malignancies are pathogenetically complex and unfit for an imatinib-CML-like treatment paradigm
- The experience, so far, from JAK inhibitor clinical trials has underscored the contribution of cytokines to cancer phenotype and symptoms. However, such drugs appear to have severe limitations as an anti-neoplastic therapy and their value is largely palliative
- A radical rather than incremental advance in the treatment of cancer requires better understanding of not only its genetic make up but also its metabolic profile and its interactions with host microenvironment and immunity
- The prospect of success with combination drug trials relies on scientifically- and not industry-driven strategies

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Regulation and Role of the Autophagy-Lysosomal Pathway in Myc-Driven Lymphomagenesis

John L. Cleveland
Professor and Chair
Department of Cancer Biology

SCRIPPS FLORIDA
THE SCRIPPS RESEARCH INSTITUTE

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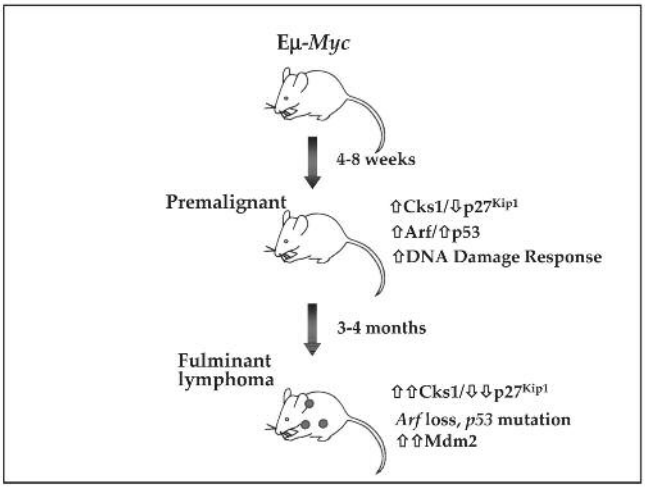

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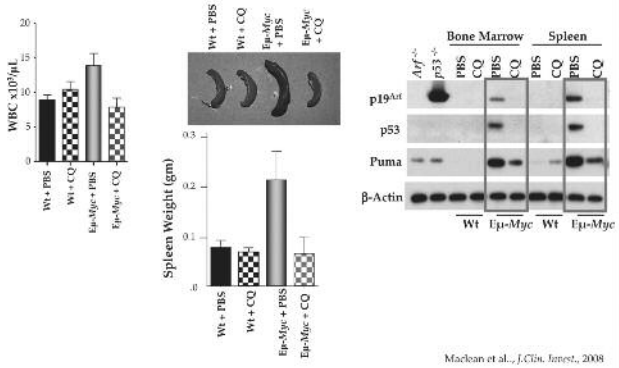
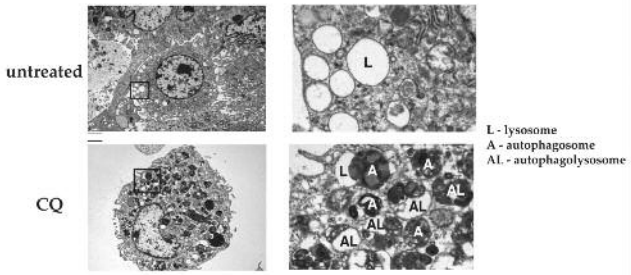
Chloroquine abolishes the pre-cancerous response



Maclean et al., *J.Clin. Invest.*, 2008

130

The anti-malarial drug Chloroquine blocks the Autophagy Pathway



- Chloroquine (CQ) blocks degradation of cargo delivered to the lysosome
- Chloroquine selectively and rapidly kills many tumor cell types; *e.g.* melanoma

Maclean et al., *J.Clin. Invest.*, 2008

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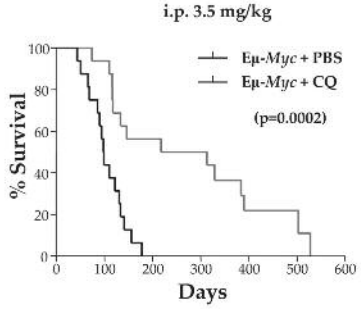
Chloroquine Prevents Myc-Driven Lymphoma Development



But, are effects of CQ protection due to disrupting the autophagy pathway?

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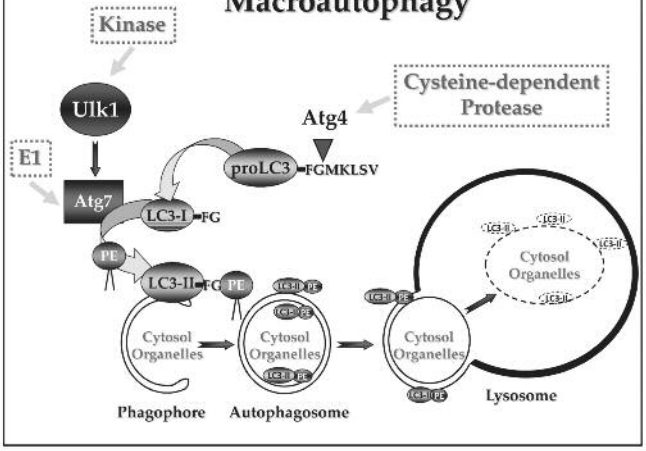
Macroautophagy



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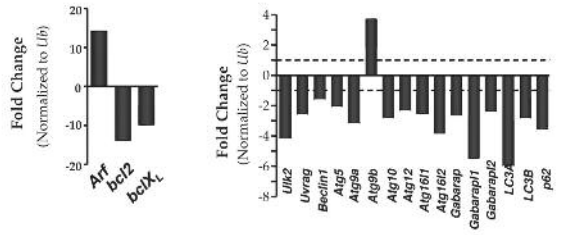
Myc suppresses expression of components of the autophagy pathway in vivo



Relative expression in wild type vs. premalignant Eμ-Myc BM B220+ B cells

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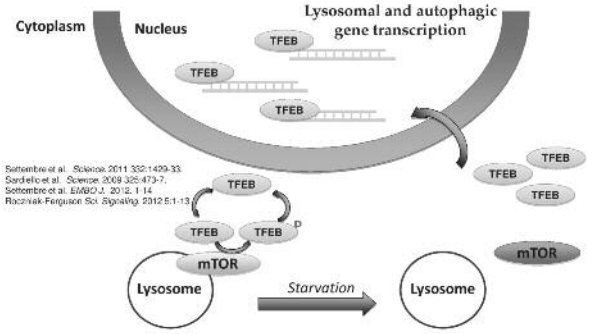
An mTOR-TFEB circuit controls lysosomal biogenesis



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* TFEB, like Myc, functions a b-HLH-Zip transcription factor that binds to and activates targets harboring E-boxes (CANNTG)

* Does Myc antagonize TFEB & is this important for lymphomagenesis?

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