Epigenetic Targeted Therapy in AML

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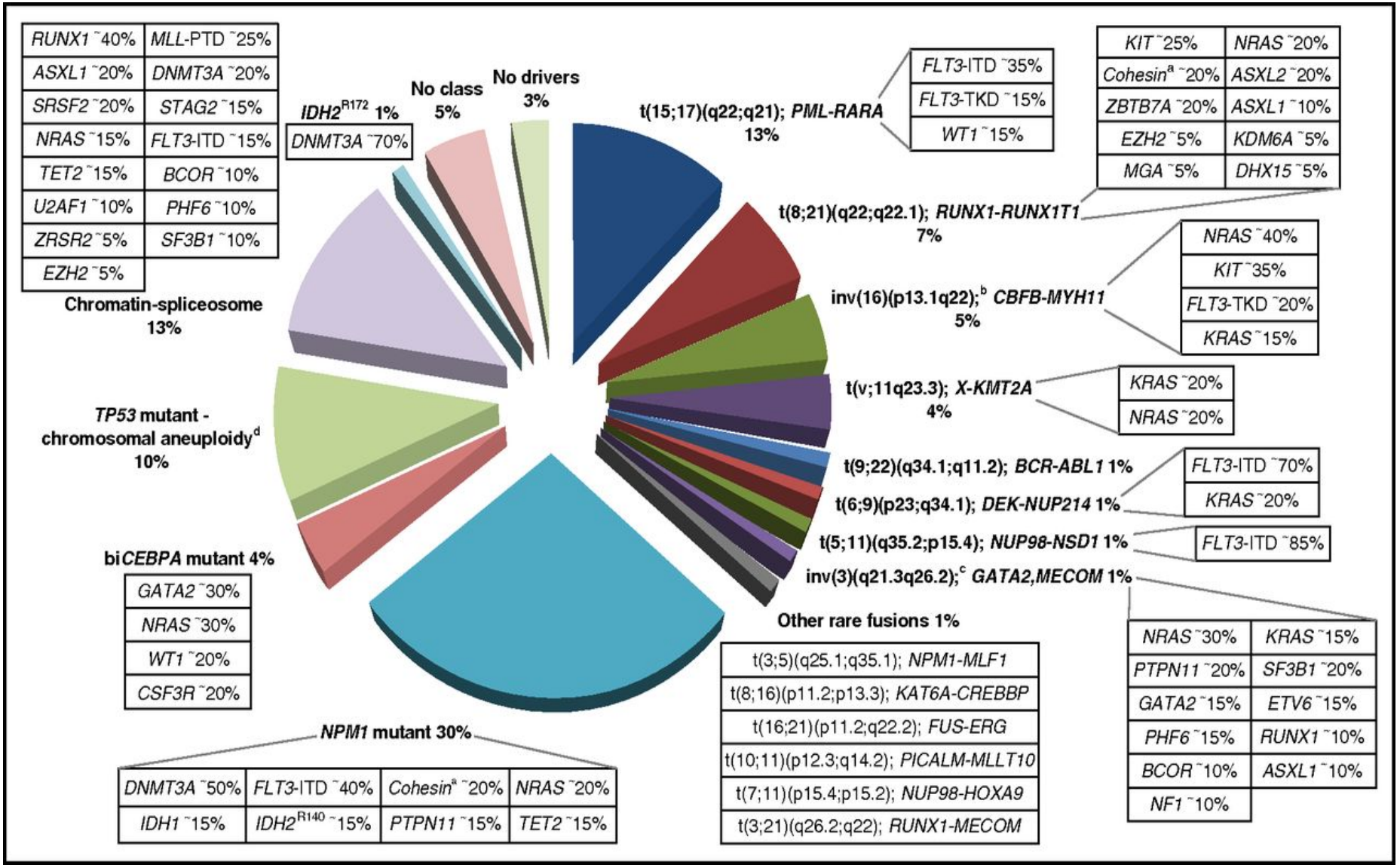
Acute Myeloid Leukemia

State-of-the-Art 2017-2018

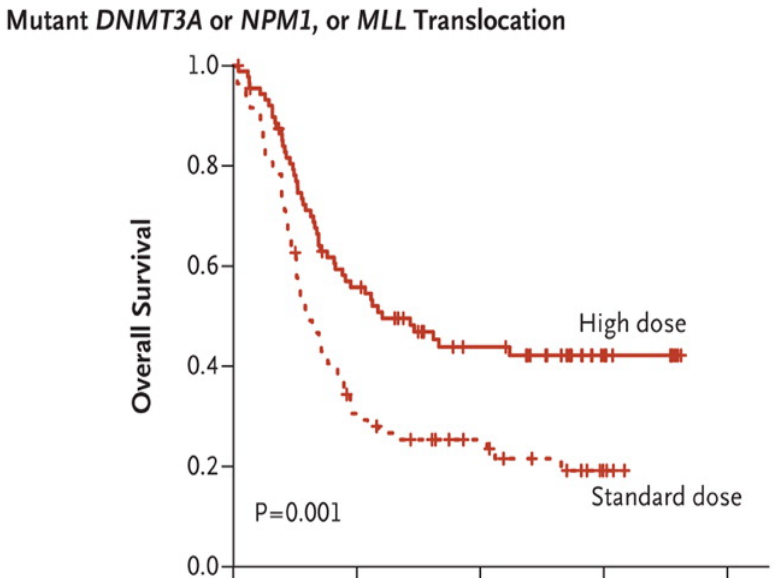
- **Defined by cytogenetic and molecular interactions**
- Intensified induction/less intensive consolidation
- Increased importance of minimal residual disease
- Expanded availability of allogeneic transplantation
- Paradigm shift in older patients
- **Incorporation of novel agents**

Molecular Classes of AML and Recurrent Gene Mutations

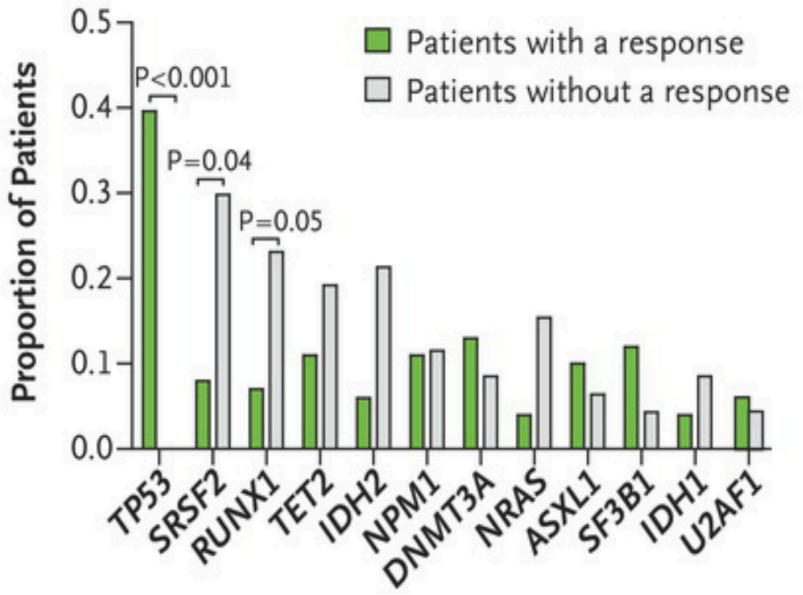


Döhner et al. Blood, 2017

Risk-Stratification and Prognostication of AML Informed by Mutational Profile



Patel et al. NEJM, 2012



Welch et al. NEJM, 2016

Gene Mutations Important in Everyday Practice Today

"Clinically Actionable"

Gene	Incidence	Associations	Impact
FLT3-ITD/TKD	30%	*NPM1*	Unfavorable
NPM1	33%	*FLT3*	Favorable
dCEBPα	8%	*FLT3*	Favorable
C-KIT	15%	*CBF*	Unfavorable [in t(8;21), but less clear in inv(16)]; [1]D816 worse than others
IDH1 and 2	22%	*NPM1*	Favorable
p53	7%	t-AML, complex karyotype (60%)	Unfavorable

[1] *Yui et al. ASH, 2016 (abstr 2785)*

Mutated Genes With Epigenetic Func in AML

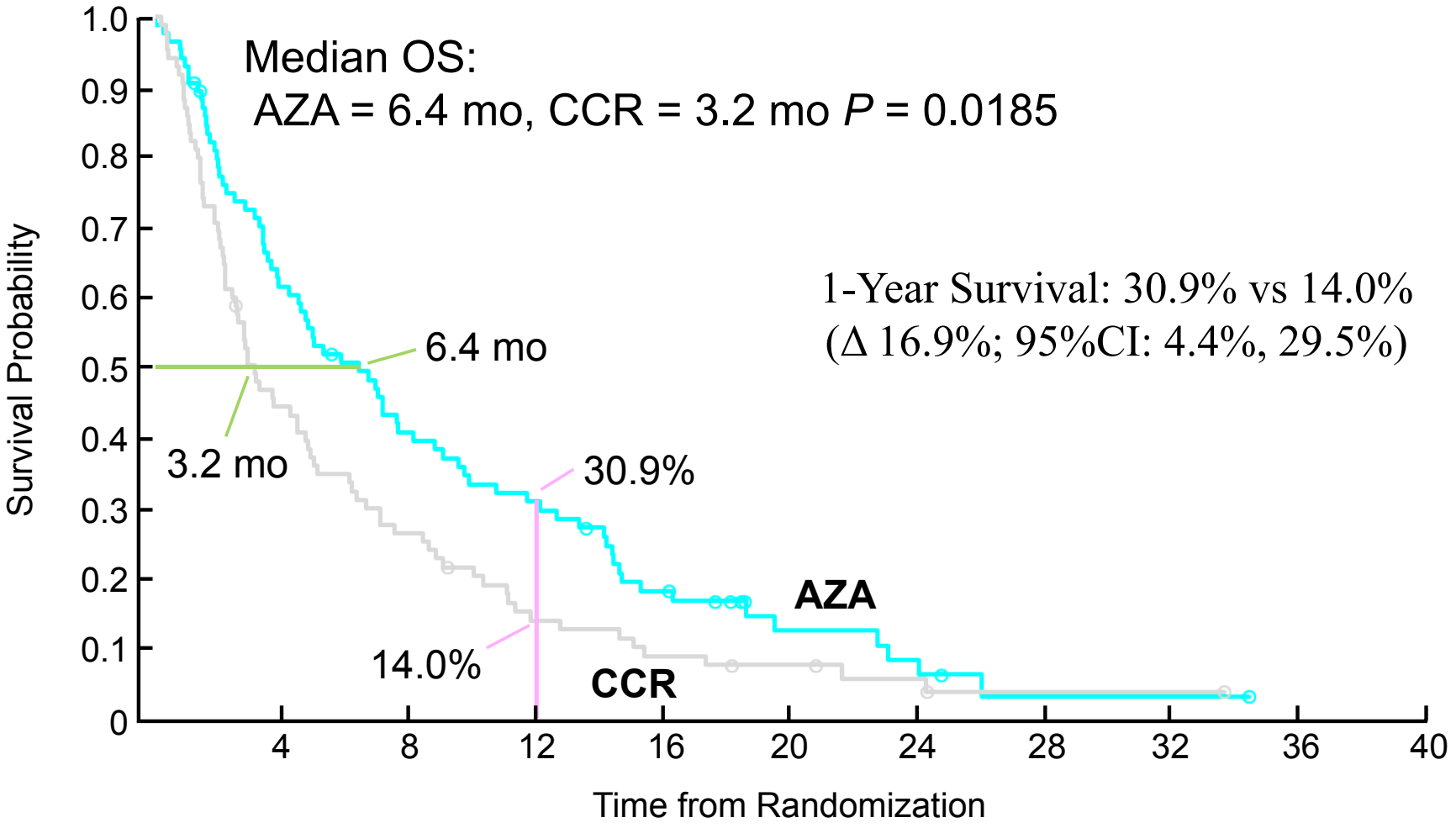
Gene	Function
IDH1/2	Converts isocitrate to alpha-KG
MLL (KMT2A)	H3K4 methyltransferase
DNMT3A	DNA methylation
ASXL1	Recruitment of PRC2 to target loci
EZH2	H3K27 methyltransferase

Adapted from Wouters and Delwel, Blood, 2016

Epigenetic Targeted Treatment

- DNMT inhibitors
  - Azacitidine
  - Decitabine
- HDAC inhibitors
  - Valproic acid
  - Vorinostat
  - Panobinostat
- Methyltransferase inhibitors
  - EPZ-5676
- BET Bromodomain inhibitors
  - CPI-0601
  - FT-1101
- EZH2 inhibitors
  - DS-3201
- **IDH1/2 inhibitors**
  - **Ivosidenib**
  - **Enasidenib**

OS in Patients with Poor-risk Cytogenetics



Dombret et al. Blood, 2015

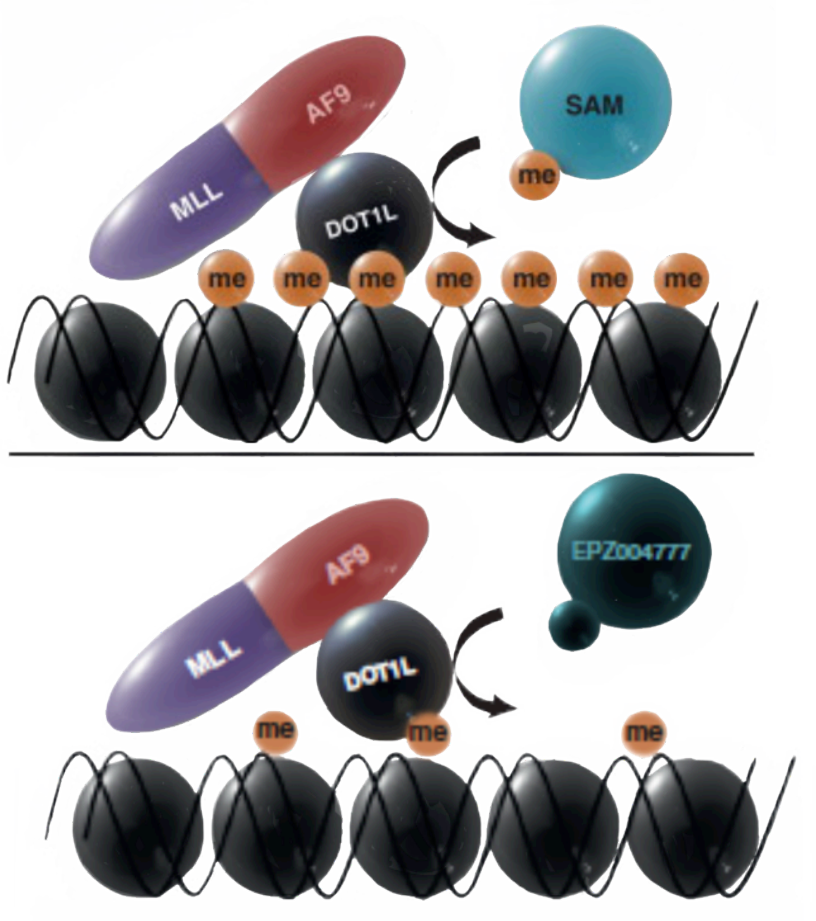
Translocations Involving *MLL* Gene

- In 70% of infant ALL (less than age 1) and has poor prognosis
- In approx. 10% of de novo adult AML
- In therapy-related AML
- More than 60 known fusion partners
  - Most common: t(4;11), t(9:11), t(11;19), t(10;11), t(6;11)

Krivtsov and Armstrong. Nat Reviews Cancer, 2007

DOT1L Inhibitor For *MLL*-Assoc. Leukemias

- *MLL*-fusion proteins interact with DOT1L
- Aberrant recruitment of DOT1L → methylation of H3K79 → sustained expression of *MLL* target genes → leukemic phenotype
- Hypothesis that inhibition of DOT1L activity may treat leukemia with *MLL* translocation



A Phase I First In Man Clinical Trial of the DOT1L Inhibitor EPZ-5676

- Objectives
  - Primary: Determine Maximum Tolerated Dose (MTD) or Rec Phase 2 Dose (RP2D) with a 21 or 28 day infusion
  - Secondary: Describe safety, pharmacokinetics & pharmacodynamics
- Study Design
  - Part 1: Dose Escalation
    - 3+3 design
    - Adult patients with advanced hematologic malignancies
    - Initial cohorts not MLL-r restricted
  - Part 2: Expansion
    - Restricted to MLL-r (translocations and PTD)

Patient Characteristics

		Total patients n=42 (%)
Median age, years (range)		52 (19 to 81)
Sex	Female	17 (40)
Disease at study entry	ALL AML / MDS MPN (CMML)	6 (14) 34 / 1 (81 / 2) 1 (2)
MLL rearrangement	t(6;11) t(11;19) PTD t(4;11) other MLL-r t(9;11) t(10;11) No MLL rearrangement	8 (19) 8 (19) 5 (12) 4 (10) 4 (10) 3 (7) 2 (5) 8 (19)
Prior attempts at remission	1	13 (31)
	2	13 (31)
	3	10 (24)
	≥4	6 (14)
Number of patients with prior allogeneic hematopoietic cell transplants (*one patient with two prior HCTs)		16* (38)

Stein et al. ASH, 2014

Safety: Treatment Related Adverse Events

- Total incidence (all grades): 16 patients (38%)
  - 10 patients ≤ grade 2
    - Majority gastrointestinal
  - 4 patients with grade 3
    - Leukocytosis (n=3)
    - Anemia (n=1)
- Dose Limiting Toxicities
  - 90 mg/m^2/d dose escalation cohort (n=6)
    - None
  - 90 mg/m^2/d expansion cohort (n=17)
    - Grade 4 reversible cardiac failure with concurrent sepsis
    - Grade 4 reversible hypophosphatemia during rapid WBC drop
- MTD not reached

Clinical Activity

- 9 patients (8/34 MLL-r) had either:
  - marrow response and/or
  - resolution of leukemia cutis and/or
  - leukocytosis or differentiation

Dose mg/m^2/day	Number of patients (n=42)	Marrow Response (n=3)	Leukemia cutis resolved (n=2)	Leukocytosis or Differentiation (n=8)
12	1	-	-	-
24	5	-	-	1
36	4	-	1	2
54	6	2 CR	1	1
80	3	-	-	2
90 (28 day CIV)	23	1 PR	-	2

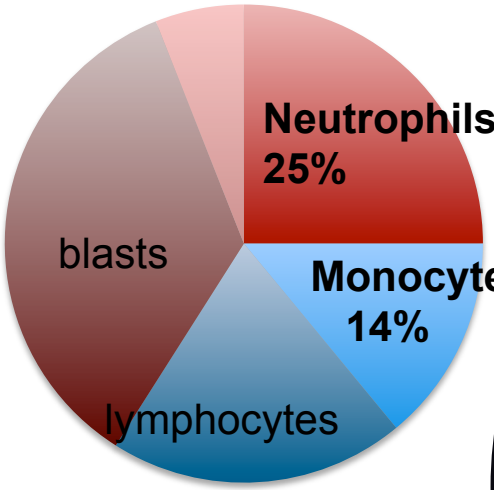
Stein et al. ASH, 2014

Clinical Activity: Marrow Response and Leukemia Cutis

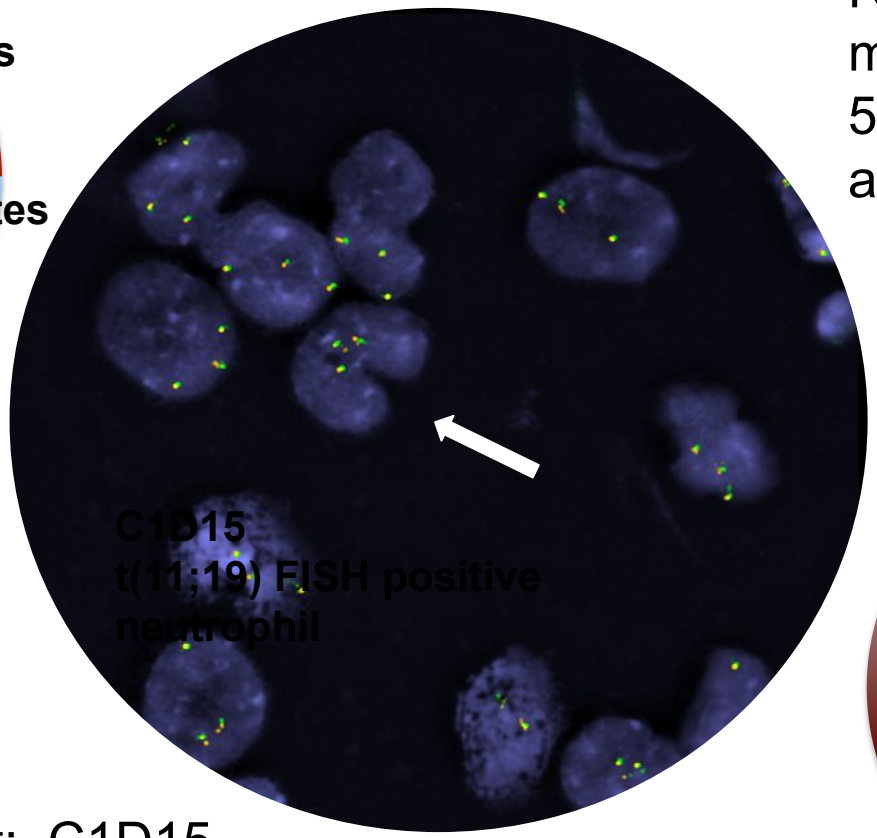
Disease	MLL-r	Dose	Response (weeks on study)	Extra-medullary Disease
MPN (CMML) 01-101	t(11;19)	54 mg/m^2/day	Cytogenetic CR (27)	Resolved leukemia cutis
AML 04-401	t(11;19)	54 mg/m^2/day	Morphologic CR (16*)	NA
AML 01-105	Other: trisomy 11	90 mg/m^2/day	PR (12)	NA
AML 03-300	t(6;11)	36 mg/m2/day	- (6)	Resolved leukemia cutis

Stein et al. ASH, 2016 * Off-study for Hematopoietic Cell Transplant

Clinical Activity: Leukocytosis and Differentiation
Patient 01-103: AML, t(11;19) at 90 mg/m$^{2/}$day

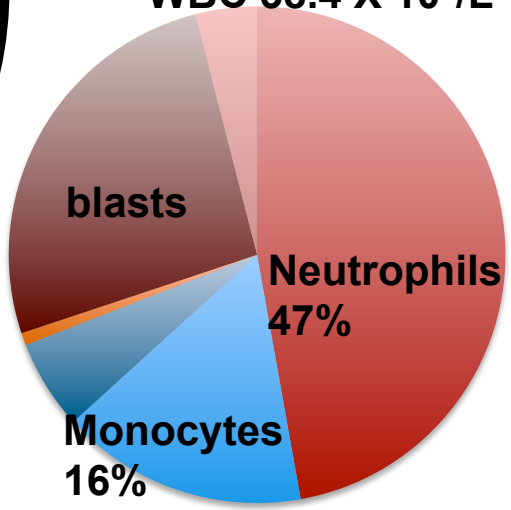


Baseline
WBC 13.2 X 10^9/L



Rise of absolute monocyte/neutrophil 50% above baseline and above ULN

C1D22
WBC 38.4 X 10^9/L

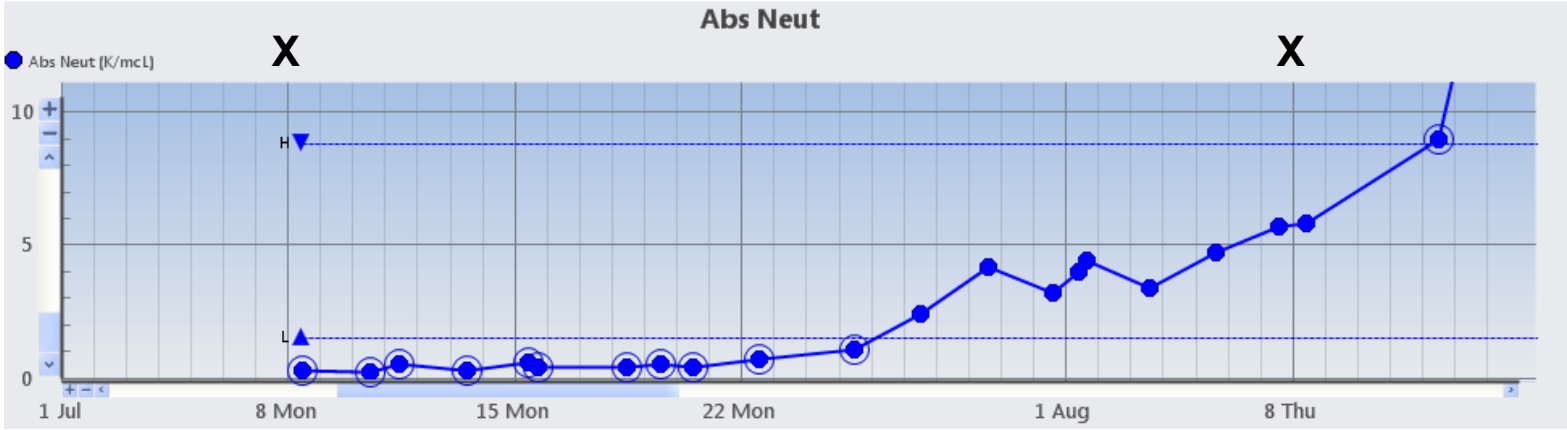


Median day of onset: C1D15
(range: 8-28 days)

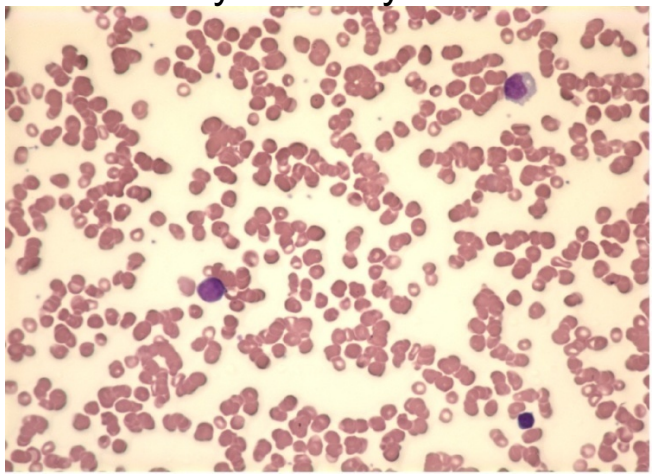
Focus on Specific Patients

- 22 yo Kuwaiti man with t-AML associated with an t(11;19) after treatment of Ewing's Sarcoma with anthracycline-based therapy in 2011.
- Primary induction failure after failing to achieve CR with HiDAC and MEC at DFCI.
- Leukemia-related cachexia, ECOG of 2 (at best)

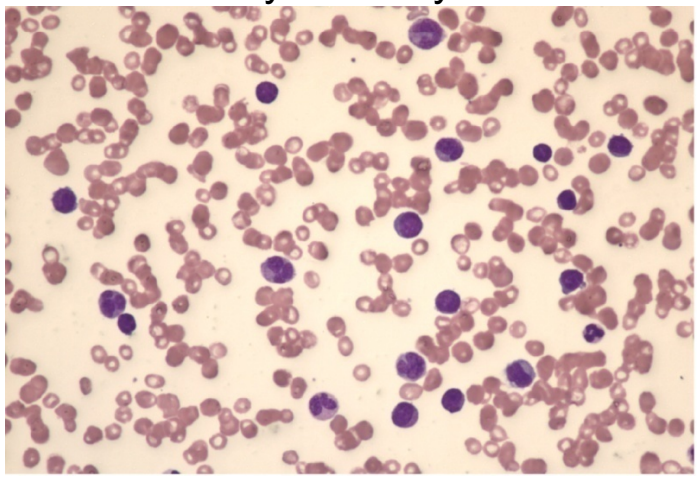
Differentiation Effects With EPZ-5676 Among Patient With *MLL*-r



Cycle 1 day 1

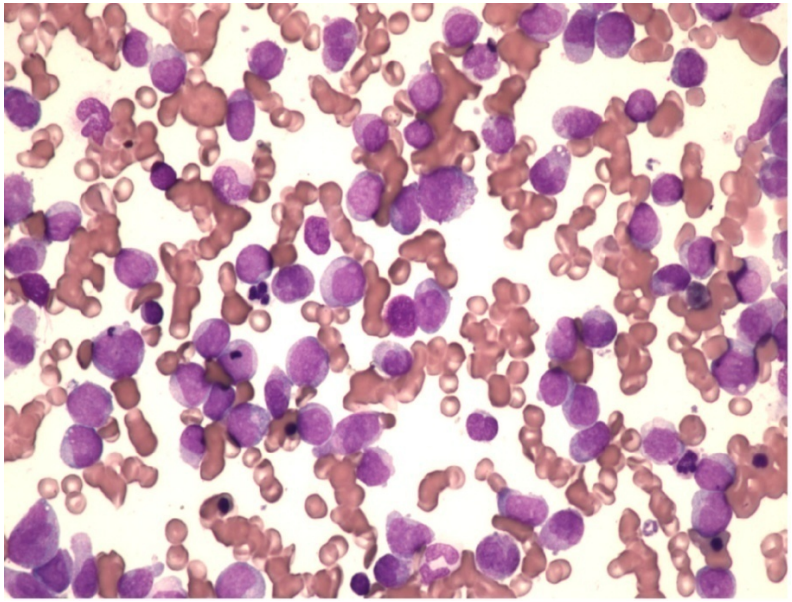


Cycle 2 day 1

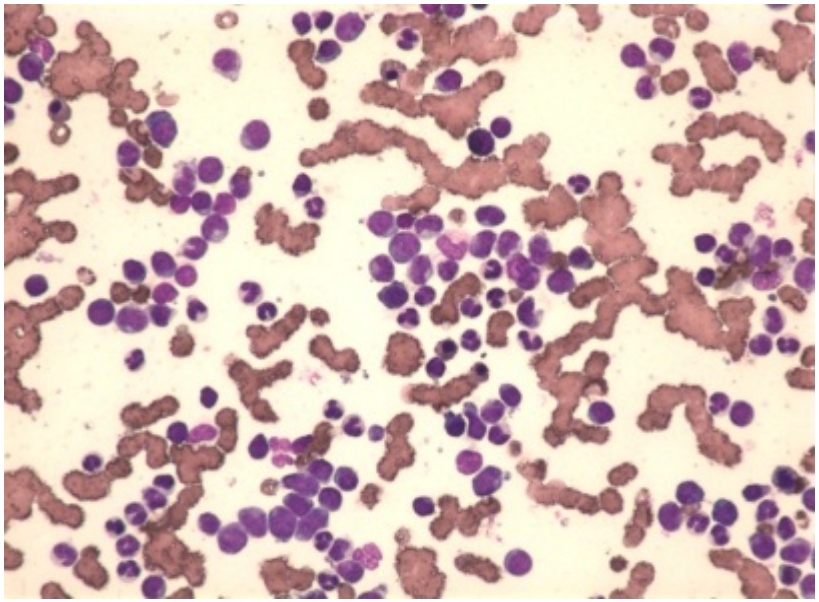


Differentiation Effects With EPZ-5676 Among Patient With *MLL*-r

Bone Marrow Aspirate



Cycle 1 day 1

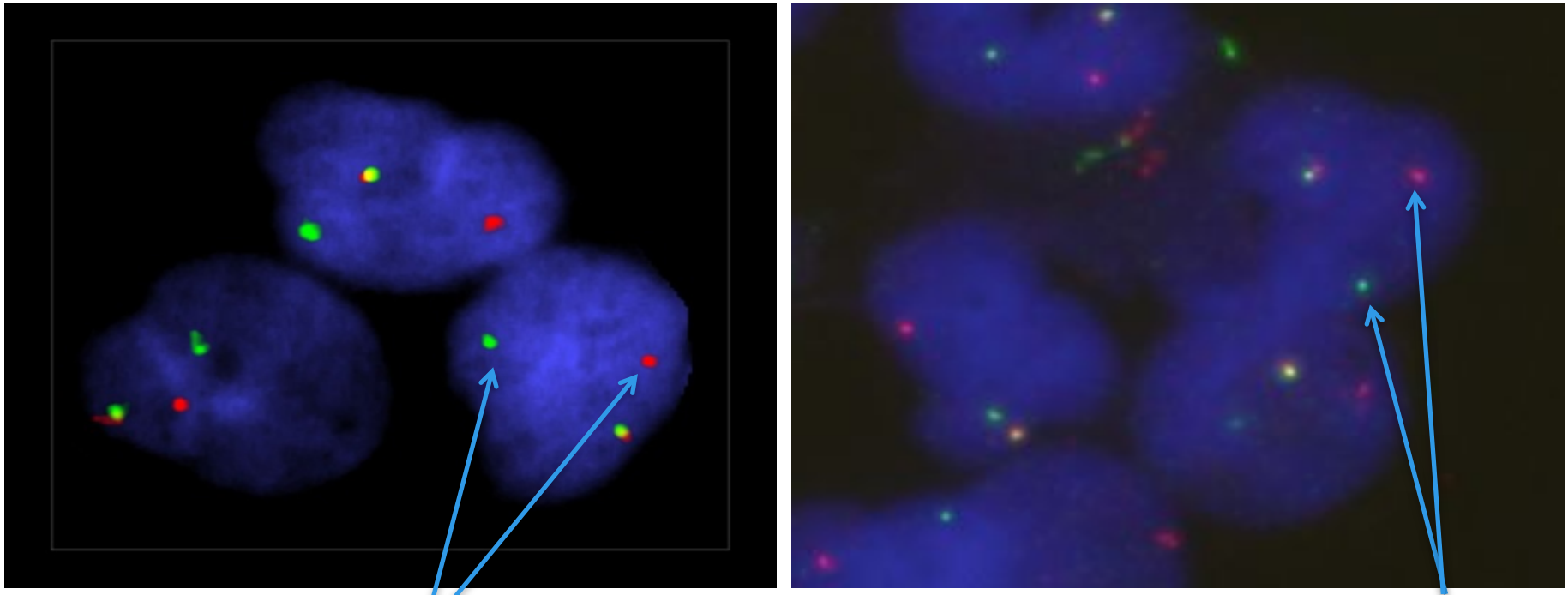


Cycle 2 day 1

Differentiation Effects of EPZ-5676 Among Patients With *MLL*-r

Leukemic blasts

Neutrophils



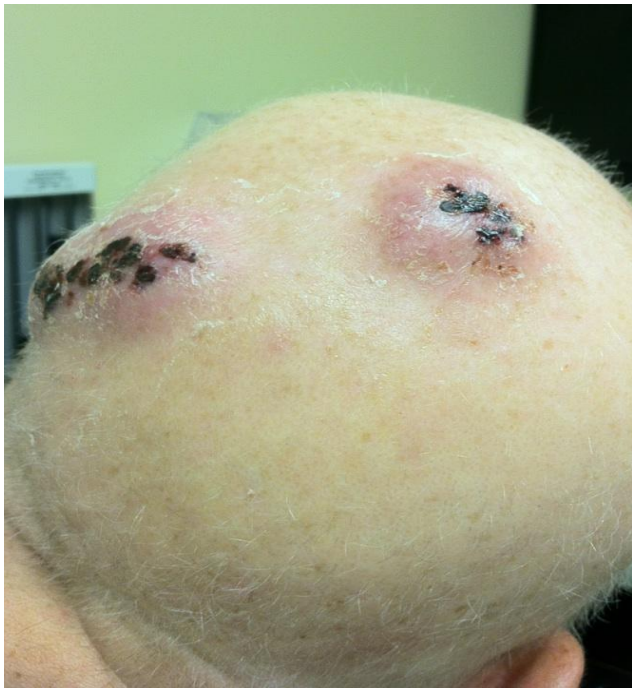
Translocation positive in blasts

Cycle 2 day 1 (break-apart FISH)

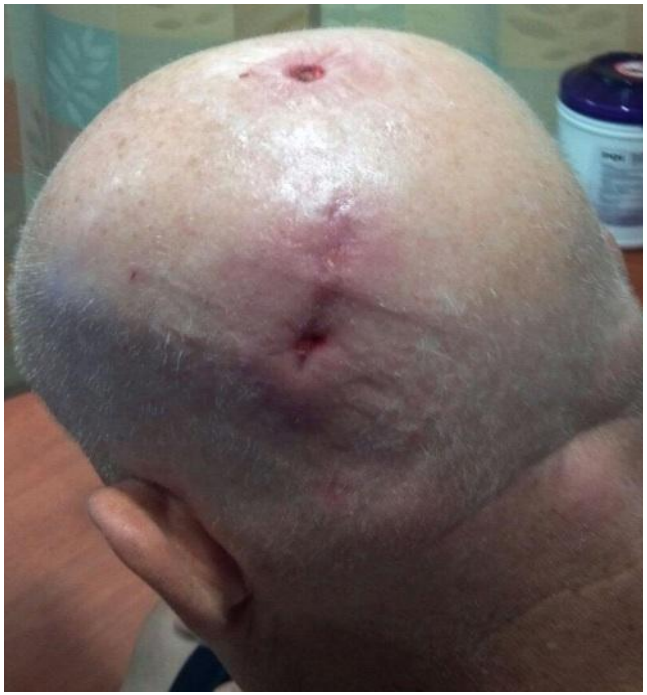
Translocation positive in differentiated neutrophils

Andrei Krivtsov, Scott Armstrong

Resolution of Leukemia Cutis With EPZ-5676 in a Patient with AML *MLL*-r



Day 0

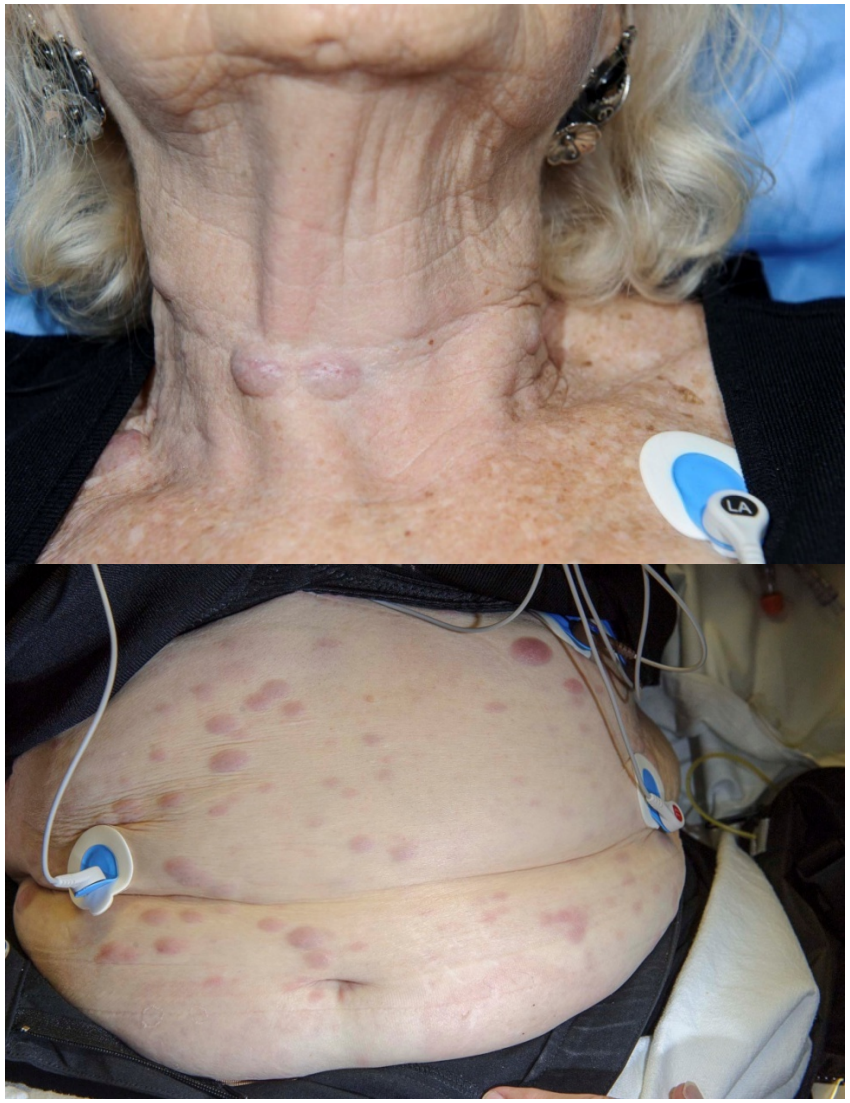


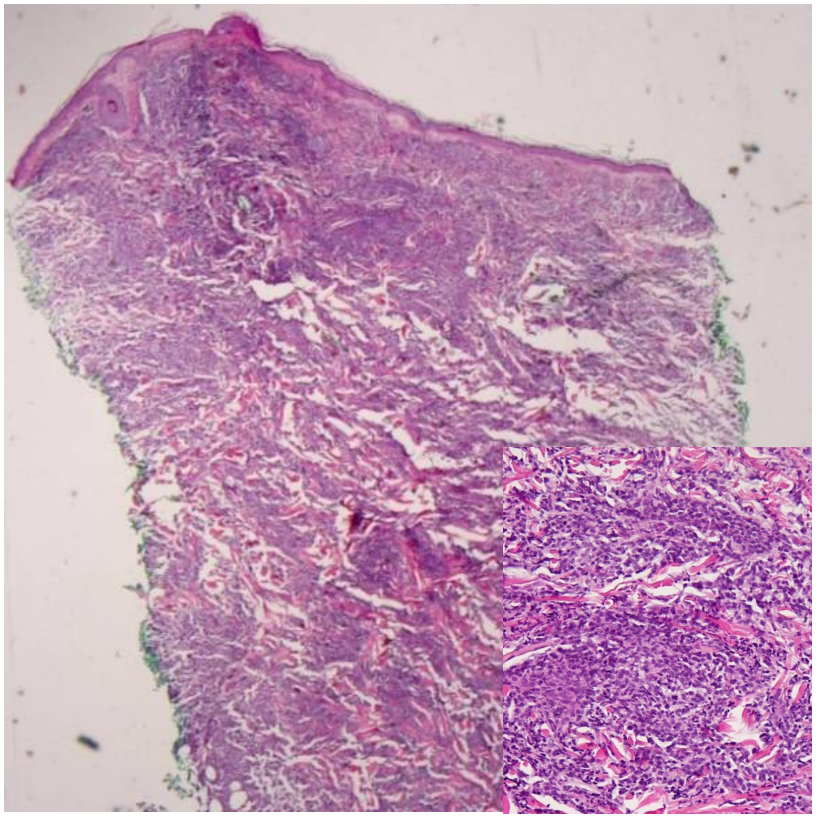
Day 28

Focus On Specific Patients

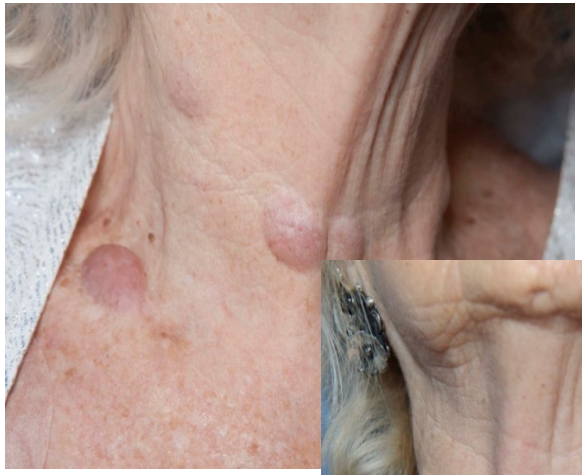
- 81 yo woman with CMML → leukemia cutis. No elevation of blasts in bone marrow at the time of diagnosis of leukemia cutis but did have 11;19 translocation in >90% of bone marrow cells.
- Received 1 cycle of 5-azacitidine. Declined further therapy because of drug side effects

Screening

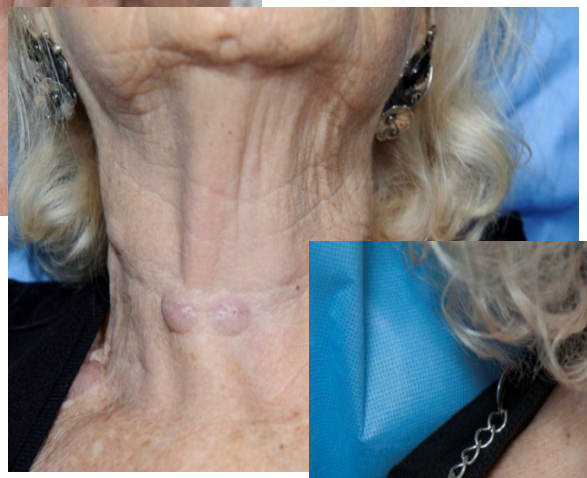




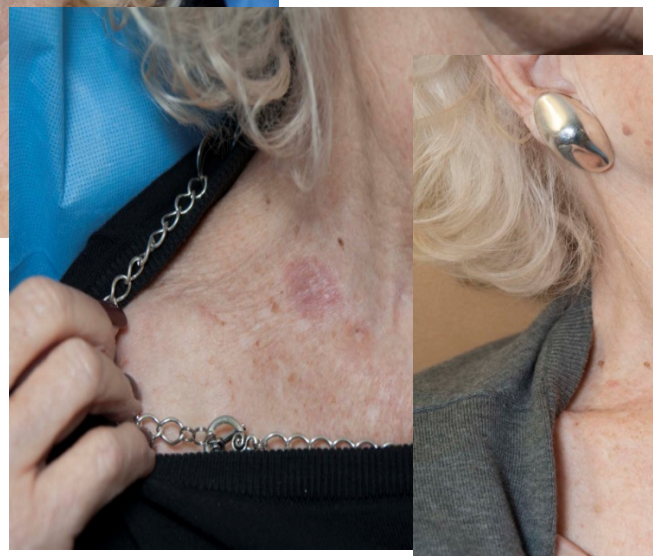
Courtesy, Dr. Klaus Busum



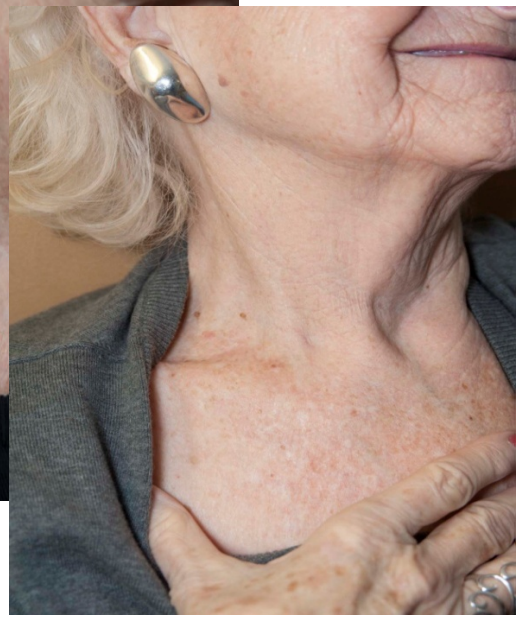
Cycle 1 day 1



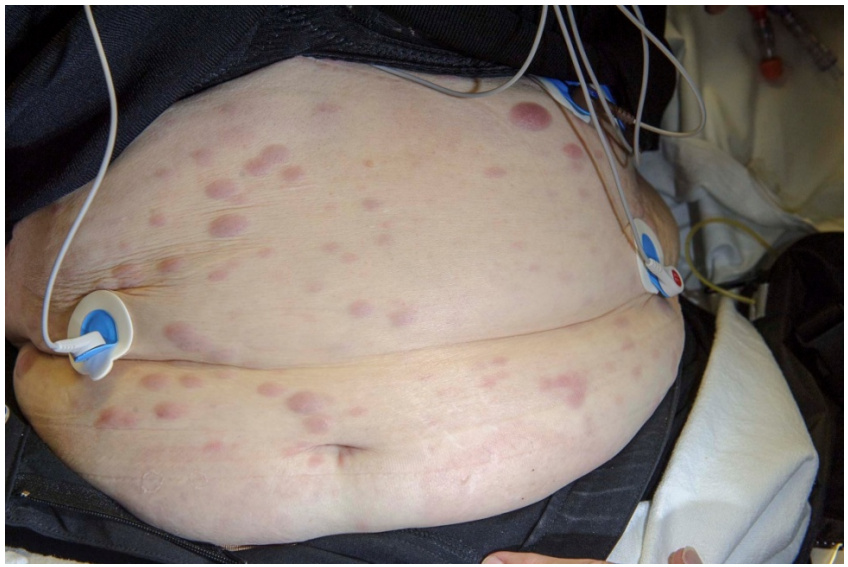
Cycle 2 day 1

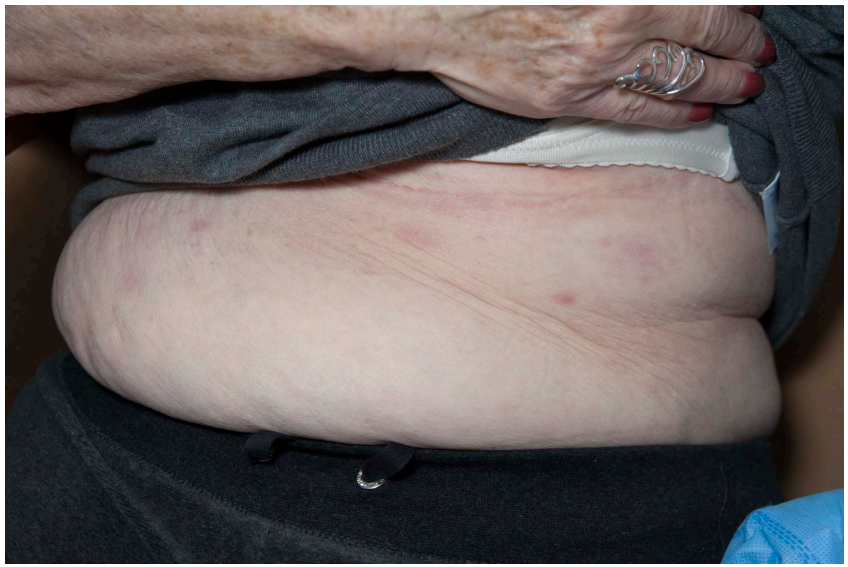


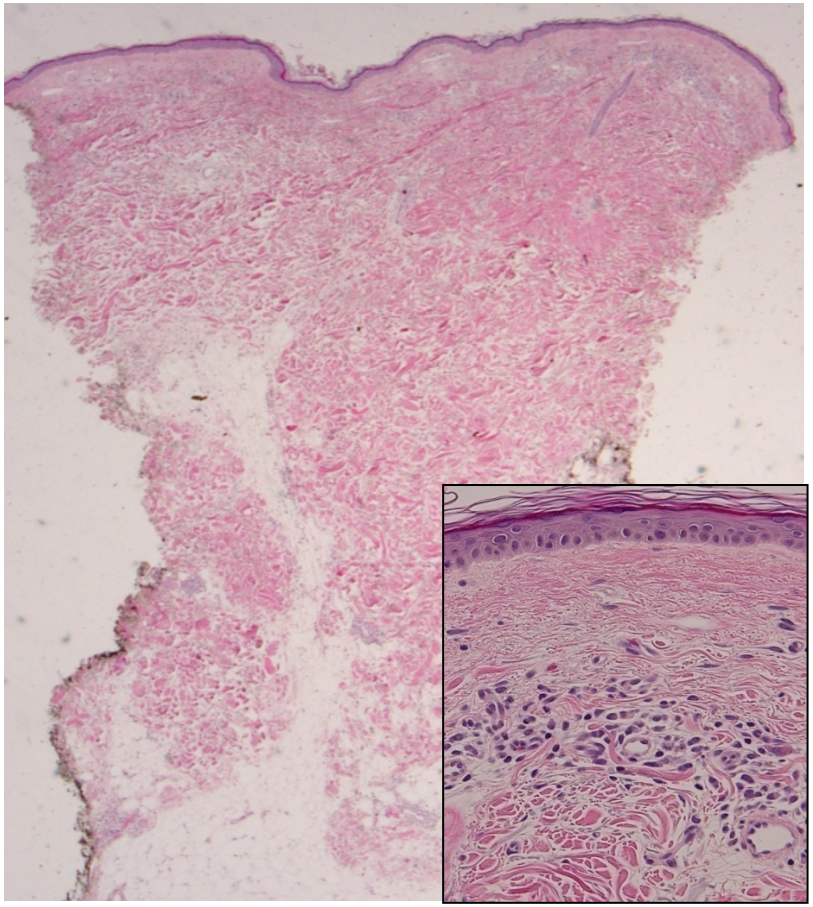
Cycle 3 day 1



Cycle 4 day



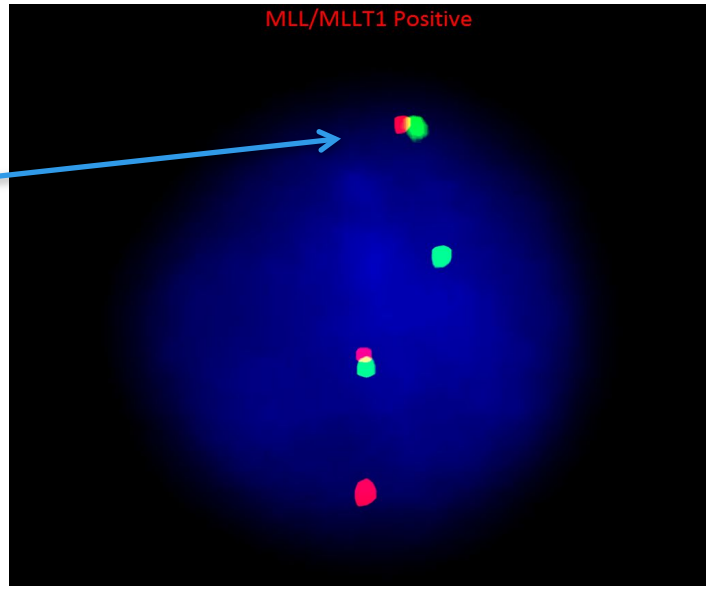




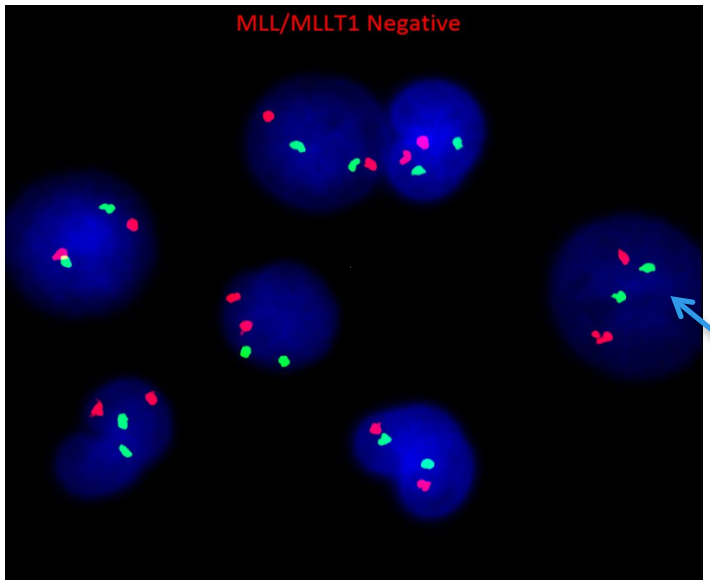
Courtesy, Dr. Klaus Busum

Changes in Peripheral Blood Counts and Decrease in Translocation Positive Cells

	WBC	Platelets	ANC	Abosolute Moncytes
Baseline	10.7	87	3.0	3.1
C1D15	1.6	138	0.4	1.1
C2D1	1.3	143	0.3	0
C3D1	2.0	157	0.6	0.2
C4D3	3.4	191	1.7	0.4



Translocation positive at screening



Translocation negative at Cycle 4

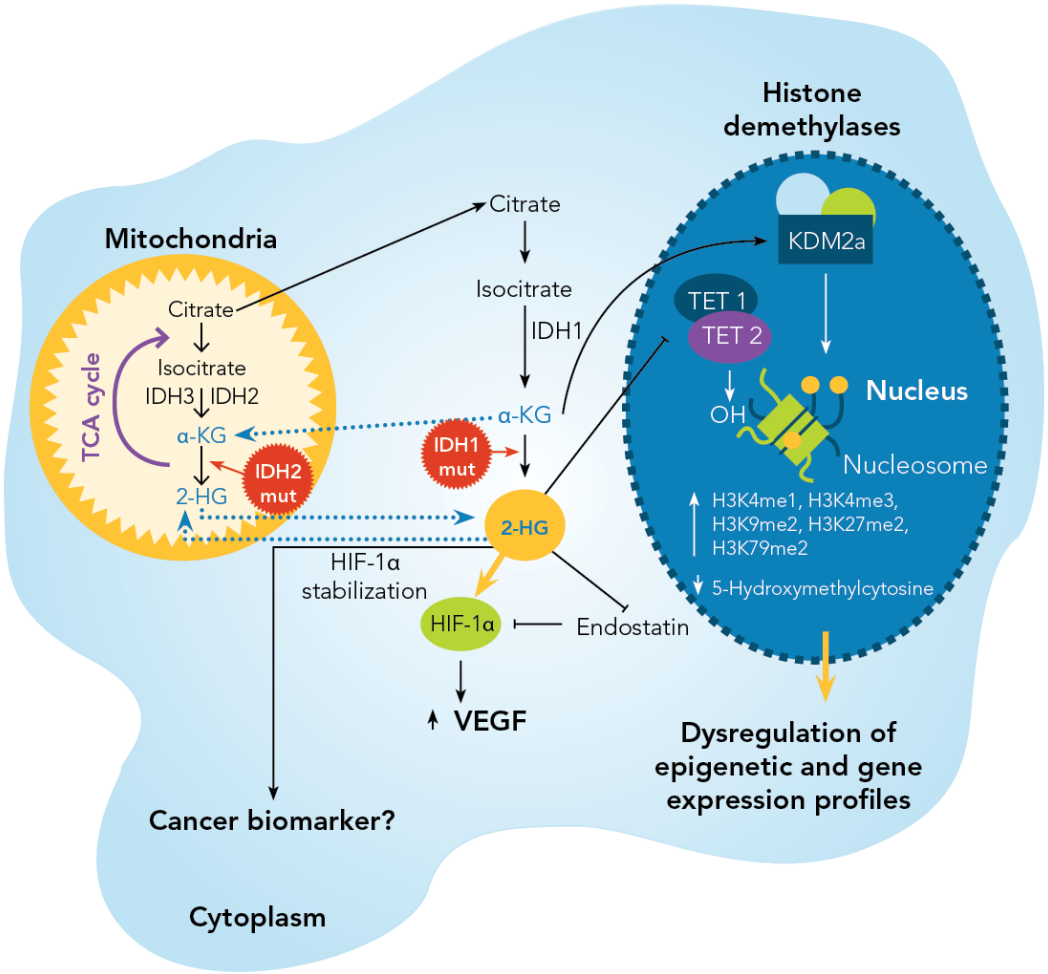
Screening – 90% positive **Cycle 4, day 1, 0.2% positive**

(Fusion FISH)

DOT1L Inhibitor in AML

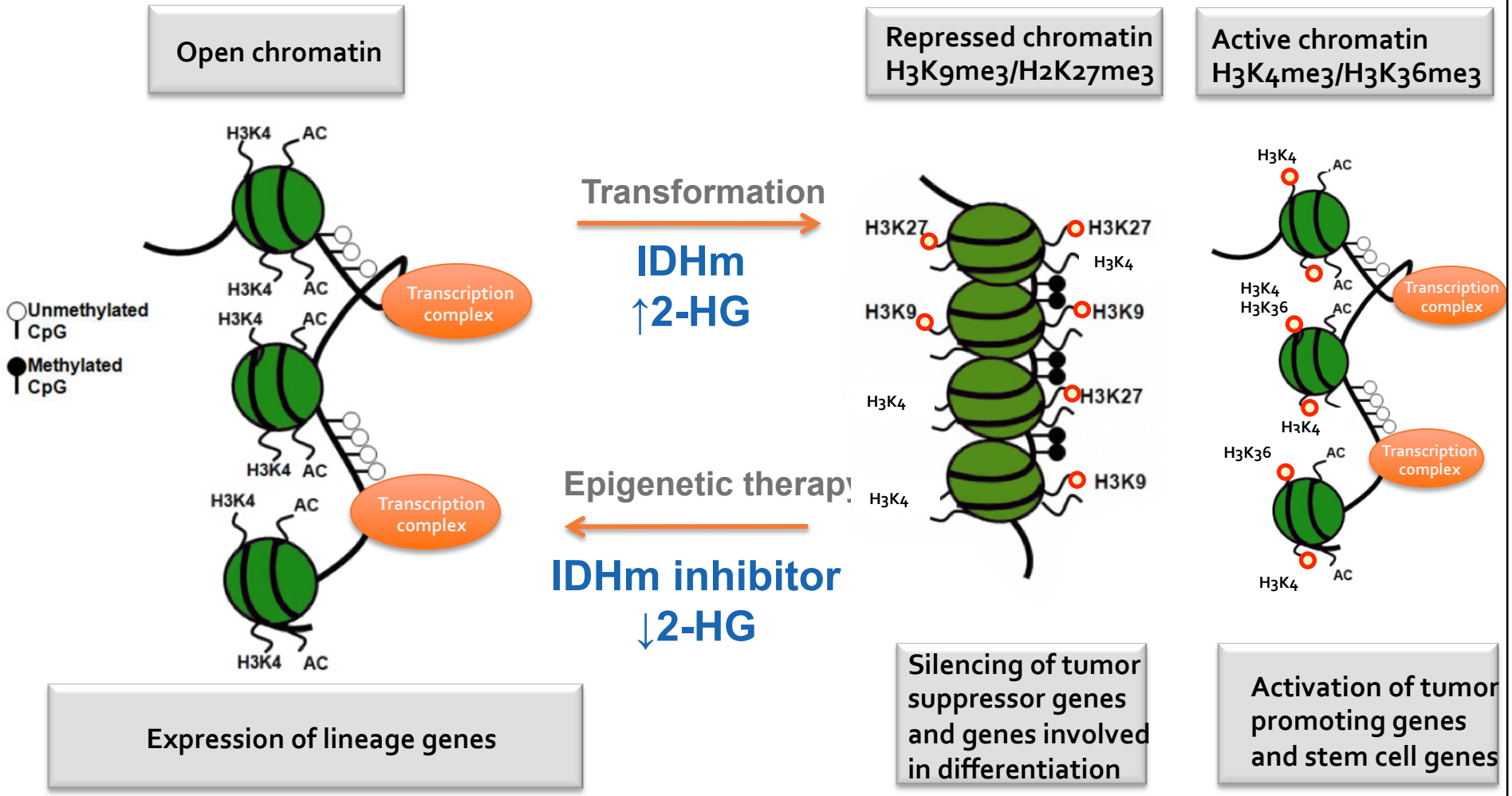
- Is active and only in *MLL*-r patients
- Appears to induce differentiation
- Is well-tolerated
- Next steps for development include combination with other novel agents and/or chemotherapy
  - Mennin (inhibitors): Ubiquitously expressed nuclear protein, tumor suppressor, cofactor of MLL fusions
  - Entospletinib + CPX-351
  - DOT1L + Aza

Role of *IDH* in Malignancy



- IDH is critical metabolic enzyme in the citric acid cycle
- IDH1 in cytoplasm and IDH2 in mitochondria
- Cancer-associated IDHm produces 2-hydroxyglutarate (2-HG) and blocks normal cellular differentiation

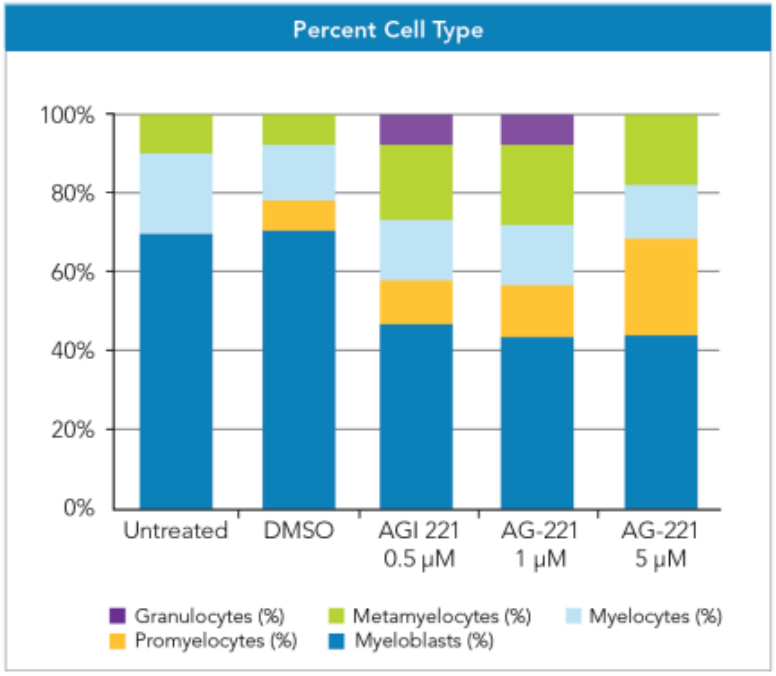
Current Working Model of 2-HG as Oncometabolite

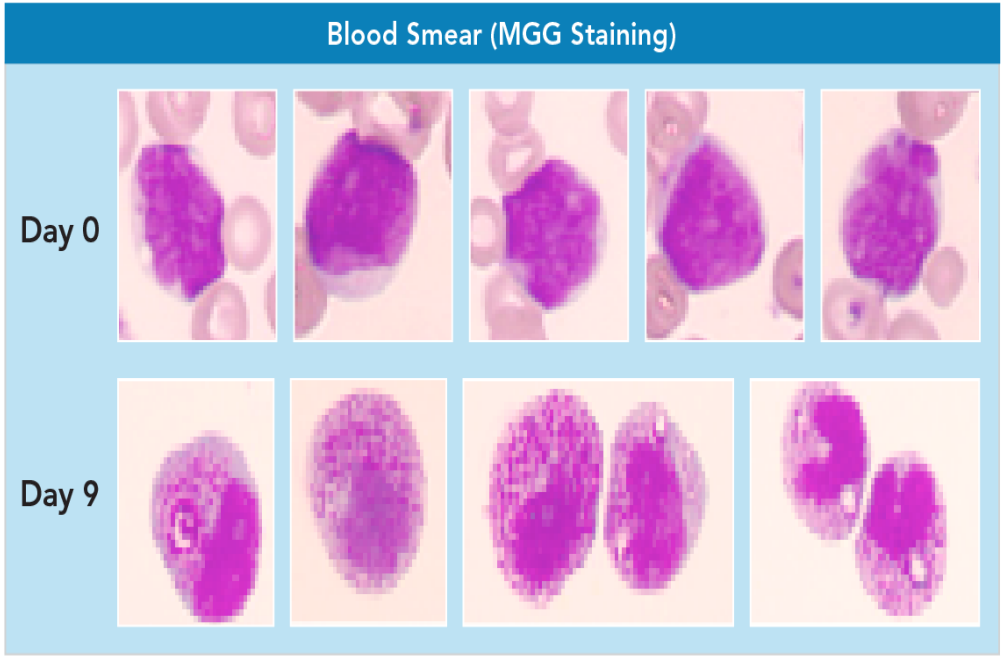


Dawson et al. NEJM, 2012;
Rrodriguez et al. Nature Rev Genet, 2014

AG-221 Reverses Differentiation Block in Primary Patient Samples

- Ex vivo dosing of an *IDH2 R140Q*, AML M1 patient sample
- Cytology following treatment with AG-221

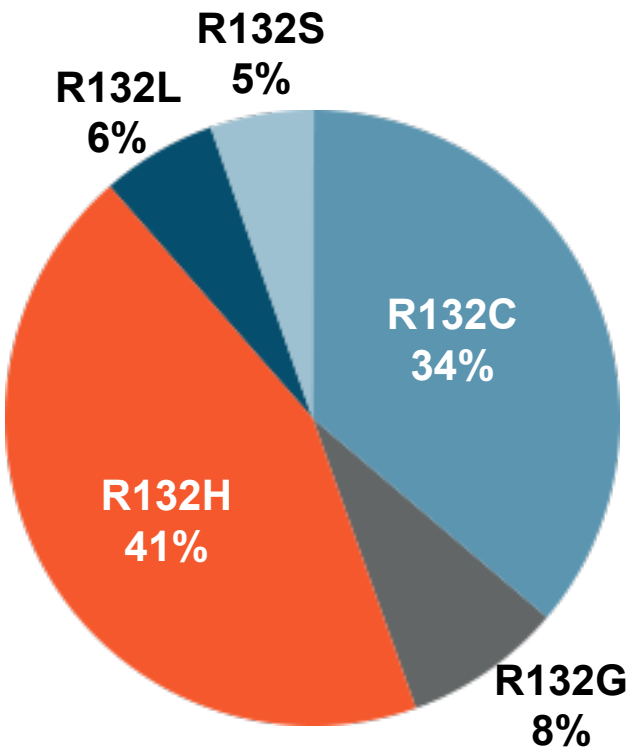




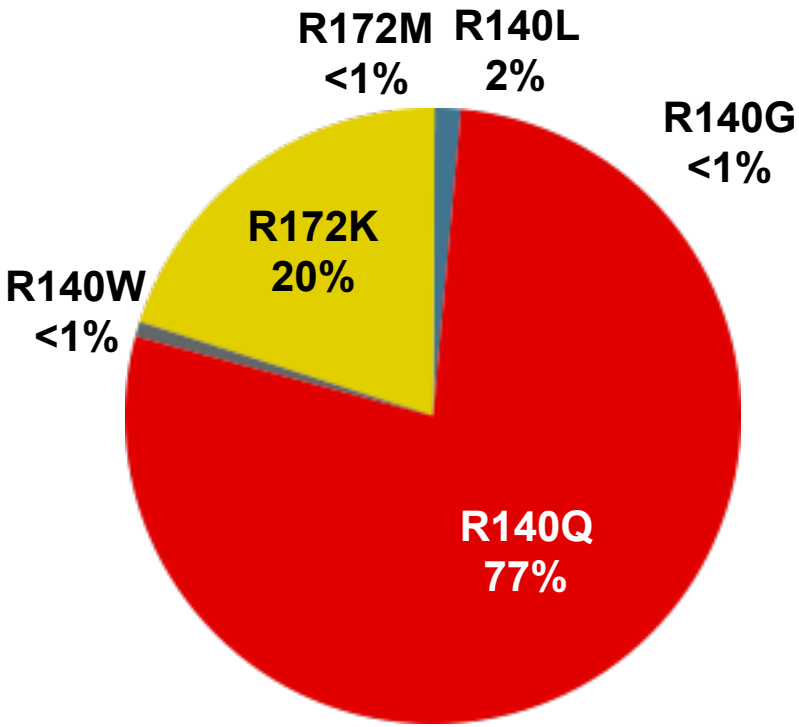
Stephane de Botton, IGR

IDH Mutations in AML

IDH1m (8%) in AML



IDH2m (15%) in AML

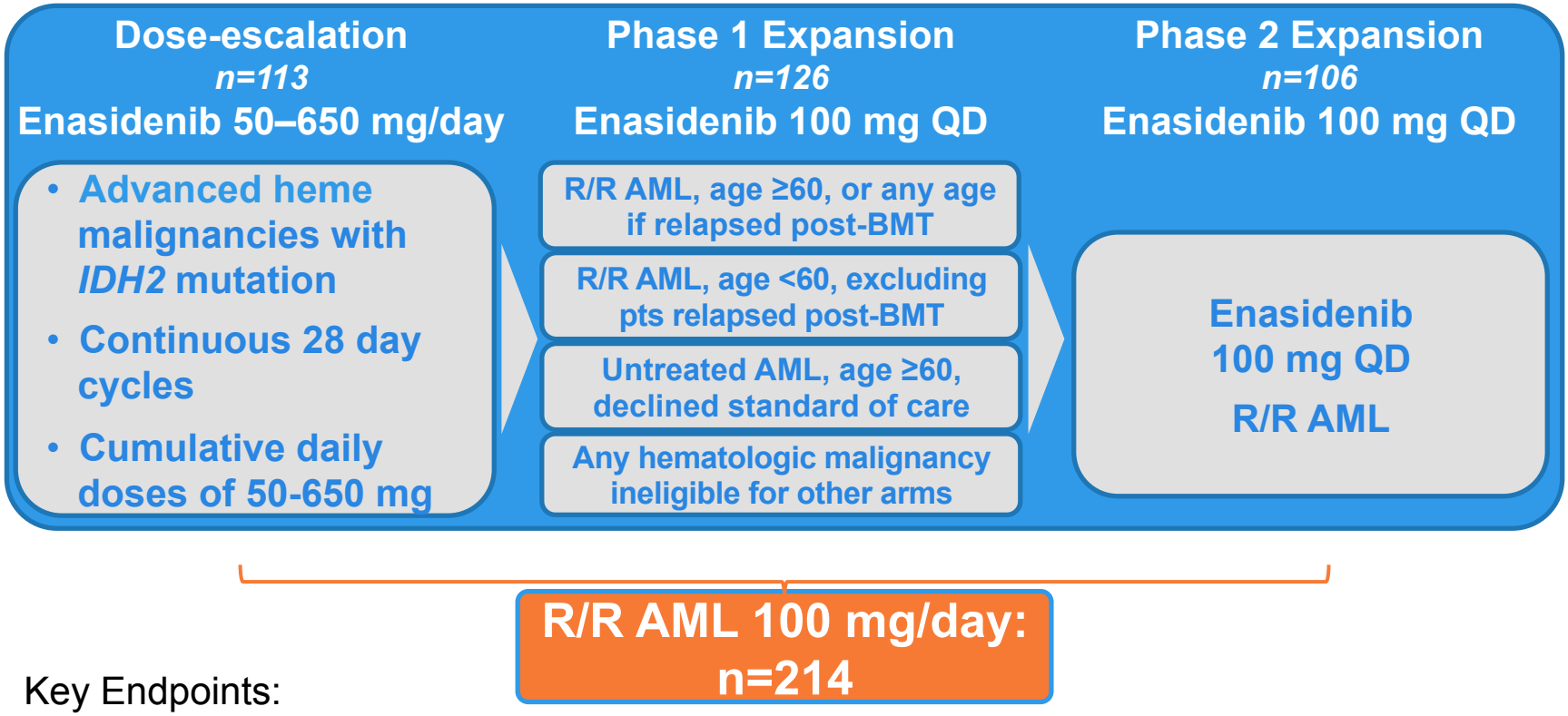


IDH2 Mutations

- Enriched in patients with NK
- Increase with advancing age
- Occur in 1 of 2 arginine residues of the enzyme, R140Q and R172K
- Generally mutually exclusive with IDH1
- R140 comutation with NPM1, R172 mutually exclusive with NPM1
- In preclinical studies inhibition decreased 2-HG by >90%, reduced histone hypermethylation and restored myeloid differentiation

Papaemmanuil et al. NEJM, 2016

Phase 1/2 Study Design



Key Endpoints:

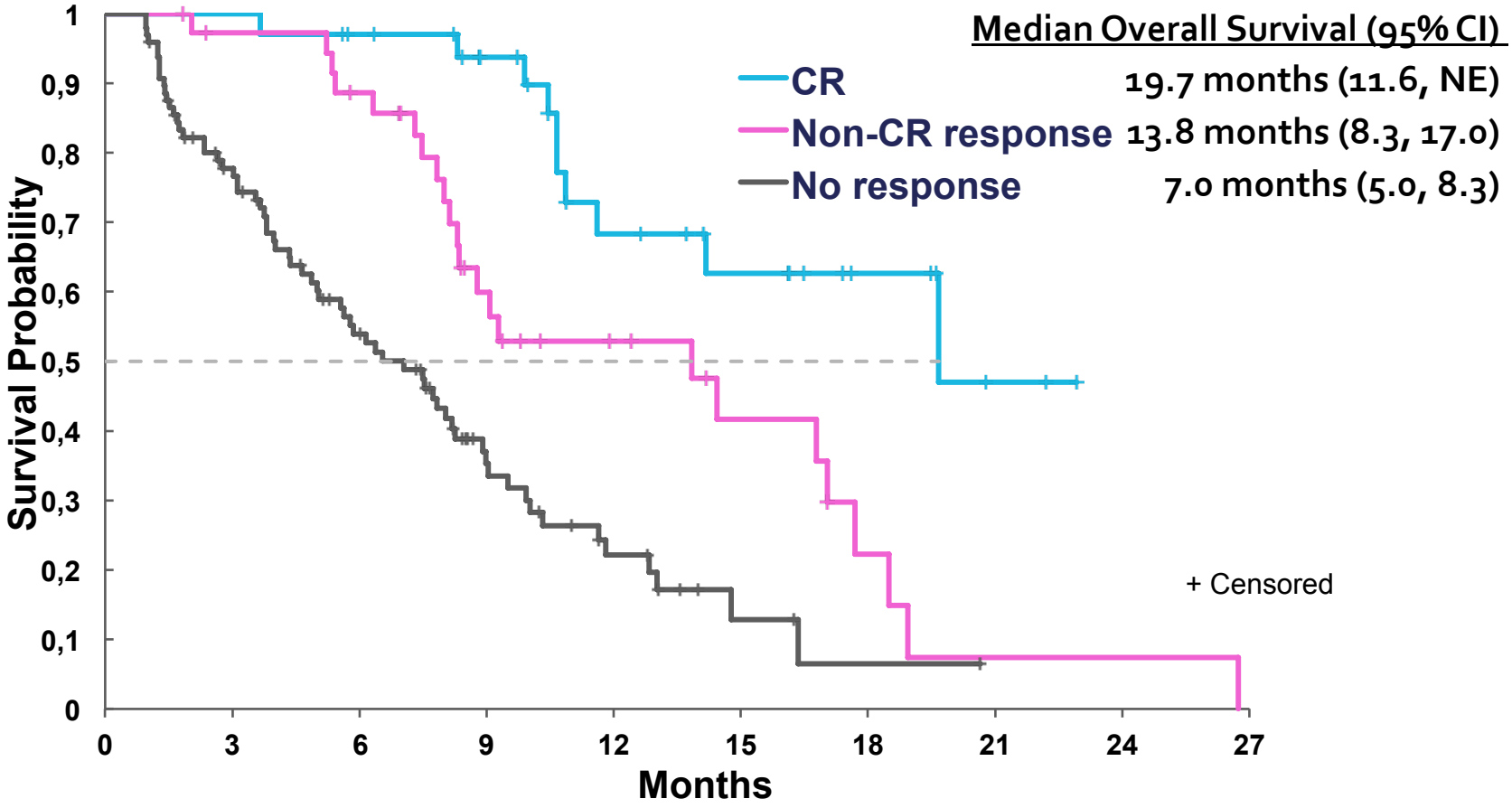
- Safety, tolerability, MTD, DLTs
  - MTD not reached at doses up to 650 mg/day
- Responses assessed by local investigator per IWG criteria
- Assessment of clinical activity, with focus on 100-mg daily dose in patients with R/R AML

AG-221: Response

	Relapsed or Refractory AML	
	Enasidenib 100 mg/day (n=109)	All doses (N=176)
Overall response rate, % [n/N] 95% CI	**38.5%**	**40.3%**
Best response		
CR, %	**20.2**	**19.3**
CRi or CRp,%	6.4	6.8
PR,%	2.8	6.3
MLFS,%	9.2	8.0
SD,%	53.2	48.3
PD,%	4.6	5.1
NE,%	1.8	1.7
Time to first response mos, median	1.9	1.9
Duration of response mos, median	5.6	5.8
Time to CR mos, median	3.7	3.8
Duration of CR mos, median	8.8	8.8

Stein et al. ASCO, 2017 (abstr 7004) and Blood, 2017

Overall Survival by Best Response



Stein et al. ASCO, 2017 (abstr 7004) and Blood, 2017

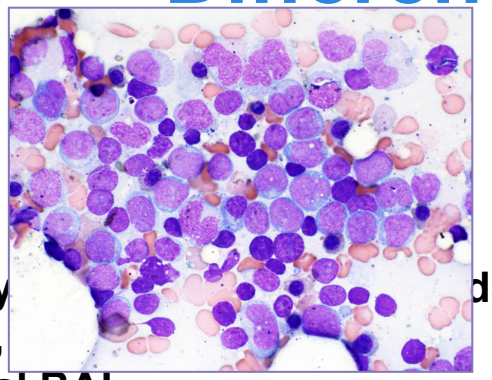
Most Common Treatment-Emergent Adverse Events (≥20% of All patients)

All Patients (N=345)	Any Grade	Grade 3-4	
		All	Treatment-related
Nausea	48%	5%	2%
Diarrhea	41%	4%	< 1%
Fatigue	41%	8%	2%
Decreased appetite	34%	4%	2%
Blood bilirubin increased	33%	11%	8%
Vomiting	33%	2%	< 1%
Dyspnea	32%	8%	3%
Anemia	32%	24%	6%
Cough	30%	8%	0
Febrile neutropenia	30%	29%	2%
Peripheral edema	29%	1%	< 1%
Pyrexia	28%	3%	< 1%
Constipation	27%	< 1%	0
Hypokalemia	26%	8%	< 1%
Thrombocytopenia	21%	18%	3%
Headache	20%	< 1%	< 1%
Pneumonia	20%	16%	0

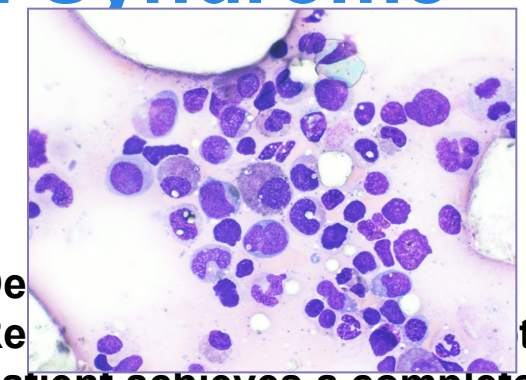
Serious treatment-related IDH-DS was reported for 7% of patients

Stein et al. EHA, 2017 and Blood, 2017

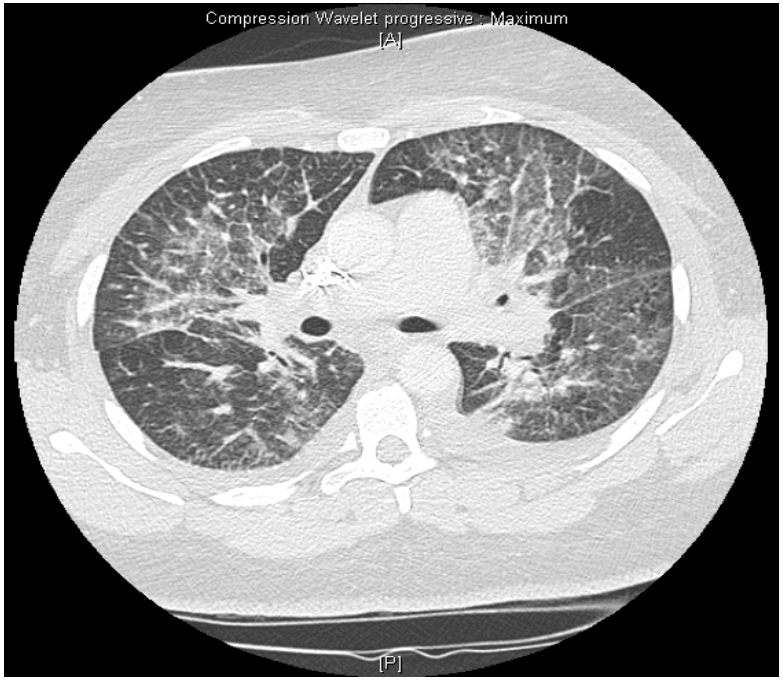
Differentiation Syndrome

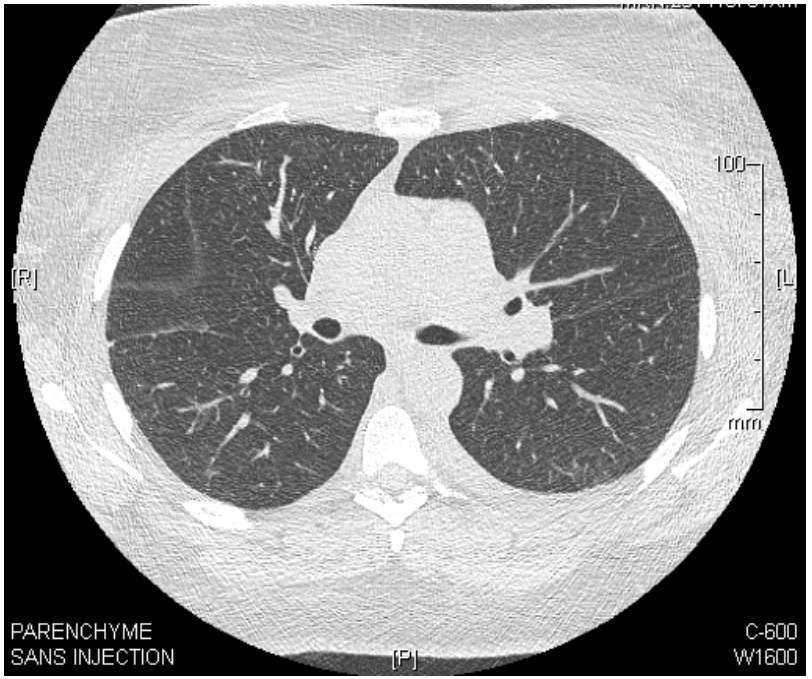


- 21 day … daily
- Fever, …
- Normal BAL



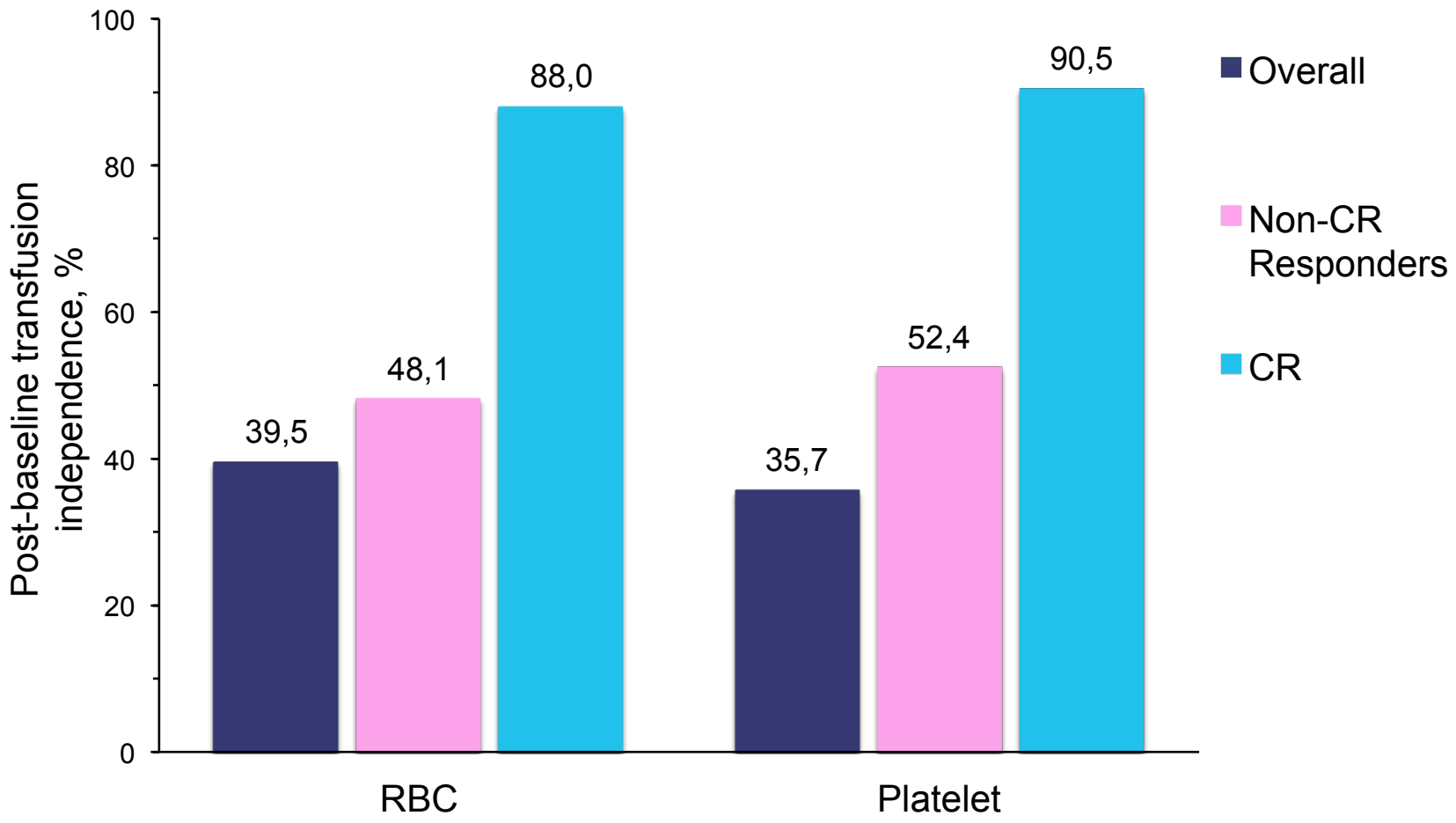
- De… for 15 days
- Re… toms
- Patient achieves a complete remission





Courtesy Dr. Stephane De Botton

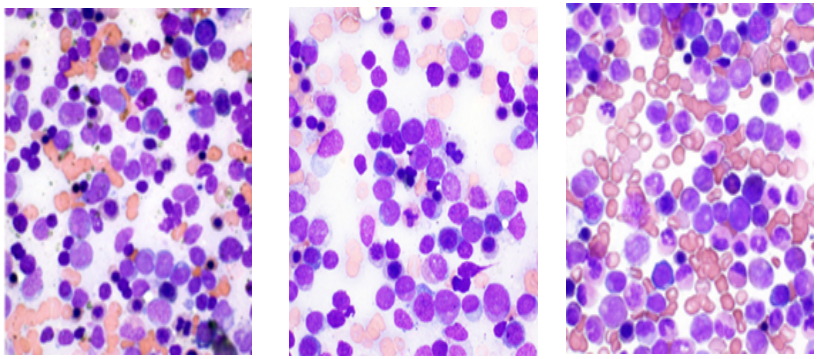
Transfusion Independence



Morphological evidence of myeloid differentiation

Patient 1

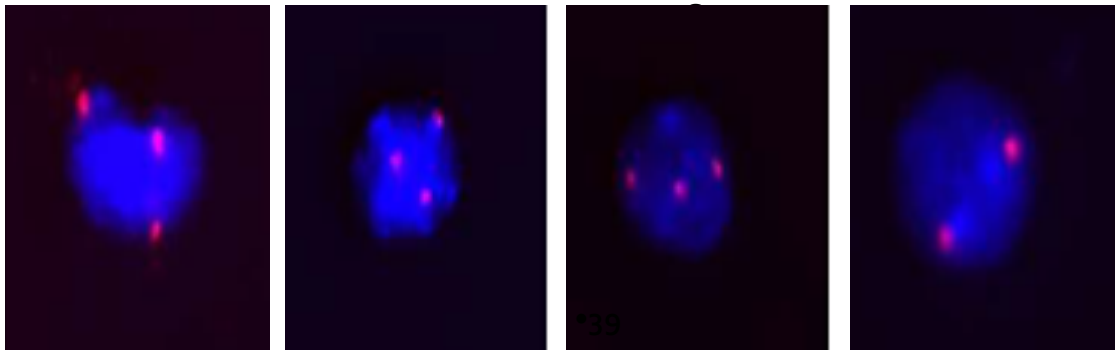
Screening	Cycle 1 Day 15	Cycle 3 Day 1
37% blasts	Evidence of cellular differentiation	4% blasts



FISH evidence of myeloid differentiation

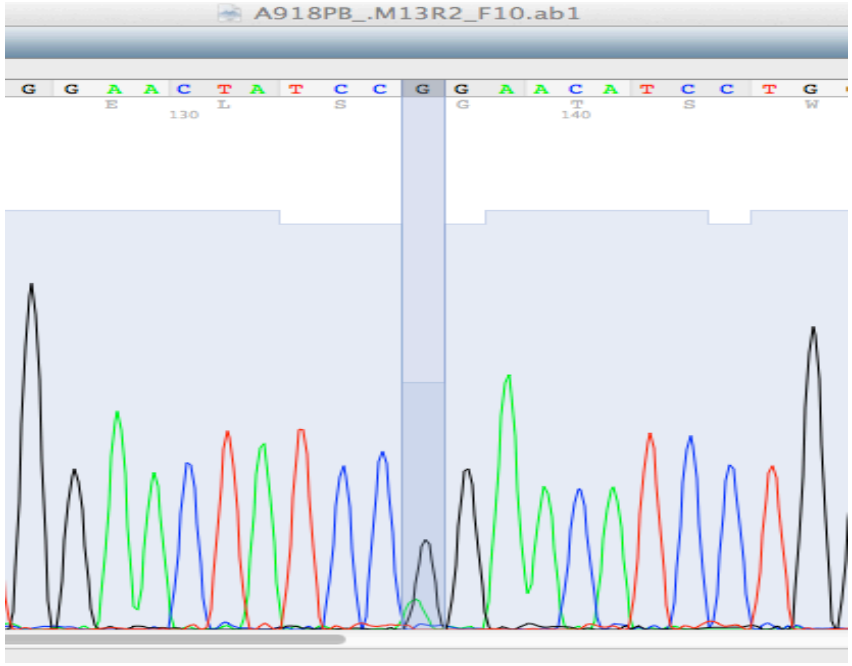
Patient 2 C2D1, trisomy 8

Blasts	Promyelocytes	Mature Granulocyte	Lymphocytes

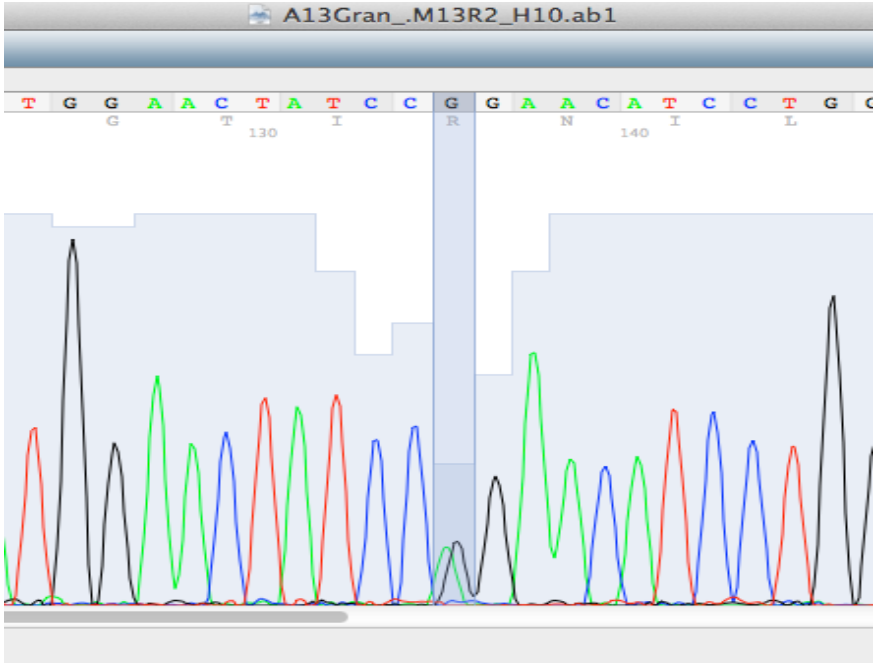


Molecular Evidence of Differentiation

Screening – PBMC



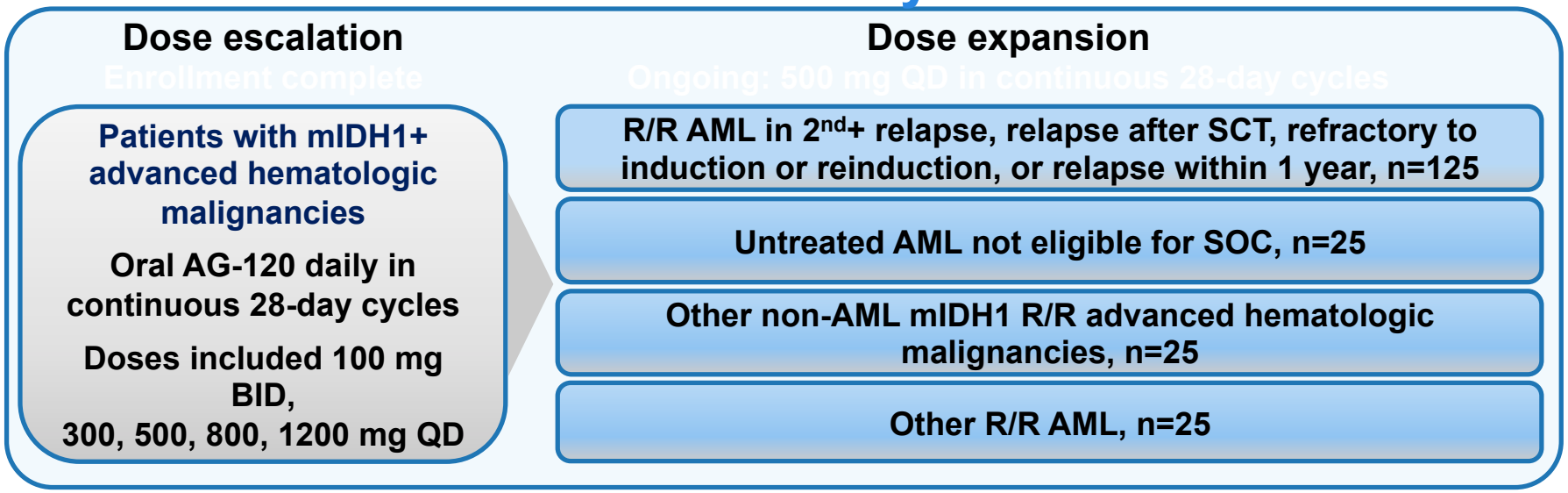
Cycle 3 day 1 – Remission - Granulocytes



Alan Shih and Ross Levine, MSKCC

Study Design

Single-arm, open-label, phase 1, multicenter study of AG-120



Dose escalation objectives

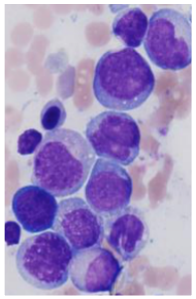
Primary	Safety and tolerability, identification of maximum tolerated dose (MTD) and/or recommended phase 2 dose
Secondary	Assessment of clinical activity by investigators using modified 2003 IWG criteria in AML
Exploratory	Determination of mIDH1 variant allele frequency (VAF) by next-generation sequencing (NGS)

Dinardo et al. ASH, 2016

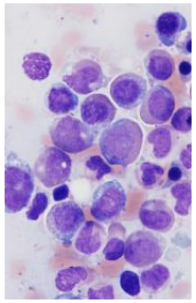
Clinical activity

Patient achieved CR by end of Cycle 1

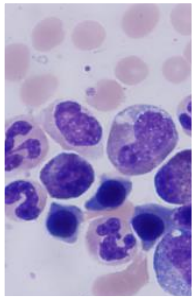
Screening
44% blasts



Cycle 1
Day 15
3% blasts



Cycle 1
Day 28
2% blasts



	Dose escalation	
	R/R AML n=63	Overall N=78
CR, n (%)	10 (16)	14 (18)
CRi/CRp, n (%)	8 (13)	8 (10)
PR, n (%)	1 (2)	2 (3)
mCR/MLFS, n (%)	2 (3)	6 (8)
SD, n (%)	27 (43)	30 (38)
PD, n (%)	8 (13)	8 (10)
NE, n (%)	7 (11)	10 (13)
ORR, n (%) [95% CI]	21 (33) [22, 46]	30 (38) [28, 50]

Dinardo et al. ASH, 2016

Determination of mIDH1 Mutation Clearance by NGS

Mutation clearance defined as:
- **mIDH1 positive at screening (VAF >1% from any sample type), AND**
- **no mIDH1 detected at ≥1 on-study time point (VAF cut off 1%)**

Genomic DNA extracted for NGS analysis of mIDH1 VAF from samples:
- **Whole PB/BM**
- **PB/BM mononuclear cells**

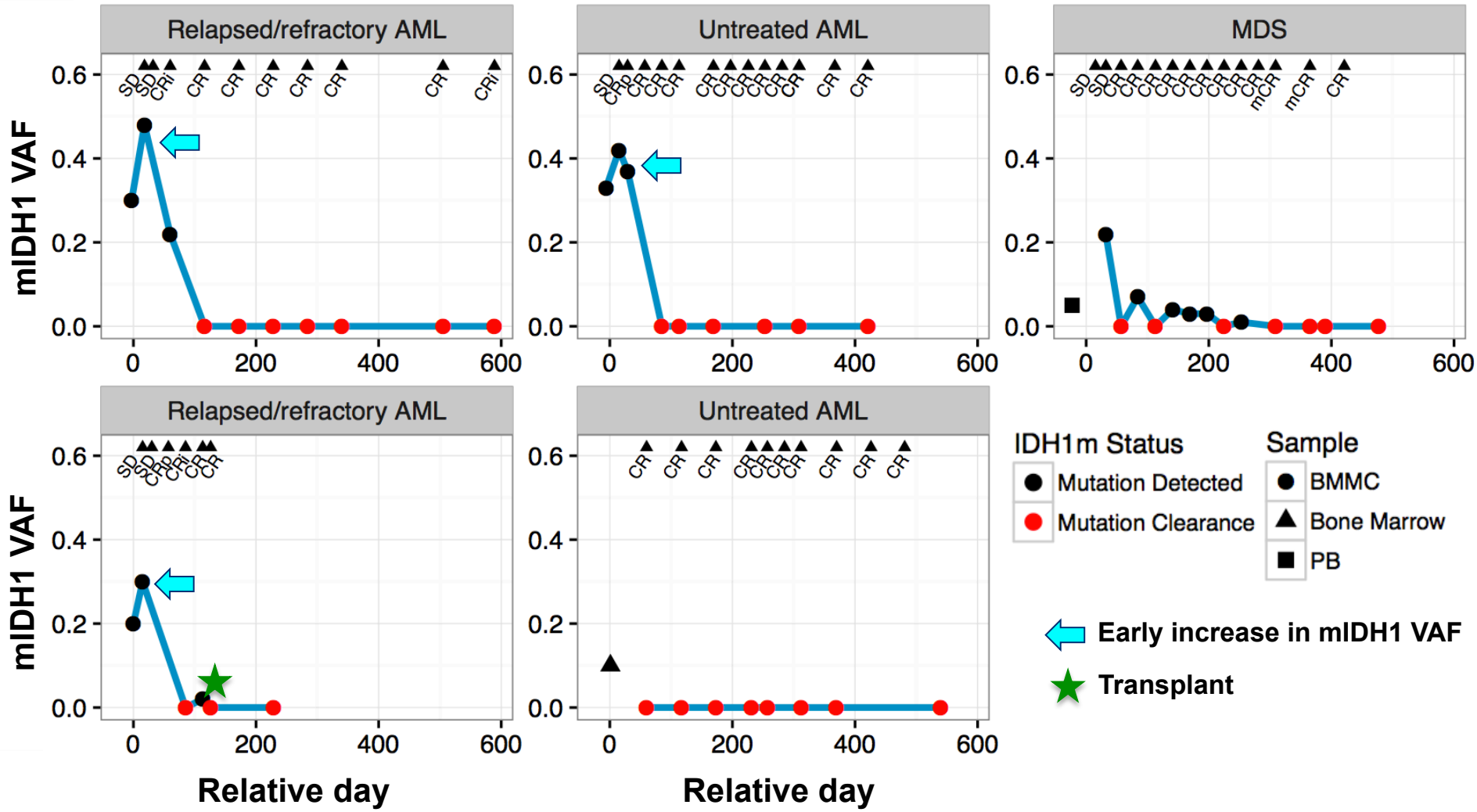
→ **67 subjects with mIDH1 VAF data at screening and at least one on-study time point**

→ **mIDH1 mutation clearance analyzed**

Best response	Number of subjects with longitudinal VAF	Number of subjects with mutation clearance
CR	14	5
Non-CR	53	2
Total	**67**	**7**

Dinardo et al. ASH, 2016

AG-120: IDH1 Mutation Clearance in Patients With CR



Dinardo et al. ASH, 2016

Frequently Asked Questions
Enasidenib

- Does molecular CR occur? Yes, about 30% yet EFS same as CR wo molec CR
- Does differentiation syndrome occur? Yes, and can occur late (med d48,10-340)
- How long does it take to achieve CR? 21% by C3, 68% by C5, 82% by C7
- Are molecular signatures predictive of response or nonresponse? RAS mutations assoc with NR
- What is the longest duration of CR? >30 months

Current Open and Planned Trials With Ivosidenib or Enasidenib

- Open
  - Phase 3 IDHENTIFY: Ena vs CCR in advanced AML
  - Phase 1: Ivo or Ena with induction and consol in newly diagnosed AML
  - Phase1/2: Ivo or Ena with sq aza in newly diagnosed AML
  - Phase 3 AGILE: Ivo vs placebo + Aza in previously untreated AML
- Planned
  - Phase 1/ 2; Ivo/Ena + gilteritinib in pts w/ IDH and FLT3 mutations
  - Phase 1/2: Ena + Trimetinib in pts w/ IDH and RAS pathway mutation
  - Venetoclax + Ena in rel/refr AML

Summary and Conclusions

- Continuous oral Ivosidenib and Enasidenib induce CRs in rel/ refr AML
- Treatment leads to lowering 2-HG (but lack of assoc between extent of suppression and response) and differentiation of leukemic blasts rather than cytotoxicity
- Ivosidenib and Enasidenib well tolerated and not myeloablative
- OS median in rel/refr AML with Ena 9 months
- May be a bridge to transplant
- Multiple combination trials underway


Acknowledgments
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