NEW DIRECTIONS IN MYELOID MALIGNANCIES

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MODERATOR Good morning everyone. Thank you for being here. My name is Gabrielle Zecha and I am a PA at the Seattle Cancer Care Alliance. Our next session is entitled New Directions in Myeloid Malignancies and it is my pleasure and honor to introduce two of the most fantastic people, who are incredibly knowledgeable about AML and myeloid malignancies. Ms. Kelda Gardner and Melinda Tran. Come on up. Good morning.

MS. GARDNER Good morning, everyone. I'm Kelda.

MS. TRAN And I'm Melinda.

MS. GARDNER There are a lot of you here this morning. That's really great to see this early on a Saturday.

MS. TRAN Just a quick question, how many of you are from the West Coast? I see a lot of West Coasters. I thought this session might be a little early for our West Coast practitioners, like myself.

MS. GARDNER So we are going to talk about what's going on in myeloid malignancies. There have been a number of updates and changes over the last couple of years and we have a couple of learning objectives we will start with. We are going to talk about outlined strategies to monitor MRD. It's noted on the slide to be minimal residual disease. At our center we often call it measurable residual disease because in the eyes of the patient, any leftover leukemia or blood disorder is not really thought to be minimal for them, so we call it

measurable residual disease. And then we will look at some novel approaches including safety and efficacy and then also describe a number of mechanism of actions of targeted agents. Neither of us have anything to disclose financially. And then just another little deeper outline, we are going to talk just very quickly about epidemiology. There have been some changes in risk classification we'll talk about. I feel anytime you talk about hematologic disorders, especially high-grade ones, it's important to talk about heme emergencies. There are just three slides on that, so not too much. And then response criteria has changed in the last couple of years and then we will talk about MRD relapse, and then therapies, new drugs, and then we will end with a little information about APL.

You have all seen this probably many, many times, either in your training or in your clinics, in the patient rooms. As the main attending, I work with Eli Estey likes to say pretty frequently, "blasts don't come from the sky, they actually come from the bone marrow," so it all starts right here inside the bone. You develop a hematopoietic stem cell and as that grows it eventually divides out into being a myeloid lineage, or lymphoid lineage, and what we are going to talk about is far on the right with the myeloid.

Acute leukemia can present in the marrow. It can show up in the blood and it also can be seen in organs or even as a mass outside of the blood and marrow system, and that would be called a granulocytic sarcoma. There are a number of clonal blood disorders with myeloid phenotypes, and this is a pretty good schematic I think that shows a whole list of these and how they can all actually develop into acute leukemia. It is estimated in 2018 there will be about

20,000 new cases of AML, estimated deaths about 10,000, and a 5-year survival rate of only about 27%, which is still pretty low in the world of oncology. It has improved over time from 6.3% in 1975. A lot of that has to do with supportive care, and we will talk about regimens that have been used over the last 40-plus years, and how we are just now seeing emergence of new therapies. The median age of diagnosis is 68. The incidence of AML goes up with age, overall about 4 cases in 100,000 patients. Etiology is generally mostly unknown. The two most important characteristics are listed in bold here, prior chemotherapy or radiation, also known as tAML for treatment-related AML, or secondary AML from having a prior hematologic disorder. There are two survival graphs, the one on the left is all patients with de novo with either secondary AML or treatment-related AML, and there is a striking difference between that and the one on the right, which shows a survival curve with patients with a favorable type risk de novo AML.

There has been a lot learned in the last couple of decades about genetic predisposition to AML, and it's thought to be about 5 to 10% of these patients have some sort of inherited background. So one of the big questions is who to test and who to look at for genetic predispositions, and in our center we are really looking and testing patients that are under the age of 45, or that have a strong family history of AML or MDS. Diagnosis of AML is by WHO criteria 20% more myeloid blasts that are in the marrow, or sometimes you can just do it off peripheral blood if there are enough blasts in the peripheral blood. With the diagnosis of AML comes the pretreatment knowledge that outcomes range from death within a few days of starting therapy to a potential cure. So there is a

mandatory testing, at least at our center that we consider doing at diagnosis, morphology obviously, cytogenetics, FISH, molecular studies either by a hot spot or next-generation sequencing, and also immunophenotyping flow cytometry. And the importance of doing the full panel workup at the initial diagnosis is not only does it give a prognostic indication for potential need of a transplant or novel therapies, it also gives us an idea of what markers we would like to follow later to see if someone is really truly in a CR and how they responded to therapy.

So as we move into cytogenetic abnormalities in AML, it is pretty overwhelming to distinguish from the variety of different abnormalities what would be considered a good marker, or what would be considered a bad marker. You can see from the slide that most patients show up with a normal karyotype. And as this is a disease of older patients, the older someone is, the more likely they are to have abnormalities with cytogenetics as we are regrowing them all the time. By the age of 70, about 10% presumed normal people—not people with AML, just normal people walking around—will have mutations in the hematopoietic cells.

This is one of my favorite slides; it is very pretty. This is a nice show of molecular abnormalities and just how tremendously heterogeneous the population is in regards to molecular. There is a lot of co-localization that happens, for instance you can see on this, NPM1-positive patients can also have three positive markers. This is from some ECOG data that looked at survival of hundreds of patients and their mutations. Last year the ELN, European Leukemia Network, came up with a new set of risk classification. This is a bit messy slide,

but it is a good demonstration of what actually has changed. In 2010 was the first year they put together cytogenetics and molecular to do risk stratification, and that continued in the redo in 2017 with the main changes being that intermediate 1 and intermediate 2 are combined. Then also it adds in the TP53 abnormality, which we know is the single most adverse factor, and it also divides out patients that are FLT3 by their allelic ratios. Here is a quick diagram of the survival stratification by ELN, the one on the left-hand side is the new one with 2017, which has intermediate combined, and the one on the right is from 2010 that has the intermediate divided out.

Just a couple of words on heme malignancies, leukocytosis; hyperleukocytosis is defined as a white cell count of over 100,000, leukostasis is the concern, and it usually affects the CNS and the lungs, which is the most concerning. The main treatment in cytoreduction with the use of hydroxyurea and/or dexamethasone for pulmonary symptoms, high dose Ara-C, one dose or two doses, and also leukapheresis. Everybody really wants to know what the exact number is, or the ideal number is, to actually initiate cytoreduction and really one of the main things is to look for symptoms like stroke-like symptoms, blurred vision, and what not to initiate intervention. The symptoms may tell you a bit more than the actual total white cell count.

Tumor lysis syndrome is probably more common in ALL and lymphomas, but certainly can be seen in the myeloid malignancies as well. It can be spontaneous or chemo induced, and also you can see hyperkalemia, hyperphosphatemia, and hypocalcemia. Hydration is one of the mainstones of

treating this, and at least with AML population we have to be a bit judicious with fluids given the chance for it rushing to the lungs and causing pulmonary insufficiency.

Hemorrhagic syndrome, very common in APL patients, and we are going to talk about that right towards the end. The big things with this are keeping platelets up, transfusing FFP to keep an INR up above 1.5, and then cryo to keep fibrinogen well above 150. Who here has heard about the treatment-related mortality line scoring system? So, Dr. Roland Walter and Dr. Eli Estey came up with this treatment-related mortality calculator, which looks at a number of different variables for a newly diagnosed AML patient and the chances of dying within 28 days of receiving intensive chemotherapy. It looks at age, platelets, albumin, whether they have secondary AML, or primary AML, white cell count, peripheral blood blasts and a creatinine. You can just Google TRM calculator, FHCRC, which is Fred Hutchinson Cancer Research Center, and it will take you to this calculator and you can start putting in numbers. This is just a picture of it. You just put in all of the different variables and then you get the TRM at the bottom. For this patient, the likelihood of dying is about 11% within the first month of starting chemotherapy.

Another update that has happened in the last couple of years is the ELN also has updated the response criteria. There is a new category which has been added, which is the one on the top. It is bolded. It is CR without MRD. In the CR category, you will see under the comment section on the right that this also now includes whether someone has MRD positive, or it's unknown if they are MRD

positive. The CRi now encompasses CRp, so whether someone hasn't recovered their neutrophils, or haven't recovered their platelet count, either way they get put into the CRi category.

Speaking of CRis, or CRps, or MRD, there is a lot of interest right now about what to do about measurable residual disease and the importance of the depth of response that a patient gets after therapy. Dr. Chen a couple of years ago at Fred Hutchinson, did a retrospective analysis of several hundred patients, showing that blood count recovery is also important for response and survival. Just less than 5% blasts in the blood is not alone sufficient, and CRps and CRis are inferior responses compared to a full CR.

Now we will move into measurable residual disease, just as a CRp or CRi are inferior responses, so is the presence of residual disease. The survival curve, the one on the left, shows the chance of relapse when there is MRD, and the chart on the right shows overall survival. With the one on the left, there is lower incidence of relapse without MRD and then the chart on the right showing the overall survival is better without MRD. Pathology has gotten more and more sophisticated. We have the multiparameter flow cytometry testing to look for MRD. We also have the next-generation sequencing looking at molecular studies and this is a nice diagram that just came out in the *New England Journal of Medicine* this year showing that if someone has flow cytometry or mutational testing that's positive or negative, how that affects the chance of relapse. So, if you look at the top line, which is the purple line, those are patients that their disease is positive by flow and positive by next-generation sequencing, and they

have the highest relapse rate of 75%. The patients in the group in the bottom in blue, shows that if someone who has a negative flow cytometry and negative molecular studies, has a lower chance of relapse at about 25%.

So, the question remains what to do about MRD. Outcomes are clearly worse. A lot of clinical trials do not allow patients to get the different study drugs with only 2% or 3% blasts, and we certainly need more novel therapies, which we will talk about later.

I think it is pretty well known that the main reason patients are not cured from AML is because of resistance to disease, which can manifest as a relapse after remission. This shows the role of transplant after someone has relapsed if they didn't have a transplant in CR1, and for those with a favorable risk group, after an allo they have an 88% 5-year survival versus 33% without getting a transplant in the CR2.

Patients that are older with AML, which is a good number of people that present, the question is, is age just a number? Medicare records from 2000 to 2009 show that only about 40% of patients that were over the age of 65 received any type of chemotherapy within 3 months of their diagnosis. Also, in this data set, it showed patients that were actually treated lived longer. So the question is, what do you do with an older patient with AML? The ELN 2017 guidelines say that no longer should just age be a reason not to treat a patient with older age, but it really should be age plus another factor against having an intensive therapy. Those things could be ECOG such as 3 and 4 and other comorbidities.

Next we are going to start talking about therapy and new drugs. Just as a reminder, our backbone, which has been the standard of care, is 7 and 3, which is Ara-C and an anthracycline. You will see the notation on the bottom sites a paper from 1973. That tells you really how far we've gone if our main backbone is still something from back then. The NCCN guidelines do recommend the best management for any cancer patient is on a clinical trial. A number of centers use 7 and 3. I know MD Anderson use a combination like FLAG-IDA. At our center we use something very similar called GCLAM. It is cladribine, Ara-C, and mitoxantrone. That takes us to the concept of alternatives to intensive regimens and Melinda is going to talk about that.

MS. TRAN I know Dr. Nelson and Dr. May will be presenting an update on new drugs and heme malignancies, so I will kind of give you an idea of the background of some of these drugs, the mechanism, but I won't go into too much detail about them, just because there is going to be an excellent session coming up at 11:20 today. The drugs that are bolded are the ones I am going to be talking about today, and you will hear, I will be pointing to Kelda for some commentary just given your extensive background with the AML and the research and the different trials that our center has been through.

The first drug I will talk about is formerly known as CPX351, or liposomal daunorubicin and cytarabine. When I was in the pharmacy, we would call it "purple rain" because if you have ever seen it, it is a 500 mL bag of a very beautiful violet color, so my technicians would call it purple rain. This is just a repackage of our traditional induction therapy of 3+7. It uses a liposomal fixed

molar ratio of cytarabine and daunorubicin. It utilizes nanoscale delivery technology that seems to be preferentially taken in by the leukemic cells, so that hypothetically increases the efficacy while decreasing the toxicities. In a lot of our older patients, giving them 7+3 is almost as toxic as it is beneficial. By formulating in this kind of repackaged liposomal formulation, we are hopefully decreasing that tissue distribution, that toxicity, and hopefully providing the same therapeutic benefit.

This drug was approved in August 2017, based on the data from this phase 3 trial, where they randomized previously untreated high-risk AML patients, older patients. The study had defined high risk as patients with therapy-related AML, patients previously with MDS or CMML, or de novo AML with MDS karyotypes. These patients were randomized to receive either daunorubicin, standard 7+3, or the CPX351. What we see is that CPX is superior to 7+3 in all endpoints that they examined, overall survival, event-free survival, as well as a response rate analyzed by CR and CRi. It also had a better 60-day mortality rate as well. One of the key takeaways of this study is that patients that receive CPX were more likely to undergo a successful allo transplant than patients who underwent 7+3. Among those patients who underwent a successful allo transplant, those patients on CPX had a better median overall survival. It wasn't reached by the end of analysis, versus 10.25 months in patients who were on 7+3. I think one of the key things that I think about is that hey, maybe this is a viable option for patients who have high-risk AML and are transplant eligible.

When this drug first came out, there was a lot of concern about whether or not it would have increased toxicity because essentially with this liposomal form, we are concerned that there is going to be a longer half-life of this drug. And indeed there is a longer half-life. But what we know now, is that the safety profile of this drug is very comparable to standard therapy. In fact, as I mentioned earlier, it had an improved 60-day mortality rate compared to 7+3. I think it was 13% versus 21%, around there.

There are some notable differences to CPX versus 7+3. The first one being that CPX had prolonged neutropenia and thrombocytopenia, so about 7 to 10 days longer than standard therapy. Then there was also increased risk of hemorrhagic and infectious adverse effects. But, fortunately those adverse effects did not result in increased risk of early mortality.

So the next drug I will talk about is gemtuzumab, and this one has a very long and unusual history. This was the first antibody drug conjugate approved for human use by the FDA. We will talk about the history in just a little bit, but just in a nutshell, it was approved in 2000, it was removed from the market in 2010, and then it makes a big comeback in 2017. It is a humanized monoclonal antibody, but you already knew that from the name, ZU humanized, an MAB monoclonal antibody, and is conjugated to a toxin calicheamicin. It targets the transmembrane protein CD-33 that is highly expressed on leukemic blast surfaces and it affects, I believe, more than 80% of patients with AML express CD33. Some of the notable adverse effects include prolonged cytopenia, as well as VOD, venoocclusive disease, which we will talk about in just a little bit as well.

As I have mentioned, it is a monoclonal antibody, it binds the CD33 receptors. Once it binds the CD33, it's internalized into the cell and under the acidic environment of the lysosome, the calicheamicin gets cleaved, that gets released into the cell, that binds to the DNA and causes double strand, single strand breaks, and eventually apoptosis. Now when this drug was first approved in 2000, it was approved based on data from accelerated interim analysis and at that time it was approved at the dose of 9 mg/m^2, on days 1 and 14, and it had a CR plus CRp of about 30%. As part of the post-approval commitment, the drug manufacturers worked with SWOG on S0106, where they randomized over 600 patients with de novo AML, older patients, to receive the standard 7+3 plus or minus gemtuzumab. Unfortunately, what they found was that there was no overall improvement in survival and there was actually an increased risk of mortality in the experimental arm. Kelda, we worked on that trial. Was there anything special about that trial that you remember?

MS. GARDNER Maybe not specifically about the SWOG S0106, but maybe in the time after gemtuzumab was taken off the market and went back on, but we can talk about that in a little bit.

MS. TRAN Now this drug, like I said, it made its big comeback last year. That approval was based on a few different things. The first thing was the data from the ALFA-0701 trial. Also, a better understanding of how we dose gemtuzumab, as well as a lot of clinical experience. Just because it was off the market in 2010 doesn't mean we stopped using it. We have been using it ever since then. There is a lot of clinical experience behind that as well. ALFA-0701

was very similar to the SWOG trial in that we randomized patients to either standard therapy plus or minus gemtuzumab, but what we did was we used a lower dose. Instead of the 6 mg/m^2 using the SWOG trial on day 4, we used 3 mg/m^2 on days 1, 4 and 7. When I say "we" I wasn't a part of this, it is the collective "we." What we found out was that patients who received gemtuzumab had a similar CR rate compared to the standard arm, but there was an improved relapse rate, there was improved relapse-free as well as overall survival at 5 years. The other thing we learned is that gemtuzumab, that VOD is really associated with high max concentrations on the first dose, so by using a lower dose we would expect a lower concentration. And we also know that at the lower dose, the CR rate is comparable to the higher dose. In the meta-analysis, it came out with similar findings as well, so same CR, but improved other outcomes. From that meta-analysis, we see three survival curves. Each of these survival curves are for the different risk factors. The first is for the favorable-risk AML, second intermediate risk, and third for adverse risk. As you can expect, patients with favorable-risk AML had the best outcomes with gemtuzumab.

The next drug we will talk about is IDH or isocitrate dehydrogenase mutations. These mutations are found in 15 to 20% of newly diagnosed AML. They generally happen in older patients, they are generally associated with worse outcomes. In normal cells, IDH converts isocitrate to alpha ketoglutarate and we will talk a little bit more about what alpha KG does in the body, but it's very important. But in mutant cells, the mutant IDH take it one step further and it converts alpha KG to alpha HG or alpha hydroxy glutarates, which is an

oncometabolite. This is a visual of what I just mentioned. So, isocitrate is converted to alpha KG by IDH1 and 2. As a throwback to our biochem days, alpha KG is very important because is it a key intermediate of the Krebs cycle. It also does a lot of different things to cellular processes. It helps oxidize different nutrients, amino acids, glucose, fatty acids. It acts as an antioxidant and nitrogen scavengers, so it does a lot of different things in our system. From there, a mutant IDH will then convert it to 2HG, an onco-metabolite. 2HG's whole purpose in life is to promote leukemic proliferation. It blocks myelodifferentiation and it does it in a lot of different ways. It causes epigenetic changes, which forces itself to lose ability to differentiate correctly.

Again, like I said, I won't talk too much about the drug itself just because there is an excellent session coming up in just a few hours, but enasidenib is previously known as AG221, and this drug was approved in 2017, so the first IDH inhibitor that was approved by FDA. It targets IDH2. From what I know, this drug is fairly well tolerated, but one of the key things about this is differentiation syndrome. Anytime you have drugs that promote differentiation, like ATRO or ATO, this drug, we are going to be concerned about differentiation syndrome. Patients might experience some symptoms like acute respiratory distress, lymphedema, peripheral edema, pleural effusion, pericardial effusions, weight gain, leukocytosis, different things like that, that you already know about. It's very, very dangerous. We would have to treat this patient with a course of steroids until those symptoms have resolved and depending on how severe those symptoms are determines whether or not you would stop the drug in the

interim. As a pharmacist, I am going to be worried about drug interactions. This drug and its metabolite are processed by a number of CYPs and UGTs, so please be aware of drug interactions.

Ivosidenib, previously known as AG-120, is very, very similar in that it is an IDH inhibitor, but inhibits IDH1 enzyme. Also, fairly well tolerated. Differentiating syndrome is a concern and it is primarily metabolized by CYP3A4, so drug interactions. I won't go too much into each of the trials, but essentially both agents were studied in patients with relapsed/refractory AML, who had an *IDH* mutation, and the primary endpoint was overall responsive, the CR plus CRh. What we see here is that both drugs were able to produce a clinically meaningful response that was also quite durable as well, so 8.2 months for each of these drugs.

Moving on to BCL2 inhibitors. Resistance of apoptosis is really a hallmark of cancer biology. Pretty much all cancer cells are going to adopt some sort of mechanism to avoid apoptosis, and leukemic cells are no exception. AML cells happen to be very dependent on BCL2 for survival. BCL2 is a family of proteins, and think of it as a gate keeper to apoptosis, and we will talk a little bit more in the next slide, but they are very dependent on BCL2, which happens to also be increased in concentration in these leukemic cells. BCL2, there is a BCL2 family of protein, but there is also a BCL2 protein itself, and it is an antiapoptotic protein. Since we just talked about *IDH* mutations, just kind of throwing a tidbit in there, what we know is that some patients with a mutated IDH are more likely to respond to BCL2 inhibition. Then we will talk about two clinical trials, very quickly,

that involved the use of BCL2 inhibitor plus a low-dose Ara-C or a hypomethylating agent.

As I mentioned, resistance of apoptosis is a hallmark of cancer biology, and there are different pathways of apoptosis. There is an extrinsic pathway, in which essentially something binds onto the cell, like a death receptor, and that causes the cell to kill itself. Then there is an intrinsic pathway, or mitochondrial pathway, and that's where the cell will kill itself based on something it senses in the environment. Typically in heme malignancies, there is a discrepancy, or a dysregulation of intrinsic pathway. I'd also mentioned that BCL2 is a family of proteins, it's considered as gate keeper to apoptosis, and that family of proteins contains both proapoptotic, as well as antiapoptotic proteins. When you have more of one or the other, that shifts the balance between cell survival and cell death. Usually antiapoptotic proteins and proapoptotic proteins live in hemostasis. They live, they are in harmony, they are bound to each other, they cancel each other out, but in malignancies you will see a shift in one way. You will see an increase in antiapoptotic stuff, and that will shift it more into cell survival mode. Drugs like venetoclax will actually displace the proapoptotic protein from the BCL protein and allow it to move on its merry way, fulfill its life, and initiate apoptosis. In this clinical trial, as well as the next one, we are utilizing venetoclax, a BCL2 inhibitor, in combination with a hypomethylating agent such as azacitidine, typically in our older patients who are not eligible for more intensive therapy. I know this is also one that we did as well. This one, as well as the next one. What we saw in these trials is that patients who received this

combination of venetoclax, as well as a hypomethylating agent, that there was a response rate, roughly about 67%, and the median overall survival of these patients was 17.5 months, median duration of response had not been reached at the time of analysis. They also did an exploratory analysis of MRD, and 29% of these patients were MRD negative as well, which is really surprising to me given kind of the intensity of this drug, so it provides a better outlook to a lot of our older patients who would not have been eligible for standard therapy.

The next trial is very similar. We looked at venetoclax and low-dose Ara-C. In this combination of drugs, we looked at older patients who were not eligible for standard therapy. Same types of outcomes as well. We see that for the most part, this combination demonstrated acceptable safety profile, and that remission was achieved in the majority of our patients. We see the overall response rate, so CR, CRi, plus a partial response, was 75%. Of those patients CR and CRi were obtained in 70% of our patients. Of the patients who responded, the overall survival had not been reached, and there was 12-month overall survival of almost 75% of all patients.

The next things we are going to talk about are FLT3 inhibitors. FLT3 is a receptor tyrosine kinase. It plays a major role in our system in cell growth and proliferation, and normally, upon binding of a ligand to FLT3, it activates a cascade of downstream signalling that promotes cell growth, inhibits apoptosis, and activates differentiation. However, in a mutated FLT3 receptor, we see that it's no longer dependent on a ligand binding to it, and it's almost always on. One of the most common *FLT3* mutations is ITD, or internal tandem duplication, and

we find that in about 23% of patients with AML. It carries a very poor prognosis, and in fact, as Kelda had mentioned earlier, patients with a high allelic ratio of FLT3 ITD, so more ITD compared to wild type FLT3, we see that these patients in the absence of an *NPM1* mutation are classified by ELN 2017 in the adverse risk category.

Midostaurin is the first multityrosine kinase inhibitor that targets FLT3 ITD and TKD, which is another type of *FLT3* mutation, not quite as common, not quite as bad. It's the first one approved by the FDA for human use in AML. It is very well tolerated. Main thing as a pharmacist I worry about is drug interactions, since it is a substrate of CYP3A4. This drug was approved based on the RATIFY trial where they randomized patients to standard 7+3 plus or minus midostaurin. The endpoint of the study was overall survival and event-free survival, and this trial demonstrated very statistically significant improvement in both overall and event-free survival in these patients compared to the placebo arm.

TP53 is one of the bad players. This is really one I am sad to see when I see it on a patient's report. TP53 is a tumor suppressor gene, and patients who have a *TP53* mutation tend to be older and tend to have very complex karyotypes. Traditionally conventional chemotherapy only provides them 4 to 6 months of survival. There was a study done, a single-arm study, single institution, where investigators provided patients with MDS and AML with deciding standard dose 20 mg/m^2 times 10 days, and these patients, what they were surprised to find out was that patients who had unfavorable-risk karyotypes had very similar outcomes, or similar survival outcomes, to patients who had favorable- or

intermediate-risk AML. Unfortunately, these responses were not durable. So, we are still yet to see what is the correct action when patients have *TP53* mutation. I am going to pass this over to Kelda.

MS. GARDNER I'm going to piggy back a little bit on what Melinda was saying with these new therapies. We did a number of the clinical trials at our center, which I'm realizing now that we didn't even say what that was. Gabrielle mentioned Seattle Cancer Care Alliance. We are also University of Washington, and Fred Hutchinson Cancer Research Center, and also Seattle Children's is also part of our collaboration. Let's talk a quick second about the CPX351, as Melinda calls it "purple rain." We were part of that trial. We found that this was really a very well-tolerated therapy. The ECOG for eligibility of this trial was from 0 to 2, and the age range was from 60 to 75. It will be potentially interesting to look further into whether this, since it was so well tolerated, could be used in patients with even high ECOGs or even age ranges outside of that 60 to 75. She also mentioned gemtuzumab, which as she mentioned made a comeback in 2007. During those years when it was off the market, we opened up an expanded access trial with this and treated 44 individual patients, most of them were adults, and a handful of them were pediatric. If any of you are going to ask, I will have a poster on this. You are welcome to come see me on Sunday evening talking about what we used in combination with gemtuzumab, which was a wide range of therapies, high dose and low dose. We used our standard backbone of GCLAM with it. We used FLAG-IDA with it. We did azacitidine and even gemtuzumab alone as well. The main criteria for being able to be treated on this

expanded access was to have relapsed or refractory AML, MDS, or APL. Initially patients had to have good-risk cytogenetics since that is what was shown probably the main benefit of gemtuzumab. Then later, once the data came out, especially in pediatric patients that despite cytogenetic abnormalities it could be beneficial in pediatric patients, we changed that criteria and Pfizer and the FDA allowed us to use this despite what the cytogenetic abnormalities were. Spoiler alert for the poster, which I can talk about because I think as of the first of November all of that's available online now. The big take away is that it was well tolerated and no one died from toxicities from the go. The observed versus expected outcomes were really about the same.

The third therapy talked about was the ABT199, venetoclax. We had it open at our center with both the Ara-C arm and also with the azacitidine arm. It will be a big topic I think at ASH this year. It looks like there is a number of oral presentations and posters in the use of venetoclax. Eventually, maybe we will see some use of it in the relapsed/refractory setting as well. As far as I know it has been used mostly up front, but I could be wrong about that. Ask me I guess after ASH. One of the things about it being low intensity is we have at least two patients that I can name off the top of my head who had poor-risk disease and that were older that are still in a full CR without relapse, from a lower intensity therapy with patients with AML, that's pretty impressive, so we will see how this all unfolds.

MS. TRAN Kelda and I were talking about it yesterday. I hadn't seen a lot of ABT199 or venetoclax in AML prior to doing the research, as well as these

clinical trials. A lot of it happens to be because the drug itself, up till that point, was not approved for AML, only CLL and SLL, so getting insurance approval would be very difficult, just because you are going to be jumping through a lot of hoops, a lot of prior authorizations, and crossing your fingers that the patient has good insurance that after a PA or two you will be able to get it approved. I think following ASH and all the research that is coming out of that drug in this realm, we may be seeing some changes in the indication as well.

MS. GARDNER Future directions. There was a nice talk yesterday by Dr. Park and Dr. King about CAR T cells. I won't go into detail; that was very well covered yesterday. Currently, we don't have a specific target for AML, at least at our center. I believe there is one center, maybe City of Hope, that does have a CAR T cell for AML, but so far it's not been widely available. Then, also there is new BiTE studies coming out by specific T-cell engager. Since the gemtuzumab has shown to have value as an antibody in AML, against antibodies in AML, the BiTE has potential also to harness some polyclonal cytotoxic T cell to lyse tumor cells. There was an AMG-330 drug that's on clinical trial right now, and the target is CD33 and CD3. It could be potentially effective in destroying CD33-positive human AML cells. We will see how that goes.

A couple other future directions I want to mention that I didn't get up on the slides. In regards to future directions, minimal residual disease is a pretty hot topic. The use of different PCR and use of flow cytometry certainly has a role in modifying what post-remission therapy patients might get if there is MRD. At our center, we are hoping to open up a trial using gemtuzumab in the MRD setting.

We also have a couple other currently open therapies for MRD. There is quite a bit of interest in personalized individual customized therapies for patients. There is the Beat AML trial that's run by the Leukemia and Lymphoma Society, and I think other centers have individual studies as well. Dr. Becker at our center has something that we call internally as "pick a drug" where she takes a sample of patients' AML cells and puts it on an assay of about 50 different anticancer drugs in general and sees what therapies could potentially be good to kill of that person's leukemia presence. It is a little difficult, the fact of insurance clearance, so if you find a drug on that panel that might look like it could be really effective, the chance of getting that can be difficult through insurance and cost prohibitive without insurance.

MS. TRAN I remember one of our patients who had failed so many different lines of therapy and the one drug that worked was bortezomib, and the insurance absolutely would not approve it, but it was based on the assay, that one showed significant ability.

MS. GARDNER I think we will see more of this in the future as well. There was a nice talk by Dr. von Gunten yesterday about palliative care, and that wraps in the idea of supportive care, which has really been one of the biggest reasons we have better outcomes now than in 1973, including the use of posaconazole or antifungals during times of significant myelosuppression. But there are also other things that are coming out. There is a company called Nohla, which stands for non-HLA matched progenitor cells. We have a clinical trial using it after intensive chemotherapy to see if we can abate some of the infectious

complications after. That's another thing we will see in our center, and actually there are another six of eight centers across the US that are running this trial.

We couldn't leave without talking about APL, acute promyelocytic leukemia. It represents about 10% of new AML cases in the year. This was pretty much a very high mortality rate, even in the first week of starting therapy, and that has changed now with the use of ATRA, all trans-retinoic acid, which is a vitamin A derivative, and the use of arsenic trioxide. Patients can now, especially if they are not high risk, if the have a lower white cell count under 10, can be potentially cured with non-chemotherapy agents alone. APL's hallmark is the translocation of 15;17, and the biggest complication is coagulopathy and bleeding at presentation. This study was published last year using ATRA and arsenic as a superior to chemotherapy in patients with APL, and you just don't see survival curves like this in most of the myeloid malignancy areas where it's right up into the 90-something percent.

We would like to thank everyone at JADPRO. This has been really a lovely experience getting ready to do this talk, very organized. Thank you all for coming and all of our preceptors who have taught us along the way.

MS. TRAN This has been a real exciting experience. I don't very often give presentations to such large groups so my heart has been pounding for the last 5 months. Thank you so much for being here. Thank you for your attention.

QUESTIONER I have a question. Kelda, do you mind elaborating a little bit on exactly what equates MRD? Is it like when the BCR-ABL is

undetectable, or if the *FLT3* mutation is now gone. Kind you go into more detail about what exactly is MRD for the patient?

MS. GARDNER Traditionally it has been use of morphology greater than 5% in the marrow, but at our center we are highly suspicious if it is anything. If the FLT3 is still positive, the NPM1 is still positive, CEBPA is still positive, then it is very curious that we would consider it. Our hands get a bit tied with being able to treat with that just because so many protocols require it be greater than 5% blasts, often by morphology in the marrow, and that really limits what we can treat with. But at our center, if someone was still FLT3 positive after initial induction, that may be a consideration of really trying to push more towards a transplant or more additional high-dose therapy. It is a hot topic right now for sure. I wish we could almost say anything that's there, let's make more therapy available, but we are not quite there yet with our knowledge.

MS. TRAN Even though I think a lot of us practice with MRD as kind of a standard of care, it is not quite fully accepted yet. If you read the NCCN guidelines, they don't recommend MRD in AML; however, if you look at ALL, you see that MRD is a crucial part of treatment.

QUESTIONER I was wondering if you could tell us more about that assay you said that could look at and tell exactly what the patient might respond to.

MS. TRAN Dr. Becker's assay.

MS. GARDNER Oh, Dr. Becker's assay. She has an oral presentation on this at ASH coming up next month, so I don't know how much I can say about

it, but she isolates about 5 million cells, it's what's required to take a look at the assay. She will take it off peripheral blood if there are enough cells, otherwise it requires bone marrow aspirate to do it. It's under a protocol, so the patient signs an informed consent to have this done. Then she takes the sample into her lab and within an average of 5 to 7 days from the sample arriving to coming up with therapies that could be potentially beneficial to the patient. I don't know off the top of my head the panel of 50 some different probes on her assay, how many often tend to be higher, but then she will take a look at the ones that are in the higher ranges and see if any of those therapies are potentially viable for using for the patient. It's interesting work.

QUESTIONER How often are you testing the MRD? Is there a frequency or a time span after they have received the treatment? Like when the counts are recovered, do you do it immediately? Are you running it on day 14 or 21 with your GCLAM?

MS. GARDNER That's a great question. When we do the recovery marrow, which is sometimes day 28, sometimes day 35 or 40, we might give them a little more time to recover their counts, which we hope would mean recovery of blood counts meaning a healthier marrow is coming in. Ideally, we would run whatever was positive again, and that's something our center is looking at, and actually a project that is on my list where I am going through all these patients and seeing if they were NPM1 to begin with were they retested. It's something that you are going to see more of a push to make sure this is done. At least, we have been talking about it, but ideally all the time there was

something positive, we would recheck for it. It is also, sometimes patients come from a different center, or they get flown in from Alaska and their marrow was done there, and they come and we confirm disease, they start therapy, and then we realize later that some of those molecular studies hadn't been done, or certain things weren't complete, then we are not able to really—unless there is a lot of disease burden—figure out what was there to begin with.

MODERATOR Thanks Melinda and Kelda for a wonderful talk. This is a Smartie presentation, so be on the lookout for your email. Thank you and have a wonderful day.

[END]