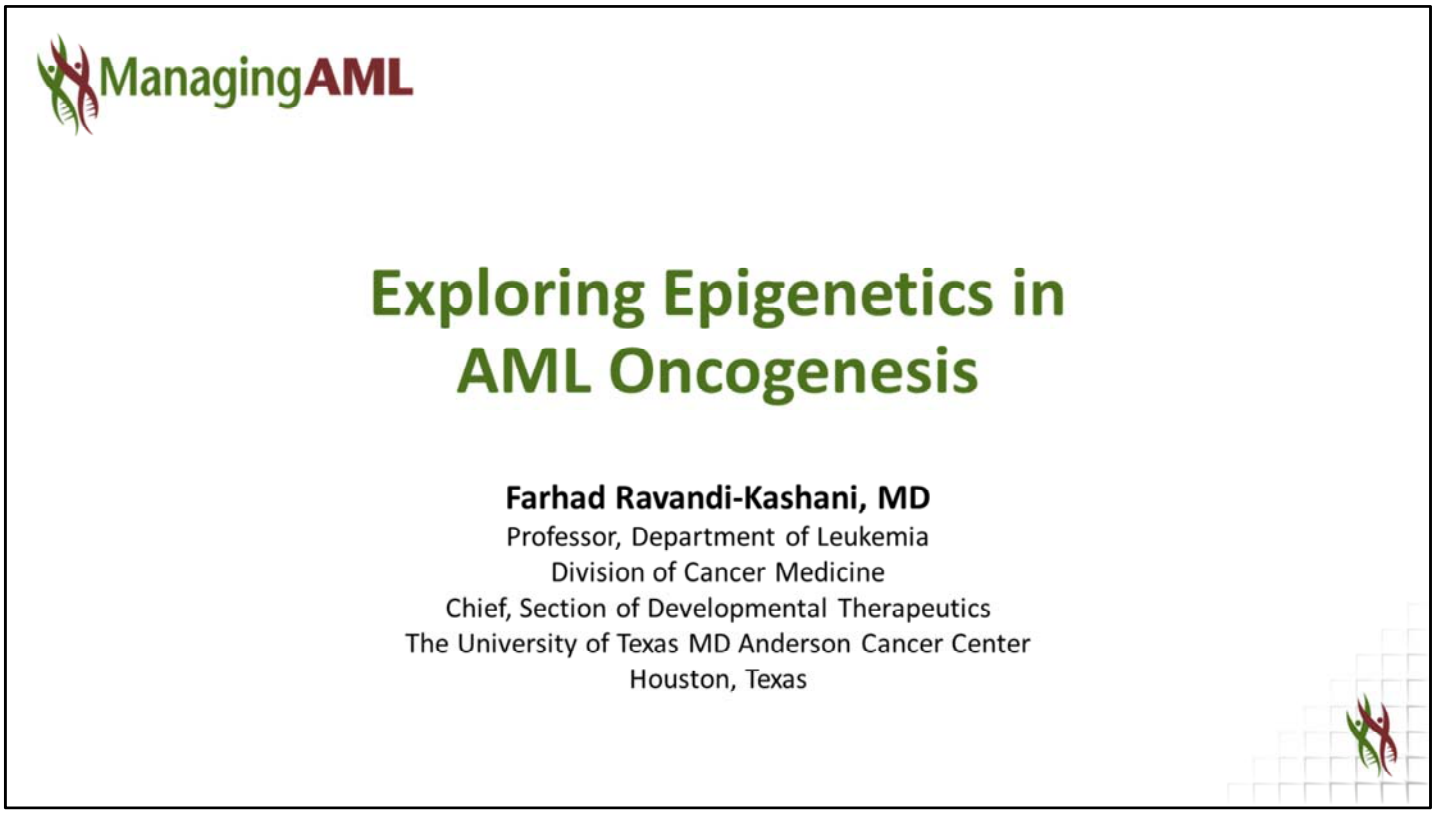
Exploring Epigenetics in AML Oncogenesis



Welcome to *Managing AML*. I am Dr. Farhad Ravandi-Kashani. In today's presentation, I will review exploring epigenetics in AML oncogenesis. In this video, I will explain the various epigenetic pathways that have been implicated in the pathogenesis of acute myeloid leukemia (AML) including DNA methylation and, to a lesser extent, histone acetylation and histone methylation. We will also explore the associated genetic mutations and what this means for clinical practice. I will also cover some of the current clinical trials and best practices focused on epigenetic therapies, and how to apply this data to the management of patients with acute myeloid leukemia. Let's begin.

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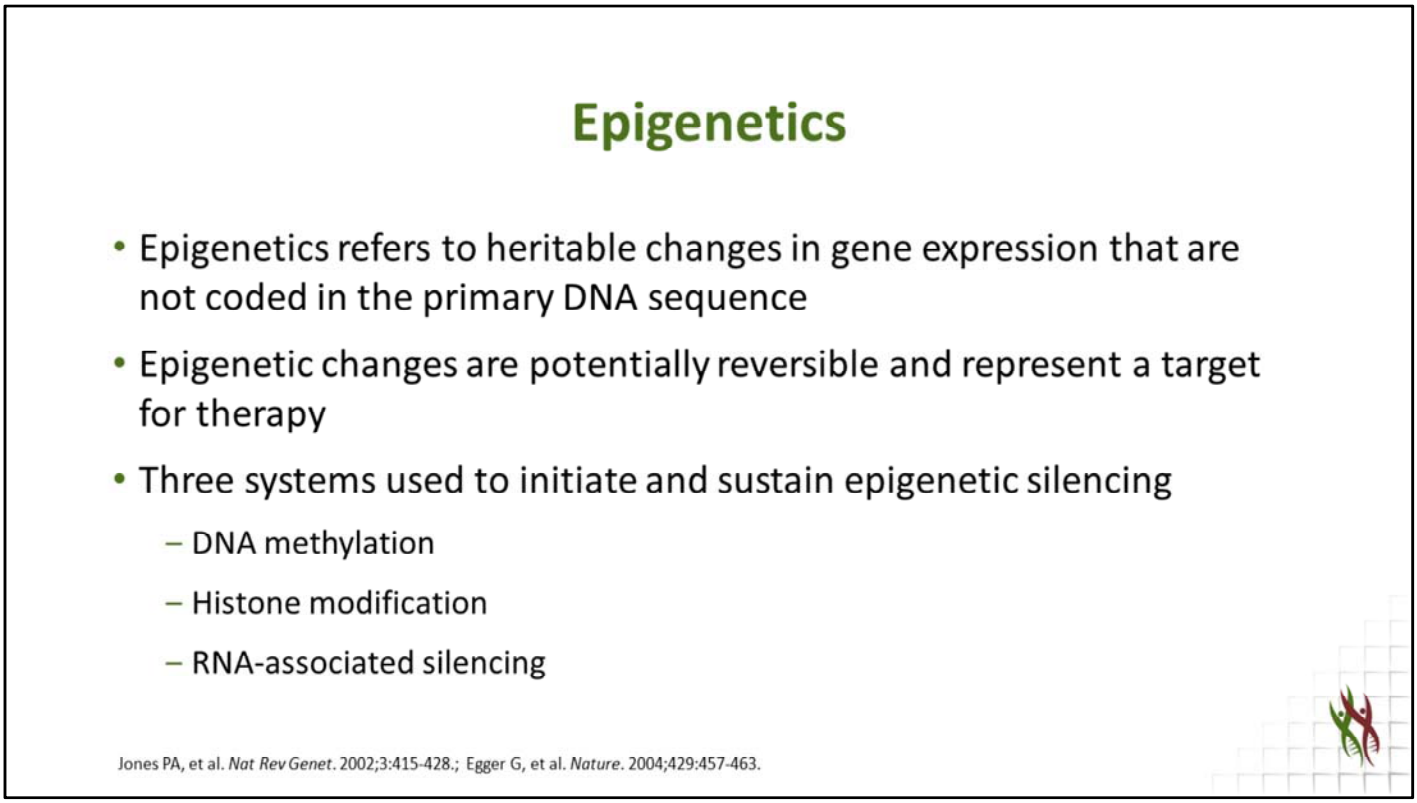

Briefly, epigenetics refers to heritable changes in gene expression that are not coded in the primary DNA sequences. Epigenetic changes are potentially reversible and represent a target for therapy. Overall, there are three main mechanisms used to initiate and sustain epigenetic silencing of genes in various cells: DNA methylation, histone modification, and RNA- associated silencing.

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Epigenetic Inheritance at the Agouti Locus in the Mouse

Morgan HD, et al. *Nat Genetics*. 1999;23:314-318.

This slide is here to show you what I mean by epigenetics. These mice are genetically identical and they have essentially identical DNA sequences; however, as you can see the coat is very different in terms of color and this is because of the different expression of genes related to epigenetic expression. Another way to describe this is that human cells all have the entirely same exact DNA sequence; however, as we all know cells in the body are very different with various functions, and this is dependent on epigenetic silencing or activation of various genes.

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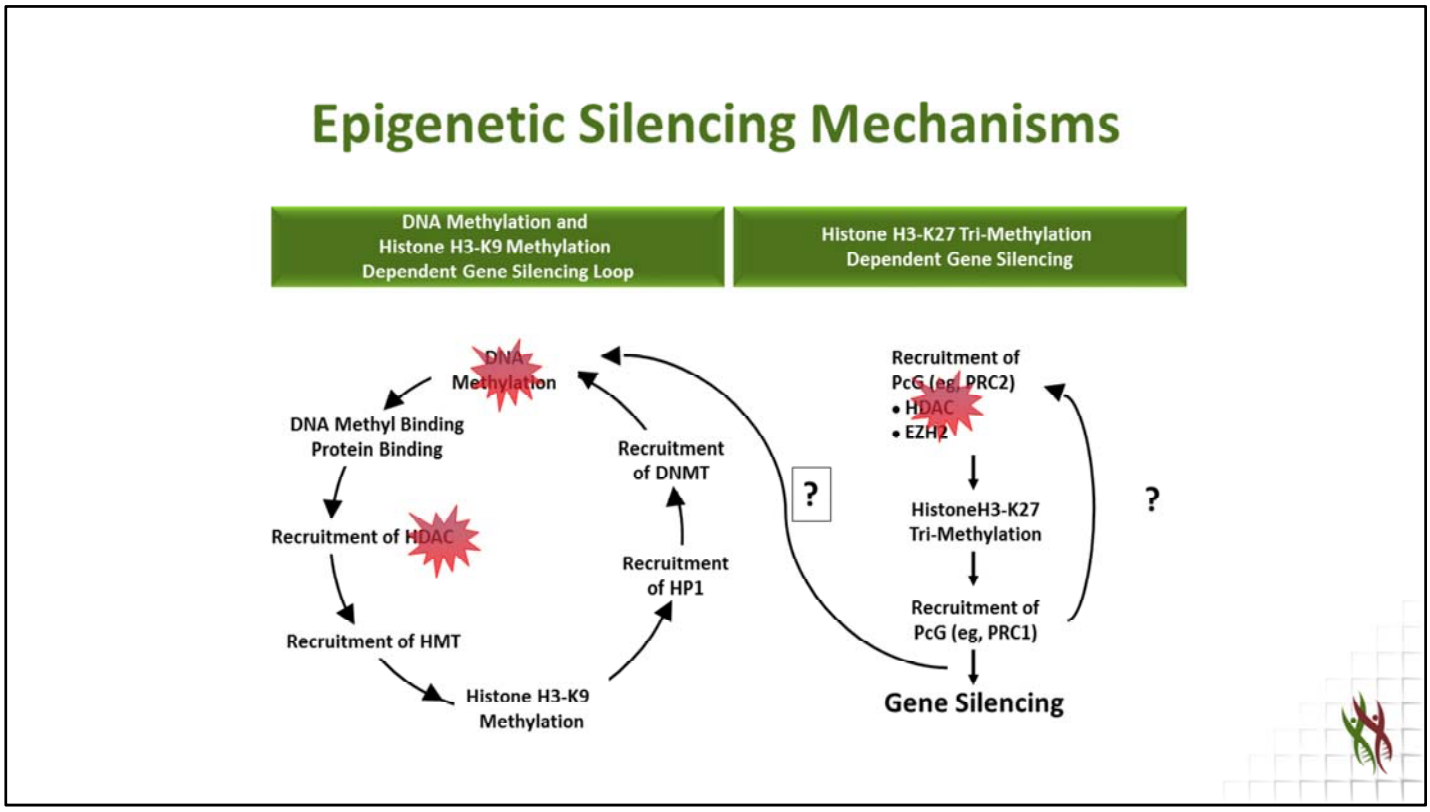

There are two major mechanisms of epigenetic silencing of the genes that have been previously described. As I mentioned, RNA silencing is also important, but DNA methylation and histone modification is something that has been studied in the lab clinically over the last couple of decades.

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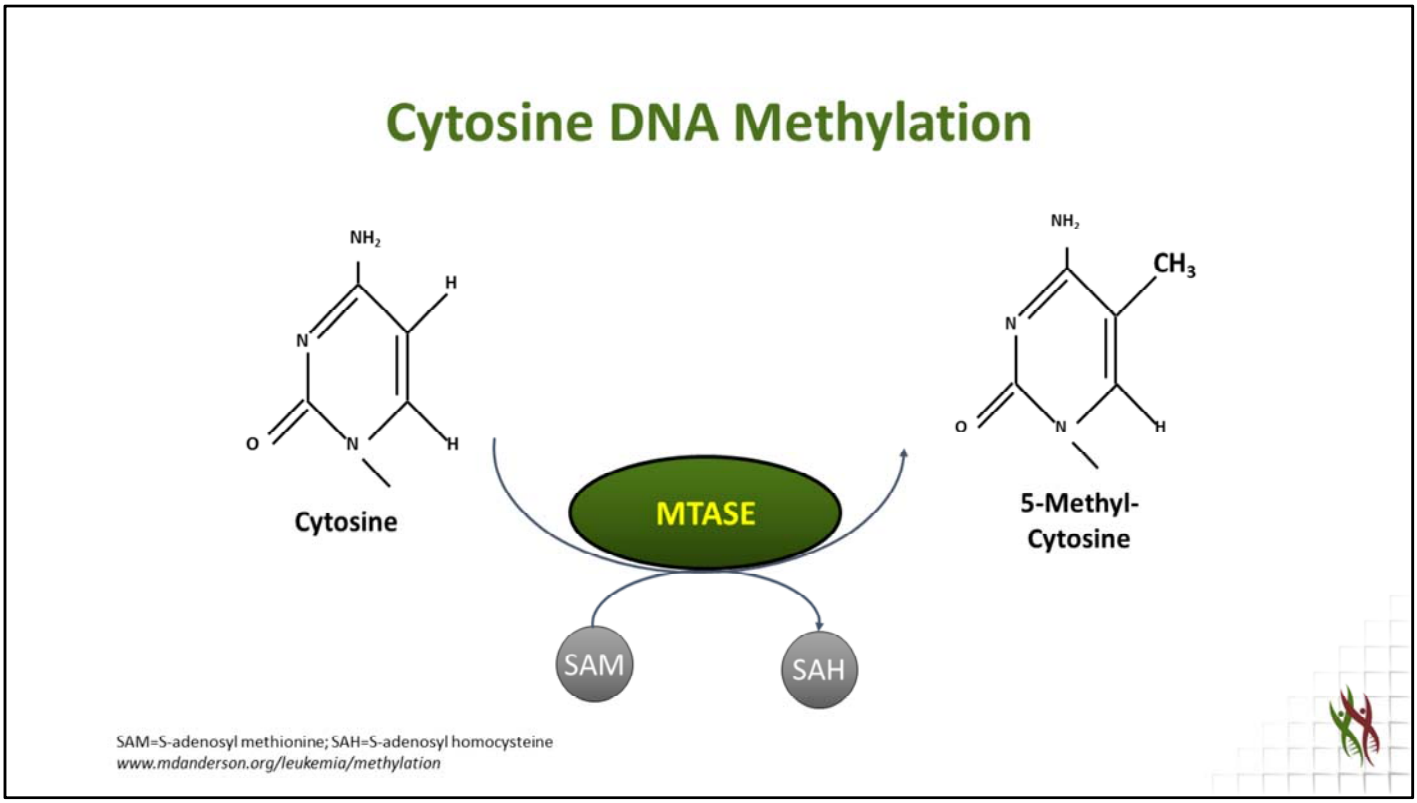
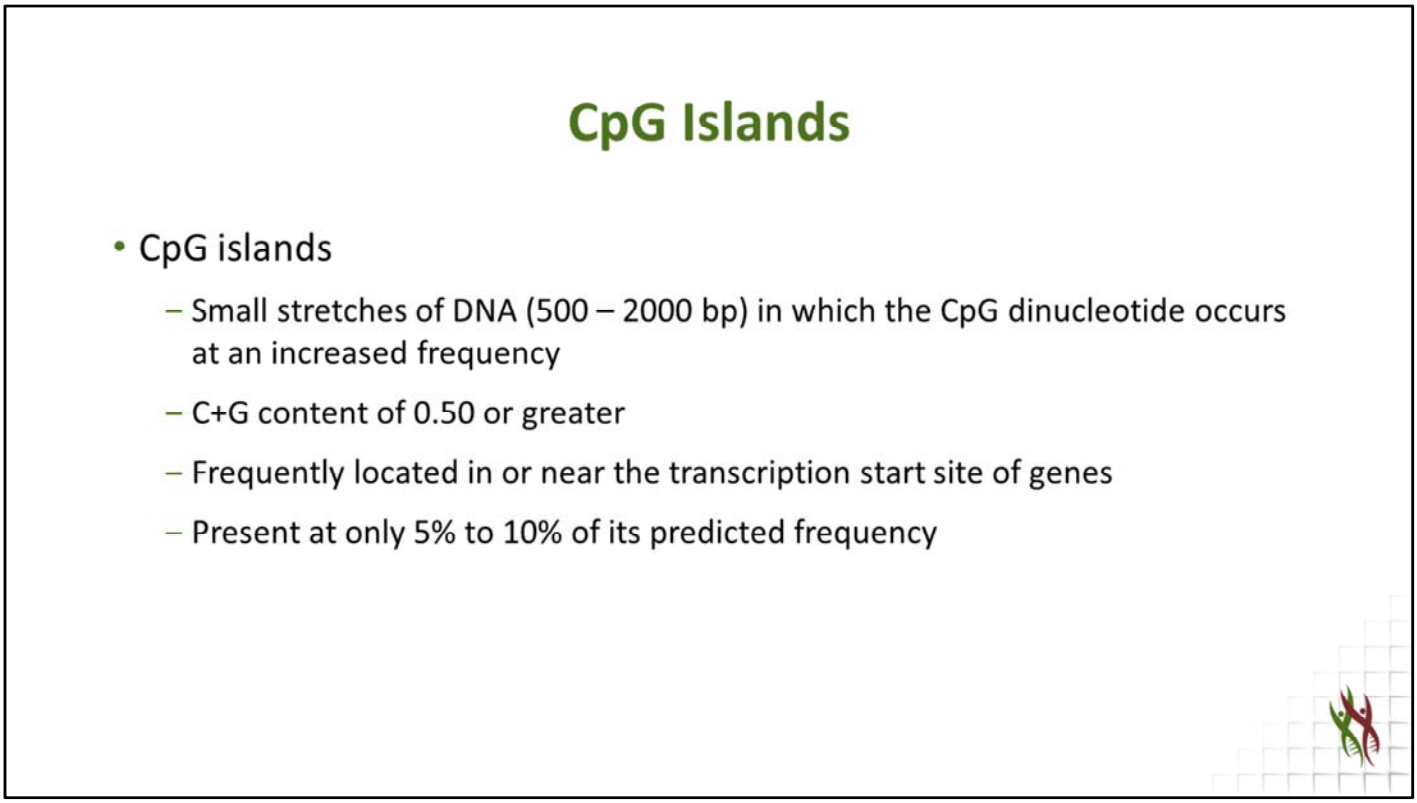


Cytosine DNA methylation is a mechanism of gene silencing. There are cytosine residues that can be methylated by DNA methyltransferases to produce methylated cytosines.



These typically occur in CpG islands, which are small stretches of DNA (about 500 to 2000 base pairs) in which the CpG dinucleotides occur at increased frequency. These areas are frequently located at the beginning of the transcription start sites of genes, and they are present at only about 5% to 10% of their predicted frequency.

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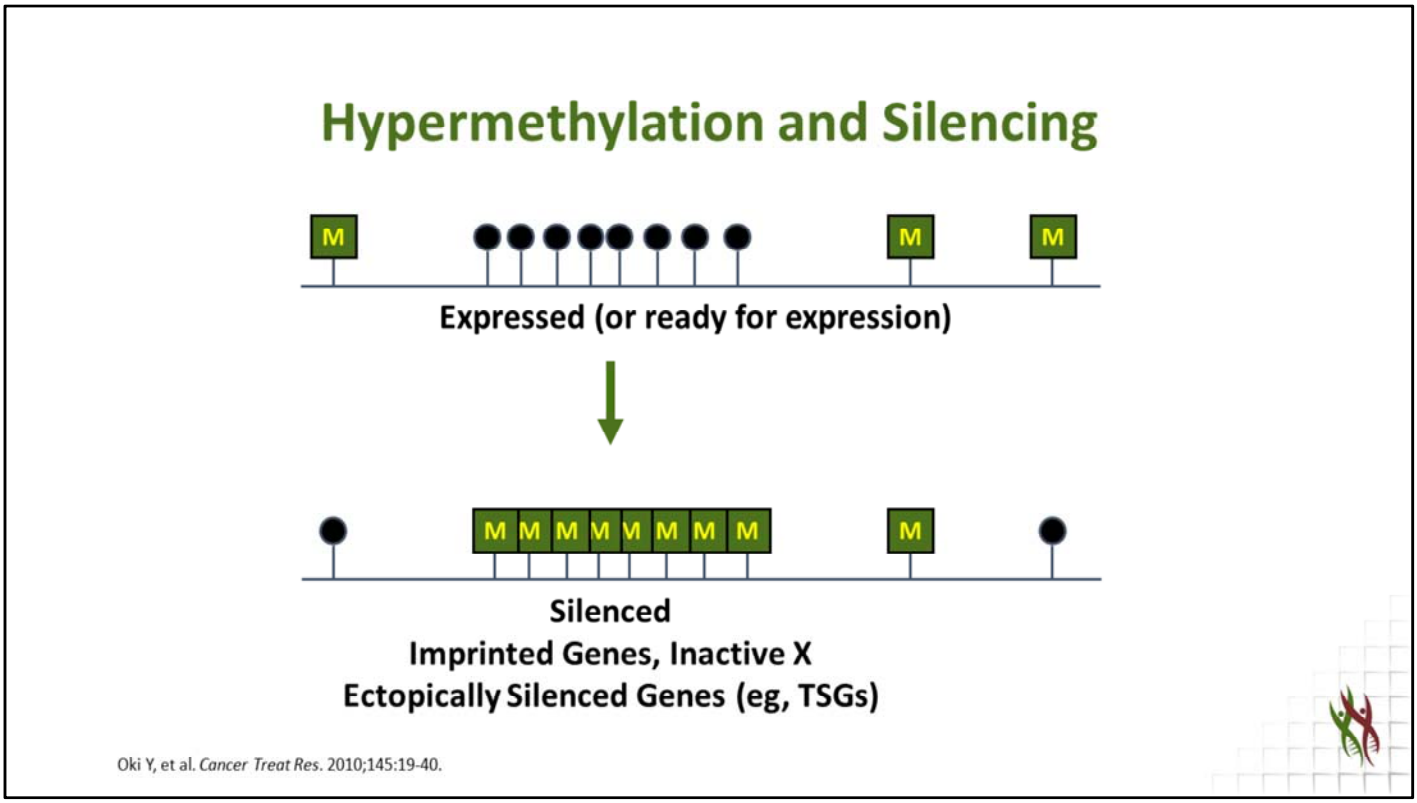

Why they are important is because, as they get methylated, they lead to gene silencing. For example, here you can see that when CpG islands are not methylated, the gene is expressed. Methylation of these transcription start sites results in silencing of the imprinted genes and activation of the genes or lack of their functions in the appropriate cell.

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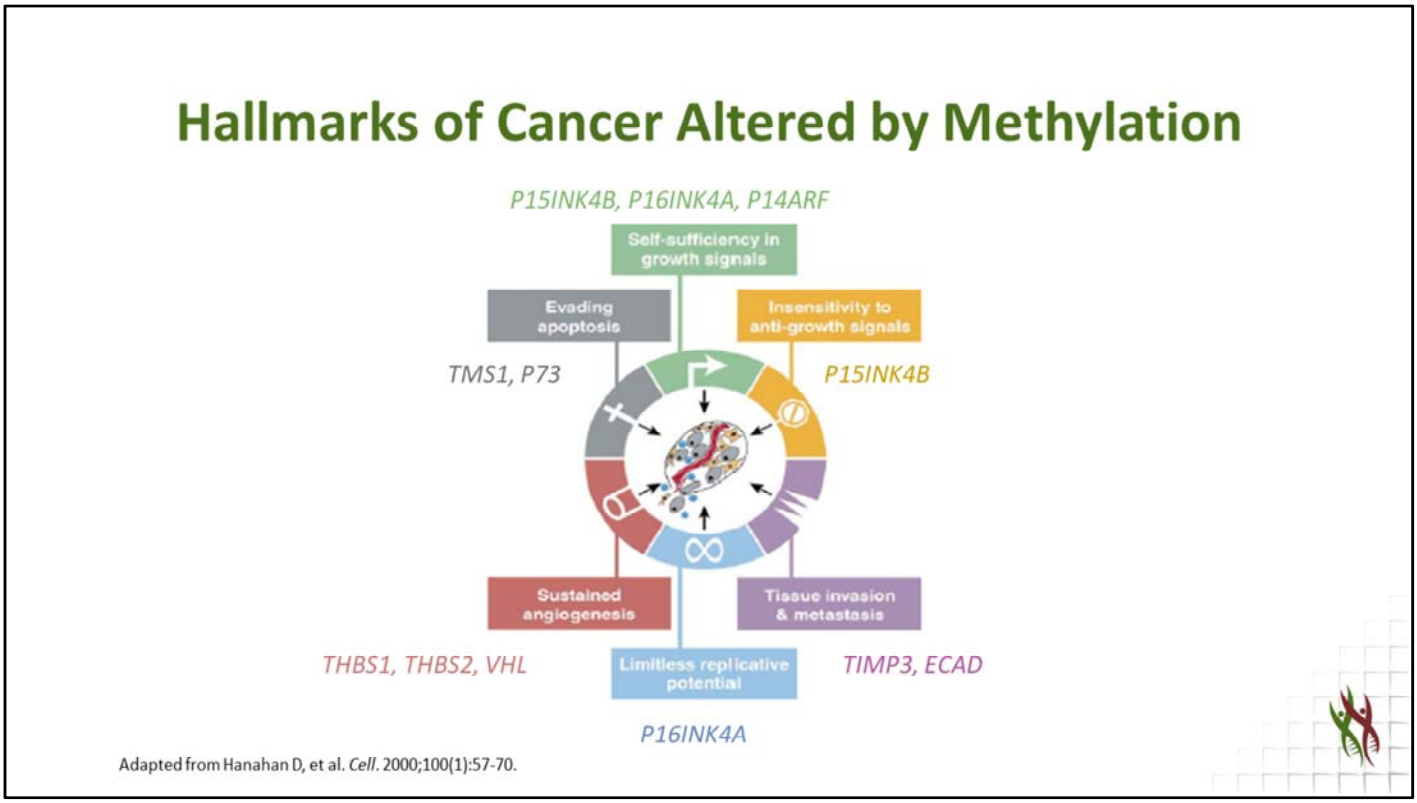

This is important in cancer because it is believed that a number of tumor suppressor genes, or genes that are essentially modulating and preventing carcinogenesis, are silenced by various silencing mechanisms such as epigenetic silencing.

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Common Genes Hypermethylated in Advanced MDS and AML

Hyper-Methylated Gene	*Gene Function*	*MDS (%)*	*AML (%)*
Calcitonin	Ca++ bone resorption	40-80	50-90
E-cadherin	Ca++-mediated cell adhesion		32-78
ER	Estrogen receptor		70-90
MyoD	Muscle cell differentiation, repair		50-80
$p15^{INK4b}$	Cyclin-dependent kinase inhibitor	20-79	30-90
$p16^{INK4a}$	Cyclin-dependent kinase inhibitor	<10	17
RARβ	Retinoic acid receptor beta		>50
WT-1	Wilms' tumor suppressor-1 protein		5

Adapted from Claus R, et al. *Oncogene.* 2003;22:6489-6496.

This is probably a fairly old slide. A number of these genes have tried to be identified. As you can see here, the number of candidate genes have been described both in myelodysplastic syndrome as well as in acute myeloid leukemia where they appear to be silenced, and their silencing is important in the pathogenesis of MDS and AML. A lot of these genes are believed to be silenced by the hypermethylation of the promoter regions as I described earlier.

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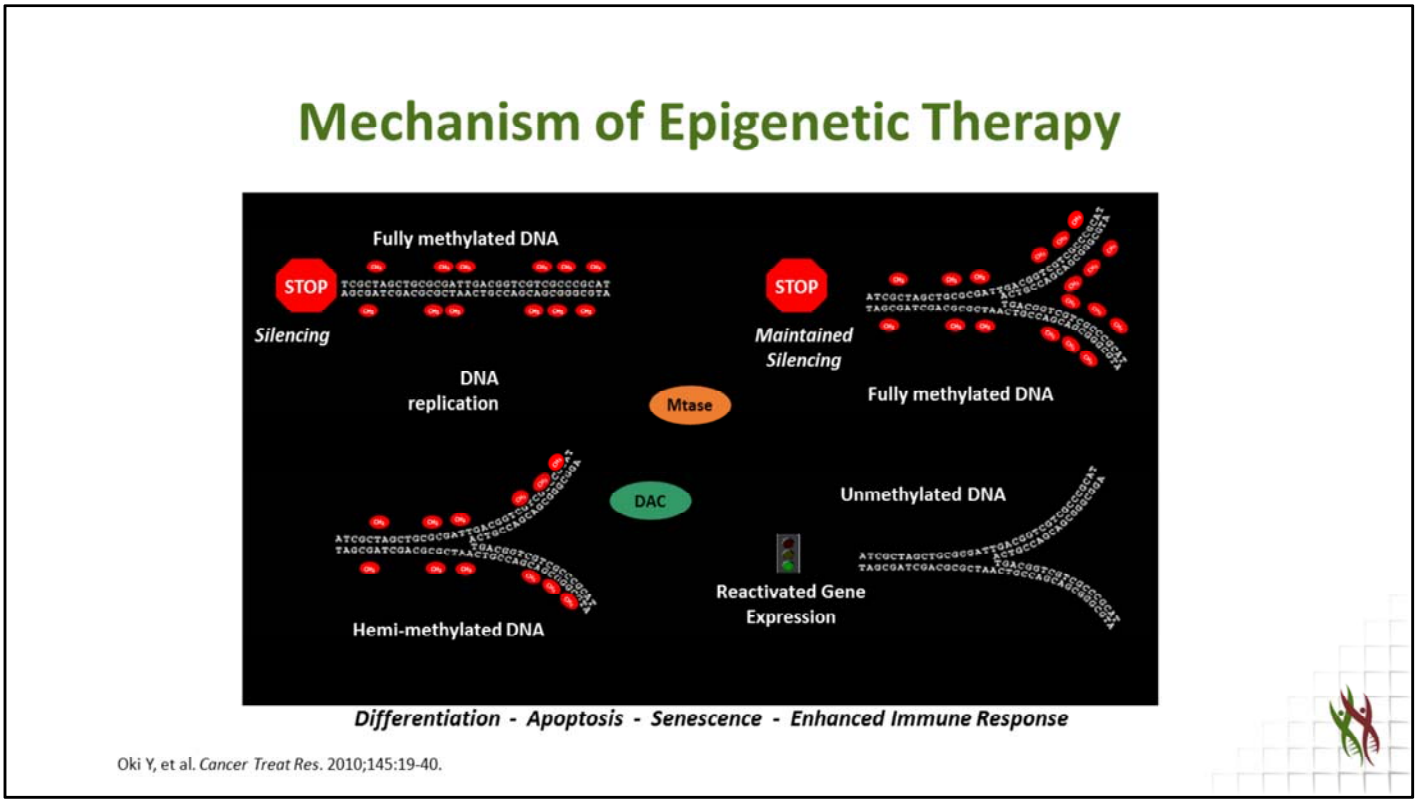

Why is this important? The enzyme that is important in the methylation of DNA is DNA methyltransferase, which sits on transcription fork and is responsible for completely copying the methylation pattern of the genes in the daughter cells when the cell divides. Now, if you have a DNA methyltransferase inhibitor, as you can see at the bottom left of the slide, you can actually prevent this methylation in half of the DNA and so the cell becomes hemi-methylated. Then if you continue to do this with subsequent cell divisions, you can eventually make that segment of DNA unmethylated. Obviously, this will lead to the activation or reactivation of the genes that have been previously silenced. Now, you can imagine if this is in a leukemic cell or an MDS cell and there are genes that have been silenced by hypermethylation and you are reactivating them, you can actually promote the differentiation of these cells and allow them to lose their AML phenotype and eventually differentiate and die of senescence. This has been the subject of hypomethylating therapy and the basis of developing hypomethylating agents.

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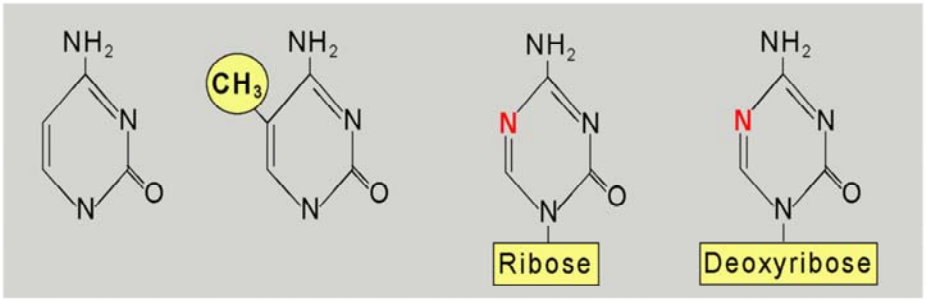
Hypomethylating Cytosine Analogues



Cytosine | 5-methyl-cytosine | 5-aza-cytidine | 5-aza-2'-deoxycytidine

Azacitidine | Decitabine

Azacitidine and decitabine are not approved by the FDA for the treatment of AML in the United States.
Azacitidine and decitabine are approved in Europe for the treatment of AML.

There are now two hypomethylating agents that are FDA approved and commercially available: azacitidine and decitabine. As I mentioned, both of these agents are FDA approved in the U.S. for treatment of patients with myelodysplastic syndrome, and they are also approved in Europe for treating AML as well as MDS. In the U.S., both of these agents, decitabine and azacitidine, are commonly used for the treatment of older AML patients who are deemed to be unfit to receive traditional cytotoxic chemotherapy in the form of cytarabine and anthracycline.

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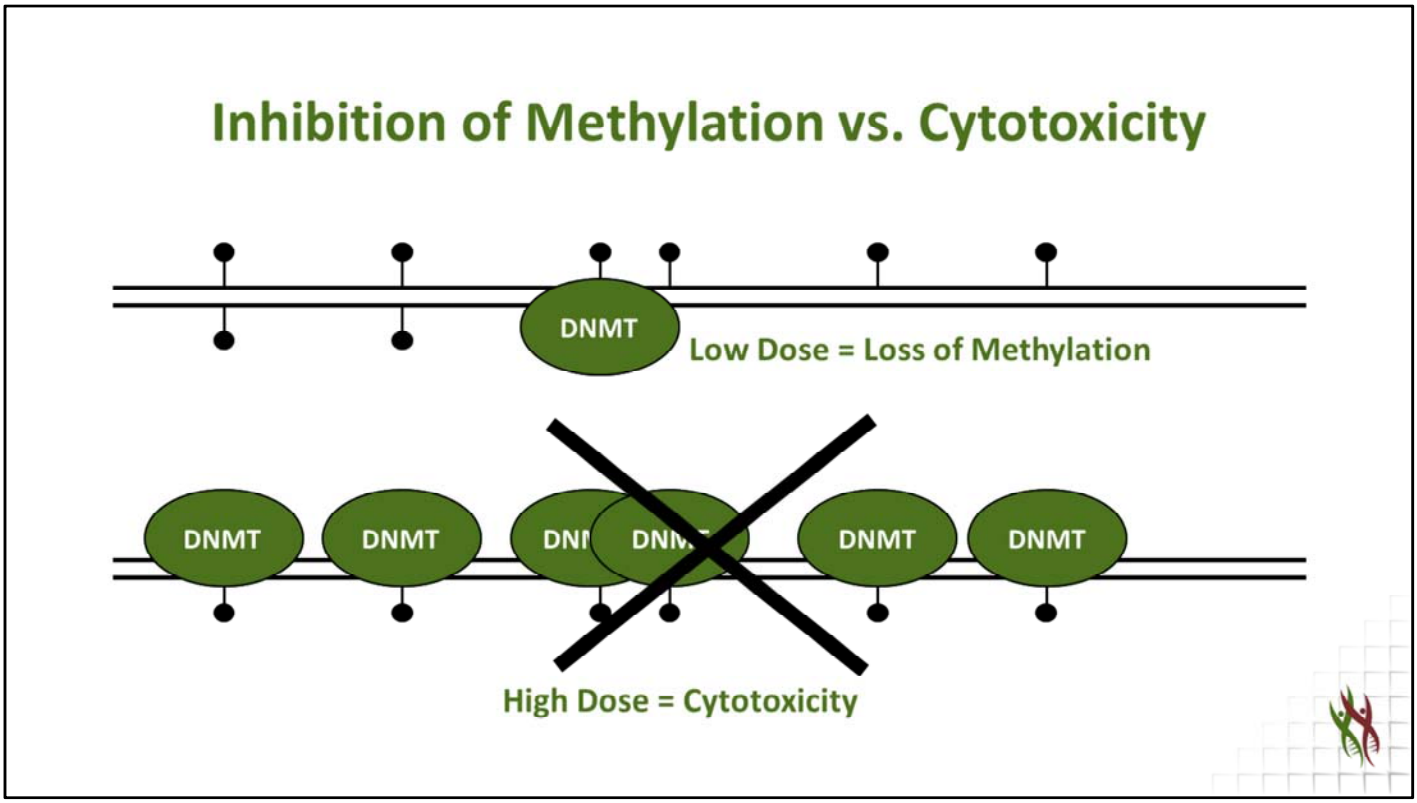

The agents work very similarly, again by inhibiting DNA methyltransferases, but please remember that these agents are nucleoside analogs, and so they do have cytotoxic properties when they are used in high doses. If you use them in high doses, they act more like nucleoside analogs and actually cause cytotoxicity; whereas at low doses, by inhibiting DNA methyltransferases as I mentioned earlier, they reactivate epigenetically silenced genes and allow differentiation of leukemic cells to mature cells.

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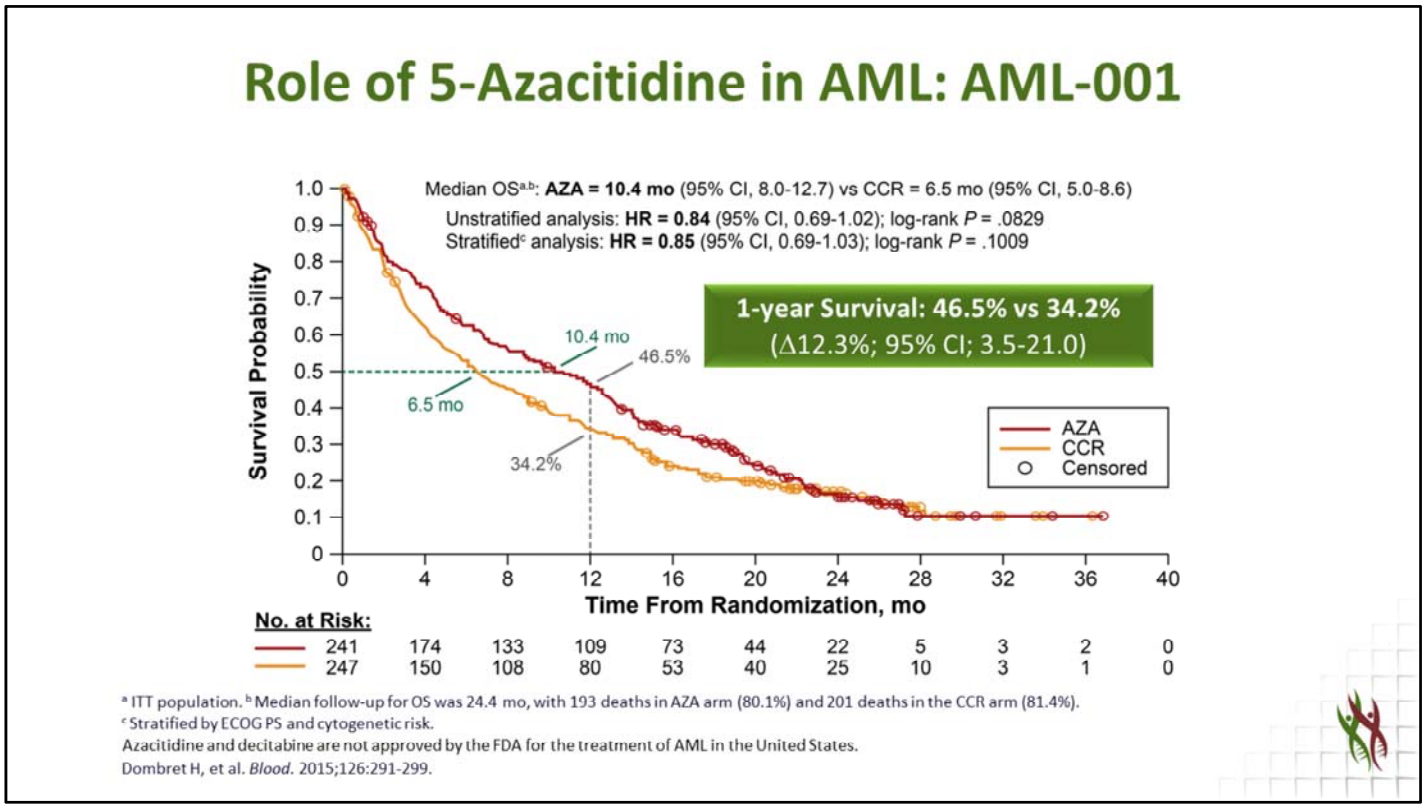

As I mentioned, both of these drugs are approved for treating MDS in the U.S. and are commonly used for treating older patients with AML. The reason for this is based on a couple of randomized studies that have clearly demonstrated that they can be beneficial in older AML patients. This one, the azacitidine AML-001 trial, randomized all the patients with AML who were considered not ideal candidates for traditional cytotoxic chemotherapy to receive either azacitidine or conventional care strategies. The conventional care regimens were either low-dose AraC or supportive care, or in some patients, chemotherapy. As you can see, this randomized study showed that using azacitidine in this specific older AML population does produce improvement in median overall survival from 6.5 months in the conventional care regimen to 10.4 months in the azacitidine-treated patients.

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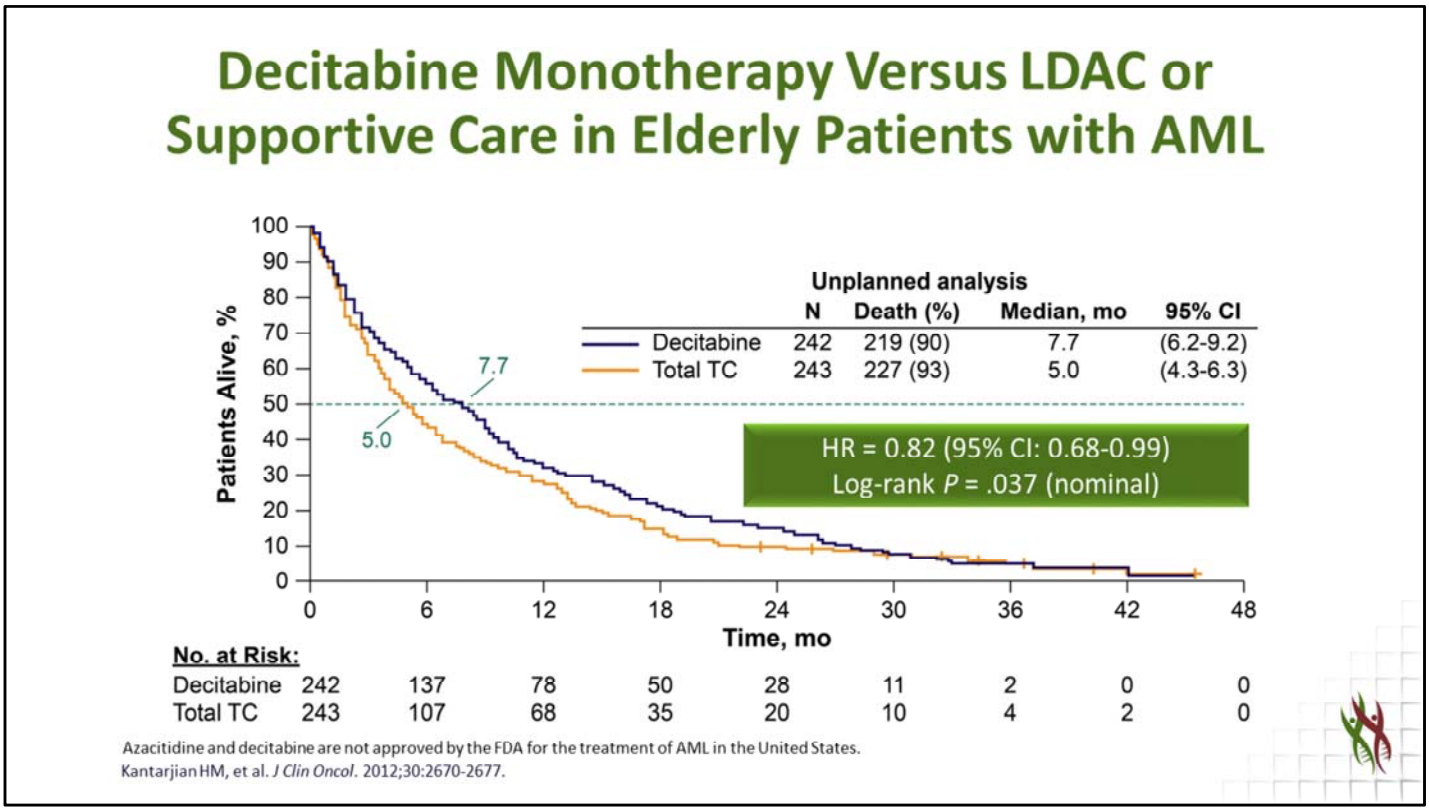

A somewhat similar study was conducted using decitabine, and patients, again older AML patients, were randomized to receive either decitabine or supportive care, perhaps with the addition of hydroxyurea. Again, this did show an improvement in overall survival when the eventual number of patients were assessed. When the original assessment was done at 90% of events, the *P*-value was not significant, but with total population and total followup, you can see there was an improvement in survival, albeit modest. Both these drugs do have some modest improvement in outcomes in older AML patients, and that is why they are both now in NCCN Guidelines for unfit elderly patients.

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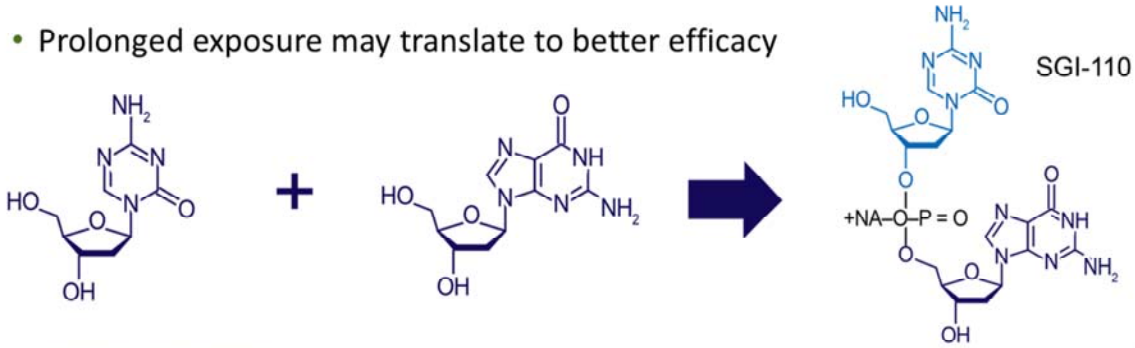
Guadecitabine (SGI-110): A Second-Generation Hypomethylating Agent

- Guadecitabine: dinucleotide of decitabine and deoxyguanosine that increases the in vivo exposure of decitabine by protecting it from deamination
- Decitabine is rapidly eliminated by cytidine deaminase, limiting drug exposure time to cancer cells in vivo
- Prolonged exposure may translate to better efficacy



Issa JP, et al. ASH 2013. Abstract 497.

There are now second-generation hypomethylating agents in development and in clinical trials. Guadecitabine is a dinucleotide of decitabine and deoxyguanosine that increases the in vivo expression of decitabine by protecting the molecule from deamination by cytidine deaminase, which is essentially the enzyme that causes limiting drug exposure time to cancer cells. This prolonged exposure is believed to improve the demethylation profile and hopefully, to improve the efficacy of the agent. Clinical trials of this agent are ongoing, including randomized trials which hopefully will let us know if this is a more potent demethylating agent.

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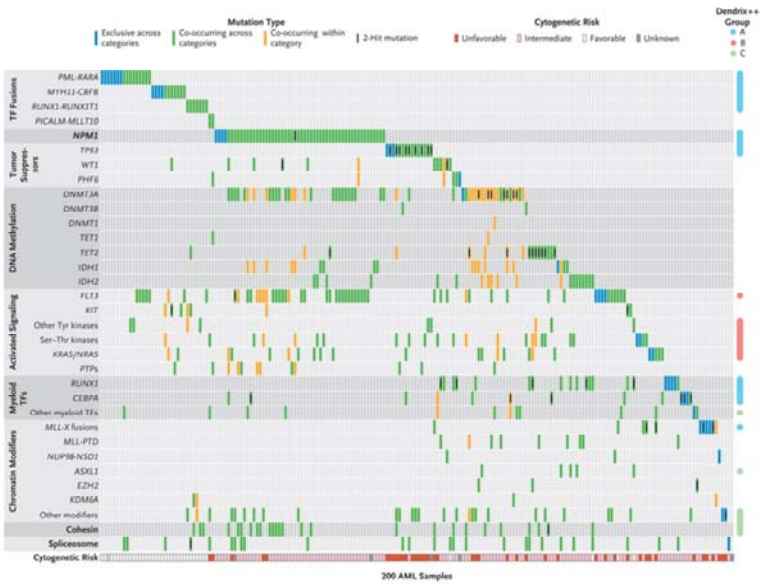
Genomic Landscape of De Novo Adult AML



Cancer Genome Atlas Research Network. *N Engl J Med*. 2013;368:2059-2074.

Also of interest is the fact that we have been able to identify a number of genetic mutations in patients with AML. This study by Washington University is pivotal. They examined cells from 200 AML patients by both whole genome and whole exome sequencing and identified about 23 commonly mutated genes that are shown in the next slide.

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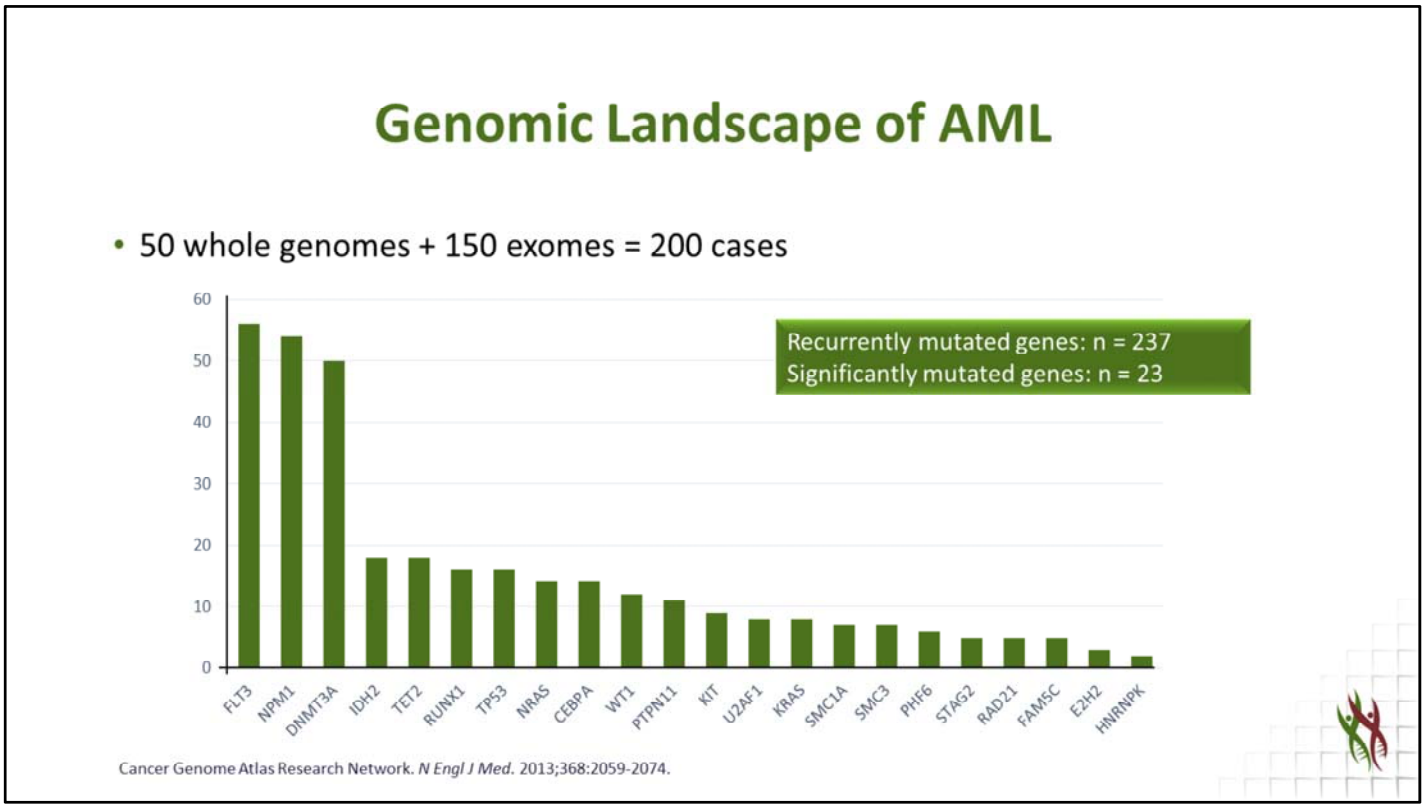

These include genes such as DNMT3A, IDH1, and IDH2 as well as TET2. Why this is important is because these genes are involved in the control and management of the epigenetic machinery of the cell.

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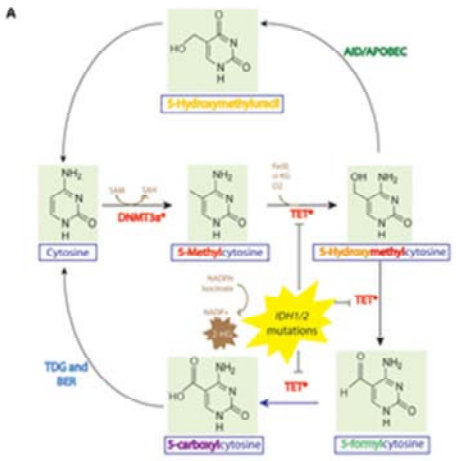
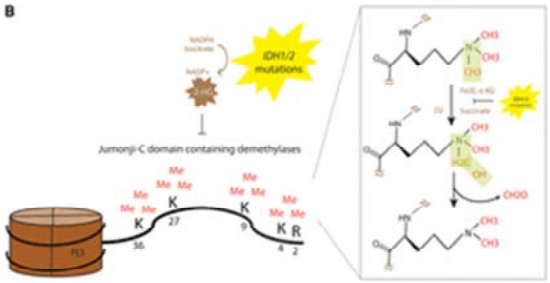
The DNA Methylation and Demethylation Pathway, and Effect of TET2 and IDH1/2 Mutations on Epigenetic DNA and Histone Modification





Abdel-Wahab O, et al. *Blood*. 2013;121:3563-3572.

TET2, IDH1 and IDH2, as well as the DNMT3A, are critical enzymes in the DNA methylation and demethylation pathways. One could imagine that in the cells where these genes are mutated, there is perturbance and disturbance of the epigenetic machinery. Potentially, these agents can be targets for drug development and modifying the epigenetic system.

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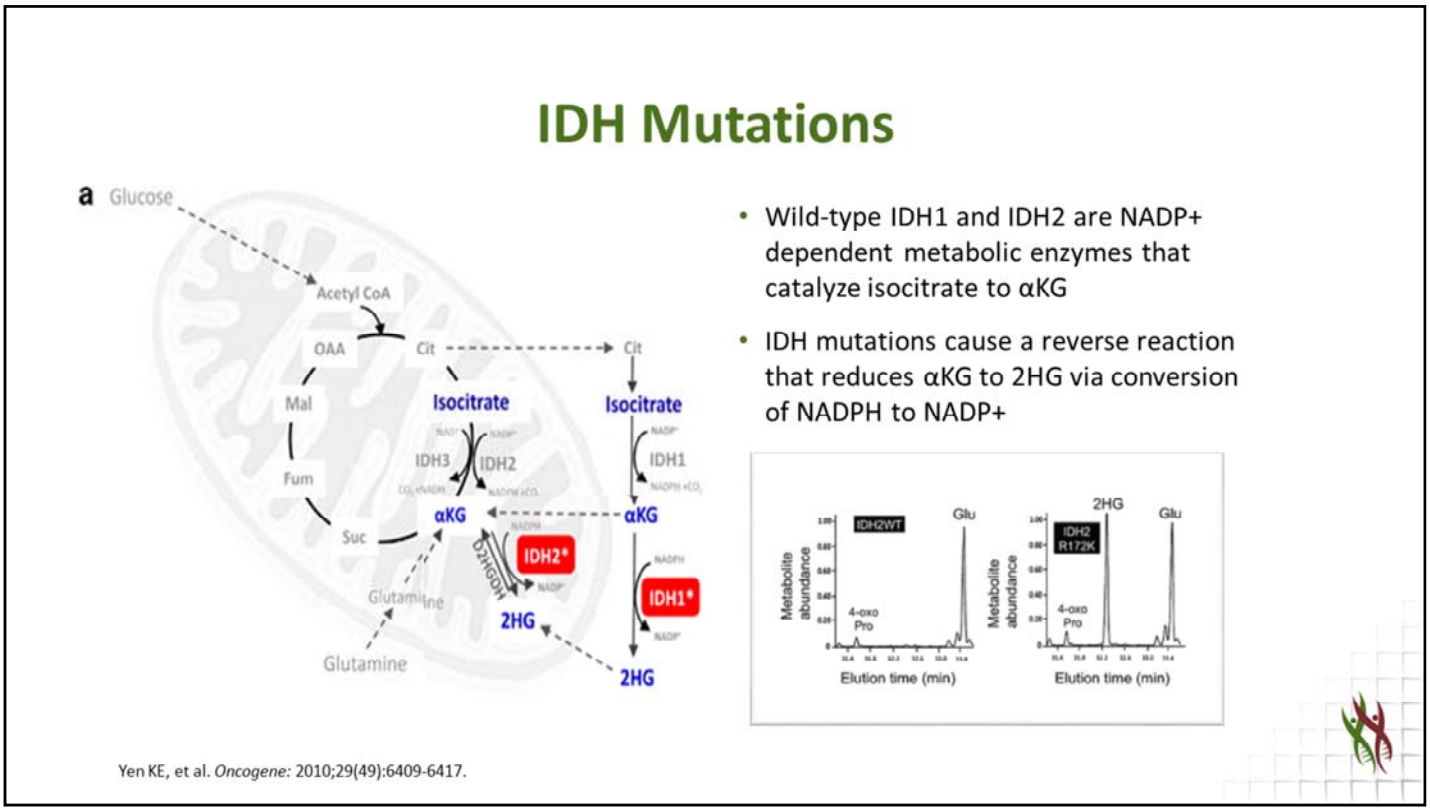

IDH mutations, for example, occur in about 10% to 20% of patients with AML. IDH1 and IDH2 are enzymes in the normal Kreb cycle. They convert isocitrate to alpha-ketoglutarate which is important in a number of enzymatic reactions within the cell, including some of the epigenetic enzymatic reactions involving TET2. When these genes are mutated, instead of the normal product alpha-ketoglutarate, an aberrant product 2-hydroxyglutarate is produced which interferes with these normal epigenetic mechanisms. Because of this, there has been interest in developing IDH inhibitors, and a number of them have already been in clinical practice.

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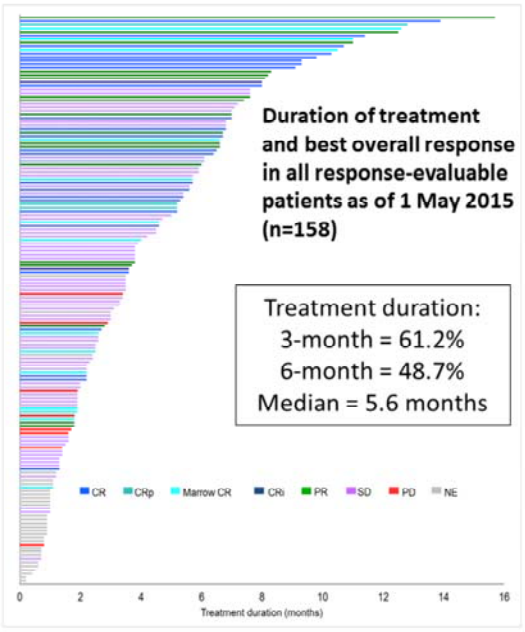
AG221: IDH 2 Inhibitor



Stein EM, et al. ASH 2015. Abstract 323.

AG-221 is an IDH2 inhibitor that has been evaluated in mainly patients with relapsed and refractory AML, who have IDH2 mutation. This study was a very important study because first, it showed that the agent is a very well-tolerated drug. AG-221 is associated with relatively little toxicity, and the patients could stay on it for a long period of time, many of them for months. More importantly, continued therapy actually led to improvement of responses in some patients. Overall, about 40% of patients had a response including about 20% with complete response in this relapsed/refractory population. That is important because again this is an oral agent and it is well tolerated, so when you produce 20% CR, that is clearly a significant response.

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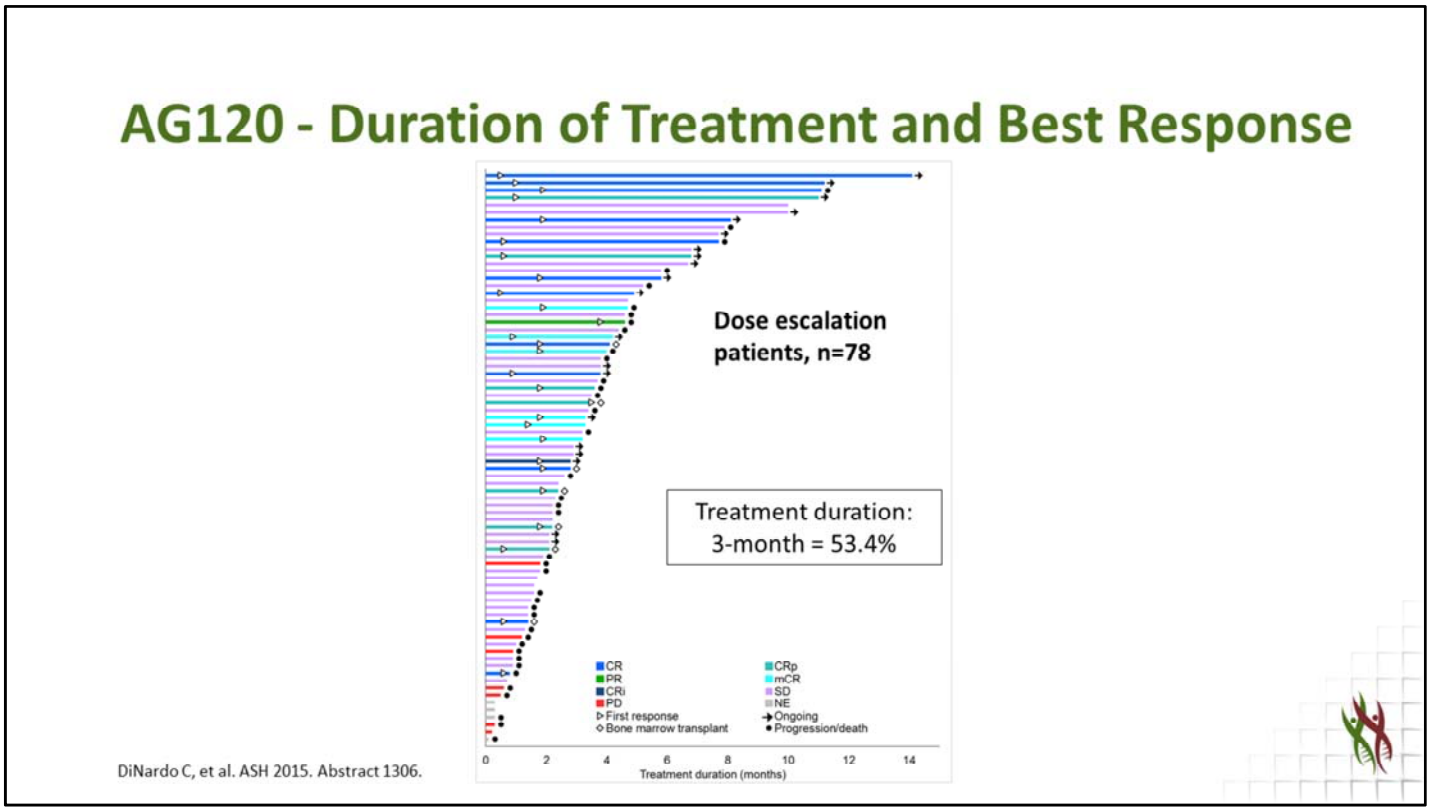

Another drug, AG-120, is an IDH1 inhibitor which is also being evaluated in clinical trials, mainly in the relapsed and refractory patients with IDH1 mutation. This is an oral drug that is well tolerated with relatively little toxicity, and the patients again remain on it and can continue taking it for a protracted time. With both of these drugs, myelosuppression can actually improve while patients continue to take the drug without achieving a response, so platelet count may improve. Patients can continue and again, some of the responses occur late, after several months of therapy.

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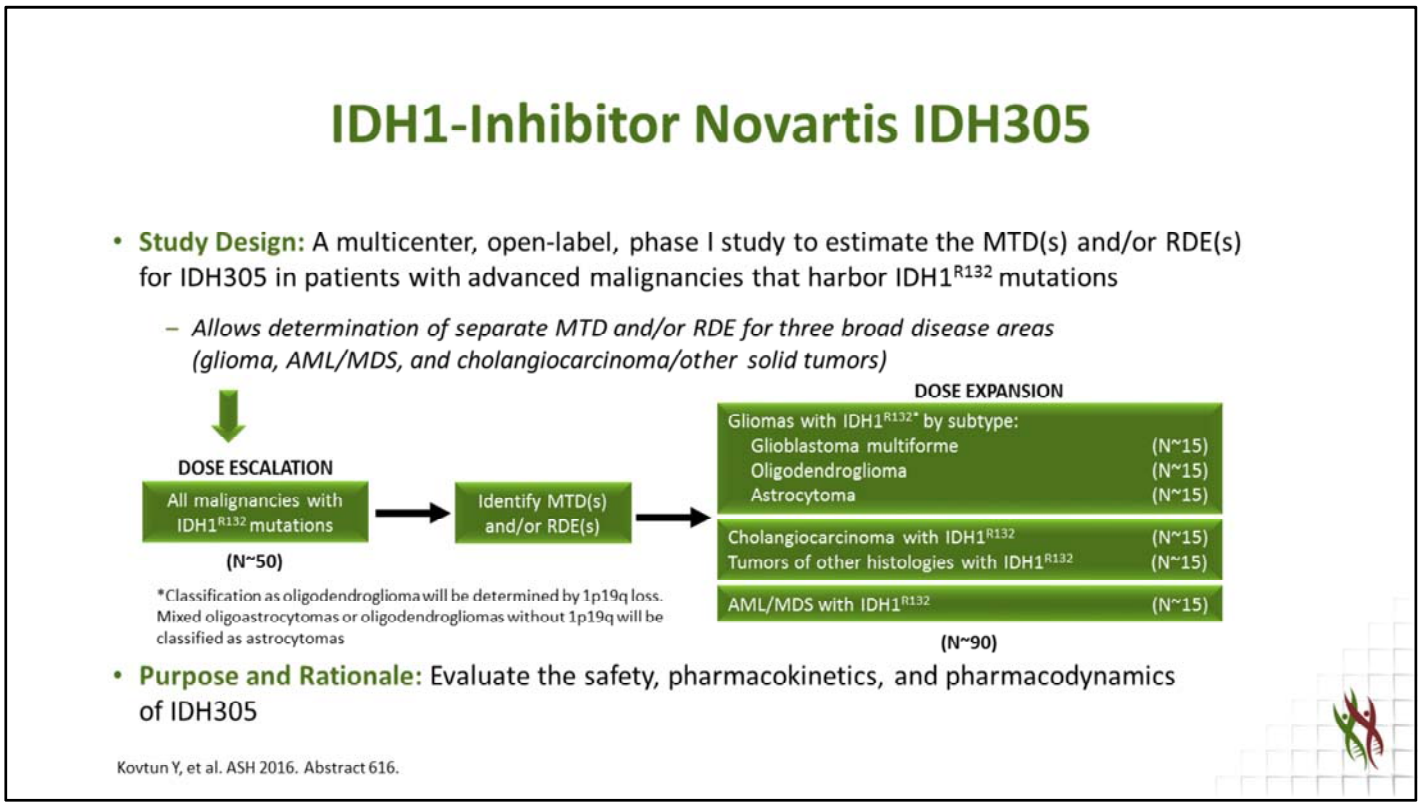

Other IDH inhibitors are also being developed. This is an agent called IDH305. Similar to the two previous agents, it is a well-tolerated drug and the early reports suggest that there is similar activity and similar tolerability with this agent in IDH mutated or IDH1 mutated agents. I think we will hear a lot more about these IDH inhibitors in the future, especially that many of them will be combined with other agents such as hypomethylating agents, and potentially with chemotherapy.

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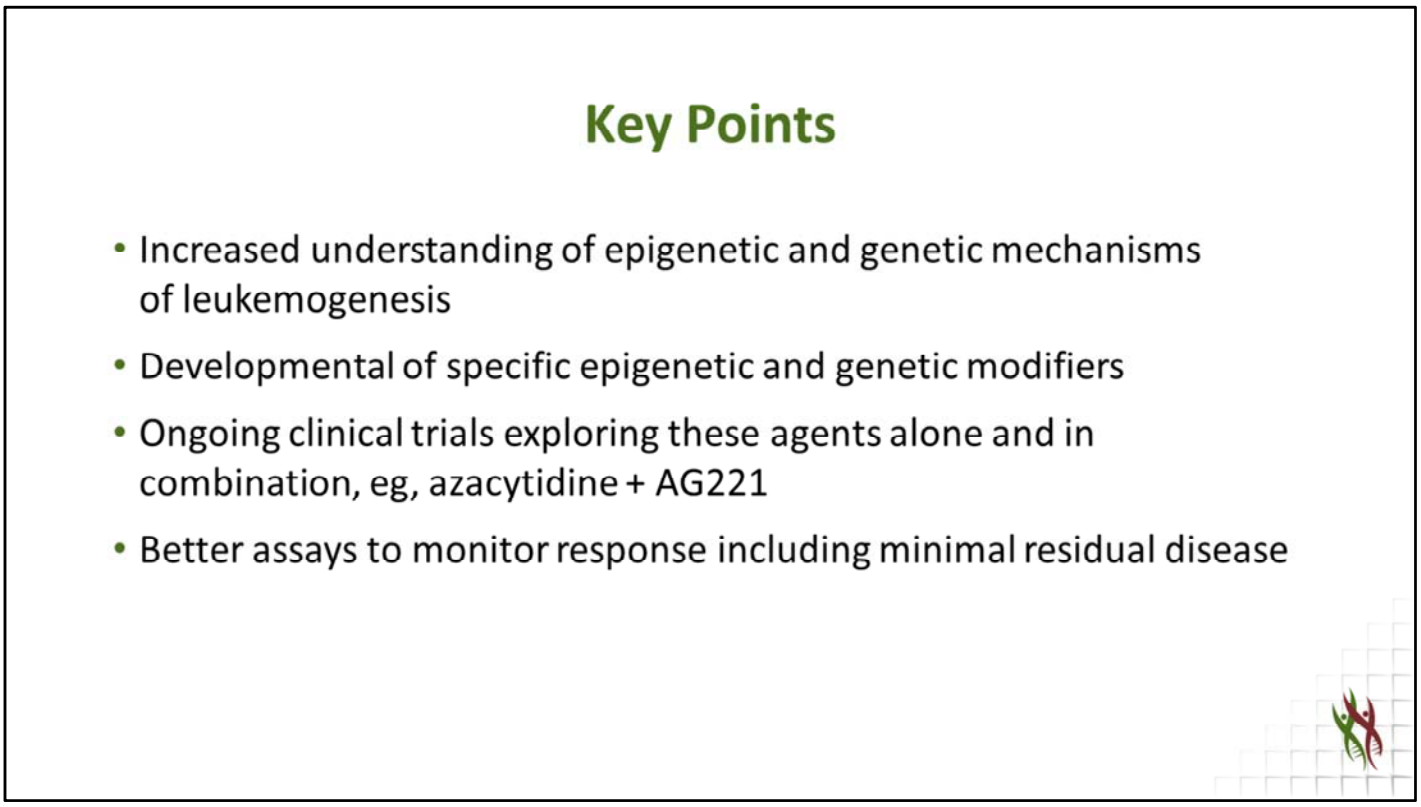

To conclude, I would like to leave you with these key take-away points. There is an increasing understanding of epigenetic and genetic mechanisms of leukemogenesis, and this has led to a number of specific epigenetic and genetic modifiers being developed and there are ongoing clinical trials exploring these agents alone and in combination. As I mentioned, we will see combinations of hypomethylating agents with IDH inhibitors. Potentially in the future when we have other agents modifying the epigenetically important mutations, we will likely see further combinations of these drugs. There are better assays to monitor response including using minimal residual disease which will help us potentially identify patients who are responding early, and removing patients who are not responding to other strategies. At the end, I would like to thank you for viewing this activity. Thank you very much.