COLLEGE of AMERICAN PATHOLOGISTS

Molecular Diagnostic Testing in Myelodysplastic Syndrome

CAP PHC Webinar

Adam Seegmiller, MD, PhD
Michael Savona, MD

Vanderbilt University
Medical Center

4/24/2018

Webinar Host

- **This series is sponsored by the Personalized Healthcare Committee (PHC)**
- **Today's webinar host is PHC chair, Dr. Sophia Yohe**

Housekeeping

- **This presentation will be recorded. The recording and PDF will go out to all registrants in one week**
- **All lines are muted during the presentation**
- **Please send in your questions as you think of them via the “Question box” in your control panel**

Adam Seegmiller, MD, PhD, FCAP

- **Associate Professor and Vice Chair, Department of Pathology, Microbiology, and Immunology at Vanderbilt University School of Medicine**
- **Executive Medical Director, Clinical Pathology at Vanderbilt University Medical Center**
- **Leads the divisions of Laboratory Medicine and Hematopathology, and actively practices clinical hematopathology**

Michael Savona, MD

- **Director, Hematology Research and Director, Hematology Early Therapy Program Vanderbilt-Ingram Cancer Center**
- **Associate Professor of Medicine, Vanderbilt University School of Medicine**
- **Translational scientist and medical oncologist specializing in myeloid malignancies, and a leader in development of novel therapies for these diseases patients**

Disclaimer

- **The CAP does not permit reproduction of any substantial portion of the material in this Webinar without its written authorization. The CAP hereby authorizes attendees of the CAP Webinar to use the PDF presentation solely for educational purposes within their own institutions. The CAP prohibits use of the material in the Webinar – and any unauthorized use of the CAP's name or logo – in connection with promotional efforts by marketers of laboratory equipment, reagents, materials, or services.**

Disclaimer, continued

- **Opinions expressed by the speaker are the speaker's own and do not necessarily reflect an endorsement by the CAP of any organizations, equipment, reagents, materials, or services used by participating laboratories.**

Learning Objectives

1. To understand the current approach to the diagnosis of MDS.
2. To describe the potential and the limitations of applying molecular diagnostic testing in MDS.
3. To discuss the impact of molecular diagnostic testing on the prognosis and treatment of patients with MDS.

Part 1: Genetic Testing in the Diagnosis and Evaluation of Myelodysplastic Syndrome

Adam Seegmiller, MD, PhD

Case Presentation

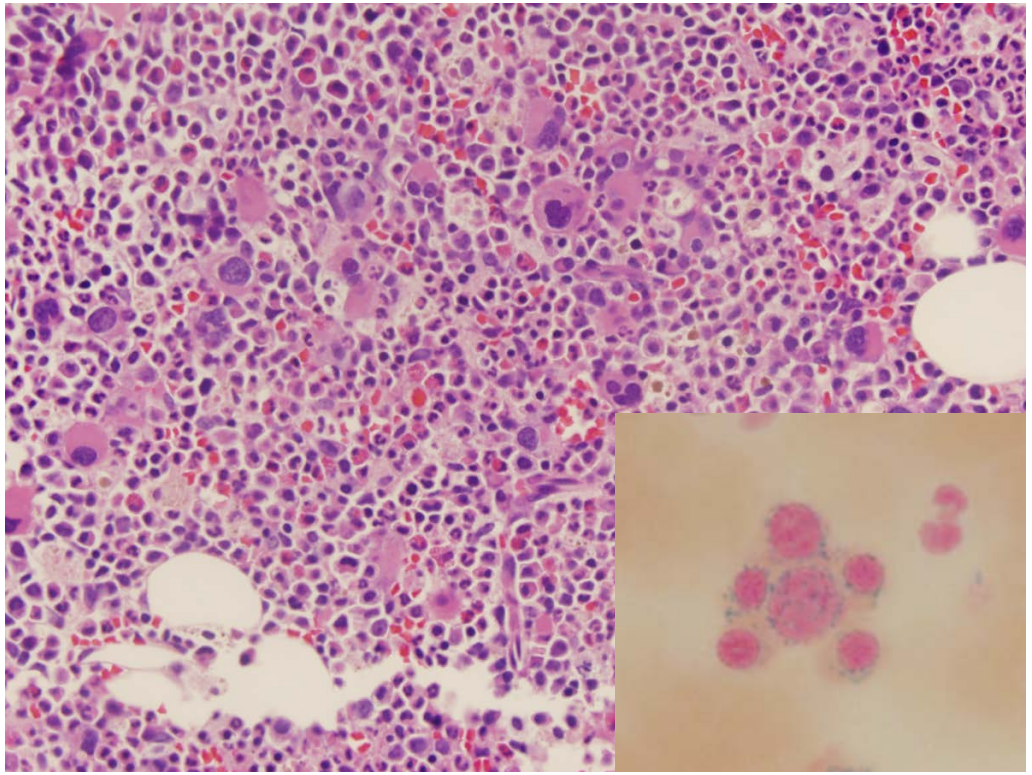
- **A 65 year-old female with history of follicular thyroid carcinoma treated with radioiodine.**
- **Presents to the emergency department complaining of shortness of breath, weakness, and fatigue.**
- **Physical examination was significant for tachycardia and pallor.**

Case Presentation

- **Complete blood count**
  - **Profound macrocytic anemia (Hbg 4.0 g/dL, MCV 113 fL)**
  - **Mild leukocytosis (WBC 17.7x10^3/µL)**
  - **Mild thrombocytosis (470x10^3/µL)**

Bone Marrow Biopsy

- **Hypercellular (90%)**
- **Increased abnormal megakaryocytes**
- **Dysplastic erythroid precursors**
- **Ring sideroblasts**



Myelodysplastic Syndrome (MDS)

- **Clonal neoplasm of myeloid precursors**
- **Characterized by:**
  - **Ineffective hematopoiesis → usually hypercellular bone marrow with peripheral cytopenias**
  - **Morphologic dysplasia in one or more myeloid cell line (granulocytic, erythroid, or megakaryocytic)**
  - **Increased risk for development of acute myeloid leukemia**

Hasserjian RP, et al. *WHO Classification.* 2017:98
Arber DA, et al. *Blood*. 2016;127(20):2391.
Steensma DP. *Mayo Clin Proc*. 2015;90(7):969

Myelodysplastic Syndrome (MDS)

- **Incidence is 10,000-40,000 per year in the US**
- **Mostly a disease of older individuals, due to accumulation of mutations over a lifetime**
- **Toxic exposures increase risk – 10-15% of cases are due to prior chemotherapy**
- **Some inherited hematopoietic disorders predispose to MDS**

Hasserjian RP, et al. *WHO Classification.* 2017:98
Arber DA, et al. *Blood*. 2016;127(20):2391.
Steensma DP. *Mayo Clin Proc*. 2015;90(7):969

Diagnosis of MDS

- **Diagnosis of MDS requires the following:**
  1. **At least one peripheral blood cytopenia*:**
     - **Anemia (Hbg <10 g/dL)**
     - **Neutropenia (absolute count <1.8x10^3/µL)**
     - **Thrombocytopenia (platelets <100x10^3/µL)**
  2. **Morphologic dysplasia: >10% dysplastic blood or bone marrow cells in any myeloid lineage**
  3. **<20% blasts in blood and bone marrow**

Hasserjian RP, et al. *WHO Classification.* 2017:98
Arber DA, et al. *Blood*. 2016;127(20):2391.
Steensma DP. *Mayo Clin Proc*. 2015;90(7):969

Differential Diagnosis

- **Cytopenias with dysplasia can be seen in many non-clonal reactive conditions:**
  - **Infections**
  - **Nutritional deficiencies**
  - **Drug effects**
  - **Immune or inflammatory disorders**
  - **Congenital syndromes**
- **These should be ruled out before a definitive diagnosis is made.**

Steensma DP. *Curr Hematol Malig Rep*. 2012;7:310

Classification of MDS

Classification is based on:

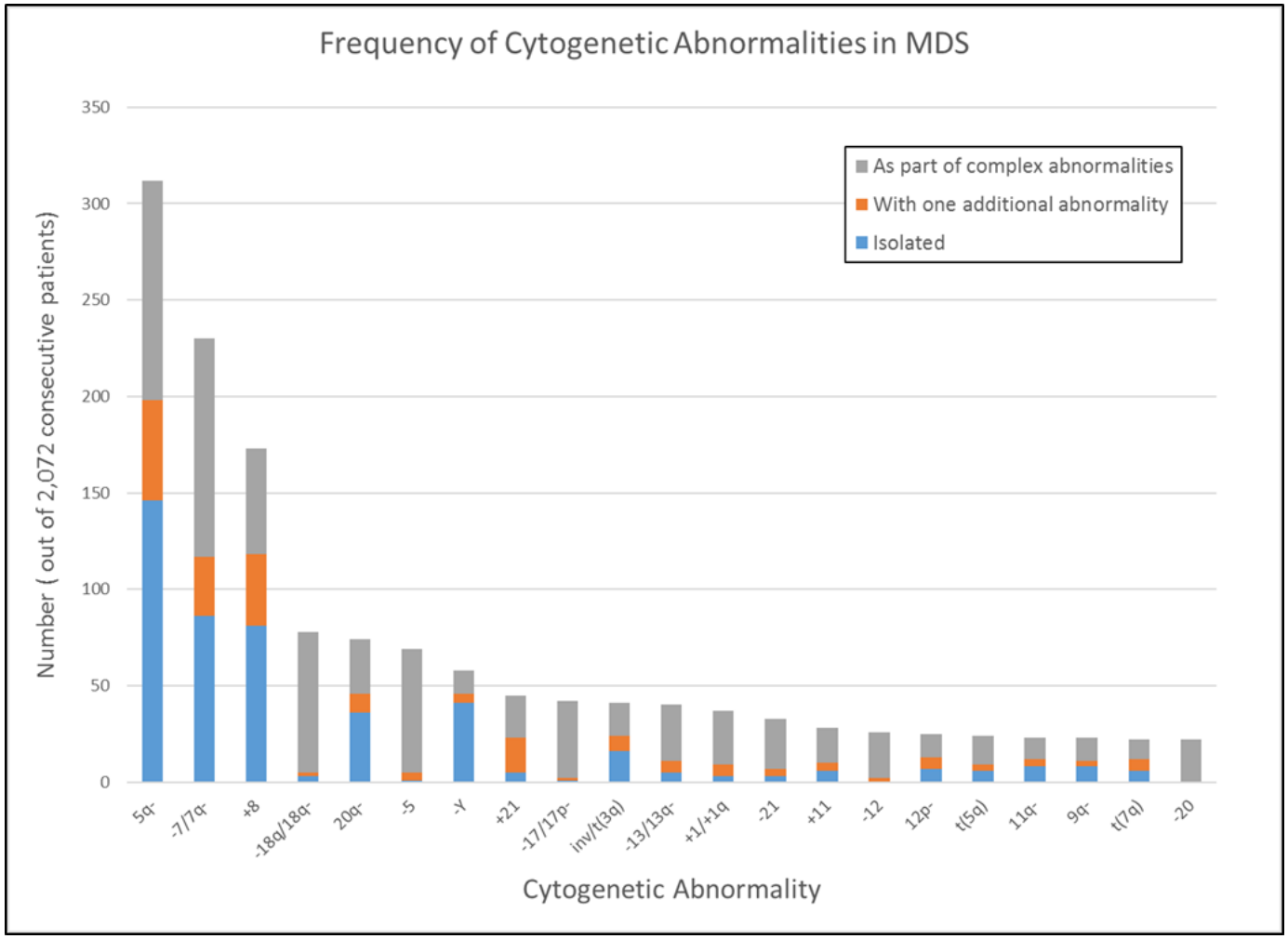
1. Number of hematopoietic lineages affected.
2. Percentage of marrow or blood blasts
3. Presence/absence of ring sideroblasts
4. Cytogenetic profile

Classification of MDS

Name	Dysplastic lineages	Cytopenias*	Ring sideroblasts as % of marrow erythroid elements	BM and PB blasts	Cytogenetics by conventional karyotype analysis
MDS with single lineage dysplasia (MDS-SLD)	1	1 or 2	<15%/<5%†	BM <5%, PB <1%, no Auer rods	Any, unless fulfills all criteria for MDS with isolated del(5q)
MDS with multilineage dysplasia (MDS-MLD)	2 or 3	1-3	<15%/<5%†	BM <5%, PB <1%, no Auer rods	Any, unless fulfills all criteria for MDS with isolated del(5q)
MDS with ring sideroblasts (MDS-RS)					
MDS-RS with single lineage dysplasia (MDS-RS-SLD)	1	1 or 2	≥15%/≥5%†	BM <5%, PB <1%, no Auer rods	Any, unless fulfills all criteria for MDS with isolated del(5q)
MDS-RS with multilineage dysplasia (MDS-RS-MLD)	2 or 3	1-3	≥15%/≥5%†	BM <5%, PB <1%, no Auer rods	Any, unless fulfills all criteria for MDS with isolated del(5q)
MDS with isolated del(5q)	1-3	1-2	None or any	BM <5%, PB <1%, no Auer rods	del(5q) alone or with 1 additional abnormality except −7 or del (7q)
MDS with excess blasts (MDS-EB)					
MDS-EB-1	0-3	1-3	None or any	BM 5%-9% or PB 2%-4%, no Auer rods	Any
MDS-EB-2	0-3	1-3	None or any	BM 10%-19% or PB 5%-19% or Auer rods	Any
MDS, unclassifiable (MDS-U)					
with 1% blood blasts	1-3	1-3	None or any	BM <5%, PB = 1%,‡ no Auer rods	Any
with single lineage dysplasia and pancytopenia	1	3	None or any	BM <5%, PB <1%, no Auer rods	Any
based on defining cytogenetic abnormality	0	1-3	<15%§	BM <5%, PB <1%, no Auer rods	MDS-defining abnormality
Refractory cytopenia of childhood	1-3	1-3	None	BM <5%, PB <2%	Any

Hasserjian RP, et al. *WHO Classification.* 2017:98
Arber DA, et al. *Blood*. 2016;127(20):2391.

Cytogenetics in MDS



Haase D. *Ann Hematol.* 2008;87(7):515-25.

Cytogenetics and Diagnosis

- **MDS-defining abnormalities allow for a diagnosis of MDS even in the absence of definitive morphologic dysplasia:**
  - **Loss of chromosomes 7 or 13**
  - **del(5q), del(7q), del(9q), del(11q), del(12p), del(13q)**
  - **t(1;3), t(2;11), inv(3)/t(3;3), t(3;21), t(6;9), t(11;16), or translocations involving 12p or 17p**
  - **i(17q), idic(X)(q13)**
- **Presence of –Y, +8, or del(20q) as a sole abnormality is insufficient for diagnosis.**

Hasserjian RP, et al. *WHO Classification.* 2017:98
Arber DA, et al. *Blood*. 2016;127(20):2391.

Case Study Patient

Abnormal female karyotype:

46,XX,del(5)(q22q35)[11]/46,XX[9]

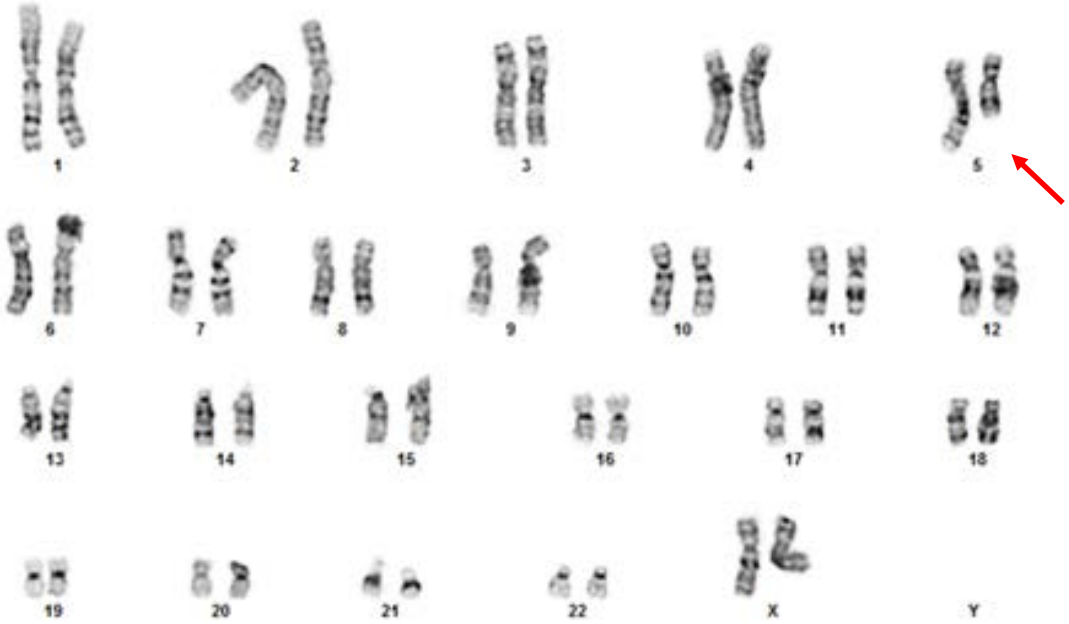


Image from atlasgeneticsoncology.org

MDS with isolated del(5q)

- **Only cytogenetic abnormality that defines a distinct diagnostic entity.**
- **Most common in older women.**
- **Typically presents with anemia, often severe and macrocytic; thrombocytosis is common.**
- **Marrow morphology usually shows erythroid hypoplasia and abnormal megakaryocytes with hypolobated nuclei.**
- **Generally favorable outcomes with low risk of transformation compared with other MDS subtypes.**

Hasserjian RP, et al. *WHO Classification.* 2017:98
Arber DA, et al. *Blood*. 2016;127(20):2391.

Other Cytogenetic/Morphologic Correlates

- **inv(3)/t(3;3) – abnormal megakaryocytes and thrombocytosis**
- **del(17p) – pseudo-Pelger-Huët anomaly, small vacuolated neutrophils**
- **del(20q) – dysmegakaryopoiesis and thrombocytopenia**

Rogers HJ, et al. *Hematologica.* 2014;99:821
Lai JL, et al. *Leukemia*. 1995;9:370.
Braun T, et al. *Leuk Res*. 2011;35:863

Cytogenetics and Prognosis

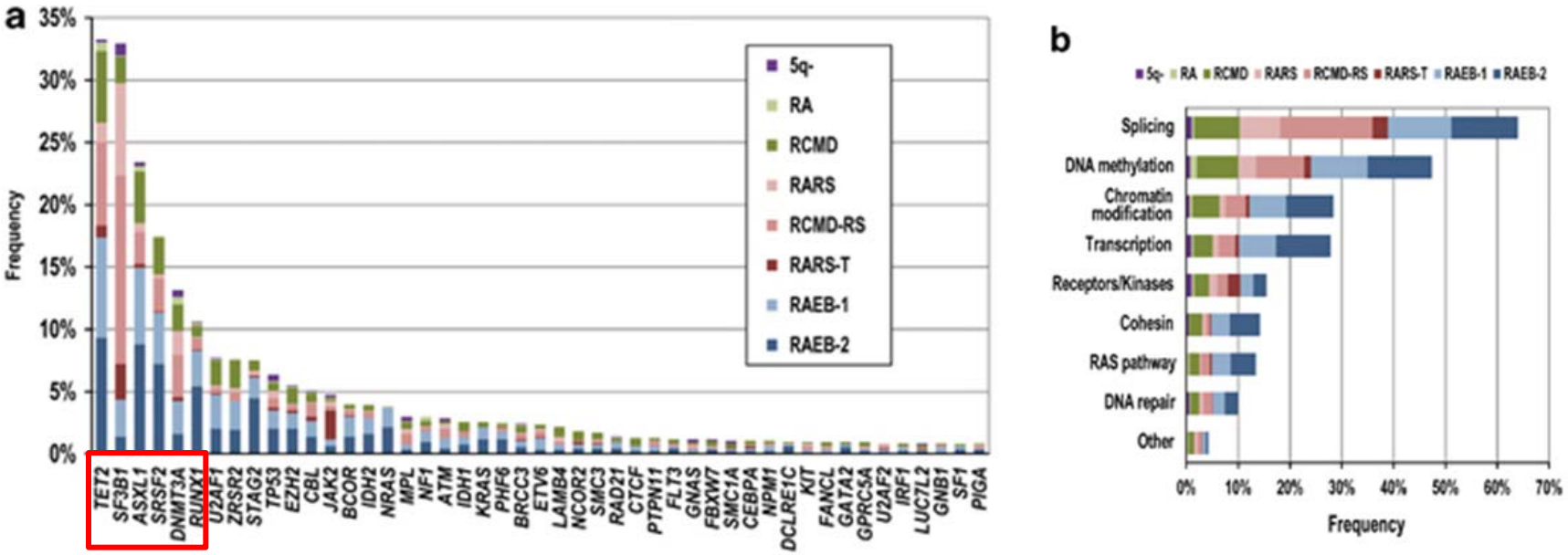
- **Cytogenetic findings are categorized according to impact on median survival.**
- **These are a component of the Revised International Prognostic Scoring System (IPSS-R) for assessment of primary MDS.**

Prognostic Subgroup	Cytogenetic Abnormalities	Median Survival (y)
Very Good	-Y, del(11q)	5.4
Good	Normal, del(5q), del(12p), del(20q), double including del(5q)	4.8
Intermediate	del(7q), +8, +19, i(17q), any other single or double independent clones	2.7
Poor	-7, inv(3)/t(3q)/del(3q), double including -7/del(7q), complex: 3 abnormalities	1.5
Very Poor	Complex: >3 abnormalities	0.7

Greenberg PL, et al. *Blood*, 2012;120:2454
Schanz J, et al. *J Clin Oncol.* 2012;30:820

Gene Mutations in MDS

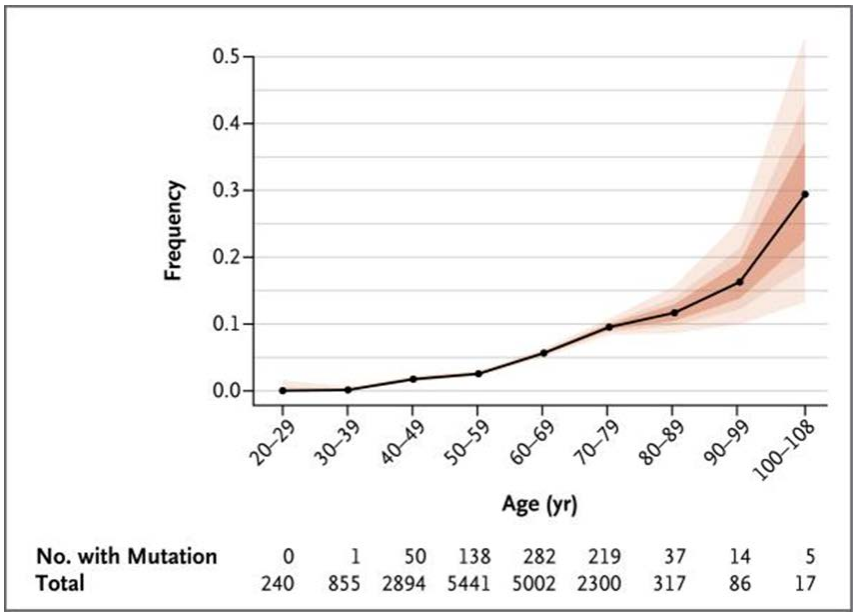
- **80-90% of MDS patients carry gene mutations**



Haferlach T, et al. *Leukemia*. 2014;28(2):241-7.
Papaemmanuil E, et al. *Blood*. 2013;122(22):3616-27.
Malcovati L, et al. *Blood.* 2017;129(25):3371-78.

Gene Mutations and Diagnosis

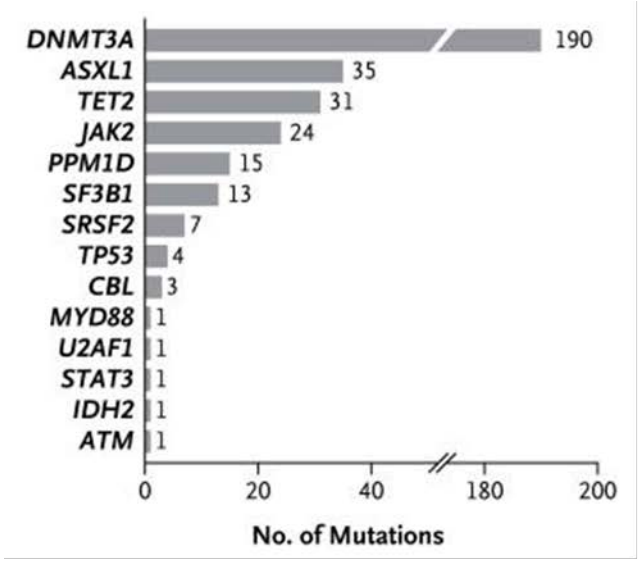
- **Can gene mutations be used as *de facto* evidence of MDS in the absence of dysplasia, like certain cytogenetic abnormalities?**
- **Problem: many otherwise healthy older adults carry low-level somatic mutations in the same genes (CHIP or ARCH).**

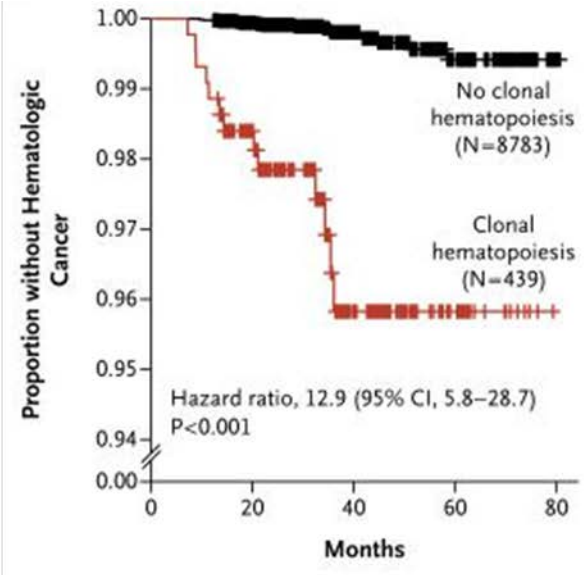


Jaiswal S, et al. *N Engl J Med.* 2014;371(26):2488-98.

Age-related Clonal Hematopoiesis

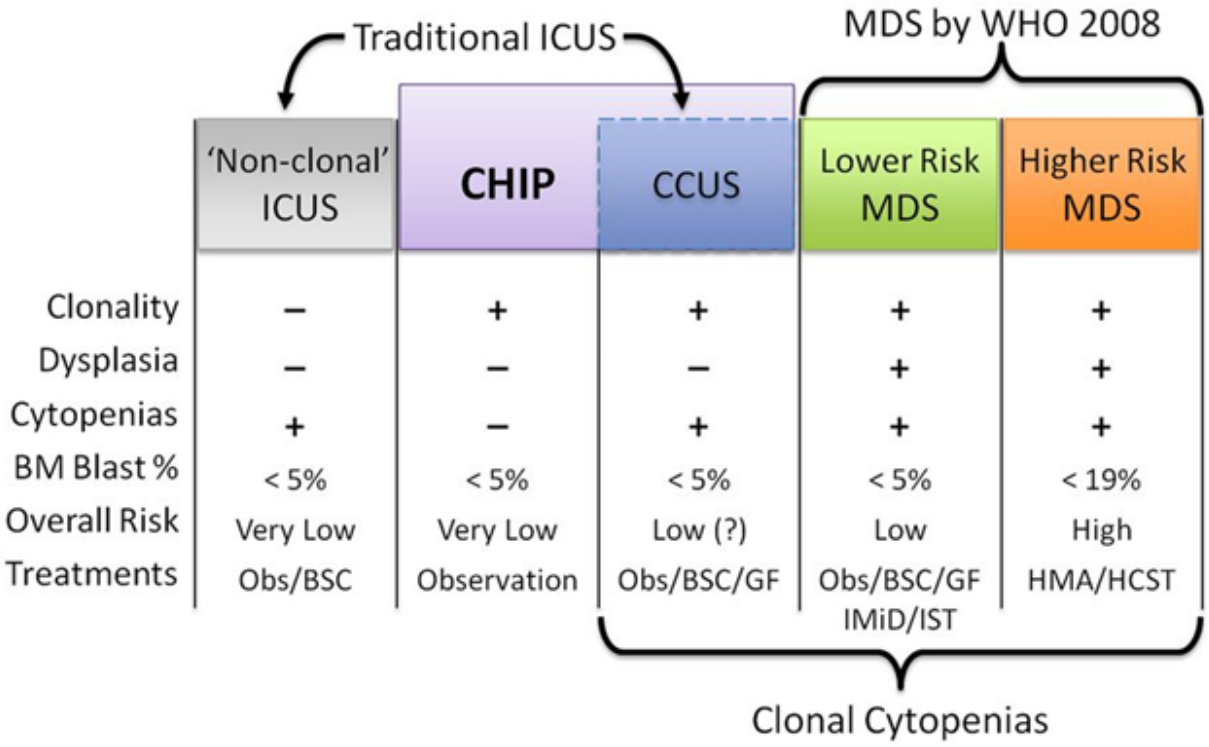
- **Present in ~10 of healthy patients >65.**
- **Mostly in genes commonly mutated in MDS.**
- **These patients are more likely to develop subsequent hematologic malignancy, and have greater risk of CVD.**





Genovese G. *N Engl J Med*. 2014;371(26):2477-87.
Jaiswal S, et al. *N Engl J Med.* 2014;371(26):2488-98.
Jaiswal S, et al. *N Engl J Med.* 2017;377(2):111-121

New Diagnostic Categories



Steensma DP, et al. *Blood*. 2015;126(1):9-16.
Kwok B, et al. *Blood*. 2015;126(21):2355-61.

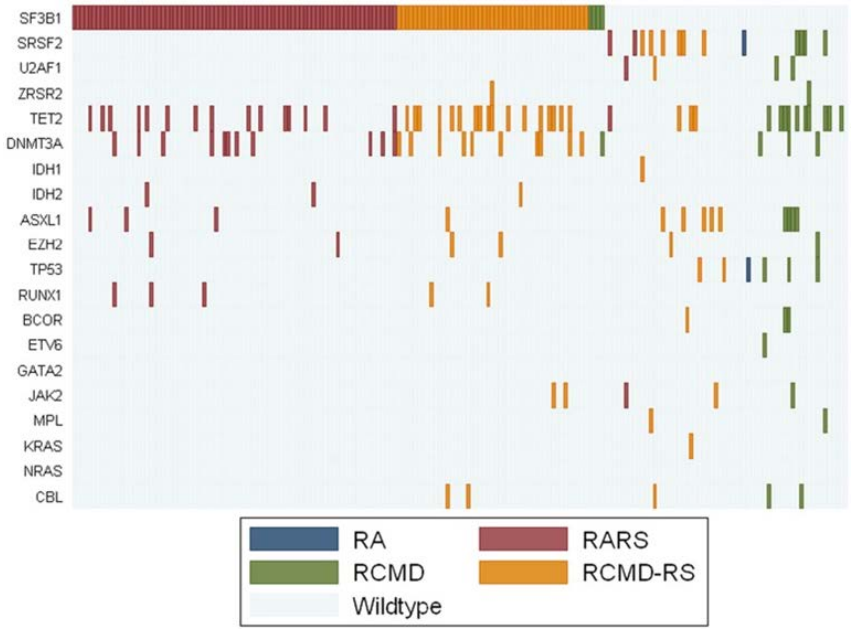
Gene Mutations and Diagnosis

- **Gene mutations cannot be used to make a diagnosis of MDS without dysplasia.**
- **Are there clues in the genotype that might predict increased likelihood of MDS?**
  - **Number of mutations: ≥2 mutations in 64% of pre-clinical MDS vs. 8% in CHIP; PPV for MDS of ≥2 mutations = 0.88.**
  - **Median variant allele fraction (VAF): 40% in pre-clinical MDS vs. 9-10% in CHIP; PPV for MDS of one mutation with ≥10% VAF = 0.86.**
  - **Particular genes: Mutations in spliceosome genes (*SF3B1, SRSF2, U2AF1*), *JAK2*, and *RUNX1* are most predictive of MDS, while mutations in *DNMT3A, ASXL1* or *TET2* are much less specific.**

Cargo CA, et al. *Blood.* 2015;126(21):2362-5.
Malcovati L, et al. *Blood.* 2017;129(25):3371-78.

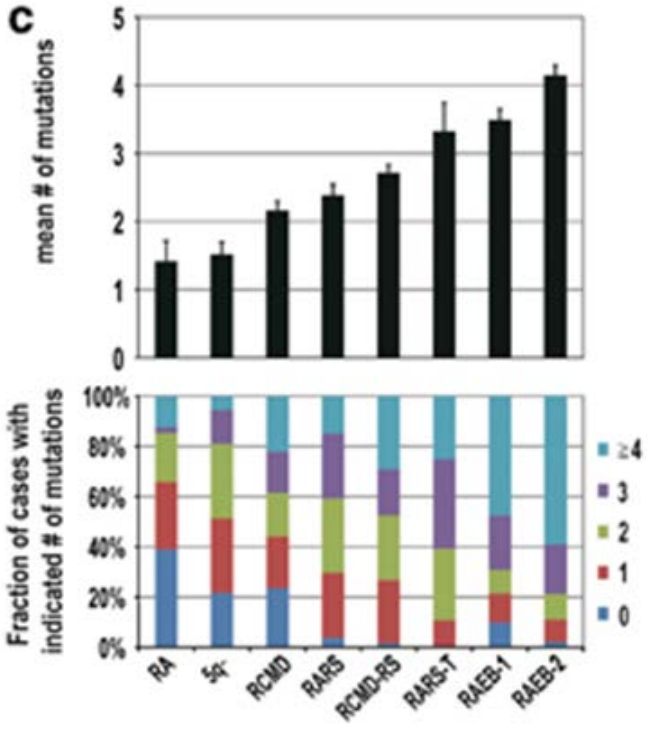
Morphologic Correlates

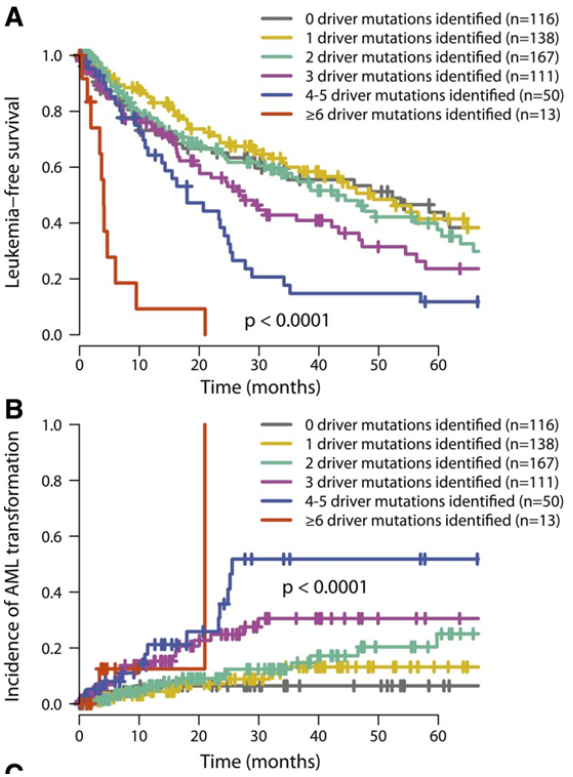
- **Mutations in *SF3B1* are highly associated with the presence of ring sideroblasts and more favorable prognosis.**
- **The 2016 WHO update reduced the through percentage of RS required to make a diagnosis from 15% to 5% in the presence of *SF3B1* mutations.**



Malcovati L, et al. *Blood.* 2015;126(2):233-41.
Papaemmanuil E, et al. *N Engl J Med.* 2011;365(15):1384-95.
Hasserjian RP, et al. *WHO Classification.* 2017:98

Gene Mutations and Prognosis

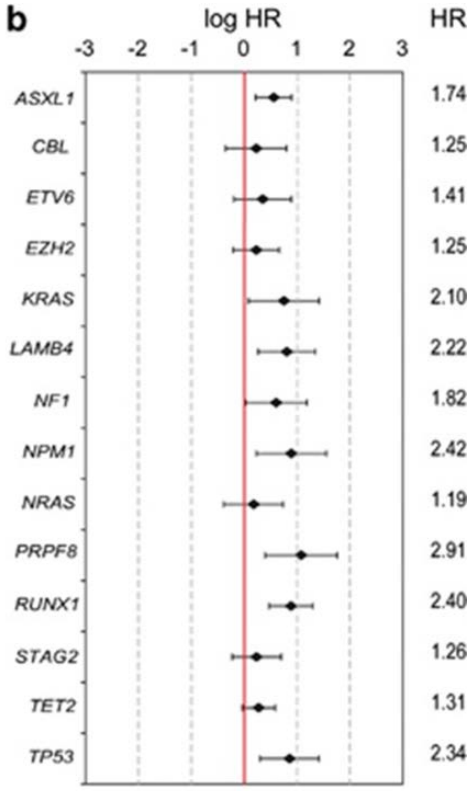




Haferlach T, et al. *Leukemia*. 2014;28:241
Papaemmanuil E, et al. *Blood*. 2013;122:3616

Gene Mutations and Prognosis

- **Mutations in particular genes impact prognosis:**
  - **Most consistently associated with poor outcome: *ASXL1*, *RUNX1,* and *TP53***
  - **In particular, *TP53* mutations often lead to genomic instability, complex abnormal karyotype, and high risk of progression to AML.**
  - **Patients with *SF3B1* mutations have better outcomes.**



Haferlach T, et al. *Leukemia*,. 2014;28:241
Bejar R, et al. *J Clin Oncol*. 2012;30:3376
Papaemmanuil E, et al. *Blood*. 2013;122:3616

Case Study Patient

- **SF3B1 p.Lys700Glu (47%)**
- **DNMT3A p.Arg882Pro (46%)**
- **RUNX1 p.? (44%)**
- **BCORL1 p.Ser706* (14%)**

Ring sideroblasts

RUNX1 +
4 mutations =
poor prognosis?

Patient rapidly progressed: bone marrow biopsy 4 months later showed increased blasts. Referred to hospice and passed away a few weeks later.

Summary – Part 1

- MDS is frequently associated with recurrent clonal somatic genetic abnormalities, detectable by karyotype and/or molecular studies.
- These have some utility in MDS diagnosis:
  - Certain clonal cytogenetic abnormalities can indicate MDS even in the absence of dysplasia.
  - Gene mutations can help diagnostically, but should be interpreted with caution due to age-related clonal hematopoiesis.
- Particular cytogenetic and molecular diagnostic findings are associated with distinctive morphologic findings and predict clinical outcomes.

Part 2: Genetic Testing in the Management and Therapy of Myelodysplastic Syndrome

Michael Savona, MD

Use of Molecular Testing in Clinical Care of MDS

- **Establishment of clonality and prognosis to guide therapy**
- **Targeted agents (clinical trials)***

- **?Future? Guided targeted therapy, and refinement of understanding of disease evolution**

Clonality and Prognosis and Molecular Lesions

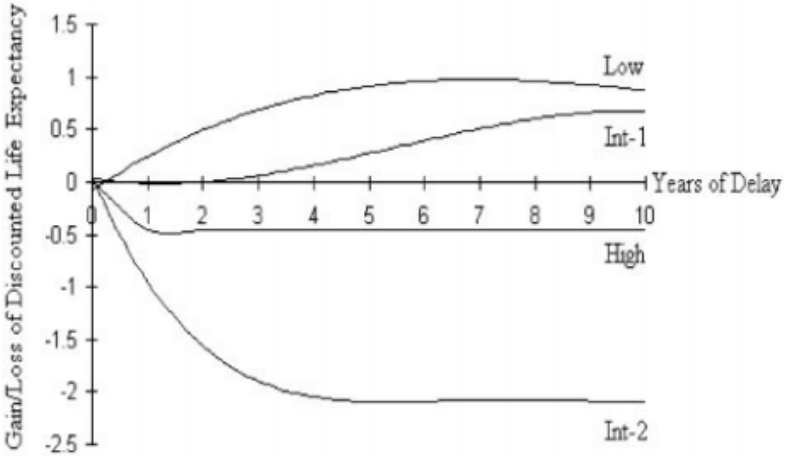
- **Most mutations have negative influence on prognosis in MDS**
  - ***SF3B1* (isolated) is associated with MDS-RS /favorable risk MDS**
  - **Combinatory influences are TBD (eg, *SF3B1+RUNX1*)**
- **Poor risk mutations often occur at loci shared in CHIP - so what does that mean?**
  - **Large majority of CHIP does not evolve to neoplasia**
  - **However, presence of CCUS with higher risk mutations changes surveillance**

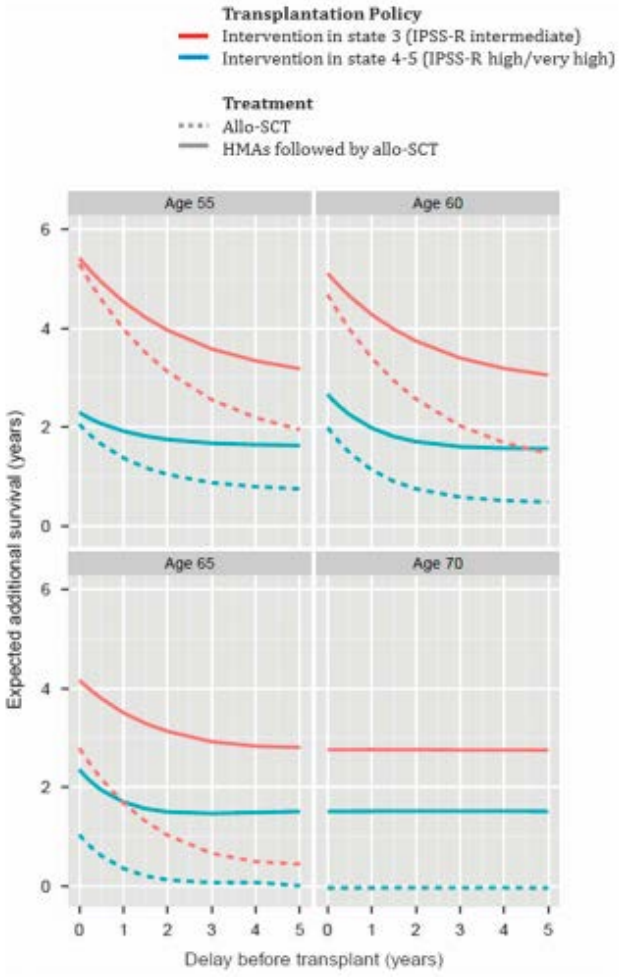
Allogenic Hematopoietic Stem Cell Transplant and Molecular Lesions

- **Usually, increased mutations occur in correlation to complexity of cytogenetic lesions and/or increased blasts (but not always)**
- **Number of mutations, high risk mutations account for high risk disease**
  - **Should this lead to HSCT?**
  - **Should mutations disqualify from HSCT?**

Decision Analysis in MDS is Largely Based on IPSS

- **Biased by deficiencies of IPSS**
- **If higher risk MDS (by IPSS) is enriched for mutations → logically, transplant patients with high risk defined by mutations**





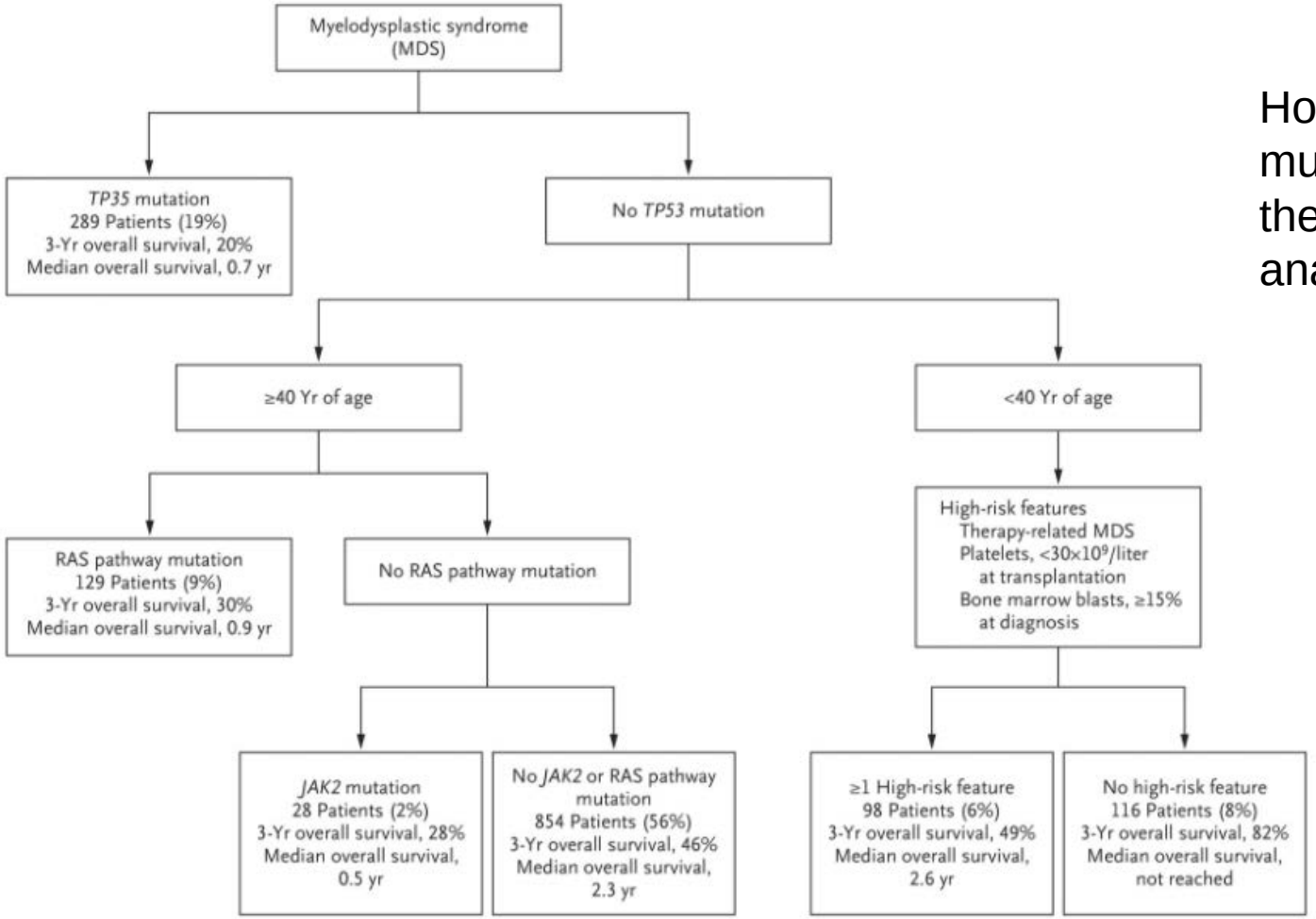
Cutler et al. *Blood*. 2004
Della Porta et al. *Leukemia*. 2017

Mutations added to AlloHSCT Decision analysis

- **Higher IPSS Risk may be associated with worse molecular profile, but not always**
- **As mentioned, shorter survival is associated with:**
  - **More mutations**
  - **Allele burden**
  - **High risk mutations**

Haferlach T, et al. *Leukemia*,. 2014;28:241
Bejar R, et al. *J Clin Oncol*. 2012;30:3376
Papaemmanuil E, et al. *Blood*. 2013;122:3616

Isolated High Risk Mutations Imply Miserable alloHSCT Outcomes

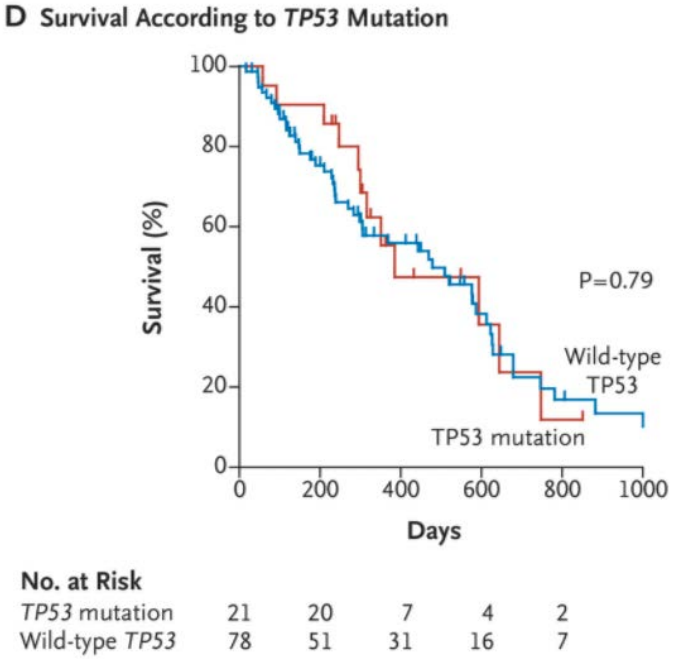


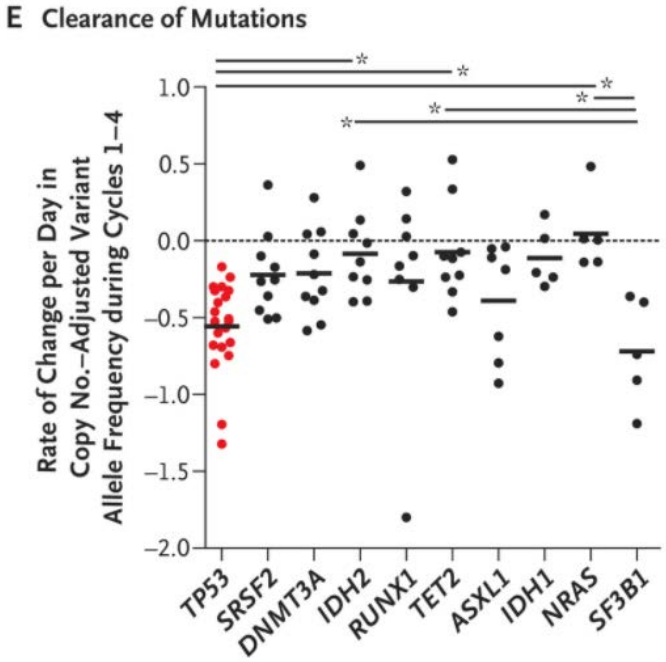
How to add mutational status to the HSCT decision analysis?

Lindsley et al, *NEJM*. 2017.

TP53 Mutations Respond to Decitabine*10days

- **Phase 2 – 84 patients**
- **21/21 patients (100%) with *TP53* mutations with mCR/CR**





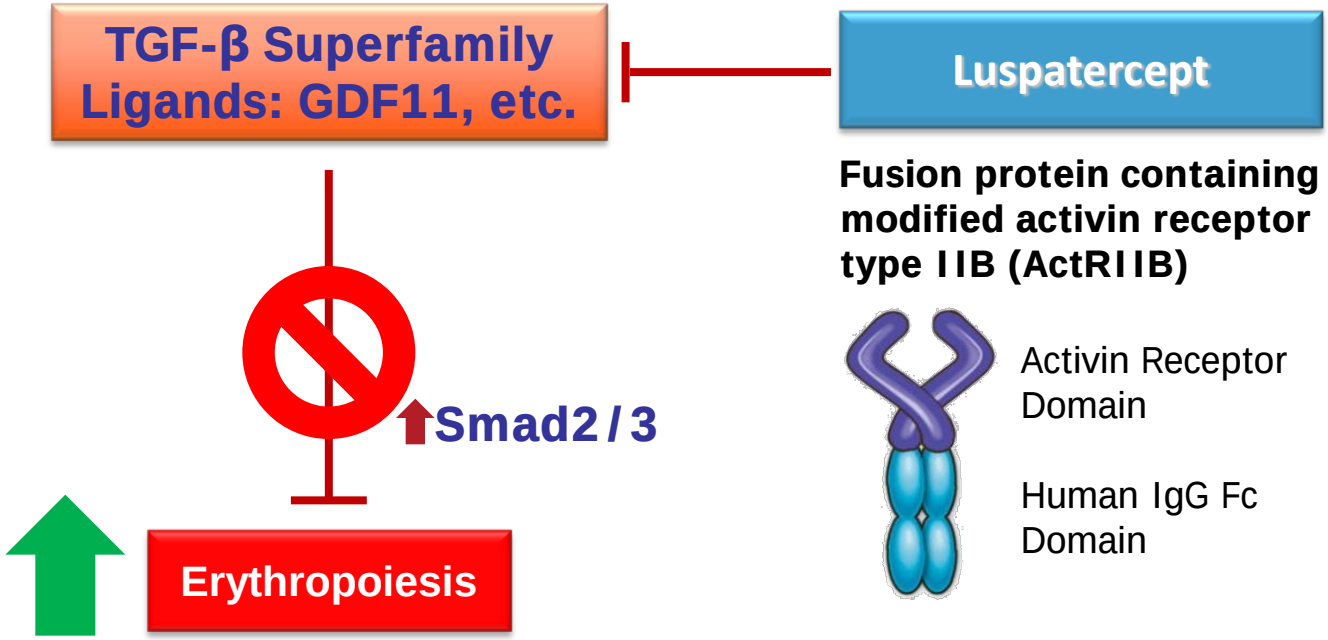
Welch et al, *NEJM*. 2016.

The Future

SF3B1 – Targeting "Good Risk"

- ***SF3B1* mutations are associated with RARS and favorable risk MDS, but are associated with chronic anemia, transfusion dependence, iron overload, and diminished quality of life**
- **Luspatercept / Sotatercept TGFb ligand trap analogues**

Luspatercept in MDS

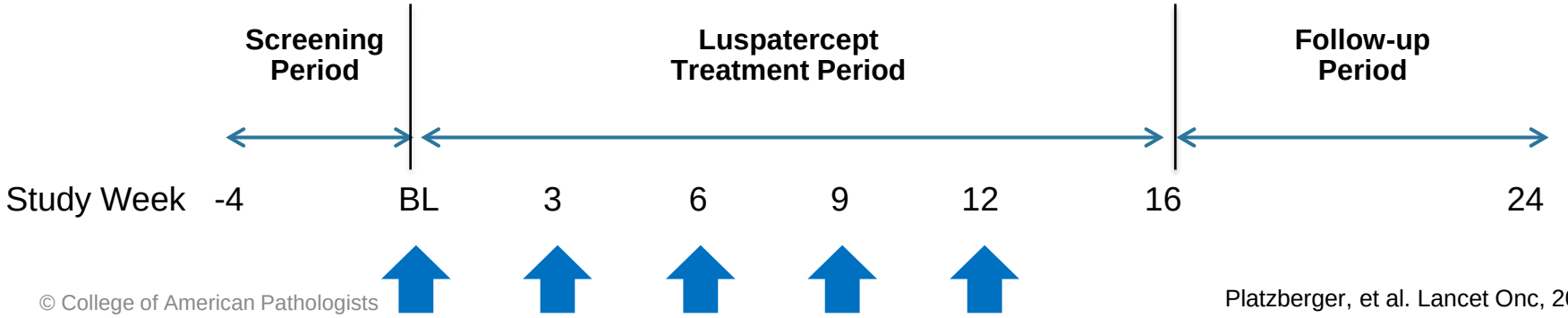


- **Mechanism is distinct from erythropoietin**
- **Acts on late-stage erythropoiesis to increase mature RBCs in the circulation**

Suragani R, et al. Nature Med 2014
Zhou L, et al., Blood 2008

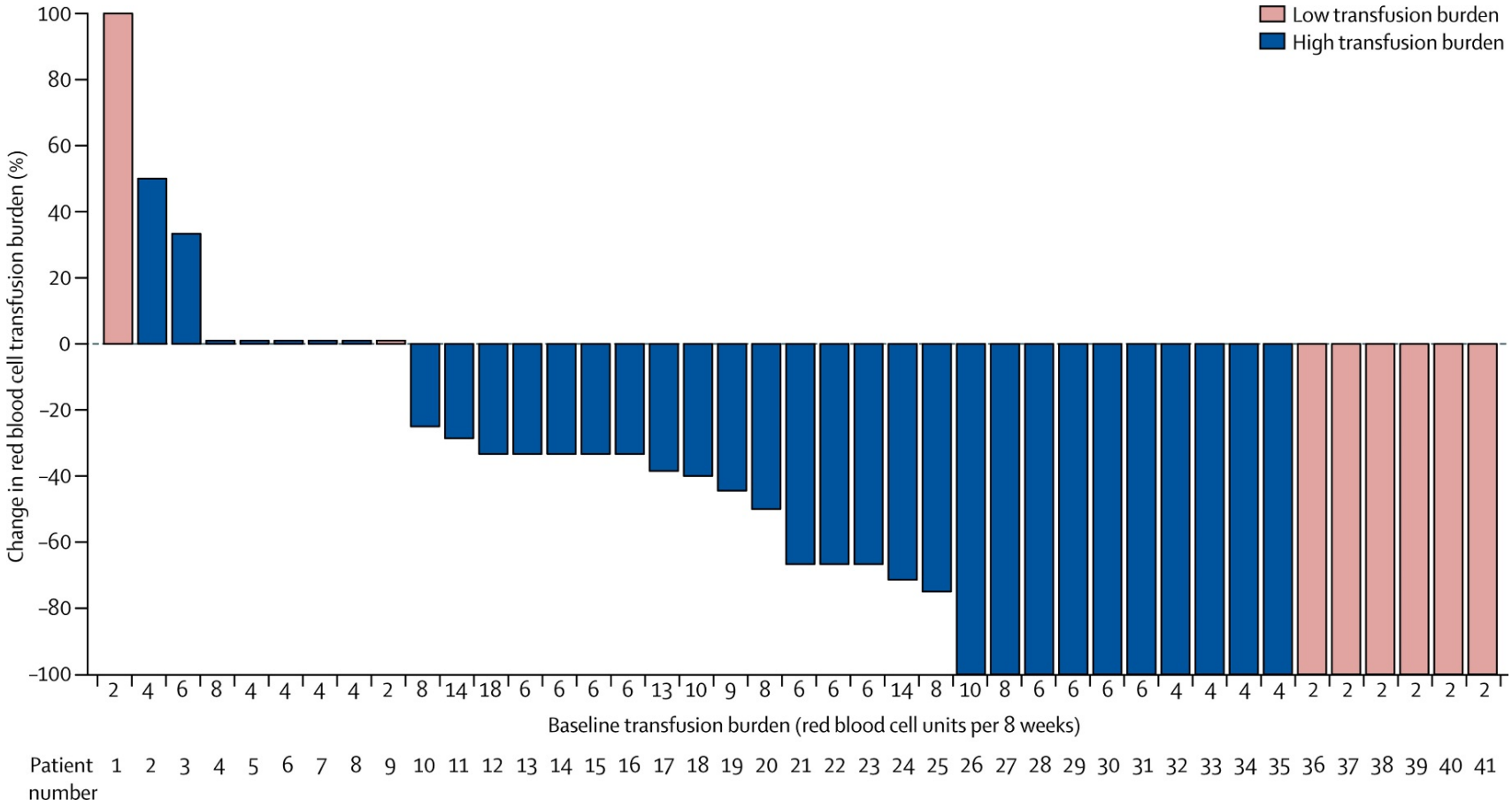
Luspatercept PACE-MDS Study Overview

- Phase 2, multicenter, open-label, dose-finding study in IPSS low/int-1 MDS
- **Eligibility criteria:** EPO >500 U/L or nonresponsive/refractory to ESA; no prior azacitidine or decitabine; no current lenalidomide, ESA, G-CSF
- **Primary efficacy endpoints**
  - Low Transfusion Burden (LTB, <4U RBC/8 weeks, Hgb <10 g/dL): Hemoglobin increase of ≥ 1.5 g/dL for ≥ 2 weeks
  - High Transfusion Burden (HTB, ≥4U RBC/8 weeks): Reduction of ≥4U or ≥50% units transfused over 8 weeks
- Luspatercept administered SC every 3 weeks for 3 months (base study→ extension



Platzberger, et al. Lancet Onc, 2017.

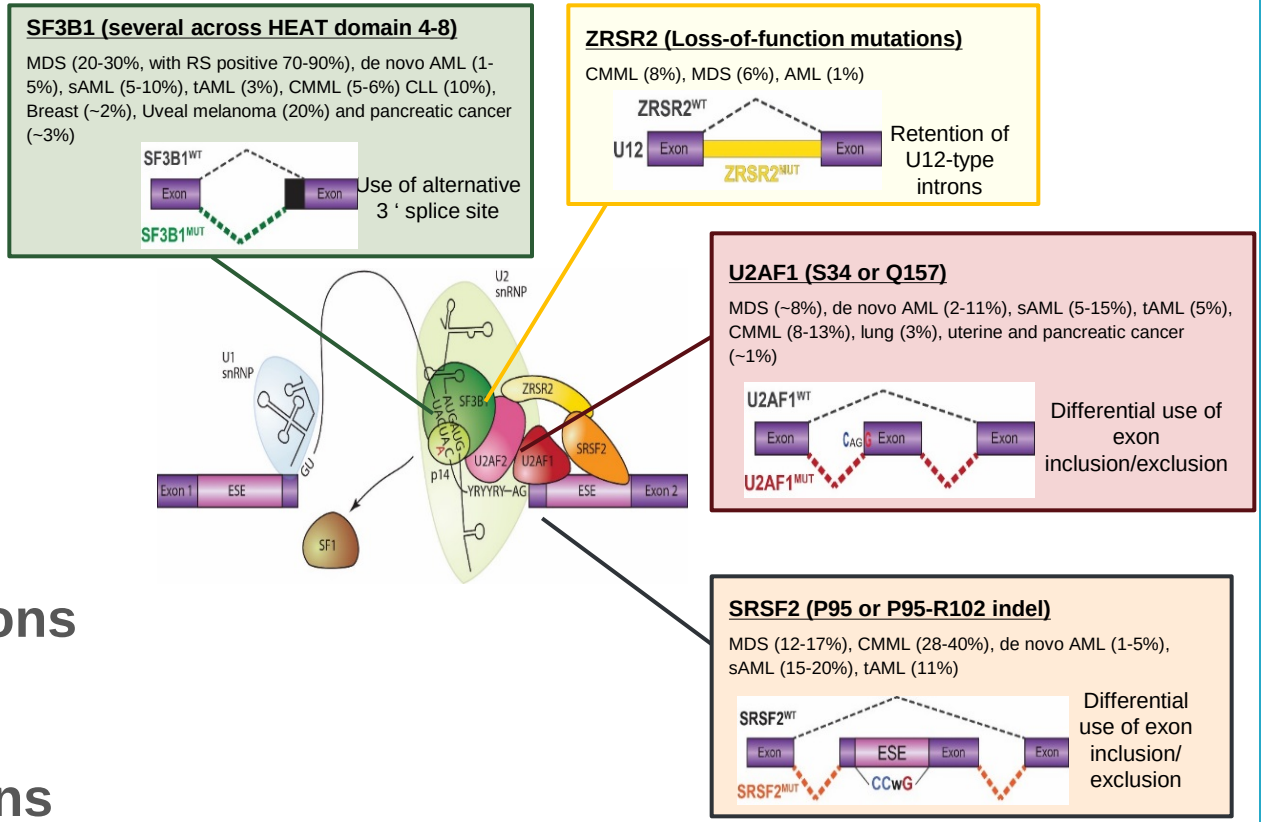
MAXIMUM PERCENTAGE CHANGE IN RBC TRANSFUSION BURDEN



Platzberger, et al. Lancet Onc, 2017.

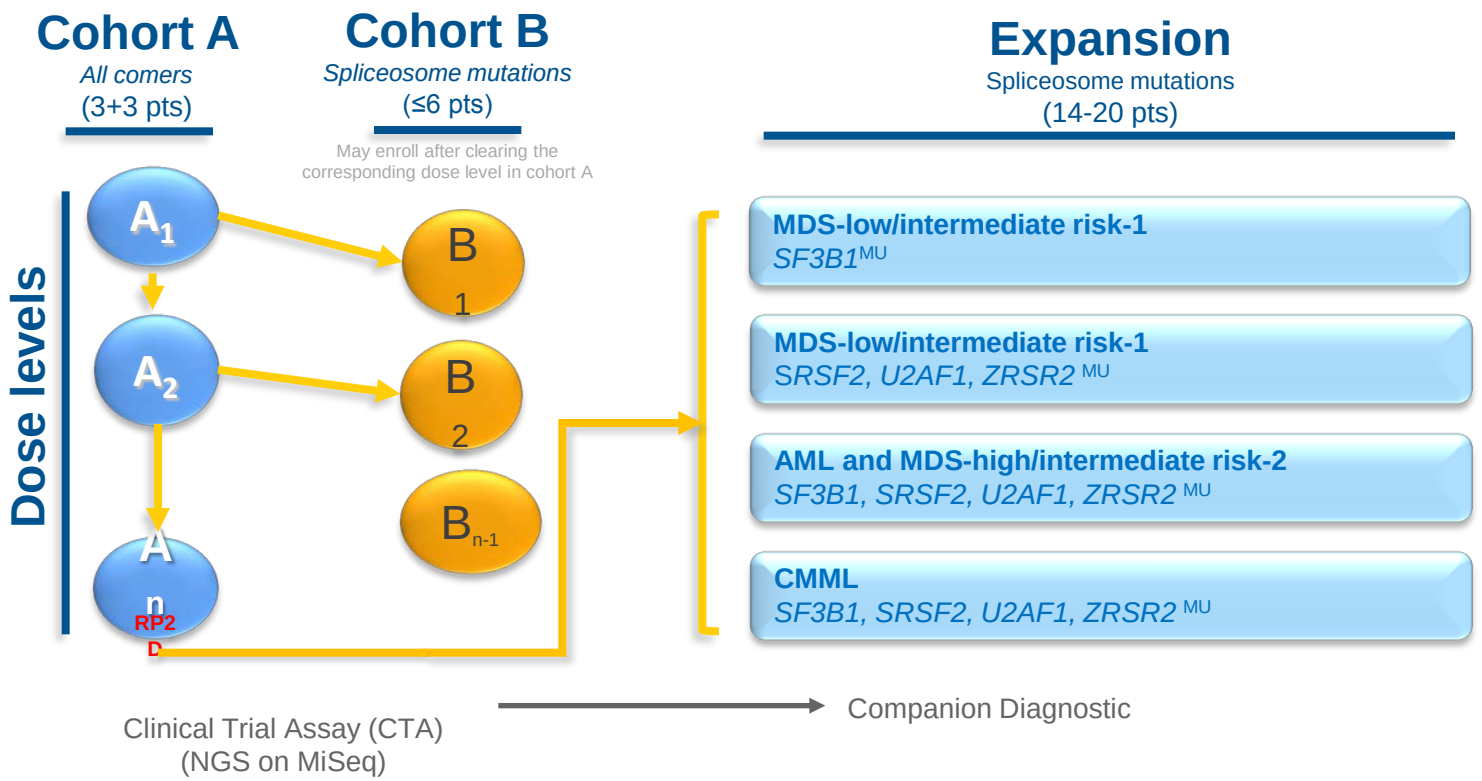
Addressing Spliceosome Malfunction

- **Recurrent mutations observed in *SF3B1, U2AF1, SRSF2* and *ZRSR2***
- **Heterozygous mutations & mutually exclusive**
- **Spliceosome mutations cause aberrant splicing**



Seileret al. Nature Med2018.

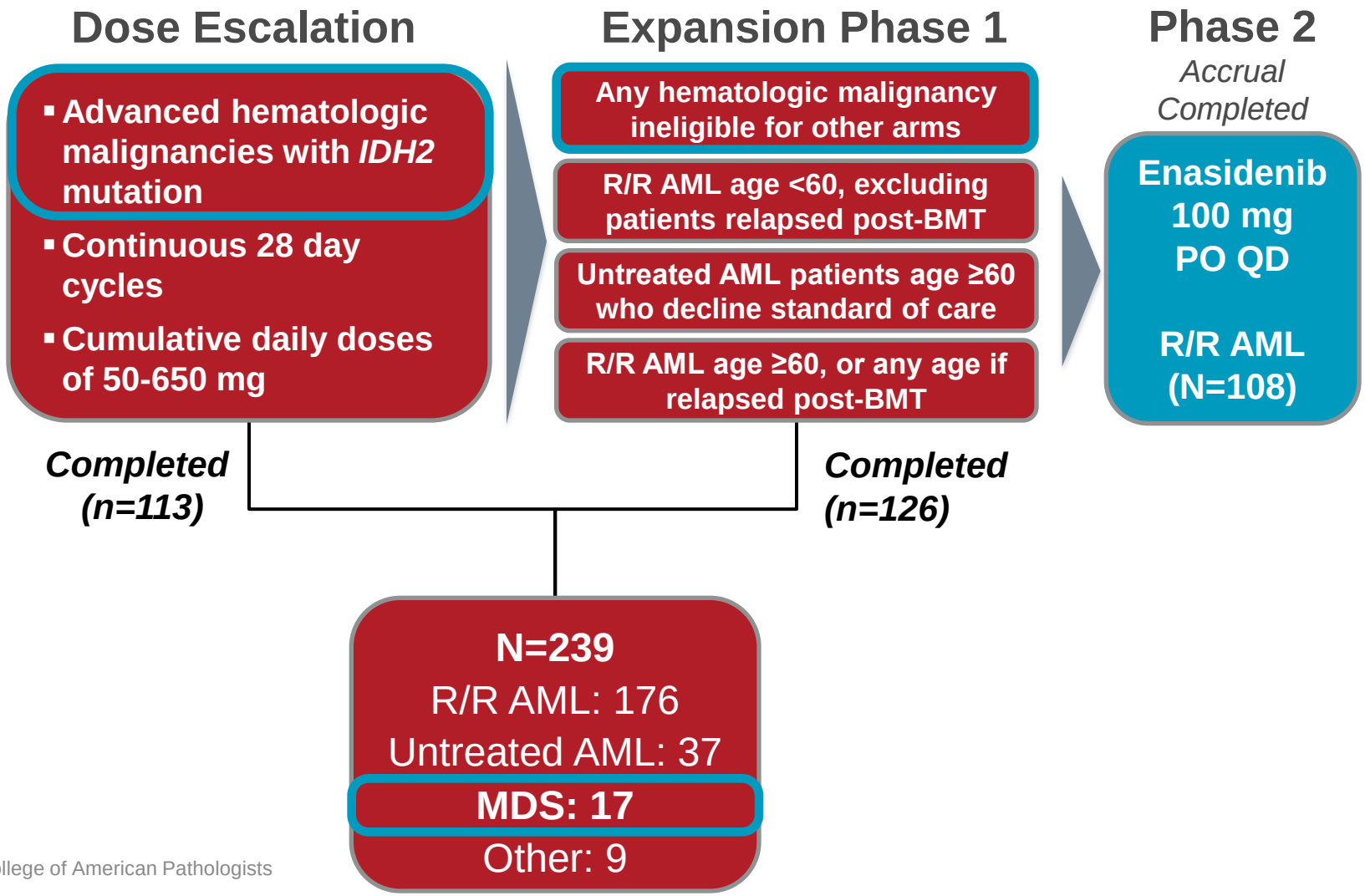
H3B-8800 Phase I Study



- **Eligibility**: Cohort A - AML, MDS, CMML; Cohort B – spliceosome mutant.

Buonamici et al. ASH 2016; abstract 966

Enasidenib (IDH2 inhibitor) Phase 1/2 Dose-escalation and Expansion



Response

	MDS Patients (N=17) n/N (%)
Overall response rate (CR + PR + mCR + HI)	10/17 (59)
Best Response	
Complete remission*	1/11 (9)
Partial remission*	1/11 (9)
Marrow CR*	3/11 (27)
Any hematologic improvement (HI)†	5/17 (29)
HI-E	3/15 (20)
HI-P	4/12 (33)
HI-N	4/10 (40)

*Investigator-assessed; evaluable patients had ≥5% bone marrow blasts at baseline

†HI was programmatically adjudicated per IWG 2006 criteria for MDS; denominators reflect eligibility for response

CR, complete remission; PR, partial remission; mCR, marrow CR; HI, hematologic improvement

- **Of 13 patients who had received prior HMA therapy, 7 (54%) had a response with enasidenib**
- **Of patients who attained HI, 2 had trilineage and 2 had bilineage improvement**
- **Median time to response was 21 days (range 10-87)**

Summary – Part 2

- Currently, the mutational profile of MDS has some impact on patient management:
  - Surveillance in patients with CCUS
  - Prognosis, risk of progression, and transplant decision
  - Decitabine in patients with TP53 mutations
- The future holds promise for targeting pathways involved in the pathogenesis of MDS

Questions?

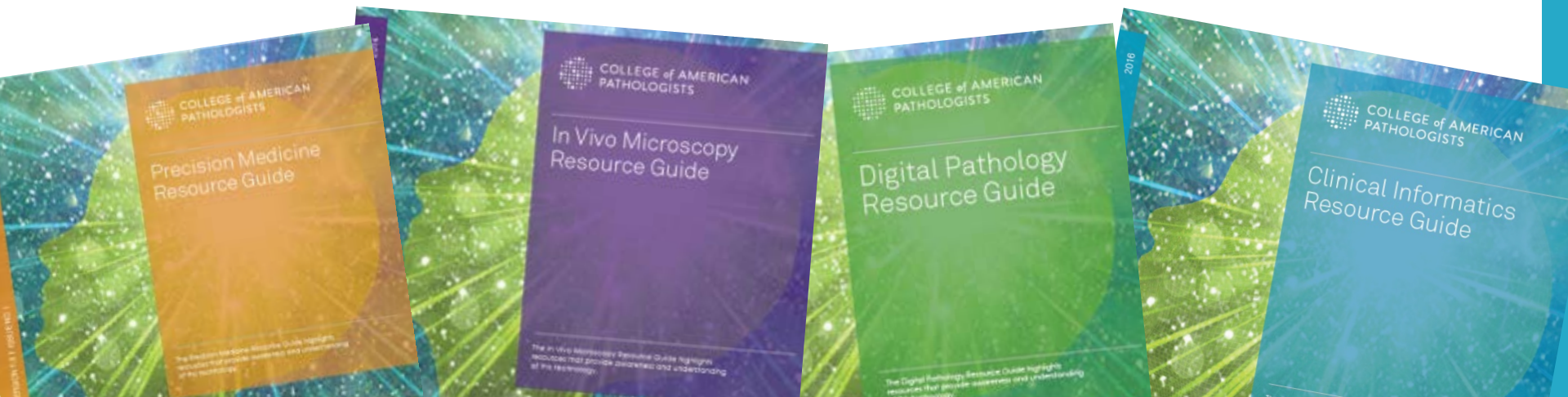
Register for Upcoming Webinars

DATE	TOPIC	SPEAKER(s)
May 2, 2018	HPV Testing on Head and Neck Carcinomas: A Review of the CAP Guidelines	Justin Bishop, MD
June 13, 2018	New Guideline for Lung Cancer Biomarker Testing: Essentials and Applications	Philip Cagle, MD & Eric Bernicker, MD

Register for upcoming & archived webinars:
www.cap.org > Calendar > Webinars > Previous

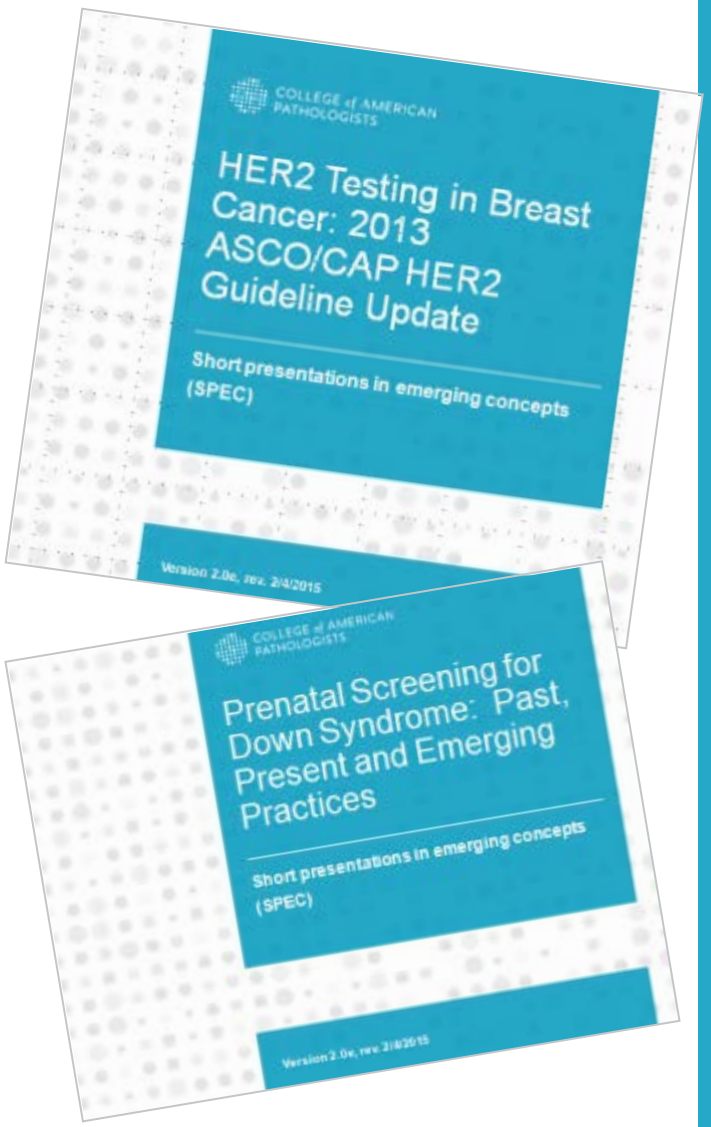
CAP's Pathology Resource Guide: Precision Medicine

- **The CAP has created the Pathology Resource Guides to assist pathologists in understanding key emerging technologies.**
  - **Printed guides are now available for members ($39) and non-members ($69)**
  - **The digital copy of the Resource Guides are a complimentary member benefit**
  - **Access them www.cap.org > Resources and Publications**



Short Presentations on Emerging Concepts (SPECS)

- **Pathology SPECs are:**
  - **short PowerPoints, created for pathologists**
  - **Focused on diseases where molecular tests play a key role in patient management**
- **Topics include Renal Tumors, cell free DNA (cfDNA), and PD-L1 as well as other emerging topics**
- **Access them www.cap.org > Resources and Publications**



capfoundation

seetest&treat

See, Test & Treat® brings cancer screenings to women in need!

- See, Test & Treat is a CAP Foundation-funded program that brings free, same-day cervical and breast cancer screening, diagnoses and follow-up care to women in medically underserved communities across the U.S.

- CAP member pathologists' partner with gynecologists, radiologists and other medical professionals to lead See, Test & Treat programs in hospitals, clinics and other facilities

- Women learn the importance of preventive care through annual exams, a Pap test, Mammogram and a healthy lifestyle

See, Test & Treat Needs Your Financial Support

Visit foundation.cap.org and click on DONATE!

THANK YOU!

Thank you for attending our webinar,

“Molecular Diagnostic Testing in Myelodysplastic Syndrome” by

Adam Seegmiller, MD, PhD &

Michael Savona, MD

For comments about this webinar or suggestions for upcoming webinars, please contact phcwebinars@cap.org.

NOTE: There is no CME/CE credit available for today’s free webinar. The PDF of the presentation will be sent out in a week.

COLLEGE of AMERICAN PATHOLOGISTS