Liquid Biopsies in Oncology Drug and Device Development Part 2
Transcript: Session III, Liquid Biopsies in Cancer Drug Development and Clinical Use

Pasi Janne:

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We're gonna get started with the afternoon session, which will focus on liquid biopsies and cancer drug development in clinical use. We have fours speakers in the session including myself, I'll give a small overview of where things are in lung cancer. Then we'll hear from Gideon Blumenthal from the FDA, David Hyman from Memorial Sloan Kettering, and Scott Kopetz from MD Anderson on different aspects of using liquid biopsies in guiding drug development. So, with that I will move on to the presentation.

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These are my disclosures. So, I wanted to give a few highlights about lung cancer and you've heard some of these from the prior speakers as to where one would potentially use liquid biopsies. Of course, there's the non-invasive diagnostic tool. Studying the evolution of resistance. And, ultimately, also as potentially a pharmacogenomic tool to guide drug development.

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Now, you heard from some of the prior speakers about using Droplet Digital PCR. This is something that our group has worked on for several years, and really a very sense that a quantitative assay is quite useful in terms of many of the topics that we talked about this morning in terms of monitoring and looking at the different evolutions of clones.

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In lung cancer, because you have genotypes that are mutually exclusive with one another, so when you have an EGFR mutation you don't have a KRAS mutation, and vice-versa. It allowed us initially, to be able to develop this assay in such a way that we would use the contrasting genotypes as a way to normalize the assay. So, meaning that if you have a tumor that has a KRAS mutation and you find an EGFR mutation in the blood of that individual, that's likely a false-positive, not a true-positive, and vice-versa. So, it allows you to refine the analytical range of the assay.

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We next validated this assay in a prospective study. We took patients who were newly diagnosed or newly progressing. This was a study led by Adrian Sacher and Jeff Oxendine Institution where we would take patients who are going to be genotype, take blood in a prospective manner and ask how good of a predictor of the tumor genotype was blood. What you could see is that in fact it was quite good and the positive-predictive value for EGFR mutations and KRAS mutations was quite high.

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Sensitivity of cfDNA genotyping by ddPCR goes up with tumor burden. So, the more metastatic sites you have, the more likely you are to find a mutation. In lung cancer, we often have patients who have chest-only disease. So, technically have Stage 4 lung cancer, but have disease in the chest only. Those tend not to be patients who shed cfDNA or shed at a much lower rate. You can see the sensitivities when you look for these different genotypes, that they range somewhere in around the seventy percent ballpark. The one exception, and I'll

get to this in a bit, is the EGFR T790M assay and this is due to clonal heterogeneity. It's not always present in every single tumor biopsy of the patient who develops resistance to an EGFR-inhibitor, which accounts for some of these differences.

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Also, in this same study we were able to demonstrate that this is much faster for the patients. So, the median turnaround time for a patient to be able to get a result is three days, whereas, tissue genotyping varies anywhere from twelve to twenty-seven days or longer depending on what kind of assay you're using for tissue-based genotypinG, be it an allele-typing assay or NGS, which tends to add time. Again, this one example can show that if you start therapy on a patient it can be effective and get to that patient much quicker when you need to make that therapeutic decision. Sometimes, especially in patients who are progressing on prior therapy, waiting around for two to three weeks to get an NGS-based result can be clinically tough. Patients may not be able to wait around for such a long period of time.

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Now, in the context of the osimertinib phase one clinical trial program, both patients who were T790M-positive and T790M-negative were treated, and plasma was collected in these patients. This paper that Jeff from our group published last year ... We're able to look at the predictive- value of plasma-based genotyping in tumor-based genotyping. So, here patients that are tumor-positive for T790M, their response rate to osimertinib at sixty-two percent and a PFS of ten months. Exactly, the same predictive-value in individuals who are plasma-positive in terms of response rate and PFS. Now, the interesting thing is that these are not overlapping or hundred percent overlapping patients and in some instances you have some patients whose tumor is negative and yet their plasma is T790M-positive.

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Here are those individuals ... I think one of the things we're learning is from some of these assays and the plasma-based assays is that they really do provide some complementarity to those individuals. So, here are patients who are plasma T790M-negative, so, presumably the patients that don't shed. If you now go back and look at their tumors, if someone has a plasma-negative but tumor-positive, they do exceedingly well as you'd expect as this is likely an individual who doesn't shed but still has T790M-positive disease. Again, the point being that these are good tests when we find the thing that we're looking for. In this case, a mutation where we can act upon with a drug. But, if you don't find it, it doesn't always mean that it's not there. Again, most of these tests hover in the seventy to eighty percent sensitivity. The tumor is always a backup and shouldn't necessarily be overlooked in these circumstances.

Now, there are a few clinical applications that are trying to look at plasma-based genotyping prospectively. In terms of choosing therapy this is a trial that's being run in our institution where we take patients who are sort of clinically-enriched for potentially having an EGFR-mutation. So, never smokers, patients of asian ethnicity, they're consented, they're blood is geno typed for an EGFR-mutation. If their tumor or if their plasma is positive for an EGFR-mutation, then they're

[00:06:30] treated prospectively in a phase two trial of an EGFR-inhibitor, in this case erlotinib. If they're negative, they're not treated. The idea, really, is to prospectively be able to demonstrate that the predictive-value in terms of response to therapy is similar, as it would be if you chose individuals based solely on tumor-based genotyping.

[00:07:00] Some other clinical trials ... This is a clinical trial run by [inaudible 00:06:51] Therapeutics on a MET inhibitor. This is focused on patients that have MET exon 14 deletions as well as MET amplification. There are essentially two cohorts, there are tissue-based cohorts as well as ctDNA-based cohorts here. All of them analyzed individually to be able to look at, again, the correlation between ctDNA-detected MET amplification compared to tumor-detected MET amplification and are they similar or different to one another. I think these kinds of prospective studies really help us further solidify the predictive-value of using cfDNA-based genotyping.

[00:07:30] Now, we talked about in the prior presentation, sort of, the monitoring of drug resistance. Here, an example of, again, monitoring the evolution of an EGFR-mutation, that resistance mutation, here are the lead-time bias between actual clinical progression and being able to detect the T790M-mutations, only a few months. I'm not sure that this is clinically something that is actionable at this juncture. Perhaps, it helps you guide what you end up doing next for the patient

[00:08:00] as opposed to switching the patient's therapy if you detect it just a few months prior to that. We don't have endless therapies and no curative therapies in lung cancer. So, I think ... At lease, I don't personally switch anyone until they have true clinical progression with using guidance from what you find in cfDNA.

[00:08:30] Now, in a study that we did with Ken Thress and the group from AstraZenica, looking at patients who had progressed on osimertinib therapy. This was an example where we were able to identify new resistance mutations, the C797S resistance mutation in somebody who'd progressed on osimertinib after having an initial response. This then gave us the opportunity to start looking at how do tumors evolve in response to osimertinib therapy, again, using the ddPCR assay. So, these are all patients that are T790M-positive. All were treated with osimertinib.

[00:09:00] But, they have sort of three different fates that happened during the course of therapy. I think you get insights to that by studying what happened their cfDNA's. So, here we're looking at the original EGFR-mutation in blue, the EGFR T790M-mutation in red, the C797S-mutation. These are patients that start out with plasma-detectable EGFR activating mutation, T790M-mutation, and then get treated with a drug. They go down, only to reappear and then now along with the C797S-mutation, there's another patient. In the middle, our examples of

[00:09:30] individuals where both mutations go down, they come up and the C797S-mutation doesn't come up, presumably because there's some other resistance mechanism that we're not capturing by the ddPCR assay.

[00:10:00] This is the one that I think piqued out interest the most because here what we're seeing is patients start out with the T790M and an EGFR activating mutation. The T790M-mutation plummets and the EGFR activating-mutation has its transient dip and come up. Here, more than likely, the T790M was in fact a sub-clone of that resistant tumor. The resistant tumor probably had other resistance mechanisms and we've subsequently been able to show that, that is indeed the case and that you've treated the sub-clone. But, again, the serial monitoring gives you insight into the fates of these individuals and it turns out that this group of individuals, in fact, does quite poorly as you could imagine because they have a sub-clone and your only treating part of the resistant disease with an effective drug. I think, analyses like these will hopefully continue to be useful in clinical guidance.

[00:10:30] Of course, with NGS you can get multiple different types of resistance mechanisms simultaneously. Here's an example of an ALC-resistant patient. You have multiple resistance mutations simultaneously. This is done by the [inaudible 00:10:37] NGS assay. Multiple resistance mutation simultaneous. Here's a patient, where you can see again, the evolution of resistance. Different EGFR-mutations. Originally, EGFR-mutation, the T790M-mutation, the C797S-mutation. Again, you can see how they appear or disappear in response to therapy. So, for

[00:11:00] here, example, under osimertinib-selection you see a selection for the C797S-mutation and when the patients taken off of that you can see that this clone starts to disappear over time.

Now, finally, let me just touch on another final topic on this before we move on to the other presenters. That is the idea of sort of, thinking about the use of cfDNA pharmacodynamic marker. The quantitative nature as you heard from the prior presenter is really ideally suited to follow disease-burden following

[00:11:30] treatment. You can ask the [inaudible 00:11:28]. You can ask whether there's a benefit in patients, will you lose the detection of cfDNA and also potentially in a use as a dose-finding opportunity in early-stage clinical trials?

So, again, in that same prospective study that we looked at, we looked at patients different outcomes of patients with their cfDNA. So, here are patients ... Again, it's sort of a variety of patients who have detectable cfDNA and following whatever treatment they're getting, they either had undetectable cfDNA at two weeks or by six weeks with a different set of kinetics. Compared to these groups

[00:12:00] of patients who had, sort of, really no effect or sort of, a transient effect or bounce-back. If you looked here in patients who had, sort of, complete resolution of plasma-cfDNA and asked what fraction of those patients discontinued their therapy, it was very little compared to here there is a much greater fraction of individuals who discontinued their therapy. This is across different kinds of treatments and not a uniform treatment. But, I think, it nevertheless, gives you the sense that this could be potentially a marker clearance of cfDNA ... Could potentially be a marker that could be used in future studies.

[00:12:30]   In this past years ASCO, Ken Thress with AstraZenica presented data looking at plasma clearance of EGFR-mutations in patients who have been treated with osimertinib. So, here a same finding that if, in fact, at six weeks you cleared your cfDNA, you had a much longer progression-free survival than if you retained the presence of detectable cfDNA at six weeks. You can see here the difference in

[00:13:00]   PFS 10.8 versus 4.2 months and the response rate difference highly statistically significant. So, very early marker of someone who's likely to not do well long-term. Again, whether we can use these kinds of assays in future clinical trial design to potentially add a second agent or do a more intensive monitoring or whatever in the patients that don't clear their cfDNA, that may be an appropriate strategy. In addition, you can ask, what fraction of the patients don't clear their

[00:13:30]   cfDNA and if you now had, for example, a combination therapy and you have a lower fraction of patients that don't clear their cfDNA, does that ultimately translate into better outcome for that population. So, again, you can potentially, start thinking about using these types of assays in this sort of context.

In just the final example, presented by Geoff Shapiro and [inaudible 00:13:44] at the AACR this year, looking at KRAS allelic burden in a phase one clinical trial of combining palbociclib and the [inaudible 00:13:51] MEK inhibitor with increasing doses. Here we're looking at the delta of the cfDNA compared to screening. So,

[00:14:00]   we're looking at the screening level of cfDNA and asking the change in the allelic burden following treatment. You do get a sense that as you get into higher doses of the drug you're starting to see more decrements in cfDNA clearance compared to [inaudible 00:14:11] and cfDNA compared to the earlier time points suggesting that there is a PEDI effect that potentially could be exploited in future such studies moving forward.

These are just the acknowledgements of the people who contributed to this

[00:14:30]   work as I highlighted them. With that background just wanted to then move on to the next presenters in the session. You'll hear from Gideon Blumenthal from the FDA on biomarkers and surrogate endpoints From David Hyman look at cfDNA-based genotyping for trial enrollment and from Scott Kopetz at MD Anderson for use of cfDNA to guide the care of colorectal cancer patients. WE won't take any questions during the presentations. Once all the speakers have

[00:15:00]   done their presentations, like with the prior session, we have about forty minutes for a panel discussion at the end. With that, I'd like to invite Gideon to come up and give his presentation.

Gideon   Thanks, Pasi. It's great to be in the session with the coveted post-prandial
Blumenthal: session, where everyone's sort of in their post-lunch food coma. But, we'll try to
             ... Never too exciting, the regulatory talk, but we'll try to spice it up. So, when we
[00:15:30]   were brainstorming on this workshop, Pasi actually made the suggestion, that the science is fascinating surrounding liquid biopsy but we need to ... Workshop number two, we need to get down to brass tacks and figure out ...
             ... my talk will help to enlighten us all. These are some of the key points. We're gonna get back into basic definitions and types of biomarkers that we use in oncology drug development. We're gonna talk about examples of validated surrogate endpoints in drug development. I use the word validate in quotes, I got

[00:00:30] this from our center director Dr. Woodcock, who doesn't love the word validation because it suggests sort of a binary on/off process. Whereas, biomarker qualification, or validation, substantiation occurs along a continuum. Then we'll talk about methods to validate biomarkers, and I'd be remiss if I gave an FDA talk and didn't give you opportunities to interact with the agency 'cause that's ... usually the bottom line is come talk to us.

[00:01:00] Basic definitions and types of biomarkers. This is from the NIH FDA best harmonization project, where a lot of times these definitions get bandied about, so this is a very useful resource for your reference. A biomarker is a measure measured as an indicator of a biologic pathogenic processes, response to
[00:01:30] exposures, or interventions. You can use molecular histologic radiographic or physiologic characteristics. Endpoints are precisely defined variables intended to address a particular research question, and surrogate endpoint is defined by the best criteria as an endpoint that is used in clinical trials as a substitute for a direct measure of how a patient feels, functions, or survives could predict, benefit, or harm based on EPI, therapeutic, pathophysiology, or other evidence.

[00:02:00] I want to just go into different regulatory definitions of surrogate endpoints. The language we use are surrogate endpoints that are reasonably likely to predict clinical benefit, versus established surrogates. For accelerated approval, we
[00:02:30] except surrogate endpoints reasonably likely to predict clinical benefit. These are for serious conditions with unmet medical needs, such as cancer, for a drug has to show that it's better than available therapy. Typically, we require confirmatory studies to confirm clinical benefit. We've used this approval pathway frequently in oncology. In fact, Dr. Beaver sitting here in the front row, has a publication in
[00:03:00] press, which shows that we've used it over 90 times in oncology since 1992 in the sub part H regulations, when it was instituted really for HIV.

The other point is that it's very rare for clinical benefit to fail to be confirmed. There are a few cased. Think Avastin and metastatic breast cancer, but very rare.
[00:03:30] Even in those instances, a lot of times those drugs do get resuscitated, such as think about Iressa and EGFR mutant lung cancer, when the proper patient population was found. Or recently Mylotarg and AML when the dose was optimized.

In contrast to accelerated approval there's regular approval. These are approvals based on established surrogates. Think about hyper blood pressure for antihypertensives, or improvements in clinical benefit endpoints, such as how a patient feels, functions, or survives.

[00:04:00] These are just some of the types of biomarkers we look at in oncology. You've heard a lot today on the four in the box monitoring, biomarkers, prognostic, predictive, and pharmacodynamic, and response biomarkers.

As far as biomarkers we use frequently in oncology. These include prognostic
[00:04:30] biomarkers. One example would be the MammaPrint 70-gene Assay in breast cancer. Or, as we've heard today, potentially using cfDNA MRD assessment.

These are useful for finding your higher risk population more likely to recur, so using enriched patient population, or potentially even stratifying your clinical trial based on these parameters to ensure that there's balance between control arms.

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Oftentimes we use predictive or selection biomarkers. We're seeing more and more of these actionable targets. Think EGFR, BRAF Ross 1 in non-small cell lung cancer. Hurt 2, ERPR in breast cancer, IDH2, C-kit, PDL1, and most recently we had our first histology or site agnostic approval for a PD1 inhibitor for MSI high refractory solid tumors. These biomarkers frequently require contemporaneous approval of companion diagnostics, if they're essential for the safe and effective use. You have to see Dr. Phillip from CDRH to talk about co-development of the companion diagnostic.

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For the rest of the talk, I'm gonna focus on pharmacodynamic, or response biomarkers, as Posse nicely illustrated with some of the examples. These are used to assess proof of concept or target engagement, and the learning phase can be useful for dose finding, as well. They can be used as surrogate endpoints both for go no-go decision-making, or for approval, either accelerated or regular, based on the context.

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Just some of the general factors to consider in assessing response biomarkers. Something that we look at often are the risk introduced by its use. For example if you have a patient with CML and you're using a novel biomarker to decide whether to stop [inaudible 00:06:39] therapy, a highly active therapy, that would be a high risk device, so we would take a close look at the analytic and clinical performance. Also other factors include the biologic rationale and understanding of its role in the disease or causal pathway, if it's highly influenced in the causal pathway, versus more of a peripheral or secondary effect. Then, of course, assay considerations, both analytic and pre-analytic, but I don't want to steal Rena's thunder, so stay tuned for session four to hear more about that.

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I was gonna touch upon two examples of validated surrogate endpoints in drug development. The first being HIV RNA as a validated surrogate. This is a paper that one of HIV colleagues at FDA shared with me as we were writing a perspective piece on lessons learned from the HIV experience to cancer. This is from 1999. Prior to 1997, the approval endpoints for HIV medications were either death, mortality, or opportunistic infections.

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Through the intensive work in validating the surrogate endpoint of HIV RNA clearance, through great cooperation amongst various stakeholders, after 1997 all HIV drugs were approved based on HIV RNA clearance. An early time point 24 week HIV RNA as an accelerated approval endpoint. Then, a later time to show durability, 48 weeks of HIV RNA clearance for regular or full approval. This has been a highly successful paradigm as combination antiretroviral therapy has transformed life expectancy to match that of age match controls in this disease.

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The other example, as mentioned earlier today, is BCR/ABL PCR, and CML just highlighted to critical papers. Again this was a function of great work by multiple stakeholders, in terms of standardizing and harmonizing the assays, coming to consensus. This first paper from 2003 indicated that in the pivotal [inaudible 00:09:21] versus [Interferon RC 00:09:22] trial, it appeared that 12 month major molecular response correlated with progression-free survival. This was confirmed with a publication in 2010, where longer-term outcomes from the same [inaudible 00:09:40] study confirmed that clearance of BCR/ABL PCR was prognostic.

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This was subsequently used in drug development. This was pulled directly from the Dasatinib and Nilotinib labels, where 12 month MMR was used as an approval endpoint for accelerated approval for both drugs in randomized controlled trials versus Imatinib. Then a 60 month MMR was used as the regular approval endpoint to confirm clinical benefit.

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I also wanted to touch upon methods to evaluate a candidate's surrogate endpoint. There are meta analyses techniques, I'm not a statistician, I don't play one on TV, but I have been involved in some meta analyses, but I rely heavily on my statistical colleagues. There are two different analyses you can do, or you should do. One would be the individual or patient level. We've seen [Olla 00:10:44] mentioned this with respect to MRD in Myeloma.

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This measures the association between a candidate surrogate and the clinical endpoint on the individual level. As you can see here that MRD negative patients do better that MRD positive patients, in terms of both PFS and OS. Then, as Olla also mentioned this morning, there are landmark approaches at sensitivity analyses to exclude those rapid progressors.

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In addition, there are also trial level surrogacy analyses that can be conducted. This measures the association of the effect on a surrogate with the clinical endpoints between treatment arms within the confines of a randomized controlled trials. This is an example of a publication that we did in JCO in 2015, looking at trial level meta analysis of response rates, odds ratio versus PFS hazard ratio, and overall survival hazard ratio. The bubbles signify the size of the clinical trial. We saw actually a pretty strong correlation between PFS hazard ratio and response rate odds ratio, but less of a correlation when we looked at either response rate and overall survival, or PFS in overall survival.

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There are some caveats to these analyses, particularly when looking at overall survival crossover can confound any detectable differences in overall survival, as well as the magnitude of effect you see within the individual trials. If you don't see big effect aisles in the individual trials, you're probably not going to be able to detect associations with these trial level surrogacy analyses.

In addition, it's important to try to determine the surrogate threshold effect, both at the patient level and at the trial level. What magnitude of effect on the surrogate is likely to produce the effect on the longer-term clinical outcome.

[00:13:00]   As far as opportunities to interact with the agency on validating, or substantiating surrogate endpoints. We do have the seeder biomarker qualification program, which can qualify drugs within their context of use as drug development tools. Admittedly, this has not been used frequently in oncology, although there have been examples in other therapeutic areas, and in the pre-

[00:13:30]   clinical space, where biomarkers have been qualified through this process. In addition, there are opportunities through the pre-IND, or IND meeting to develop surrogate endpoints, and opportunities to share data with the agency within the construct of a pre-IND, or and IND meeting.

                Then, lastly there are opportunities and there have been examples of various multi-stakeholder consortia that have formed. One example is the CTNeoBC

[00:14:00]   pooled analysis, looking at pathologic CR for neoadjuvant breast cancer. This was led by Pat Cortazar and published in the Lancet in 2014.

                Just some parting thoughts. Liquid biopsy technology has exciting potential applications for risk stratification, prediction monitoring, potentially for

[00:14:30]   surrogate endpoints for drug development, and for clinical practice decision-making. There have been successful examples of establishing blood based as surrogate endpoints, such as an HIV RNA, and BCR/ABL CML. Although admittedly, these are relatively simple diseases. HIV infection being the lone

[00:15:00]   ideology for AIDS, BCR/ABL CML being a monogenic, single oncogene driver malignancy, and could be much more complex within the context of solid tumors.

                Developing surrogate endpoints requires careful planning, embedding these endpoints. Secondary endpoints into pivotal trials requires data sharing, assay, standardization and a great deal of cooperation amongst the various

[00:15:30]   stakeholders. At FDA, The Oncology Center of Excellence were a committed partner to advance these technologies potentially a surrogate endpoints to foster innovation and more rapidly deliver safe and effective drugs for patients.

                With that, I'll stop. I just wanted to share a picture from Dr. Pazdur's home with some other international regulators. We have a monthly teleconference with other international regulators and we meet with them once or twice a year. Also,

[00:16:00]   a shameless plug for our FDA disco podcast for our drug approvals. Thank you very much.

David Hyman: Thank you. It's always great to give talks at joint sessions of FDA academia and industry, because once the FDA representative is gone, everyone tunes out what else happens, so I'm off the hook.

[00:00:30]   But anyway, thank you very much for inviting me. So I thought I would start out with a slide of just what are we talking about here when we're talking about enrolling patients to clinical trials based on their genomic alterations. We recently did a careful look at that, at our institutional experience using a tumor-based assay. We call it "MSK-IMPACT." This is a tissue-based assay, like I said, the

[00:01:00] sequences, tumor, and match normal, that includes now upwards of 460 genes, but when it started, it was in the 300 range.

When we looked at the initial 5,000 patients that we sequenced, we found that about 38% of that cohort had gone to any therapeutic clinical trial at our center, so kind of establishing a baseline for clinical trial enrollment for therapeutic studies during that time period. And 11% of the patients had gone on to be enrolled on the explicit basis of a genetic alteration being matched to a therapy.

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[00:02:00] I think this kind of establishes a realistic baseline within the context of a center that has a very active clinical-trial portfolio. But equally important is to look at the diversity of alterations that patients are enrolling on. You can see that not only were patients enrolled on the basis of missense variants or single nucleotide variants, but also copy number alterations and gene fusions. Although the gene fusions may be the smallest subset, in many ways we find that they're among the most actionable alterations we see, and I think that plays a role in as we think about cell-free DNA enrollment as well.

[00:02:30] So I think the key message when I was asked to talk about this, and I wanted to start out by saying, is that molecular allocation and targeted therapy studies based on cell-free DNA is already happening. So this is not discussing how we approach this in the future, but this is the world that we live in today. And as a PI in a lot of these studies, it is quite common now to see commercial lab reports that do cell-free DNA testing being used as evidence to support the presence of alteration, and to support eligibility for a study.

[00:03:00] I do think, with that being said, that these results are not completely interchangeable in all cases with tissue-based testing, and there are some special considerations, both things that are obviously very positive aspects of this technology, and others that we could spend some time thinking about the limitations of. And I just listed some of the ones that came to top of mind here, and I'll touch on some, but not all, of these, but these are essentially ... This first one's purely operational. There are issues of assay content; there are issues of somatic variant culling methodologies; the ability to move beyond single gene variants to gene signatures, or other broader computational analyses; the ability ... the sensitivity of detecting fusions, I've alluded to this; indels and copy-number alterations.

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[00:04:00] What I find to be very tricky when I look at these reports is trying to understand whether the alteration that we're being asked to clinically action on is plausibly a dominant clone in the patient. I think you could also ask me, "Well, how do we know that's important?" And I think you've ... Posse showed some data from the T790 world where this has really been, I think, very well worked out. And then obviously alluded to patients that don't shed. And then I think there is clearly a unique opportunity in drugging required resistance mechanisms detected this technology.

[00:04:30] So I thought I would just start out running down this list by looking at this issue of the operational advantages of cell-free DNA. We've looked at this at Memorial on the tissue level, and when we did the analysis, we had about 11,000 sequenced patients in the cohort. And when it was all said and done of ... This is ... A patient has been consented to testing, and the physician enters the order, how many of them ultimately have a report signed out that meets the QC specifications of our lab? And it's 86% of patients.

[00:05:00] Now when you look at what goes into this, you can see that the likelihood of signing out, successful or poor, ... And these are ... This is where we have sequencing failures ... is very low with surgical resections; is actually fairly high with biopsies; and drops a little bit with cytology. I will tell you, if the cytology is obtained at Memorial, the rates are actually equal to that of a core biopsy. If the cytology is outside Memorial, it's lower.

[00:05:30] Now I think what we have to recognize when we look at this experience is that this does not represent the real world. Memorial, as much as I hate to admit it, is not the real world. So I think that ... I showed this number, 86%, as establishing kind of what a center of excellence can achieve with really world-class medical pathologists, but I think we can assume that the rest of the world is not achieving this level of success. So this would, I think, set the kind of upper bounds of what

[00:06:00] we can expect in terms of feasibility of tissue testing or availability of tissue-based testing. And also, turn-around time in this series was just under three weeks.

I want to spend a little bit of time talking about the robustness of the assays, or the genomic content that is really required for these assays to maximize the opportunity to enroll patients in clinical trials. I want to show you one gene that I've been working on for a while, which is mutations in ERBB2 or HER2, and what you can see here is that HER2 doesn't behave like some other oncogenes, like

[00:06:30] BRAF, where you have a single predominant mutation that accounts for the overwhelming proportion of activating variants in that gene. This was patients enrolled to a summit study, which was a basket study of HER2 mutant tumors, non-amplified tumors.

You can see the mutations occur across multiple domains: extracellular domain; all throughout the kinase domain; in the transmembrane domain. And not only

[00:07:00] do they occur in geographically dispersed regions of the gene, but they have multiple different classes of variants. So you can see insertions and deletions; we even have a fusion enrolled. So you can see that in order to detect all these patients and enroll them on clinical trial, you need an assay that provides coverage for much of the coding region of ERB2, as well as the ability to detect multiple different classes of variants.

[00:07:30] So we have actually sat down and looked carefully at what we think a fairly comprehensive assay would look like: what is kind of the minimum that we think is fairly comprehensive, if that makes sense. And this is what we came to. And I wanted to take you through the process here of how we thought about this.

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So we have an institutional knowledge base around variants that we call "OncoKB." You can access this publicly at oncokb.org. And if you look at ... We started off saying, "Okay, we need to include all the kinase domains of every kinase that has an OncoKB alteration." So these are alterations for which clinical evidence exists, even if anecdotal of the variant being actionable. And then we went on and said, "Okay, well, we should broaden this to include, really, at least select regions of any gene that has annotation of clinical actionability," again, having a fairly permissive filter. So some of these are really based on, for example, phase I clinical trial reports, but not robust phase II or III data. And then also saying, "Well, we think that if you're in one of the top 25 most-mutated tumor suppressor genes, that may be important, and so we would like to include those as well."

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And then we have put in a lot of effort institutionally through the Taylor Laboratory of looking at how we define mutations which are positively selected for. So these are statistically significantly recurrent mutations that you can analyze in an unbiased fashion looking at large genomic datasets. We've done this, multiple iterations, including a cohort of 50,000 sequenced tumors now, and we added in all of those kind of hot-spot regions. I think this was any gene that has 30 or more mutations in our series. And then SNPs.

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So when you come all down to it, we [averaged 00:09:17] about 1,300 exons covering 128 genes, or about 200,000 kilobases. This is the rough design of what we planned to undergo clinical validation now, to bring into our clinical lab, but we stood up in our research lab, and I'll touch on that a little bit later. One of the points I want to raise, and this will lead me to my next slide, is that we do sequence normal from buffy coats in this assay.

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So why do we do that? We have obviously ... I think we probably have the largest cohort of prospectively sequenced, matched tumor-normal specimens in the world. When we looked at that in the first thousand or so cases that we did, and we said, "Okay, in this thousand cases," ... Again, this was just a panel of 341 genes at the time. You can imagine how this scales to a whole exome. We had about 16,000 unique coding variants. If you look just at those in cosmic, you get down to 874; but if you look at what was truly somatic, again, by looking into the germline, it's about 5,000. Using cosmic as a rough filter, you'd actually throw out almost 80% of this somatic variance. Now I don't think anybody suggests that cosmic is the appropriate filter to put on an unmatched informatics pipeline. I know there are people in this room that are much more expert at this than me. But I would essentially propose that there's no way, with perfect accuracy, to develop informatic filters that sequence ... that throw out all germline and retain all somatic variants. And for a discovery effort, this is important.

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Now cell-free DNA is obviously a different issue. When you look at a large clinical validation paper published by a commercial vendor cited here, you can see that the majority of the variants that they sign out occur in this kind of 1% range of allel frequency, and obviously germline mutations would be expected to occur

[00:11:30] closer to 50% allele fraction. And so, simply by kind of looking at the variant allele fraction, you have additional information on how you might filter germline variants. However, we've looked at this across individual studies at our center, and one of the points I want to make is that the mutant allele fraction is obviously affected by the overall tumor burden in the patient, so the percent of cell-free DNA that's contributed by the tumor, but also by the genomic configuration of the variant within the tumor.

[00:12:00] And one of the things that we noticed when we were looking at an AKT basket study we were running is that we were seeing incredibly high variant allele fractions of AKT. And this was a validation we did looking at cell-free DNA using our MSK-IMPACT platform versus DBPCR, with near-complete concordance. And we've now repeated this experiment with a hundred more samples, and there are a lot more dots, but the trend is the same.

[00:12:30] But there are a substantial proportion of patients where the variant allele fraction of the AKT1-E17K mutation exceeded 50% in plasma. When we looked into why that was the case, we think part of this is because AKT1 exhibits this pattern of allelic imbalance, where the tumors lose the wild-type copy and either gain another mutant copy, or have single mutant copy. Now this is obviously phenomenon that's quite relevant to tumor-suppressor genes. So if you have a P53 mutation in a tumor and you've lost the other copy, and you have a high-

[00:13:00] burden disease patient, you might have a difficulty distinguishing a germline [inaudible 00:13:00] mini- patient from a PP3 mutation that is somatic in the tumor.

[00:13:30] I know Nick Turner is in the room. This is a paper that he published with my colleague Britta Weigelt looking at revertant mutations. This is obviously now a well-documented event. He may have even showed this slide this morning; if it is, I apologize. But I want to make a point about this, which is that if we don't sign out the presence of a germline BRCA allele, you could actually have the counter-intuitive result of signing out a revertant mutation as a potential sensitizing biomarker to PARP inhibitor therapy, when in fact the revertant mutation is restoring reading frame for the original germline mutation, and is actually a resistance allele.

[00:14:00] I think different commercial laboratories have addressed this in different manners, but I think what I'm kind of getting to multiple different ways is that we think that looking at the germline confirming what is somatic, but also looking at the germline for actionability, because that's where a lot of action is as well, is important. So I don't want this issue of germline to fall off the table when we talk about cell-free DNA sequencing.

[00:14:30] I thought these were very interesting data that were showed through ... These are Roche data presented at ESMO looking at a atezolizumab versus docetaxel, presented at ESMO and looking at the outcome by tumor mutational burden as defined in cell-free DNA. And what you can see here is clearly the high-cell ... the high tumor mutational burden patients in a cell-free DNA assay seem to do

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better. This kind of correlates with what we see in tissue. Obviously if you ... Oh, sorry ... If you add in IHC staining for PD-L1, then you get potentially increased sensitivity. So you not only look for high mutation burden patients, but high PD-L1 expressors.

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However, when you dive into the data a little bit further, what you see here is that the negative predictive value is quite good, but the positive predictive value is ... maybe has room for improvement. I think this relates to number of factors. You can see here: these are the negative ... These are essentially the so-called "false negative" by comparing tumor mutational burden from blood (B) versus tumor (T). These are the concordant samples, and these are where blood detected hypermutation, but tumor didn't.

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I think ... Like I said, I alluded to, there's a number of potential reasons for this. One is that the genomic content in cell-free DNA assay tends to be smaller, and so its ability to scale to the larger content of tumor may not be quite as robust. There's a number of other factors that were cited by the investigators, but I would put forth maybe one more factor, which is that the tumor burden in the patient, which is obviously difficult to measure in a clinical trial database, or quantify, as well as the shedding characteristics of that tumor, may play a role. Simply put: if there isn't a lot of tumor drive DNA in the blood, the ability to detect the full diversity of mutations may be lower. And we see the ability to detect subclonal mutations grow as the burden of disease in that patient grows.

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And actually this is data through GRAIL Collaboration presented by Pedram Razavi at ASCO. Looking at this, where we looked at cancer cell fraction of mutations in various tumor types, and looked at the detection rate by cell-free DNA assay that obviously includes very deep coverage with all the technical specifications, like UMIs and others that have the ability to error-correct, sequencing error versus true mutations or somatic mutations, and what you can see is not unexpected, is that the sensitivity for detection in plasma rises if the mutation clonal in the tumor. And I think that's not surprising, but it give you a sense of kind of where we are. And you can also see that mutations that we detect as clonal in tumor are not always detected in plasma. Now it may be that that mutation is clonal to that metastatic site, but not clonal to the patient writ large, and so it really kind of gets to "what is truth?" in these patients.

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But nonetheless, you can see that these are orthogonal techniques that will never have perfect concordance.

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I want to just touch on low shedders. So we alluded to this study, this AKT study that we recently published in JCO. If you look here, there was a lot of data in this figure: this is the waterfall plot. This was digital droplet PCR for E17K and AKT1. You can see that some of our best responders in gynecologic cancers did not shed, or did not have detectable AKT ... And these are patients, some of whom that went on, were ongoing for more than a year and a half of therapy at the time that the data analysis was pulled. And when you really dive into it, you find, for example, that AKT mutations co-occur with low-grade endometrial cancers.

[00:18:30] So they kind of enrich for two to three wild-type, low-grade endometrial cancers. And these lower-grade endometrial cancers appear to have lower shedding dynamics into plasma. And we've looked at this multiple ways to make sure this wasn't a sequencing artifact: we looked at other somatic mutations, looked at NGS sequencing, and they just don't shed any tumor-derived DNA that we can detect by really any methodology into the blood.

So purely cell-free DNA-based testing will miss some patients. Frankly, so does tumor-based testing. So there is no perfect test.

[00:19:00] I want to end by discussing acquired resistance, and a particular focus of mine recently have been drugging kinase fusions. I think this is really a beautiful story for, and a beautiful space for cell-free DNA because, as Posse already alluded to, much of the resistance in this space is driven by on-target mutations in the previously wild type kinase domain of the fusion product. And this was a nice review published by Ali Schramm from Memorial and Alex Drilon showing the different variety of mutations. This is obviously [inaudible 00:19:22], but also

[00:19:30] been published in Ross, Wan, and others. And what's really nice is you can see that these mutations often align into [paralogous 00:19:31] parts of the genome.

[00:20:00] We actually exploited this recently in the track-fusion space, where we presented data on larotrectinib, which is a selective track inhibitor across a variety of track fusion positive cancers. We were able to detect in the small proportion of patients that developed acquired resistance, through either tissue and/or cell-free DNA sequencing, a dominant resistance mechanism very early on, almost immediately in real time as this developed ... actually in some cases, even before you can observe it clinically ... and were able to rush a drug into the clinic, in this case LOXO-195, that actually salvaged responses. And interestingly, through this the first two patients who actually developed acquired resistance to the first-generation track inhibitor were sequenced to the second-generation track inhibitor. And I think cell-free DNA plays an important role in kind of accelerating that.

[00:20:30] These are data out of Boston looking at BDG, the FGFR inhibitor and FGFR fusion-positive cholangiocarcinomas, and again, you can see ... This shows a slightly different pattern, where you see [pichronal 00:20:36] resistance mechanisms. And again, I would draw your attention to the allele fraction here generally being less than 1%. You have a sense of what you're asking these to perform to be able to detect these.

[00:21:00] So at Memorial, we have been collecting cell-free DNA prospectively in our patients through the [inaudible 00:20:54] environment for a while now. Began in 2014; this is all done on automated systems, and we're projected to draw about 5,000 samples this year. We don't yet have a clinical assay; we're going through the validation now, but this allows us to do a variety of research using only assays while, in parallel, validating a cell-free DNA panel.

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So in conclusion, from a clinical trialist's perspective, I think the utility of single-analyte cell-free DNA testing is there, but will be limited to very select clinical scenarios like T790M. Broader cell-free DNA next-generation sequencing is operationally efficient, especially in the community, and will be important for enrolling to clinical trials. And although the content of these assays is generally smaller, they're typically still adequate for most of the targets, and I believe this will scale over time as sequencing costs drop. And the technical validity of things like tumor mutational burden, MSI status, other signatures like BRCAness, really need to undergo ongoing validation. I think we're getting there, but we're maybe not quite where we want to be. And we have to consider things like low shedding, the analytic sensitivity of various things, like fusions being the most problematic ... I didn't touch on that, but we actually see sometimes patients where the ... You miss the fusion entirely, but you capture the acquired-resistance mutation, because the threshold for detecting the fusion is that much lower than the variant. And then the potential for detecting clinically relevant and irrelevant subclones, and eligbilities to evolve to address this.

So really, this is my last slide. This is already happening in both routine practice and clinical trial enrollment, and we need to understand these technologies as clinical trialists and physicians, to make best use of them. Thank you.

Pasi Janne:

What are the opportunities and needs? Scott?

Scott Kopetz:

[00:00:30]

All right. Thank you. And thank you for the opportunity to present on this topic. Why don't I first start with some disclosures? What I wanted to touch on is really trying to think about some of these from the current state of clinical care. What are the low hanging fruits? To augment some of the things that you've heard already. Of course we've heard about the opportunities in screening, recurrence detection, metastatic disease. What I wanted to do is just touch on some of the immediate opportunities in the area of recurrence, but then spend most of it talking about metastatic disease, where I think the clinical implication and applications are at hand now.

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So, we've heard a very nice session before this session on recurrence and detection in minimal residual disease. But I wanted to make a few points, and I think, importantly, reiterate a few of the things that we heard about. I think one thing that I think we should really think about as a field is how do we define this disease state? THere's been talk about this as a prognostic biomarker, but I really think that what we're seeing is a, probably better defined as a Stage IV MRD. All right? This idea that we're talking about minimal residual disease that is very high specificity for recurrent disease. And it's not really a marker of high risk of recurrence, but I'd actually argue that this IS recurrent disease. Right? So we don't talk about a visible liver metastasis on a CT as a marker of high risk of recurrence. Right? We say it is recurrence. I think, likewise, when we're starting to see data that is this strong, this convincing, we should be thinking about this as a marker of residual disease and not a biomarker of risk.

[00:02:00] And certainly this idea, as you've heard, has been adapted in human pathologic malignancies for quite a while. And I think it's time to start thinking about it and adopting some of that terminology in solid tumors.

[00:02:30] So, off of my soapbox for that, and now onto some data. I just wanted to share some nice data. This is actually data of a Max's that he presented at ASCO this year in colon cancer. So we're glad that he's looking outside of his other tumor types and doing colon cancer work as well. But, beautiful data and I think, also, as we talked about, Jeanne Tie and others from Australia and Hopkins have demonstrated this as well. And importantly, here, we're talking about Stage II, 5% prevalence, 16% prevalence. These are not insignificant numbers in these patients. And really seeing curves that look like this are very clinically compelling.

[00:03:00] But this idea, how do you demonstrate utility? Right? And I wanted to share one effort ongoing in the NCI community for colon cancer. This is and NRG and inner group supported Stage II adjutant study that is under review. And really highlighting some of the areas that we talked about before where this design is taking a Stage II patient, whee the standard of care would be to do no further adjutant therapy, a randomization to applying the test or not, and the setting of a positive ctDNA, then all patients going on to receive adjutant therapy.

[00:03:30] But I think, while these are the first wave of studies that we need to do, I think there, as questions that are raised before and why we need to do these studies, is the question about given that we can reduce the risk of recurrence by 50% with adjutant chemotherapy? Is this the 50% that we can cure? Is it those that are detectable by ctDNA as opposed to those other patients that we still can't

[00:04:00] detect with current assays? One slide that I wanted to reiterate some of the things that were brought up, and like Turner had mentioned before. But really this opportunity, and I think it's a tremendous opportunity for the field, to really enable some of the Phase II proof of concept studies. Right? Where you can identify very high risk patients that have high event rates, which is really what we

[00:04:30] need to drive proof of concept signals, but with this idea that a ctDNA clearance as a surrogate, potential surrogate, endpoint maybe a necessary, but not sufficient biomarker benefit. Right? So that we can use these to really help with those go-no go decisions as was talked about before, as one of those immediate implications of this.

This is something that we've not been able to do as a community. There's been reticence to get into the adjutant space because it requires 1,000 patients and a Phase III commitment, and there really has not been anything to really de-risk

[00:05:00] that next step. So I really think that by doing this, we may see a lot more of this kind of Phase II studies that then can either lead into a de-risked Phase III, with a more traditional design, or potentially, as you heard before, a ctDNA positive.

So, with that kinda discussion of the idea of where we can apply this clinically in recurrence, really moving then on to metastatic disease. And in metastatic

[00:05:30] disease, really, the utility that we see I think falls into four different categories here. And I'll touch on each of these. The first two, one may think and look at as fairly mundane from a technology advancement. But this is really where there's a

lot of immediate application. We ask where are we using these tests now clinically outside of the research infrastructures? These are the questions that we are now finding ourselves applying this to. And then touch on some of these other aspects as well.

[00:06:00] So when I talk about access to tumor, so you've heard, certainly, and we know that we have to do biopsies in a number of patients in order to get sufficient material for testing, and just some numbers to reiterate what we all know, that these can be expensive tests. And they have real complications rates. So we've reported on 750 about research biopsies where we had complication rates of 5%

[00:06:30] overall, and 17% for lung biopsies. There's certainly real risks, and those come with morbidity, but also costs to the healthcare system when we have these complications.

So, I think we're a bit beyond the point of discussing concordance. But I did want to acknowledge that there is one very nice prospective study to demonstrate concordance done by and presented at ASCO this last, Pierre Laurent-Puig, who

[00:07:00] had demonstrated in colon cancer kind of a 93% overall accuracy here, and identified a sub-set that did not have detectable ctDNA. And the question then, for the field, is: is 93%, you know this idea of above 90% sufficient for clinical utilization? Right? Is this enough for us to move forward with in the clinic? And really, it really depends on the context of the false negatives. I think you see this

[00:07:30] very nicely in the lung cancer. Right? Where the idea is do tissue, up front perhaps, but then in the resistance do ctDNA first and the reflux to tissue as needed? But this idea that false positives are very rare, but it really is the false negatives are the limitation of the ctDNA technology. The ones that we miss. And certainly we know in some settings that may be missed opportunities for novel therapies or proof therapies.

[00:08:00] But in the colon cancer field, it's actually the inverse because what we have is a negative biomarker. You know, K-res, N-res mutations are associated with lack of response to EGFR inhibitors. I think, importantly, this idea that we know now from studies that if you treat a patient who has a mutation with an EGFR inhibitor, you can harm them. Right? You can do worse than if you didn't do anything at all with monoclonal antibodies. I think that has, that is a higher bar

[00:08:30] for the clinicians to overcome when there's the potential thought in the back of your head: is 90% enough? Right? If there's one out of ten patients I'm gonna be harming by treating with an EGFR inhibitor, based on this. So, one thing I think we need to do better as a community is really try to communicate this information in the clinical reports. Right? Right now we get a report back that

[00:09:00] says "no K-ras or N-ras detected." I think as we're now moving towards the panels that are multiplexed, where we have NGS panels that are now reporting back from this, I think we have and opportunity and an obligation to do a better job of reporting this out for clinical utility.

So what does a ctDNA negative mean? I think we can take, for example, colon cancer example and continue that where we have these truncal mutations that are present in a vast majority of patients, very early on in the carcinogenesis

[00:09:30] pathway, that occur prior to the K-ras mutation. So there's an informative negative where you can see the P53 APC mutations, but no K-ras mutation. Or the uninformative negative whee you just don't see anything at all. None of these other truncal mutations. And I think one of the things that encourages the field to do, is we really need to do a better job of communicating these two

[00:10:00] different scenarios back to the clinicians. I think the clinicians have to be more comfortable with what do these tests mean, when should I trust them and when don't I? Right now there's still a lot of reticence because of the idea of these false negatives.

So, part of this is when do you collect? Right? So just some unpublished data from our Center, of 416 patients on a commercial NGS panel where overall we can detect circulating tumor DNA with 84%. Remembering that the lung, as the

[00:10:30] lung cancer community has shown us, and likewise in colon cancer, when we have lung only disease, that's lower rates. When we have lymph node, liver involvement, those rates go up. But it turns out the most critical component is really when you collect it relative to their treatment. Right? So this idea that newly diagnosed or recently progressing patients, when you collect that plasma

[00:11:00] and do the test, then you have very high detection rates. But this idea of giving a dose of chemotherapy and then sending off plasma really is sub-optimal. So this is also something that, as a community, we're gonna need to learn how to communicate to the clinicians that are doing the testing. When should I be doing the testing and when will I get the best results?

So what about speed of results? So I think you saw some very nice data from the, a few different settings. This idea of speed is really not so much the sequencing

[00:11:30] time itself, but I think a lot of times, it's that time to actually get the right tissue in the right place. There's a striking number, that 70% of the patients in the U.S. actually have chemotherapy administered in a different healthcare system than their initial tissue was obtained. Right? So that's just a comment on our healthcare system, that we tend to be a bit fractured. So, there's a whole lot of tissue moving back and forth from different centers and different requests, and

[00:12:00] that can add time. Certainly, in a lot of times, patients don't have this tissue available when they first meet with a medical oncologist. And increasingly, this information is required in order to assign that first treatment.

And so an experience we had of about 400 some patients, whee the order was place. The median time was six days. Once you got it, it was kinda of another, these were calendar days, another two weeks to get the results turned around.

[00:12:30] Then, of course, you gotta sit back down with the patient. So you've got an overall of twenty-seven day median time frame from when you order the test until you communicate this back. But the difficulty here is also this distribution. So while six may be the median, you can see that the tail here also drives this. These are the times you have to ask again and again to get the tissue from the outside. So this immediate application then, potentially think, can ctDNA may

[00:13:00] not be better or give more insights, but if they can do it faster and enable a better decision in the clinic, that is a substantial win.

[00:13:30]   So we did a head-to-head study, prospective study, 107 patients, a simple study where the patients came. We sent off both plasma and tissue, both with CLIA compliant assays, and 89% of the time plasma came back first. So probably not a surprise to those, because we can get the plasma that day and really have a substantial headstart in getting that information.

[00:14:00]   All right. So, what about the dynamics? And I think this is another area where there's a lot of interest in using this in some immediate clinical applications. Reiterate what we had discussed before, which is that ctDNA has a very short half-life. Right? So, unlike protein markers that may change on the order of days to weeks, the ctDNA in circulation has a half-life of minutes to hours, depending on the source. Very short. And we know that these levels can fall fairly rapidly. Right? These are not subtle changes, but really dramatic changes that happen on log scales. And, for example, here's some data. Jeanne Tie's data demonstrating that the levels fall greater than 90% in two weeks in responding colorectal cancer patients. So fairly rapid changes that can occur.

[00:14:30]   Again, in colon cancer, our first prospective study to demonstrate this, the Placol study, Pierre Laurent-Puig and colleagues had published this. Demonstrated again that if you had a fall of greater than 80% in your ctDNA, that here in blue you had much higher response rates, you had longer PFS and longer overall

[00:15:00]   survival. And again, those are really quick onset assays. So these are nice, but the question is: how do we use this? Right? How do we actually apply that information and change our clinical practice as a result of this type of data?

[00:15:30]   So, I highlighted at least three different areas, but I'm sure we could come up with a few different additional ones to add onto this. But one, I think, is this recognition that in many of our tumor types, we're dealing with very active, initial regimens, but that risk-benefit ratio starts to slip in later lines of therapy. Certainly in colorectal cancer, we have agents that have very low to near zero response rates, with a fair bit of toxicity, and a very short improvement and progression pre-survival or overall survival on a population level. So the question is: could you use these things in settings where your adverse event rates are still

[00:16:00]   high, but where you response rates and clinical benefit rates are very low? Could you design studies where you are looking for these early changes, even with standard of care chemotherapy? And what would the implications be of making changes based on a rising ctDNA before radiographic progression? I think that may not always result in improved outcomes, but these are questions that are now starting to be asked in some prospective efforts.

[00:16:30]   And, finally, there's settings where you have a limited window to intervene. And in colon cancer, for example, we try to shrink the cancer so that we can resect metastatic disease in the liver, while we're accumulating toxicities to the liver with these agents. So there's a kind of a limited window there to intervene. And if we over-treat the patient, or treat him with the wrong agents, we miss this window because we accumulate liver toxicity.

[00:17:00] So I think there's kinda the lowest hanging fruit in what we're seeing in the first wave, are really this idea of how can we limit ineffectual therapies. Right? And I think that may offer some potential opportunities forward. It may not always be about improving progression for survival, but really looking at some of these other end points that Dr. Blumenthal had mentioned that can be part of the approval process, about patient reported outcomes, adverse events, and so on. So I really, I think we have to keep that on the radar as well as the traditional improvements.

[00:17:30] And finally, assessment of heterogeneidial resistance. In colorectal cancer, we do get alterations in heterogeneidae. YOu've seen these curves to present it already at this conference, where you can see the architecture that may be maintained with the clone, the different clones. But just the overall burden increasing or decreasing with time. But we also see curves like this, where the substantial

[00:18:00] clonal diversity that occurs at different time points, where new clones evolve at each time point. And when we looked at a series of these, 84 patients, there was a majority of patients that had a gain or loss of clones over time in colon cancer. And so, we see this heterogeneidae, and as clinicians we start to scratch our head and bit and think what does this mean? One of the things we see is that these sub-clonal alterations, when we do see them, they tend to be one of the

[00:18:30] ones that tends to be less frequently mutated in colon cancer, and are more likely the variants of unknown significance. So, as you kinda heard about before, this idea that there is some sub-clonal architecture, but we're still not at the point where we know full what do to with some of these alterations.

And importantly, because we see sub-clonal mutations N-ras that occur in untreated patients, a very low lever, the question is: does this preclude

[00:19:00] response? I think was a very insightful study done by AMGEN out of the aspects study, 1,000 patients. At baseline, the Ras clones were detected in untreated patients, sub-clonal Ras clones in 20% of patients who had stable disease with treatment, single agent treatment. And even in 6% of patients that had a response. Right? So this idea that just because we see these sub-clonal

[00:19:30] mutations, doesn't mean that the patients don't have clinical benefit. So we have to, you know, why is that? It's certainly the sub-clonality, the benefit of the Ras wild-type population. But I think this simple idea that just having a mutant sub-clone means that the tumor's gonna behave as if it was a clonal mutation. We have to recognize that's probably not gonna be the case.

After EGFR inhibitors, we know we can develop resistance mutations, like in lung cancer with EGFR inhibitors, but what we see are multiple different acquired

[00:20:00] alterations and down stream of the EGFR pathway, we're in the ector domain region of EGFR. And we can see multiple mechanisms in the same patients. So as was starting to be described, as you heard this morning, in lung cancer, we certainly can see this even more relevantly here. So the resistance mutations

[00:20:30] rarely travel alone in colorectal cancer. So this is a demonstration of each of the patients here in a row. And although it's small and hard to identify, these are all the different mutations and alterations. So you can see multiple different mechanisms in one patient.

[00:21:00]	So we are involved and exploring this. How do we tackle this in the clinic? So the clinical opportunities are to do re-challenge studies. So this is the Cricket study that's looking at that. Or look at novel therapies using ctDNA selection to try to improve outcomes. So stay tuned in this space. But there's certainly opportunities.
[00:21:30] [00:22:00]	In conclusion, I think we can argue there's several clinical needs that handle this logistic hurdle. All right? That this really are major issues in clinical care and these are opportunities to use the technology, access to tissue, timely results. I think that's where we're seeing ctDNA really being utilized right now in the clinic, outside of the research environment. There's studies using the ctDNA for treatment response, planned and ongoing. The utility remains to be defined. And again, we may be talking about doing studies that limit ineffective therapies. Targeting resistance mechanisms studied in many settings, but it's a whole other area to talk about. How do you really handle that when you have multiple different clones that are developing in these agents. And then reiterate what we discussed before, ctDNA in minimal residual disease has tremendous opportunities for development and clinical application. But we really just have to do the studies, because with the simple answer of deploying standard chemotherapy in these patients may not actually be the one that provides the most benefit.
	I'll end there with acknowledgment to the lab and some of the collaborators I did present. Thank you.
Pasi Janne: [00:00:30]	Answer session and I'll encourage you to ask questions and direct them to the particular speakers. I'll start ask ... Gideon a question you mentioned about surrogacy for HIV, RNA load and for [BCRA ball 00:00:20]. Do you think we will ever get to that point in solid tumors or do you think that, in order to get there, we need some fundamental additional advances in technology or other aspects?
Gideon Blumenthal: [00:01:00] [00:01:30]	Yeah, good question. I don't know. Your data suggested that for highly refractory disease, perhaps as an efficacy as an end point, you might not need it as much because, unfortunately, patients progress quickly and the radiographs can serve as the end point. As Scott alluded to, there might be certain advantages in the resistance setting apart from analyzing efficacy such as decreasing toxicity or de-escalating therapy in that setting. Where it may have most use may be in earlier stage disease. We've already heard some potential trial designs, for example, in the adjuvant setting, where if you detect cfDNA and then you intervene and perhaps you could follow that and that may predict prior to disease recurrence. That could be the space where this could be applied as a surrogate end point.
[00:02:00] Pasi Janne:	Microphone two.
Speaker 1:	Actually I'm going to follow up to that question. There was an effort a few years back using CTCs in the CellSearch platform as a surrogate in clinical trial for

overall survival. Do you know whatever happened to that effort or anything similar that might be coming on the pipeline?

Gideon Blumenthal: [00:02:30]	I think maybe the panelists might know. Some of those studies have been published. I think there was a SWOG study in metastatic breast cancer, which didn't show that there was necessarily clinical utility.
Speaker 4:	I know there was some in prostate because the correlation was clear that the decline below the five cut off clearly correlated with overall survival. And they had been used in the data with [inaudible 00:02:52] and others.
[00:03:00] Gideon Blumenthal:	Yeah, there are ongoing efforts. I know Howard Scher from Memorial, for example, is looking at qualifying changes in CTCs as a surrogate end point in prostate cancer trials.
Scott Kopetz: [00:03:30]	I think that's the key question is, how do you use that to help, then move the field forward as a surrogate marker, because I think part of the hurdles of getting that in, and I think in colon cancer, there's also very nice data about changes in CTCs being associated with prognosis. The question, at the end of the day, became, how do you treat the patient different as a result of that. I think the part of that slow effort to get that moving forward is probably related to the clinical applicability and I think if we can establish it as surrogacy in that setting, that can really open up more rapid development options.
Gideon Blumenthal:	Number four.
Nick Turner:	I've got a general question to the panel.
[00:04:00]	The same topic came up in most of the talks, which is, at the moment, we've got no robust way of picking up purity, working out how much circulating [chemo 00:04:06] DNA is there. When we don't find a KRAS mutation in colorectal cancer, we don't know if it's just there's not circulating chemo DNA there or if the tumor's truly negative.
[00:04:30]	Then, for T79TM in plasma, some are subclonal, but we've got no good way of working that out. So, the question to the panel is, what do they think is the right way forward here? How do you think we should be technically trying to do this with circulating chemo DNA to get a much better feel of whether we've got clonally dominant mutations?
Scott Kopetz: [00:05:00] [00:05:30]	There's a few different approaches. I think, certainly, if we follow the direction of tissue testing where we move a large panel, testing fairly rapidly, I think we'll see the same in NGS, so at least having large panel testing allows you to address some of those potential other truncal mutations. Not a complete solution, but I think in some settings. We are also seeing efforts where there are orthogonal methodologies either methylated DNA markers that may be a little more agnostic. Also, efforts to look at the characteristic or the size fragment, some of

the characteristics of the DNA size to help understand some of that, too. I'm not sure if I have a good answer, other than to say, this is absolutely something we need to do. I think this idea that we can just leave these tests as it's a negative without providing the end users, the clinicians and patients, some guidance on what that means is the critical next step.

[00:06:00]

Pasi Janne: I would say, on the EGFR front, we like using the presence of the activating mutation as a control, so if you have a negative T790M test, but you can still detect the L858R mutation, that's more likely to be a true negative, as opposed to, in a patient where you can't detect either the resistance mutation or the activating mutation. That just could be a low shedder. I think that does provide some context. In one of your slides, you showed, looking for APC mutations in

[00:06:30]   colon cancer, so if you can detect that and no KRAS mutation, that gives you some context of, did that actually come from the tumor. I think your point is well-taken that, interpreting a negative, the devil's in the details. 6;37

David Hyman: I guess I would just add one point is that, I'm not sure this is actually unique issue with [inaudible 00:06:45] DNA. I actually remember I was at a conference maybe two years ago with you discussing this where we said, "Well, we're being asked, we submitted protocols targeting BRAF mutations." The FDA says to us, "Well,

[00:07:00]   you're not using FDA-approved tests, how do you know your tests performs well?" When we look at it, our tests performs better than the gold standard, so essentially, we had a situation where the established, the cobas assay, for example, actually there were negative patients who turned positive when we subject them to deep tissue based next generation sequencing. I do think it's an

[00:07:30]   issue. The other point is is that I think physicians have a really hard time interpreting these tests. I don't even just mean [inaudible 00:07:32] DNA, I mean sequencing tests in general. We've put all manner of warnings on low-tumor content sample reports. "Don't trust this, this is a negative," you know, "The CC repeated," whatever, and when you actually subject this to more rigorous

[00:08:00]   analysis of the comprehension of physicians, even at large, academic centers, it's not what we want them to be. I think part of this is just a broader training and education purpose.

Gideon   Microphone one.
Blumenthal:

Speaker 2:   It's along the same lines. Do we really need to have some minimal amount of DNA retreat before we say ... Because Scott, you're description of what is a true negative is very important. That's a very good way of looking at it. Not to put

[00:08:30]   other work down, but personally, I've seen data from your lab come out where, there's been a slew of T790 positives and the activating mutation is not in it, so I assume that that's been a specimen which has been inadequate. So, should we have that threshold, but say it had enough that we can do and call a true negative that Scott pertained to.

                If I'm not technical enough, tell me.

[00:09:00]
Scott Kopetz: I think, certainly, there should be some quality metrics, in terms of what goes in, and I think the next session actually has some very nice ... I'm looking forward to some of those presentations to address some of those key points. At the same time, the technology is such, at least my interpretation of as such that, when you

[00:09:30]   see the mutation present, it's there with a fair degree of certainty. So, when you see a T790, even if you don't see that other truncal mutation, I think we'd have, at least I'd have, confidence in that result, either way.

Speaker 2:   But you couldn't really, in any way, determine how dominant it is.

Scott Kopetz: Right, you couldn't assess the subclonality, correct. That's a good point.

[00:10:00]
Speaker 2:   And just one other quick question. Scott, the data you showed at [Max 00:10:01], I assume that since they're all stage three colons, we're really looking at following adjuvant chemotherapy, so despite adjuvant chemotherapy, they still all recurred and all it did was delay it.

Scott Kopetz: Right, so that's a good question, and that really gets back to that point of, are
[00:10:30]   these the cohort of patients that we're really curing? A lot of times, these studies are done in that initial post-adjuvant, so you're asking, after the adjuvant therapy, what remains? But I think the big question is, how many of those can we go from ctDNA positive to negative with adjuvant therapy and then the other point that was made, not just transiently, but even a year a later, are they still ctDNA negative. Maybe there's some additional time that needs to lapse to have increased confidence in what that means clinically.

Gideon   Carlos?
Blumenthal:
[00:11:00]
Carlos Arteaga: Yeah, this is a question for Scott. I'm thinking of the randomized scenarios that Nick Turner presented earlier. You presented a study where, it was not randomized. You had ctDNA, after colon cancer primary therapy, I presume you would go to adjuvant. And if you did not, you did not get anything further. That's
[00:11:30]   a good study, but I'm thinking, how would you control for that? How you interpret any outcome of that single arm, ctDNA positive arm?

Scott Kopetz: This actually was, and I apologize for not going into that in a little more detail, but it was actually a randomization, but it was a randomization to having the testing, the ctDNA testing done or not. Then, for those that did have the testing done, you can then, if you're positive, go on to get treatment. This was a
[00:12:00]   discussion with some of the patient advocates and trying to understand that, because there is a lot of concern, given those recurrence curves, what do you do if you test everyone for ctDNA and then try to randomize the positives? I think we can all wear our own hat as a potential patient. I'm not sure I'd wanna go on that study if I knew I was ctDNA positive and wasn't getting chemotherapy. That
[00:12:30]   design is really a randomization to the testing or not, with the idea that, of

course, samples banked and at some point later you can go back and look at both arms.

Pasi Janne: Daniel.

Daniel Haber: I'm struck that the discussion that we've had has always been this idea that we're targeting mutations, then ultimately biopsies will become replaced by plasma DNA analyses. As we look to the future of immunotherapy, I'm wondering if the clock is moving back to the left, because in some way, everybody needs a biopsy.

[00:13:00]   If you look at patients with even BRAF positive melanoma, the first line of treatment is often immunotherapy and the first question is, really, is the tumor inflamed, or what's the level of infiltration in the tumor? I wonder, as we plan the future of plasma DNA, if we need to imagine that we're going back to a world where the initial biopsy will be quite relevant?

Pasi Janne: Why don't you take that on?

Scott Kopetz: Yes, sorry.

[00:13:30]   I'd agree that you need that information. I think there is still limited settings that has been tied to, right now, has been tied to efficacy with the agents, but it very well may be in the future that we'll start to get into inflammation scoring with companion diagnostics. In which case, you're right, we're back to square one with the caveat of some of the discussions about what are the tumor mutation

[00:14:00]   burden, for example, that you can correlate out of that, and there are some actually, fairly, now sophisticated methodologies to really try to understand what some of the histone marks, for example, about what's the inflammatory state of the host.

Pasi Janne: There certainly are of course, outside of those examples, obviously, instances where ctDNA isn't useful. For example, histologic transformation is gonna be very difficult to pick up on ctDNA. Even detecting fusions, as David mentioned,

[00:14:30]   continues to be a challenge. If that is a mechanism of resistance or something that you follow, I think that's a limitation. I think we still need to recognize that it isn't going to replace biopsies, not just for the example that you mention, but perhaps for multiple other examples.

Gideon
Blumenthal: Microphone two.
Speaker 3:   I just want to clarify something related to the CTC as a surrogate end point since
[00:15:00]   there's a question come out. SWOG trial did show that CTC failed to be as predictive marker when you change the treatment at a certain point, I think that's true. But that doesn't mean that CTC cannot be used as a surrogate end point for the clinical trial as a measurement of the response to drug. To my knowledge, actually, the work that validate this as surrogate end point, the work
[00:15:30]   is still ongoing. So, if you need more information so I can share more. I just want to make sure that predictive marker and the surrogate end point or surrogate marker has total different utility and a lot of predictive marker actually are not

surrogate biomarker. On the other hand, surrogate marker may not necessarily be the predictive marker.

Pasi Janne: Fair enough, thank you for those comments.

[00:16:00]
Speaker 2:   Could I just again agree again with something Scott said. I really think that despite immunotherapy, [inaudible 00:16:03] DNA will be here for specifically what Scott mentioned, and that was identification of utility. In most tumors, immunotherapy fails over the first six months in well over the majority of patients except maybe melanoma, maybe a few lungs, but nevertheless, it's been [00:16:30] shown with immunotherapy, it's been shown with every therapy that if you don't move that [inaudible 00:16:32] DNA, if you get the 80%, but if you don't move it, you flame and burn, and you can tell that in two weeks. So, I think it's gonna take a lot of useless drugs. Now, maybe you haven't got something else to go to, but it would save an awful lot of useless money.

Scott Kopetz: Yeah, I would agree. I think it could be widely applied in that center.

[00:17:00]
Pasi Janne: David, you mentioned there's an effort at Memorial to collect CFDNA on all patients? Can you talk a little bit about that and how does it get operationalized at an institutional level and-

David Hyman: It's not really all patients. We've done it universally across our clinical trial program and then we've set up a number of specific projects. The key has really [00:17:30] been involving laboratory medicine. They're the group within hospitals that tend to operate with the greatest analytic validity and scale. The other huge advantage has been the development of tubes, obviously, Streck and others that preserve [inaudible 00:17:44] DNA and are stable over some period of time and so essentially, it's like ordering a CBC. You order in a computer, the consent [00:18:00] comes from an institution-wide biospecimen consent and it's routed through the normal, clinical structures. It's extracted in an automated machine in a LIMS system in a CLIA environment. We haven't had the CLIA test yet with the exception of T790M and a couple of other ddPCR Assays, but the infrastructure, it certainly has helped with collection and scale. When clinical test become [00:18:30] available, we'll be routed as such. I think it's hard to do this with a bunch of research coordinators running around. It doesn't scale well.

Pasi Janne: It automatically is done, or it still has to be ordered by a physician?

David Hyman: It has to be ordered by a physician. It can be built into order sets that are part of protocols or routine care. Obviously, you have to make sure there is patient consent in place. But the tubes are stocked by general supply. They're available in all the lab locations, including the regional network site, so it's really been an [00:19:00] institution-wide commitment to being able to make capturing this sample as easy as drawing a CBC, essentially.

Pasi Janne:	And are you doing it serially or once in time, or again there's options-

David Hyman: [00:19:30]	It depends on the project, yeah. Obviously, I think the problem we have in the clinical trial world is that you never know the patient who's gonna be the one you should've collected on until the event's happened. We definitely overcollect. I think our view is that the cost is driven by what you do with the samples, not so much the sample acquisition. Although, clearly there is cost in the sample acquisition, the extractions and the storage, but it's really project by project. As always, it's good to have a good project and a good study design at the outset.

Gideon Blumenthal:	Carlos?
Carlos Arteaga:	I have a question for David.

[00:20:00] [00:20:30]	Your slide on AKT, had almost 100% concordance, not 100%, but close to 100% concordance between tumor presence of AKT1 mutation and detectability in plasma. I know that for other genotypes like HER2 mutations, the correlation is almost close to 100%. These are really great tumor markers. Are we at a point that we can start using the decline or not upon treatment with a targeted therapy as a way of stopping therapy in those patients where you see, don't see a drop in the titre and continuing the treatment of those where you see? That way you really am reaching for a population that is going to be really responsive and some that may not be at all.

David Hyman: [00:21:00] [00:21:30]	I resisted the urge to include data on serial monitoring in my talk, I figured that other people would and I think that's been more robustly presented in other contexts. I guess I could speak from a couple narrow examples. Actually one of the most surprising examples in the AKT story is that within the first two weeks, you saw almost complete elimination of AKT in all patients, even those that didn't respond. But those that go on not to respond rebound very quickly. You almost see, what I would describe as a pharmacodynamic effect. Again, this is a highly selected population. We clearly perturbed the biology of these tumors in ways that is not evident clinically or radiographically. Then there's innate resistance mechanisms. Again, we've found what everyone has, more or less, reported, which is, when you look at it a slightly further time point, that it correlates with outcome at a population scale.
[00:22:00]	I really agree with what you said. These patients don't have unlimited therapies and although these are population scale statistics, there are individual outliers and I'm not personally eager to take people off of therapy more quickly. I'm an ovarian cancer doctor when I'm not treating patients on clinical trials and we've had C125 forever. Really, I use that trigger scans, but I don't take people off therapy the first time their C125 rises. I think what we're really describing, in essence, in monitoring, is using this as a universal tumor marker. I'm cautious about that.

Carlos Arteaga:	Okay, thanks.

Pasi Janne:
[00:22:30]

Scott, let me ask you a related question to that. There some data published, mostly from [Alberta-Bardelli's 00:22:29] group, looking at retreating patients with EGFR directed antibodies after the loss of a KRAS mutant clone that you detect in CFDNA. Are there additional studies that are trying to validate that or is that a common approach now or can you comment on that?

Scott Kopetz:

[00:23:00]

[00:23:30]

Yes, so there's been a series of small studies including the cricket study that I alluded to at the very end of my presentation that had just taken the very simple clinical question and said, if somebody had a good response and it's been a while since they had it before, let's just retreat them in a homogeneous way and there are, indeed, responders. Then there's been efforts, now, prospective studies to actually integrate that with ctDNA, to really ask the question about, can you monitor the loss of these resistance clones? Because we, and others, have shown that, indeed, over time, they'll drop off. There is a study right now that is used in Europe that is used doing first line EGFT inhibitor and then they go on to get some other therapies in between and then the patients are being rechallenged once the ctDNA has fallen. These are still single arm type of efforts at the moment, but I think pending those, we may see that move forward.

Pasi Janne:

Any other questions from our audience?

Comments from the panel?

[00:24:00]

If not, we'll close the session, we have a break now before the session four begins. Thank you, everybody.