ManagingAML

Molecular Abnormalities in AML

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It is real honor to be here tonight and certainly put into context some of our recent findings in terms of what we are learning from molecular characterization of patients with AML and how we can slowly start to interpret them and most importantly translate them into clinical practice. So, I need to learn how to use this first. So, this is what I am going to touch on today, and it very much stems on the concept that the cancer genome and the mutations found in the tumor of every patient essentially dictates the tumor's biology and that in turn determines the clinical presentation and the overall clinical course and treatment response of the disease, and this is very much the premise upon which now that benchtop molecular profiling technologies in the clinic are becoming more and more routine across the world. We want to set up a platform upon which we can learn from those mutations and deliver treatment decisions that are tailored to the individual patient, and one of the promises comes from early successes in leukemia where we assume that we are going to identify the one mutation in a patient for which we are going to have the one drug for which we will be able to treat them and ultimately cure them. But we know that this narrative is much more complicated because cancer is never the consequence of one mutation, but most importantly, every cancer patient or leukemia patient at presentation has had a natural history that has resulted from an early initiating event that led to a first clonal expansion and mutation that is present in older cells and that one cooperates with additional events, secondary and tertiary events that ultimately dictate disease progression, clonal diversification, treatment response, and ultimately treatment resistance.

So, how does that lie together? Now that we are completing cancer genome sequencing profiling across most tumor types, we learn that most patients have three or five main driver mutations that they can be present in different subsets of the cells, some in older cells, some in smaller subsets of the cells. So, how would that tie in and translate with the precision medicine paradigm that we are envisioning or dreaming of where we are hoping to have the one mutation that we will treat. And bringing this into context of the clinical challenges that we are facing now in AML, AML treatment has not significantly changed over the past decade, although we are now seeing a significant surge of potential therapeutic options. It is an aggressive disease, and in standard of care, we use standard demographic and bone marrow morphology and peripheral blood counts. We are more recently tying this with karyotype data to make specific clinical decisions. It is an acute disease. We need to treat fast, and we need to consider how what we are learning and how quickly we can deliver molecular profiling data can be incorporated within clinical practice. So, the early characterization of the genomic profiles of AML have been instrumental in supporting those decisions, and this has happened from the early identification of cytogenetic abnormalities that were recurrent, and one thing that was very specific about the

cytogenetic abnormalities that they are most frequently mutually exclusive. They separate AML patients into distinct groups, and we have learned over the years that these cytogenetic subgroups also segregate with very distinct clinical outcomes, and these have enabled, over the years, refinement of both diagnostic classification and prognostication algorithms that define patients into favorable or adverse or unknown risk categories upon which we can base clinical decisions such as where do we need to intensify chemotherapy, who do we need to transplant at first CR, who do we need to transplant post relapse. These have been very much the mainstay of both diagnosing and prognosing patients, supporting clinical decisions along the way, and we have also seen the characterization of those molecular abnormalities resulting into very effective therapeutic intervention protocols. The delineation through a combination of parameters of these patients into risk groups has been very successful in one part, but on the other hand, it has been also challenging because variable clinical response, as you see here, we have separated patients between the four previous risk categories in AML and we looked how the top performers within each category perform, and you can see that the worst performers within the favorable group do pretty much as bad as patients that fall within the adverse risk group. So, while we are refining molecular risk groups to support these clinical decisions, we know that there is a lot more work that needs to be done. We have patients with favorable risk performing really well, and others that do very poorly.

So, how can we refine and improve this characterization of those patients so that we can deliver the best treatment decisions for those? So, this is a summary of how the clinical picture of AML looks. These are patients from three clinical trials from the AML-SG group in Europe, and we started with 1540 patients and you see that just over 1200 patients will achieve complete remission, but then 600 of those patients will relapse, and only 140 patients would be salvaged after relapse. How then we take each one of the risk groups and try and determine specific patients route along those different stages of the disease can be quite challenging, and also how with emerging new therapeutics we can put this into context. So, particularly, more recently, we have uncovered many more molecular alterations that through the TCGA effort there were 200 recurrently mutated genes in AML, and one thing that was immediately noticeable in this case is that contrary to the cytogenetic abnormalities, which you see on the left of the panel which are mutually exclusive, patients with gene mutations tend to have a lot of those gene mutations. These mutations like to hang out together. We are not defining discrete and non-overlapping molecular subgroups, and they seem to affect a number of pathways that implicate both transcriptional regulation, epigenetic modifiers, chromatin regulators, and more recently, the spliceosome machinery. So, a lot of new pathways that were previously unrecognized in cancer are becoming the mainstay of the interest of their abnormalities in AML and how we can incorporate them into both diagnostic prognostication, most importantly therapeutic protocols, is with this complexity and heterogeneities becoming increasingly complex. So, we have more than 100 recurrently mutated genes, many genes per patients, and also very diverse prognostic relationships emerging from each one of those genes. So, quite a lot of the work that we have been doing over the past years is to take large population studies and try and uniformly profile patients with treatment and clinical outcome annotations to try and understand what are the genomic interrelationships that define the backbone of AML, and most importantly how we can then learn from them to try and build clinical algorithms both for diagnoses as well as clinical decisions.

So, we have recently taken those 1540 patients for which we had cytogenetic data, peripheral blood counts, clinical outcome, treatment data, and we performed deep targeted resequencing for the 100 or so most frequently mutated genes in AML. These let us identify 5234 mutations. What is quite critical here is that for a disease that is predominantly stratified on the basis of cytogenetic abnormalities, mutations in genes are accounted for 75% of the overall mutation burden seen in AML patients. What we saw was cytogenetic abnormalities also accounted for just over 50% of the patients. We found at least one abnormality, one in 97% of the cases and two or more in 86% of the cases, and this suggests that we now have at least one if not more biomarkers for pretty much every patient that comes in to the clinic. So, how can we use this information? We performed a statistical and supervised analysis to see whether these gene mutations, if we consider secondary and tertiary genetic groups interactions, segregate distinct and non-overlapping groups. Remember, the TCGA study of 200 or so cases could not really delineate distinct molecular groups. There was a very heterogenous landscape, but with the analysis of 1540 patients, we have significantly more power to study both patterns of cooperativity as well as mutual exclusivity. This led us to characterize 11 non-overlapping molecular subgroups. We validated many of the previous and very well-recognized cytogenetic groups. We validated two provisional categories, one AML defined by NPM1 mutations as well as C/EBP alpha balletic mutations, but most importantly, our analysis identified three distinct molecular subgroups. One which overlaps very much with one of complex karyotype or monosomies, but here it includes in general chromosomal aneuploidies and TP53 mutations which was a very distinct group. The second which in fact was the second largest group in AML was characterized by mutations in the chromatin and spliceosome machinery, and another group defined by IDH2 mutations in a particular codon. This has now been validated in an extra set of 3000 AML patients from the UK MRC trials. Here I present to you the 11 groups where every column represents a patient and every row represents one of the lesions. The advantage of next-generation sequencing data is that we can use very intelligent fraction matrix to estimate the proportion of cells that carry each one of those mutations. If you remember the early map that I showed of the first, second cooperative events that lead to disease overall in cancer, we were able to ask, can we see similar patterns in AML? Within those groups, can we identify which gene is mutated first, which mutation comes second, and which mutations come third? And quite strikingly, what we saw here is that amongst 1540 patients, we identified 1060 distinct genotypes, but those 1060 distinct genotypes segregate in 11 common themes, those 11 molecular subgroups, and each molecular subgroup in turn seemed to have a very consistent pattern of which genes were mutated early, like the founder clones, the genes that generate the founder clone, the secondary, and the late events. This can become very important as we are thinking the clinical management of AML whether we want to treat the late lesions, the early lesions, or the combination of the two lesions that come together most frequently. And this is schematic that represents while we consider AML as one disease, we are now learning that there are distinct paths that can lead to AML. Each of the paths can invoke a very distinct pathway, and we can now learn what are the critical nodes of each one of those pathways that we can both learn to use, both for disease surveillance protocols, but most importantly therapeutic protocols.

So, beyond the biological significance of those molecular groups, we wanted to test whether they were clinically relevant, and indeed, each one of those molecular subgroups segregated distinct clinical outcomes and this is published data. So, if you want to digest it in more detail, please do. You can find it available. So, now, whereas previously we could categorize 50% of

AML patients in a risk group, now 85% of the patients are accounted by one molecular subgroup for which we have recognized very distinct clinical overall survival outcomes. Beyond overall survival, we have here asked the question. We have this bar plots where we indicate on the top bar the proportion of patients within the group that achieved complete remission and on the bottom bars, the lower bars, the proportion of patients that relapsed in light blue that was alive after relapse or alive without relapse at last follow up. What we found was that we now are seeing these common themes emerging for each one of those subgroups beyond overall survival as well as in the patterns in which patients might achieve complete remission in the first instance, the proportion of patients within each group that might relapse or not, and then the proportion of patients that will be alive within 3 or 5 years. Bringing it into more context of novel and emerging therapies that we are very interested in, and we are going to hear a lot more about today, I took the review from Ross Levine, which indicates potential gene markers that are currently in either phase 1 to phase 3 clinical trials or that result in stratification of patients in clinical trials, and I asked how many patients have at least one, and this is like the upper level of what could be considered in the clinic and an optimistic level, but how many patients have at least one mutation that could stratify them if the worlds of clinical trial was available to them, how many have two and how many have three? I was actually pretty stricken by the results in that, in this overestimate of potential targets that we could explore clinically, we had 74% of the patients that had at least one that could be a FLT3 mutation and IDH mutation, 45 that have at least two, and 16% that have three or more. This is important because we need to start, if there are consistent patterns in which these mutations come together, perhaps we can start using this information on both how we interpret outcomes from clinical trials, but also how we might in the future rationalize combination therapies. So, this is the representation of each one of the molecular subgroups, just to give you a flavor how the commutation and the overall composition within the group is important and how we can use this in the NPM1 context. We see that 75% of patients are mutated in one of the genes affected in DNA hydroxy methylation machinery or receptor tyrosine kinase pathways or GTPase of which there are numerous targets both through IDH inhibitors or FLT3 inhibitors or RAS inhibitors that we could consider for that subgroup. If we were to subset the patients that carry these alterations, and while NPM1 is considered a favorable prognostic group, here we see that patients with NRAS mutations, particularly codon 12, seem to have consistently good outcomes in accordance with what we know and expect from NPM1 mutations. However, patients that have mutations in IDH, one of the IDH genes, show increased refractory disease in patients that have the combination of DNMT3A and FLT3-ITD, have increased refractory disease and increased relapse rates. Clearly, the numbers of each of those subsets are small, but we can now start validating these and learn which ones of the subgroups, as we are doing with FLT3 for example in the NPM1 context, we can stratify and consider additional therapeutic modalities.

I would like to draw the attention to the chromatin spliceosome group which accounted for 18% of the patients in our cohort. So, this was the second largest molecular group. This group performed really poorly, has very poor survival. The majority of those patients would be currently considered as intermediate-risk AML that present with de novo disease, they are generally older with a median age of 58 years old, and they are associated with poor survival and a high relapse-related mortality, and less than 20% were alive at the last follow up. So, this group is one that we immediately recognized as a novel or high-risk group in AML, and there has been a lot of population genomic studies to show that there is a number of gene mutations within the spliceosome machinery that determined the backbone of this disease, and these

mutations are mutually exclusive. This observation has recently led us to postulate that two mutations within the spliceosome machinery are probably not viable within the cell, and this has led to the development of spliceosome complex inhibitors that are currently being trialed for both MDS and AML because there are recurrent molecular abnormalities in both diseases. These are showing early promising results, and here, we see the on-target effects of two of the agents that are currently underinvestigated, an SF3B1 mutated context as well as the preferential effects that they have on splicing factor mutated CMML in this context which you see in patients with the blue line and the same is here where they have a very specific and selective effect in reducing the overall blast count in F3B1 and splicing factor mutated CMML. So, we are going to hear a lot more about IDH mutations and FLT3 mutations, but we are all startled by the fact that we are dealing with hundreds of molecular alterations that we need to learn and incorporate within our clinical algorithms. But with population studies, we can now learn how these gene mutations come together, how they define distinct clinical groups, how mutations and key pathways that we can target come together, they help us rationalize potential clinical trial protocols, and while we may have 1500 individual patients, we are now recognizing more and more distinct molecular and clinical subgroups that we can deliver more personalized or group-tailored therapy that is more relevant to the biology of the disease. This is an example whereby joining forces through collaborative efforts we can learn from a large proportion of AML patients to individually treat each of the patients and how we are thinking and learning as we are expanding the studies of incorporating the molecular markers and the pathways into logical algorithms upon which we can interpret. So, I would like to thank all of my collaborators and the funding bodies and everyone in the lab. It is impossible to just mention everyone, particularly all the physician scientists that enroll patients in trials and then submit samples and clinical information into such population studies because without this effort it would be impossible to extract messages that we can then bring back into every individual clinic. So, thank you very much.