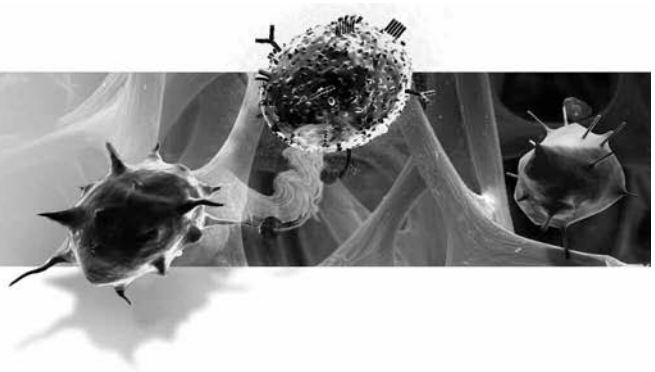
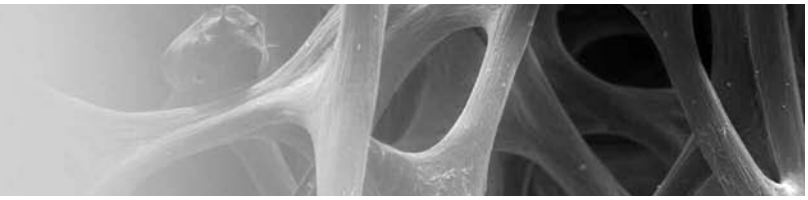
CONTENTS



WELCOME



The Leukemia & Lymphoma Society®
Fighting Blood Cancers

On behalf of The Leukemia & Lymphoma Society (LLS), thank you for joining us for ***Significance of the Tumor Microenvironment in Hematological Malignancies***, a continuing education activity originally presented in Orlando, Florida.

LLS would also like to thank our esteemed speakers for sharing their time and expertise. Through this activity, our presenters will explain the complex interactions between stromal cells and tumor cells; discuss the role of bone marrow microenvironment in leukemia stem cell survival; describe the mechanisms by which bone marrow stroma can confer net chemotherapy resistance in diverse hematologic malignancies; and discuss the therapeutic potential of targeting one or more stromal components to reverse malignant cell resistance to chemotherapy and improve clinical outcomes for hematologic malignancies.

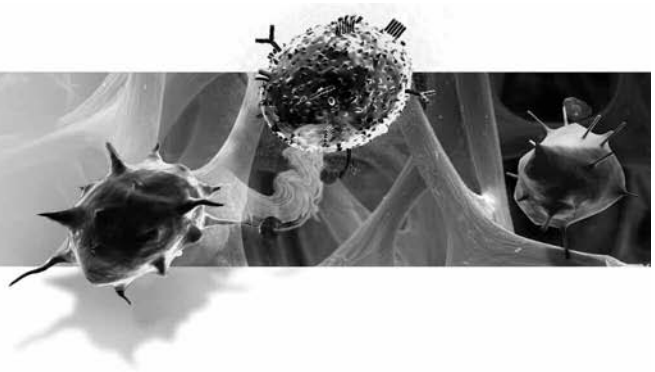
This workbook includes the presenters' slides to help guide you through the activity. If you would like to receive 3.5 *AMA PRA Category 1 Credit(s)*™, please complete the online learning assessment and evaluation.

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

Sincerely,

Louis J. DeGennaro, PhD
Executive Vice President
Chief Mission Officer
The Leukemia & Lymphoma Society

AGENDA



Program Overview
Louis J. DeGennaro, PhD
Executive Vice President
Chief Mission Officer
The Leukemia & Lymphoma Society

The Hematopoietic Stem Cell Niche
Sean J. Morrison, PhD

Cytokines and the Microenvironment of Lymphoma
Louis M. Staudt, MD, PhD

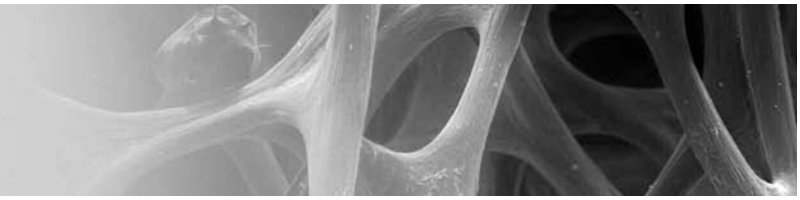
Molecular Control of Leukemic Cell Infiltration Into the CNS
Iannis Aifantis, PhD

Clinical and Translational Studies of Stroma-Leukemia Interactions
Michael P. Rettig, PhD

Cell Trafficking in Multiple Myeloma
Irene M. Ghobrial, MD

Question-and-Answer Session

OVERVIEW



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 research and clinical practice for hematological malignancies. The program subject matter may be of interest to those who provide specialized care in the diagnosis, treatment and monitoring of patients with malignancies such as leukemia, lymphoma or myeloma.

ACTIVITY PURPOSE

This activity is designed to educate hematologists, oncologists, nurses, social workers and other healthcare professionals on the evolving role of the tumor microenvironment in the pathogenesis and management of various malignancies.

STATEMENT OF NEED

Much of the cancer research conducted over the past two decades has focused on the tumor and its various characteristics. However, recent data suggest that the microenvironment in which a tumor originates plays a critical role in tumor propagation as well as the development of drug resistance.[1] The National Cancer Institute has launched The Tumor Microenvironment Initiative, which focuses on expanding our current understanding of the role of the tumor microenvironment in cancer initiation, progression and metastases.[2] As research continues, the potential for components of the tumor microenvironment to serve as therapeutic targets in the management of hematologic malignancies becomes more promising.[3,4] It is critical that healthcare professionals who treat patients with various hematologic malignancies remain abreast of key findings regarding the importance of the tumor environment and its implications in future research, management and patient outcomes.

[1] Weinberg RA. *Nat Genet*. 2008;40:494-495.

[2] National Cancer Institute. http://plan2010.cancer.gov/Tumor_Microenvironment.htm. Accessed March 6, 2010.

[3] Dalton W, Anderson KC. *Clin Cancer Res*. 2006;12:6603-6610.

[4] Burger JA, Peled A. *Leukemia*. 2009;23:43-52.

EDUCATIONAL OBJECTIVES

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

- Explain the complex interactions between stromal cells and tumor cells
- Discuss the role of bone marrow microenvironment in leukemia stem cell survival
- Describe the mechanisms by which bone marrow stroma can confer net chemotherapy resistance in diverse hematologic malignancies
- Discuss the therapeutic potential of targeting one or more stromal components to reverse malignant cell resistance to chemotherapy and improve clinical outcomes for hematologic malignancies

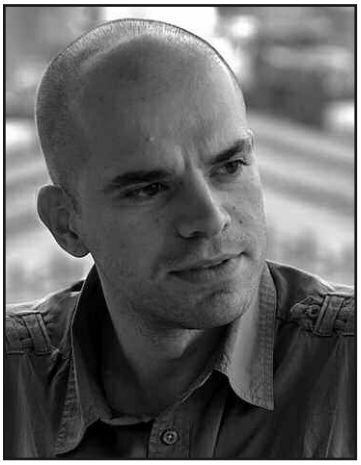
STATEMENT OF SUPPORT

This activity is jointly sponsored by Robert Michael Educational Institute LLC and Postgraduate Institute for Medicine and supported by educational grants from Millennium Pharmaceuticals, Inc., Celgene Corporation and Allos Therapeutics.

FACULTY BIOGRAPHIES

Iannis Aifantis, PhD

***Associate Professor*, Department of Pathology**
New York University School of Medicine
***Co-Director*, Cancer Stem Cell Program**
New York University Cancer Institute
Early Career Investigator
Howard Hughes Medical Institute
New York, NY



Dr. Aifantis is Associate Professor of Pathology at the New York University School of Medicine and Co-Director of the Cancer Stem Cell Program of the New York University Cancer Institute. He is an Early Career Investigator at the Howard Hughes Medical Institute. Dr. Aifantis received a master of science degree in molecular biology and genetics from the University of Crete in Iraklion, Greece, and a doctor of philosophy in immunology at the University of Paris in Paris, France. He completed post-doctoral fellowship training in immunology at the Dana-Farber Cancer Institute / Harvard University, in Boston, Massachusetts. Dr. Aifantis serves on the editorial boards of *Immunology & Cell Biology* and *Oncogene* and has published extensively in peer-reviewed journals. He is the Principal Investigator on several National Cancer Institute and foundation-supported projects. His laboratory is focused on mechanisms of differentiation and transformation of hematopoietic stem cells and progenitors.

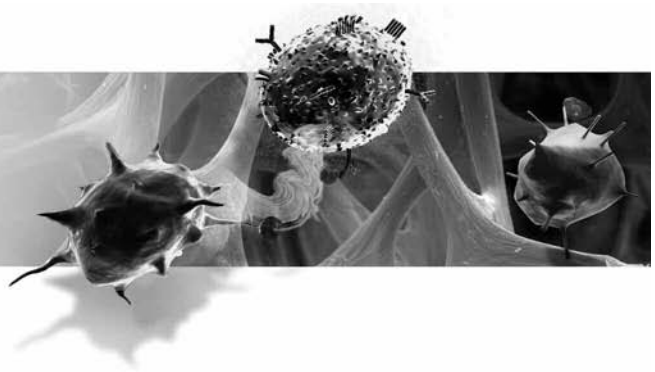
FACULTY BIOGRAPHIES

Irene M. Ghobrial, MD

Director of Laboratory
Dana-Farber Cancer Institute
Assistant Professor, Department of Medicine
Harvard Medical School
Boston, MA

Dr. Ghobrial is Laboratory Director at Dana-Farber Cancer Institute and Assistant Professor in the Department of Medicine at Harvard Medical School in Boston, Massachusetts. Dr. Ghobrial received her medical degree from Cairo University School of Medicine in Cairo, Egypt, completed residency training in internal medicine at Wayne State University in Detroit, Michigan, and fellowship training in hematology/oncology at the Mayo Clinic College of Medicine in Rochester, Minnesota. She joined Dana-Farber Cancer Institute in the field of multiple myeloma and Waldenström macroglobulinemia in 2005. She received the Clinical Investigator Award from Dana-Farber Cancer Institute in 2006. Dr. Ghobrial is the Principal Investigator for National Cancer Institute–funded projects as well as for foundation grants. She has published extensively in peer-reviewed journals. Her research is focused on cell trafficking and homing of multiple myeloma and Waldenström macroglobulinemia.

FACULTY BIOGRAPHIES



Sean J. Morrison, PhD

***Director*, University of Michigan Center for Stem Cell Biology**
***Professor*, Departments of Internal Medicine and Cell and Developmental Biology**
***Research Professor*, Life Sciences Institute**
***Investigator*, Howard Hughes Medical Institute**
University of Michigan
Ann Arbor, MI

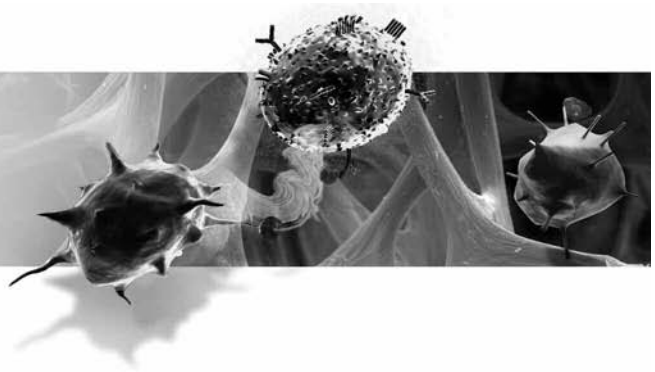
Dr. Morrison is Director of the University of Michigan Center for Stem Cell Biology, Professor in the departments of Internal Medicine and Cell and Developmental Biology and Research Professor in the Life Sciences Institute at the University of Michigan. Dr. Morrison completed his undergraduate studies at Dalhousie University in Halifax, Canada. He received a PhD in immunology from Stanford University in Stanford, California, and completed a post-doctoral fellowship at the California Institute of Technology in Pasadena, California. Dr. Morrison is the recipient of the McCulloch and Till Award from the International Society for Hematology and Stem Cells, the Harland Winfield Mossman Award from the American Association of Anatomists, and a MERIT award from the National Institute on Aging. He is a Howard Hughes Medical Institute investigator, has published extensively in peer-reviewed journals and has received funding from the National Institutes of Health, the Department of Defense and various private foundations. Dr. Morrison's research is focused on the mechanisms that regulate stem cell function in the nervous and hematopoietic systems, particularly the mechanisms that regulate stem cell self-renewal and stem cell aging, as well as the relationship between stem cell self-renewal and cancer cell proliferation.

FACULTY BIOGRAPHIES

Michael P. Rettig, PhD
Research Assistant Professor of Medicine
Section of Bone Marrow Transplant
Division of Oncology
Washington University School of Medicine
St. Louis, MO

Dr. Rettig is Research Assistant Professor of Medicine in the Section of Bone Marrow Transplant, Division of Oncology at Washington University School of Medicine in St. Louis, Missouri. Dr. Rettig received his doctorate of philosophy in chemistry from Purdue University in West Lafayette, Indiana. He has served as Principal Investigator on several foundation-supported projects and Co-Investigator on several National Cancer Institute-supported projects. In 2005, Dr. Rettig received the American Society of Hematology Fellow Scholar Award. In collaboration with Dr. John DiPersio, Dr. Rettig's laboratory at the Washington University School of Medicine studies the biology of hematopoietic stem and leukemia cell mobilization.

FACULTY BIOGRAPHIES



Louis M. Staudt, MD, PhD
Deputy Chief, Metabolism Branch
Center for Cancer Research
National Cancer Institute, National Institutes of Health
Bethesda, MD

Dr. Staudt serves as the Deputy Chief of the Metabolism Branch in the Center for Cancer Research at the National Cancer Institute. He received his doctor of medicine and doctor of philosophy in immunology from the University of Pennsylvania School of Medicine in Philadelphia, Pennsylvania. Following training in internal medicine, he completed a post-doctoral fellowship at the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts. In 2009 he received the William Dameshek Prize for outstanding contribution in hematology from the American Society of Hematology and in 2010 he received an NIH Director's Award for his work with the Lymphoma/Leukemia Molecular Profiling Project. Dr. Staudt serves on the editorial boards of *Genome Biology* and *Cancer Cell* and is associate editor of the *Journal of Experimental Medicine*. His research is focused on the study of the molecular basis of human lymphoid malignancies.

ACCREDITATION & CREDIT

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 (ACCME) through the joint sponsorship of Postgraduate Institute for Medicine (PIM) and Robert Michael Educational Institute LLC (RMEI). PIM is accredited by the ACCME to provide continuing medical education for physicians.

Credit Designation

Postgraduate Institute for Medicine designates this educational activity for a maximum of 3.5 *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 January, 20, 2011, through January 20, 2012, 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 7831. 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 3.5 continuing education contact hours through the California Board of Registered Nursing.

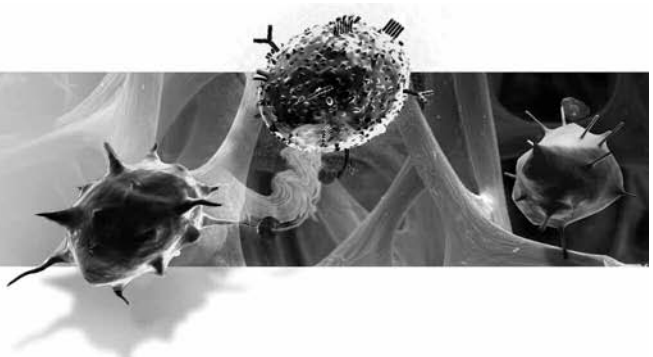
The Leukemia & Lymphoma Society (LLS), provider number 1105, is approved as a provider for social work continuing education by the Association of Social Work Boards (ASWB) www.aswb.org Approved Continuing Education Program (ACE). Approval Period: 12/2008-12/2011. LLS maintains responsibility for the program. Social workers should contact their regulatory board to determine course approval. Social workers will receive 3.5 CE clinical clock hours.

Upon completion of this program and submission of the CE 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.

DISCLOSURES & DISCLAIMER



DISCLOSURE OF CONFLICTS OF INTEREST

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:

- **Iannis Aifantis, PhD,** has no affiliations with commercial interests to disclose.
- **Irene M. Ghobrial, MD,** has affiliations with Millennium Pharmaceuticals, Inc., Celgene, Novartis and Genzyme *(Advisory Board)*.
- **Sean J. Morrison, PhD,** has affiliations with Hospira *(Consulting)* Merck/Schering-Plough *(Speakers' Bureau)* and OncoMed *(Stockholder)*.
- **Michael P. Rettig, PhD,** has an affiliation with Genzyme *(Honoraria)*.
- **Louis M. Staudt, MD, PhD,** 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

- **Louis J. DeGennaro, PhD,** has no affiliations with commercial interests to disclose.

ROBERT MICHAEL EDUCATIONAL INSTITUTE LLC

- **Sherri Kramer, MD,** has no affiliations with commercial interests to disclose.
- **Laura Altobelli, MS,** has no affiliations with commercial interests to disclose.
- **Nora Duffy** has no affiliations with commercial interests to disclose.

POSTGRADUATE INSTITUTE FOR MEDICINE

- **Jan Hixon, RN, BSN, MA,** has no affiliations with commercial interests to disclose.
- **Trace Hutchison, PharmD,** has no affiliations with commercial interests to disclose.
- **Julia Kimball, RN, BSN,** 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

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), Robert Michael Educational Institute LLC (RMEI), Millennium Pharmaceuticals, Inc., Celgene Corporation and Allos Therapeutics do not recommend the use of any agent outside of the labeled indications.

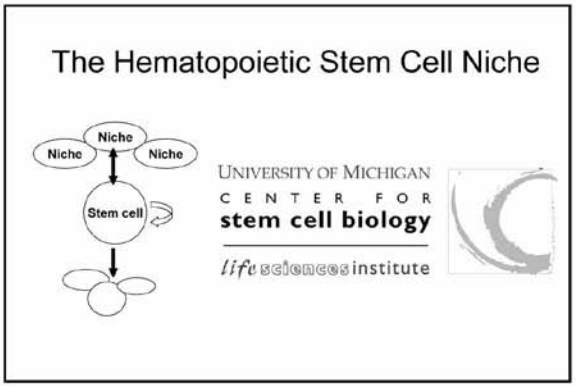
The opinions expressed in the educational activity are those of the faculty and do not necessarily represent the views of PIM, RMEI, Millennium Pharmaceuticals, Inc., Celgene Corporation or Allos Therapeutics. Please refer to the official prescribing information for each product for discussion of approved indications, contraindications, and warnings.

DISCLAIMER

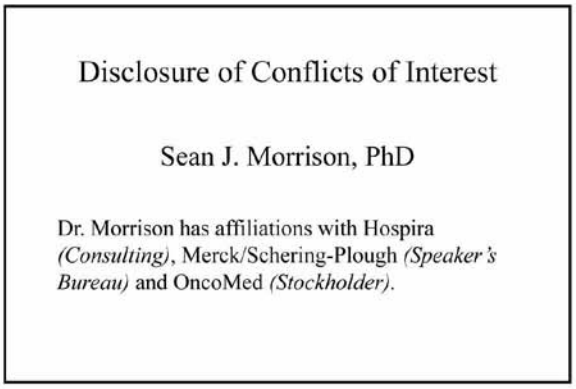
Participants have an implied responsibility to use the newly acquired information to enhance patient outcomes and their own professional development. The information presented in this activity is not meant to serve as a guideline for patient management. Any procedures, medications, or other courses of 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.


Significance of the
TUMOR MICROENVIRONMENT
in Hematological Malignancies
The Leukemia & Lymphoma Society
Fighting Blood Cancers

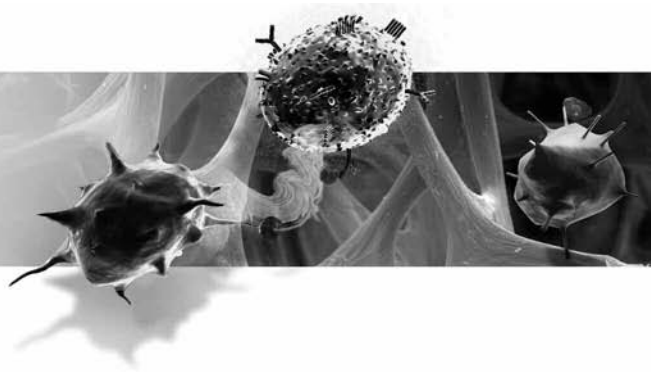
1


The Hematopoietic Stem Cell Niche
Niche
Niche
Niche
Stem cell
UNIVERSITY OF MICHIGAN
CENTER FOR
stem cell biology
life sciences institute

2


Disclosure of Conflicts of Interest
Sean J. Morrison, PhD
Dr. Morrison has affiliations with Hospira (Consulting), Merck/Schering-Plough (Speaker's Bureau) and OncoMed (Stockholder).

3

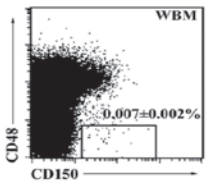


The nature of the hematopoietic stem cell niche remains uncertain

- Data are not consistent with the N-cadherin-mediated osteoblastic niche model
- It remains possible that osteoblasts directly or indirectly regulate HSC maintenance through other mechanisms
- Data suggest the possibility of a perivascular niche in bone marrow but the precise identity of cells that secrete factors that regulate HSC maintenance remains uncertain
- Many niche models remain consistent with existing data

4

$CD150^+CD48^-CD41^-$ cells in bone marrow and spleen are highly purified HSCs

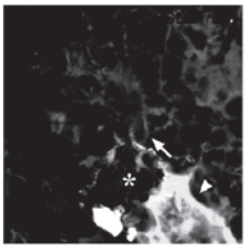


1. 45% of single $CD150^+CD48^-CD41^-$ bone marrow cells give long-term multilineage reconstitution
2. 33% of single $CD150^+CD48^-CD41^-$ cytokine mobilized spleen cells gave long-term multilineage reconstitution
3. Possible to localize HSCs in tissue sections using a 2-color stain

Kiel et al. Cell 121:1109, 2005

5

HSCs in bone marrow are usually adjacent to sinusoids



- 0.0067% of cells were $CD150^+CD48^-CD41^-Lineage^-$
- 57% in the trabecular zone
- 14% at endosteal surface
- 60% adjacent to sinusoids
- 95% near sinusoids

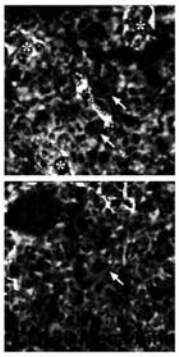
Kiel et al. Cell 121:1109, 2005

6

HSCs in mobilized spleen were usually associated with sinusoids

- 0.006% of cells were CD150^{+} CD48^{-}CD41^{-}Lineage^{-}
- 62% adjacent to sinusoidal endothelium
- 38% were not

Kiel et al. Cell 121:1109, 2005



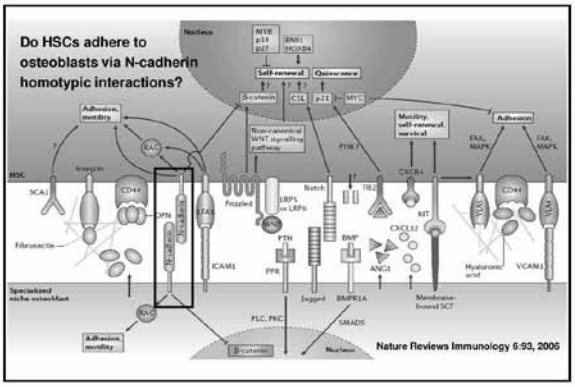
7

HSC localization using validated markers suggests that most HSCs are perivascular

- These data contrast with the model that most HSCs reside on the surface of osteoblasts
- Are HSCs regulated by osteoblasts by direct or indirect interactions?
- We re-examined the evidence for a direct interaction between HSCs and osteoblasts

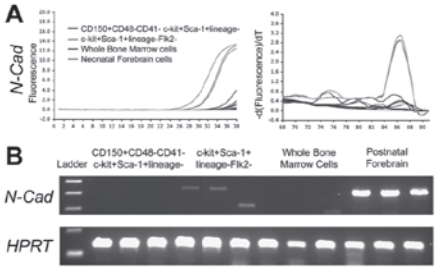
8

Do HSCs adhere to osteoblasts via N-cadherin homotypic interactions?



9

We could not detect *N-Cadherin* expression in highly purified HSCs by quantitative PCR



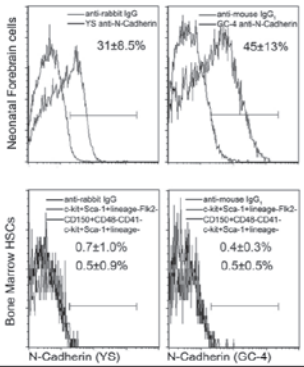
Kiel et al. Cell Stem Cell 1:204

10

We are not able to detect any staining of highly purified HSCs with any anti-N-cadherin antibody

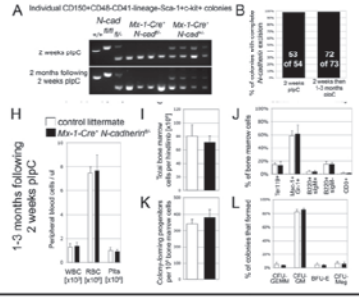
Also unable to detect N-cadherin in HSCs by western blot, microarray, or analysis of gene trap mice

Kiel et al. Cell Stem Cell 1:204
See also:
Foudi et al. Nat. Biotech. 27:84
Morita et al. JEM 207:1173
Li and Zon Cell Stem Cell 6:19



11

Conditional deletion of *N-cadherin* in vivo does not affect hematopoiesis



***N-cadherin* deletion was complete**

No effect on blood cell counts, bone marrow cellularity, composition, or CFUs, even 5 months later

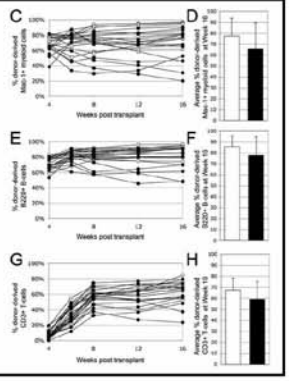
Kiel et al. Cell Stem Cell 4:170

12

Deletion of *N-cadherin* does not impair HSC reconstituting capacity in primary or secondary recipients

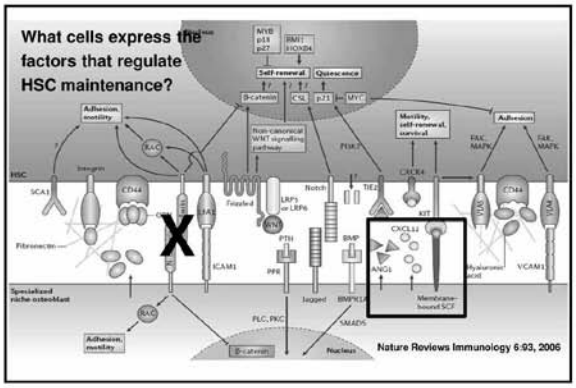
***N-cadherin* was conditionally deleted before transplantation into irradiated mice**

Kiel et al. Cell Stem Cell 4:170, 2008



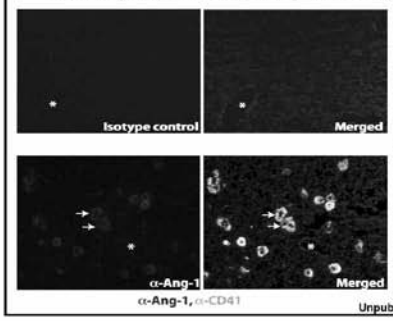
13

What cells express the factors that regulate HSC maintenance?



14

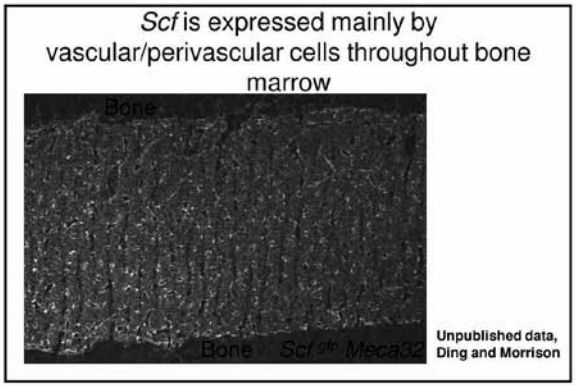
Perivascular cells, including megakaryocytes, are the major sources of Ang-1 in the bone marrow



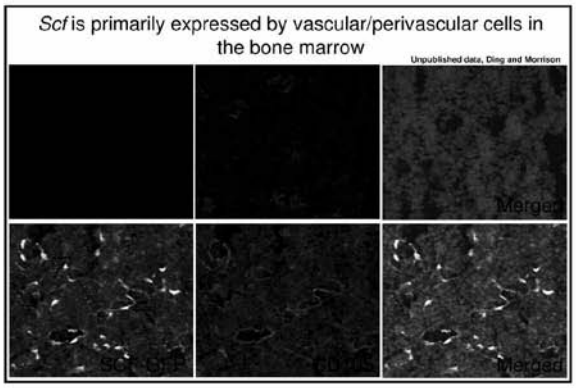
- We have not been able to detect Ang-1 in osteoblasts or at the endosteum (*)
- Ang-1 must be conditionally deleted from different cell types in the bone marrow to test whether it regulates HSC maintenance

Unpublished data, Ding and Morrison

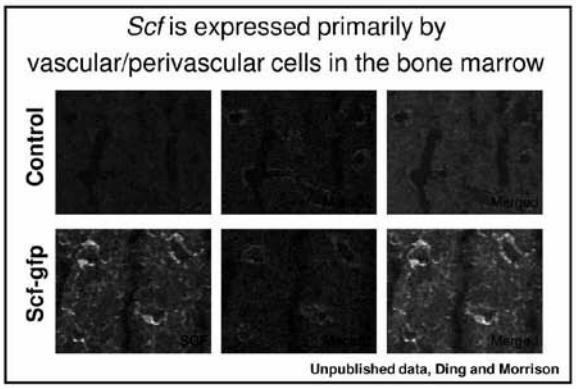
15


Scf is expressed mainly by vascular/perivascular cells throughout bone marrow
Bone
Bone
Unpublished data, Ding and Morrison

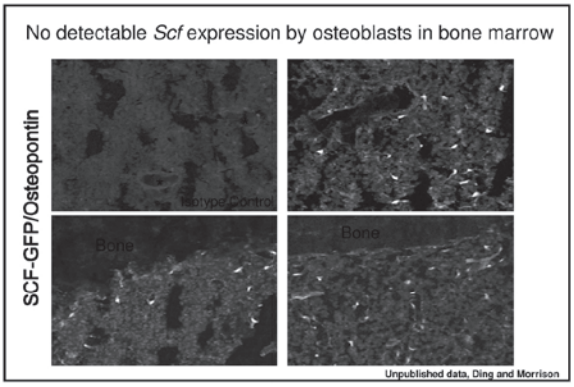
16


Scf is primarily expressed by vascular/perivascular cells in the bone marrow
Unpublished data, Ding and Morrison
Merged
Merged

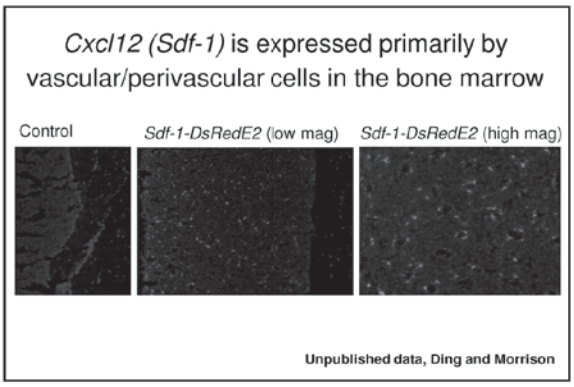
17


Scf is expressed primarily by vascular/perivascular cells in the bone marrow
Control
Scf-gfp
Unpublished data, Ding and Morrison

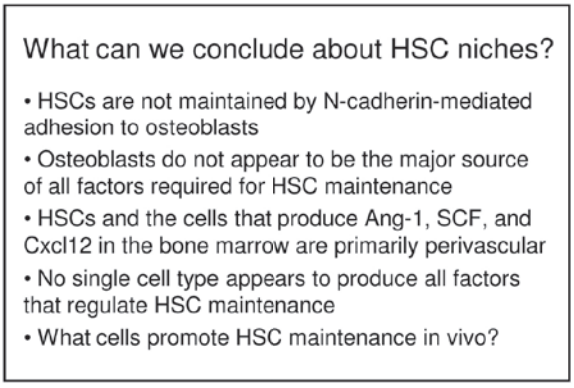
18


No detectable *Scf* expression by osteoblasts in bone marrow
SCF-GFP/Osteopontin
Isotype Control
Bone
Bone
Unpublished data, Ding and Morrison

19

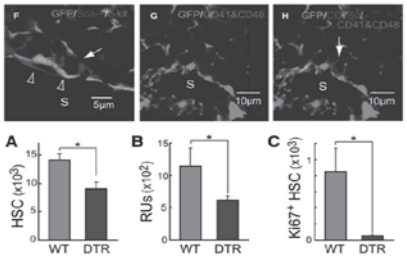

Cxcl12 (Sdf-1) is expressed primarily by vascular/perivascular cells in the bone marrow
Control
Sdf-1-DsRedE2 (low mag)
Sdf-1-DsRedE2 (high mag)
Unpublished data, Ding and Morrison

20


What can we conclude about HSC niches?
• HSCs are not maintained by N-cadherin-mediated adhesion to osteoblasts
• Osteoblasts do not appear to be the major source of all factors required for HSC maintenance
• HSCs and the cells that produce Ang-1, SCF, and Cxcl12 in the bone marrow are primarily perivascular
• No single cell type appears to produce all factors that regulate HSC maintenance
• What cells promote HSC maintenance in vivo?

21

CXCL12-expressing perivascular cells are required for HSC maintenance (Nagasawa and colleagues)

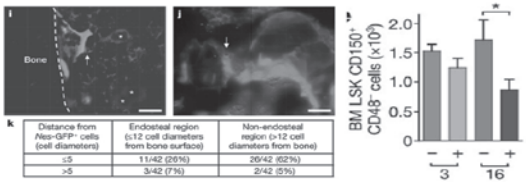


Sugiyama *et al.* Immunity 25:977, 2006

Omatsu *et al.* Immunity 33:387, 2010

22

Perivascular Nestin+ mesenchymal stem cells in the bone marrow are required to maintain HSCs (Frenette and colleagues)

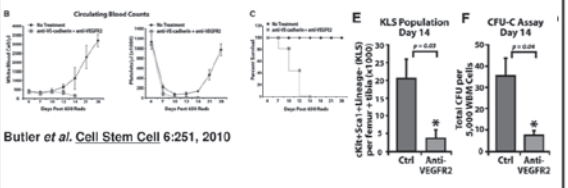


Are the Nestin+ MSCs the same cells that secrete Cxcl12?

S Méndez-Ferrer *et al.* Nature 466:829, 2010

23

Endothelial cells are essential for the recovery of HSCs after sublethal irradiation (Rafii and colleagues)



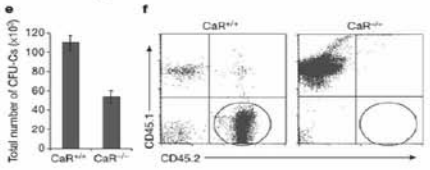
Butler *et al.* Cell Stem Cell 6:251, 2010

Hooper *et al.* Cell Stem Cell 4:263, 2009

Butler, JM *et al. Cell Stem Cell* 2010, 6:251-64

24

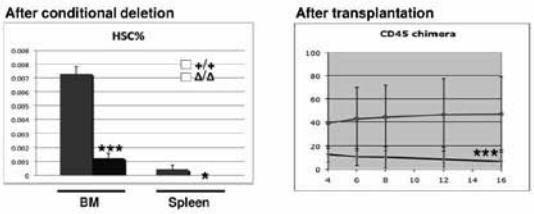
Osteoblasts are likely to regulate HSC localization/maintenance, though this may or may not involve cell-cell contact (Scadden and colleagues)
Ca-sensing receptor is required in HSCs for engraftment in bone marrow



Adams *et al.* Nature 439599, 2006

25

What cells secrete the factors that promote HSC maintenance?
Conditional deletion of *Scf* from HSCs and endothelial cells using *Tie2-Cre* leads to HSC depletion

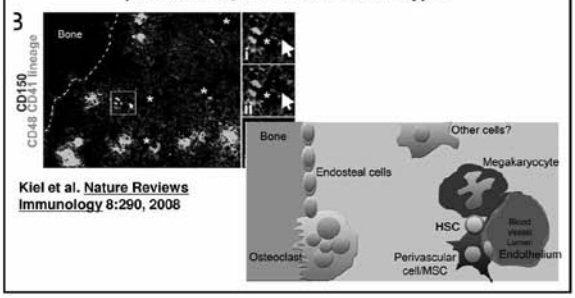


Endothelial cells are likely to promote HSC maintenance by secreting SCF into a perivascular niche

Unpublished data, Ding and Morrison

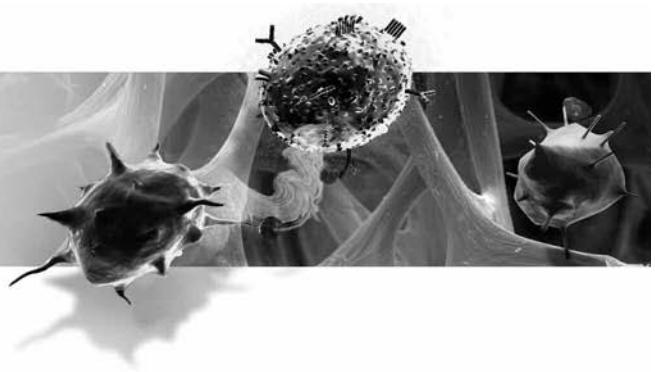
26

The precise nature of the HSC niche remains uncertain but it involves direct or indirect regulation by multiple vascular, perivascular, and endosteal cell types



Kiel et al. Nature Reviews Immunology 8:290, 2008

27

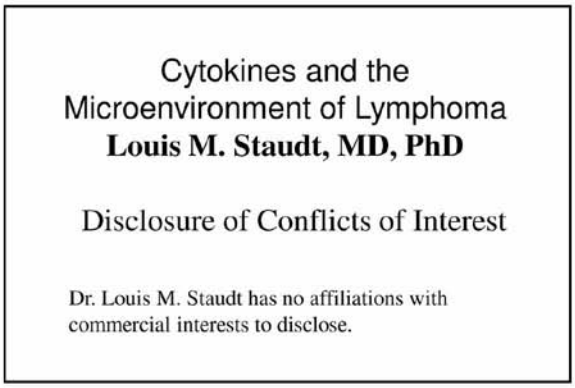



Mark Kiel
Omer Yilmaz
N-cadherin
Melih Acar
Glenn Radice
Published data from other labs
David Scadden
Shahin Rafii
Paul Frenette
Takashi Nagasawa

28


Thank You

29


Cytokines and the
Microenvironment of Lymphoma
Louis M. Staudt, MD, PhD
Disclosure of Conflicts of Interest
Dr. Louis M. Staudt has no affiliations with
commercial interests to disclose.

30

Dissecting Cancer into Molecularly and Clinically Distinct Subgroups by Gene Expression Profiling

Diffuse large B cell lymphoma

~40% of Non-Hodgkin lymphomas

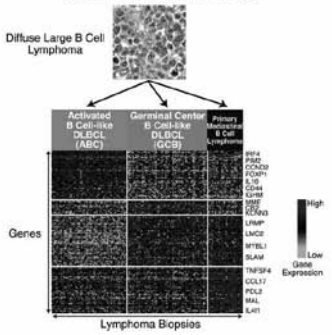
~23,000 new diagnoses/yr

~50% cure rate

~10,000 deaths/yr

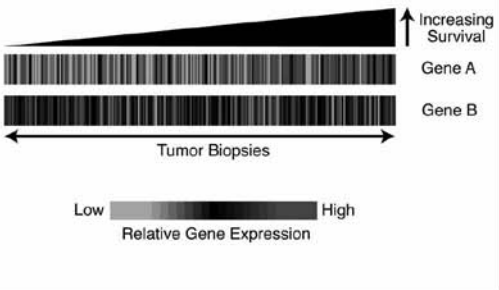
31

Dissecting Cancer into Molecularly and Clinically Distinct Subgroups by Gene Expression Profiling

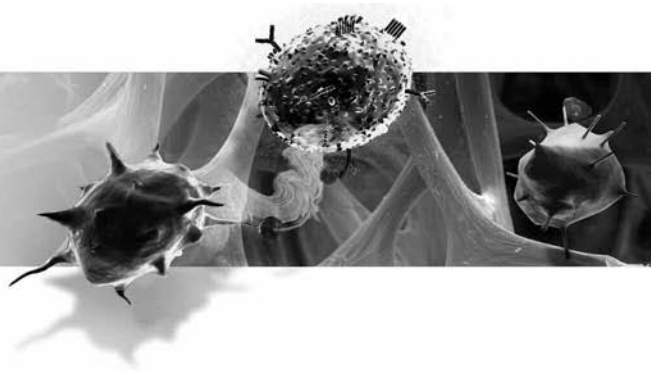


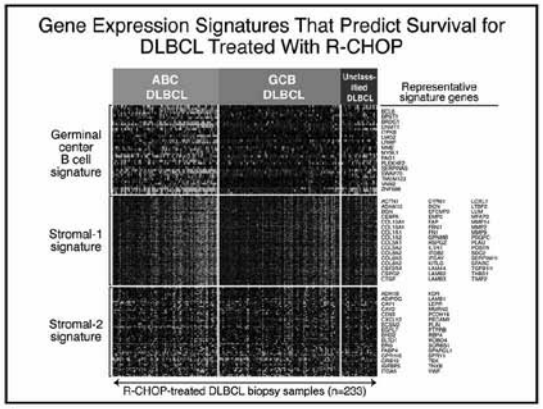
32

Supervised Discovery of Genes that Influence Clinical Outcome in Cancer

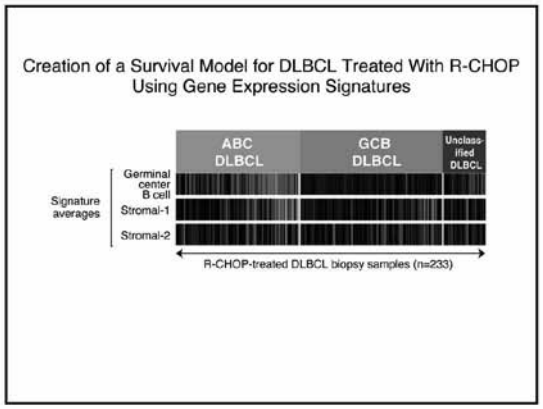


33

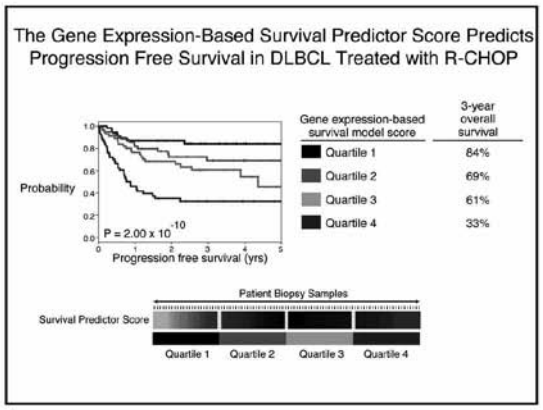



Gene Expression Signatures That Predict Survival for DLBCL Treated With R-CHOP
ABC DLBCL
GCB DLBCL
Unclassified DLBCL
Representative signature genes
Germinal center B cell signature
Stromal-1 signature
Stromal-2 signature
R-CHOP-treated DLBCL biopsy samples (n=233)

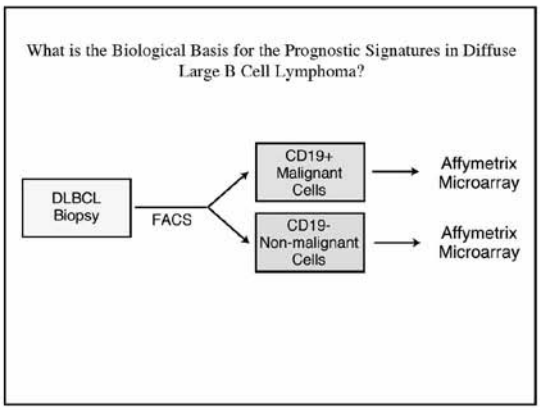
34


Creation of a Survival Model for DLBCL Treated With R-CHOP Using Gene Expression Signatures
ABC DLBCL
GCB DLBCL
Unclassified DLBCL
Signature averages
Germinal center B cell
Stromal-1
Stromal-2
R-CHOP-treated DLBCL biopsy samples (n=233)

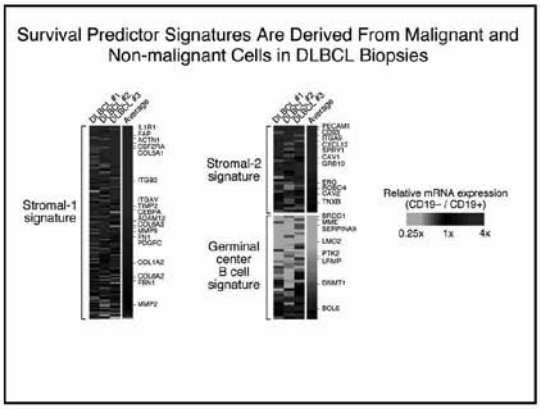
35


The Gene Expression-Based Survival Predictor Score Predicts Progression Free Survival in DLBCL Treated with R-CHOP
Probability
P = 2.00 x 10-10
Progression free survival (yrs)
Gene expression-based survival model score
3-year overall survival
Quartile 1
84%
Quartile 2
69%
Quartile 3
61%
Quartile 4
33%
Patient Biopsy Samples
Survival Predictor Score
Quartile 1
Quartile 2
Quartile 3
Quartile 4

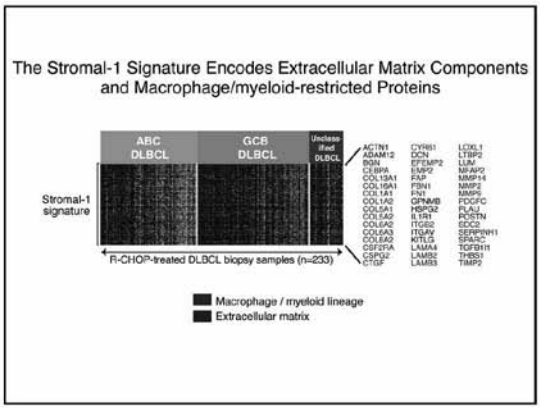
36


What is the Biological Basis for the Prognostic Signatures in Diffuse Large B Cell Lymphoma?
DLBCL Biopsy
FACS
CD19+ Malignant Cells
CD19- Non-malignant Cells
Affymetrix Microarray
Affymetrix Microarray

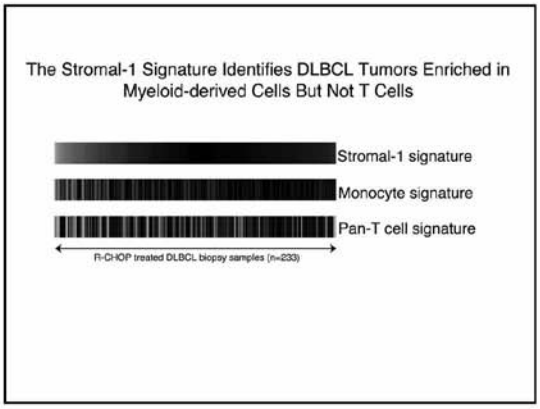
37


Survival Predictor Signatures Are Derived From Malignant and Non-malignant Cells in DLBCL Biopsies
Stromal-1 signature
Stromal-2 signature
Germinal center B cell signature
Relative mRNA expression (CD19− / CD19+)
0.25x
1x
4x

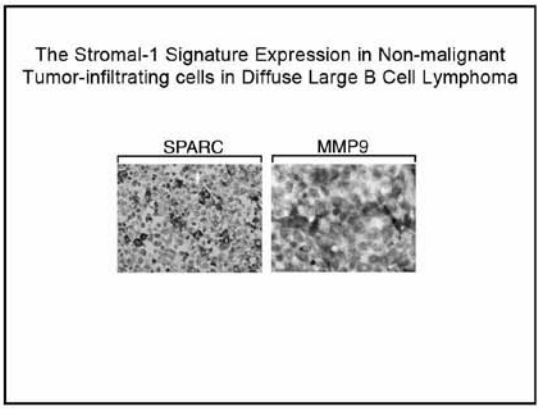
38


The Stromal-1 Signature Encodes Extracellular Matrix Components and Macrophage/myeloid-restricted Proteins
ABC DLBCL
GCB DLBCL
Unclassified DLBCL
Stromal-1 signature
R-CHOP-treated DLBCL biopsy samples (n=233)
Macrophage / myeloid lineage
Extracellular matrix

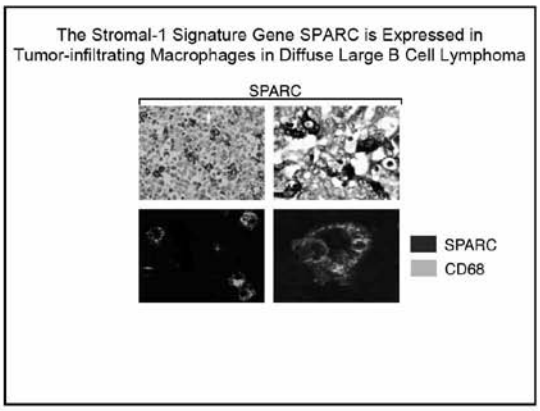
39


The Stromal-1 Signature Identifies DLBCL Tumors Enriched in Myeloid-derived Cells But Not T Cells
Stromal-1 signature
Monocyte signature
Pan-T cell signature
R-CHOP treated DLBCL biopsy samples (n=233)

40


The Stromal-1 Signature Expression in Non-malignant Tumor-infiltrating cells in Diffuse Large B Cell Lymphoma
SPARC
MMP9

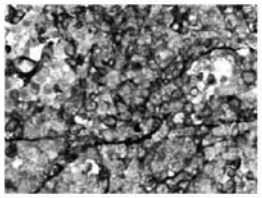
41


The Stromal-1 Signature Gene SPARC is Expressed in Tumor-infiltrating Macrophages in Diffuse Large B Cell Lymphoma
SPARC
SPARC
CD68

42

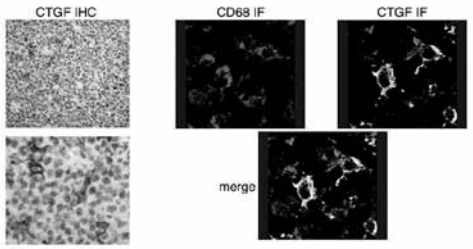
Stromal-1 Signature Genes Encode Components of the Extracellular Matrix in Diffuse Large B Cell Lymphoma

Fibronectin



43

CTGF Protein is Localized to Tumor Infiltrating Macrophages in Diffuse Large B Cell Lymphomas

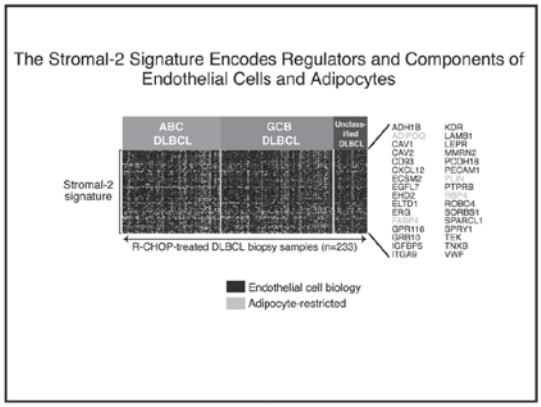


44

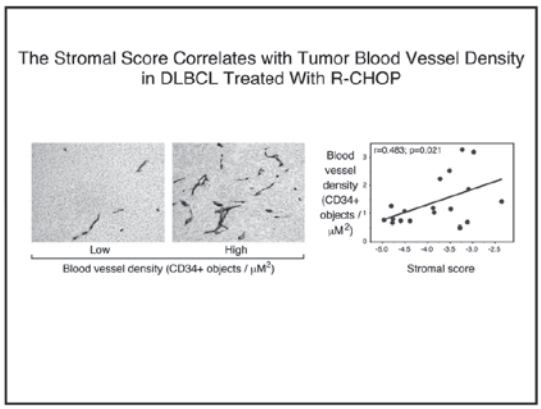
Connective Tissue Growth Factor

1. CTGF (aka CCN2) is a member of the CCN family of secreted proteins that also includes CYR61 and NOV.
2. CTGF binds heparin, fibronectin and integrins $\alpha4\beta1$ and $\alpha5\beta1$, => may bridge matrix components and cell surface receptors.
3. CTGF is pro-fibrotic and has been implicated in pathogenic fibrosis of the skin, lung and kidney.
4. CTGF is present in metastatic lesions in breast cancer and anti-CTGF antibodies block pancreatic cancer metastasis.
5. CTGF may control the production of extracellular matrix in DLBCLs with high stromal-1 signature expression.

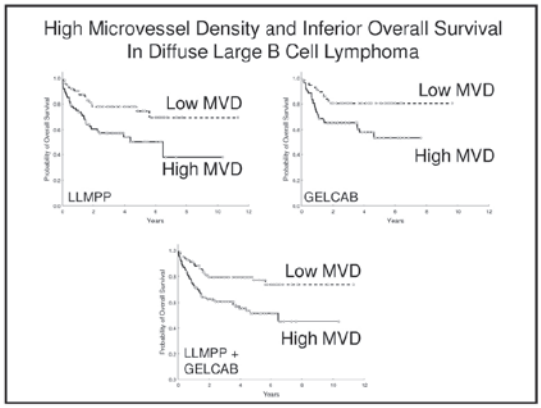
45


The Stromal-2 Signature Encodes Regulators and Components of Endothelial Cells and Adipocytes
ABC DLBCL
GCB DLBCL
Unclassified DLBCL
Stromal-2 signature
R-CHOP-treated DLBCL biopsy samples (n=233)
ADH1B
AMPDQ
CAV1
CAV2
CDH5
CXCL12
ECSM2
EGFL7
EHD2
ELTD1
ERG
FABP4
GPR116
GRB10
IGFBP5
ITGA9
KDR
LAMB1
LEPR
MMRN2
PCDH18
PECAM1
PLVAP
PTPRB
RGS5
ROBO4
SORBS1
SPARCL1
SPRY1
TEK
TNXB
VWF
Endothelial cell biology
Adipocyte-restricted

46

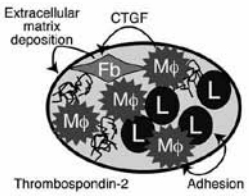

The Stromal Score Correlates with Tumor Blood Vessel Density in DLBCL Treated With R-CHOP
Low
High
Blood vessel density (CD34+ objects / μM²)
Blood vessel density (CD34+ objects / μM²)
r=0.483; p=0.021
Stromal score

47


High Microvessel Density and Inferior Overall Survival In Diffuse Large B Cell Lymphoma
Low MVD
High MVD
LLMPP
Low MVD
High MVD
GELCAB
Low MVD
High MVD
LLMPP + GELCAB

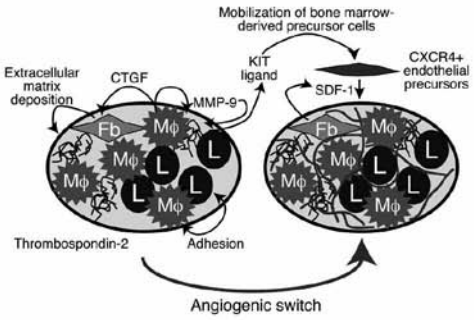
48

A Stromal Cell Dependence Model of Diffuse Large B Cell Lymphoma



49

A Stromal Cell Dependence Model of Diffuse Large B Cell Lymphoma

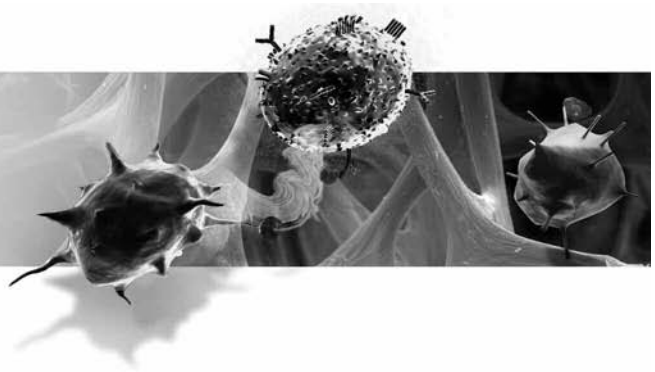


50

Survival-associated Signatures: Implications for Therapy of DLBCL

1. Clinical trials in DLBCL need to assess these signatures to enable the comparison of patient cohorts in different trials.
2. Anti-angiogenic therapy may be selectively active in cases with high expression of the stromal-2 signatures.
3. The stromal-2 signature chemokine SDF-1 is pro-angiogenic - blocking its receptor CXCR4 should be considered.
4. Targeting innate immune cells (macrophages) may eliminate trophic signals in cases with high stromal-1 expression.
5. CTGF inhibitors may block trophic signals from the fibrotic microenvironment of DLBCLs with high stromal-1 expression.

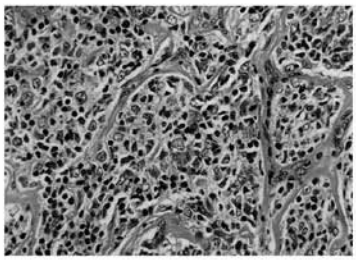
51



Genetic Aberrations in the Malignant Lymphoma Cell that May Influence the Immune Response

52

Primary Mediastinal B Cell Lymphoma

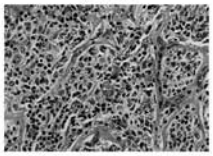


A Functional Role for PD-L1 / PD-L2?

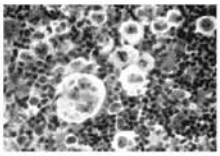
53

Molecular Similarities between PMBL and Hodgkin's Lymphoma

Primary Mediastinal B Cell Lymphoma (PMBL)

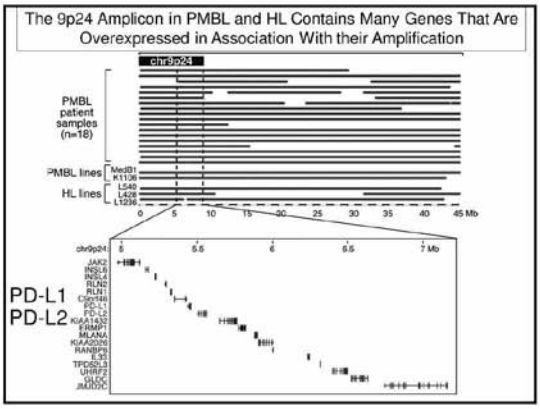


Hodgkin's Lymphoma (HL)

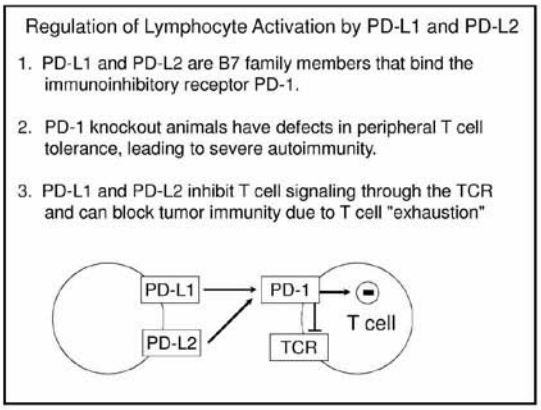


– Over one third of PMBL signature genes also expressed in HL
– Genomic locus on chromosome band 9p24 amplified in 30-50% of PMBL and HL cases

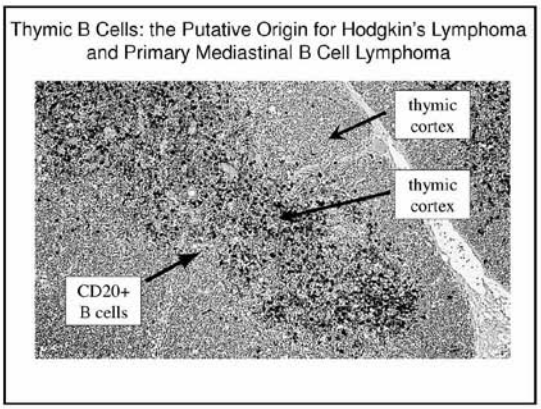
54


The 9p24 Amplicon in PMBL and HL Contains Many Genes That Are Overexpressed in Association With their Amplification
chr9p24
PMBL patient samples (n=18)
PMBL lines
HL lines
PD-L1
PD-L2

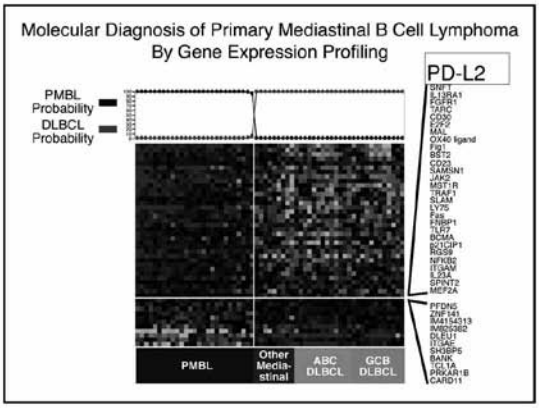
55


Regulation of Lymphocyte Activation by PD-L1 and PD-L2
1. PD-L1 and PD-L2 are B7 family members that bind the immunoinhibitory receptor PD-1.
2. PD-1 knockout animals have defects in peripheral T cell tolerance, leading to severe autoimmunity.
3. PD-L1 and PD-L2 inhibit T cell signaling through the TCR and can block tumor immunity due to T cell "exhaustion"
PD-L1
PD-L2
PD-1
TCR
T cell

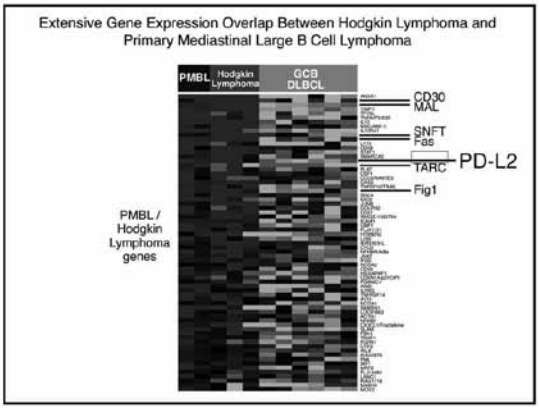
56


Thymic B Cells: the Putative Origin for Hodgkin's Lymphoma and Primary Mediastinal B Cell Lymphoma
thymic cortex
thymic cortex
CD20+ B cells

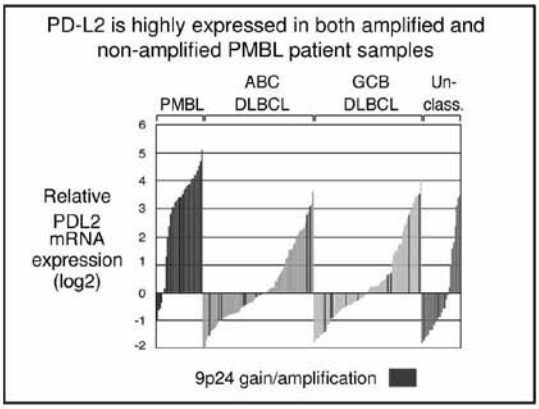
57


Molecular Diagnosis of Primary Mediastinal B Cell Lymphoma
By Gene Expression Profiling
PD-L2
PMBL Probability
DLBCL Probability
PMBL
Other Media-stinal
ABC DLBCL
GCB DLBCL

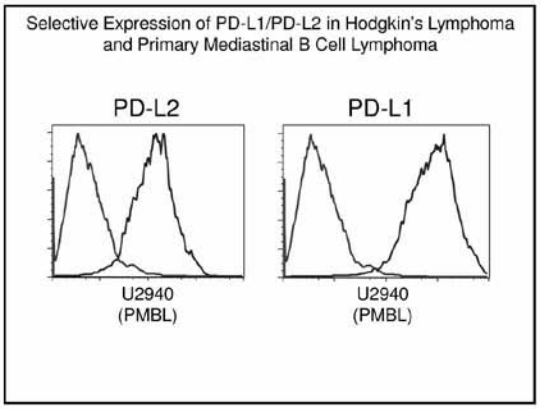
58


Extensive Gene Expression Overlap Between Hodgkin Lymphoma and
Primary Mediastinal Large B Cell Lymphoma
PMBL
Hodgkin Lymphoma
GCB DLBCL
CD30
MAL
SNFT
Fas
PD-L2
TARC
Fig1
PMBL / Hodgkin Lymphoma genes

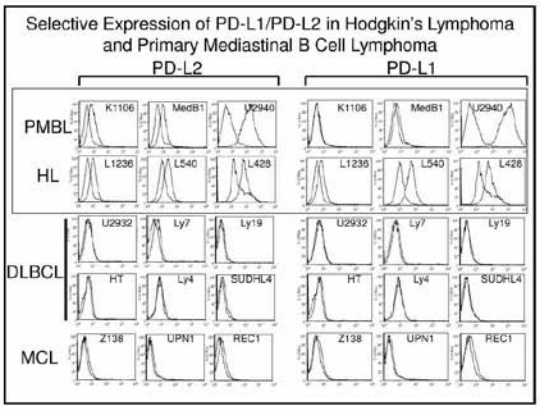
59


PD-L2 is highly expressed in both amplified and
non-amplified PMBL patient samples
PMBL
ABC DLBCL
GCB DLBCL
Un-class.
Relative PDL2 mRNA expression (log2)
6
5
4
3
2
1
0
-1
-2
9p24 gain/amplification

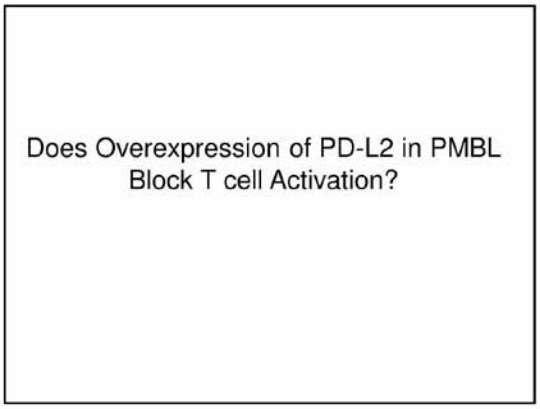
60


Selective Expression of PD-L1/PD-L2 in Hodgkin's Lymphoma and Primary Mediastinal B Cell Lymphoma
PD-L2
PD-L1
U2940 (PMBL)
U2940 (PMBL)

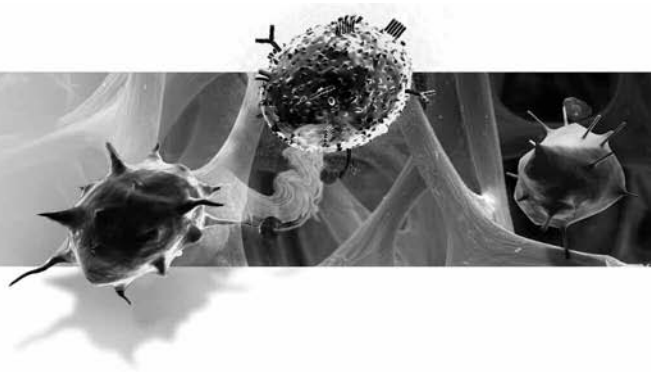
61

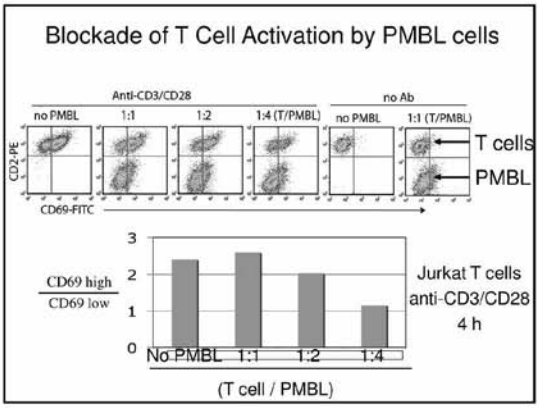

Selective Expression of PD-L1/PD-L2 in Hodgkin's Lymphoma and Primary Mediastinal B Cell Lymphoma
PD-L2
PD-L1
PMBL
HL
DLBCL
MCL
K1106
MedB1
U2940
L1236
L540
L428
U2932
Ly7
Ly19
HT
Ly4
SUDHL4
Z138
UPN1
REC1

62

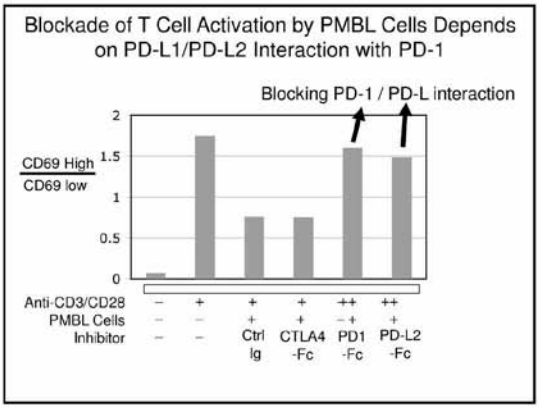

Does Overexpression of PD-L2 in PMBL Block T cell Activation?

63

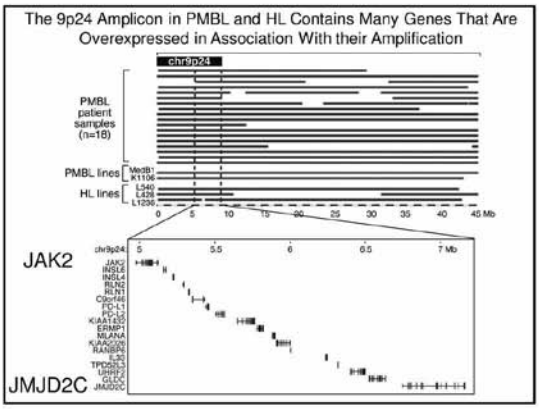



Blockade of T Cell Activation by PMBL cells
Anti-CD3/CD28
no PMBL
1:1
1:2
1:4 (T/PMBL)
no Ab
no PMBL
1:1 (T/PMBL)
CD2-PE
CD69-FITC
T cells
PMBL
CD69 high
CD69 low
Jurkat T cells
anti-CD3/CD28
4 h
No PMBL
1:1
1:2
1:4
(T cell / PMBL)

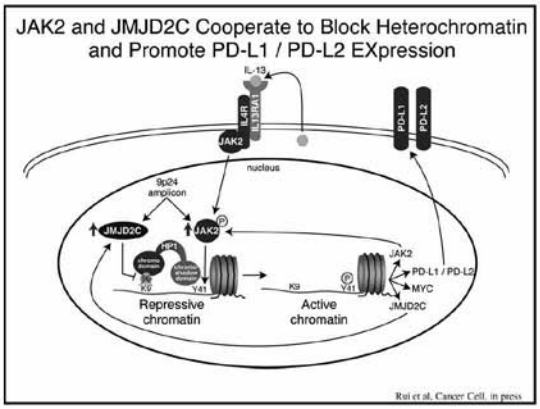
64


Blockade of T Cell Activation by PMBL Cells Depends on PD-L1/PD-L2 Interaction with PD-1
Blocking PD-1 / PD-L interaction
CD69 High
CD69 low
Anti-CD3/CD28
PMBL Cells
Inhibitor
Ctrl Ig
CTLA4 -Fc
PD1 -Fc
PD-L2 -Fc

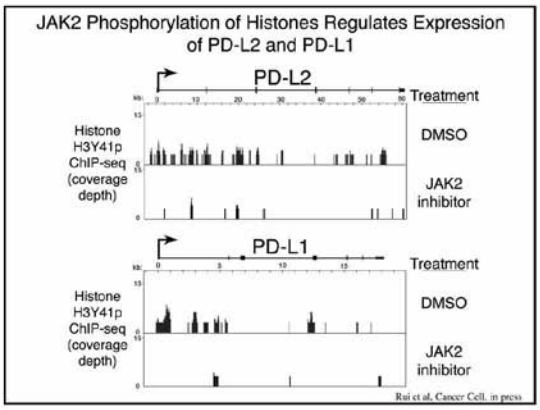
65


The 9p24 Amplicon in PMBL and HL Contains Many Genes That Are Overexpressed in Association With their Amplification
chr9p24
PMBL patient samples (n=18)
PMBL lines
HL lines
JAK2
JMJD2C

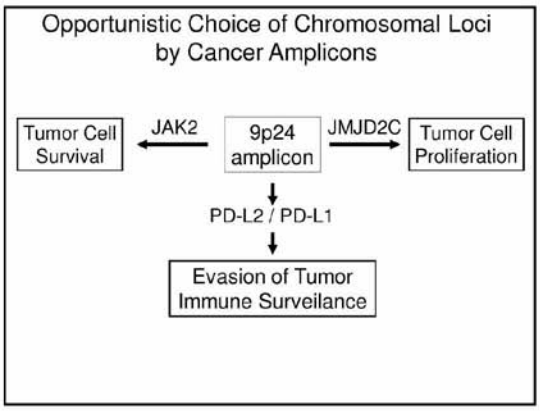
66


JAK2 and JMJD2C Cooperate to Block Heterochromatin and Promote PD-L1 / PD-L2 EXpression
IL-13
IL4R
IL13RA1
JAK2
PD-L1
PD-L2
nucleus
9p24 amplicon
JMJD2C
JAK2
HP1
K9
Y41
K9
Y41
JAK2
PD-L1 / PD-L2
MYC
JMJD2C
Repressive chromatin
Active chromatin
Rui et al, Cancer Cell, in press

67


JAK2 Phosphorylation of Histones Regulates Expression of PD-L2 and PD-L1
PD-L2
Treatment
Histone H3Y41p ChIP-seq (coverage depth)
DMSO
JAK2 inhibitor
PD-L1
Treatment
Histone H3Y41p ChIP-seq (coverage depth)
DMSO
JAK2 inhibitor
Rui et al, Cancer Cell, in press

68


Opportunistic Choice of Chromosomal Loci by Cancer Amplicons
Tumor Cell Survival
JAK2
9p24 amplicon
JMJD2C
Tumor Cell Proliferation
PD-L2 / PD-L1
Evasion of Tumor Immune Surveilance

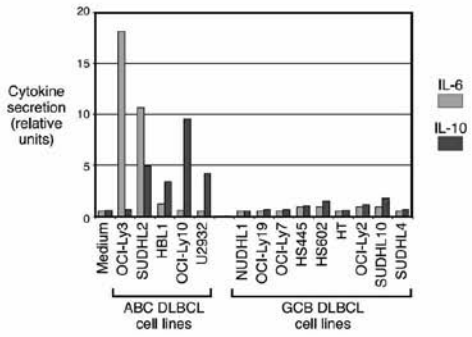
69

Oncogenic Mutations that Influence Lymphoma Cytokine Secretion

70

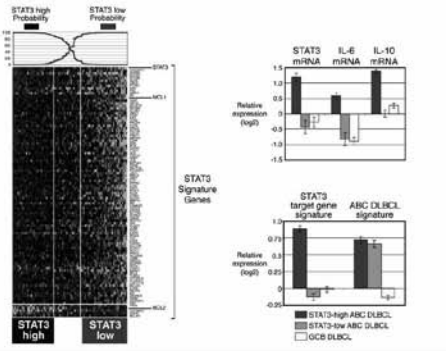
71

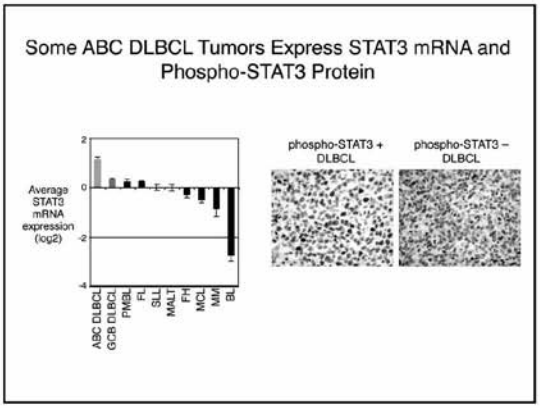
Secretion of IL-6 and IL-10 by ABC DLBCL Cell Lines



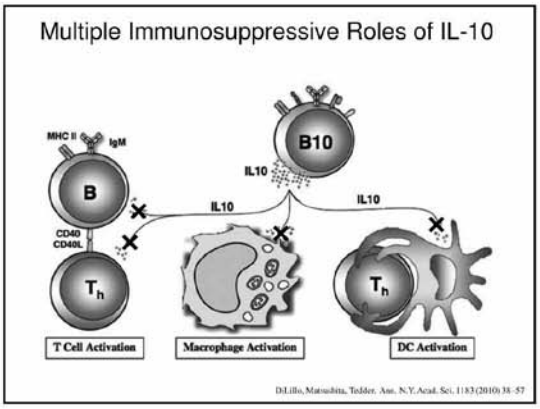
72

Definition of a STAT3-high Subgroup of ABC DLBCLs

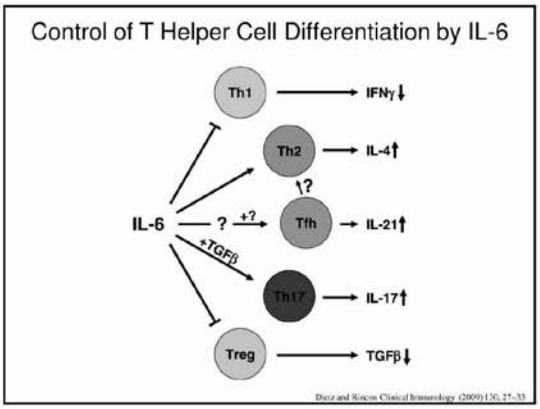



Some ABC DLBCL Tumors Express STAT3 mRNA and Phospho-STAT3 Protein
Average STAT3 mRNA expression (log2)
2
0
-2
-4
ABC DLBCL
GCB DLBCL
PMBL
FL
SLL
MALT
FH
MCL
MM
BL
phospho-STAT3 + DLBCL
phospho-STAT3 – DLBCL

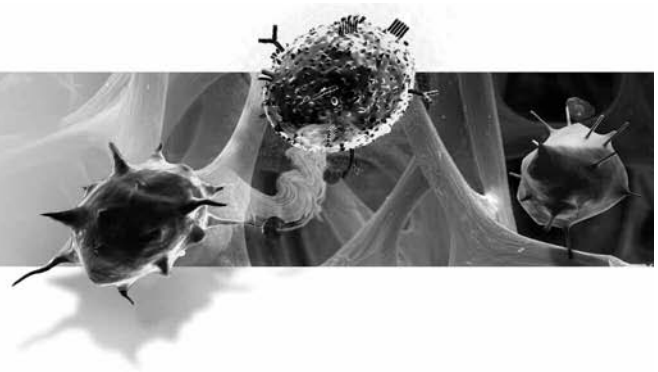
73

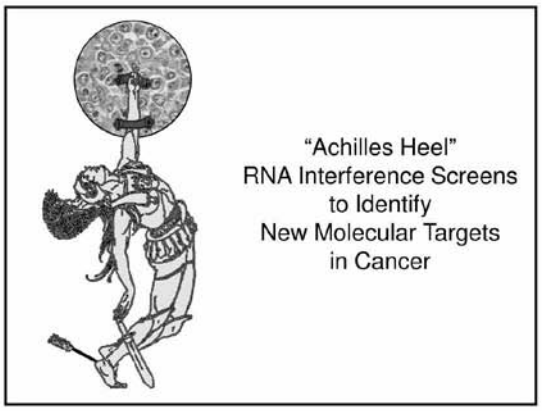

Multiple Immunosuppressive Roles of IL-10
MHC II
IgM
B
B10
IL10
IL10
IL10
CD40
CD40L
Th
Th
T Cell Activation
Macrophage Activation
DC Activation
DiLillo, Matsushita, Tedder. Ann. N.Y.Acad. Sci. 1183 (2010) 38–57

74

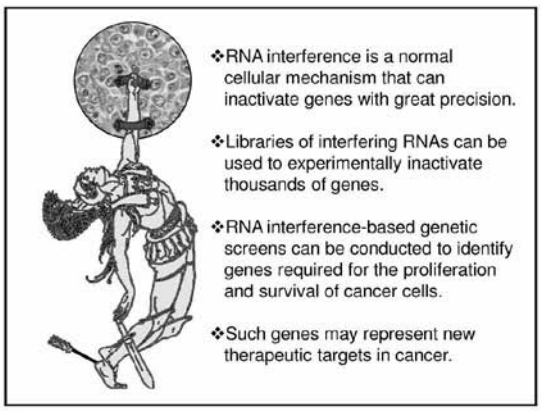

Control of T Helper Cell Differentiation by IL-6
Th1
IFNγ↓
Th2
IL-4↑
IL-6
?
+?
Tfh
IL-21↑
+TGFβ
Th17
IL-17↑
Treg
TGFβ↓
Dietz and Rincon Clinical Immunology (2009) 130, 27–33

75

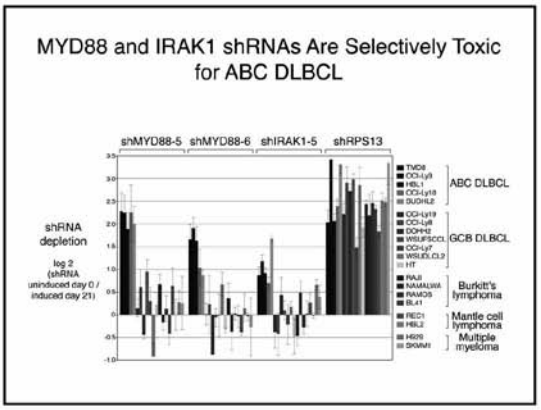



"Achilles Heel"
RNA Interference Screens
to Identify
New Molecular Targets
in Cancer

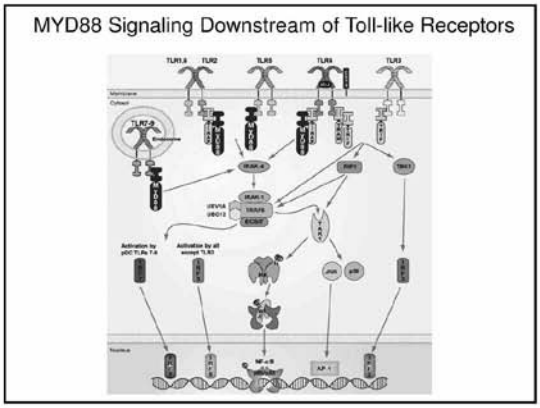
76


❖RNA interference is a normal cellular mechanism that can inactivate genes with great precision.
❖Libraries of interfering RNAs can be used to experimentally inactivate thousands of genes.
❖RNA interference-based genetic screens can be conducted to identify genes required for the proliferation and survival of cancer cells.
❖Such genes may represent new therapeutic targets in cancer.

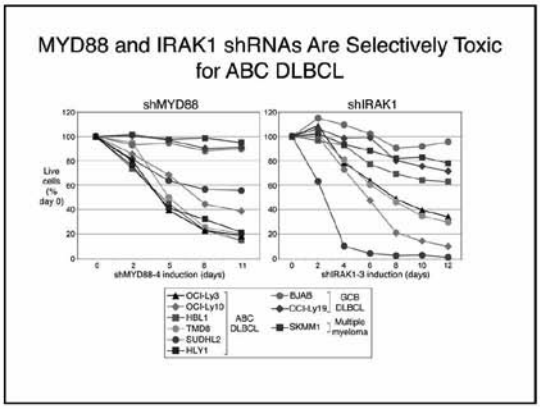
77


MYD88 and IRAK1 shRNAs Are Selectively Toxic for ABC DLBCL
shMYD88-5
shMYD88-6
shIRAK1-5
shRPS13
shRNA depletion
log 2 (shRNA uninduced day 0 / induced day 21)
ABC DLBCL
GCB DLBCL
Burkitt's lymphoma
Mantle cell lymphoma
Multiple myeloma

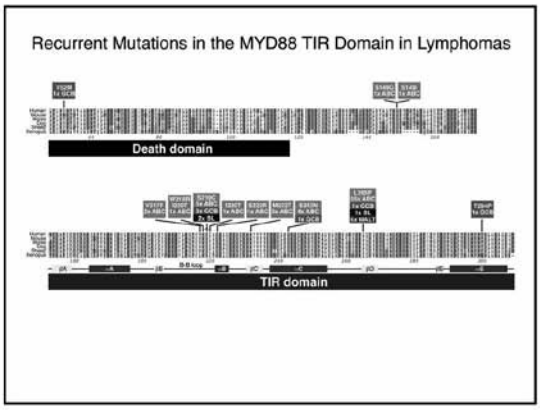
78


MYD88 Signaling Downstream of Toll-like Receptors

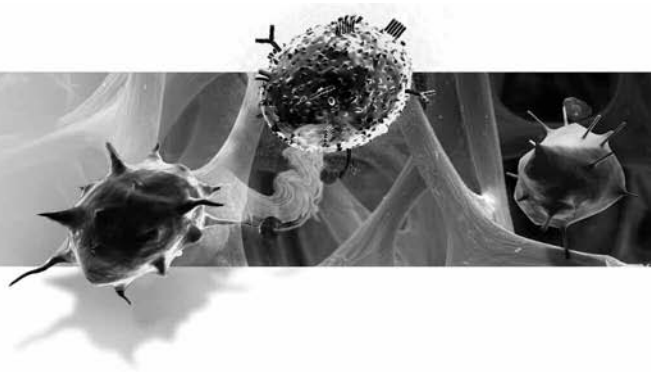
79

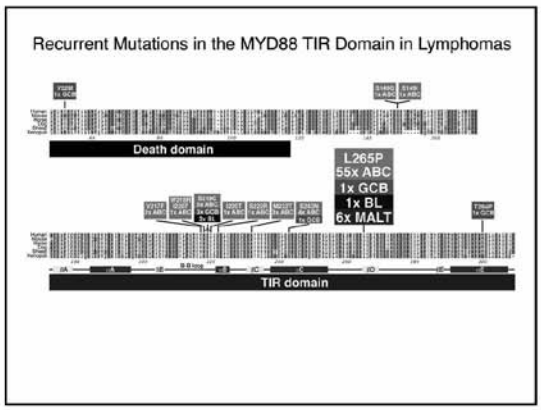

MYD88 and IRAK1 shRNAs Are Selectively Toxic for ABC DLBCL
shMYD88
shIRAK1
Live cells (% day 0)
shMYD88-4 induction (days)
shIRAK1-3 induction (days)
OCI-Ly3
OCI-Ly10
HBL1
TMD8
SUDHL2
HLY1
ABC DLBCL
BJAB
OCI-Ly19
SKMM1
GCB DLBCL
Multiple myeloma

80

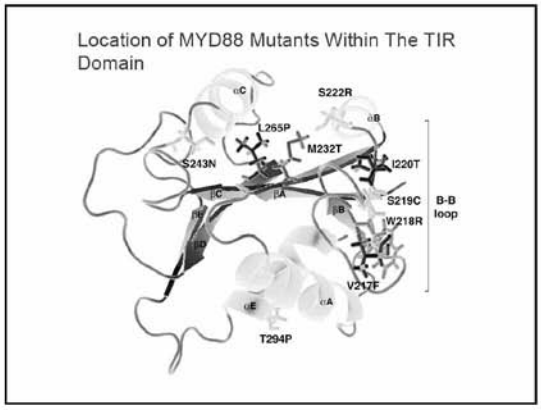

Recurrent Mutations in the MYD88 TIR Domain in Lymphomas
Death domain
TIR domain

81

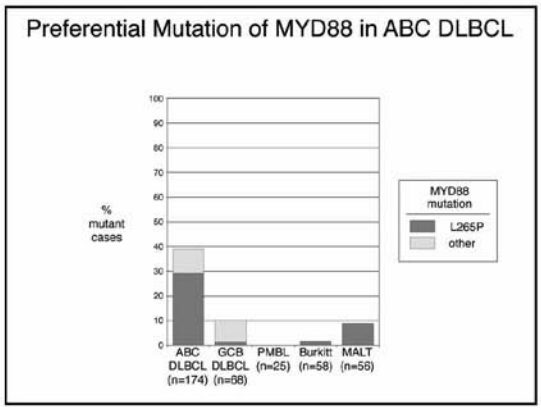



Recurrent Mutations in the MYD88 TIR Domain in Lymphomas
Death domain
L265P
55x ABC
1x GCB
1x BL
6x MALT
TIR domain

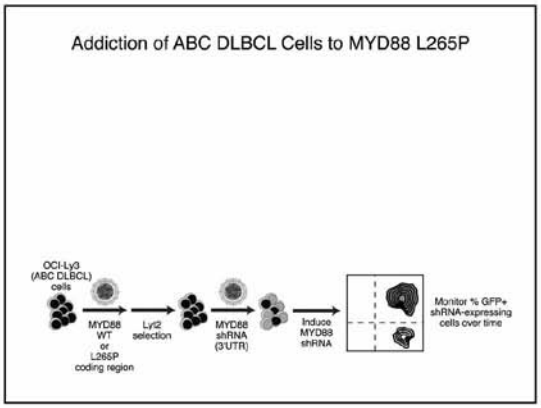
82


Location of MYD88 Mutants Within The TIR Domain
S222R
L265P
M232T
I220T
S243N
S219C
W218R
B-B loop
V217F
T294P

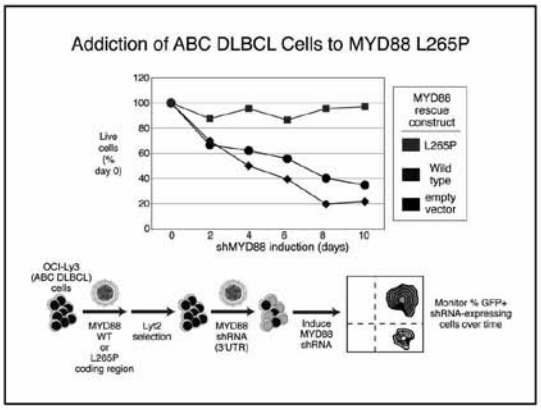
83


Preferential Mutation of MYD88 in ABC DLBCL
% mutant cases
MYD88 mutation
L265P
other
ABC DLBCL (n=174)
GCB DLBCL (n=68)
PMBL (n=25)
Burkitt (n=58)
MALT (n=56)

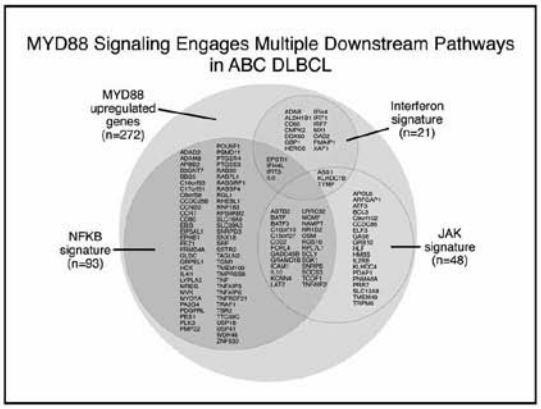
84


Addiction of ABC DLBCL Cells to MYD88 L265P
OCI-Ly3 (ABC DLBCL) cells
MYD88 WT or L265P coding region
Lyt2 selection
MYD88 shRNA (3'UTR)
Induce MYD88 shRNA
Monitor % GFP+ shRNA-expressing cells over time

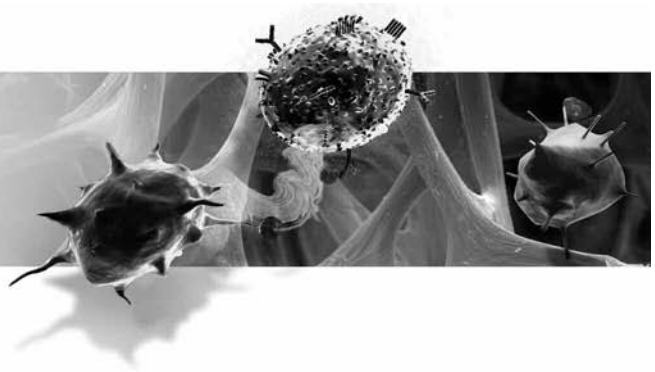
85

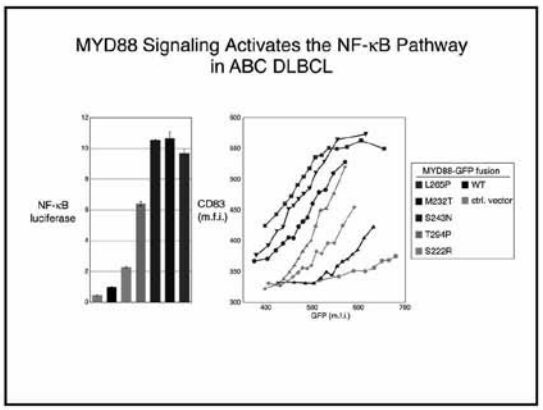

Addiction of ABC DLBCL Cells to MYD88 L265P
Live cells (% day 0)
shMYD88 induction (days)
MYD88 rescue construct
L265P
Wild type
empty vector
OCI-Ly3 (ABC DLBCL) cells
MYD88 WT or L265P coding region
Lyt2 selection
MYD88 shRNA (3'UTR)
Induce MYD88 shRNA
Monitor % GFP+ shRNA-expressing cells over time

86

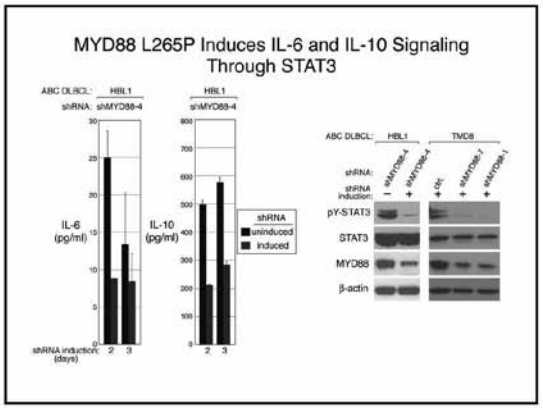

MYD88 Signaling Engages Multiple Downstream Pathways in ABC DLBCL
MYD88 upregulated genes (n=272)
Interferon signature (n=21)
NFKB signature (n=93)
JAK signature (n=48)

87

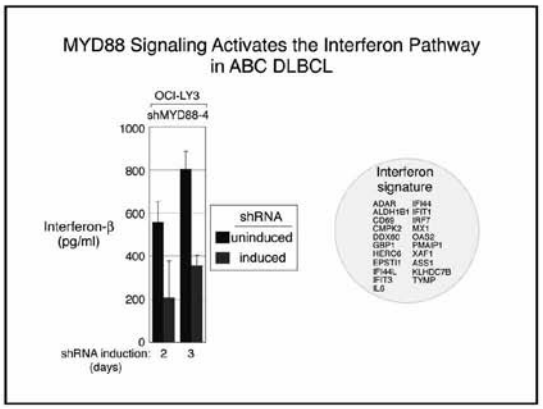



MYD88 Signaling Activates the NF-κB Pathway in ABC DLBCL
NF-κB luciferase
CD83 (m.f.i.)
GFP (m.f.i.)
MYD88-GFP fusion
L265P
WT
M232T
ctrl. vector
S243N
T264P
S222R

88

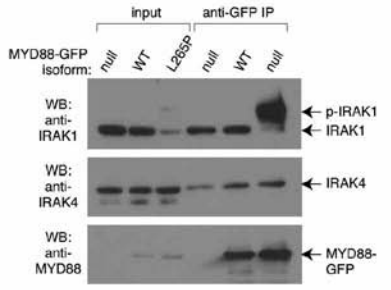

MYD88 L265P Induces IL-6 and IL-10 Signaling Through STAT3
ABC DLBCL:
HBL1
shRNA: shMYD88-4
IL-6 (pg/ml)
IL-10 (pg/ml)
shRNA induction: (days)
shRNA
uninduced
induced
TMD8
pY-STAT3
STAT3
MYD88
β-actin

89


MYD88 Signaling Activates the Interferon Pathway in ABC DLBCL
OCI-LY3
shMYD88-4
Interferon-β (pg/ml)
shRNA induction: (days)
shRNA
uninduced
induced
Interferon signature
ADAR
ALDH1B1
CD55
CMPK2
DDX60
GBP1
HERC6
EPSTI1
IFI44L
IFIT3
IL6
IFI44
IFIT1
IRF7
MX1
OAS2
PMAIP1
XAF1
AS51
KLHDC7B
TYMP

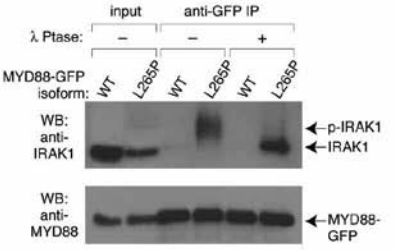
90

MYD88 L265P Associates with Phosphorylated IRAK1 and IRAK4



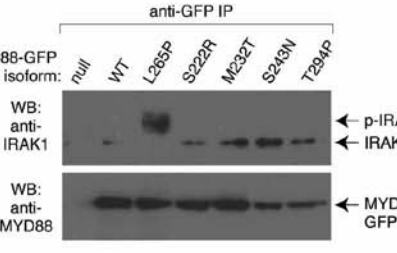
91

MYD88 L265P Associates with Phosphorylated IRAK1 and IRAK4

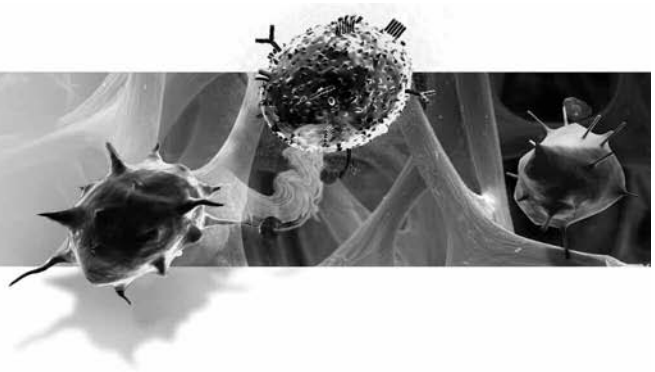


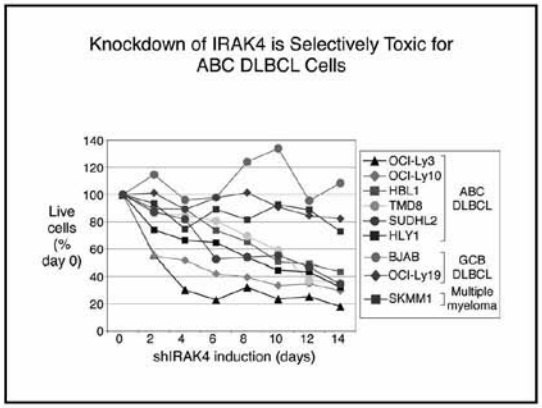
92

MYD88 L265P Associates with Phosphorylated IRAK1 and IRAK4

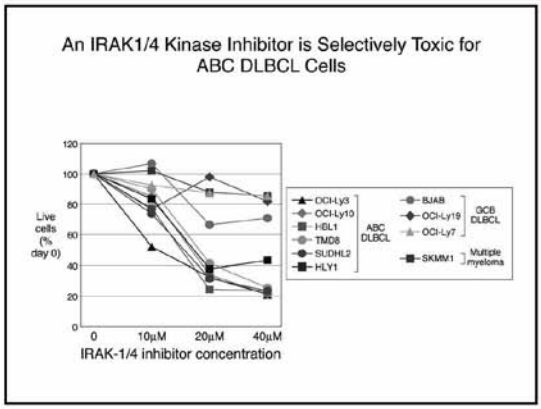


93

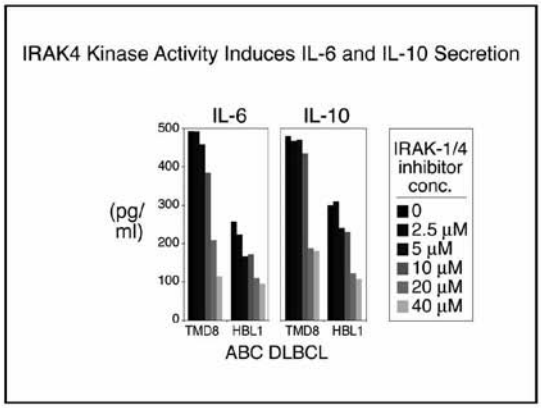



Knockdown of IRAK4 is Selectively Toxic for ABC DLBCL Cells
Live cells (% day 0)
shIRAK4 induction (days)
OCI-Ly3
OCI-Ly10
HBL1
TMD8
SUDHL2
HLY1
ABC DLBCL
BJAB
OCI-Ly19
GCB DLBCL
SKMM1
Multiple myeloma

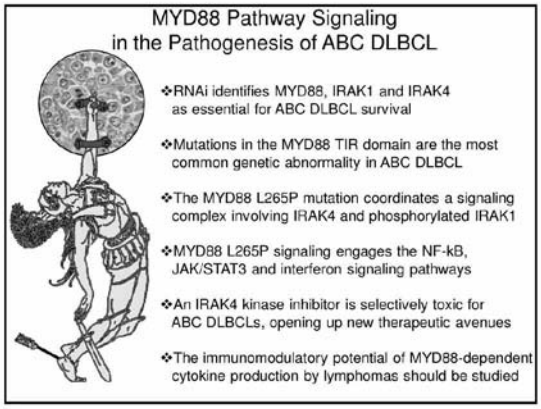
94


An IRAK1/4 Kinase Inhibitor is Selectively Toxic for ABC DLBCL Cells
Live cells (% day 0)
IRAK-1/4 inhibitor concentration
OCI-Ly3
OCI-Ly10
HBL1
TMD8
SUDHL2
HLY1
ABC DLBCL
BJAB
OCI-Ly19
OCI-Ly7
GCB DLBCL
SKMM1
Multiple myeloma

95


IRAK4 Kinase Activity Induces IL-6 and IL-10 Secretion
IL-6
IL-10
(pg/ml)
IRAK-1/4 inhibitor conc.
0
2.5 µM
5 µM
10 µM
20 µM
40 µM
TMD8
HBL1
ABC DLBCL

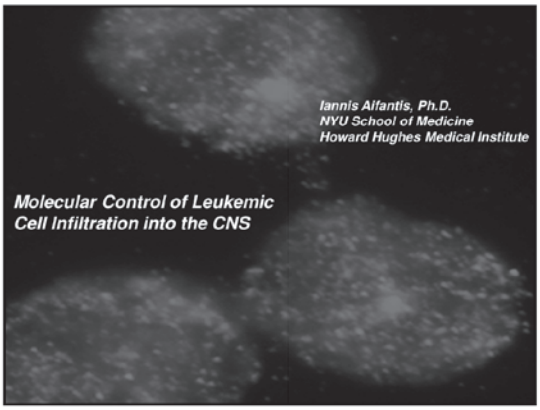
96


MYD88 Pathway Signaling
in the Pathogenesis of ABC DLBCL
❖RNAi identifies MYD88, IRAK1 and IRAK4 as essential for ABC DLBCL survival
❖Mutations in the MYD88 TIR domain are the most common genetic abnormality in ABC DLBCL
❖The MYD88 L265P mutation coordinates a signaling complex involving IRAK4 and phosphorylated IRAK1
❖MYD88 L265P signaling engages the NF-kB, JAK/STAT3 and interferon signaling pathways
❖An IRAK4 kinase inhibitor is selectively toxic for ABC DLBCLs, opening up new therapeutic avenues
❖The immunomodulatory potential of MYD88-dependent cytokine production by lymphomas should be studied

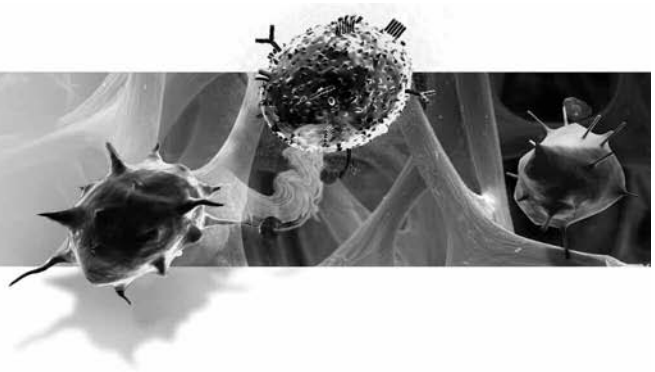
97


Acknowledgements
Christian Steidl
Project

98


Iannis Aifantis, Ph.D.
NYU School of Medicine
Howard Hughes Medical Institute
Molecular Control of Leukemic
Cell Infiltration into the CNS

99



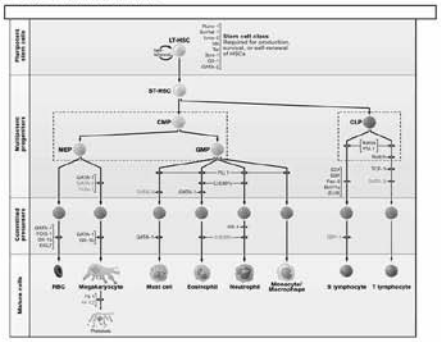
Disclosure of Conflicts of Interest

Iannis Aifantis, PhD

Dr. Iannis Aifantis has no affiliations with commercial interests to disclose.

100

A simplified view of hematopoiesis.....



101

...however, Notch *gain-of-function* leads to T cell acute lymphoblastic leukemia

Accumulation of blasts in the bone Marrow and peripheral blood.

Massive expansion of lymph nodes and spleen

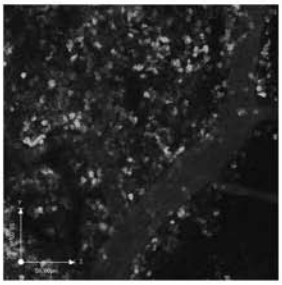
Infiltration of central nervous system

T-ALL specifically afflicts children.

Notch1 is mutated in >50% of patients

Notch pathway components (Fbw7) Are also mutated

Notch pathway activation is the main Oncogenic trigger in T-ALL



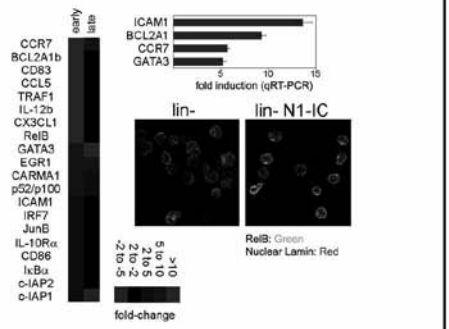
Viiimas et al. Nature Medicine 2007; Buonamici et al. Nature 2009; Espinosa et al. Cancer Cell 2010

102

(part 1): Why is Notch a blood oncogene?

103

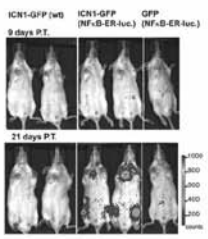
Notch1 activation targets the NF-kB pathway



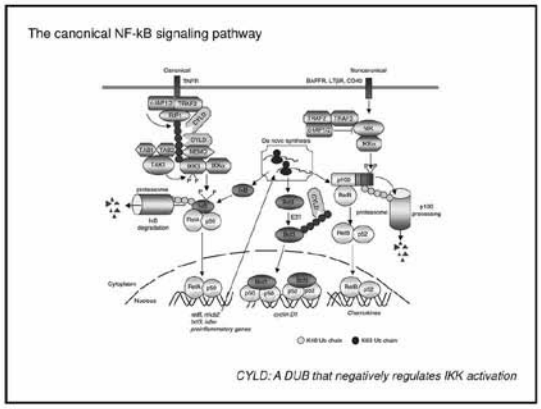
Vilimas et al. Nature Medicine 2007

104

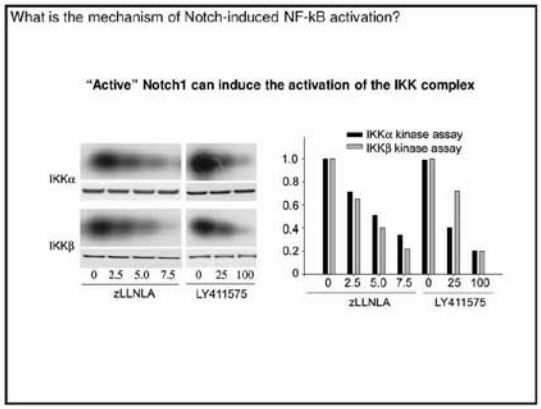
Rapid *in vivo* induction of the NF-kB pathway in an animal model of T-ALL



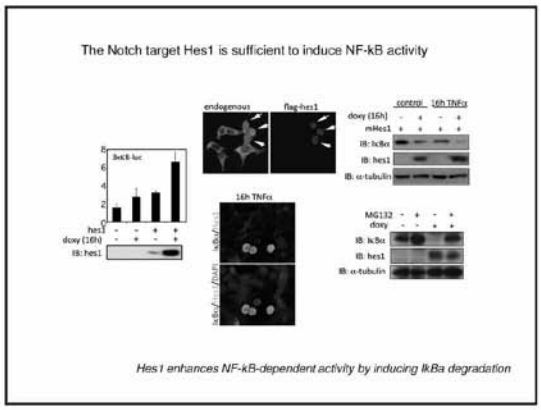
105


The canonical NF-kB signaling pathway
CYLD: A DUB that negatively regulates IKK activation

106

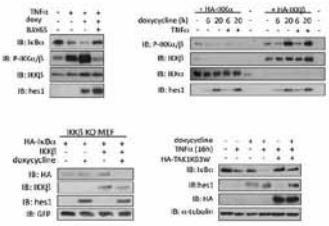

What is the mechanism of Notch-induced NF-kB activation?
"Active" Notch1 can induce the activation of the IKK complex
IKKα
IKKβ
0 2.5 5.0 7.5 0 25 100
zLLNLA
LY411575
IKKα kinase assay
IKKβ kinase assay
1.0
0.8
0.6
0.4
0.2
0

107


The Notch target Hes1 is sufficient to induce NF-kB activity
endogenous
flag-hes1
control
16h TNFα
doxy (16h)
mHes1
IB: IκBα
IB: hes1
IB: α-tubulin
3xκB-luc
16h TNFα
MG132
doxy
IB: IκBα
IB: hes1
IB: α-tubulin
Hes1 enhances NF-kB-dependent activity by inducing IkBa degradation

108

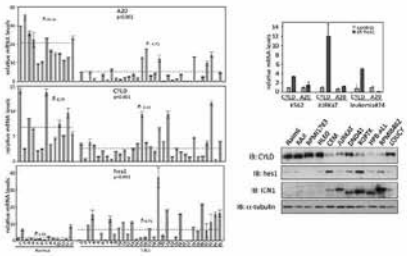
Hes1 facilitates IκBa degradation by regulating directly IKKb activity



Hes1 functions upstream of TAK1

109

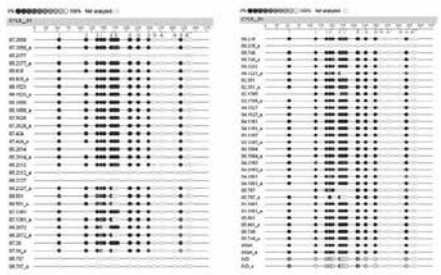
The expression of CYLD, a negative regulator of IKK function, is suppressed in T-ALL



Espinosa-Cathelin et al. Cancer Cell 2010

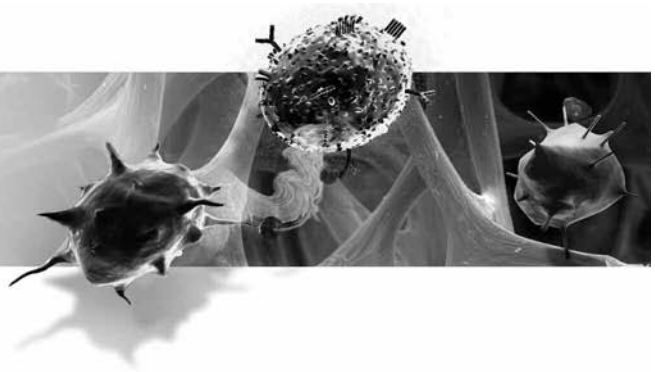
110

Promoter methylation is not the mechanism of CYLD expression suppression

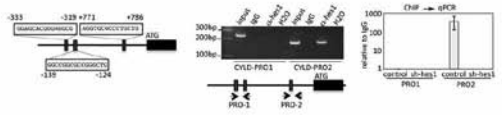


CYLD and A20 exon sequencing revealed no mutations in T-ALL

111

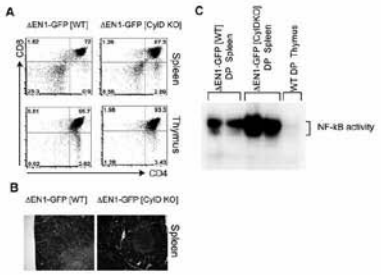


Notch signaling suppresses CYLD, though direct binding of Hes1



112

CYLD deletion enhances NF-kB activity in a mouse model of T-ALL



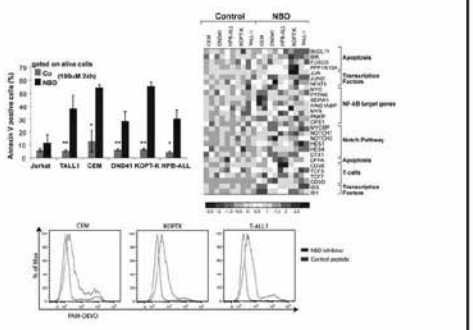
113

Is IKK/NF-kB targeting a putative T-ALL therapy?

.....(Is IKK signaling essential for T-ALL maintenance?)

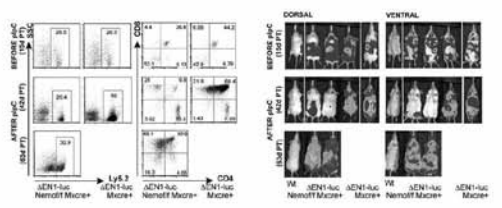
114

IKK/NEMO activity silencing is able to induce rapid T-ALL cell line death



115

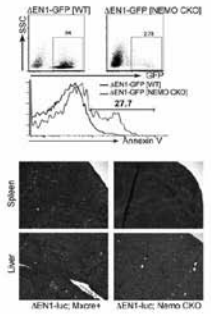
IKK/NEMO signaling is essential for the maintenance of T-ALL



Espinosa-Cathelin et al. Cancer Cell 2010

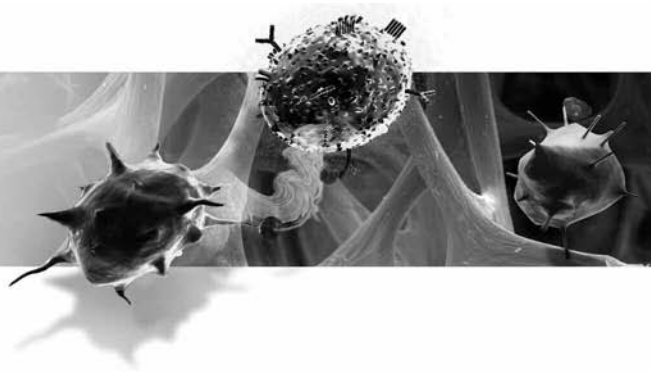
116

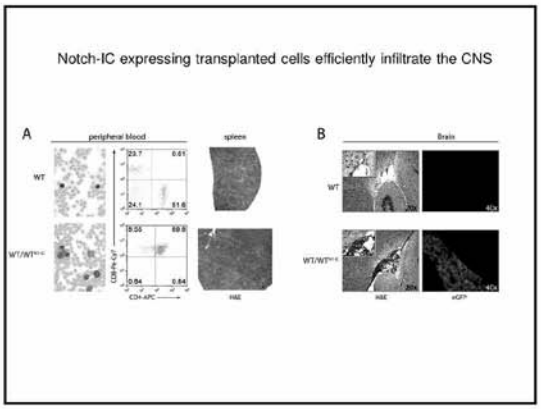
NEMO/IKK silencing is inducing rapid T-ALL cell apoptosis *in vivo*



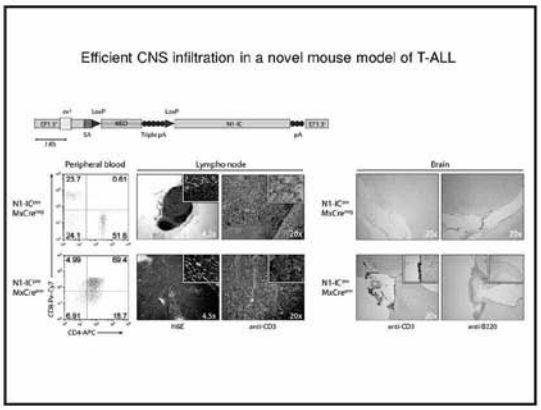
Can we use inhibition of IKK signaling as a T-ALL therapeutic?

117

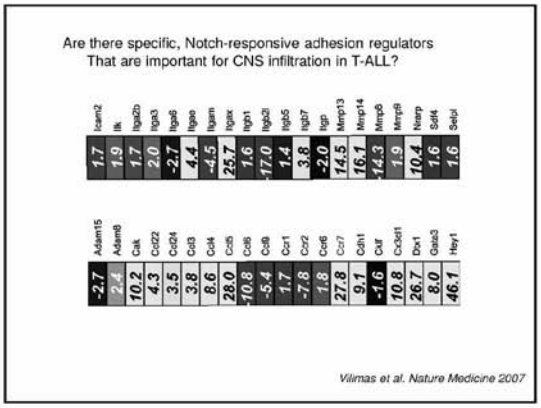



Notch-IC expressing transplanted cells efficiently infiltrate the CNS
A
peripheral blood
spleen
WT
WT/WT^N1-IC
23.7
0.61
24.1
51.6
9.55
89.8
0.84
0.84
CD8-FN-Cy7
CD4-APC
H&E
B
Brain
WT
WT/WT^N1-IC
40x
H&E
eGFP

118


Efficient CNS infiltration in a novel mouse model of T-ALL
Peripheral blood
Lympho node
Brain
N1-IC^ires MxCre^wt
N1-IC^ires MxCre^tet
23.7
0.61
24.1
51.6
4.99
69.4
6.91
18.7
CD8-FN-Cy7
CD4-APC
H&E
anti-CD3
anti-B220
4.5x
20x

119

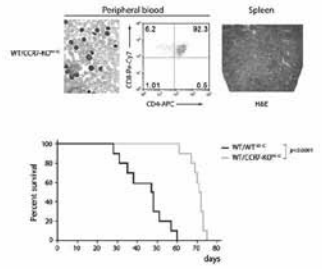

Are there specific, Notch-responsive adhesion regulators
That are important for CNS infiltration in T-ALL?
Icam2 1.7
Ik 1.9
Itga2b 1.7
Itga3 2.0
Itga5 -2.7
Itgae 4.4
Itgam -4.5
Itgax 25.7
Itgb1 1.6
Itgb2l -17.0
Itgb5 1.4
Itgb7 3.8
Itgp -2.0
Mmp13 14.5
Mmp14 16.1
Mmp8 -14.3
Mmp9 1.9
Ntsrp 10.4
Sdc4 1.6
Sdcbp 1.6
Adam15 -2.7
Adam9 2.4
Cak 10.2
Cd22 4.3
Cd24 3.5
Cd3 3.8
Cd4 8.6
Cd5 28.0
Cd8 -10.8
Cd9 -5.4
Ccr1 1.7
Ccr2 -7.8
Ccr6 1.8
Ccr7 27.8
Cdh1 9.1
Cer -1.6
Cldn1 10.8
Dlx1 26.7
Gata3 8.0
Hey1 46.1
Vilimas et al. Nature Medicine 2007

120

What will happen if we switch-off CCR7 expression in leukemic cells?

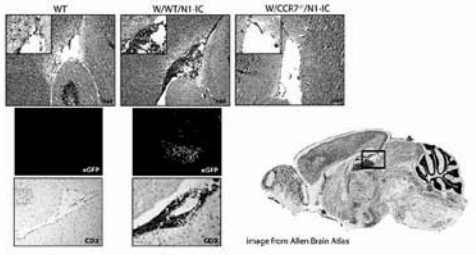
121

CCR7 deletion slows down leukemia progression



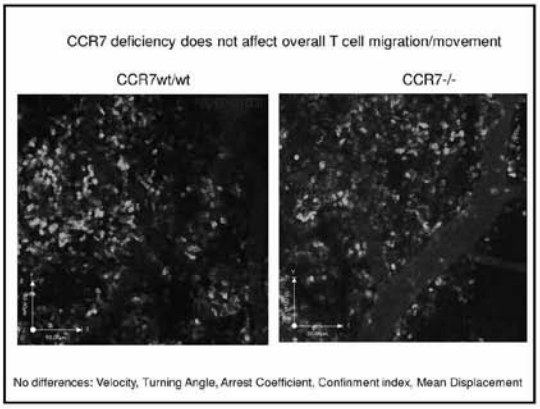
122

Deletion of CCR7 suppresses T-ALL CNS infiltration

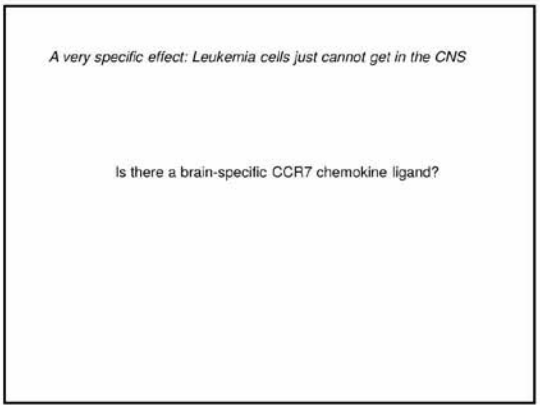


Buonamici et al. Nature, 2009

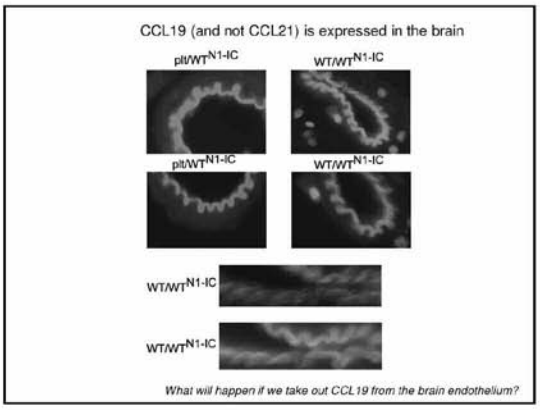
123


CCR7 deficiency does not affect overall T cell migration/movement
CCR7wt/wt
CCR7-/-
No differences: Velocity, Turning Angle, Arrest Coefficient, Confinment index, Mean Displacement

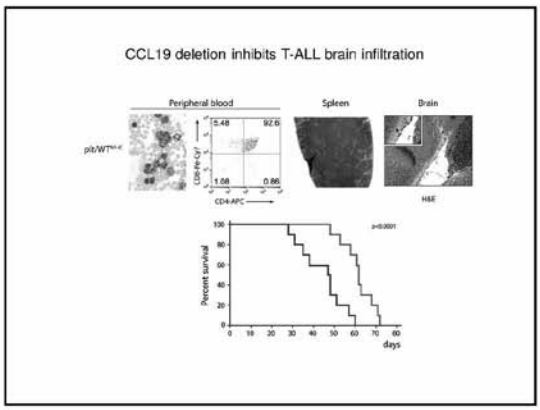
124


A very specific effect: Leukemia cells just cannot get in the CNS
Is there a brain-specific CCR7 chemokine ligand?

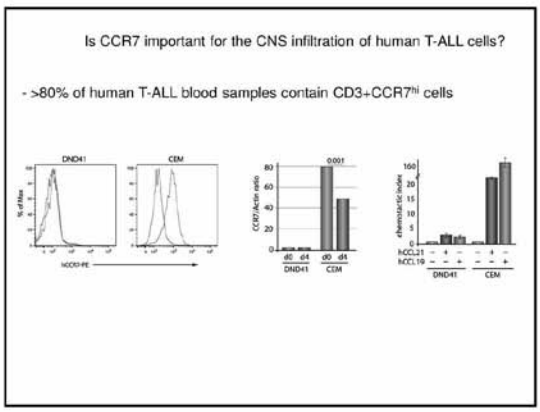
125


CCL19 (and not CCL21) is expressed in the brain
plt/WTN1-IC
WT/WTN1-IC
plt/WTN1-IC
WT/WTN1-IC
WT/WTN1-IC
WT/WTN1-IC
What will happen if we take out CCL19 from the brain endothelium?

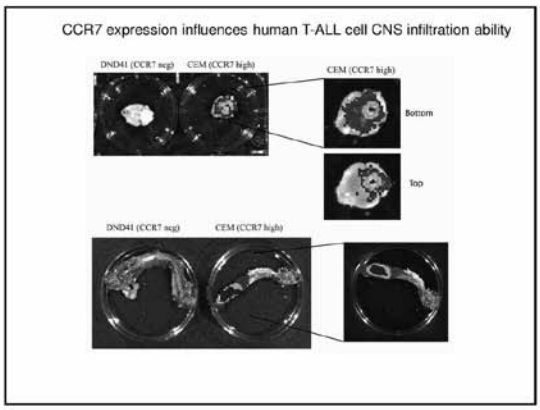
126


CCL19 deletion inhibits T-ALL brain infiltration
Peripheral blood
Spleen
Brain
5.48
92.6
1.08
0.86
CD8-Pe Cy7
CD4-APC
H&E
Percent survival
days

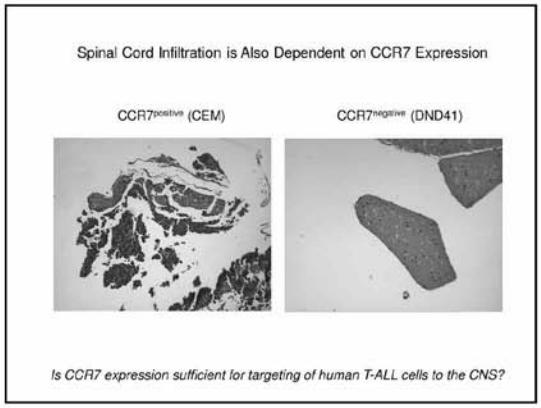
127


Is CCR7 important for the CNS infiltration of human T-ALL cells?
- >80% of human T-ALL blood samples contain CD3+CCR7hi cells
DND41
CEM
% of Max
hCCR7-PE
CCR7/Actin ratio
0.001
d0 d4
d0 d4
DND41
CEM
chemotactic index
hCCL21
hCCL19
DND41
CEM

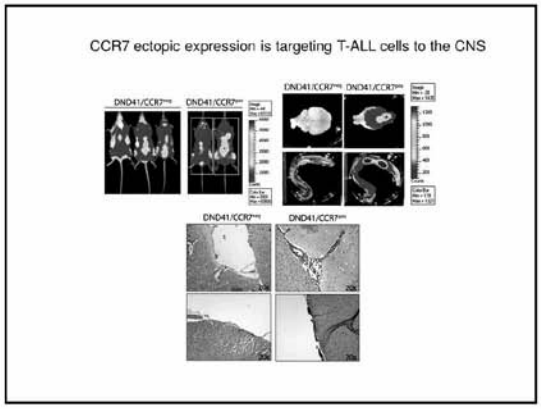
128


CCR7 expression influences human T-ALL cell CNS infiltration ability
DND41 (CCR7 neg)
CEM (CCR7 high)
CEM (CCR7 high)
Bottom
Top
DND41 (CCR7 neg)
CEM (CCR7 high)

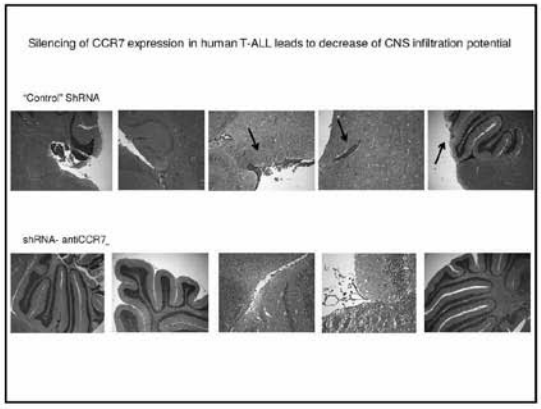
129


Spinal Cord Infiltration is Also Dependent on CCR7 Expression
CCR7positive (CEM)
CCR7negative (DND41)
Is CCR7 expression sufficient for targeting of human T-ALL cells to the CNS?

130


CCR7 ectopic expression is targeting T-ALL cells to the CNS

131


Silencing of CCR7 expression in human T-ALL leads to decrease of CNS infiltration potential
"Control" ShRNA
shRNA- antiCCR7_

132

Down to a gene: Specific Notch targets are important for disease progression:

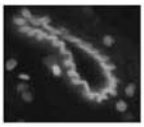
CCR7 is a Notch target essential for CNS infiltration

The NF-kB signaling is essential for T-ALL survival (and migration)

CCR7/CCL19 interactions could be an attractive therapeutic target

Integrins activated by CCR7/CCL19 could be also attractive targets

The IKK complex is another attractive drug target



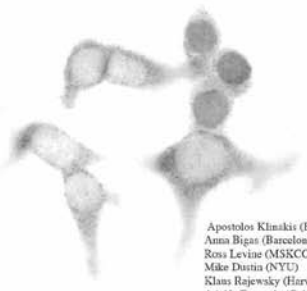
Buonamici et al. Nature, 2009; Espinosa et al. Cancer CELL, 2010

133

http://www.aifantislab.com

Severine Cathelin
Silvia Buonamici
Jelena Nedjic

Philmo Oh
Camille Lobry
Thomas Trimarchi
Linsey Reavie
Shannon Buckley
Bryan King
Jie Gao
Kelly Moran
Camille Lobry
Beatriz Aranda
Katie Sawai
Anna Vanilyeva
Panos Ntziachristos
Alex Strikoudis
Jasper Mullenders
Delphine Lobry

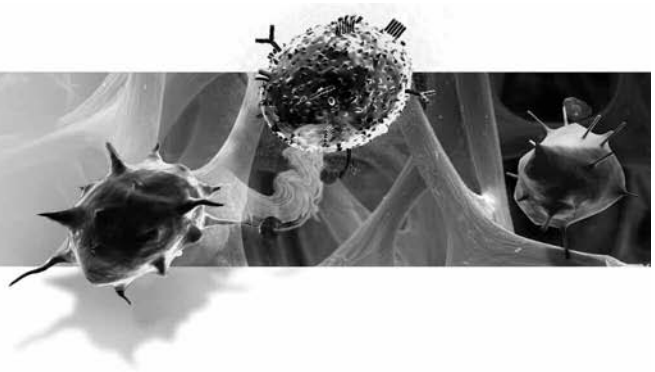


Apostolos Klinakis (Bioacademy Athens)
Anna Bigas (Barcelona)
Ross Levine (MSKCC)
Mike Dustin (NYU)
Klaus Rajewsky (Harvard)
Adolfo Ferrando (Columbia)
Charles Mullighan (St. Jude's)

134

Thank You

135



Clinical and Translational Studies of Stroma-Leukemia Interactions

Michael P. Rettig, PhD
Research Assistant Professor of Medicine
Division of Oncology
Washington University School of Medicine

Presenting for:

John F. DiPersio MD, PhD
Division of Oncology
Siteman Cancer Center
Washington University School of Medicine

136

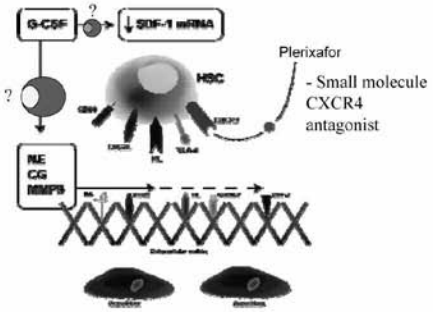
Disclosure of Conflicts of Interest

Dr. Michael P. Rettig has an affiliation with Genzyme (Honoraria).

Dr. John F. DiPersio has an affiliation with Genzyme (Honoraria).

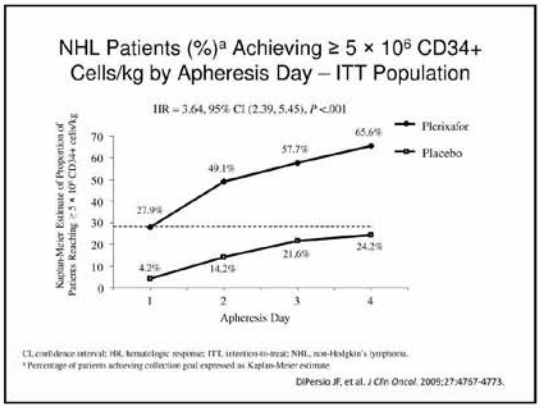
137

Stem Cell Mobilization: AMD3100 + G-CSF

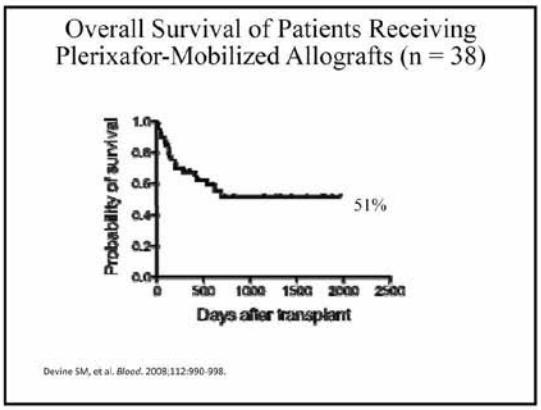


Pusic I, DiPersio JF. *Curr Pharm Des.* 2008;14:1950-1961.

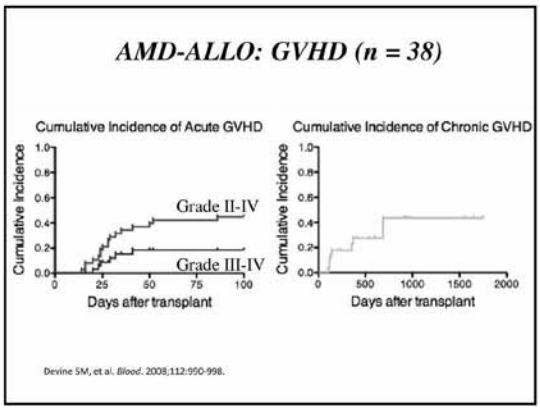
138


NHL Patients (%)[a] Achieving ≥ 5 × 10⁶ CD34+ Cells/kg by Apheresis Day – ITT Population
HR = 3.64, 95% CI (2.39, 5.45), P <.001
Plerixafor
Placebo
Kaplan-Meier Estimate of Proportion of Patients Reaching ≥ 5 × 10⁶ CD34+ cells/kg
27.9%
49.1%
57.7%
65.6%
4.2%
14.2%
21.6%
24.2%
Apheresis Day
CI, confidence interval; HR, hematologic response; ITT, intention-to-treat; NHL, non-Hodgkin's lymphoma.
[a] Percentage of patients achieving collection goal expressed as Kaplan-Meier estimate
DiPersio JF, et al. J Clin Oncol. 2009;27:4767-4773.

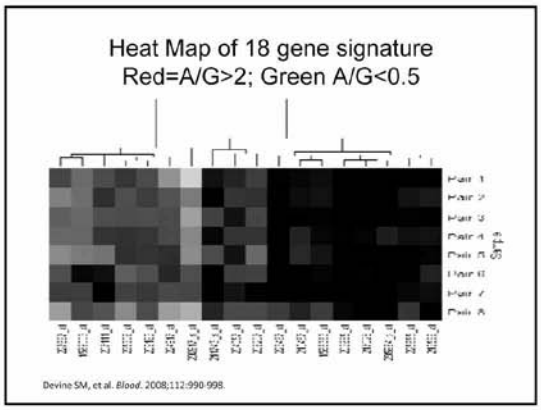
139


Overall Survival of Patients Receiving Plerixafor-Mobilized Allografts (n = 38)
Probability of survival
51%
Days after transplant
Devine SM, et al. Blood. 2008;112:990-998.

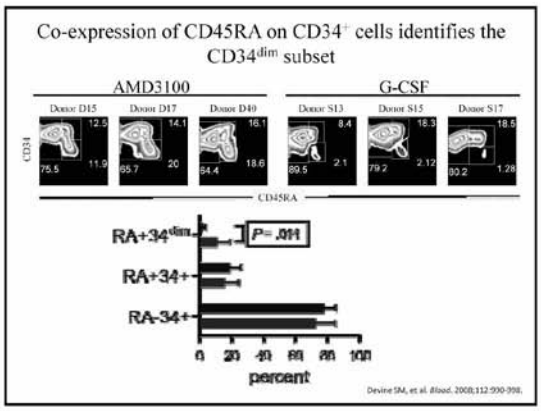
140


AMD-ALLO: GVHD (n = 38)
Cumulative Incidence of Acute GVHD
Cumulative Incidence of Chronic GVHD
Cumulative Incidence
Grade II-IV
Grade III-IV
Days after transplant
Devine SM, et al. Blood. 2008;112:990-998.

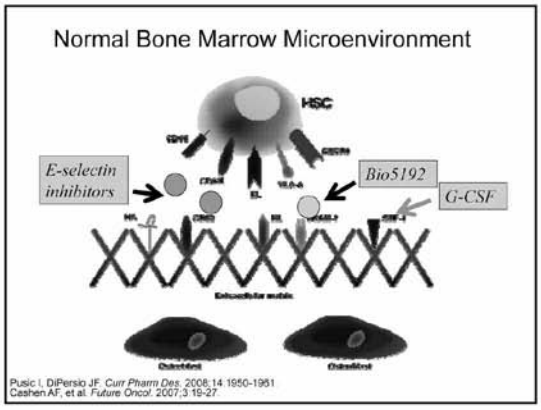
141


Heat Map of 18 gene signature
Red=A/G>2; Green A/G<0.5
Devine SM, et al. Blood. 2008;112:990-998.

142

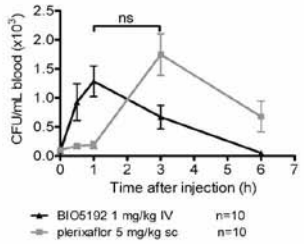

Co-expression of CD45RA on CD34+ cells identifies the CD34dim subset
AMD3100
G-CSF
Donor D15
Donor D17
Donor D40
Donor S13
Donor S15
Donor S17
CD34
CD45RA
12.5
75.5
11.9
14.1
65.7
20
16.1
64.4
18.6
8.4
89.5
2.1
18.3
79.2
2.12
18.5
80.2
1.28
RA+34dim
P= .011
RA+34+
RA-34+
0
20
40
60
80
100
percent
Devine SM, et al. Blood. 2008;112:990-998.

143


Normal Bone Marrow Microenvironment
HSC
E-selectin inhibitors
Bio5192
G-CSF
Pusic I, DiPersio JF. Curr Pharm Des. 2008;14:1950-1951
Cashen AF, et al. Future Oncol. 2007;3:19-27

144

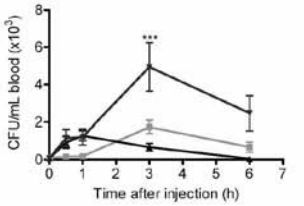
Kinetics of Murine Progenitor Mobilization in Response to BIO5192 and Plerixafor



Ramirez F, et al. Blood. 2009;114:1340-1343.

145

Additive Mobilization of Murine Progenitors After Combination of Plerixaflor SC and BIO5192 IV



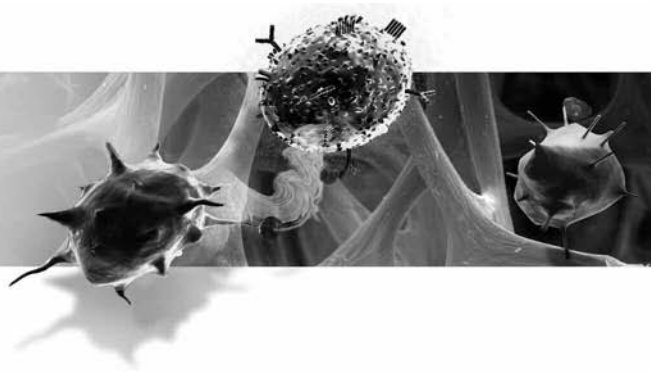
Ramirez P, et al. Blood. 2009;114:1340-1343.

146

Hypothesis of chemosensitization

- The interaction of leukemia cells with the BM stroma may provide a survival benefit to leukemia cells
- The interruption of this interaction may enhance the sensitivity to genotoxic stress such as chemotherapy or radiation therapy:
  - Others have shown modest benefit using G-CSF or GM-CSF to enhance the sensitivity of leukemia cells to chemotherapy

147



BLOOD, 1 SEPTEMBER 2003 • VOLUME 102, NUMBER 5

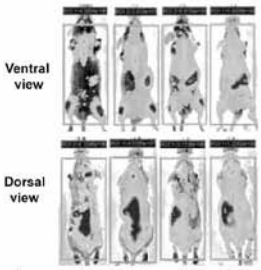
High-penetrance mouse model of acute promyelocytic leukemia with very low levels of PML-RARα expression

Peter Westervelt, Andrew A. Lane, Jessica L. Pollock, Kristie Oldfather, Matthew S. Holt, Drazen B. Zimonje, Nicholas C. Popescu, John F. DiPersio, and Timothy J. Ley

- To develop a KI mouse for APL, the human PML-RARα transgene was targeted to a single allele of the murine cathepsin G locus in ES cells.
- 90-100% penetrance.
- Death from leukemia at 150-400 days.
- Adoptive transfer of APL splenocytes into genetically compatible mice results in a rapidly fatal leukemia.

148

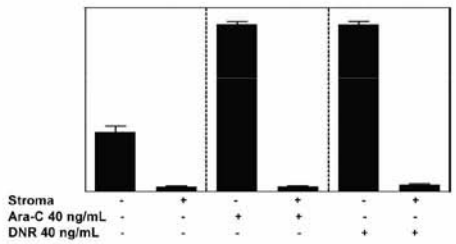
APL ENGRAFTMENT



	%blasts	%blasts	%blasts	%blasts
Blood	0%	2%	10%	40-50%
Spleen	1-2%	10%	40%	80-90%
BM	1-2%	20%	40%	90%
WBC/ul	3,000	8,000	15,000	75,000
Spleen	50 mg	150 mg	750 mg	1000 mg

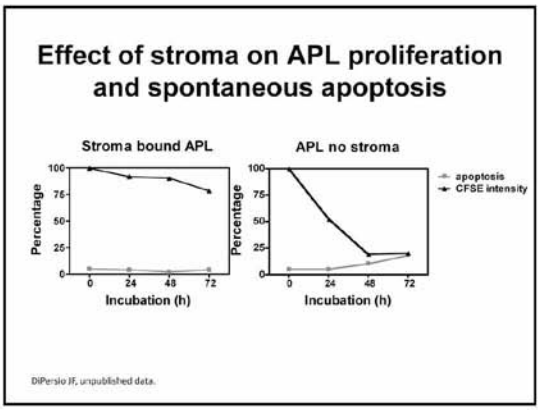
149

Effect of direct contact between APL and stromal cells on APL viability

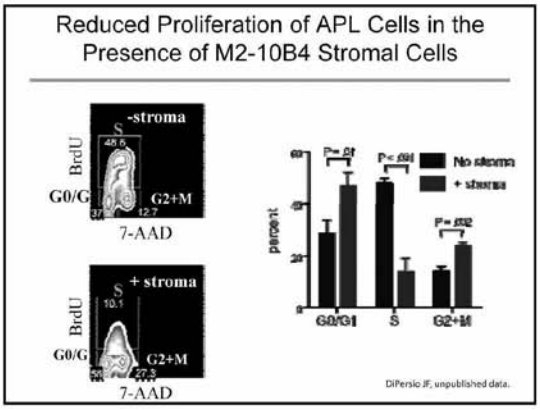


DiPersio JF, unpublished data.

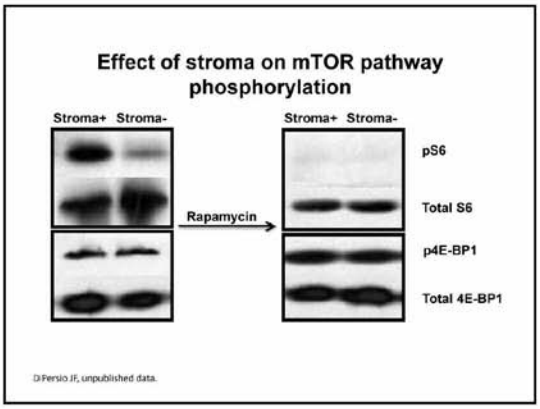
150


Effect of stroma on APL proliferation and spontaneous apoptosis
Stroma bound APL
APL no stroma
Percentage
Incubation (h)
apoptosis
CFSE intensity
DiPersio JF, unpublished data.

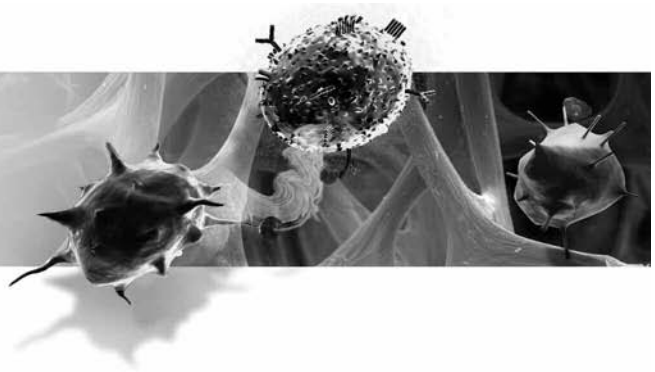
151

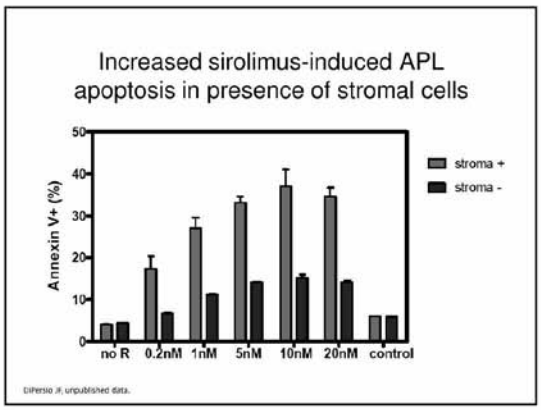

Reduced Proliferation of APL Cells in the Presence of M2-10B4 Stromal Cells
-stroma
+ stroma
BrdU
7-AAD
G0/G1
S
G2+M
No stroma
+ stroma
percent
DiPersio JF, unpublished data.

152

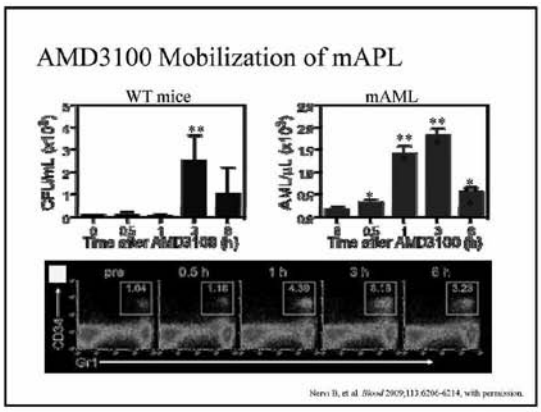

Effect of stroma on mTOR pathway phosphorylation
Stroma+ Stroma-
Rapamycin
pS6
Total S6
p4E-BP1
Total 4E-BP1
DiPersio JF, unpublished data.

153

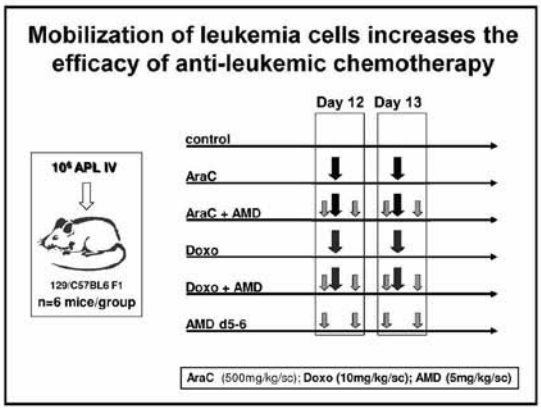



Increased sirolimus-induced APL apoptosis in presence of stromal cells
Annexin V+ (%)
50
40
30
20
10
0
stroma +
stroma -
no R
0.2nM
1nM
5nM
10nM
20nM
control
Diersio JF, unpublished data.

154

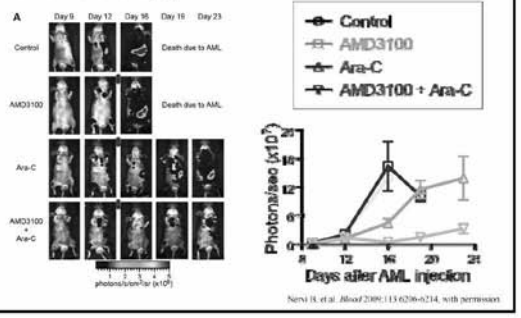

AMD3100 Mobilization of mAPL
WT mice
mAML
CFU/mL (x10⁶)
AML/µL (x10⁶)
Time after AMD3100 (h)
Time after AMD3100 (h)
pre
0.5 h
1 h
3 h
6 h
1.64
1.16
4.39
6.16
5.29
CD34
Gr1
Nervi B, et al. Blood 2009;113:6206-6214, with permission.

155


Mobilization of leukemia cells increases the efficacy of anti-leukemic chemotherapy
Day 12
Day 13
control
AraC
AraC + AMD
Doxo
Doxo + AMD
AMD d5-6
10⁶ APL IV
129/C57BL6 F1
n=6 mice/group
AraC (500mg/kg/sc); Doxo (10mg/kg/sc); AMD (5mg/kg/sc)

156

AMD3100 enhances effect of chemotherapy



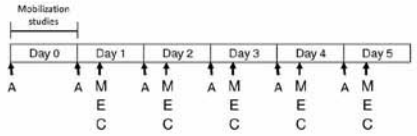
Nervi B, et al. *Blood* 2009;113:6206-6214, with permission.

157

Phase I/II Study of AMD3100 + MEC in Relapsed or Refractory AML

Eligibility Criteria
1.Dx of AML and either 1st refractory or relapsed disease (For pts in 1st relapse, must have CR1 duration < 12 mo)
2.Age 18-65, ECOG PS ≤ 2
3.Blast ct < 30,000/mm³
4.No previous MEC salvage

AMD3100
Dose level 1 80 mcg/kg SQ on d0-5
Dose level 2 160 mcg/kg SQ on d0-5
Dose level 3 240 mcg/kg SQ on d0-5
Mitoxantrone 8 mg/m² IV on d1-5
Etoposide 100 mg/m² IV on d1-5
Cytarabine 1000 mg/m² IV on d1-5



DiPersio JF, unpublished data.

158

Patient Characteristics (n=49)

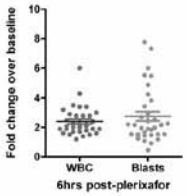
Age, median yrs (range)	51 (19-71)
Male / Female	25/24
Cytogenetics	
Favorable	8
Intermediate	28
Poor	13
Secondary AML	
Therapy related	3
Previous MDS/MPD	5
FLT-3	11
Mutated (ITD/D835)	11 (10/1)
Unmutated	19
Unknown	17
Prior HSCT (allo/auto)	8 (6/2)

159

Treatment Indication	n (%)
1st Relapse, 1st Salvage	36 (73%)
CR1 < 6 months	13 (27%)
CR1 6-12 months	13 (27%)
CR1 > 12 months	10 (20%)
1st Relapse, 2nd salvage	2 (5%)
2nd Relapse	1 (3%)
Primary refractory	10 (20%)
1 induction	8 (16%)
2 inductions	2 (5%)

160

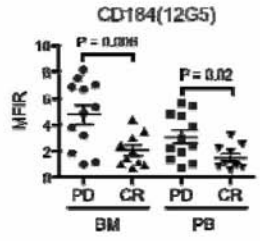
Mobilization of AML Blasts



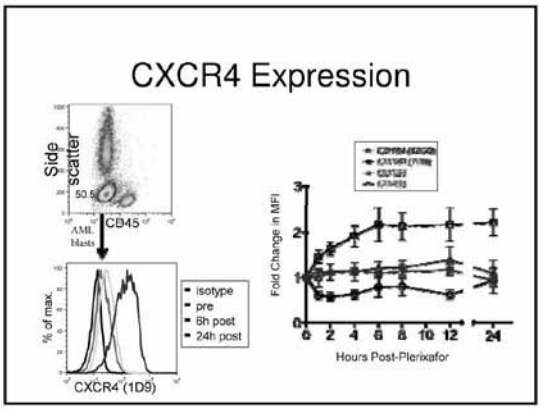
Probe	Post Plerixafor (# FISH+ cells/ total) 0 Hrs	6 Hrs	24 Hrs
MLL	128/200	106/200	136/200
MLL	142/200	130/200	120/200
CBFB	12/200	15/200	11/200
AML-ETO	151/200	150/200	162/200
AML-ETO	47/200	33/200	41/200
8	111/200	94/200	126/200
EGR1	92/200	146/200	152/200
EGR1	3/200	0/200	0/200
D20S108	192/200	199/200	196/200

161

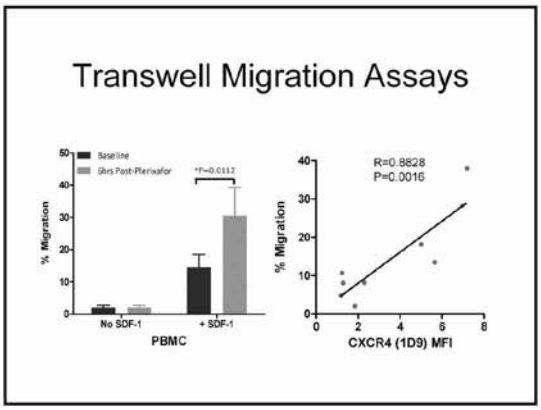
Surface CXCR4 Expression & Response



162



163

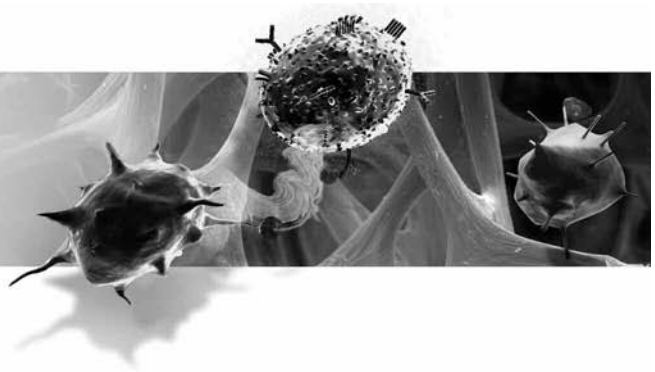


164

Safety & Toxicity

- No evidence of hyperleukocytosis
- Median time to hematopoietic recovery
  - ANC > 500/mm^3: 26 days (21-37 days)
  - Platelets > 50,000/mm^3: 26 days (14-40 days)
- Adverse events
  - no dose limiting toxicities in phase I
  - AEs primarily hematologic, febrile neutropenia
- Two early deaths (< 30 days) due to complications of sepsis

165



Response Evaluation (n=49)

	Patients		Responses		Treatment Failures		Response Rate	
Plerixafor Dose	Total	# Eval	CR	CRi	Death	PD	CR+CRi	CR
80 mcg/kg/day	3	3	1	0	0	2	33%	33%
160 mcg/kg/day	3	3	1	0	0	2	33%	33%
240 mcg/kg/day	43	40	17	3	2	19	50%	40%
Overall	**49**	**46**	**19**	**3**	**2**	**23**	**48%**	**39%**

166

Response Evaluation (240mcg/kg cohort)

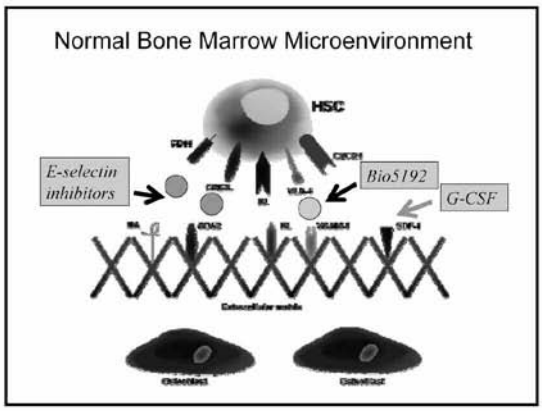
	Patients		Responses		Treatment Failures		Response Rate		Pred CR% Estey Blood '96	
	Total	# eval	CR	CRi	Death	PD	CR+CRi	CR	All pts	Trad salvage
CR > 2yrs, 1st salvage	0	0	0	0	0	0		N/A	73%	79%
CR 1-2 yrs, 1st salvage	9	7	6	0	1	0	86%	86%	47%	64%
CR<1 yr or no CR, 1st salvage	30	29	9	3	1	16	41%	31%	14%	20%
CR1<1yr and >2nd salvage	4	4	1	0	0	2	25%	25%	0%	0%

167

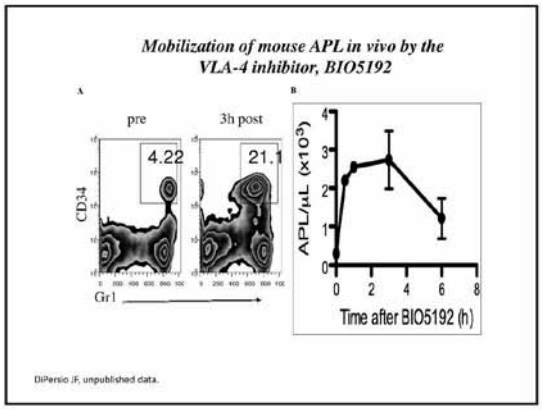
Conclusions

1. Plerixafor can be safely administered in combination with cytotoxic chemotherapy in patients with AML.
2. Effects of CXCR4 blockade are observed in AML blasts *in vitro* and *in vivo* following treatment with plerixafor.
3. CR + CRi rate of 50% compares favorably to historical controls with this regimen.

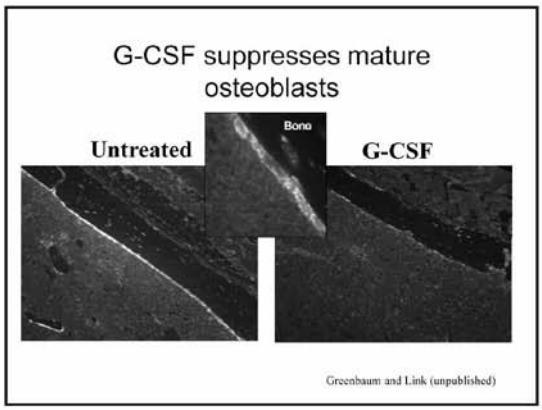
168


Normal Bone Marrow Microenvironment
HSC
E-selectin inhibitors
Bio5192
G-CSF

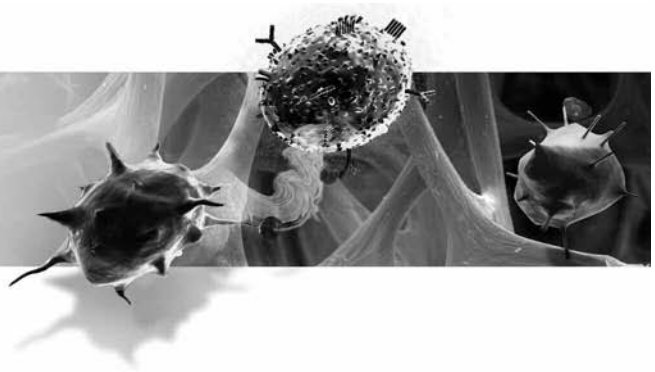
169

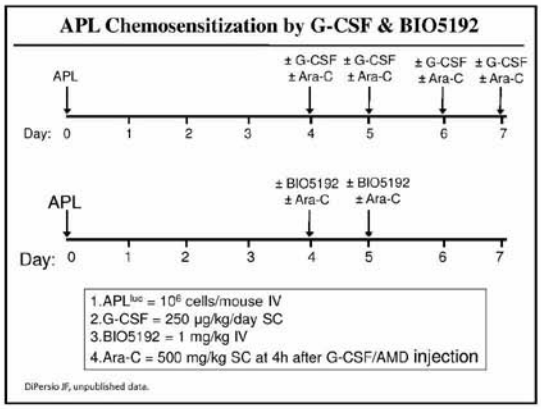

Mobilization of mouse APL in vivo by the VLA-4 inhibitor, BIO5192
A
B
pre
3h post
4.22
21.1
CD34
Gr1
APL/μL (x10³)
Time after BIO5192 (h)
DiPersio JF, unpublished data.

170

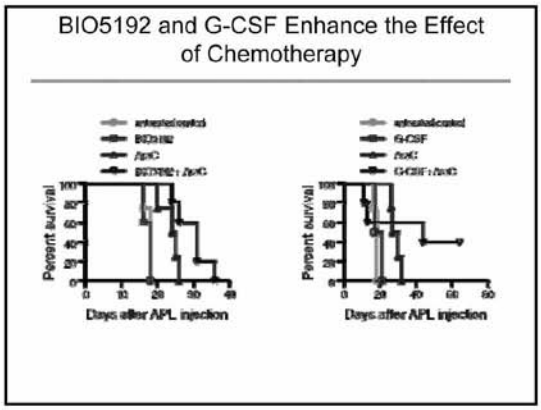

G-CSF suppresses mature osteoblasts
Untreated
Bone
G-CSF
Greenbaum and Link (unpublished)

171

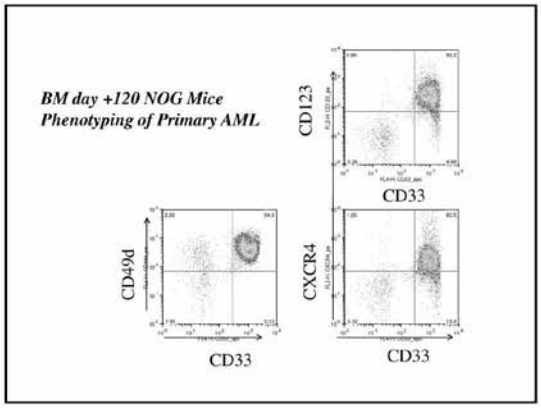



APL Chemosensitization by G-CSF & BIO5192
APL
± G-CSF ± Ara-C
± G-CSF ± Ara-C
± G-CSF ± Ara-C
± G-CSF ± Ara-C
Day: 0 1 2 3 4 5 6 7
APL
± BIO5192 ± Ara-C
± BIO5192 ± Ara-C
Day: 0 1 2 3 4 5 6 7
1.APL^luc = 10^6 cells/mouse IV
2.G-CSF = 250 µg/kg/day SC
3.BIO5192 = 1 mg/kg IV
4.Ara-C = 500 mg/kg SC at 4h after G-CSF/AMD injection
DiPersio JF, unpublished data.

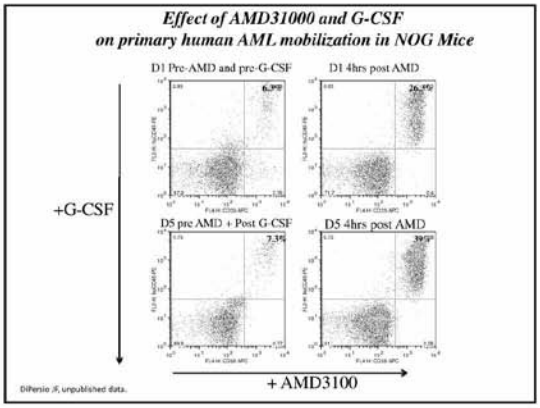
172


BIO5192 and G-CSF Enhance the Effect of Chemotherapy
Percent survival
Days after APL injection
Percent survival
Days after APL injection

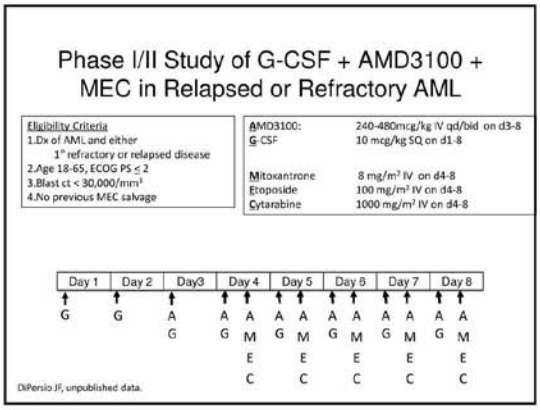
173


BM day +120 NOG Mice
Phenotyping of Primary AML
CD123
CD33
CD49d
CD33
CXCR4
CD33

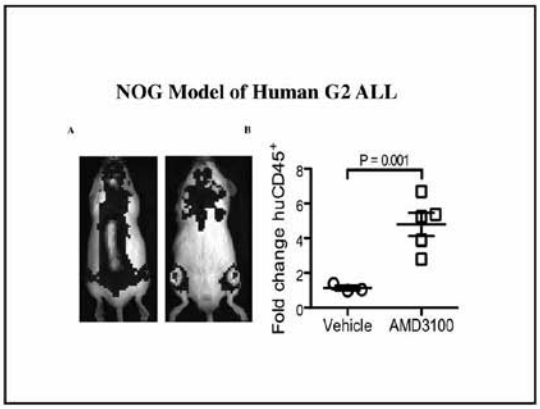
174


Effect of AMD31000 and G-CSF
on primary human AML mobilization in NOG Mice
D1 Pre-AMD and pre-G-CSF
D1 4hrs post AMD
D5 pre AMD + Post G-CSF
D5 4hrs post AMD
+G-CSF
+ AMD3100
DiPersio JF, unpublished data.

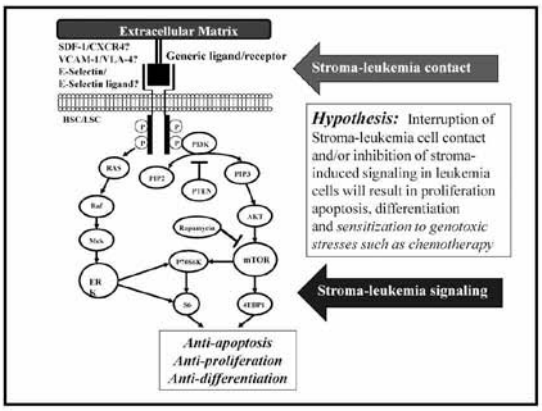
175


Phase I/II Study of G-CSF + AMD3100 +
MEC in Relapsed or Refractory AML
Eligibility Criteria
1.Dx of AML and either
1st refractory or relapsed disease
2.Age 18-65, ECOG PS ≤ 2
3.Blast ct < 30,000/mm³
4.No previous MEC salvage
AMD3100: 240-480mcg/kg IV qd/bid on d3-8
G-CSF 10 mcg/kg SQ on d1-8
Mitoxantrone 8 mg/m² IV on d4-8
Etoposide 100 mg/m² IV on d4-8
Cytarabine 1000 mg/m² IV on d4-8
Day 1 Day 2 Day3 Day 4 Day 5 Day 6 Day 7 Day 8
DiPersio JF, unpublished data.

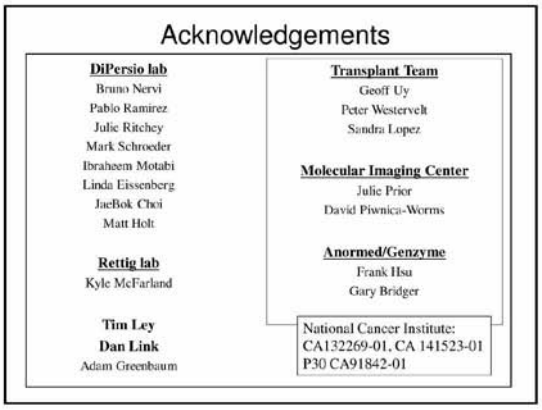
176


NOG Model of Human G2 ALL
A
B
P = 0.001
Fold change huCD45+
Vehicle
AMD3100

177


Extracellular Matrix
SDF-1/CXCR4?
VCAM-1/VLA-4?
E-Selectin/
E-Selectin ligand?
Generic ligand/receptor
HSC/LSC
Stroma-leukemia contact
Hypothesis: Interruption of Stroma-leukemia cell contact and/or inhibition of stroma-induced signaling in leukemia cells will result in proliferation apoptosis, differentiation and sensitization to genotoxic stresses such as chemotherapy
RAS
Raf
Mek
ER
PI3K
PIP2
PIP3
PTEN
AKT
Rapamycin
mTOR
4E-BP1
Stroma-leukemia signaling
Anti-apoptosis
Anti-proliferation
Anti-differentiation

178


Acknowledgements
DiPersio lab
Bruno Nervi
Pablo Ramirez
Julie Ritchey
Mark Schroeder
Ibraheem Motabi
Linda Eissenberg
JaeBok Choi
Matt Holt
Rettig lab
Kyle McFarland
Tim Ley
Dan Link
Adam Greenbaum
Transplant Team
Geoff Uy
Peter Westervelt
Sandra Lopez
Molecular Imaging Center
Julie Prior
David Piwnica-Worms
Anormed/Genzyme
Frank Hsu
Gary Bridger
National Cancer Institute:
CA132269-01, CA 141523-01
P30 CA91842-01

179


Thank You

180

Cell Trafficking in Multiple Myeloma

Irene Ghobrial, MD
Harvard Medical School
Dana Farber Cancer Institute
Boston, MA

DANA-FARBER CANCER INSTITUTE

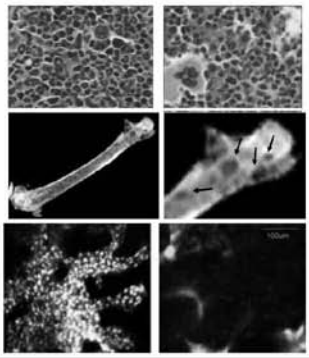
181

Conflict of Interest

- Advisory board of Millennium, Celgene, Novartis and Genzyme Inc.

182

Multiple Myeloma is a dynamic interaction of MM cells with TME



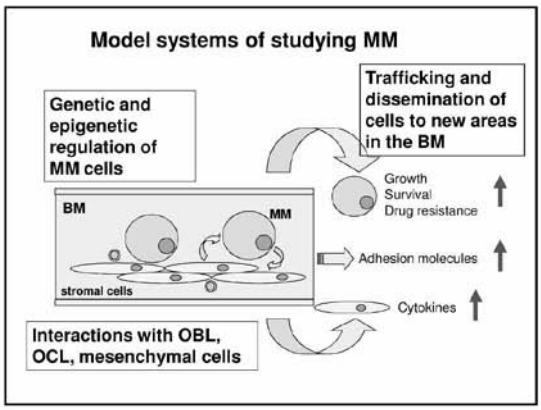
MM cell proliferation

Lytic lesions dues to activation of OCL and inhibition of OBL

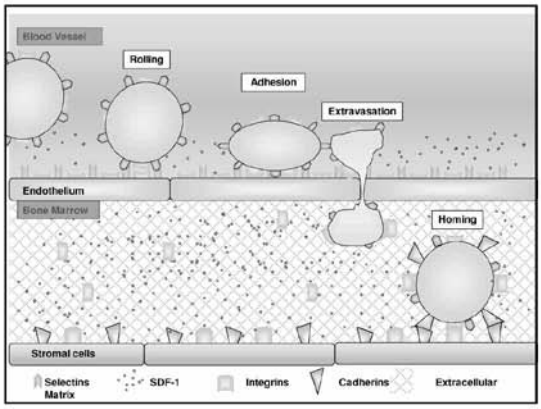
Trafficking and dissemination

70% of patients have circulating cells

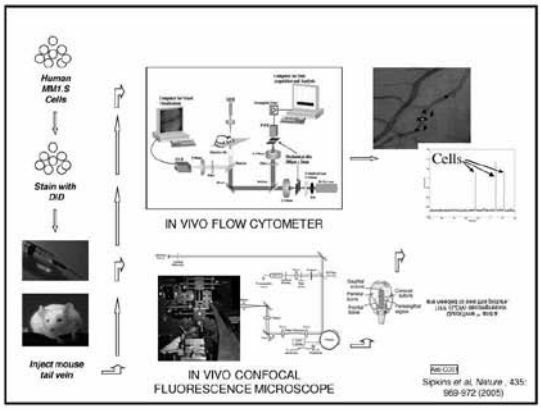
183


Model systems of studying MM
Genetic and epigenetic regulation of MM cells
Trafficking and dissemination of cells to new areas in the BM
BM
MM
stromal cells
Growth
Survival
Drug resistance
Adhesion molecules
Cytokines
Interactions with OBL, OCL, mesenchymal cells

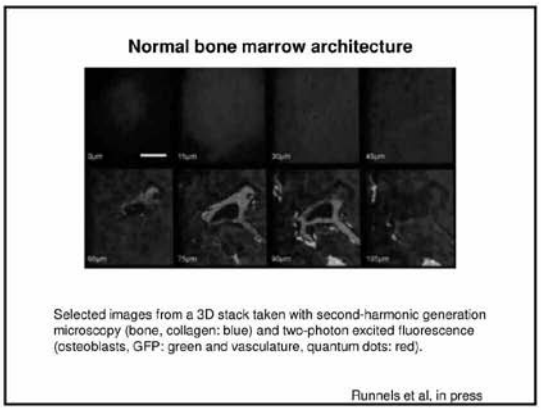
184


Blood Vessel
Rolling
Adhesion
Extravasation
Endothelium
Bone Marrow
Homing
Stromal cells
Selectins
Matrix
SDF-1
Integrins
Cadherins
Extracellular

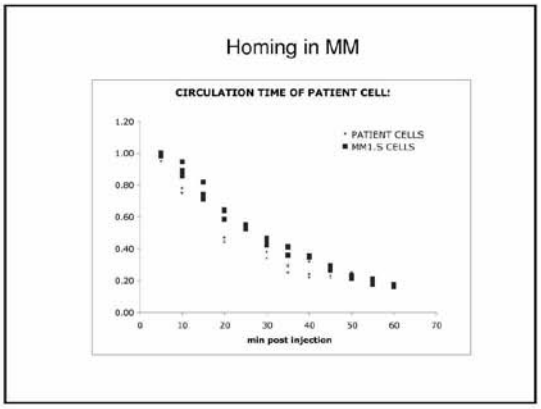
185


Human MM1.S Cells
Stain with DiD
Inject mouse tail vein
IN VIVO FLOW CYTOMETER
Cells
IN VIVO CONFOCAL FLUORESCENCE MICROSCOPE

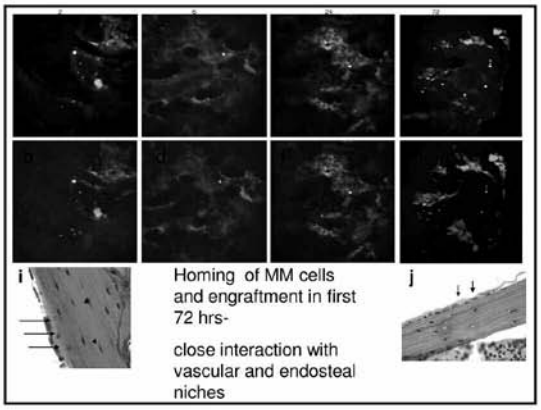
186


Normal bone marrow architecture
Selected images from a 3D stack taken with second-harmonic generation microscopy (bone, collagen: blue) and two-photon excited fluorescence (osteoblasts, GFP: green and vasculature, quantum dots: red).
Runnels et al, in press

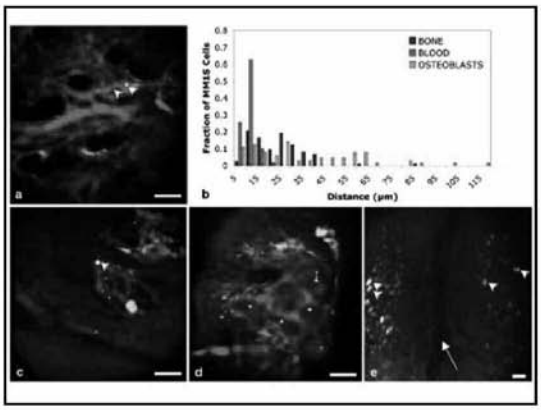
187


Homing in MM
CIRCULATION TIME OF PATIENT CELL!
PATIENT CELLS
MM1.S CELLS
1.20
1.00
0.80
0.60
0.40
0.20
0.00
0
10
20
30
40
50
60
70
min post injection

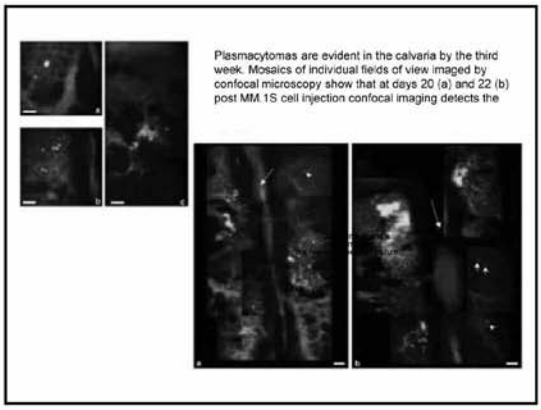
188


i
j
Homing of MM cells and engraftment in first 72 hrs-
close interaction with vascular and endosteal niches

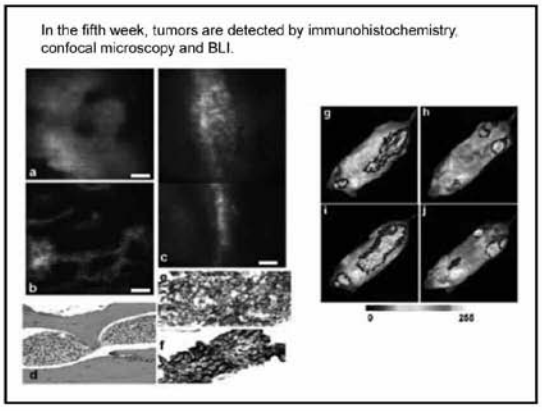
189


BONE
BLOOD
OSTEOBLASTS
Fraction of MM1S Cells
Distance (μm)
a
b
c
d
e

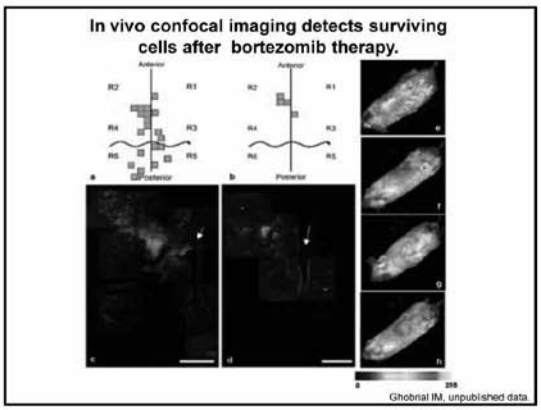
190


Plasmacytomas are evident in the calvaria by the third week. Mosaics of individual fields of view imaged by confocal microscopy show that at days 20 (a) and 22 (b) post MM.1S cell injection confocal imaging detects the

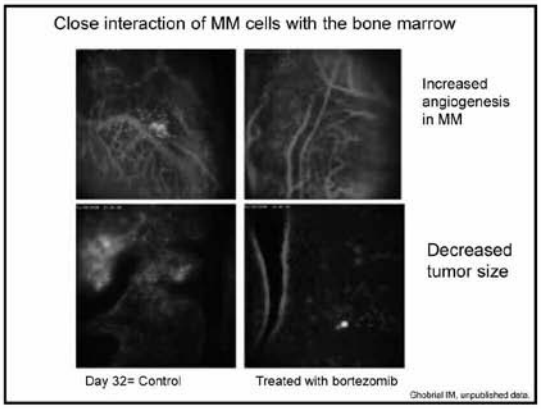
191


In the fifth week, tumors are detected by immunohistochemistry, confocal microscopy and BLI.
a
b
c
d
e
f
g
h
i
j
0
255

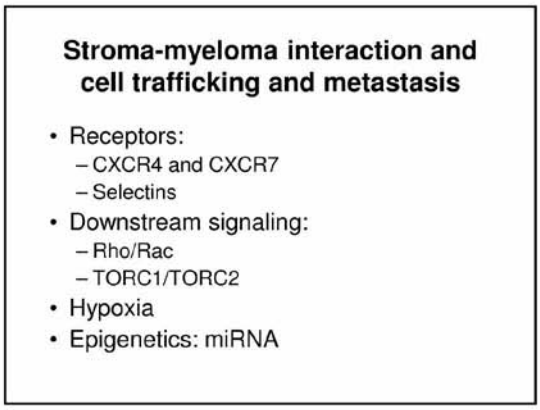
192


In vivo confocal imaging detects surviving cells after bortezomib therapy.
Ghobrial IM, unpublished data.

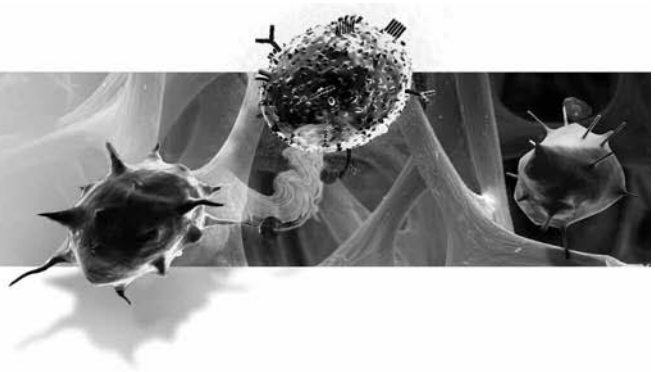
193

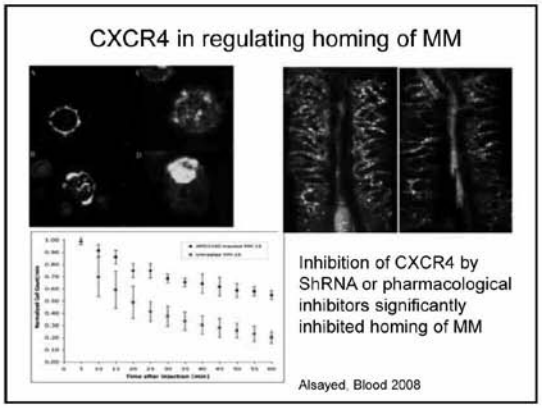

Close interaction of MM cells with the bone marrow
Increased angiogenesis in MM
Decreased tumor size
Day 32= Control
Treated with bortezomib
Ghobrial IM, unpublished data.

194

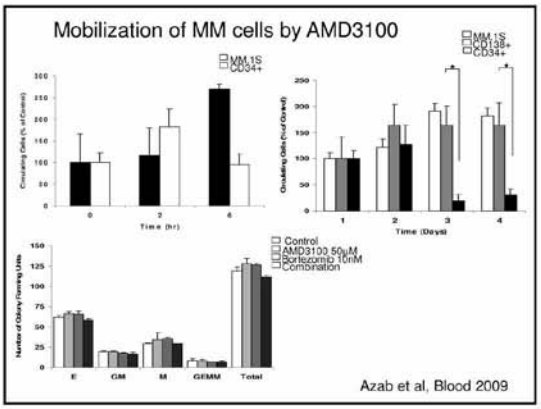

Stroma-myeloma interaction and cell trafficking and metastasis
• Receptors:
– CXCR4 and CXCR7
– Selectins
• Downstream signaling:
– Rho/Rac
– TORC1/TORC2
• Hypoxia
• Epigenetics: miRNA

195

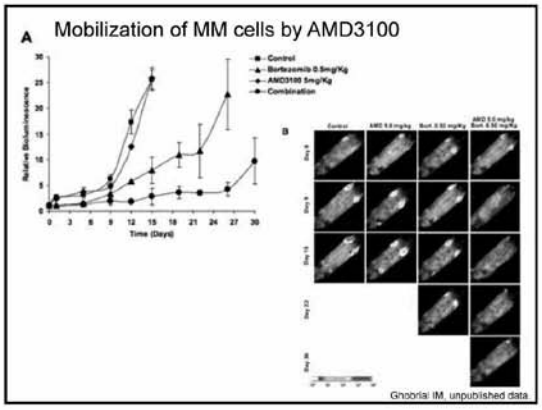



CXCR4 in regulating homing of MM
Inhibition of CXCR4 by ShRNA or pharmacological inhibitors significantly inhibited homing of MM
Alsayed, Blood 2008

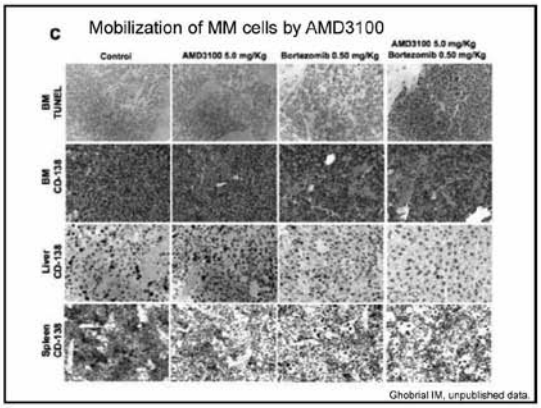
196


Mobilization of MM cells by AMD3100
MM.1S
CD34+
MM.1S
CD138+
CD34+
Time (hr)
Time (Days)
Control
AMD3100 50μM
Bortezomib 10nM
Combination
E
GM
M
GEMM
Total
Azab et al, Blood 2009

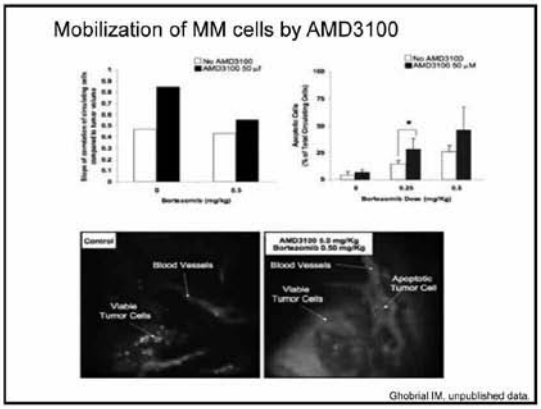
197


A
Mobilization of MM cells by AMD3100
Control
Bortezomib 0.5mg/Kg
AMD3100 5mg/Kg
Combination
Relative Bioluminescence
Time (Days)
B
Ghobrial IM, unpublished data

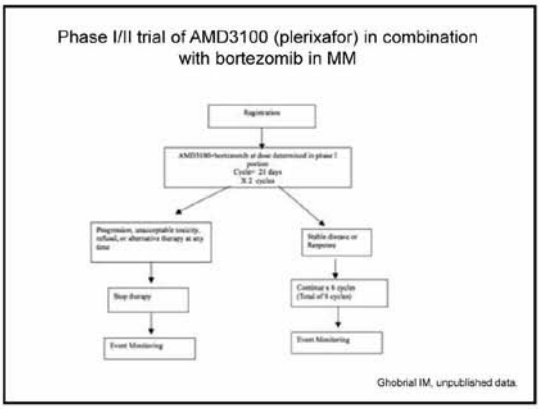
198


C
Mobilization of MM cells by AMD3100
Control
AMD3100 5.0 mg/Kg
Bortezomib 0.50 mg/Kg
AMD3100 5.0 mg/Kg
Bortezomib 0.50 mg/Kg
BM TUNEL
BM CD-138
Liver CD-138
Spleen CD-138
Ghobrial IM, unpublished data.

199


Mobilization of MM cells by AMD3100
No AMD3100
AMD3100 50 μM
Bortezomib (mg/kg)
Bortezomib Dose (mg/Kg)
Control
Blood Vessels
Viable Tumor Cells
AMD3100 5.0 mg/Kg
Bortezomib 0.50 mg/Kg
Blood Vessels
Viable Tumor Cells
Apoptotic Tumor Cell
Ghobrial IM, unpublished data.

200


Phase I/II trial of AMD3100 (plerixafor) in combination with bortezomib in MM
Registration
Cycle= 21 days
Stop therapy
Event Monitoring
Continue x 8 cycles
Event Monitoring
Ghobrial IM, unpublished data.

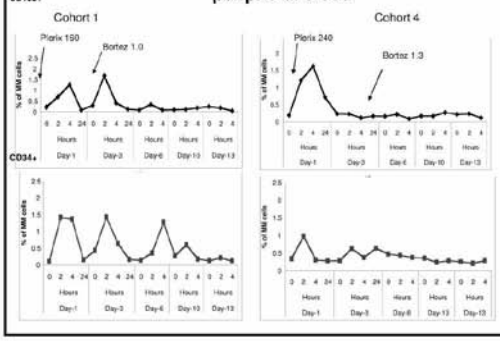
201

Schedule and dose escalation

Dose Level	Assigned therapy. **A cycle = 21 days**
Level 1	Plerixafor sq 160 µg/kg daily from day 1 to 6 and bortezomib IV push 1 mg/m^2 Days 3, 6, 10, and 13.
Level 2	Plerixafor sq 160 µg/kg daily from day 1 to 6 and bortezomib IV push 1.3 mg/m^2 Days 3, 6, 10, and 13.
Level 3	Plerixafor sq 240 µg/kg daily from day 1 to 6 and bortezomib IV push 1 mg/m^2 Days 3, 6, 10, and 13.
Level 4	Plerixafor sq 240 µg/kg daily from day 1 to 6 and bortezomib IV push 1.3 mg/m^2 Days 3, 6, 10 and 13.
Level 5	Plerixafor sq 320 µg/kg daily from day 1 to 6 and bortezomib IV push 1.3 mg/m^2 Days 3, 6, 10 and 13.
Level 5B	Plerixafor sq 320 µg/kg days 1, 2, 3, 6, 10 and 13. Bortezomib IV push 1.3 mg/m^2 Days 3, 6, 10 and 13.
Level 6	Plerixafor sq 400 µg/kg days 1, 2, 3, 6, 10 and 13. Bortezomib IV push 1.3 mg/m^2 Days 3, 6, 10 and 13.
Level 7	Plerixafor sq 480 µg/kg daily days 1, 2, 3, 6, 10 and 13. Bortezomib IV push 1.3 mg/m^2 Days 3, 6, 10 and 13.

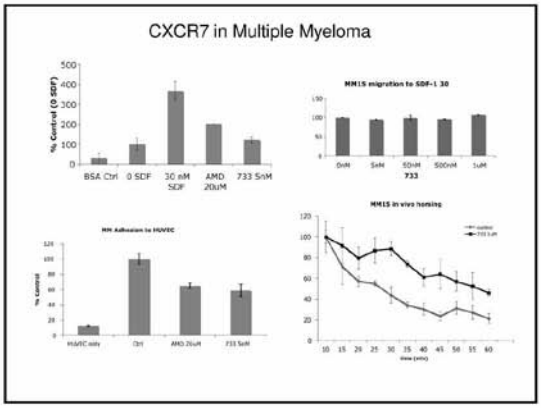
202

Mobilization of MM and CD34 cells in the peripheral blood

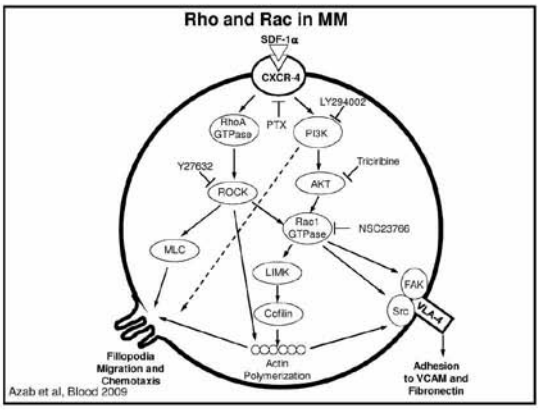


203

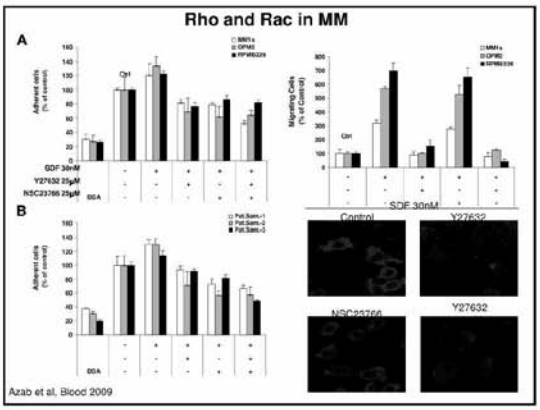
CXCR7 in Multiple Myeloma



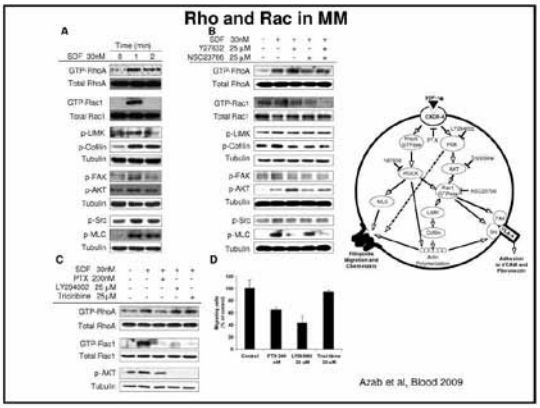
204


Rho and Rac in MM
SDF-1α
CXCR-4
LY294002
RhoA GTPase
PTX
PI3K
Y27632
Triciribine
ROCK
AKT
Rac1 GTPase
NSC23766
MLC
LIMK
FAK
Src
VLA-4
Cofilin
Filopodia Migration and Chemotaxis
Actin Polymerization
Adhesion to VCAM and Fibronectin
Azab et al, Blood 2009

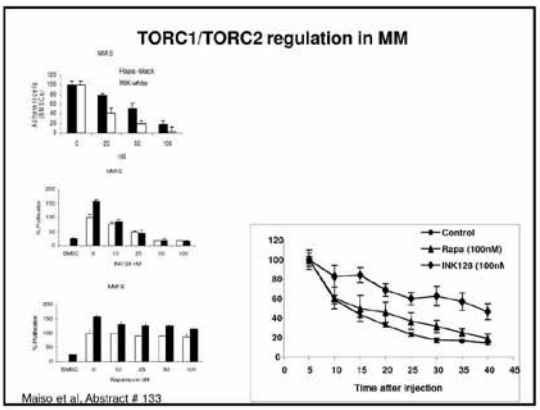
205


Rho and Rac in MM
A
B
Control
SDF 30nM
Y27632
NSC23766
Y27632
Azab et al, Blood 2009

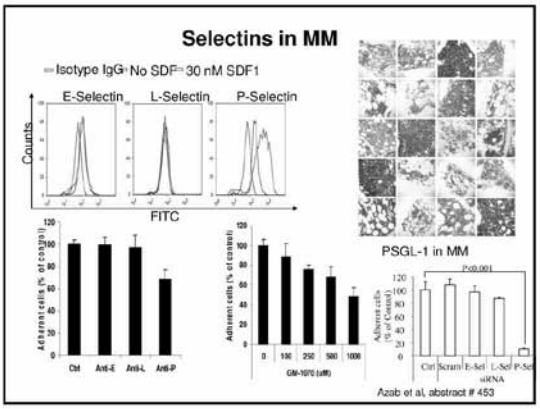
206


Rho and Rac in MM
A
B
C
D
Azab et al, Blood 2009

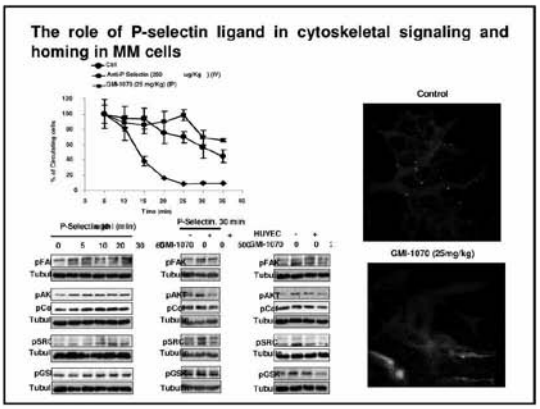
207


TORC1/TORC2 regulation in MM
Control
Rapa (100nM)
INK128 (100nM
Time after injection
Maiso et al, Abstract # 133

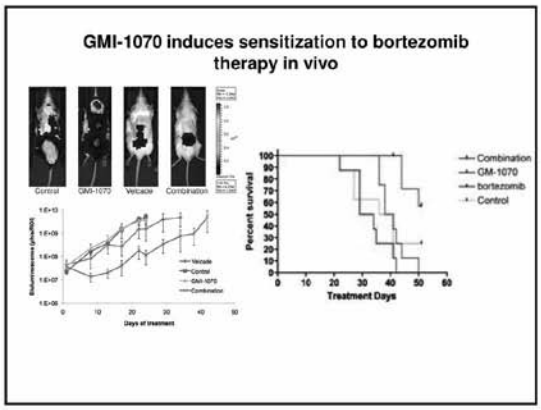
208


Selectins in MM
Isotype IgG
No SDF
30 nM SDF1
E-Selectin
L-Selectin
P-Selectin
Counts
FITC
PSGL-1 in MM
Ctrl
Anti-E
Anti-L
Anti-P
GM-1070 (uM)
Azab et al, abstract # 453

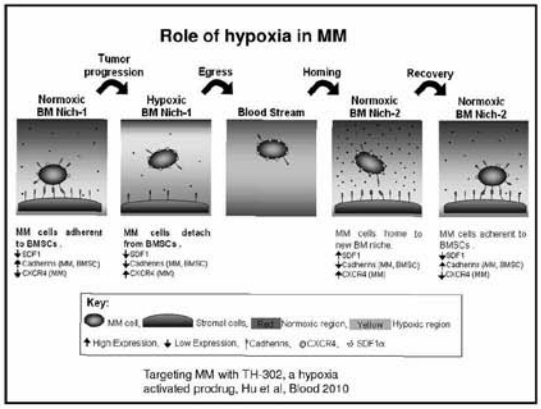
209


The role of P-selectin ligand in cytoskeletal signaling and homing in MM cells
Control
GM-1070 (25mg/kg)

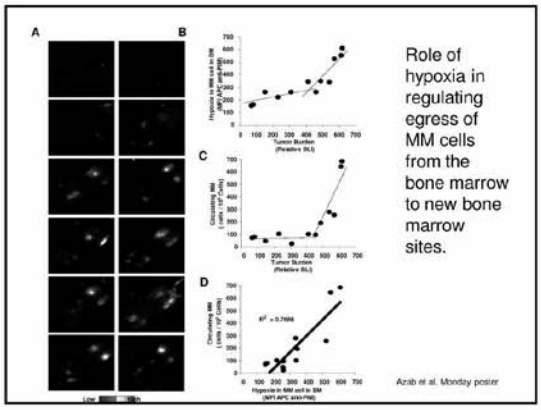
210


GMI-1070 induces sensitization to bortezomib therapy in vivo
Control
GMI-1070
Velcade
Combination
Percent survival
Treatment Days
Combination
GM-1070
bortezomib
Control

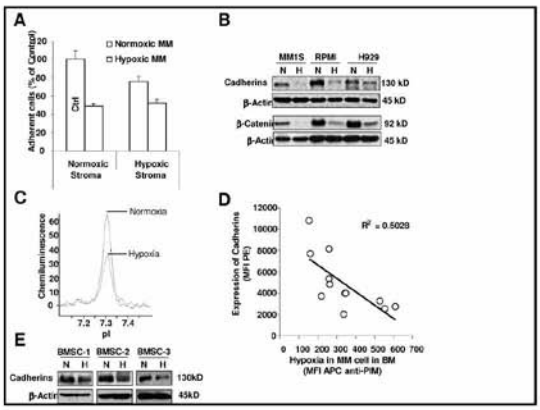
211


Role of hypoxia in MM
Tumor progression
Egress
Homing
Recovery
Normoxic BM Nich-1
Hypoxic BM Nich-1
Blood Stream
Normoxic BM Nich-2
Normoxic BM Nich-2
MM cells adherent to BMSCs ,
MM cells detach from BMSCs ,
MM cells home to new BM niche
MM cells adherent to BMSCs
Key:
MM cell
Stromal cells
Red: Normoxic region
Yellow: Hypoxic region
High Expression
Low Expression
Cadherins
CXCR4
SDF1α
Targeting MM with TH-302, a hypoxia activated prodrug, Hu et al, Blood 2010

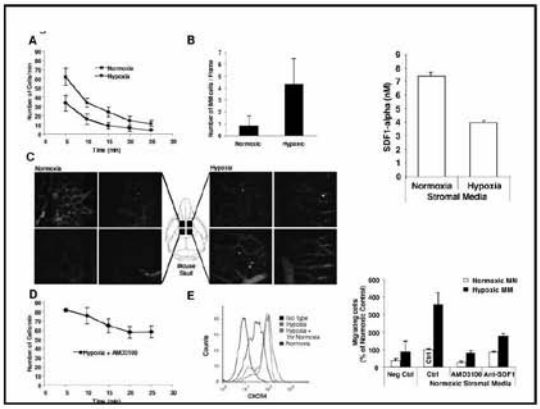
212


A
B
C
D
Role of hypoxia in regulating egress of MM cells from the bone marrow to new bone marrow sites.
Azab et al, Monday poster

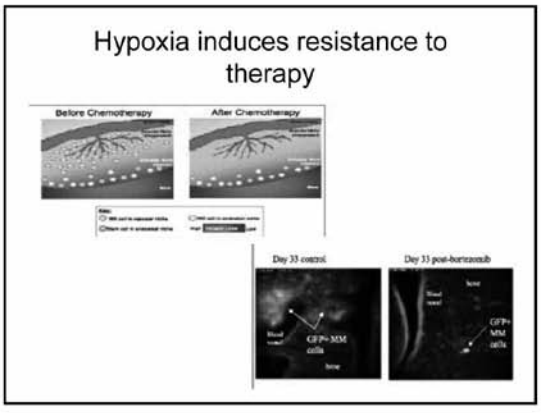
213


A
Normoxic MM
Hypoxic MM
Adherent cells (% of Control)
Ctrl
Normoxic Stroma
Hypoxic Stroma
B
MM1S RPMI H929
N H N H N H
Cadherins
β-Actin
130 kD
45 kD
β-Catenin
92 kD
45 kD
C
Normoxia
Hypoxia
Chemiluminescence
7.2 7.3 7.4
pI
D
R² = 0.5029
Expression of Cadherins (MFI FC)
Hypoxia in MM cell in BM
(MFI APC anti-PIM)
E
BMSC-1 BMSC-2 BMSC-3
N H N H N H
Cadherins
β-Actin
130kD
45kD

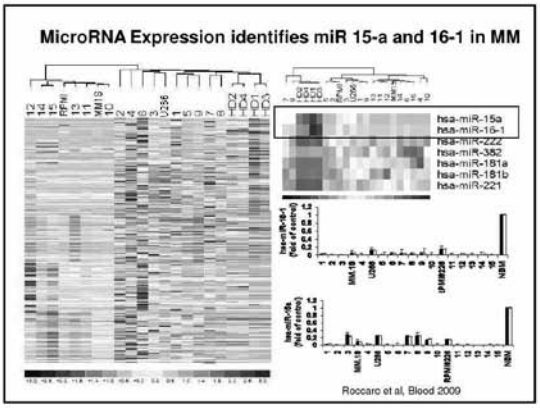
214


A
B
C
D
E
Normoxia
Hypoxia
Normoxia Stromal Media
Hypoxia Stromal Media
Mouse Skull
Neg Ctrl
Ctrl
AMD3100 Anti-SDF1
Normoxic Stromal Media
Normoxic MM
Hypoxic MM

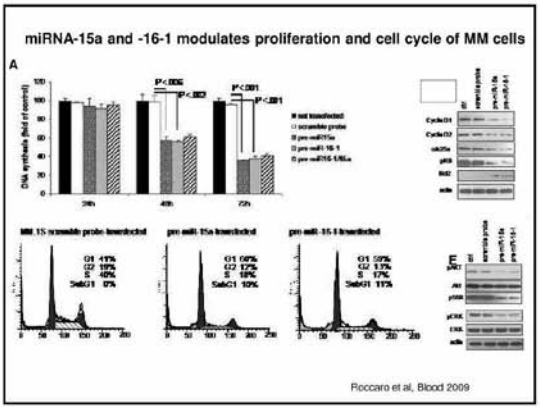
215


Hypoxia induces resistance to therapy
Before Chemotherapy
After Chemotherapy
Day 33 control
Day 33 post-bortezomib
GFP+ MM cells
bone
blood vessel

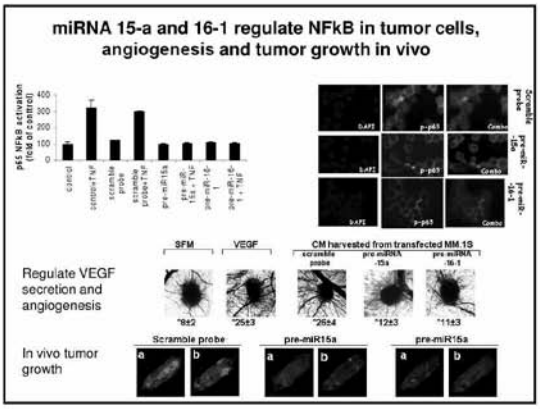
216


MicroRNA Expression identifies miR 15-a and 16-1 in MM
hsa-miR-15a
hsa-miR-16-1
hsa-miR-222
hsa-miR-382
hsa-miR-181a
hsa-miR-181b
hsa-miR-221
Roccaro et al, Blood 2009

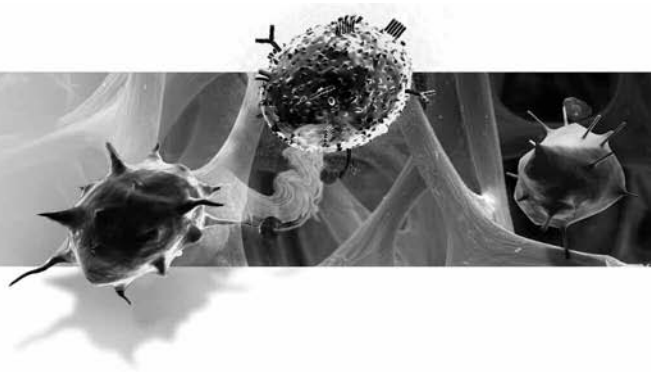
217


miRNA-15a and -16-1 modulates proliferation and cell cycle of MM cells
A
Roccaro et al, Blood 2009

218


miRNA 15-a and 16-1 regulate NFkB in tumor cells, angiogenesis and tumor growth in vivo
Regulate VEGF secretion and angiogenesis
SFM
VEGF
In vivo tumor growth
Scramble probe
pre-miR15a

219



Conclusion

- Cell trafficking in MM regulates dissemination and metastasis.
- Developing drugs that target these pathways: CXCR4/CXCR7/SDF-1, selectins, Rho/Rac, TORC, hypoxia, miRNA.
- Sensitization to therapy as a new modality of therapy.
- Prevent dissemination and progression.
- Role of the stroma in regulating cell metastasis.

220

Acknowledgement

- Dana-Farber Cancer Institute
  - Ken Anderson, MD, Nikhil Munshi, and Lab team
  - Barrett Rollins
  - Paul Richardson, MD, Ruben Carrasco, Charles Lin, Andrew Kung,

  - **Lab Team:** Aldo Roccaro, MD, PhD, Kareem Azab,PhD, Judith Runnels, PhD, Antonio Sacco, Xavier Leleu, MD,PhD, Hai Ngo, BS, Xiaoying Jia, BS, Feda Azab, Patricia Maiso, Phong Quang, Brian Thompson, Emanuel Husu, Mena Farag, Yong Zhang, Yang Liu, Brittany Morgan, Jennifer Stedman.
  - **Clinical Team:** Stacey Chuma, Janet Kunsman, Renee Leduc, Meghan Rourke, Brianna Harris, Amy Sam, Ranjit Banwait, Tiffany Poon, Amber Walsh.
  - NIH support (1R01CA125690, 1R01CA152607, R21CA126119), Leukemia and Lymphoma Society, MMRF, IWMF, Kirsch Lab for WM

221

Question-and-Answer Session

222

REFERENCES

Sean J. Morrison, PhD

Adams GB, Chabner KT, Alley IR, et al. Stem cell engraftment at the endosteal niche is specified by the calcium-sensing receptor. *Nature*. 2006;439(7076):599-603.

Butler JM, Nolan DJ, Vertes EL, et al. Endothelial cells are essential for the self-renewal and repopulation of Notch-dependent hematopoietic stem cells. *Cell Stem Cell*. 2010;6(3):251-264.

Hooper AT, Butler JM, Nolan DJ, et al. Engraftment and reconstitution of hematopoiesis is dependent on VEGFR2-mediated regeneration of sinusoidal endothelial cells. *Cell Stem Cell*. 2009;4(3):263-274.

Kiel MJ, Yilmaz OH, Iwashita T, Yilmaz OH, Terhorst C, Morrison SJ. SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. *Cell*. 2005;121(7):1109-1121.

Kiel MJ, Radice GL, Morrison SJ. Lack of evidence that hematopoietic stem cells depend on N-cadherin-mediated adhesion to osteoblasts for their maintenance. *Cell Stem Cell*. 2007;1(2): 204-217.

Kiel MJ, Acar M, Radice GL, Morrison SJ. Hematopoietic stem cells do not depend on N-cadherin to regulate their maintenance. *Cell Stem Cell*. 2009;4(2):170-179.

Kiel MJ, Morrison SJ. Uncertainty in the niches that maintain haematopoietic stem cells. *Nat Rev Immunol*. 2008;8(4):290-301.

Wilson A, Trumpp A. Bone-marrow haematopoietic-stem-cell niches. *Nat Rev Immunol*. 2006 Feb;6(2):93-106.

Méndez-Ferrer S, Michurina TV, Ferraro F, et al. Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. *Nature*. 2010 ;466(7308):829-834.

Louis M. Staudt, MD, PhD

Canioni D, Salles G, Mounier N, et al. High numbers of tumor-associated macrophages have an adverse prognostic value that can be circumvented by rituximab in patients with follicular lymphoma enrolled onto the GELA-GOELAMS FL-2000 trial. *J Clin Oncol*. 2008;26(3):440-446.

Farinha P, Masoudi H, Skinnider BF, et. Analysis of multiple biomarkers shows that lymphoma-associated macrophage (LAM) content is an independent predictor of survival in follicular lymphoma (FL). *Blood*. 2005;106(6):2169-2174.

Iannis Aifantis, PhD

Buonamici S, Trimarchi T, Ruocco MG, et al. CCR7 signalling as an essential regulator of CNS infiltration in T-cell leukaemia. *Nature*. 2009;459(7249):1000-1004.

Espinosa L, Cathelin S, D'Altri T, et al. The Notch/Hes1 pathway sustains NF- B activation through CYLD repression in T cell leukemia. *Cancer Cell*. 2010;18(3):268-281.

Vilimas T, Mascarenhas J, Palomero T, et al. Targeting the NF-kappaB signaling pathway in Notch1-induced T-cell leukemia. *Nat Med*. 2007;13(1):70-77.

Michael P. Rettig, PhD

Cashen AF, Nervi B, DiPersio JF. AMD3100: CXCR4 antagonist and rapid stem cell-mobilizing agent. *Future Oncol*. 2007;3:19-27.

Devine SM, Vij R, Rettig M, et al. Rapid mobilization of functional donor hematopoietic cells without G-CSF using AMD3100, an antagonist of the CXCR4/SDF-1 interaction. *Blood*. 2008;112:990-998.

DiPersio JF, Micallef IN, Stiff PJ, et al. Phase III prospective randomized double-blind placebo-controlled trial of plerixafor plus granulocyte colony-stimulating factor compared with placebo plus granulocyte colony-stimulating factor for autologous stem-cell mobilization and transplantation for patients with non-Hodgkin's lymphoma. *J Clin Oncol*. 2009;27:4767-4773.

Nervi B, Ramirez P, Rettig MP, et al. Chemosensitization of acute myeloid leukemia (AML) following mobilization by the CXCR4 antagonist AMD3100. *Blood*. 2009;113:6206-6214.

Pusic I, DiPersio JF. The use of growth factors in hematopoietic stem cell transplantation. *Curr Pharm Des*. 2008;14:1950-1961.

Ramirez P, Rettig MP, Uy GL, et al. BIO5192, a small molecule inhibitor of VLA-4, mobilizes hematopoietic stem and progenitor cells. *Blood*. 2009;114:1340-1343.

Westervelt P, Lane AA, Pollock JL, et al. High-penetrance mouse model of acute promyelocytic leukemia with very low levels of PML-RAR expression. *Blood*. 2003;102:1857-1865.

Irene M. Ghobrial, MD

Alsayed Y, Ngo H, Runnels J, et al. Mechanisms of regulation of CXCR4/SDF-1 (CXCL12)–dependent migration and homing in multiple myeloma. *Blood*. 2007;109:2708-2717.

Hayashi T, Hideshima T, Akiyama M, et al. Molecular mechanisms whereby immunomodulatory drugs activate natural killer cells: clinical application. *Br J Haematol*. 2005;128(2):192-203.

Lapidot T, Dar A, Kollet O. How do stem cells find their way home? *Blood*. 2005;106(6):1901-1910.

Nakayama T, Hieshima K, Izawa D, Tatsumi Y, Kanamaru A, Yoshie O. Cutting edge: profile of chemokine receptor expression on uman plasma cells accounts for their efficient recruitment to target tissues. *J Immunol*. 2003;170(3):1136-1140.

Roccaro AM, Sacco A, Thompson B. MicroRNAs 15a and 16 regulate tumor proliferation in multiple myeloma. *Blood*. 2009;113(26):6669-6680.

Sipkins DA, Wei X, Wu JW. In vivo imaging of specialized bone marrow endothelial microdomains for tumour engraftment. *Nature*. 2005;435(7044):969-973.