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On behalf of The Leukemia & Lymphoma Society (LLS), thank you for joining us for Epigenetics in Hematologic Malignancies: Pathogenesis and Therapy, a continuing education activity originally presented in San Diego, California. LLS would also like to thank our esteemed speakers for sharing their time and expertise. Through this activity, our presenters will explain how epigenetic changes affect gene expression and contribute to malignant changes in blood cancer cells; discuss new strategies for the development of blood cancer therapies which inhibit factors that produce epigenetic changes in blood cancer cells; discuss the therapeutic potential of targeting epigenetic changes as they relate specifically to leukemia, lymphoma and myeloma; and utilize the information presented from current trials and best practices focusing on epigenetic therapies to better manage their patients with hematologic malignancies.

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.

Sincerely,

Richard C. Winneker, PhD
Senior Vice President, Research
Program Overview
Richard C. Winneker, PhD
The Leukemia & Lymphoma Society
Irwin D. Bernstein, MD
Fred Hutchinson Cancer Research Center
Overview of Epigenetics and its Role in Malignant Transformation
Stephen B. Baylin, MD
Drug Discovery for Epigenomic and Transcriptional Targets in Hematologic Diseases
James E. Bradner, MD
Research and Epigenetic Influence in Myeloma and Lymphoma
Jonathan D. Licht, MD
Role of Mutations in Epigenetic Modifiers in Leukemia Pathogenesis and Therapy
Ross L. Levine, MD
Epigenetic Therapy of Hematologic Malignancies
Jean-Pierre Issa, MD
TARGET AUDIENCE
This activity is designed for hematologists, oncologists, nurses, social workers and other healthcare professionals who wish to enhance their knowledge of advances in epigenetic research and implications in treating patients with hematologic malignancies.

STATEMENT OF NEED
Conventionally, scientists have paid close attention to how mutations in DNA sequence can develop into disease. However, new technologies and approaches have begun to focus on the consequences of DNA modifications altering gene expression, known as epigenetics, as having a critical role in tumorigenesis. Epigenetic changes are more common and persistent than genetic lesions in cancer, and these epigenetic markers may have the potential to be independent clinical predictors. The successes that epigenetic therapy has had in hematopoietic malignancies not only drives home the point of the importance of DNA alterations at a therapeutic level but also will be essential to understanding how these modifications can aid in prevention, diagnosis, risk stratification and outcome. It is critical that healthcare professionals who treat patients with various hematological malignancies remain abreast of key findings regarding the importance of epigenetics and its implications in future research, management and patient outcomes.


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

• Describe how epigenetic changes affect gene expression and contribute to malignant changes in blood cancer cells
• Discuss new strategies for the development of blood cancer therapies which inhibit factors that produce epigenetic changes in blood cancer cells
• Discuss the therapeutic potential of targeting epigenetic changes as they relate specifically to leukemia, lymphoma and myeloma
• Utilize the information presented from current trials and best practices focusing on epigenetic therapies to better manage their patients with hematologic malignancies

STATEMENT OF SUPPORT
This activity is jointly sponsored by RMEI, LLC and Postgraduate Institute for Medicine in collaboration with The Leukemia & Lymphoma Society and is supported by educational grants from Millennium: The Takeda Oncology Company, Celgene Corporation and Allos Therapeutics, Inc.
Stephen B. Baylin, MD
Deputy Director, The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins
Research Professor of Oncology
Johns Hopkins University
Baltimore, MD

Born in 1942 in Durham, North Carolina, Stephen Baylin attended Duke University, and earned his MD degree at its Medical School, where he completed his internship and first year residency in Internal Medicine. Then he worked for two years at the National Heart and Lung Institute of the National Institutes of Health (NIH). In 1971 he joined the departments of Oncology and Medicine at the Johns Hopkins University School of Medicine, an affiliation that still continues.

Presently, he is Deputy Director of the Cancer Center, Professor of Medicine and Professor of Oncology. He is also Chief of the Cancer Biology Division of the Johns Hopkins Oncology Center, and Associate Director for Research of The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins. He has been a member of committees of the American Cancer Society and of NIH, and his honors include a Research Career Development Award from NIH, the Edwin Astwood Lectureship of the Endocrine Society, and appointment to the Virginia and D.K. Ludwig Professorship in Cancer Research and most recently the 2009 Kirk A. Landon-AACR Prize for Basic Cancer Research, also shared with Peter A. Jones, PhD.

Dr. Baylin was recently awarded the 14th NCI Alfred G. Knudson Award in Cancer Genetics and, most recently, the Nakahara Memorial Lecture prize at the 2010 Princess Takematsu Symposium and the 2011 American Cancer Society’s Medal of Honor. Currently, he leads, with Peter Jones, the Epigenetic Therapy Stand up to Cancer Team (SU2C).

So far, during his highly productive career, Stephen Baylin has authored or co-authored over 300 full-length publications.
James E. Bradner, MD

Attending Physician, Hematology-Oncology
Assistant Professor of Medicine
Dana-Farber Cancer Institute
Harvard Medical School
Boston, MA

James E. Bradner, MD, is a Staff Physician in the Division of Hematologic Malignancies at Dana-Farber Cancer Institute as well as an Assistant Professor in Medicine at Harvard Medical School. The present research focus of the Bradner laboratory concerns the discovery and optimization of prototype drugs targeting cancer gene regulation. The clinical objective of the Bradner group is to deliver novel therapeutics for human clinical investigation in hematologic diseases.

Dr. Bradner’s awards and honors include the Damon Runyon-Rachleff Innovation Award, the Smith Family Award for Excellence in Biomedical Research, the Dunkin Donuts Rising Star Award and the HMS Distinguished Excellence in Teaching Award. He is a member of the American Society of Clinical Investigation, the American Society of Hematology, the American Chemical Society and the American Association of Cancer Research. His recent research has been published in Nature, Cell, Nature Chemical Biology and the Journal of the American Chemical Society. He has authored more than sixteen United States Patent applications, licensed to five pharmaceutical companies, and is a scientific founder of Acetylon Pharmaceuticals, Shape Pharmaceuticals and Tensha Therapeutics.

Dr. Bradner received his AB from Harvard University, his MD from the University of Chicago, and a MMS from Harvard Medical School. He completed his postgraduate training in Internal Medicine at Brigham & Women’s Hospital, followed by a fellowship in Medical Oncology and Hematology at Dana-Farber Cancer Institute. Following additional post-doctoral training in Chemistry at Harvard University and the Broad Institute with Prof. Stuart Schreiber, Dr. Bradner joined the research faculty of Dana-Farber in 2008.
Jonathan D. Licht, MD

Johanna Dobe Professor and Chief
Division of Hematology/Oncology
Associate Director, Clinical Sciences
Northwestern University
Robert H. Lurie Comprehensive Cancer Center
Northwestern University
Chicago, IL

Dr. Licht is the Johanna Dobe Professor of Medicine, Chief, Division of Hematology/Oncology, and Associate Director for Clinical Sciences of the Robert H. Lurie Comprehensive Cancer Center of Northwestern University. Dr. Licht received his MD from Columbia University, performed his internal medicine residency at Beth Israel, Boston and trained in medical oncology and molecular biology at the Dana-Farber Cancer Institute. From 1991-2006 he served on the faculty at the Mount Sinai School of Medicine where he rose to the rank of Professor of Medicine and Associate Dean for Cancer Programs.

Dr. Licht’s laboratory studies aberrant transcriptional regulation as a cause of hematological malignancy, including acute leukemia and multiple myeloma, and is exploring multiple strategies to reverse this process. Dr. Licht is the Principal Investigator of a Leukemia and Lymphoma Society Specialized Center of Excellence grant and Senior Investigator of the Northwestern Physical Sciences Oncology Center studying epigenetic mechanisms in malignancy. Dr. Licht is a Senior Editor of Clinical Cancer Research and serves on the editorial boards of Oncogene, Clinical Epigenetics and Cancer Biology and Therapy. Dr. Licht is a member of the American Society for Clinical Investigation and a member of the Association of American Physicians.
Ross L. Levine, MD  
*Associate Member, Human Oncology and Pathogenesis Program*  
*Associate Attending Physician, Leukemia Service, Department of Medicine*  
*Geoffrey Been Junior Faculty Chair*  
Memorial Sloan Kettering Cancer Center  
*Assistant Professor of Medicine and Cell/Developmental Biology*  
Weill Cornell Medical College  
New York, NY

Ross L. Levine, MD, is an Associate Member in the Human Oncology and Pathogenesis Program, Associate Attending Physician in the Leukemia Service, Department of Medicine, and the Geoffrey Been Junior Faculty Chair at Memorial Sloan Kettering Cancer Center. In addition, he serves as Assistant Professor of Medicine and Cell/Developmental Biology at Weill Cornell Medical College in New York.

Dr. Levine received his medical degree from Johns Hopkins University in Baltimore, MD, completed residency training at Massachusetts General Hospital and fellowship training at Dana Farber Cancer Institute/Partners Cancer Care in Boston, MA.

Dr. Levine’s awards and honors include the 2011 Boyer Award for Clinical Research from Memorial Sloan Kettering Cancer Center, the Howard Hughes Medical Institute Early Career Award, and the Doris Duke Charitable Foundation Clinical Scientist Development Award. He is a member of the American Society of Hematology and the American Association for Cancer Research. Dr. Levine has published extensively and also serves on the editorial boards of the *American Journal of Hematology*, *Blood* and *BBA Reviews on Cancer*. The focus of the Levine lab at Memorial Sloan Kettering Cancer Center is to improve understanding of the genetic basis for activation of signal transduction pathways in myeloid malignancies, and to use this knowledge to improve therapies for patients with these disorders.
Jean-Pierre J. Issa, MD
Professor of Medicine
Director, Fels Institutes for Cancer Research
Temple University
Philadelphia, PA

Jean-Pierre J. Issa, MD, trained in medical oncology at Johns Hopkins, where he started his research career in the field of epigenetics and cancer and was assistant professor of oncology. Dr. Issa was also professor at the University of Texas MD Anderson Cancer Center in Houston, Texas, and he is currently serving as professor and director of the Fels Institute for Cancer Research at Temple University in Philadelphia, Pennsylvania.

Dr. Issa’s laboratory and clinical/translation interests are in the area of epigenetics, with particular emphasis on the role of DNA methylation and histone modifications in aging, cancer development, and as a target for prevention and therapy for cancer. His honors have included the Frei Award for Translational Research from the Division of Medicine of MDACC, the Dallas Fort Worth Living Legend Faculty Achievement Award in Basic Science Research, and, most recently, the Richard and Hinda Rosenthal Memorial Award from the American Association for Cancer Research. Dr. Issa has been published in over 170 peer-reviewed journals and contributed to numerous research projects as leader, collaborator, and consultant.
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 Postgraduate Institute for Medicine and RMEI, LLC. 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 enduring material for a maximum of 3.25 AMA PRA Category 1 Credit(s)™. Physicians should claim only the 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 22, 2012 through February 22, 2013, 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 8064. 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.

NURSE AND SOCIAL WORKER CONTINUING EDUCATION INFORMATION
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 via email or US mail within 30 days.

FEE INFORMATION
There is no fee for this educational activity.

AMERICANS WITH DISABILITIES ACT
Event staff will be glad to assist you with any special needs (physical, dietary, etc.).
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Postgraduate Institute for Medicine (PIM) assesses conflict of interest with its instructors, planners, managers and other individuals who are in a position to control the content of continuing medical education (CME) activities. All relevant conflicts of interest that are identified are thoroughly vetted by PIM for fair balance, scientific objectivity of studies utilized in this activity and patient care recommendations. PIM is committed to providing its learners with high-quality CME activities and related materials that promote improvements or quality in healthcare and not a specific proprietary business interest of a commercial interest.

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:

- **Stephen B. Baylin, MD**, has affiliations with Johns Hopkins University (Salary); and MDx Health, Bionumerick Pharmaceuticals, and Constellation Pharmaceuticals (Consultant).
- **James E. Bradner, MD**, has affiliations with Shape Pharmaceuticals, Acetylon Pharmaceuticals, and Tensha Therapeutics (Patent and Stock).
- **Jonathan D. Licht, MD**, has affiliations with Abcam and EMD Biosciences (Royalties); and Epizyme, Inc. (Research).
- **Ross L. Levine, MD**, has an affiliation with Agios (Research).
- **Jean-Pierre Issa, MD**, has affiliations with Celgene (Research and Honoraria) Glaxo-Smith Kline and SYNDAX (Consultant); Merck and Eisai (Research); and Novartis and Johnson & Johnson (Honoraria).

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:

- **Richard C. Winneker, PhD**, has no affiliations with commercial interests to disclose.
- **Sherri Kramer, MD**, has no affiliations with commercial interests to disclose.
- **Nora Hartley** has no affiliations with commercial interests to disclose.

POSTGRADUATE INSTITUTE FOR MEDICINE
The following PIM staff serve as clinical content reviewers and/or participate in planning CME/CE activities in a manner that may affect content: Laura Excell, ND, NP, MS, MA, LPC, NCC; Trace Hutchison, PharmD; Samantha Mattucci, PharmD, CCMEP; Jan Schultz, RN, MSN, CCMEP; and Patricia Staples, MSN, NP-C, CCRN hereby state that they or their spouse/life partner do not have any financial relationships or relationships to products or devices with any commercial interests related to the content of this CME/CE activity of any amount during the past 12 months.

DISCLOSURE OF UNLABELED USE
This educational activity may contain discussion of published and/or investigational uses of agents that are not indicated by the FDA. Postgraduate Institute for Medicine (PIM), RMEI, LLC, The Leukemia & Lymphoma Society, Millennium; The Takeda Oncology Company, Celgene Corporation and Allos Therapeutics do not recommend the use of any agent outside of the labeled indications.

The opinions expressed in the educational activity are those of the faculty and do not necessarily represent the views of PIM, RMEI, Millennium: The Takeda Oncology Company, Celgene Corporation or Allos Therapeutics. 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.
Presentation

"Overview of Epigenetics and its Role in Malignant Transformation"

Stephen B. Baylin, MD

Disclosure of Conflicts of Interest

Stephen B. Baylin, MD

Stephen B. Baylin, MD, has affiliations with Johns Hopkins University (Salary); and MDxHealth, Bionumerick Pharmaceuticals, and Constellation Pharmaceuticals (Consultant).
Considerations Key to Probing the Functional Significance of Epigenetic Abnormalities in Cancer

Matching with genetic changes – for genes that control the epigenome and for individual genes

The vulnerability of genes to undergo the changes – emerging role for gene location and/or for groups of genes

Understanding the epigenetics of development and extrapolating the knowledge to cell renewal in adult systems - where do specific tumors and their subtypes come from – key to cancer specificity

Must think in terms of groups of genes that are altered and their contribution to pathways - challenges the mentality which is prevalent in interpreting genetic abnormalities in cancer

Familial Cancer Genes

Methylated

- Rb
- P16
- VHL
- MLH1
- E-cadherin
- BRCA1
- APC
- PI (LK81)

Typical Gene Methylation Pattern Within a Partially Methylated Domain in Cancer

Berman et al, Nat Genet, in press, 2011
Epigenetics in Hematologic Malignancies: Pathogenesis and Therapy
Dynamics of CpG island DNA Hypermethylation - the 2011 View?

**Genomic vulnerability**

SNF, EZH2, ARID1a, DNMT3a

**Loss of CpG Island Protection**

**Evidence for an Instructive Program**

Polycomb 

H3K79me3 in 

stem cells

Genes Hypermethylated in CLL

Significant overlap (>50%)

Ohm et al., 2007
Laird and colleagues, 2007
Cedar and colleagues, 2007

Epigenetic marks in precursor stem cells instructive in defining aberrant methylation?
Epigenetics in Hematologic Malignancies: Pathogenesis and Therapy
Pre-clinical Studies of Low Dose Aza and DAC

Cindy Zahn

Forty years after its discovery, 5-AC may prove to be a drug that works at low mg levels, to hit the designated target in multiple ways, and ingiving therapeutic efficacy.

Hsing Tsai
Laundor Van Nostre

Memory Effects of Low Dose 5-Aza-ctydine

5 to 10 mM

DAC or Aza
T14 treatment

Scultered and primary cells

Orthotopic implantation

Sphere assay for self-renewal

Growth curves of MLL-1, MLL-0, and K562 cell lines with or without 5-Aza or DAC treatment.
Drug Discovery for Epigenomic and Transcriptional Targets in Hematologic Disorders

James E. Bradner, MD

Disclosure of Conflicts of Interest

James E. Bradner, MD, has affiliations with Shape Pharmaceuticals, Acetylom Pharmaceuticals, and Tensha Therapeutics (Patent and Stock).

Targeting Gene Regulatory Complexes
- Transcription Factors
- Histone-Methylating Enzymes
- Histone Binding Domains

Platforms for drug discovery
- Chemical probes for basic research
- Lead compounds for therapeutic development
- Rationales for human clinical investigations

Bradner Laboratory
- Dana-Farber Cancer Institute
- Harvard Medical School
Gene Regulatory Protein Alterations and Dependencies in Cancer

Pharmaceutical Inhibitors of Gene Regulation in Cancer

Platforms of Ligand Discovery

Epigenetics in Hematologic Malignancies: Pathogenesis and Therapy
Presentations

Epigenetics in Hematologic Malignancies: Pathogenesis and Therapy

Direct Inhibition of the Notch Complex

Chromatin-Mediated Transcriptional Signaling

Chromatin-Mediated Transcriptional Signaling
Bromodomain

- Acetyllysine binding modules
- Four conserved motifs: I, II, III, IV
- Interactions between the bromodomain and acetylated lysine
- Two interfaces known to be acetylated
- Conserved arginine residues leading to reduced enzyme specificity
- Frequent participants in oncogenic translocations
- Modulation of bromodomain function

BRD4
- Epigenetic regulator of active gene
- Facilitates nuclear chromatin remodeling
- Proteins, such as P300, are translocated
- C/EBP delta interacts with BRD4 in various contexts

Bromodomain Inhibition
PDB 3JKK

Bromodomain Biochemical Platform
Strader and Knapp Laboratories
Epigenetics in Hematologic Malignancies: Pathogenesis and Therapy
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Barriers to Clinical Translation

- Limited sample size
- Disease not studied prospectively
- Often treated in a localized manner

JQ1 as a chemical probe
- Not optimized for DMF properties
- Intellectual property concerns

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www.tmoregistry.org

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Chromatin-Mediated Transcriptional Signaling
target selection irrespective of perceived druggability • facilities appropriate for discovery research • immediate use of emerging animal models • collaborative support of our patients • flexibility in licensing for further clinical development • access to a massive research infrastructure • research motivated by creative impact insights derived from clinical experience • lack of profit motive • a sense of urgency and immediacy • broad dissemination of chemical probes • ability to crowd-source new indications • long timelines • toward an open-source model of drug discovery • increased velocity of knowledge around a new chemical probe • generation of relatively inexpensive assets • flexible development of clinical candidates • a tradition of creativity and research excellence • peer-review of research at every stage • support of institutional leadership at the dana-farber cancer institute • maximum return of revenue to research and development • no bureaucracy • a culture of collaboration and scholarship • generation of inexpensive assets • publication of chemical probes more relaxed legal restrictions on sharing and discussing research
Epigenetics in Hematologic Malignancies: Pathogenesis and Therapy
Research and Epigenetic Influence in Myeloma and Lymphoma

Jonathan D. Licht
Hematology/Oncology
Robert H. Lurie Comprehensive Cancer Center

Disclosure of Conflicts of Interest

Jonathan D. Licht, MD

Jonathan D. Licht, MD, has affiliations with Abcam and EMD Biosciences (Royalties); and Epizyme, Inc. (Research).
Hypothesis

Mutations in Epigenetic Regulators in Cancer Cause Global Shifts In Chromatin Modification, Structure and Function

Histone Post-translational Modifications

Histone Lysine Modifications

Lysines are the most diversely modified residues in histones.
Global Chromatin Anomalies in Lymphoid Malignancy

EZH2 and H3K27

- EZH2 - Major Histone Methyl Transferase for H3K27
  - Enzymatic component of PRC2
  - Muted/Lost in Myeloid malignancy
  - Other PRC2 Components can be mutated as well
    - ASXL1, SUZ12, EED
  - Loss of H3K27 Methylation

UTX Mutation

- JmJC Class Histone Demethylase
  - Specific for H3K27
  - Alpha Keto Glutarate Dependent
**UTX Mutation**

- UTX Deletion/Mutation in AML, MM
- UTY tends to be mutated as well (2-hit)
- Possible Gain in H3K27me

<table>
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<tr>
<th>UTX Mut</th>
<th>M0522</th>
<th>L026</th>
<th>RPMI2650</th>
<th>L363</th>
<th>FLM</th>
<th>M1L</th>
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<td>+</td>
<td>+</td>
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- H3K27me3
- Total H4

**EZH2 Point Mutation in Lymphoma**

- 20% of Germinal Center Lymphoma
- 8% Follicular
- Not in Activated B Cell
- Recurrent Heterozygous Mutation Suggests Gain of Function

**Structural Model of SET Domain**
t(4;14) Myeloma - A Global Chromatin Anomalies

The MMSET Protein Contains Domains Involved in Chromatin Function

- **PWWP**: Pro-Trp-Trp-Pro, found in nuclear proteins.
- **HMG**: High Mobility Group, possible DNA binding domain.
- **PHD**: Plant Homeodomain, methyllysine binding module.
- **SET**: Suvar, Enhancer-of-Zeste and Trithorax domain, catalyzes lysine methylation in histones and transcription factors.
- **NLS**: Nuclear Localization Sequence

MMSET Overexpressed in t(4;14) Myeloma

What Are the Consequences?
MMSET in Myeloma

- Biological Function - Is MMSET Overexpression Important?

- Can Biochemical Analysis Lead to Insights into Disease Pathogenesis and Therapy?

Hypothesis: MMSET is a Reader and a Writer of the Histone Code

What Mark(s) Does MMSET Read?
What Mark(s) Does MMSET Make?
How Does This Mark Affect Gene Expression?
Can MMSET Be Inhibited?
MMSET: \textit{in vitro} Methyltransferase Activity For Histone H3 and Histone H4

In Vitro- MMSET Can methylated Many Different Histone Sites

KNOCK OUT SYSTEM FOR MMSET
MMSET Expression Causes Global Changes in Histone Modifications

MMSET Re-Addition Reverses the Switch

MMSET Methylation Activity Critical to Myeloma Growth
HMTs Gone Wild! -- K36/K27 Switch

METHYL

Hypothesis: MMSET is a Reader and a Writer of the Histone Code

What Mark(s) Does MMSET Read?
What Mark(s) Does MMSET Make?
How Does This Mark Affect Gene Expression?
Can MMSET Be Inhibited?

Which Genes are Affected by MMSET Expression?

<table>
<thead>
<tr>
<th>Condition</th>
<th>Gene(s)</th>
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<tbody>
<tr>
<td>Knock down</td>
<td>BCL2, HDAC1, PDCD10, H3K4</td>
</tr>
<tr>
<td>Cell repair</td>
<td>MLL, H3K7, HAT1, SETDB2</td>
</tr>
<tr>
<td>ChIP-associated remodeling</td>
<td>ATMS, MPP, H3K4, H3K27</td>
</tr>
<tr>
<td>+ MMSET IIB</td>
<td>H3K4, H3K7, HAT1, SETDB2</td>
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<tr>
<th>CGHtile</th>
<th>p-value</th>
<th>Examples</th>
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<td>-0.42</td>
<td>0.02</td>
<td>BCL2, HDAC1, PDCD10, H3K4</td>
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<tr>
<td>0.38</td>
<td>0.03</td>
<td>MLL, H3K7, HAT1, SETDB2</td>
</tr>
<tr>
<td>0.50</td>
<td>0.03</td>
<td>ATMS, MPP, H3K4, H3K27</td>
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<tr>
<td>+0.42</td>
<td>0.01</td>
<td>H3K4, H3K7, HAT1, SETDB2</td>
</tr>
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MMSET- Genome Wide Profiling

KMS11 cells- double crosslinking, ChIPseqer software;
4736 Peaks identified

MMSET Targets

- SET Dependent
  - Cell Cycle, Adhesion, Apoptosis Genes
  - Chromatin Regulators Among Targets
  - This Activity Critical for Growth

Which Targets are involved in Pathogenesis?

HMTs Gone Wild- Non Transcriptional Effects?
MMSET effects on chromatin accessibility

γH2AX in Knock out system 30min after radiation

MMSET- An Effect on DNA Damage Response
Hypothesis: MMSET is a Reader and a Writer of the Histone Code

What Mark(s) Does MMSET Read?
What Mark(s) Does MMSET Make?
How Does This Mark Affect Gene Expression?

Can MMSET Be Inhibited?
**PRESENTATIONS**

**BIX 01294**
G9a/GLP Inhibitor
Inhibits H3K9me3
Reprograms Somatic to P8 cells

**H3K36 Me3 Ab**

**HMT Inhibitor Killed MMSET+ Cells More Readily**

**Mass Spec Based Screen for MMSET Inhibitors**
**MMSET Inhibitor Screen Summary**

- 10,000 compounds screened in pools of 8
- 5 μM MMSET, 2 μM peptide, 1 mM SAM, 12.5 μM each compound
- Total of 11 pools with significant inhibition (20-30%)
- Deconvoluted 11 pooled hits to singletons (Data below)
- 4 compounds with >40% inhibition at 10 μM

**Validation of Compounds 1-7**

**Conclusions**

- Disorders of Histone Methylation are a Recurrent Theme in Hematological Malignancy
- H3K27 Up or Down regulation can occur
  - Other Marks (H3K36) affected too
- Alterations in HMT Activity Alter Cell Growth
  - Exact Targets to be Determined
- HMT Dysfunction May Have Effects on other Chromatin-Dependent Processes
  - DNA Repair
  - DNA replication
- HMT inhibitors might restore chromatin function and growth control
Role of Mutations in Epigenetic Modifiers in Leukemia Pathogenesis and Therapy

December 9, 2011
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Leukemia Service, Department of Medicine
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Weill Cornell School of Medicine
Disclosure of Conflicts of Interest

Ross L. Levine, MD

Ross L. Levine, MD, has an affiliation with Agios (Research).

Two-hit model of AML Pathogenesis

Class I Mutations (FLT3, JAK2, RAS)
- Enhance proliferation and survival
- No effect on differentiation

Class II Mutations (RUNX1, CEBPA)
- Impair differentiation
- No effect on proliferation/survival

MPN

Class II Mutation

MDS

Class I Mutation

AML

Gilliland and Griffin Blood 2002

• But not all patients have mutations in class I and class II genes
• Does not reflect role of novel AML mutations in leukemogenesis

Discovery of novel mutations in AML patients

• Whole genome sequencing has identified novel recurrent disease alleles in AML
  - IDH1 mutations (Mardis et al. NEJM 2009)
  - DNMT3A mutations (Loy et al. NEJM 2010)

• Candidate gene/array based studies have identified novel disease alleles in AML, MDS, MPN
  - ASXL1 (Stinbaurn et al. BJM 2009)
  - PHF5 (Van Vlierberge et al. Leukemia 2011)

• Biologic and prognostic relevance of these novel disease alleles has not been fully delineated—but some of these mutations are thought to have a role in regulating the epigenetic state of leukemic cells
We performed mutational profiling of the 18 genes known to be mutated in AML in the 1900 phase II trial cohort:
- Identify novel genes with prognostic relevance
- Integrate mutational data with epigenetic analysis of cohort
- Make novel insights about AML biology
- Determine if specific genetically defined subsets benefit from high dose induction chemotherapy

*Jay Patel, Malhotra Gonen, Ken Figueroa/AI/MSKCC, ECOG

Mutational Profiling of ECOG 1900 Cohort*

Mutation Summary

- Including cytogenetic abnormalities, 591 somatic mutations in 398 samples:

  - 355 (60%) samples had no mutations or cytogenetic abnormalities
  - 167 (22%) samples had 1 mutation
  - 146 (21%) samples had 2 mutations
  - 45 (6%) samples had 3 mutations
  - 6 (1%) samples had 4 mutations
  - 1 sample had 5 mutations

  - 91.2% had a clonal somatic abnormality
  - Number of mutations did not affect outcome

Cooperativity Matrix Reveals Marked Mutational Heterogeneity in AML Patient Samples

FLT3-mutant AML
Relevance to Clinical Practice

- Do these novel mutations have prognostic value in AML?

- If so can integrated mutational analysis be used to refine our ability to risk-stratify AML patients

- E1900 tested standard dose vs. high dose daunorubicin - do any genetic factors influence effects of induction dose intensity?

Effects of Mutations on Overall Survival

- Specific Mutations Associated with adverse overall survival
  - KIT-ITD (p=0.001)
  - ASXL1 (p=.002)
  - PHF6 (p=.02)
  - MLL PTD (p<0.001)

IDH2 R140Q Mutations Associated With Improved Overall Survival

- IDH2 R172K and IDH1 mutations no effect on overall survival
Intermediate Risk AML

- Specific Mutations Associated with Adverse Outcome
  - FLT3-ITD (p<0.002)
  - TET2 (p<0.04)
  - ASXL1 (p<.02)
  - PHF6 (p<0.001)
  - MLL-PTD (p<0.001)

- Specific Mutations Associated with Favorable Outcome
  - IDH2 (p<0.02) but not IDH1
  - CEBPA

IDH/NPM1 mutations define favorable outcome in FLT3-negative, intermediate risk AML

- IDH/NPM1-mutant patients, but not NPM1-mutant/IDH-WT patients have a favorable outcome

Modified risk model for FLT3-ITD negative, intermediate risk AML

- Group 1 (favorable): IDH2/NPM1 mutant
- Group 3 (poor-risk): TET2, PHF6, ASXL1, MLL-PTD mutations
- Group 2: all others
Multivariate Model for FLT3-ITD-positive intermediate risk AML

- Group 1 (favorable): CEBPA
- Group 3 (poor-risk): TET2, trisomy 8, DNMT3A R882, or MLL-PTD
- Group 2: all others – similar to CEBPA-mutant
- Can discriminate a set of patients with FLT3-mutant disease with much poorer outcome than others
Human genetics is always right: using mutational studies to elucidate AML pathogenesis

- By profiling primary patient samples we can improve our understanding of AML biology
- We can identify lesions that commonly occur together (NPM1/IDH) to guide development of new models, pathways to transformation...
- But... we can also identify mutations which NEVER occur together and define novel complementation groups/mutational classes
- Would suggest that specific genes function in a pathway
- Or that specific genes have a “synthetic lethal” interaction
- We hypothesized that we could elucidate the function of IDH mutations in AML by identifying mutations exclusive of IDH mutations of AML

ECOG 1900 Cohort: IDH1/2 mutations mutually exclusive of TET2 mutations

<table>
<thead>
<tr>
<th>TET1 Wildtype</th>
<th>TET1 Mutant</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPM1 Wildtype</td>
<td>120</td>
</tr>
<tr>
<td>NPM1 Mutant</td>
<td>0</td>
</tr>
</tbody>
</table>

(P-value = 0.005 [2-sided Fisher's exact test])

Conversion of 5-Methylcytosine to 5-Hydroxymethylcytosine in Mammalian DNA by MLL Partner TET

Cytosine (unmethylated) → Methyl-Cytosine (homomethylated) → α-KG → TET 1-3 → Hydroxy-methyl-Cytosine

Inactivating TET2 mutations may result in hypermethylation of DNA and promotion of myeloid neoplasia— is this true in AML patients?
TET2 Mutations Are Associated with Hypermethylation

IDH1 mutations define a hypermethylator phenotype in GBM

- Defined a hypermethylator phenotype (CIMP) in GBM enriched in proneural tumors based on expression profiling
- This group was markedly enriched for IDH1 mutations
- No IDH2 mutations in this cohort
- Suggested hypermethylation leads to a context which favors IDH mutations

IDH1/2 Mutations Are Associated with Hypermethylation

Do IDH mutations induce hypermethylation or does hypermethylation favor acquisition of IDH mutations?

Figueroa, Abdel-Wahab, Lu et al, Cancer Cell 2010
IDH mutations or TET2 shRNA in primary hematopoietic cells:
deCREASED 5-OH-METHYLCYTOSINE AND INCREASED METHYLATION*

AML patient samples:
DECREASED 5-OH-METHYLCYTOSINE AND INCREASED CYTOSINE METHYLATION WITH IDH/TET2 MUTATIONS*

**METHYLCYTOSINE**

**HYDROXY-METHYLCYTOSINE**

Done using LC/MS -- critical as not all methods distinguish mC from HmC.

Functional and genetic data suggest IDH mutations inhibit TET2 function.

Lucy Godley, Kim Figueroa (Melnick), Rasjill Rampal

IDH1/2 Mutations Inhibit TET2-mediated 5-OH-Me-Cytosine formation

Chao Lu, Pat Ward (Craig Thompson)
**IDH1/2 and TET2: convergent mechanism of transformation by mutations in metabolic enzymes and epigenetic modifiers**

How do these alleles contribute to hematopoietic transformation?

**Conditional TET2 KO mouse (Vav, Mx-Cre)**

Alan Shih (Lovine), Linsey Rasvich/Kelly Moran (Alfamis)

**TET2 deletion leads to serial replating in vitro of cells with a myeloid progenitor phenotype and to increased self-renewal in vivo**

Outcompetes WT BM in competitive transplant assays
TET2 KO cells resemble gene expression profile of progenitors>HSC

Loss of a single Tet2 allele sufficient to confer self-renewal and malignant phenotype

Cooperativity Studies in Vivo: TET2 loss leads to transplantable AML in cooperation with NRASG12D*

Increased splenomegaly

Worsened anemia

*Alan Shih
Niccastrin deletion (loss of Notch) and Tet2 loss cooperate to induce AML*

Amel mutations and their effect on the epigenetic state

- mutations can indirectly or directly alter the epigenetic state of AML cells
- in addition recent studies suggest kinase signaling can perturb chromatin state

We are likely to identify additional AML genes which contribute to hematopoietic transformation by corrupting the epigenetic state of hematopoietic stem/progenitor cells

Do these mutations have prognostic and therapeutic relevance?

Not so straightforward: divergent effects of IDH and TET2 on outcome in AML

Intermediate-Risk AML patients overall

P = 0.0006

TET2 WT
TET2 Mutant

* TET2 have an adverse prognostic impact in AML.
* Differential effects of TET2/IDH mutations on AML outcome need further investigation.
Complications, part 2....IDH/NPM1 co-mutations associated favorable outcome in FLI1-negative, intermediate risk AML

- IDH/NPM1-mutant patients, but not NPM1-mutant/IDH-WT patients have a favorable outcome

Newest Epigenetic Mutation: DNMT3A mutations in AML

- Frameshift mutations and recurrent mutations at R882 = Limited functional data consistent with loss of function
- Occur in 25-30% of patients with AML
- Associated with poor outcome in two retrospective cohorts

DNMT Mutational Status Does not Affect Outcome in E1900 Cohort

- Better outcome than in other trials, not different than DNMT3A-WT patients
- Patients were randomized to standard vs. high dose daunorubicin induction therapy—might induction dose intensity improve outcome in DNMT3A mutant patients?
High Dose Daunorubicin Improves Outcome in DNMT3A Mutant Patients, but not DNMT-WT patients

- Standard Dose
- High Dose

- Are there other mutations whose impact on survival is affected by induction dose?

High Dose Daunorubicin Improves Outcome in Patients with DNMT3A mutations, MLL Fusions, or NPM1 mutations

- High Dose
- Standard dose

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- Mithat Gonen
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- Peter Valk
- Bob Lowenberg
- Ruud De Kevel
Chicago
- Lucy Godley
Epigenetic Therapy of Hematologic Malignancies

Jean-Pierre Issa

Fels Institute for Cancer Research
Temple University

Disclosure of Conflicts of Interest

Jean-Pierre Issa, MD

Jean-Pierre Issa, MD, has affiliations with Celgene (Research and Honoraria) GlaxoSmithKline and SYNDAX (Consultant); Merck and Eisai (Research); and Novartis and Johnson & Johnson (Honoraria).

- and -

I will discuss off label use and/or investigational use of hypomethylating drugs in my presentation.

Multiple Defects in the Leukemia Epigenome

* The epigenetic signals are abnormal and affect the expression of key genes
  - DNA methylation, histone modifications, microRNAs

* The readers and writers of the marks are often mutated in cancer
  - AML/MDS: DNMT3a, TET2, EZH2, ASXL1, MLL, CBP etc.
**DNA Methylation is Abnormal in Cancer**

-41,000 CpG sites

Jelinek, AACR 2013

**MDS: Genetically and Epigenetically Heterogeneous**

DNA methylation analysis of a panel of genes in MDS

Methylation is independent of cytogenetics

Shen JCO 2010

**Epigenetic Therapy:**

Therapeutic targeting of epigenetic modifiers to achieve epigenetic reprogramming in-vivo
Hypomethylating Cytosine Analogues

<table>
<thead>
<tr>
<th>Cytosine</th>
<th>5-methylcytosine</th>
<th>5-aza-cytidine</th>
<th>5-aza-2’-deoxycytidine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Azacitidine</td>
<td>Decitabine</td>
</tr>
<tr>
<td>Ribose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deoxyribose</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mechanism of Action

Azacitidine in MDS
CALGB Study Group and Therapy

- 191 patients with MDS, median age 68 yrs, randomized to:
  1. Azacitidine (N=99)
  2. Supportive care (N=92)
  3. Cross-over design
- Azacitidine 75mg/m^2 D x 7Q mo SC
Azacitidine - Phase III Overall Survival

- Median survival:
  - Azacitidine: 20 months
  - Supportive Care: 14 months

p = 0.10

Azacitidine Prolongs Survival in MDS

- 358 pts with higher risk MDS (RAEB, RAEBT, CMML) IPSS int2 or high
- Randomization
  1) AZA 75 mg/m² SQ/ Dx7 Q4 wks (n=179)
  2) Conventional Care (n=179): support (n=105); LD ara-C 20 mg/m²/Dx14 Q4 wks (n=49); Chemotherapy (n=25)
- Median age 69 yrs; IPSS: high 47%, int-2 40%, NC 13%; AZA median 9 cycles; median FU 21 mo

Azacitidine vs CC in MDS

<table>
<thead>
<tr>
<th>Therapy</th>
<th>% CR</th>
<th>% PR</th>
<th>% OR</th>
<th>Median Survival (MOS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azacitidine</td>
<td>17</td>
<td>12</td>
<td>29</td>
<td>24.4</td>
</tr>
<tr>
<td>BSC</td>
<td>1</td>
<td>4</td>
<td>5</td>
<td>11.5</td>
</tr>
<tr>
<td>LD ara-C</td>
<td>8</td>
<td>4</td>
<td>12</td>
<td>15.3</td>
</tr>
<tr>
<td>3+7</td>
<td>36</td>
<td>4</td>
<td>40</td>
<td>15.7</td>
</tr>
</tbody>
</table>
**Survival With Azacitidine vs CC**

- **Azacitidine**
- **Conventional care**

<table>
<thead>
<tr>
<th>Month</th>
<th>Azacitidine</th>
<th>Conventional</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>124</td>
<td>120</td>
</tr>
<tr>
<td>1</td>
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<td>3</td>
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<tr>
<td>24</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>30</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Source: Lancet Oncol. 2009*  
*Mar. 1(3): 222-32*

---

**Decitabine Dose vs. Methylation**

![Graph showing the relationship between Decitabine dose and LINE methylation percentage.](chart)

*Qie, CCR, 2007*

---

**Decitabine Reduced-Dose Schedule (100 mg/m²/course): 3-Arm Dosing Study**

- 3 decitabine treatment arms:
  - 10 mg/m² IV over 1 hr daily x 10 days
  - 20 mg/m² IV over 1 hr daily x 5 days (double dose-intensity)
  - 20 mg/m² SQ (10 mg SQ BID) daily x 5 days
- Study group:
  - 95 patients treated (77 MDS, 18 CMML)
  - 65% patients Int-2/High Risk
  - 69% male, 65% were ≥ 60 yrs of age

*Source: Blood 2007*
3-Arm Dosing Study Data Responses By Treatment Arm

<table>
<thead>
<tr>
<th>Schedule</th>
<th>No. CR/Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 mg/m² IV x 5 days</td>
<td>25/64 (39)</td>
</tr>
<tr>
<td>20 mg/m² SQ x 5 days</td>
<td>3/14 (21)</td>
</tr>
<tr>
<td>10 mg/m² IV x 5 days</td>
<td>4/17 (24)</td>
</tr>
<tr>
<td>Total</td>
<td>32/95 (34)</td>
</tr>
</tbody>
</table>

Overall (IWG) CR: 34%, PR/RI= 39%, Total Response-74%

Decitabine vs. Intensive Chemotherapy in HR MDS

Side-Effects

- Myelosuppression and neutropenic fever in 10-50%
- Recommend prophylactic antibiotics
- Growth factor support
- Injection site side-effects and nausea with azacitidine
- Rare cytotoxic side-effects (mucositis, diarrhea, hair loss)
Histone Deacetylase Inhibitors

<table>
<thead>
<tr>
<th>Class</th>
<th>Compound</th>
<th>Logdds Selectivity</th>
<th>Study Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydramide</td>
<td>SAHA</td>
<td>1, 4, 4b, 4c, 4d</td>
<td>Phase II</td>
</tr>
<tr>
<td></td>
<td>LBQ684</td>
<td>1, 4, 4b, 4c, 4d</td>
<td>Phase II</td>
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<tr>
<td></td>
<td>Remdesivir</td>
<td>1, 4, 4b, 4c, 4d</td>
<td>Phase II</td>
</tr>
<tr>
<td></td>
<td>DAC/AYH</td>
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</tr>
<tr>
<td></td>
<td>DAC/UCY</td>
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<tr>
<td>Short-chain acyl acids</td>
<td>SAHA</td>
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<td>Phase II</td>
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<td>Phase II</td>
</tr>
<tr>
<td></td>
<td>Remdesivir</td>
<td>1, 4, 4b, 4c, 4d</td>
<td>Phase II</td>
</tr>
<tr>
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<td>DAC/AYH</td>
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</tr>
<tr>
<td></td>
<td>DAC/UCY</td>
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<tr>
<td></td>
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<td>Benzamides</td>
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<td>Phase II</td>
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<tr>
<td></td>
<td>Remdesivir</td>
<td>1, 2, 3, 4c, 4d</td>
<td>Phase II</td>
</tr>
</tbody>
</table>

HDAC Inhibitors: Clinical Results

* Response rates of 20-60% in CTCL and Hodgkin’s lymphoma
* Side-effects include fatigue, diarrhea, effects on QT interval
* In myeloid leukemias, single agent DNA methylation inhibitors are more effective than single agent HDAC inhibitors

Evidence for an Epigenetic Mechanism of Action

* Clinical: Response patterns
* Pharmacodynamic: Correlations between response and
  * Sustained hypomethylation
  * Sustained gene activation
Epigenetic Therapy - Unknowns

- Patient selectivity
  - Limited DNA methylation analysis at baseline shows no correlation with response
  - Mutation testing (DNMT3a, TET2, EZH2 etc.) underway
- Mechanisms of response
  - Differentiation, stem cell effects, cytotoxicity, immune response etc.
- Mechanisms of relapse and resistance
  - Primary resistance: pharmacologic in some patients
  - Secondary resistance: independent of DNA methylation

Epigenetic Therapy - Unknowns

- Long term side-effects, tumorigenesis etc.
- Activity in solid tumors
  - Limited testing in previously untreated patients
  - Pharmacodynamic barriers
- Azacitidine vs. Decitabine

The Next Generation

- Combinations
**Clofarabine and Low-Dose Cytarabine Alternating with Decitabine**

- **Induction:**
  
  Clo 20mg/m² IV daily x 5 d, plus  
  Ara-C 20mg SC twice daily x 10 d  

- **Consolidation:**
  
  Clo 20mg/m² IV daily x 3 d  
  Ara-C 20mg SC twice daily x 5 d  
  alternating with  
  DAC 20 mg/m² IV daily x 5 d  

  Courses:  
  1-2, 6-8, 12-14  

  Courses:  
  3-5, 9-11, 15-17  


---

**Survival in AML**

![Survival graph](image)


---

**The Next Generation**

- **Combinations**

- **Better drugs for similar targets**
  - SGI-110, oral azacitidine  
  - Panobinostat, entinostat, belinostat etc.

- **New drugs for new targets**
Up and Coming Epigenetic Targets

- EZH2 - H3K27 histone methyltransferase
  - Activating mutations in lymphomas
  - Overexpressed in poor prognosis prostate/breast cancer

- DOT1L - H3K79 histone methyltransferase
  - Required for the growth of MLL1 mutated leukemias

Epigenetic Silencing Mechanisms

Summary

- Epigenetic therapy with DNMT inhibitors is clinically useful in MDS and active in AML but many questions remain
- Therapy is not curative - resistance commonly develops
- Combination DNMT/HDAC inhibitors and sequential, non-cross resistant therapy trials are under investigation
- New epigenetic drugs are in development and/or early stage clinical trials
Stephen B. Baylin, MD


James E. Bradner, MD


Jonathan D. Licht, MD


Ross L. Levine, MD

Jean-Pierre J. Issa, MD