Practical Interpretation of Molecular Testing in Myeloid Neoplasms

Annette S. Kim, MD, PhD
Associate Professor, Harvard Medical School
Brigham and Women's Hospital
Boston, MA

Mass General Brigham

HARVARD MEDICAL SCHOOL

Disclosure of Relevant Financial Relationships

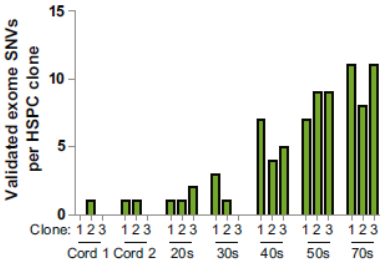
Annette S. Kim, MD, PhD reported the following relevant financial relationship(s) during the content development process for this activity:

***Consultant*, LabCorp, Inc.**
***Consultant*, Quanterix, Inc.**
***Consultant*, Papgene, Inc.**
***Research funding,* Multiple Myeloma Research Foundation**

Passengers

Clonal Hematopoiesis of Indeterminate Potential

Passengers: Schuff happens… with age to all of us



•Approximately 20 mutations acquired each year of life, a fraction of which are in exons.

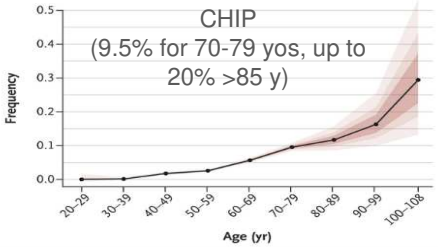
x = variable number of passenger mutations

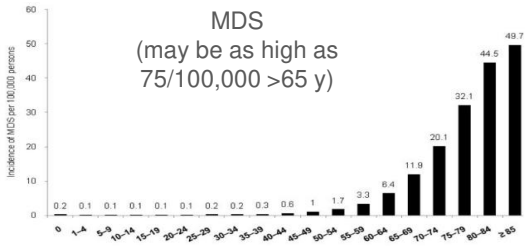
Welch *et al. Cell* (2012) 150:246-278.

CHIP- The Usual Suspects

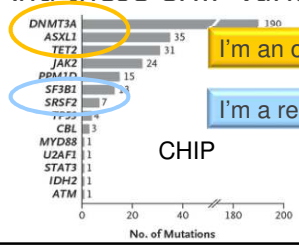
Clonal Hematopoiesis of Indeterminate Potential

An age-related increase in mutations found in individuals with no evidence of hematologic malignancies





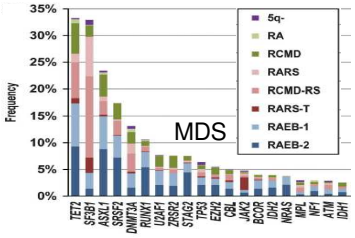
…And these CHIP variants are also commonly found in MDS



I'm an old stem cell…

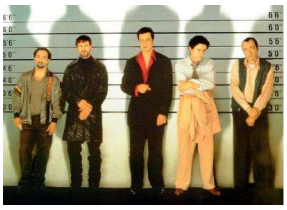
I'm a really old stem cell…

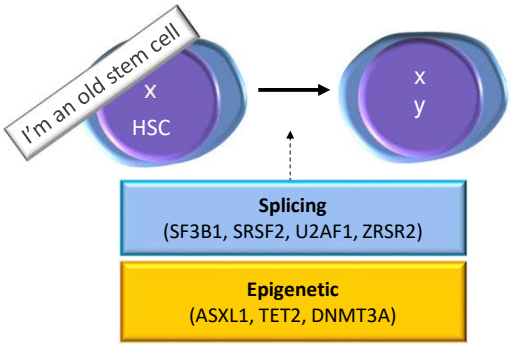
CHIP



Cogle *et al. Blood.* 2011;**117**: 7121-7125.
Ma *et al. Am J Med.* 2012;**125**:S2-S5.
Jaiswal *et al. NEJM.* 2014;371: 2488-2498.
Genovese *et al. NEJM.* 2014;371: 2477-2487.
Xie *et al. Nat Med.* 2014;20: 1472-1478.
Steensma *et al. Blood.*2015;126: 9-16.
Haferlach *et al. Leukemia.* 2014;28: 241-247.
Matsunawa *et al.* Leukemia. 2014;28:1844–1850.

Founding Drivers

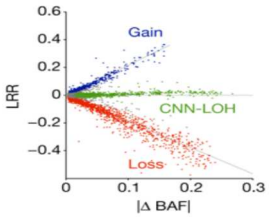




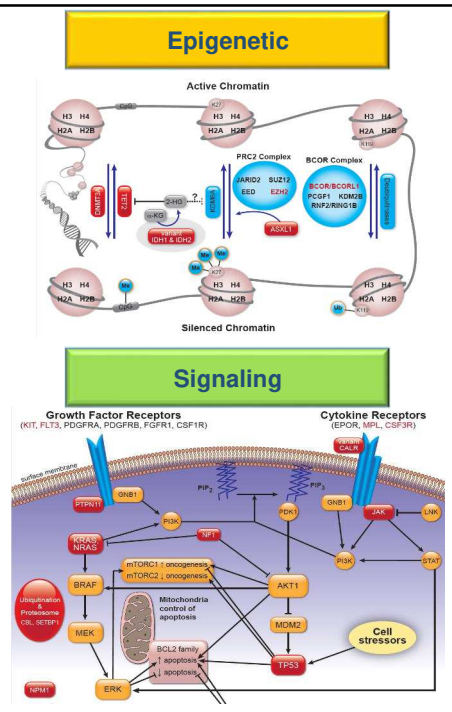
x = variable number of passenger mutations
y = founder (driver) mutation(s)

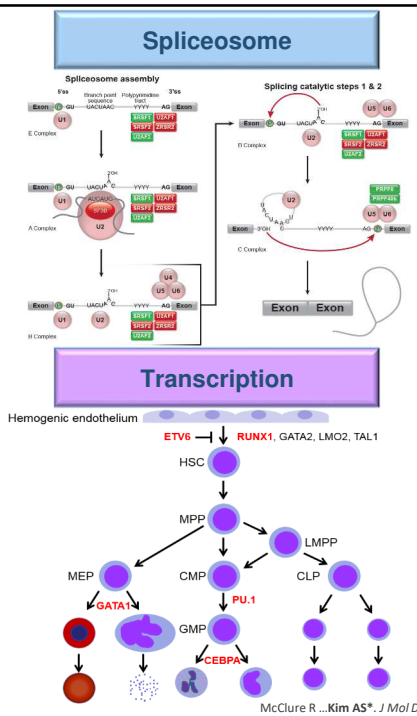
Passengers and Founding Drivers

- Genes involved: typical drivers in myeloid neoplasms, but also TP53 mutations and CNVs (and TERT predisposition?)
- Can wax and wane, typically single, low VAF
- May provide a competitive stem cell advantage



McClure R ...**Kim AS***. *J Mol Diagn*. 2018;20(6):717-737.
Loh *et al. Nature*. 2018;**559**:350-355.
Zink *et al. Blood*. 2017;**130**:742-752.

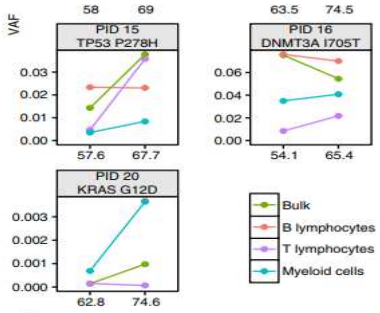


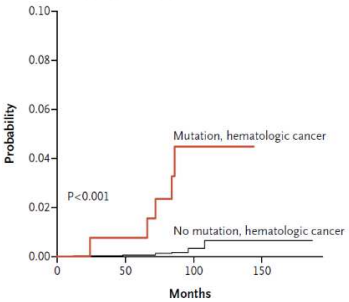


McClure R ...**Kim AS***. *J Mol Diagn*. 2018;20(6):717-737.

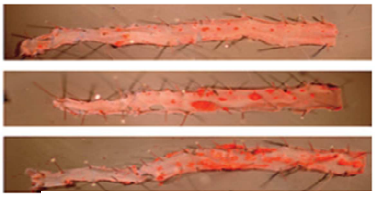
What do you mean that CHIPs aren't good for your heart?

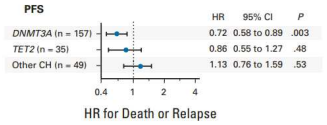
- CHIP can be found in all cellular compartments
- The presence of CHIP is associated with coronary heart disease and ischemic stroke
- CH is associated with RDW and subsequent HM (0.5-1%/y)
- Donor CH associated with donor cell leukemia, GVHD, and maybe GVL?





TET2 KO mice models:

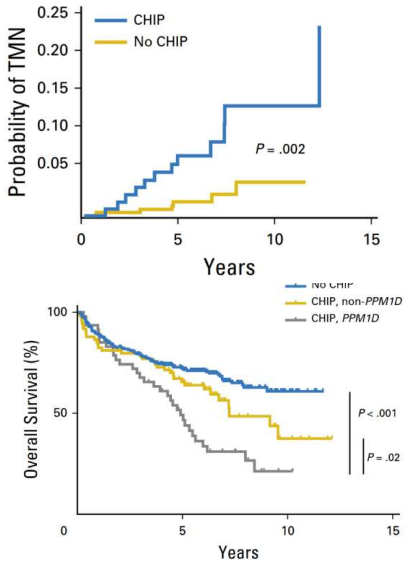


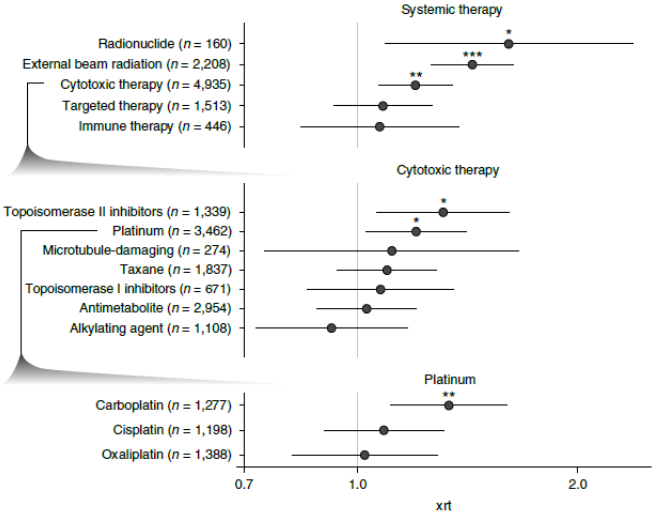


Jaiswal *et al. Nat Med.* 2014;**371**: 2488-2498; Jaiswal *et al. NEJM.* 2017;377: 111-121; Genovese *et al. NEJM.* 2014;**371**: 2477-2487; Xie *et al. Nat Med.* 2014;**20**: 1472-1478.
Young *et al. Nat Commun.* 2016;**7**:12484; Fink *et al. JCO.* 2018;37:375-385; Bolton, et al. *medRxiv.* 2020 Nov 27;2020.11.25.20233163.Gibson *et al. J Clin Oncol,* 2022;40(2):189-201.

CHIP Before Therapy

Cancer survivors have 4-fold increased risk of CHIP, associated with risk of t-MN!

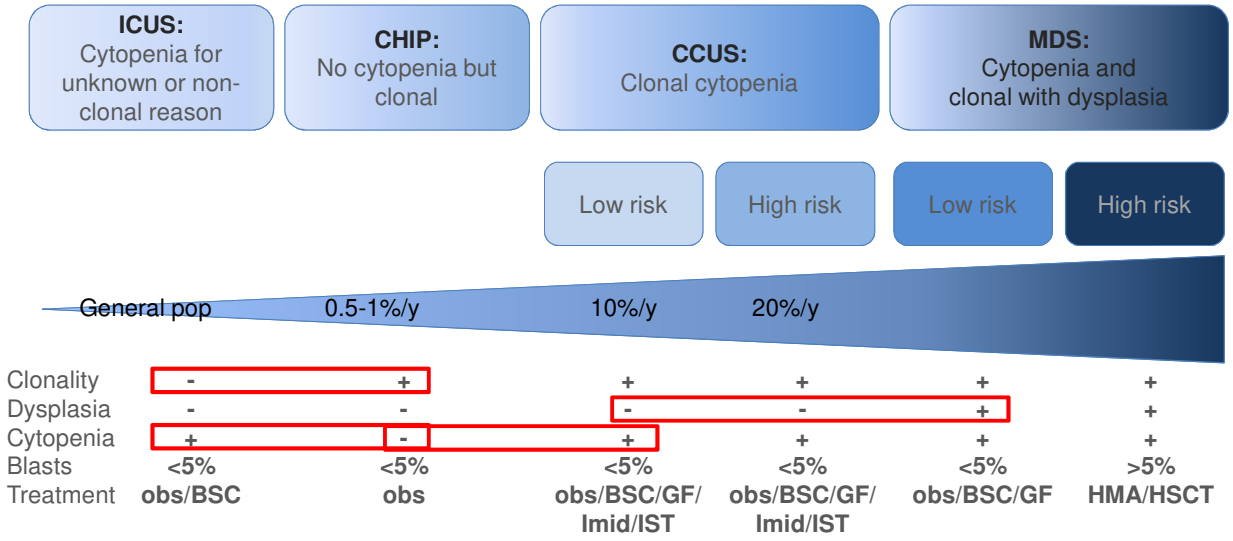




Olszewski…**Kim AS.**.*et al. BJH.* 2019;186(3):e31-e35.
Gibson *et al. JCO* (2017)2017;35:1598-1605.
Gillis *et al. Lancet Oncol.* 2017 Jan;18(1):112-121.
Bolton *et al. Nat Genet.* 2020 Nov;52(11):1219-1226.

ICUS, UCUS, we all CCUS…

Idiopathic cytopenias of uncertain significance, clonal cytopenias of uncertain significance…



Adapted from Steensma *et al. Blood.* 2015;126: 9-16 and Malcovati et al. *Blood*. 2017;22:3371-3378.

Cases 1 and 2: A Tale of Two Cytopenias

- 61 yo F presenting with pancytopenia
  - History of sarcoid and triple positive IDC dx 2011, s/p chemoradiation + Herceptin
  - 2.82 > 10.3 (92.4) < 83; ANC 1.80, no blasts
  - PB RHP:

No Pathogenic Variants Detected

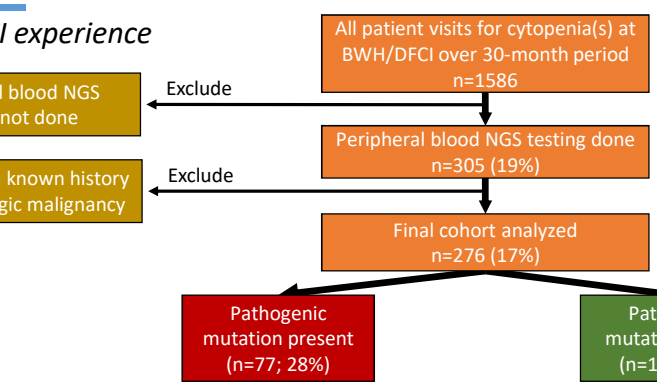
How are clinicians using NGS data from PB?

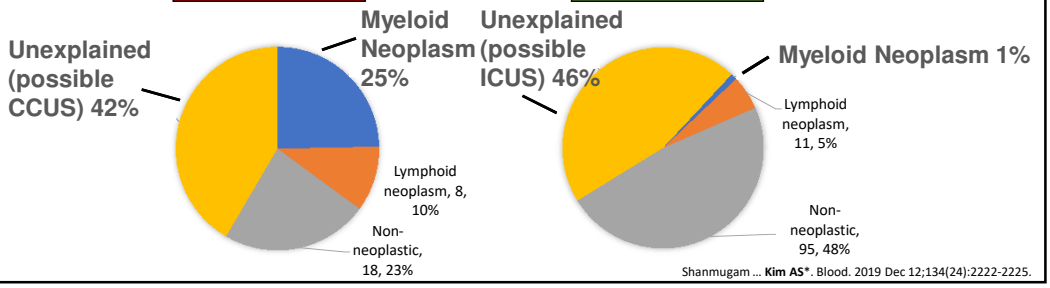
- 73 yo F with presenting with pancytopenia
  - History of uterine carcinosarcoma dx 2009, s/p chemoradiation
  - 2.52 > 8.4 (65.6) < 52; ANC 0.73, no blasts
  - PB RHP:

Somatic Variant			Variant allele fraction
Gene	Variant (c.)	Variant (p.)	Dx
DNMT3A	c.1933A>G	p.T645A	5.9%
TET2	c.4393C>T	p.R1465*	5.2%
TP53	c.831T>A	p.C277*	29.4%
CNVS: loss 3p, loss 7p, loss 7q			

Lack of mutations predict absence of **CURRENT** myeloid dz

The BWH/DFCI experience





Shanmugam … **Kim AS***. Blood. 2019 Dec 12;134(24):2222-2225.

Is bigger better?

The BWH/DFCI experience

- Negative predictive value in cases with a concurrent BMbx was 95% with a 95 gene panel
- Negative predictive value in cases with a concurrent BMbx was 95% ***with a 20 gene panel***

Gene	N. of cases with mutation	95-gene panel	22-gene panel	20-gene panel	15-gene panel	10-gene panel	5-gene panel
TET2	9	x	x	x	x	x	x
DNMT3A	9	x	x	x	x	x	x
SRSF2	7	x	x	x	x	x	x
ASXL1	8	x	x	x	x	x	x
SF3B1	4	x	x	x	x	x	x
U2AF1	3	x	x	x	x	x	
TP53	3	x	x	x	x	x	
GNAS	2	x	x	x	x	x	
SH2B3	2	x	x	x	x	x	
IDH2	2	x	x	x	x	x	
PHF6	1	x	x	x	x		
SETD2	1	x	x	x	x		
NRAS	1	x	x	x	x		
JAK2	1	x	x	x	x		
GNB1	1	x	x	x	x		
NPM1	1	x	x	x			
WT1	1	x	x	x			
IDH1	1	x	x	x			
ATM	1	x	x	x			
STAT3	1	x	x	x			
KIT	1	x	x				
RUNX1	1	x	x				
Other genes	0	x					
NPV (95% CI)		95% (83%-99%)	95% (83%-99%)	95% (83%-99%)	93% (82%-97%)	93% (82%-97%)	89% (79%-95%)
PPV (95% CI)		58% (46%-68%)	58% (46%-68%)	58% (46%-68%)	60% (47%-72%)	60% (47%-72%)	62% (47%-74%)

These patients DO NOT need a BMBx!

Shanmugam … **Kim AS***. Blood. 2019 Dec 12;134(24):2222-2225.

Using NGS to Predict a **FUTURE** Myeloid Neoplasm

CCUS Progression

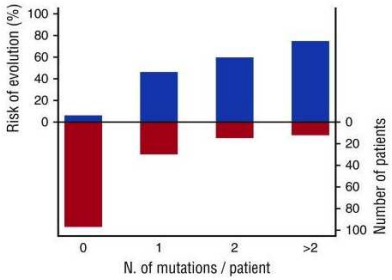
- How many mutations matter

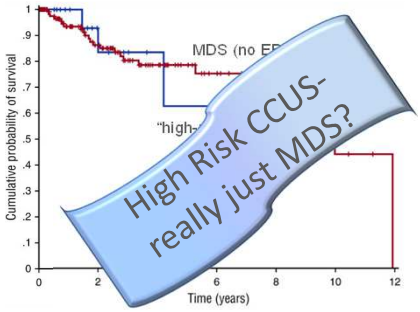
  ≥2 genes PPV 0.88, OR 4.69

- How much of the mutation matters

  ≥ 0.087 VAF PPV 0.86

- Which mutation(s) matters
  - Spliceosome genes, *JAK2*, and *RUNX1* mostly highly a/w MN
  - *DNMT3A, TET2, ASXL1 (DTA genes)* (and *PPM1D**) most often co-occur with other mutations, resulting in high PPV for MN
  - Spliceosome, *DNMT3A, TET2,* and *ASXL1* account for 73% of MNs
  - *SF3B1* alone has OR 4.83 of MN
  - Not *DNMT3A* alone (more CH-like)





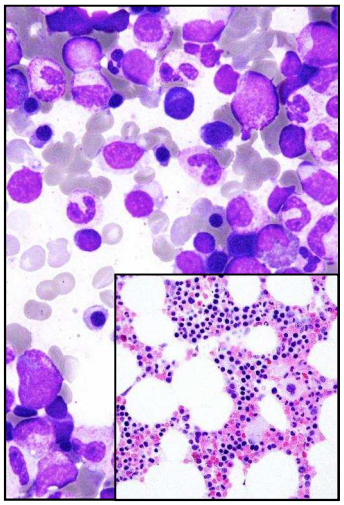
Malcovati et al. *Blood.* 2017;22:3371-3378.
Galli *et al. Blood.* 2021;138(11):965-976.

Case 3: CCUS

Incidental cytopenias

- 73 yo M incidentally found to have abnormal CBC
  - CBC: **3.9** > 12.6 (**100.5**) < **91**
- PMH: NC
- SH: daily drinker, 4-5 drinks per night for many years
- Labs: normal chemistries, SPEP, etc…
- BM Bx: NC, MTH, no dysplasia, nl KT
- RHP:

Somatic Variant			Variant Allele Fraction Time 0
Gene	Variant (c.)	Variant (p.)	Dx
RUNX1	c.802C>T	p.Q268*	41%
SF3B1	c.1998G>C	p.K666N	16%



- Diagnosis: HR CCUS (*SF3B1*, *RUNX1*, 2 mutations, >8.7% VAF, Non-*DNMT3A*)

Case 3: …3 years later

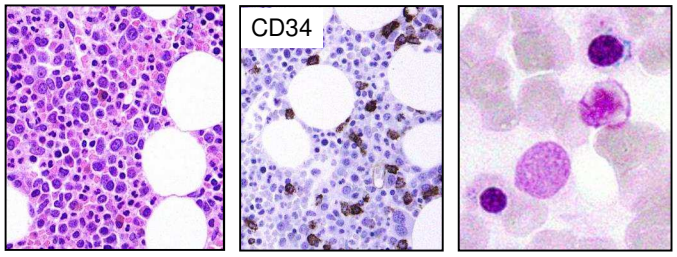
CCUS Progression

- 3 years later: CBC

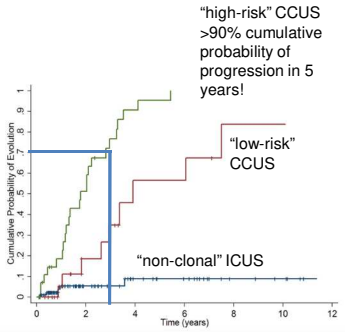
  28.8 > 11.3 (104.2) < 69;

  29% monos, AMC 4.9 K/uL

- BM Bx: CMML-1 with RS
- RHP:



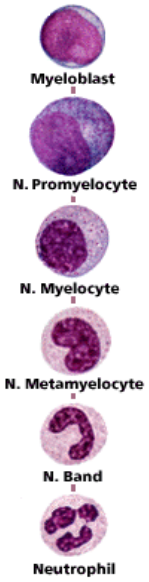
Somatic Variant			Variant allele fraction	
Gene	Variant (c.)	Variant (p.)	Dx	
RUNX1	c.802C>T	p.Q268*	41%	
SF3B1	c.1998G>C	p.K666N	14%	

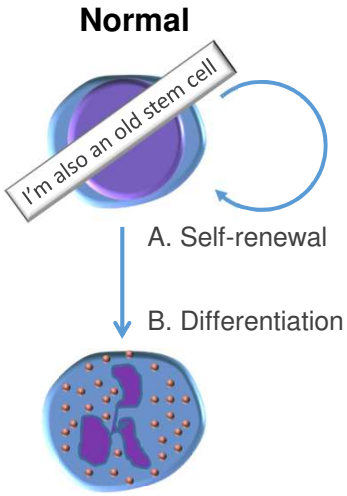


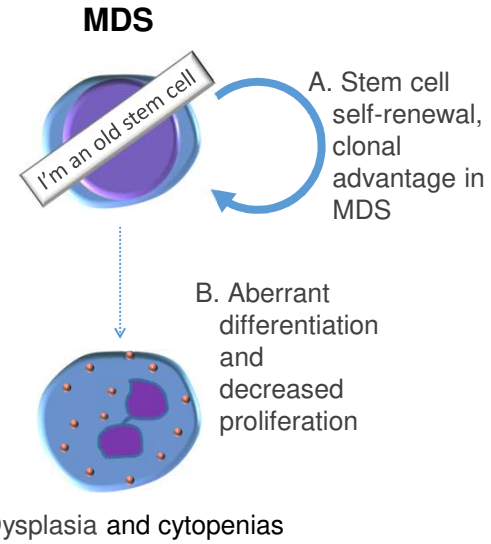
Vignesh Shanmugam and Olga Pozdnyakova

Malcovati *et al. Blood.* (2017)22:3371.

MDS Made Ridiculously Simple… or not so Simple?







MDS: A Little Bit of Dys Plasia, a Little Bit of Dat

MDS Diagnostic Criteria Have Changed Minimally in the Past Decades…

Table 15. PB and BM findings and cytogenetics 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

Cytopenias: Hgb <10 g/dL, PLT <100K/uL, ANC < 1.8 K/uL

MDS:
Cytopenia and clonal with dysplasia

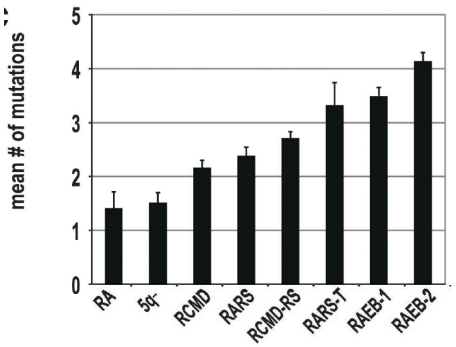
Clonality +
Dysplasia +
Cytopenia +

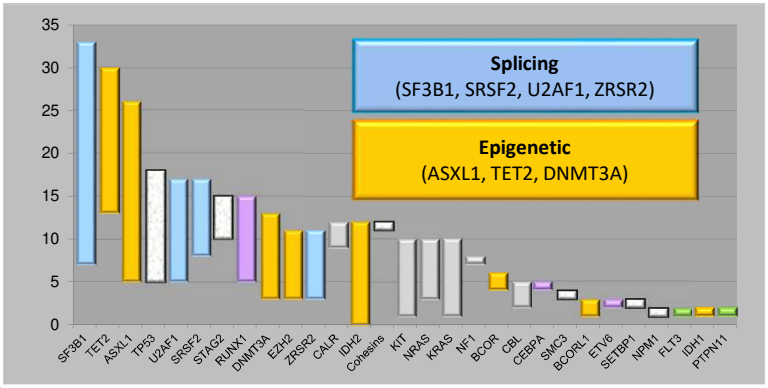
- ~50% of MDS with a normal karyotype

Arber et al. *Blood*. 2016;127:2391-2405.

MDS Has Lots of Mutations

- ~90% of MDS patients have a mutation using a myeloid-directed panel
- 47 genes statistically significantly recurrently mutated
- Median number of mutations = 3 mutations/sample (more is worse)
- >100,000 combinatorial possibilities (47x47x46)

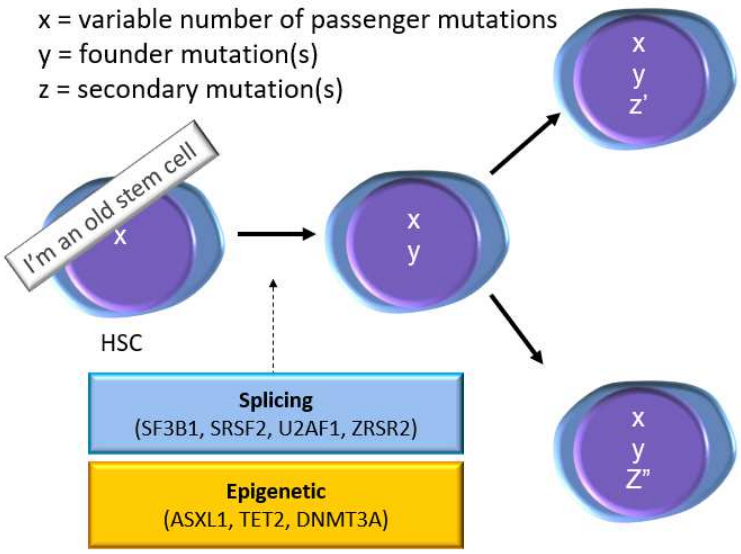




Haferlach T et al. *Leukemia*. 2014;28(2): 241-247.
Wang SA… **Kim AS** et al. Am J Hematol. 2021;96(11):E420-E423.

McClure R …**Kim AS***. *J Mol Diagn*. 2018;20(6):717-737.

MDS: Passengers, Founding Drivers, and more Mutations!

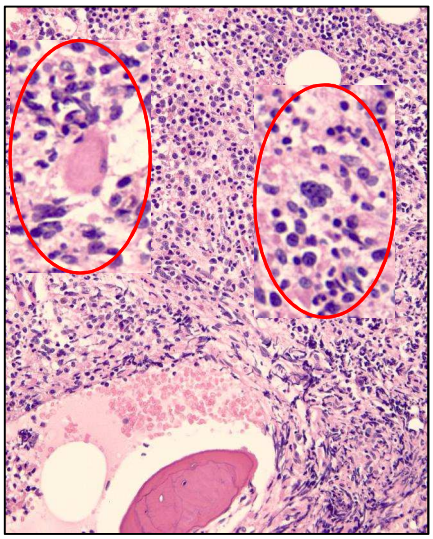


Case 4: MDS

Molecular studies and initial diagnosis

- 71 yo F admitted for HSV encephalitis, found to be leukopenic
- PMH: possible delta-beta thalassemia with baseline microcytic anemia
- CBC: **2.62** > **9.8** (**69.0**) < 366; nl diff
- Normal Karyotype
- Molecular

Somatic Variant			Variant allele fraction
Gene	Variant (c.)	Variant (p.)	Dx
U2AF1	c.470T>C	p.Q157R	45.70%
ASXL1	c.1888_191	p.E635fs*	20.20%



- Diagnosis: MDS-MLD
- Mutations = clonality

Case 5: *PPM1D* and *TP53* in setting of prior chemotherapy and XRT

- 59 F with h/o BRCA2+, TN breast cancer (s/p resection, s/p chemo-XRT), ovarian cancer (s/p adjuvant carboplatin/paclitaxel) with platinum sensitive recurrence treated with carboplatin/gemcitabine and PARP inhibitor maintenance now with new cytopenias
- CBC: **2.81** > **9.8** (**113.9**) < **112**
  - Diff: Neut**38.9**%, Ly44.8%, Mo13.2%, Eos1.1%, Basos 0.7%, imm grans0.4%
- BMBx: not diagnostic of MDS (typical HP disclaimer)

	Somatic Variant			Variant allele fraction
	Gene	Variant (c.)	Variant (p.)	Dx
In trans	***PPM1D***	c.1427delA	p.N477Ifs*6	9.1%
In trans	***PPM1D***	c.1632delC	p.L546*	8.5%
In trans	***PPM1D***	c.1654C>T	p.R552*	1.6%
Phasing cannot be determined	***TP53***	c.559+2T>C	splice site	2.6%
Phasing cannot be determined	***TP53***	c.711G>A	p.M237I	0.5%

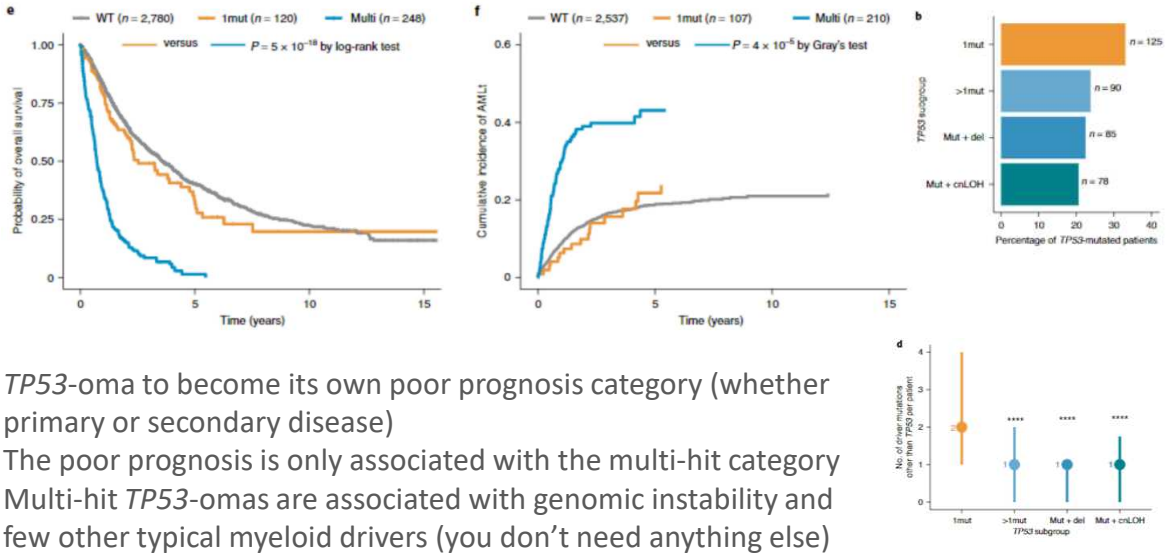
- Diagnosis: Presumed t-MDS
- Multi-hit *TP53*-omas a/w poor prognosis

Bernard *et al. Nat Med.* 2020;26(10):1549-1556.

Bernard *et al. Nat Med.* 2020;26(10):1549-1556.

TP53-oma

New category in a classification scheme coming soon to you!

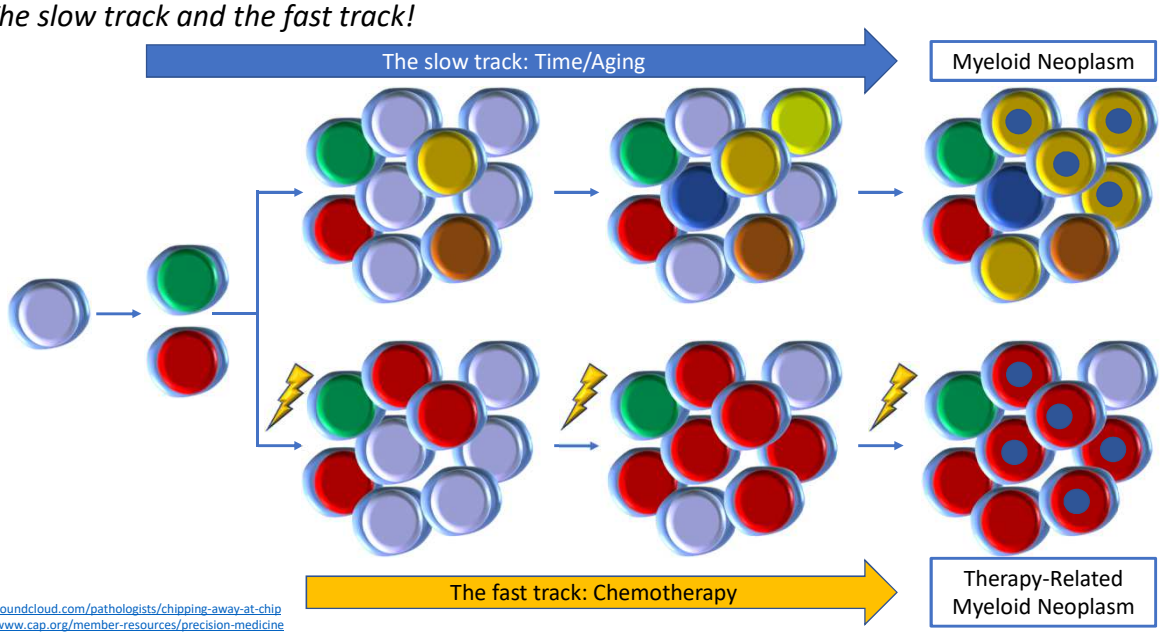


- *TP53*-oma to become its own poor prognosis category (whether primary or secondary disease)
- The poor prognosis is only associated with the multi-hit category
- Multi-hit *TP53*-omas are associated with genomic instability and few other typical myeloid drivers (you don't need anything else)

Bernard *et al. Nat Med.* 2020;26(10):1549-1556.

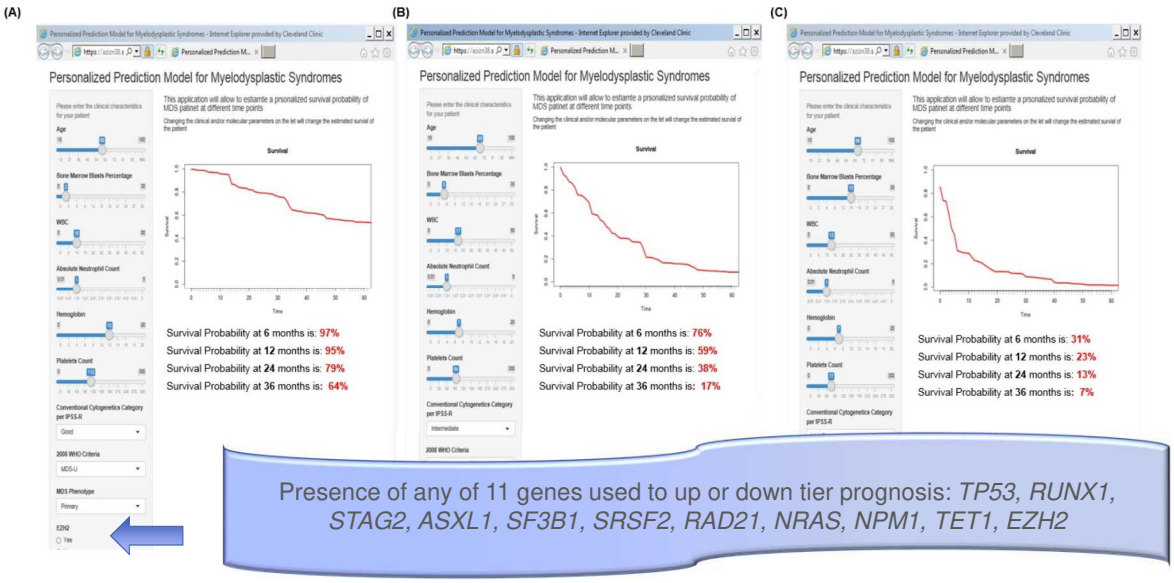
Two Paths to Neoplasia

The slow track and the fast track!



https://soundcloud.com/pathologists/chipping-away-at-chip
https://www.cap.org/member-resources/precision-medicine

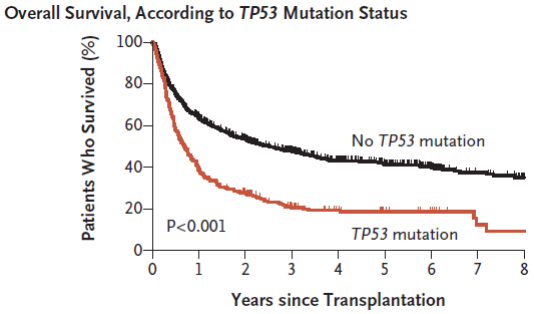
Mutations in MDS Prognostic Indices



Nazha A, et al. Proceedings from the 2018 ASH Annual Meeting and Exposition; December 1-4, 2018; San Diego, California. Abstract 793.

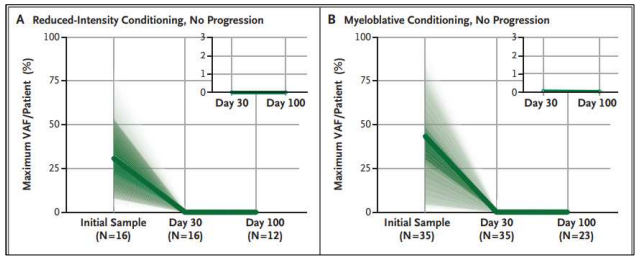
Implications of MRD after Transplant

Therapy and Monitoring Uses of NGS in MDS

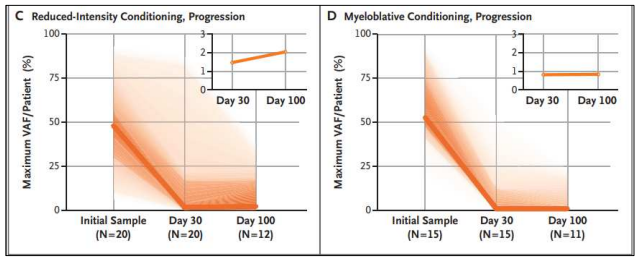


- *TP53* associated with poor OS after SCT, genomic complexity, and t-MDS.
- *RAS* mutations associated with early relapse post-SCT that can be overcome by MAC conditioning.

No Progression



Progression

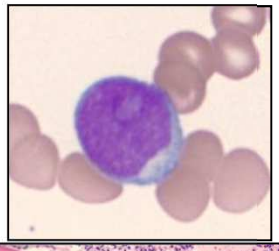


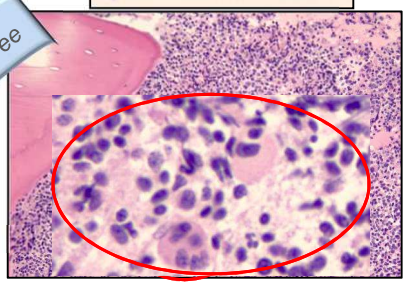
Duncavage et al. NEJM.2018;379:1028-1041.
Lindsley et al. NEJM 2017;376:536-547.

Case 4: Back to our Patient: 13 months later…

- 13 months later, patient noted to have circulating blasts
- CBC: **2.53** > **5.1** (85.1) < **61** (**3% blasts**)
- Molecular

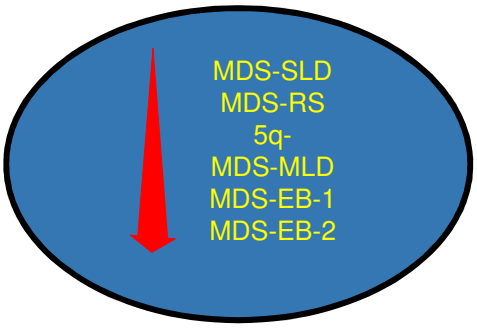
Somatic Variant			Variant allele fraction	
Gene	Variant (c.)	Variant (p.)	Dx	13 mo
U2AF1	c.470T>C	p.Q157R	45.70%	38.40%
ASXL1	c.1888_191	p.E635fs*	20.20%	24.70%

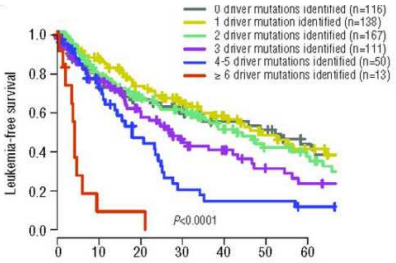




- Diagnosis: AML-MRC (secondary AML)

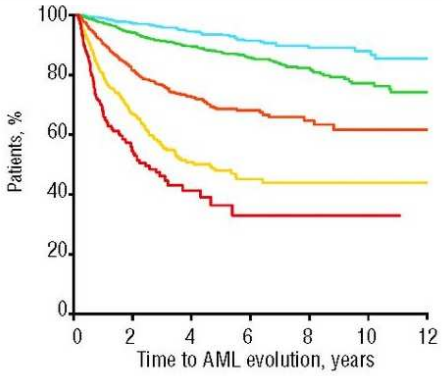
MDS Progression to AML





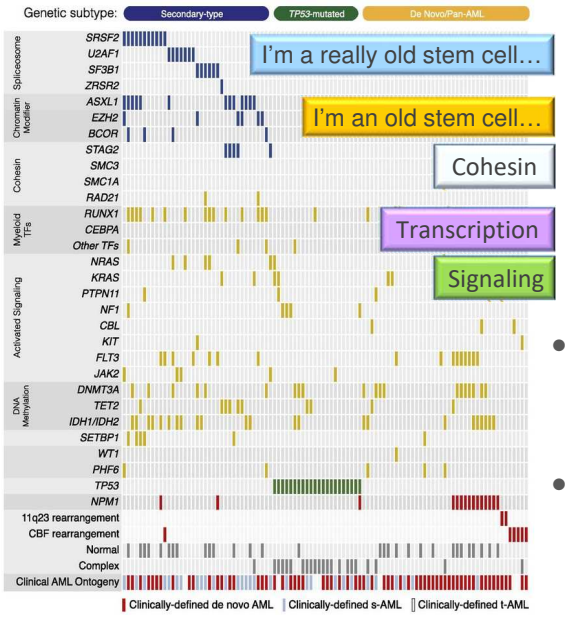
assign IPSS-R risk group

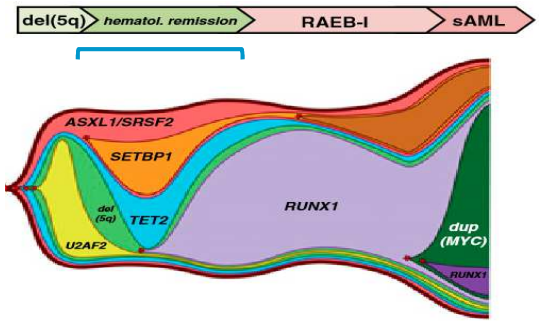
total score	% of patients	median survival, years	time to 25% with AML, years	IPSS-R risk group
≤ 1.5	19%	8.8	not reached	very low
> 1.5 - 3	38%	5.3	10.8	low
> 3 - 4.5	20%	3	3.2	intermediate
> 4.5 - 6	13%	1.6	1.4	high
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]



Bejar *et al. Haematologica.* 2014;99:956-964.

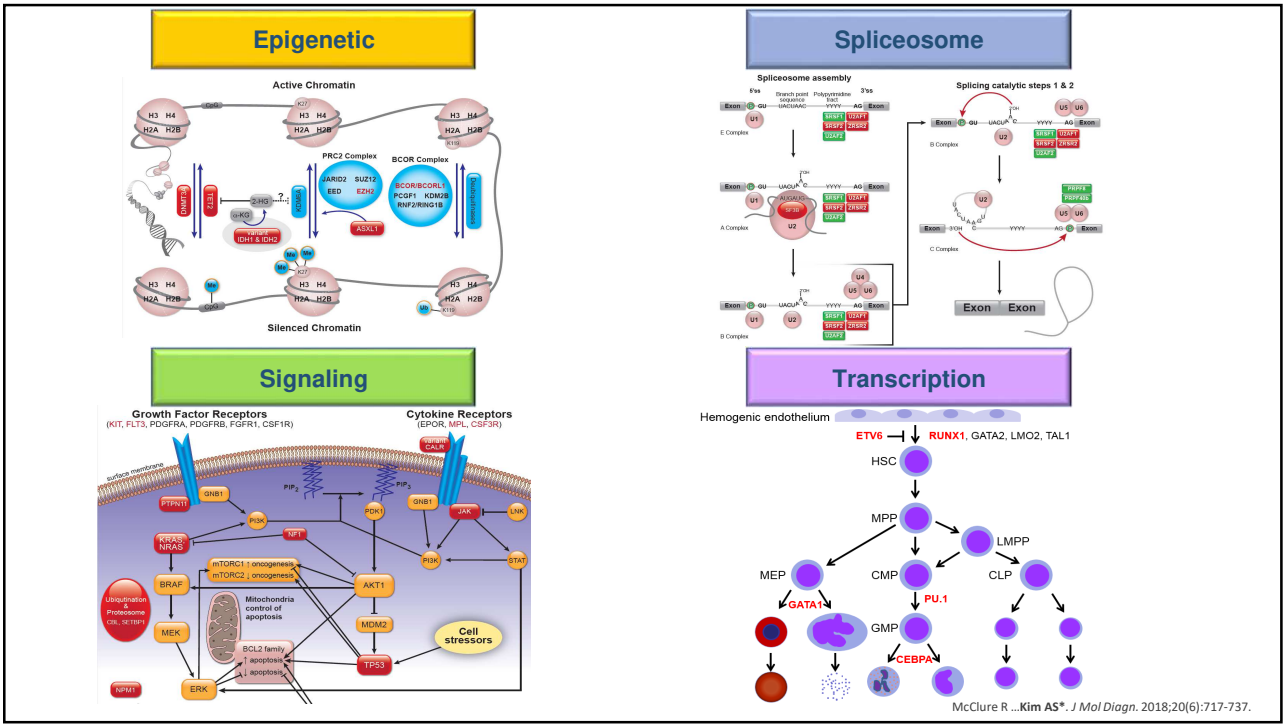
Backwards and Forwards: MDS Progression





- Presence of a mutation in one of 8 genes was >95% specific for diagnosis of s-AML with poor prognosis
- New mutations at s-AML are drivers (myeloid transcription factors or signal transduction proteins)

Mossner et al. *Blood.* 2016;128: 1246-59.
Lindsley et al. *Blood.* 2015;125:1367-1376.



McClure R ...**Kim AS***. *J Mol Diagn*. 2018;20(6):717-737.

ELN Guidelines for AML Testing

Risk category*	Genetic abnormality
Favorable	t(8;21)(q22;q22.1); *RUNX1-RUNX1T1*
	inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
	Mutated *NPM1* without *FLT3*-ITD or with *FLT3*-ITD^{low}†
	Biallelic mutated *CEBPA*
Intermediate	Mutated *NPM1* and *FLT3*-ITD^{high}†
	Wild-type *NPM1* without *FLT3*-ITD or with *FLT3*-ITD^{low}† (without adverse-risk genetic lesions)
	t(9;11)(p21.3;q23.3); *MLLT3-KMT2A*‡
	Cytogenetic abnormalities not classified as favorable or adverse
Adverse	t(6;9)(p23;q34.1); *DEK-NUP214*
	t(v;11q23.3); *KMT2A* rearranged
	t(9;22)(q34.1;q11.2); *BCR-ABL1*
	inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2,MECOM(EVI1)*
	−5 or del(5q); −7; −17/abn(17p)
	Complex karyotype,§ monosomal karyotype‖
	Wild-type *NPM1* and *FLT3*-ITD^{high}†
	Mutated *RUNX1*¶
	Mutated *ASXL1*¶
	Mutated *TP53*#

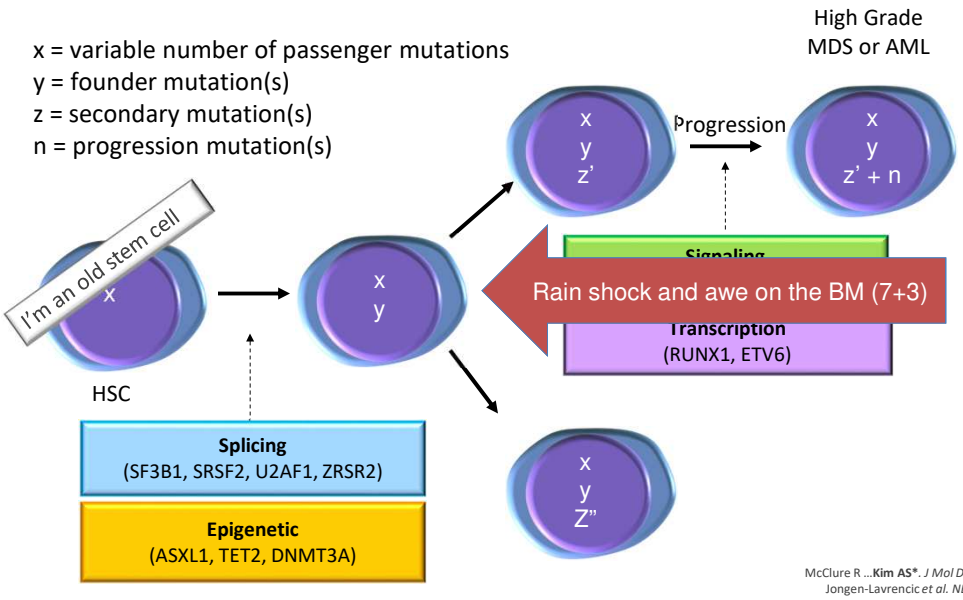
Somatic Mutation Testing

- Required for prognostication:
  - *FLT3-ITD*
  - *NPM1* (WHO category)
  - *CEBPA* (WHO category)
  - *RUNX1* (WHO category)
  - *ASXL1*
  - *TP53*
- Potentially required for monitoring:
  - *KIT* (prognostic in t(8;21))
  - *DNMT3A* (controversial?)
- Required for therapy:
  - *FLT3*
  - *IDH1/2*

Dohner H et al. *Blood*. 2017;129(4):424-447.

Patterns of MDS: Passenger, Drivers, and Boston Drivers

Who are highly dysregulated and have trouble signaling…

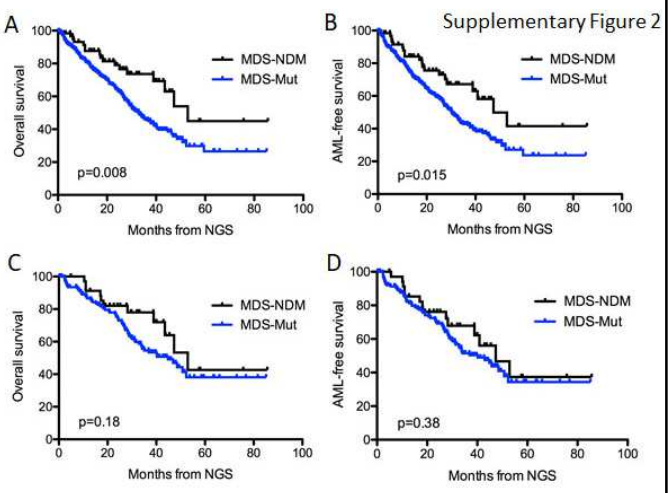


McClure R ...**Kim AS***. *J Mol Diagn.* 2018;20(6):717-737.
Jongen-Lavrencic *et al. NEJM.* 2018; 378:1189-1199.

What about MDS without mutations?

- MDS with no detected mutations (NDM) were younger (P<0.001), were more likely to go to SCT, and had better OS and LFS
- When selected for those cases with a normal karyotype, the survival differences was no longer significant
- So, what do we miss by doing PB-only NGS screening?

Cytopenia: 17,000/100,000 over age of 65
MDS: 75/100,000 over age of 65
MDS-NDM: 7.5/100,000



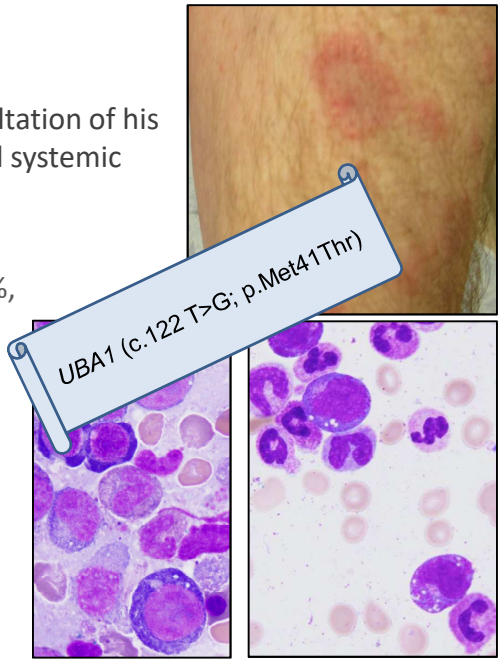
So, cases missed would be 7.5/17,000 = 0.4% or 99.6% specificity for cytopenic patients

Wang SA... **Kim AS** et al. Am J Hematol. 2021;96(11):E420-E423.
Shanmugam ... **Kim AS***. Blood. 2019 Dec 12;134(24):2222-2225.

Case 6: Mutation-Negative MDS?

Clinical history

- 70 M with h/o HTN and HLD who presented for consultation of his MDS in the context of whole-body rash and suspected systemic inflammatory process
- CBC: 5.26 > **8.6** (**102.9**) < **143**
  - Neut67.6%, Ly22.3%, Mo3.9%, Eos0.0%, Basos0.8%, **Metas1.5%, Myelos3.8%**
- BMBx: hypercellular marrow, dysplasia, <5% blasts
  E: cytoplasmic vacuoles, irregular nuclear contours, budding, binucleation, late mitoses
  M: cytoplasmic vacuoles
  MGK: small hypolobated forms
- Heme NGS panel: no pathogenic variants
- Cytogenetics: MDS FISH with normal results, 46,XY,inv(9)(p12q13)[20]

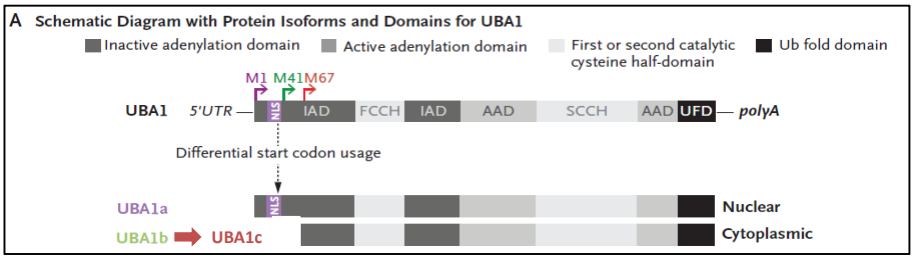


Case and images courtesy of Drs. Jacqueline Garcia, Vignesh Shanmugam, and Damodaran Narayanan

Case 6: VEXAS

Vacuoles, E1 enzyme, X-linked, Autoinflammatory, Somatic

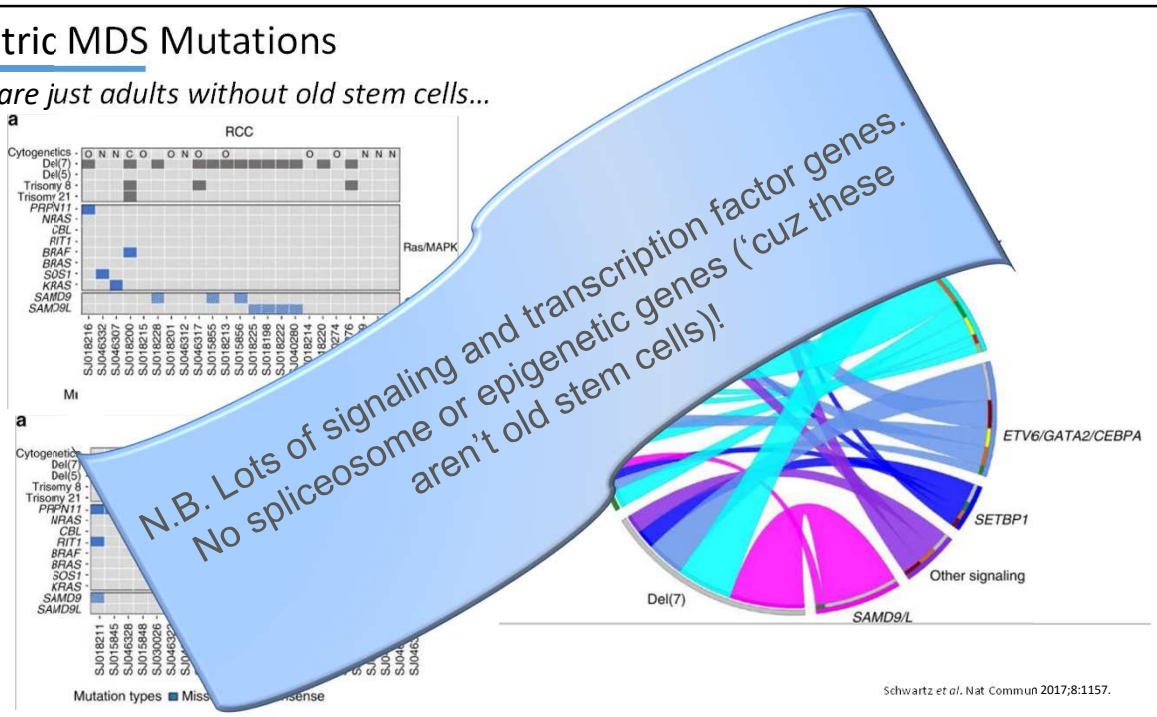
- Adult male onset inflammatory syndromes (mean 64y)
  - Heme: Macrocytic anemia, thrombocytopenia (ddx: MDS- some cases even have myeloid-y mutations)
  - Other systems: Cutaneous and pulmonary inflammation, chondritis, alveolitis, vasculitis, thromboembolic disease, recurrent fevers (ddx: Sweet's, PAN, GCA)
- *UBA1* encodes an E1 ubiquitin conjugation enzyme (on chrX)
  - All known mutations are p.M41V/T/L somatic variants at low VAF (<5%), resulting in alternate use of the M67 start site, resulting in a catalytically deficient UBA1
  - Mutation in myeloid but not lymphoid cells (can result in PB lymphocytopenia)



Beck *et al. NEJM.* 2020;282(27):2628-263.

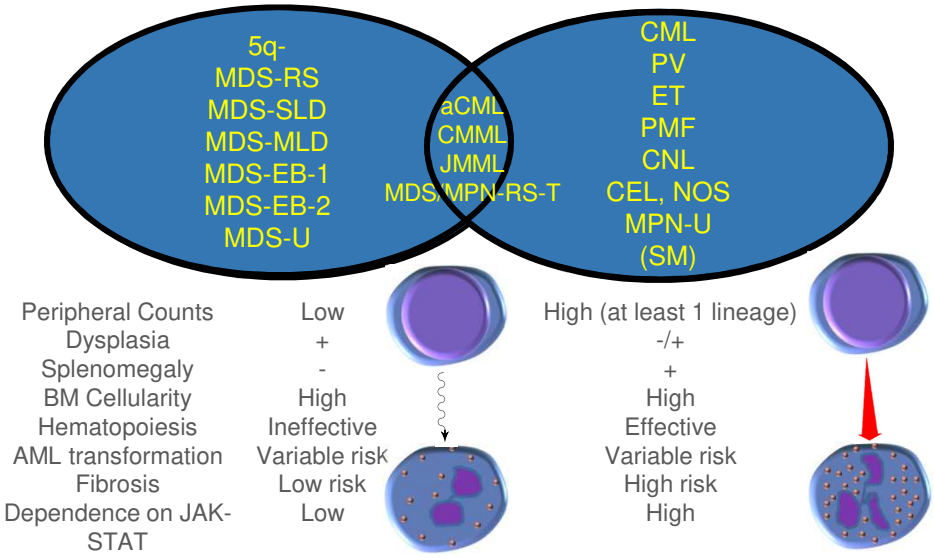
Pediatric MDS Mutations

Kids are just adults without old stem cells…



More Dys Plasia and More Dat

Other chronic myeloid neoplasms…



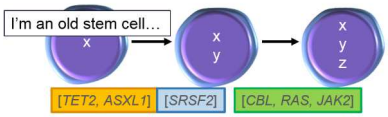
wRASsling with MDS/MPNs

Table 1. Known frequency of genetic mutations seen in MDS/MPN (% mutated)

Gene	CMML21,30-32,45,55-57	JMML26,27,58-61	RARS-T29,36,62-64	aCML35,54,65,66	MDS/MPN-U46,53
Cell signaling					
JAK2 V617F	5-10	—	58.7	7	—
JAK3	<1	9	—	—	—
CALR	—	—	13	—	—
MPL	0	—	2	—	—
NRAS	4-10	12	—	8-35	2-14
KRAS	7-10	12	—	2	0
PTPN11	2	40	—	—	—
NF1	1	11	—	—	—
FLT3	<5	—	—	—	—
CSF3R	<5	—	—	<10	—
CBL	10-14	14	—	7	2
KIT	<1	—	—	—	—
Epigenetic regulators					
TET2	50-60	—	9-26	25	18
ASXL1	35-40	4	10	25	14
DNMT3A	<5	—	17	—	3
IDH1	<1		—	—	0
IDH2	<1		—	—	0
UTX	8		—	—	—
EZH2	5-13		25	13-15	6-10
SETBP1	5-10		—	25	—
RNA splicing					
SF3B1	5-10		72	—	1
U2AF1	5		—	—	1
SRSF2	50		—	—	2
Other					
NPM1	<1-3		—	—	—
TP53	5		—	—	4
RUNX1	15		—	2	—

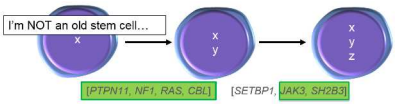
Savona *et al. Blood.* (2015) **125**: 1857-1865.
Meggendorfer *et al.* Haematologica. 2014;99:e244.
Piazza *et al.* Nature Genetics. 2013;45:18–24.

CMML

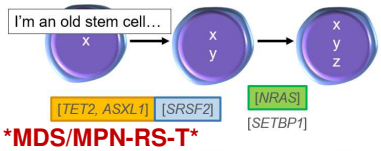


- TET2, SRSF2, or ASXL1 in 90% of cases

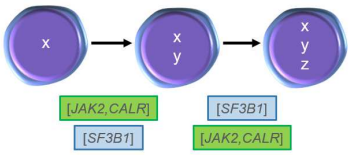
JMML



aCML

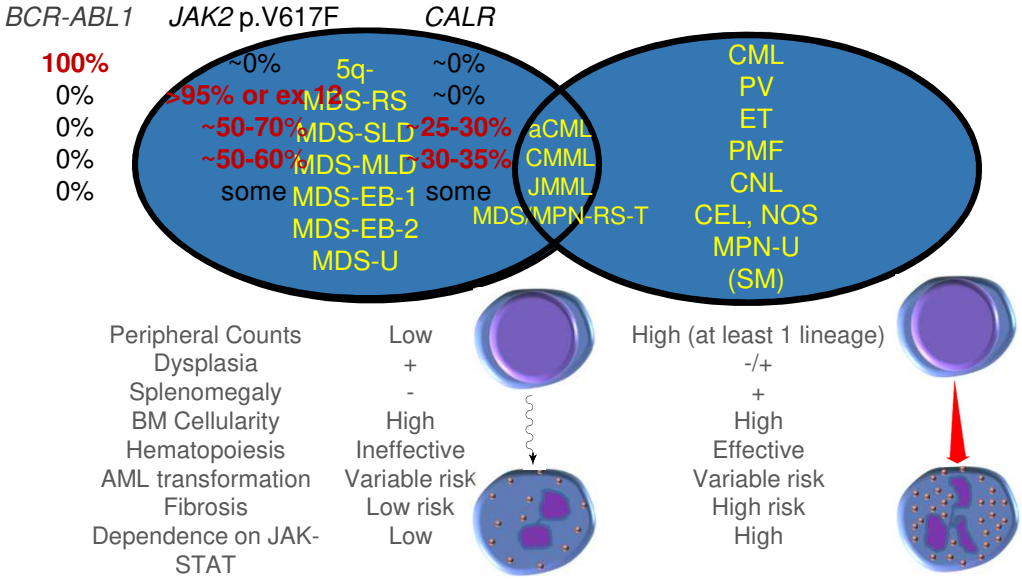


MDS/MPN-RS-T



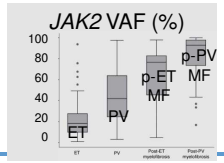
More Dys Plasia and More Dat

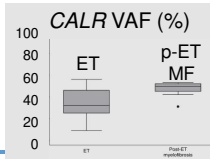
Other chronic myeloid neoplasms…



You don't know JAK!

MPN backseat mutations





JAK2

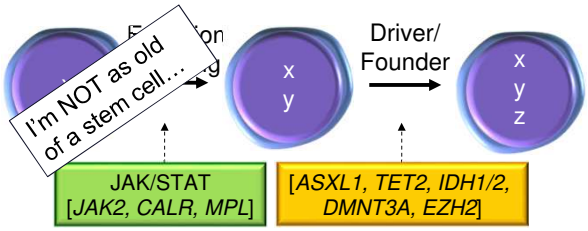
- Thrombosis
- Erythrocytosis
- CN LOH of JAK2 on 9p common
- Poor prognosis, fibrosis, and progression

- No significant differences in:
  - Major bleeding events
  - Risk of transformation to post-ET/PV MF
  - Transformation to AML

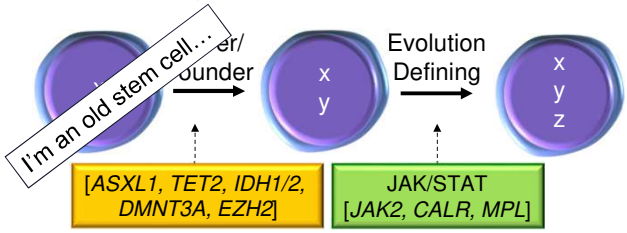
CALR

- Higher PLT counts
  - Especially CALR Type II mutations
- CN LOH of CALR on 19p rare
- Better prognosis

PV; Some ET; Some PMF



Some ET; Some PMF

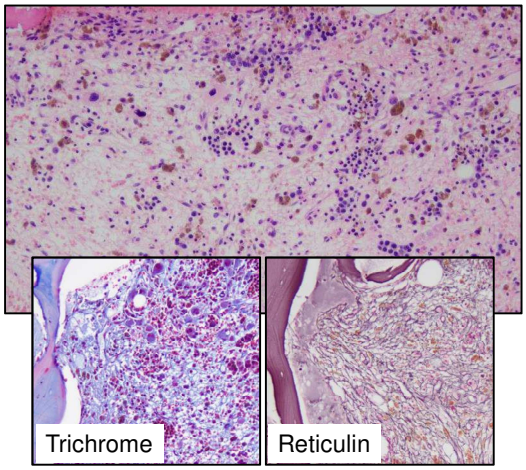


Rumi E *et al. Blood* 2014;123:1544.
Ortmann *et al. NEJM* 2015;372:601-612.

Case 7: Triple Negative MPN

We are just as cool as the breast cancer pathologists…

- 61 F with triple negative PMF s/p RIC MUD allo SCT 1 year prior, with relapse
- Now on Jakafi
- CBC: 1.08 > 5.6 (77.8) < 8; left-shifted, but no blasts
- BMBx: persistent PMF, no blasts

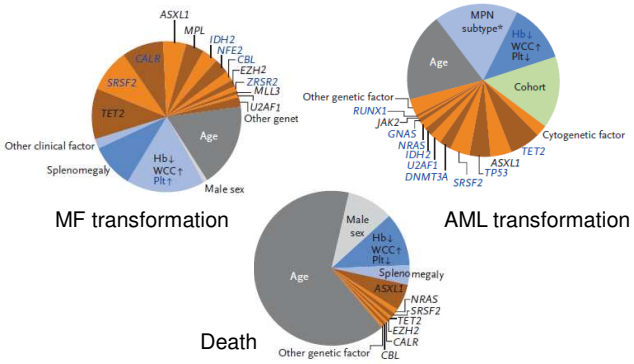


Somatic Variant			(old assay)
Gene	Variant (c.)	Variant (p.)	Pre-SCT
PRPF8	c.4792G>A	p.D1598N	26.8%

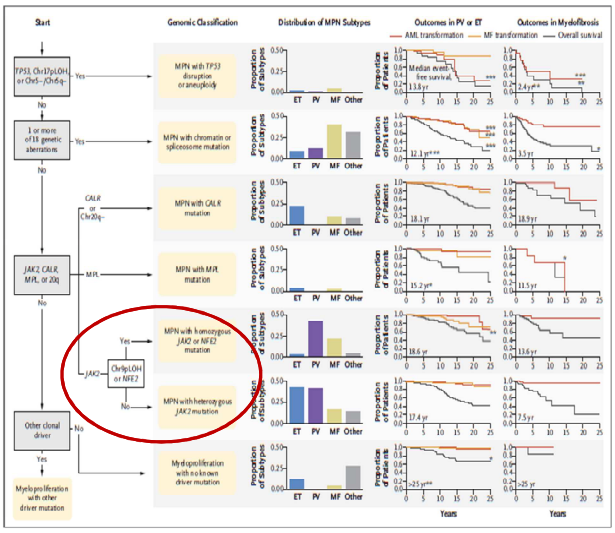
Triple Negative MPN Mutations

MPN backseat mutations

- *NFE2* mutations are truncating or missense in codon 297-300 region.
- Mutations cross morphologic categories
- Mutations more prognostically helpful than morphology



- In addition to the well-known codon 515 and 505 *MPL* mutations, other mutations at codons 204 and 230.

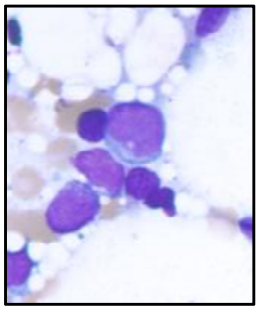


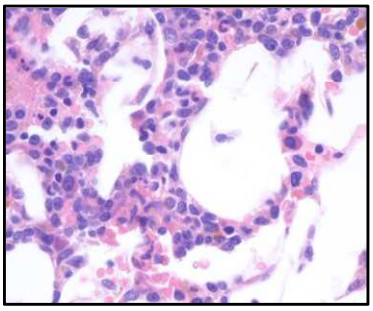
Grinfeld J et al. *N Engl J Med*. 2018;379(15):1416-1430.

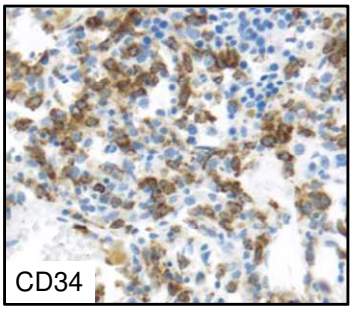
Case 8: PMF with dropping counts

Clonal Evolution in MPNs

- 71 yo M with a 2 year history of *JAK2*+ PMF, treated with ruxolitinib and stem cell transplantation, who presented with dropping counts after transplantation
- CBC: **1.59** > 13.2 (92.9) < **45**
  - N 49, Ly 45, Mo 5, Eo 1
- A bone marrow biopsy was performed.







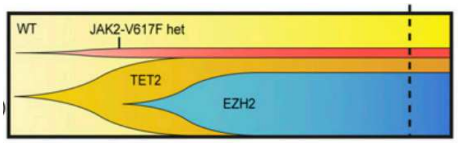
Case 8: Clonal Evolution in MPNs

Molecular and cytogenetic results

Somatic Variant			Variant allele fraction		
Gene	Variant (c.)	Variant (p.)	Pre-SCT	Post-SCT	AML
JAK2	c.1849G>T	p.V617F	10.9%		
SRSF2	c.284C>A	p.P95H	29.3%		
ASXL1	c.1771_1772insA	p.Y59fs*	25.7%		

- Normal limited karyotype: 46,XY[12]

[Note the VAFS for BCOR and STAG2!]

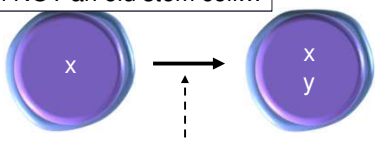


Lundberg *et al. Blood* 2014;123:2220.

Mast Cell Neoplasms

Kids are just adults without old stem cells…

I'm NOT an old stem cell…



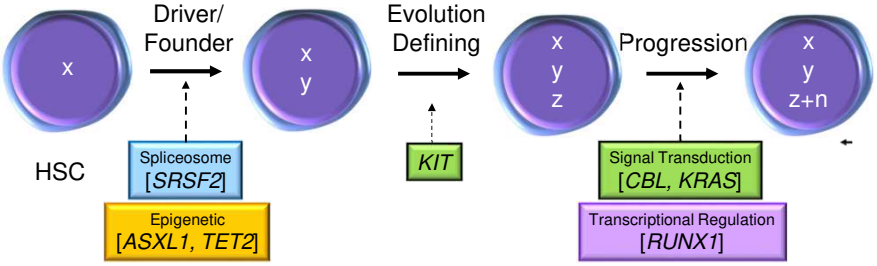
HSC — *KIT* in Mast cells only

Cutaneous Mastocytosis

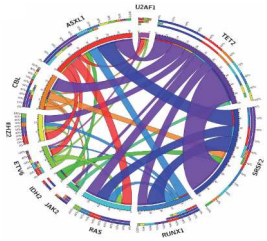
x = passenger mutations
y = founder mutation(s)
z = secondary mutation(s)
n = progression mutation(s)

Gene	Frequency
* *TET2*	47%
* *SRSF2*	43%
* *ASXL1*	29%
* *RUNX1*	23%
JAK2	16%
N/KRAS	14%
CBL	13%
* *EZH2*	10%

I'm an old stem cell…

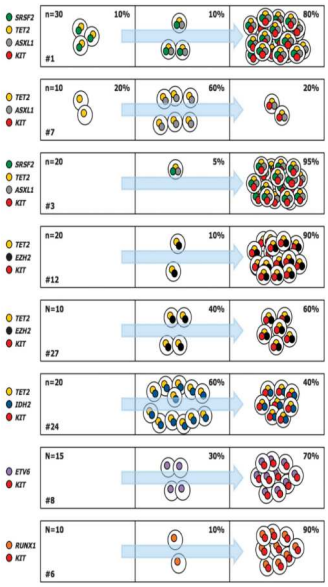


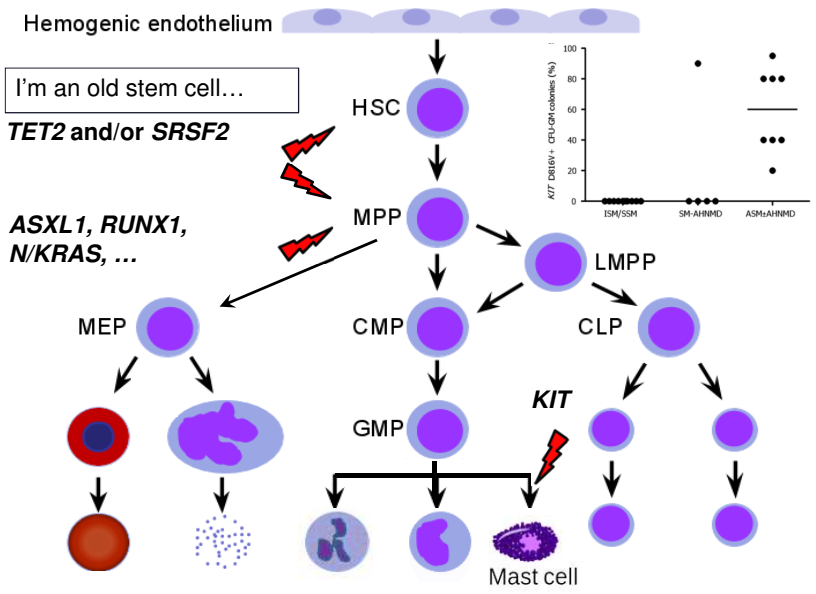
SM, Aggressive SM; SM-AHN



Jawhar M et al. Leukemia 2016;30:136.
Jawhar M et al. Leukemia 2015;29:1115-1122.

Horizontal and Vertical: SM Progression

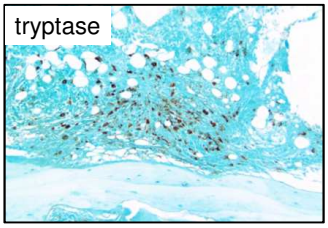




Jawhar *et al. Leukemia*. 2015;29:1115-1122.

Case 9: An Incidentaloma

- 72 M with IgG kappa monoclonal gammopathy
- CBC: 5.53 > **10.3** (83.4) < **129**
  - Diff: Neut**40.0**%, Ly**7.0**%, Mo**36.0**%, Eos6.0%, Basos0.0%, Bands1.0%, Metas9.0%, Myelos**1.0**%
- BM FC: kappa monotypic plasma cells
- BMBx: plasma cell neoplasm (30%)



- Diagnosis:

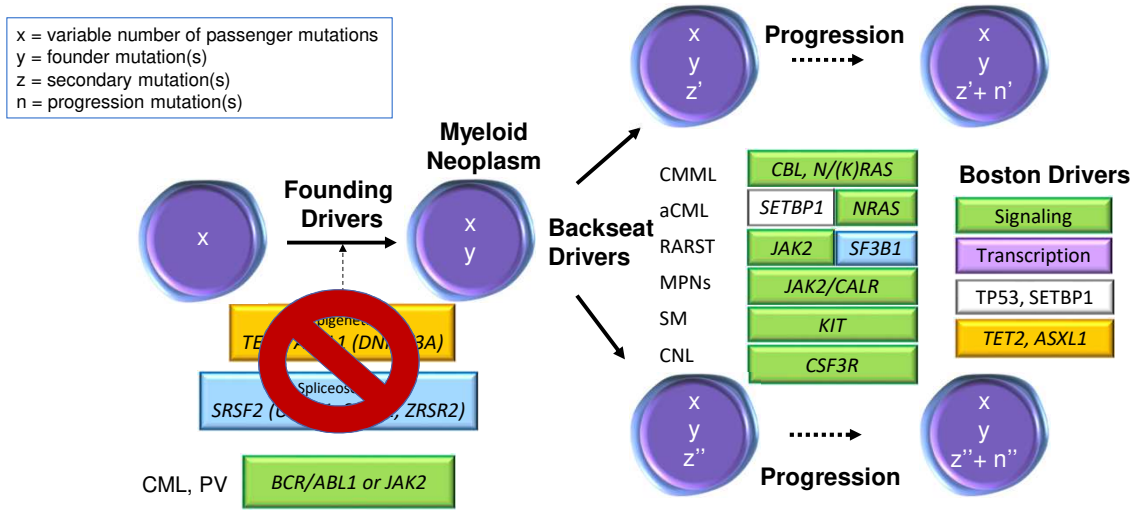
1. ACK! There is an advanced CMN here!
2. There is KIT mutation here!
3. Final diagnosis: PCN, SM-AHN, MDS

Somatic Variant			Variant allele fraction
Gene	Variant (c.)	Variant (p.)	Dx
ASXL1	c.1926_1927insG	p.G642fs*	53.10%
BRCC3	c.239_240insA	p.L80fs*	6.80%
CBL	c.1145A>G	p.K382R	31.80%
CUX1	c.2472G>A	p.W824*	46.30%
KIT	c.2447A>T	p.D816V	5.80%
NRAS	c.34G>C	p.G12R	5.40%
SRSF2	c.284C>T	p.P95L	23.70%
TET2	c.1639G>T	p.E547*	46.40%
TET2	c.4138C>T	p.H1380Y	45.70%

Craig JW…**Kim AS,** *et al. Mod Pathol*. 2020;33(6): 1135-1145..

Passengers, Drivers, Backseat Drivers, Boston Drivers

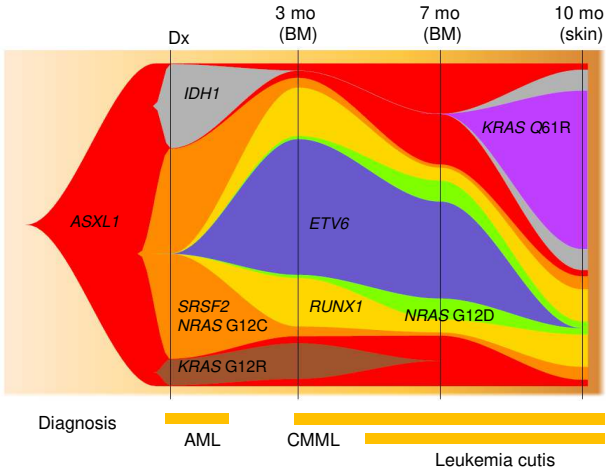
Who are highly dysregulated and have trouble signaling…



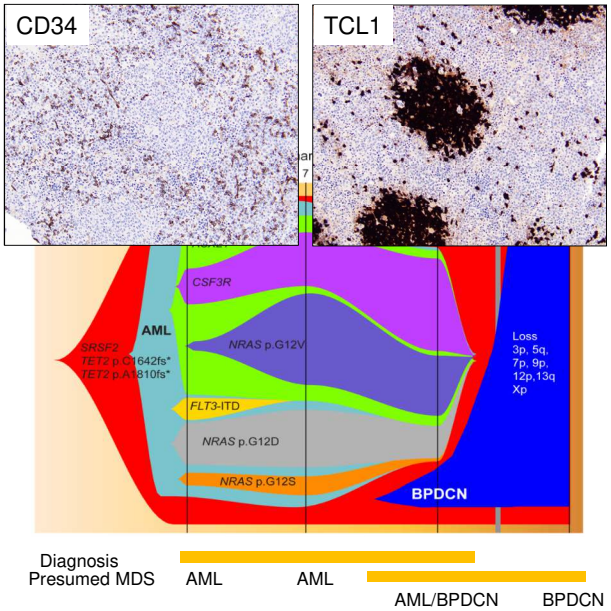
McClure R …**Kim AS***. *J Mol Diagn*. 2018;20(6):717-737.

Cases 10 and 11

Clonal Evolution: It is the Same Darn Thing!



- 55 yo M with AML -> CMML -> leukemia cutis (mixed histiocytic/Langerhans phenotype)



- 78 yo M with AML -> AML/BPDCN in BM and BPDCN in skin

Luskin MR, **Kim AS**, et al. *Leuk Lymphoma*. 2020; May 4: 1-4.
Shanmugam …. **Kim AS*** *J Clin Pathol*. 2019;272:93-96.

Take Home Messages

A. The presence of a pathogenic mutation does not equate with neoplasia (e.g., CHIP). Accordingly, these mutations do NOT always make it into the diagnostic criteria.

B. Pathologic mutations can be used as a measure of clonality as part of diagnostic criteria.

C. Nonetheless, the presence of these pathogenic mutations- in particular *mutational patterns*- is of great diagnostic, prognostic, therapeutic, and monitoring significance.

D. All chronic myeloid neoplasms share common *mutational patterns* but very complex individual panoplies of mutations with abundant clonal heterogeneity.

   A. Founders/Drivers in the same pathway in the same "clone" may be mutually exclusive.

   B. Subclonal progression mutations may show convergent evolution (buy-one-get-one-free).

   C. Pediatric myeloid neoplasms follow the SAME pattern, just without the old stem cell!

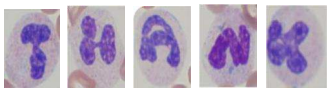
E. The more pathogenic mutations you have, the worse the prognosis, with acquisition of the "Boston Driver" progression mutations.

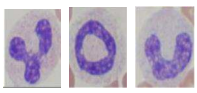
F. Clonal evolution is common and informative.

Acknowledgments

Frank Kuo
Neal Lindeman
R. Coleman Lindsley (DFCI)
Harrison Tsai
Valentina Nardi (MGH)
Vignesh Shanmugam
Elizabeth A. Morgan
Aric Parnes
Samantha Shilit
Alissa Keegan
Olga Pozdnyakova
Marlise Luskin (DFCI)
Andrew Lane (DFCI)
Kyle Wright
Sanjay Patel
Adam Fisch
Mike Wood
Jeff Craig
Robert Hasserjian (MGH)
Damodaran Narayanan
Jacqueline Garcia (DFCI)
Sam Sadigh
Dan DeAngelo (DFCI)
Adam Olszewski (RIH)

Association for Molecular Pathology Chronic Myeloid Neoplasms Working Group





Mass General Brigham

HARVARD MEDICAL SCHOOL