ManagingAML

Mutation-Targeted AML Strategies

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Hello, everyone. Welcome to this educational session on acute myeloid leukemia. I'm going to be discussing a new therapeutic era in AML. My name is Amir Fathi. I'm the Director of the Leukemia Program at Massachusetts General Hospital. I'm also Associate Professor of Medicine at Harvard Medical School.

Disclosures

- Dr. Amir Fathi has relevant financial relationships related to advisory activities and consulting from AbbVie Inc., Agios, Inc. (now Servier Laboratories), Amgen Inc., Astellas Pharma US, Inc., Blueprint Medicines, Bristol-Myers Squibb Company, Celgene Corporation – A Bristol-Myers Squibb Company, Foghorn Therapeutics, Genentech, Inc., Ipsen, Kite Pharma, Kura Oncology, Inc., MorphoSys, Pfizer Inc., Seattle Genetics, Inc., Takeda Oncology, and Trillium Therapeutics Inc., as well as clinic trial support from Abbvie, Agios (now Servier), and Celgene Corporation – A Bristol-Myers Squibb Company.

This first slide lists my disclosures

AML – Numbers (2020)

- New cases each year: 19,940
- Deaths each year: 11,180
- Median age: ~67

American Cancer Society. *Cancer Facts & Figures 2020.*

As a way of background, at least as of 2020, AML is luckily a relatively uncommon malignancy, approximately 20,000 new cases per year in the United States. Unfortunately, we continue to have more than 10,000 deaths a year from AML in the United States, and it continues to be a challenging, often lethal disease.

Part of the challenge related to AML is the median age is 67. Every year, this median age creeps up a little bit as the population gets older. What that also means is that half of our patients are predominantly in their 70s, 80s, and 90s, which makes aggressive intensive therapy difficult.

Mutation-Targeted AML Strategies

ELN Molecular and Cytogenetic Risk Groups

Genetic Group	Subsets
Favorable	t(8;21)(q22;q22); *RUNX1-RUNX1T1*
	inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
	Mutated *NPM1* without *FLT3*-ITD (normal karyotype)
	Mutated *CEBPA* (normal karyotype)
Intermediate-I	Mutated *NPM1* and *FLT3*-ITD (normal karyotype)
	Wild-type *NPM1* and *FLT3*-ITD (normal karyotype)
	Wild-type *NPM1* without *FLT3*-ITD (normal karyotype)
Intermediate-II	t(9;11)(p22;q23); *MLLT3-MLL*
	Cytogenetic abnormalities not classified as favorable or adverse
Adverse	inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *RPN1-EVI1*
	t(6;9)(p23;q34); *DEK-NUP214*
	t(v;11)(v;q23); *MLL* rearranged
	–5 or del(5q)
	–7
	abnl(17p)
	Complex karyotype*

AML, acute myeloid leukemia; ITD, internal tandem duplication
*Complex karyotype is defined as three or more chromosome abnormalities in the absence of one of the WHO designated recurring translocations or inversions: t(8;21), inv(16) or t(16;16), t(15;17), t(9;11), t(v;11)(v;q23), t(6;9), inv(3), or t(3;3).
European LeukemiaNet Standardized Reporting System for Correlation of Cytogenetic and Molecular Genetic Data in AML with Clinical Data.; Mrozek K, et al. *J Clin Oncol*. 2012;30(36):4515-4523.

Let's briefly talk about the prognostication of AML based on molecular data. AML traditionally, at least according to the European LeukemiaNet and other categorizations, is categorized according to adverse-, intermediate- or favorable-risk disease. Favorable-risk disease is related to certain chromosomal or mutational data that render the disease more susceptible to traditional chemotherapy. Unfortunately, there are many more categories of adverse-risk AML, including complex karyotype and inversion 3, isolated FLT3 mutations, deletion of 7, deletion of 5. All of these abnormalities render the disease either more resistant to therapy or more likely to relapse. If you do not have a favorable risk feature or an adverse risk signature, there's a large chunk of AML patients who are considered intermediate risk.

Mutation-Targeted AML Strategies

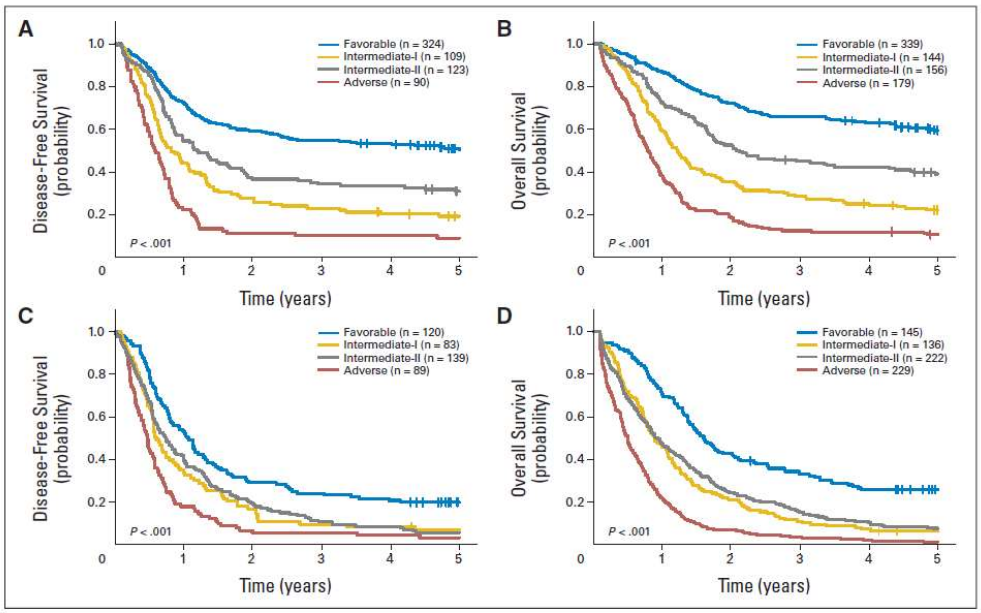
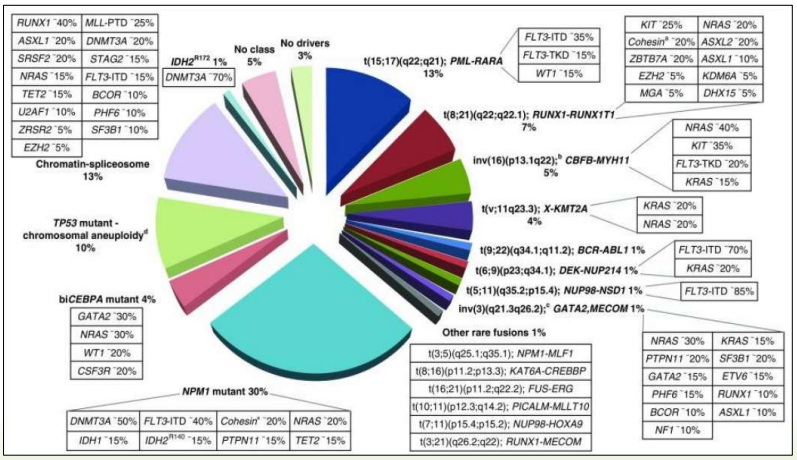


Fig 4. Outcome of patients with primary acute myeloid leukemia classified into the four European LeukemiaNet genetic groups according to the European LeukemiaNet recommendations. (A) Disease-free survival and (B) overall survival of patients younger than age 60 years; (C) disease-free survival and (D) overall survival of patients age 60 years or older.

Mrozek K, et al. *J Clin Oncol*. 2012;30(36):4515-4523.

Based on your risk, from this molecular and cytogenetic data, the survival curves can be splayed out. The top two curves, A and B, look at disease-free and overall survival among younger patients. Those at the bottom are those individuals who are 60 years or over. As you can see, patients who are older have a substantially worse prognosis, regardless of the favorable, intermediate or adverse risk.

AML Gets More Complex!

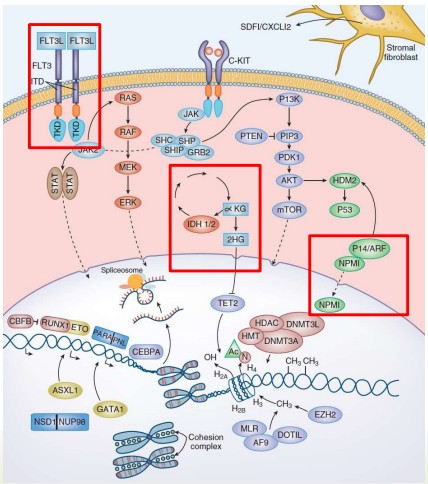


Dohner H, et al. *Blood*. 2017;129(4):424-447.

That's kind of where we've been for some time, but AML has also been substantially more complex. It's always been more complex, but we've learned much more about it. Here is a pie graph of all the different subtypes of AML and the features they share in terms of molecular features.

Mutation-Targeted AML Strategies

AML Gets More Complex!



Brunner AM, Graubert TA. *Nat Med*. 2018;24(1):7-9.

The targets that we have learned about in recent years have been FLT3-mutated disease, there is actually multiple small molecule inhibitors of FLT3. FLT3 is a receptor tyrosine kinase that resides on the surface of the cell, and the mutation of the gene causes the alteration of that receptor, causing rapid uncontrolled proliferation of the myeloid blast. IDH mutations are also another target of therapy within the small molecule inhibitors of IDH1 and IDH2. IDH alterations lead to the uncontrolled production of an oncometabolite called 2HG that leads to the myeloid malignancy phenotype.

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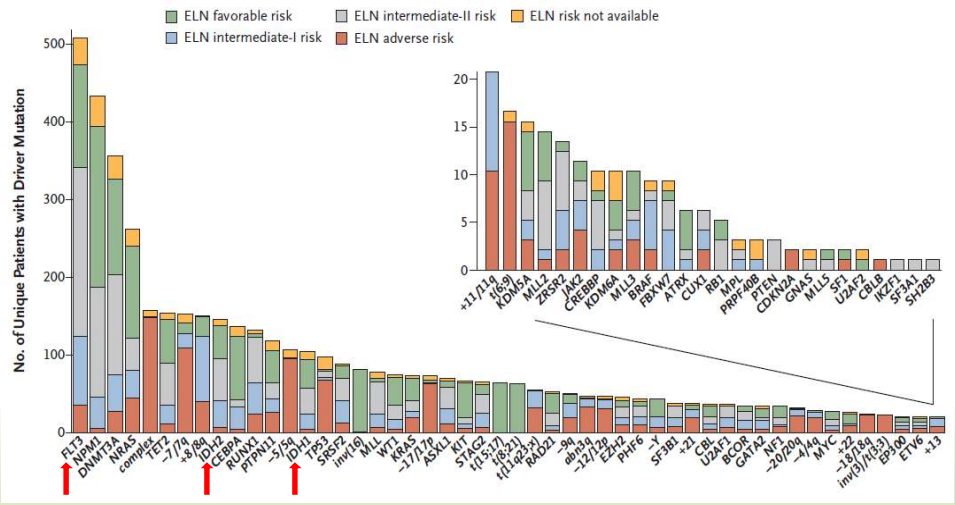
Genomic Characterization of AML

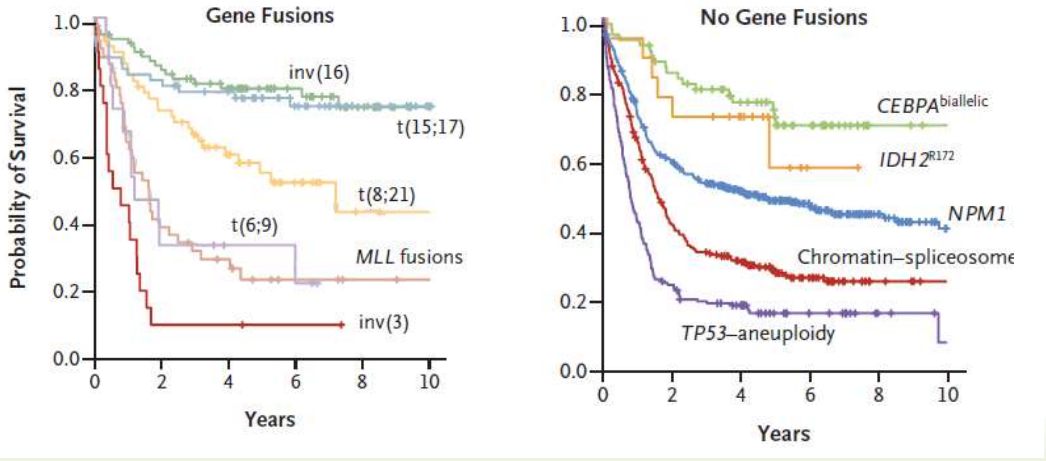
Driver Mutations in Acute Myeloid Leukemia (AML)



Papaemmanuil E, et al. *N Engl J Med*. 2016;374:2209-2221.

The more common alterations in AML are FLT3, NPM1, and DNMT3A. There is a long list of alterations that are seen in patients with favorable-risk, intermediate-risk, and adverse-risk disease, as can be seen in the bar graph here.

Kaplan-Meier Curves for Overall Survival Among Patients in the 11 Genomically Defined Subgroups

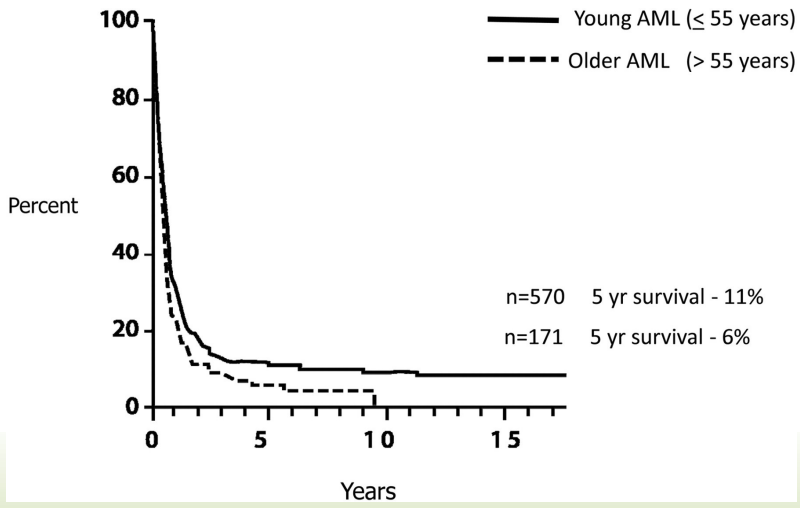


Papaemmanuil E, et al. *N Engl J Med*. 2016;374:2209-2221.

More recently, we've been able to look not just at categorizations according to disease risk, but also specifically look at mutations and chromosomal changes individually. Alterations such as inversion 3, and mutations such as P53 as can be shown in these curves, if harbored in a patient's MDS or AML, are risks that are very adverse and lead to a shortened lifespan and really poor survival.

Mutation-Targeted AML Strategies

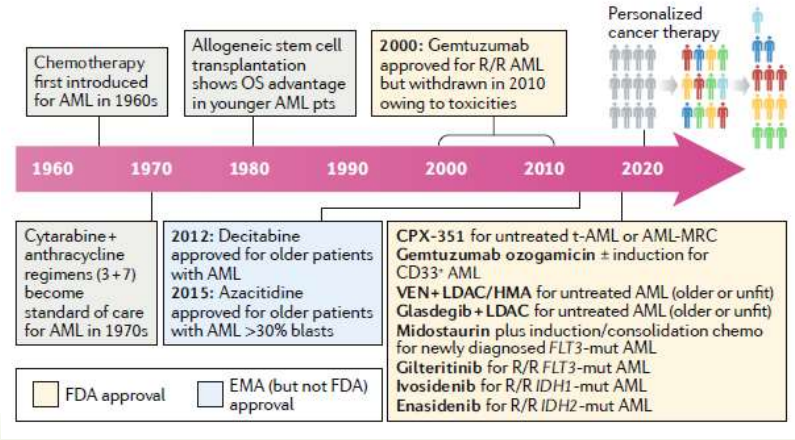
Relapsed AML



Rowe JM, Tallman MS. *Blood.* 2010;116:3147-3156.

Regardless of the risk, if a patient has relapsed AML or AML that is refractory, their outcomes are dismal. This is a curve that I borrowed from a paper from about 10 years ago. I don't think much has changed since then, perhaps it's a little bit better. Five-year survival in patients younger than 55 or over 55 remains quite poor.

Development in the Treatment of AML



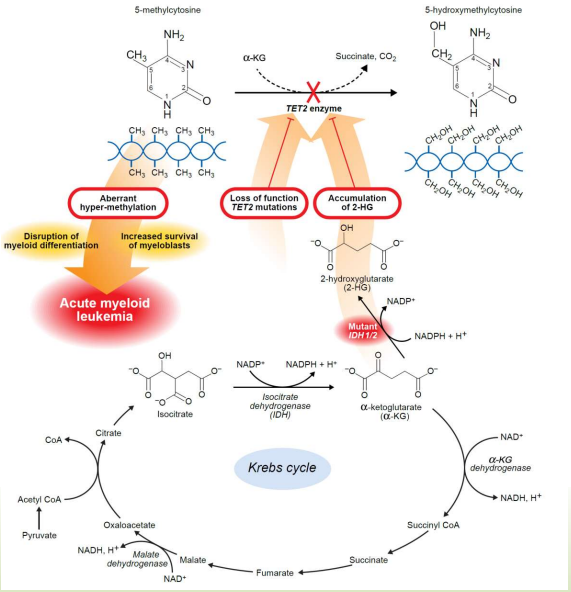
DiNardo CD, Perl AE. *Nat Rev Clin Oncol.* 2019;16(2):73-74.

There is some reason for hope. For decades the therapeutic landscape of AML had not changed since the late 1960s. There were two or three chemotherapies that were used as upfront on relapsed regimens for patients with AML. However, I was lucky enough when I joined the faculty at Mass General, in approximately 2010, soon thereafter, there were multiple drugs that entered clinical trials, and many of which ended up being highly promising, leading to FDA approvals, including IDH inhibitors, FLT3 inhibitors, antibody-drug conjugates, liposomal products. Ultimately, we've had, I believe, nine approvals in the last few years in AML. It's a very exciting time to be a part of this field.

Mutation-Targeted AML Strategies

IDH1/2-Mutant AML

- First description of IDH1 mutations in ~8% of patients with AML, associated with normal cytogenetic status (cn-AML)[1]
- Subsequent studies found a larger subset, ~15%, of patients with mutations in the *IDH2* gene
- IDH proteins, essential to the Krebs Cycle, catalyze decarboxylation of isocitrate to α-ketoglutarate (α-KG) in cytoplasm (IDH1) and mitochondria (IDH2)
- Mutant IDH enzymes catalyze an NADPH-dependent reduction of α-KG to 2-hydroxyglutarate (2-HG)
- This leads to accumulation of 2-HG onco-metabolite in IDH-mutant tumors



1. Mardis E, et al. *N Engl J Med.* 2009;361(11):1058-1066.; Ward P, et al. *Cancer Cell*. 2010;17(3):225-234.; Gross J, et al. *J Exp Med.* 2010;207:339-344.; Dang L, et al. *Nature.* 2009;462:739-744.

Let's talk about IDH-mutated disease first. The first description of IDH mutations occurred approximately 10-12 years ago. IDH1 mutations were discovered in 8% of patients with AML in a cohort of patients that were evaluated and were associated with a normal cytogenetic status. Patients with normal chromosomal cytogenetics are enriched for IDH mutations. A subsequent study found a larger subset, 15% had IDH2 mutations.

What are IDH proteins? We've all learned the Krebs cycle in high school, college, perhaps graduate school. IDH stands for isocitrate dehydrogenase. It's a key enzyme that converts isocitrate to α-ketoglutarate, leading to the production of ATP and energy for the cell in the case of IDH1, and mitochondria in the case of IDH2.

When there is an alteration as can be shown in the figure here, in the genes of IDH1 and IDH2, leading to the development of IDH1 and IDH2 mutations, there is actually the altered IDH proteins lead to catalysis of an alternative reaction. The conversion of α-ketoglutarate to 2-hydroxyglutarate, 2HG. As a result, 2HG, a normally suppressed metabolite, is built up in the cell's mitochondria as well as body fluids of patients who have IDH-mutated AML. 2HG is an oncometabolite which is normally low in patients who do not have AML. In AML with an IDH mutation, 2HG builds up, suppresses key enzymes such as TET2, leads to aberrant hypermethylation of key promoter regions that are important for differentiation and maturation of myeloid cells. This hypermethylation suppresses normal maturation, and as a result, an emergence of myeloid malignancy is seen.

Mutation-Targeted AML Strategies

Molecular Remission and Response Patterns in Patients with Mutant-*IDH2* AML Treated with Enasidenib

Eytan M Stein, Courtney D DiNardo, Amir T Fathi, Daniel A Pollyea, Richard M Stone, Jessica K Altman, Gail J Roboz, Manish R Patel, Robert Collins, Ian W Flinn, Mikkael A Sekeres, Anthony S Stein, Hagop M Kantarjian, Ross L Levine, Paresh Vyas, Kyle J MacBeth, Alessandra Tosolini, Jason VanOostendorp, Qiang Xu, Ira Gupta, Thomas Lila, Alberto Risueno, Katharine E Yen, Bin Wu, Eyal C Attar, Martin S Tallman, Stéphane de Botton

Blood. 2019 Feb 14;133(7):676-687. doi: 10.1182/blood-2018-08-869008. Epub 2018 Dec 3.

Durable Remissions with Ivosidenib in *IDH1*-Mutated Relapsed or Refractory AML

Courtney D DiNardo, Eytan M Stein, Stéphane de Botton, Gail J Roboz, Jessica K Altman, Alice S Mims, Ronan Swords, Robert H Collins, Gabriel N Mannis, Daniel A Pollyea, Will Donnellan, Amir T Fathi, Arnaud Pigneux, Harry P Erba, Gabrielle T Prince, Anthony S Stein, Geoffrey L Uy, James M Foran, Elie Traer, Robert K Stuart, Martha L Arellano, James L Slack, Mikkael A Sekeres, Christophe Willekens, Sung Choe, Hongfang Wang, Vickie Zhang, Katharine E Yen, Stephanie M Kapsalis, Hua Yang, David Dai, Bin Fan, Meredith Goldwasser, Hua Liu, Sam Agresta, Bin Wu, Eyal C Attar, Martin S Tallman, Richard M Stone, Hagop M Kantarjia

N Engl J Med. 2018 Jun 21;378(25):2386-2398. doi: 10.1056/NEJMoa1716984. Epub 2018 Jun 2.

Not only do we learn about the biology of IDH mutations and how they can lead to the myeloid malignancy phenotypes, such as in MDS and AML, but they also lead to drug development. Soon after the discovery of IDH mutations in malignancy, drug companies have started to develop small molecules that inhibit the aberrant IDH2 and IDH1 proteins. The first drug that emerged was enasidenib, which effectively and specifically inhibited mutant IDH2, and was then studied in phase 1 and phase 2 and expansion studies. Similarly, ivosidenib was a specific and highly potent inhibitor of the IDH1 altered protein and was studied in IDH1-mutated relapsed or refractory AML.

Mutation-Targeted AML Strategies

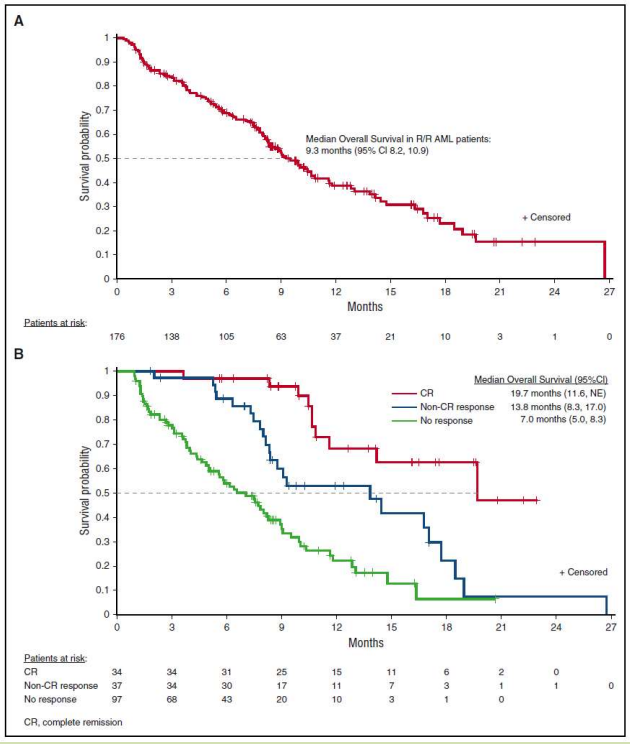
	R/R AML	
	Enasidenib, 100 mg/d (n = 214)	All doses (N = 280)
ORR, % (n/N) [95% CI]*	38.8 (83/214) [32.2%-45.7%]	39.6 (111/280) [33.9%-45.6%]
CR + CRi/CRp rate, % (n/N)	29.0 (62/214)	27.9 (78/280)
Best response		
CR, n (%) [CR rate 95% CI]	42 (19.6) [14.5-25.6]	53 (18.9) [14.5-24.0]
CRi/CRp, n (%)	20 (9.3)	25 (8.9)
PR, n (%)	9 (4.2)	17 (6.1)
MLFS, n (%)	12 (5.6)	16 (5.7)
SD, n (%)†	98 (45.8)	122 (43.6)
PD, n (%)‡	19 (8.9)	26 (9.3)
Not evaluable, n (%)	3 (1.4)	4 (1.4)
Time to first response, median (range), mo	1.9 (0.5-9.4)	1.9 (0.5-9.4)
Duration of response, median (95% CI), mo	5.6 (3.8-7.4)	5.6 (4.6-6.5)
Time to best response, median (range), mo	3.7 (0.6-14.7)	3.7 (0.5-14.7)
Time to CR, median (range), mo	3.7 (0.7-14.7)	3.8 (0.5-14.7)
OS, median (95% CI), mo	8.8 (7.7-9.6)	8.8 (7.8-9.9)
EFS, median (95% CI), mo§	4.7 (3.7-5.6)	4.6 (3.7-5.6)

	Refractory to intensive chemotherapy (n = 40)	Refractory to lower-intensity therapy (n = 44)†	Relapsed following any prior AML therapy (n = 130)
ORR, n (%) [95% CI]*	15 (37.5) [22.7-54.2]	19 (43.2) [28.4-59.0]	49 (37.7) [29.4-46.6]
CR, n (%)	4 (10.0)	12 (27.3)	26 (20.0)
CRi/CRp, n (%)	4 (10.0)	2 (4.5)	14 (10.8)
OS, median (95% CI), mo	12.4 (8.2-22.9)	8.0 (5.6-11.7)	8.1 (7.0-9.3)

Let's briefly talk about IDH2-mutated AML and enasidenib in that patient cohort. As mentioned, the first studies of enasidenib were in dose escalation, dose expansion, and phase 2 studies of enasidenib. Ultimately, a dose of 100 milligrams daily was settled on and was studied in multiple cohorts. One of those cohorts in a larger relapsed/refractory, phase 2 cohort assessed the promise of enasidenib and found an overall response rate approximating 40%, and a composite remission rate in these relapsed/refractory patients of 30%.

Now, these are the rates you see with traditional intensive therapy for relapsed/refractory treatment that come with high morbidity. Enasidenib was highly well-tolerated. Not only did it lead to these high response rates, patients also had other improvements such as improvements in transfusions, quality of life, and longevity, so median overall survival was also quite long. These overall response rates and composite remission rates, as can be shown in the lower table, were preserved in patients who had refractory disease to either higher intensity treatment or lower intensity therapy.

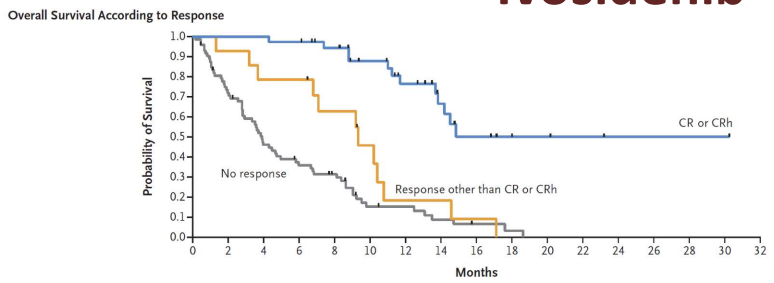
Mutation-Targeted AML Strategies



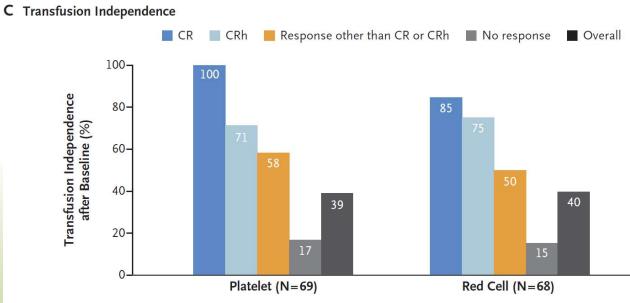
This is the median overall survival of patients receiving this IDH2 inhibitor. The median overall survival was nine months and in individuals, who had CRs at approximating 20 months. Even in those who did not achieve CR, a complete remission, but had lesser responses such as improvement in blood counts, decrease in marrow blasts that didn't quite meet the bar of CR, the median overall survival remained quite impressive at approximating 14 months.

Mutation-Targeted AML Strategies

Ivosidenib



No. at Risk																	
CR or CRh	38	38	38	37	32	25	19	13	8	5	4	3	1	1	1	1	0
Response other than CR or CRh	14	13	11	11	8	5	2	2	1	0							
No response	73	51	32	24	19	8	7	4	2	1	0						



Ivosidenib, the IDH1 inhibitor, selective and potent inhibitor of the IDH1 protein. Similarly, you have this improvement in overall survival in patients who responded and a similar rate of response. The key thing to think about with both enasidenib and ivosidenib is that not only that patients achieve response, but a substantial proportion of them, even in those who did not achieve the marrow remissions, had improvements in transfusions, as can be shown in the bar graph, with decreased red cell and platelet transfusion dependencies.

Ivosidenib or Enasidenib with Induction Chemotherapy in *IDH1/IDH2*-Mutated AML: Best Overall Response

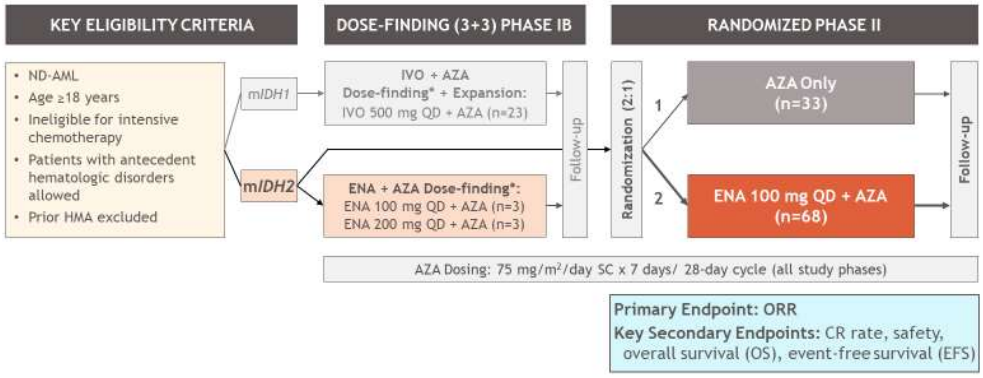
Response,[b] n (%)	Ivosidenib (AG-120) + chemotherapy			Enasidenib (AG-221) + chemotherapy		
	All (n=49)	*De novo* (n=34)	sAML (n=15)	All (n=89)	*De novo* (n=56)	sAML (n=33)
CR+CRi/CRp	**39 (80)**	31 (91)	8 (53)	**64 (72)**	43 (77)	21 (64)
CR	**35 (71)**	27 (79)	8 (53)	**50 (56)**	36 (64)	14 (42)
CRi/CRp	**4 (8)**	4 (12)	-	**14 (16)**	7 (13)	7 (21)
MLFS	**3 (6)**	1 (3)	2 (13)	**11 (12)**	6 (11)	5 (15)
PR	**1 (2)**	-	1 (7)	**1 (1)**	-	1 (3)
Treatment failure	**6 (12)**	2 (6)	4 (27)	**13 (15)**	7 (13)	6 (18)

*Evaluated in those with ≥1 postbaseline response assessment on or after induction Day 21 or who discontinued therapy prior to response assessment
Stein E, et al. ASH 2018. Abstract 460.

Enasidenib and ivosidenib IDH inhibitors can be combined also with induction chemotherapy and studied. In fact, clinical studies of the combination of 7+3 with either of these IDH inhibitors were launched. We were a part of these studies and led to very promising rates of composite remission. Now, we can't really say more than that, because this was not a comparator study versus induction chemotherapy by itself, but additional data is needed to really assess the promise of the combination of IDH inhibitors with induction chemotherapy.

Mutation-Targeted AML Strategies

AG221-AML-005: Study Design



Dose-finding for ENA or IVO; AZA remained constant
DiNardo C, et al. ASCO 2020.

IDH inhibitors have also been combined with other conventional drugs for AML including hypomethylating agents, which for years have been used as therapy for older patients or those who are not eligible for intensive therapies. This demonstrates the multiple studies that were done with ivosidenib plus AZA as well as enasidenib with azacitidine, a key hypomethylating drug.

Response

- ORR and CR rate were both significantly higher with ENA + AZA vs AZA only

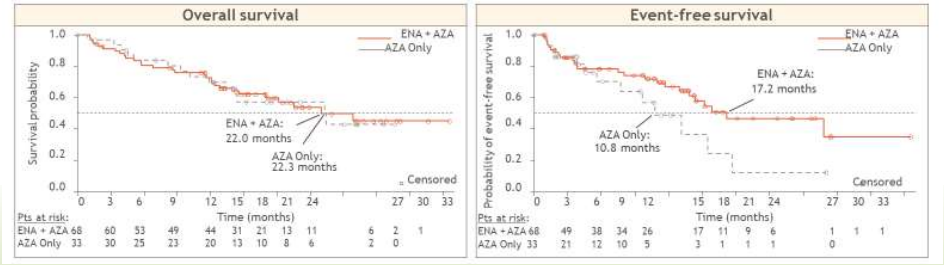
	ENA + AZA (n=68)	AZA Only (n=33)
Overall response (CR, CRi/CRp, PR, MLFS), n (%)	48 (71)	14 (42)
[ORR 95%CI]	[58, 81]	[26, 61]
P value	0.0064	
CR, n (%)	36 (53)	4 (12)
[CR rate 95%CI]	[41, 65]	[3, 28]
P value	0.0001	
CRi/CRp, n (%)	7 (10)	4 (12)
PR, n (%)	3 (4)	4 (12)
MLFS, n (%)	2 (3)	2 (6)
Stable disease, n (%)	13 (19)	13 (39)
Disease progression, n (%)	2 (3)	1 (3)
Not evaluable / Missing, n (%)	5 (7)	5 (15)
Time to first response, months, median (range)	1.9 (0.7-9.0)	2.0 (0.8-5.8)
Time to CR, months, median (range)	5.5 (0.7-19.5)	3.7 (3.0-4.1)
Duration of response, months, median [95%CI]	24.1 [11.1, NR]	12.1 [2.8, 14.6]

Data cutoff: August 19, 2019.
DiNardo C, et al. ASCO 2020. Abstract 7501.

This is the randomized phase 2 portion of enasidenib plus azacitidine versus azacitidine alone. As can be seen, the overall response rate and the composite remission rate of enasidenib and azacitidine was markedly higher than with azacitidine alone, allowing this phase 2 study to meet its primary endpoint and to be deemed successful.

Survival

- Median follow-up was 14 months in both treatment arms
- Median OS was 22.0 months in the ENA AZA group and 22.3 months in the AZA only group (HR 0.99 [95% CI 0.52, 1.87]; *P*=0.9686)
  - Among patients in the ENA + AZA arm who achieved CR, median OS was not reached and one-year survival was over 90%
- Median EFS was 17.2 months with ENA + AZA vs 10.8 months with AZA only (HR 0.59 [95% CI 0.30, 1.17] *P*=0.1278
- In the AZA only arm, 8 patients (24%) received subsequent treatment with enasidenib monotherapy

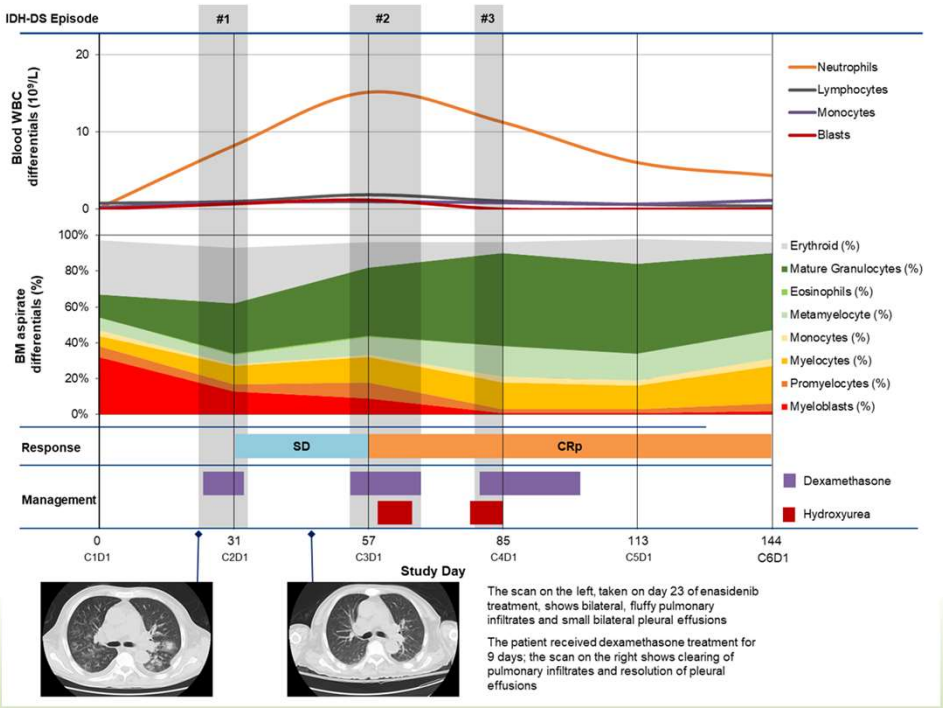


Data cutoff: August 19, 2019.
DiNardo C, et al. ASCO 2020.

However, the overall survival, which was a secondary endpoint, did not meet statistical significance in terms of superiority. Many reasons could be brought up for it and multiple patients in the azacitidine alone arm subsequently went on to receive enasidenib. There was some fallout in the azacitidine arm. It's hard to really know the specific reason why the higher composite remission rate did not translate into an improvement in overall survival. Although there was some indication of improvement in event-free survival as can be seen there, again, not reaching statistical significance.

Mutation-Targeted AML Strategies

Differentiation Syndrome Associated with Enasidenib, A Selective Inhibitor of Mutant IDH2 (Analysis of a Phase 1/2 Study)



Fathi A, et al. *JAMA Oncol*. 2018;4(8):1106-1110.

One key point to bring up is that IDH inhibitors are quite well tolerated. Ivosidenib is associated with QT prolongation, and enasidenib is associated with a benign elevation of bilirubin. Otherwise, these drugs are well tolerated. They can cause some cytopenias, but they're quite mild. However, both drugs can be associated with a condition called differentiation syndrome. That is a feature of the underlying mechanism of action of these IDH inhibitors. IDH inhibitors, as I mentioned, are differentiating drugs, meaning they inhibit 2HG, suppress TET, lead to a release of the block on differentiation that is seen in AML, and therefore these AML cells begin to differentiate and normalize and normally mature.

Well, that process of maturation and differentiation can be associated with a cytokine-mediated inflammatory process that can manifest itself in a pleomorphic diverse manner, such as patients coming in with unexplained fevers or pulmonary infiltrates, or pleural effusions, or rash, or mild renal failure. These symptoms can mimic other common things that you see with AML, secondary causes such as leukemic progression itself, or an infection, or a cardiopulmonary manifestation.

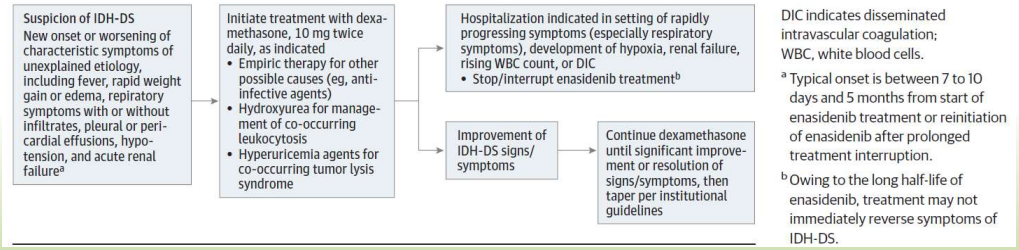
Mutation-Targeted AML Strategies

IDH-Differentiation Syndrome

Sign or Symptom	Patients With IDH-DS, No. (%) (n = 33)[b]
Dyspnea	28 (85)
Unexplained fever (body temperature of 38.0°C for 2 d)	26 (79)
Pulmonary infiltrates	24 (73)
Hypoxia	19 (58)
Acute kidney injury (CTCAE grade ≥2)	14 (42)
Pleural effusion	14 (42)
Bone pain or arthralgia	9 (27)
Lymphadenopathy	8 (24)
Rash	8 (24)
Disseminated intravascular coagulopathy	7 (21)
Edema or weight gain of >5 kg from screening	7 (21)
Pericardial effusion	5 (15)

[a] Signs and symptoms included in this table are based on retrospective differentiation syndrome review committee review of clinical records.

[b] Patients may have had multiple symptoms.



DIC indicates disseminated intravascular coagulation; WBC, white blood cells.

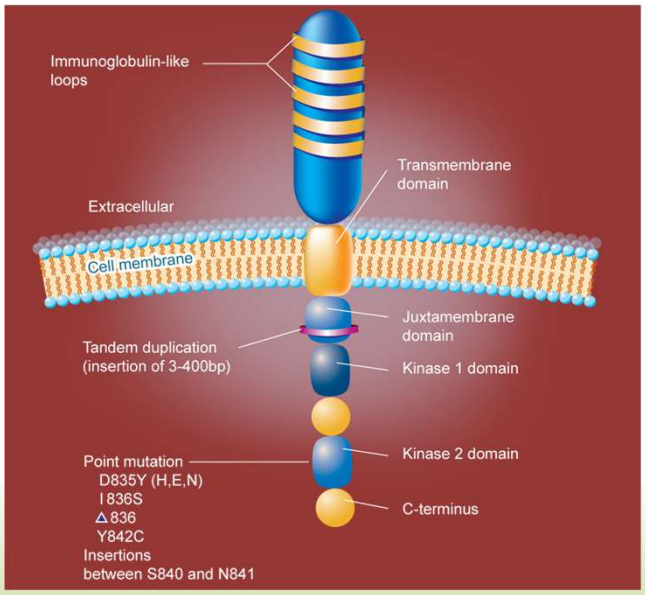
[a] Typical onset is between 7 to 10 days and 5 months from start of enasidenib treatment or reinitiation of enasidenib after prolonged treatment interruption.

[b] Owing to the long half-life of enasidenib, treatment may not immediately reverse symptoms of IDH-DS.

Nevertheless, because differentiation syndrome can be quite aggressive and lethal if not recognized, we recommend very close monitoring for it. And treatment, if it is suspected, even if a patient comes in and one suspects an infection as the cause but cannot truly rule out differentiation syndrome, I think it is entirely appropriate to treat the infection, but also treat for potential differentiation syndrome if the symptoms at the timing makes sense. Generally, differentiation syndrome arises anywhere between two weeks and six months after exposure to IDH inhibitor therapy, if it does occur. Typically, the severest forms of differentiation syndrome impact anywhere between 13% to 20% of patients who are treated with differentiation syndrome. It's not a small number. The real therapy is dexamethasone, typically given at 10 milligrams twice daily. Stopping the drug by itself won't do the trick because of the long half-life of these agents. Sometimes you have to stop the drug, but the first option is always steroid. Now differentiation syndrome can co-occur with hyperleukocytosis as the cells differentiate, in which case hydroxyurea may be necessary. It can also co-occur with disseminated intravascular coagulation and tumor lysis syndrome, and both of those conditions also need to be managed as well if they occur.

Mutation-Targeted AML Strategies

Activating *FLT3* Mutations in AML



ITD: 25-30%

High relapse, poor prognosis

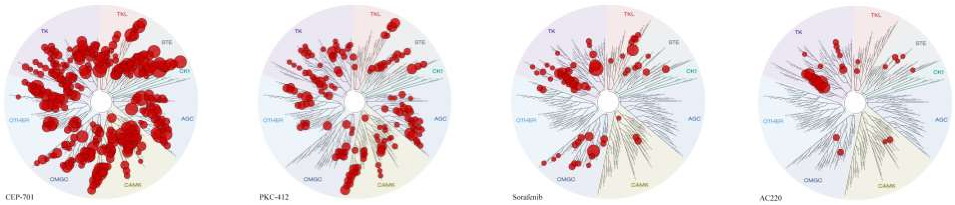
TKD: 5-10%

Litzow MR. *Blood*. 2005;106:3331-3332.

Let's move on to FLT3 inhibitors and FLT3 mutations. FLT3 mutations come in two major flavors. The more common type of a FLT3 mutation is the ITD mutation. The less common variant are the multiple different tyrosine kinase domain or the TKD mutations impacting the FLT3 receptor tyrosine kinase, like many other receptor tyrosine kinases like, EGFR, VEGF, and KIT. The FLT3 receptor resides on myeloid precursors in the marrow. When there is a mutation, this FLT3 receptor, unfortunately, becomes less and less ligand-dependent and becomes more active. As a result, the myeloid cells begin to proliferate in out-of-control fashion, leading to a proliferative form of AML that oftentimes relapses and is difficult to control, even after a transplant.

Potency and Selectivity of FLT3 Inhibitors

Drug	IC_{50} (medium)[a]	IC_{50} (plasma)[b]
Lestaurtinib	2 nM	700 nM
Midostaurin	6 nM	~1000 nM
Sorafenib	3 nM	~265 nM
Quizartinib	1 nM	18 nM



Lestaurtinib (CEP-701) | Midostaurin (PKC-412) | Sorafenib | Quizartinib (AC220)

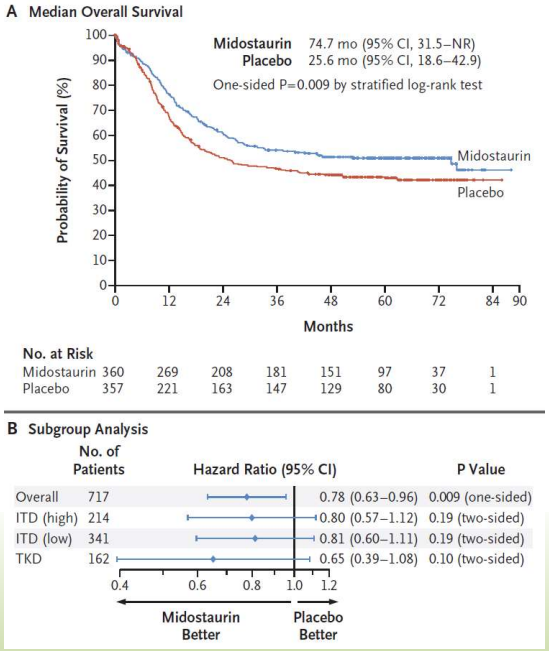
a–Molm-14 cells incubated in RPMI/10% FBS; b-Molm-14 cells incubated in plasma.; Human kinome image generated using TREEspot™ software tool and reprinted with permission from KINOMEscan™, a division of DiscoveRx Co.
Pratz K, et al. *Blood*. 2010;115(7):1425-1432.; Courtesy of Mark Levis, ASH, 2013.

Over the course of the last 10 to 15 to 20 years, folks have attempted to learn more about FLT3 and develop small molecule inhibitors of FLT3. The initial series of first-generation of FLT3 inhibitors were actually developed for other receptor tyrosine kinases and therefore were quite nonspecific and promiscuous, drugs like lestaurtinib and midostaurin. More recently, agents such as quizartinib and gilteritinib are more potent and more sensitive for FLT3 and are currently being studied in clinical trials.

Midostaurin Plus Chemotherapy for AML with a *FLT3* Mutation

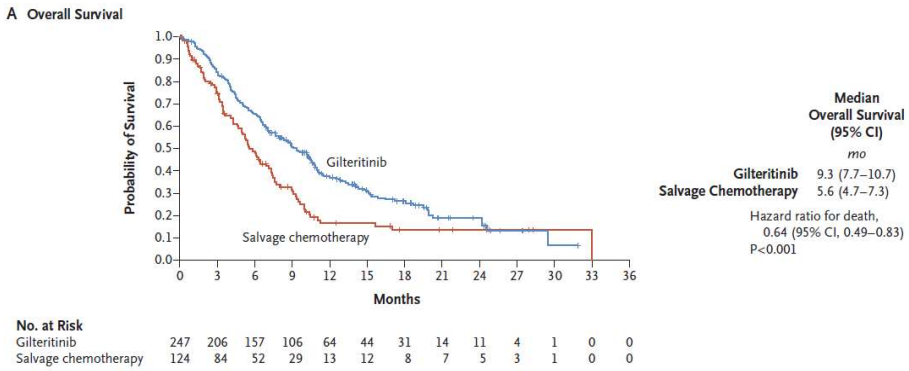
- Overall survival was significantly longer in the midostaurin group than in the placebo group (hazard ratio for death, 0.78; one-sided *P* = 0.009)
- There was a 23% reduced risk of death in the midostaurin arm
- At 4 years, 51.4% were alive in the midostaurin arm as opposed to 44.2% in the placebo arm

Stone R, et al. *N Engl J Med.* 2017:37(5):454-464.



Midostaurin, as I mentioned one of the first generation of FLT3 inhibitors, relatively nonspecific, was studied as single agent in clinical trial, but also combined with chemotherapy. As a single agent, not particularly active, but with chemotherapy it showed promise, and a subsequent phase 3 study compared the combination of the FLT3 inhibitor midostaurin with chemotherapy versus chemotherapy and placebo and found the midostaurin arm to be superior to the placebo-containing arm with a substantially longer median overall survival, so 23% reduction in risk of death and at four years, 51% were alive in the midostaurin arm as opposed to 44% in the placebo arm.

Gilteritinib or Chemotherapy for Relapsed or Refractory *FLT3*-Mutated AML



Perl A, et al. *N Engl J Med*. 2019;381(18):1728-1740.

Gilteritinib, one of the newer generation of FLT3 inhibitors, much more potent than selective, has been studied in AML as well, but in the relapsed/refractory setting as monotherapy, and in this phase 3 study was compared to multiple different salvage chemotherapy options that were deemed to be standard at the time and showed to be again markedly superior with an improvement in median overall survival, leading to the approval of both midostaurin for a frontline setting in combination with induction chemotherapy, and gilteritinib as monotherapy for relapsed/refractory AML.

HCT with and without Sorafenib Maintenance for Patients with FLT3-ITD AML in First CR

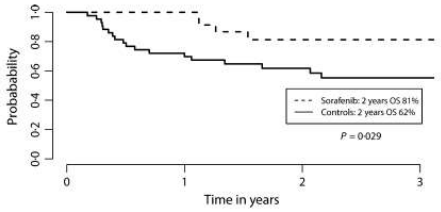


Fig 1. Kaplan Meier estimate of overall survival among sorafenib patients (dashed line) and controls (solid line). In this landmark analysis, only controls alive and without disease relapse at the median date of sorafenib initiation (+68) were included. Patients given sorafenib maintenance had a significantly higher overall survival (OS) compared to controls at 2 years (P = 0·029).

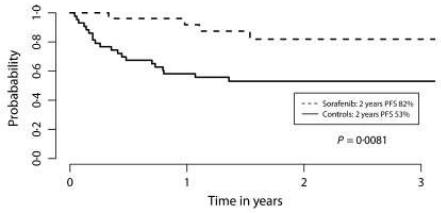


Fig 2. Kaplan Meier estimate of progression-free survival among sorafenib patients (dashed line) and controls (solid line). In this landmark analysis, only controls alive and without disease relapse at the median date of sorafenib initiation (+68) were included. Patients given sorafenib maintenance had a significantly higher progression-free survival (PFS) compared to controls at 2 years (P = 0·0081).

Brunner AM, et al. *Br J Haematol.* 2016;175(3):496-504.

Let's move on to other potential points of utility for FLT3 inhibitors in AML. At our institution, we've looked at FLT3 inhibitors as therapy following bone marrow transplant. As I mentioned, AML with a FLT3 mutation is a highly proliferative aggressive disease, marked by multiple relapses typically and historically. We thought that these patients who get transplanted with FLT3 mutations may have a reduction in risk if they received FLT3 inhibitor oral therapy as maintenance following the transplant and in fact, we found looking at a FLT3 inhibitor called sorafenib given after a transplant led to a marked reduction in the risk of relapse in patients who received the FLT3 inhibitor.

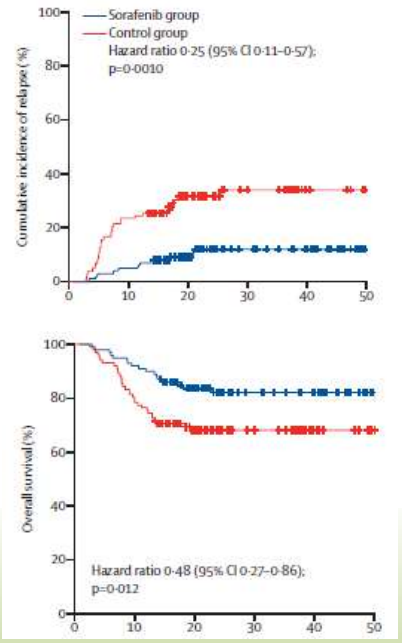
Randomized Post-Transplant Sorafenib Maintenance Studies

PH 2 SORMAIN Trial[1]

- Two-year RFS in sorafenib and placebo arm, respectively: 85.0% vs 53.3% (HR = 0.39, *P* = 0.013)
- OS significantly longer in sorafenib arm (HR = 0.447, *P* = 0.03)
- Common grade ≥3 adverse events were acute GVHD, GI toxicity, and infections

PH 3 Study[2]

- One-year relapse rate: 7.0% vs 24.5% (HR = 0.25, *P* = .0010)
- Common grade ≥3 adverse events were infection and acute GVHD



1. Bouchert A, et al. *J Clin Oncol.* 2020;38(26):2993-3002.;
2. Xuan L, et al. *Lancet Oncol.* 2020;21(9):1201-1212.

Subsequent studies have looked at post-transplant sorafenib maintenance in randomized fashion. The SORMAIN trial run in Europe compared sorafenib versus control following transplant, and found that in patients who received sorafenib, there was a marked improvement in two-year relapse-free survival, as well as overall survival. There was no substantial increase in graft versus host disease or GI toxicity. There was another phase 3 study by Xuan and colleagues published in *Lancet Oncology*, that too revealed a markedly decreased relapse rate in patients who receive sorafenib after bone marrow transplant.

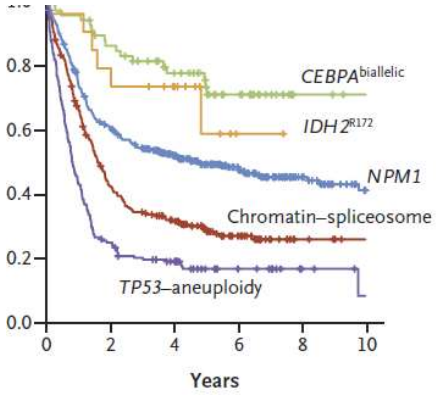
FLT3 Maintenance Trials

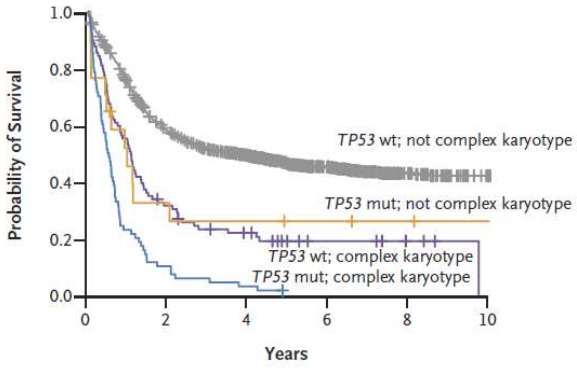
Agent	Clinical Trials in Maintenance Post-Frontline Setting
Gilteritinib	MORPHO: phase 3 trial of maintenance following HSCT
Quizartinib	QuANTUM-First: phase 3 trial during induction, consolidation, and up to 1 year of maintenance

Clinicaltrials.gov. NCT02997202.; Clinicaltrials.gov. NCT02668653.

I would say that most academic institutions now incorporate FLT3 inhibitor therapy following transplantation. Around the same time, a large phase 3 study looked at the more potent and selective FLT3 inhibitor gilteritinib following transplant, a randomized study versus placebo. That study has now finished accrual and we're looking very much forward to results. Quizartinib, another FLT3 inhibitor, was studied in the QuANTUM-First phase 3 trial that incorporated a year of maintenance following induction and consolidation.

P53 Mutations in AML



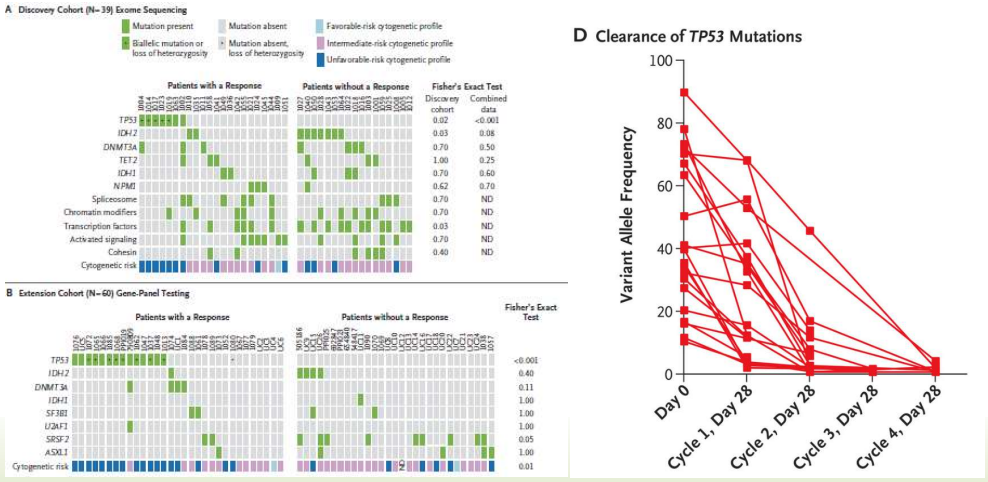


Papaemmanuil E, et al. *N Engl J Med.* 2016;374:2209-2221.

We've talked about IDH and FLT3 mutations in AML. Let's talk about P53. P53 is among the ugliest mutations in human cancer. It's no exception in AML. The presence of a P53 mutation is associated with poor outcomes in both MDS and pre-leukemic conditions as well as AML.

Mutation-Targeted AML Strategies

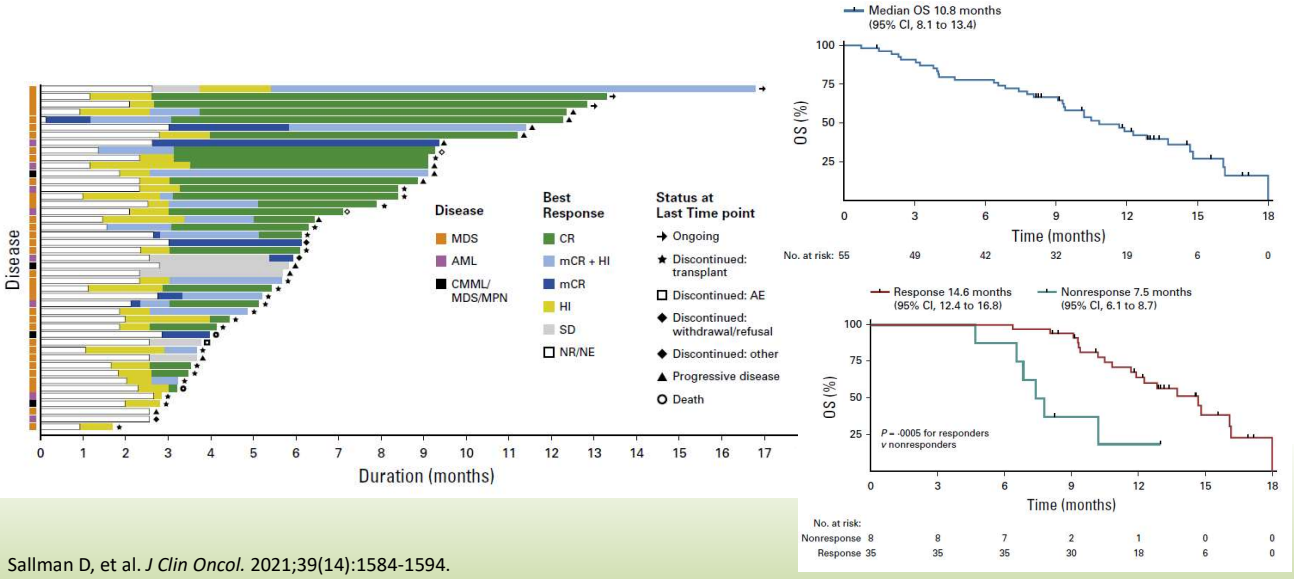
TP53 and Decitabine in AML and MDS



Welch J, et al. *N Engl J Med*. 2016;375(21):2023-2036.

However, there is some suggestion of therapeutic activity with certain agents. Let's start with the hypomethylating therapy such as azacitidine and decitabine, a study published by Welch and colleagues from Washington University and published in 2016 in the *New England Journal of Medicine* found that patients who had P53 mutations seem to respond to hypomethylating therapy quite well with a reduction in the allelic fraction of P53 mutations in treated patients. Up until very recently, the approach to patients with P53-mutated MDS and AML was treatment with a hypomethylating agent, such as decitabine or azacitidine. I have to say that subsequent studies that have looked at P53 mutations on hypomethylating agents have not seen as quite an impressive response as was seen in this *New England Journal of Medicine* article. Nevertheless, it's something to consider.

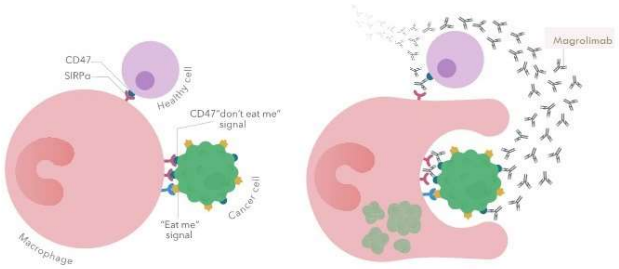
Eprenetapopt (APR-246) and Azacitidine in *TP53*-Mutant MDS

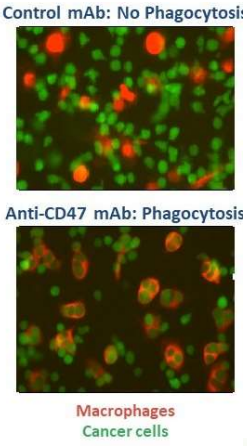


Sallman D, et al. *J Clin Oncol.* 2021;39(14):1584-1594.

APR-246, a P53 activating molecule, also has shown substantial promise in single-arm studies and has been studied in both MDS and AML. Here's a swimmer plot showing that multiple patients that received the combination of azacitidine and APR-246 had prolonged responses and long-term overall survival. Data is ongoing in both MDS and AML in randomized studies. We shall see what these results demonstrate, but a promising drug.

Magrolimab (Formerly 5F9) is a First-in-Class Macrophage Immune Checkpoint Inhibitor Targeting CD47





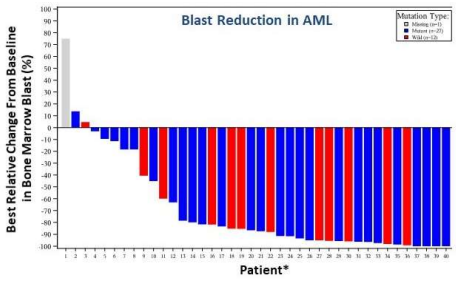
- CD47 is a "do not eat me" signal that is overexpressed in multiple cancers, including AML, leading to macrophage immune evasion
- Magrolimab, an IgG4 anti-CD47 monoclonal antibody (mAb), eliminates tumor cells through macrophage phagocytosis
- Magrolimab is being investigated in multiple cancers with >500 patients dosed

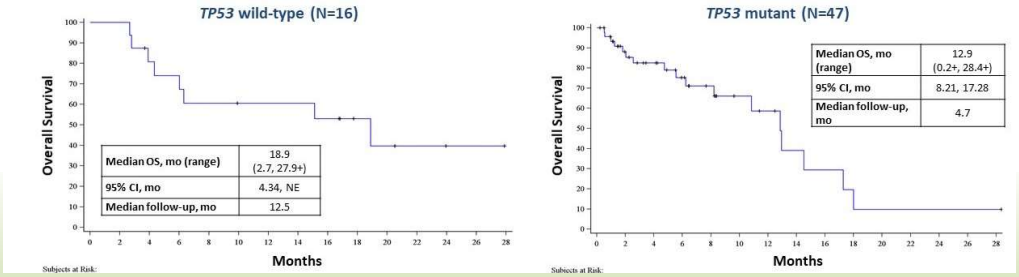
Sallman D, et al. *Blood.* 2019;134(suppl 1). Abstract 569.; Courtesy of Dr. Naval Daver.

Another agent is magrolimab, a CD47 antibody that basically interferes with the 'do not eat me' signal that is overexpressed in myeloid malignancies, leading to macrophage phagocytosis of leukemic cells, who no longer express that signal.

Magrolimab + AZA Induce High Response Rates in AML

Best Overall Response	All AML (N=43)	*TP53*-mutant AML (29)
ORR	27 (63%)	20 (69%)
CR	18 (42%)	13 (45%)
CRi	5 (12%)	4 (14%)
PR	1 (2%)	1 (3%)
MLFS	3 (7%)	2 (7%)
SD	14 (33%)	8 (28%)
PD	2 (5%)	1 (3%)





Sallman D, et al. *Blood.* 2019;134(suppl 1). Abstract 569.; Courtesy of Dr. Naval Daver.

By itself, magrolimab does not have substantial activity in MDS and AML, but when combined with azacitidine, there is a substantial increase in composite remission rate of approximately 60% to 70%. Not only do we see this in AML in total, in a smaller subset of patients with P53 mutations, there is also a high rate of response which is quite intriguing, including a substantial reduction in blasts, and an improvement in median overall survival.

Again, the number of P53-mutated patients studied in these cohorts of AML are small. We need a larger dataset hopefully, to be presented at meetings in years ahead and published to tell us more about whether this combination of magrolimab and azacitidine has activity that is robust in P53-mutated disease.

Reasons for Optimism

- Improved outcomes due to better prognostication, patient selection, and supportive care
- Emergence of effective, targeted therapies
- Novel combinations for older patients that can maintain tolerability and enhance outcomes
- Will the next decade see more approved AML therapies than the last four decades combined?

Therefore, overall, I would say there are reasons for optimism. We have improved outcomes due to better prognostication, patient selection, and supportive care. We have emergence of effective and targeted therapies such as IDH inhibitors, such as FLT3 inhibitors, such as antibody-drug conjugates, but also perhaps newer and emerging drugs for the most resistant diseases we treat, such as those with P53 mutations. There are novel combinations that are available for older patients that we didn't talk about today, but the combination of azacitidine and venetoclax was approved for patients who are older or not are eligible for intensive therapy.

There are also newer combinations that we've talked about, such as the combination of azacitidine with APR-246, or with magrolimab. Ultimately, the most intriguing question is whether the upcoming decade we'll see even more approved therapies than the last four decades combined. I very much appreciate your attention. Thank you so much.