Liquid Biopsies in Oncology Drug and Device Development Part 2

Transcript: Session II, Liquid Biopsies for Early Diagnosis

Carlos Arteaga: Good morning. Good morning. Please take your seats. We're ready to start Session 2: Liquid Biopsies for Early Diagnosis. My name is Carlos Arteaga from UT

[00:00:30] Southwestern Medical Center. We'll have three speakers today in this session, and we'll cover the spectrum of the use of liquid biopsies coming from the patients without any cancer to the patient with established cancer with the use of this approach would be to try to detect minimal residual disease before we see it clinically.

[00:01:00] Each speaker will have 20 minutes. There'll be no questions after each talk, but after that, we'll have a Q&A 40-minute session. We'll all be able to, again, have a conversation about their presentations and other aspects of this meeting. The first talk is by Dr. Anna-Renee Hartman from GRAIL. The title is Circulating Cell-Free Nucleic Acids for Early Cancer Diagnosis. Dr. Hartman.

[00:01:30]

Anne-Renee Hartman: Thank you, Carlos. I am honored to be here today on behalf of GRAIL to talk to you about early detection of cancer using cell-free nucleic acid technology. GRAIL is a relatively young company and many of you probably have not heard of GRAIL, but the scientific foundation behind GRAIL has been in development for many years.

[00:02:00] GRAIL spun out of Illumina a year and a half ago, and Illumina was offering non-invasive prenatal testing for women with advanced maternal age. In a large registry study, that Max actually viewed earlier this morning, of 125,000 women, 10 women were found to have copy number alterations indicative of cancer and upon delivery, all 10 women were diagnosed with cancer at late stages.

[00:02:30] What this demonstrates is that you could use this technology to find evidence of cancer in asymptomatic individuals, but it was going to require extensive technological advancement and large-scale clinical trials to demonstrate clinical utility for a population-level screening test, and so there was a desire to form a new company, GRAIL, with that sole purpose in mind.

This audience knows that when we find cancers at early stages, stage 1 and 2

[00:03:00] versus 3 and 4, we can significantly improve overall survival. GRAIL's vision is to improve upon existing screening paradigms such as mammography for breast cancer and also to develop early detection tests for cancers that are currently not screened, but which present at advanced stages, stage 4, and are highly lethal such as pancreatic cancer.

We know that this is really, really hard and that we face many, many challenges, so in today's talk, I'm going to talk to you about some of those challenges and

[00:03:30] how we are addressing them at GRAIL.

Why is this so hard? Well, we believe that developing this technology for an early detection application is very different than that of a liquid biopsy application. The term "liquid biopsy," we believe that you need to be able to identify a set of predefined mutations in the blood that will allow you to select therapy for an individual.

[00:04:00] For early detection, you have to be able to find all different types of heterogeneous tumors across the population for adequate sensitivity of a test. You also need to be able to filter out the significant biological noise that we know exists in the cell-free DNA for adequate specificity.

[00:04:30] We also know that current screening paradigms, while efficacious, are thought to identify a whole host of indolent tumors that may never harm an individual. Recent modeling has suggested that mammography-detected breast cancers, up to 25% of them, are overdiagnosed and may not require an intervention.

The cancer incident rate in the US, while relatively high, is still low, such that this mandates large-scale clinical trials to find enough cancer events to show performance, and in these clinical trials, we need to collect robust amounts of data to control for pre-analytical factors.

[00:05:00] GRAIL understands the magnitude of these challenges, and we're addressing it in a very scientifically rigorous way, and our approach is based on three scientific pillars: high-intensity sequencing, large-scale clinical trial programs, and rigorous data science.

We know that roughly 10 to 30 nanograms of cell-free DNA exist in a 10 ml tube of blood, but as Max and Dan have articulated, much less than 1% of this is likely to be derived from early stage cancers. Therefore, this presents a significant signal to noise challenge.

[00:05:30] We believe we need to sequence very broadly across the genome to find evidence of this heterogeneous group of cancers that would present in a population. We also need to sequence very deeply to find this faint signal that's present in early stage tumors, so we call this ultra-deep sequencing.

[00:06:00] We also have to be platform agnostic and we have to look across all the abnormal features that might be present in the blood, not just looking at genomic mutations. Additionally, we have to perspectively build cohorts, large-scale clinical trials across populations for adequate events, for clinical validation and clinical utility.

We have to be able to distinguish cancer from individuals without cancer and to be able to differentiate among different cancer types. This is really challenging and I'll be talking a little bit more about that when I discuss our ongoing clinical trials.

[00:06:30] Lastly, we have to be able to make sense of the vast amounts of information, both genomic information and clinical information, that we're obtaining. We believe that technology is really vital to our ability to classify variants across the genome, and we are developing infrastructures and pipelines at GRAIL to address this.

[00:07:00] An example of how we're addressing the signal-to-noise challenge is looking at single nucleotide variants. We believe that the presence of single nucleotide variants is going to be potentially below the sequencing noise level.

As we can see here from TCGA is we increased the number of mutations or genes in a given panel. We increased the number of variants that we can identify per tumor. We believe that leveraging this multiplicity of independent genetic variants will allow us a better chance of finding all of the diverse tumors that we need to find.

[00:07:30] We also again need to sequence very deeply to be able to find evidence of any one of these mutations that might be present across tumors, and as we increase our breadth and we increase our depth, we can increase our test sensitivity, but we are not just looking at single nucleotide variants at GRAIL. As I mentioned, we're looking across multiple platforms to look for features that can classify cancer.

[00:08:00] These are a list of our platforms. We're doing targeted sequencing of cell-free DNA, as well as sequencing of genomic DNA derived from white blood cells. We're doing whole genome sequencing of cell-free DNA, as well as whole genome sequencing of genomic DNA derived from white blood cells. We're looking very broadly at methylation patterns across the whole genome, as well as gene-specific methylation patterns. Lastly, we're looking at whole transcriptome sequencing of RNA.

[00:08:30] I'm now going to tell you about our clinical development plan at GRAIL for the development of a detection test for breast cancer using all of these platforms. I'm going to describe a discovery study, evaluation, and validation study for a breast cancer screening test.

I'm going to talk about three studies. The first two were presented at ASCO earlier this year, in collaboration with our colleagues at Memorial Sloan Kettering, looking at concordance of tumor variants to variants that were identified in the cell-free compartment.

[00:09:00] I'm then going to tell you about GRAIL's first study, which is building out a reference library of cell-free nucleic acids across a diverse set of tumors and in individuals without cancer. This is GRAIL's foundational study that's going to allow us to classify signal and drive our product development for early detection test.

Lastly, I'm going to tell you about a very large perspective cohort in a group of women undergoing mammography screening, which we are using to train and then validate a test for breast cancer to be complementary to mammography.

[00:09:30] I'm pleased to be able to share the data that we presented at ASCO earlier this year, again, with our colleagues at Memorial Sloan Kettering. This study was really the first of its kind to look at ultra-deep sequencing in individuals with advanced metastatic disease, without any preknowledge of variants that were identified in the tumor.

What this study showed in patients with advanced prostate, breast, and lung cancer is that we were able to identify the majority of variants that were present
[00:10:00] in the tumor, in the cell-free compartment without any knowledge of the composition of the tumor.

I'm going to focus specifically on our breast cancer cohort. Individuals in this study were enrolled and they had blood and tissue banked within six weeks of each other with no intervening therapy.

GRAIL's platform was a 508-gene panel that sequences to a depth of 60,000 and
[00:10:30] covers 2.1 megabases of DNA. This includes coding regions of genes, as well as non-coding regions that are implicated in cancer.

The tissue assay that we used is MSK's impact assay, which covers 410 genes and sequences tumor tissue, as well as adjacent normal tissue, and sequences to a depth of 500 to 1,000X, so variants were called in tumor tissue independently
[00:11:00] and also called in the cell-free DNA independently and then merged after to look for concordance.

What I'm showing you here is the data in breast cancer patients. What we found at a high level is that the majority of variants that were identified in the tumor tissue were also independently verified in the plasma.

What we can see here, patients across the x axis in this plot and in blue are all of the variants that were called independently, both in the tumor tissue and in the
[00:11:30] plasma compartment. What this told us is that this ultra-deep sequencing approach can be used to find evidence of cancer and advanced disease without any preknowledge of the genomic alterations in the tumor.

Additionally, in this study, we also sequenced the white blood cell genomic DNA. I think, as many of you are familiar here in the audience, there's a phenomenon called clonal hematopoiesis in which white blood cells acquire mutations as an
[00:12:00] individual ages, and this is called somatic mosaicism.

Others have shown that this is a age-dependent phenomenon, and we actually observed the same thing in this study. We saw more variants as an individual aged that were derived from the white blood cells, but importantly, we found no

difference in the number of variants that were found in individuals with cancer from individuals without cancer.

What this told us is that the somatic variants are really biological confounders.
[00:12:30] They're not being derived from the tumor, and we have to be able to account for them in the overall signal that we see in the plasma. Otherwise, you could mistakenly call them as being cancer-defining.

Using this ultra-deep sequencing approach, we're generating roughly one to two terabytes of data on an individual patient sample. We also need to come up with new approaches to be able to mine this data to come up with classifiers of cancer versus non-cancer.

[00:13:00] Traditional bioinformatic approaches have used hard filters and heuristics to call variants and abnormal features across the genome, but given the vast amount of data that we're collecting, we believe we need different approaches, more automated learning approaches that can differentiate signal versus noise for adequate sensitivity and specificity for an early detection test.

[00:13:30] We believe that our learning systems are going to be able to take all of the information that they see in a model and then distinguish cancer versus non-cancer. We've been using these approaches at GRAIL, and we've already found that they significantly outperform traditional approaches.

Now I'm going to talk a little bit more about GRAIL's first study and how we're doing and applying ultra-deep sequencing across platforms, as well as our machine-learning algorithms, to help us detect cancer at early stages.

[00:14:00] The Circulating Cell-Free Genome Atlas was GRAIL's first study, and we enrolled our first patient in August of 2016 and we're on track to enroll 10,000 participants with our collaborators in just 17 months, and this study is open at over 130 centers across the United States.

In this study, we're enrolling 7,000 participants with cancers across a diverse range of cancers, more than 15 different cancer types. We're enrolling 3,000
[00:14:30] participants that don't have cancer, but will have the same distribution to the cancer patients according to smoking status, age, gender, site of enrollment.

We are collecting blood samples, actually, eight tubes of blood on all individuals in this study, and we're applying all of the platforms that I just talked about to allow us to build out classifiers for early detection.

We're also collecting tissue on all participants with cancer, and we're doing
[00:15:00] whole genome sequencing, so we're going to use that information to help us optimize our panels for early detection.

Importantly, we're having a robust data collection on this entire cohort. For people with cancer, did they recur and what type of treatment did they have and

did they die from their cancer? Individuals without cancer, we want to know, did they develop cancer? What kind and at what stages?

[00:15:30] We are using this study to help us build out classifiers and algorithms that are going to allow us to differentiate cancer from people without cancer and importantly, to be able to say, "If we see cancer in an individual, where did this cancer come from?" We have to be able to differentiate tissue of origin for any early detection screening test.

[00:16:00] As I mentioned, we are going to be taking classifiers from this study and going further into the intended-use population to validate a test for breast cancer. I'm going to tell you about our STRIVE Study, which is our validation study for our cell-free nucleic acid test for the detection of invasive breast cancer. This is a perspective, longitudinal cohort study enrolling 120,000 women at their point of screening mammography.

[00:16:30] The goal of this study is to be complementary to mammography in two ways. One is to identify cancers that are currently missed by screening paradigms, so we call these interval cancers. They're cancers that present in between two screens. They tend to be higher stage and more aggressive and more lethal.

Additionally, we know that 10% of women that get a screening mammogram go on to some type of additional workup and sometimes, a biopsy, when in fact only 10% of those women have cancer. We believe we can add value by reducing the number of women that have to go on to this diagnostic workup.

[00:17:00] We are following the entire cohort for five years and our endpoints here are the diagnosis of invasive breast cancer, as well as the diagnosis of other types of cancers. As we can see here, in the first year, we anticipate finding 650 cancers across the cohort, as well as 650 non-breast cancers across the cohort. These non-breast cancer events are quite important because they're going to allow us to build out tests for early detection of other types of cancer.

[00:17:30] Our current partners are the Mayo Clinic and Sutter Health, and we've recently included a third partner, Sarah Cannon, to help us enroll this study on a very ambitious timeline with complete enrollment by the end of 2018.

[00:18:00] This study design is a case cohort design. This design allows us to select a random subcohort after enrollment and it also allows us to include the number of cancers that occur within, for example, here, the first two years. Using this type of study design, we anticipate only having to sequence roughly 10% of the cohort to meet our primary objectives.

This study design is different than a case-controlled design and it has many advantages. We don't have to wait for all of the cases to develop cancer and then match them on specific variables to the controls.

[00:18:30] This type of study design allows us to evaluate multiple endpoints without the need for matching. It also allows us to enroll the random subcohort without the need to wait for everyone to develop cancer.

In this study design, we're going to be using the first third of cancer events for final training and the final two-thirds for validation of a test. As I mentioned, we're collecting extensive amount of data. We have patient-reported items in the form of an electronic-only questionnaire, which is comprehensive for all cancer risks.

[00:19:00] We are also collecting imaging data, so that incudes not only the imaging report, but raw and structured images from mammography and MRI, and we're also collecting pathology reports, of course, but as well as tissue on all individuals that developed cancer.

[00:19:30] There are a couple of groups in this cohort that are getting serial blood draws. Any individual that develops invasive breast cancer in this test, in this study will get a second blood draw at the time of diagnosis, so we'll be able to look at the change in cfNA velocity from an asymptomatic state to a cancer state. There's also a group of roughly 5,000 participants that are high risk that will be getting serial sampling over to additional imaging events.

In summary, we talked a little bit today about the requirements for the development of an early detection test for breast cancer. We believe there are three important scientific components that are very important.

[00:20:00] We believe we need to have ultra-deep sequencing across many platforms to have adequate sensitivity for an early detection test. We also believe that we are going to need rigorous data science approaches and machine-learning algorithms to allow us to interpret this signal to account for both biological confounders and technical noise for adequate specificity for our screening test. Lastly, this requires population-scale, perspective longitudinal studies for adequate events to demonstrate performance of a test.

[00:20:30] Lastly, I'd really like to acknowledge all of the participants in our clinical studies who have given their time and their effort to help us on this quest, as well as all of our clinical partners who have been amazing and collaborative in working with us on these ambitious and aggressive goals and timelines. Thank you for your attention.

Carlos Arteaga: Thank you, Dr. Hartman.

Carlos Arteaga: [inaudible 00:00:00] from Memorial Sloan Kettering, titled "Minimal Residual Disease Detection in Multiple Myeloma, Outcomes and Implications"

Ola Landgren: Hi, doing well. Thank you. Thank you.

Thank you very much for those kind words. I'd like to thank the organizers for inviting me, it is a great honor being here. So I will update you on where we are
[00:00:30] in the field of multiple myeloma. The title I was assigned is to talk about MRD detection in myeloma with regard to outcomes and implications. In myeloma, the old drugs, they only provided a complete response in a very small proportion of patients that we treated. Today, when we treat patients in the clinic, 100% of our patients almost have a response, and close to 80% of patients that are newly
[00:01:00] diagnosed will obtain a complete response. This is dramatically different from a lot of other cancers. So a necessary and logical step going forward is to focus on minimal residual disease detection, and try to correlate that with clinical outcomes. That has been going on for several years in the field of multiple myeloma, and both progression-free and overall survival has been found to be associated with MRD in single studies.

In September of 2016, the first meta analysis was published, and the second
[00:01:30] analysis was published in January of this year. I will very briefly walk you through these as a background for the reason that MRD is important in myeloma. So these are the two publications, they are published in Bone Marrow Transplants and JAMA Oncology. So the first paper is a systematic search for trials using the standard Cochrane approach. It was done in collaboration with the National Library of Medicine here in Washington, D.C. A total of 390 studies were first
[00:02:00] identified and each of these were reviewed, the abstract for each of these studies. And as usual when you take this approach, most of them turn out to not be clinical trials, for the purpose of the pre-specified protocol. A total of 374 of them were non fitting the criteria. So there were 16 trials that focused on newly diagnosed multiple myeloma who have information on MRD and clinical outcomes. However, I'm going to go over and show you that a couple of these trials don't have all the details required for a statistical analysis.

[00:02:30] The statistical analysis is, again, based on the gold standard with the random effects model, where you estimate on the scale of the logarithm, you do the hazard ratio for the corresponding standard error. And this is done on the OAR statistical platform. Upon careful review of these 16 trials, 7 of them were clinical cohorts that did not provide information on hazard ratios and corresponding
[00:03:00] confidence intervals between MRD and PFS and overall survival. Also, looking through the literature very carefully, there were actually four papers that were published on the same cohorts in different ways. And there was one of these studies that did not provide timing on the MRD analysis. So these had to be excluded.

So here you see the same information in a graphical manner. So very similar to how most large meta analysis are done, you go through large numbers, you go down to a small number, and then you end up with very few.

[00:03:30] So there are four studies with information on MRD status that do have hazard ratio and progression-free survival included in the final analysis. For three of the four studies there is information on overall survival, but only two of the studies

had people dying, so only those two studies could be used for the purpose of studying overall survival correlations.

So here are the main results. The meta analysis showed that in newly diagnosed
[00:04:00] multiple myeloma patients, for those patients who obtain an MRD negativity, they had a hazard ratio of 0.35 or 65% reduced risk of progression. There were three of these studies that were based on multi-parameter flow cytometry, and one of them used a little specific QPCR, and they all lived up to the sensitivity of one cell in 10,000 or 10 to minus 4. The largest study was a Spanish study,
[00:04:30] carried 60% of the weight in the total estimate, while the smallest study, by Cord et al., done here in Washington DC at the NIH, only carried 2% weight about because of the sample size. So here you see, again, the overall results with the random effects model showing hazard ratio of 0.35, and you also see the hazard ratios for the individual studies.

As I pointed out, there were three of the four studies that had information on
[00:05:00] overall survival, but only two of these studies, the two Spanish studies by [Paivan 00:05:02] and Mateos, had people dying on the study, so they were the only two studies that were used for the purpose of studying overall survival. And the hazard ratio was 0.48, again highly significant, and here you see the same thing in a graphical manner.

So, as I've showed you on multiple slides, these meta analyses found MRD negativity to be associated with both better progression-free and overall survival
[00:05:30] in newly diagnosed multiple myeloma patients. And a second analysis that was published in January of this year shows virtually identical results. That had a few extra studies included.

So what are the implications? And that's what I'm going to spend the rest of my time talking about. So the implications are already out there, they are happening in the field. In the mid 2016 the International Myeloma Working Group criteria
[00:06:00] included MRD as part of the response criteria. And these are the criteria that all clinical trials are following around the world. So, in multiple myeloma beyond complete response, we can now talk about MRD, and we have to first make sure the patient has reached a complete response, and then secondly, per these criteria, a bone marrow biopsy has to be done, and you have to use an assay that can detect one cell in 100,000 or 10 to minus 5. It could be either sequencing or
[00:06:30] flow-based, and if negative you can declare MRD negativity. Obviously there was a lot of fine print, how many events you have to go through, et cetera. But you have to have an assay of ten to minus five.

So what are the regulatory implications? I will go over regulatory and clinical implications. So the regulatory implications, I think are very important. The median PFS, and the median overall survival for a younger patient diagnosed with multiple myeloma in the United states is five and ten years respectively. I do
[00:07:00] think a young patient treated today probably could live 15, 20 years with myeloma, which is very different than when I started my career. Patients used to live for three years. This has changed so fast. So there is urgent need for

surrogate endpoints to facilitate drug development, and of course to speed up patients' access to new drugs.

So this is from the book of the regulatory documents, by the federal government.
[00:07:30] If you have a surrogate endpoint, it could lead to accelerated approval, and then you could provide information on progression-free survival and then you could get your regulatory approval. Or overall survival also, but that's probably going to take a very long time. So how do we get there? And we have been walking around the elephant in the room for a long time. I think these are the key questions that we are still talking about, and I do think we are going to get there very soon in myeloma. We are working on this together with collaborators.

And these are some of the key points that were published earlier this year in
[00:08:00] JAMA Oncology by the FDA group by Nicole Gormley, Ann Farrell and Rick Pazdur. So as pointed out in this paper, the level of MRD sensitivity is prognostic, so if you go beyond ten to minus five, you have better PFS, so that needs to be sorted out in a little bit more sophisticated way. Also, we need to see how MRD
[00:08:30] plays out in the newly diagnosed versus the relapse setting. What's the role of the biology of the disease? What's the impact of the timing on when you check MRD? If you do it at complete response, as the clinical criteria states, you will inherently build in bias in studies for patients who have different duration of therapy until MRD. So, maybe you need to have some landmark analysis, you probably do. And also, if you want to take it to a regulatory endpoint, you
[00:09:00] certainly need to have individuals' survival and sensing times. You need to have individual patient data, not only hazard ratios, as this meta analysis shows.

There are already clinical implications of MRD testing in myeloma. At our center, and we treat a lot of patients, we have several clinical trials, focusing on MRD, and I know that's true around the country and around the world as well. So I give you a couple of high points. This paper was published very recently, and we were collaborating with Dana-Farber, the FDA, and the NCI, and other centers around
[00:09:30] the country in this paper, which outlines pretty much the roadmap going forward for how to use MRD in myeloma. In this paper there are four different scenarios. So, they are very provocative but I think a lot of this were probably going to happen whether we like it or not. MRD negativity, after combination therapy, the hypothesis here is that if a patient reaches MRD negativity early, maybe that
[00:10:00] patient doesn't need to do a transplant. The patient who undergoes a transplant and now is negative afterwards, maybe there is no need for consolidation therapy and the patient can go straight to maintenance.

A patient who has been on maintenance for a long time, and repeatedly is MRD negative, could you stop maintenance? And for a patient who is not a transplant candidate, who is currently treated with just continued therapy, if that person again is negative over an extended period of time could therapy be stopped. So
[00:10:30] these are just four obvious questions that patients are asking us every day in the clinic and there are a lot of trials looking into this right now. The answer might be no, in different settings, but the field is moving very fast in this direction to try to understand this a little bit better.

So what else is out there that I would like to share with you today? There are actually a couple of papers in the New England Journal and as we all know here in the room, everything in New England Journal is always right, so let's look at a
[00:11:00] couple of papers. So, there is ongoing prospective data showing that MRD negativity is more important than the treatment. That's a very bold statement. So, that's my extrapolation from this New England Journal paper. So this is a newly diagnosed setting. Patients were randomized between ... First they got three drugs: Velcade, Revlimid and dexamethasone for three cycles. And then they were randomized between continued such therapy for five more cycles, or
[00:11:30] to do a transplantation and get two more cycles. And patients in the blue box, they collected their stem cells so they could do a transplantation later if they needed so. Both the groups were put on a lenalidomide maintenance for one year. And the study was designed to look at progression-free survival.

And it was initially presented at the big American Society of Hematology meeting, and half the people who saw this presentation heard that the transplant won and half heard that it lost, so how could that happen? So, I'll
[00:12:00] show you why. So the people who heard that transplant won, was because they listened to the primary objective of the study. So the primary objective of the study was to look at progression-free survival at three years. That was 61% for the transplant arm. That's why I circled that. It was 48% for the other one. So, how could you not hear that transplant won? Well I think the people who heard that transplant lost, were the people who asked themselves "why is that?" So "why is that" is because there are more people reaching a complete response, but not only that, there are also people going into MRD negativity.

[00:12:30] And there was a second presentation at this American Society of Hematology meeting when this was first presented, where they actually had broken down the results by MRD status. And as you can see here, for patients who were MRD negative on both the two arms, they have very similar progression-free survival. There are more patients reaching an MRD negative state on the transplant arm, because that was the more powerful regimen. But not very, very many. I think it was about 30% versus 20%. Something of that nature.

[00:13:00] Also, there is information in the relapse setting. I mentioned to you that a couple of years ago, the average survival for myeloma was only three years. That's overall survival. So, what you're looking at now is the setting of relapse myeloma, and I'm showing you that patients can actually reach MRD negativity even in this setting, which is completely new. This is the first time ever published.

Another large phase three randomized trial here, randomizing between the anti-
[00:13:30] CD38 monoclonal antibody daratumumab in combination with lenalidomide dexamethasone, versus control arm lenalidomide dexamethasone patients with one or more prior lines of therapy. The overall progression-free survival between these two groups at 18 months was 78% versus 52% and this led to the FDA approval for the combination with daratumumab Revlimid dexamethasone in

[00:14:00] this setting. Again, if you look at MRD status, you will see very similar patterns as I showed you in the newly diagnosed setting.

Here you see MRD broken down by sensitivity: ten to minus four, ten to minus five, and ten to minus six. All this MRD data was generated by VGJ sequencing and this is based on statistical thresholds. The assay can do ten to minus six, and the reason they did it this way is that ten to minus five is from the International
[00:14:30] Myeloma Working Group guideline I showed you earlier. So if you stick to the guideline, 24.8% of the patients in this relapse group actually reach MRD negativity, which is unheard of up to this paper. Here you see MRD negative-positive for the two arms, and this is a slide I received from my friend in France, Ervay [Avelouisson 00:14:46] and I split it up in two, he had put the orange and blue in the same but I did it in two panels to show you how it's very similar to the other study I showed you before.

[00:15:00] So, again, is MRD more important than the treatment modality? I put a little question mark there, so I don't upset everybody here in the room. So what have we been thinking about at Sloan Kettering? So, we obviously have been thinking about a lot of these things also. So first of all, we know in myeloma you have IGH translocations in about 50% of patients involving partner oncogenes. You have 4-
[00:15:30] 14, 14-16, 14-20 et cetera. You also have copy number variations involving mostly the odd chromosomes, odd number of chromosomes. This has been known for 30 years with cytogenetics in fish. What's much less known in this disease is the somatic mutational landscape. There are several studies that are coming out now, but it's not really very well known, and for sure not well known in the context of MRD.

So we have developed a targeted exome sequencing assay to try to address this.
[00:16:00] So we have looked through all the mutations that have been captured in more than 2000 patients, and tried to make sense of this. And we have honed in on those who have a prevalence of 2% or more. Also we have focused on those genes that are in pathways for where we have the main drugs. So we have developed the assay My Type, which basically can replace fission cytogenetics.
[00:16:30] We have precision baits throughout the genome so we can capture the entire IGH locus, and also throughout the whole genome so we can capture all the gains and losses that I mentioned that you would normally pick up in fission cytogenetics. In addition, we also have, in shrinking the list of 2000 down to about 120 genes.

And to give you some high points, these are the frequently mutated genes I mentioned to you with the prevalence of 2% or higher. We have genes in the NF-kappa-B pathway, there are other treatment targets such as proteasome subunit
[00:17:00] steroid receptors, there are immune targets, BCMA, BCMA related, CD-38, and our checkpoint targets. And also there are genes with susceptibility.

Other things that we have been thinking about is obviously what this meeting is really all about, to try to understand capturing low levels of disease. I think the myeloma field is just, basically, around this time, moving from ten to minus five

into ten to minus six. There are many papers coming out. The data shows that
[00:17:30] there is a big difference in terms of progression-free survival if you go from ten to minus five to ten to minus six. So I think it's going to be necessary to go from flow into sequencing and other types of assays. What we have done, we have set up a real-time lab so we can actually track the VGJ sequences, and here is one of my patients. For those of you who are familiar with this, you know you generate all the sequences for all of the B-cells. For those of you who are not familiar with
[00:18:00] this, all the B-cells have their unique signatures. And this very sequence that I've highlighted here in red represents about 50% of the total sample here that was sequenced, and it easily stands out here so we can see it.

Now, if we treat this patient, and you adjust the y-axis accordingly, now you start seeing all the sequences for the normal B-cells, so does this patient have MRD, yes or no? And this is only 2% but because you know the sequence you can still see that it's there so this patient obviously is MRD positive. So we are trying to
[00:18:30] understand the dynamics of this, and we are basically doing this as our standard of care for all the patients. And just as a point of reference, I think we had about 600 new patients coming last year, so we have a pretty big database.

We are also working in the peripheral blood. We are using MALDI TOF mass spec which allows us to track fragments of light chains and heavy chains. And this is a
[00:19:00] pretty cool technology, because it's only ten seconds read. You only use 20 microliter of serum, you can also use urine. It's not very specific, and it has a lot of drawbacks, but it is very high throughput. Here you see on the left, an immunofixation electrophoresis gel from one of my patients, IGG-lambda, and you can see there is a little kappa band also. And here you see the readout with this MALDI TOF mass spec.

[00:19:30] Beyond the fact that it's fast, it's probably hundred to thousand times more sensitive than conventional technologies that are used around the world for the purpose of tracking of patients. And also another very important aspect is as we start moving into the use of monoclonal antibodies, when you give antibodies to patients with myeloma, you give them the same type of marker that we use for monitoring the disease. So it's like treating prostate cancer with a drug that contains PSA and then you follow PSA. That's what happens when you give these
[00:20:00] antibodies you see the antibody in the blood and you don't know if it's drug or disease. This technology can take care of that because you can see the molecular weight of the protein.

And the last slide I want to show you, is that we're also working in the laboratory and here is a immunoPET assay that we have developed. On top you see two mice we have injected with OPM-2 cell lines. On the right you see with BLI 24 hours how the myeloma is growing and the lower right there is no myeloma. And
[00:20:30] then we have taken radio label CD-38 and it lights up on the left as you can see while it is negative for the other. And we hope to get into patients probably in the coming few months with this approach.

I would like to thank all my collaborators at the myeloma service and throughout Sloan Kettering and our external collaborators, as well as our funding and support from NCI, FDA, MMRF, IMF, and Leukemia Lymphoma Society. Thank you so much for your attention.

Carlos Arteaga: Thank you, Dr. Landgren. Now, moving from liquid or mushy to solid tumors. The next talk is by Dr. Nick Turner from the Royal Marsden Hospital in London. Residual Disease Detection in Breast Cancer: Implications for Solid Tumors. Nick.

Nick Turner: [00:00:30] Thank you very much, Carlos, and thanks very much to the organizers for inviting me to talk about detecting residual disease in breast cancer and other solid tumors. Here are my disclosures.

I'm going to talk about residual disease detection with circulating tumor DNA, which is a topic that Max has already touched on. I'm going to give the background of why this is important, especially in breast cancer. Current data in using ctDNA to detect residual disease in solid tumors, and then discuss the challenges in a similar way to Ola has just done for future clinical trial designs, [00:01:00] using residual disease detection.

Now, why is this important? Now, I'm a Brit, so I've got U.K. statistics for breast cancer here, but it's, of course, the same in the U.S. This is breast cancer over the last 40 years, and this is the rare incidents and mortality rates per population size. As we know that the incidence of breast cancer has gone up for a variety of societal factors, but because of substantial advances in treatment, the mortality [00:01:30] has been going down substantially. That means the vast majority of ladies with breast cancer are cured by their current treatment, and we need much better ways at trying to identify which ladies aren't cured by their current treatment.

This improvement in treatment presents a real problem for clinical trial design as exemplified as, perhaps, the most important Phase 3 to come out in breast [00:02:00] cancer this year, which is of the HER2-targeting antibody pertuzumab, which required a Phase 3 study of almost 5,000 women because we've made substantial advances already, and we have a real challenge in showing improvements on what is already very good for these women with previously poor prognosis: HER2 positive breast cancer. We clearly need better ways of identifying who's at risk of relapse.

[00:02:30] This comes onto a data with residual disease. I'm first going to show you our data in breast cancer, and then come onto other tumor types. We did a Proof of Principle study with 55 women who all presented with early stage breast cancer, who were going to have standard neoadjuvant chemotherapy and surgery, and then they had blood samples for residual disease detection out into the adjuvant [00:03:00] setting. We sequence the tumor to identify mutations specific to that patient's tumor, and then we, in this study, used digital PCR to look for those mutations in the blood samples out in followup.

[00:03:30] Now, first of all, blood sample at original diagnosis ... Well, of course, the breast tumor is still inside you, has a pickup rate of about 70% for the techniques we used, and is not greatly prognostic for recurrence. The level of circulating tumor DNA, at this point, is very different between different tumor subtypes. This is, obviously, telling us something about the ability to detect circulating tumor DNA. For triple negative breast cancer, the pickup rate is high, and much higher than that with AR-positive HER2-negative breast cancer, which is a more indolent, less proliferative subtype and is clearly more challenged for sensitivity of circulating

[00:04:00] tumor DNA assays, clearly telling us that the ability to detect cancer and residual disease will differ between different subtypes of cancer.

[00:04:30] Of course, what we then find is when we look in the blood samples after the patients have completed their treatment with chemotherapy and surgery, detecting circulating tumor DNA at that point has a very high risk of recurrence, both disease-free survival and then going on to overall survival in this single post-surgical time point.

[00:05:00] In this single post-surgical time point, after the patients have relatively recently finished their chemotherapy, you can see you miss a lot of patients with the assays we used, but we built in serial sampling into the study. If you take serial sampling, you much more robustly pick up those ladies who are going to relapse. In this Proof of Principle study, we had a 100% positive predictive value, so all those ladies who we picked up ctDNA out in followup subsequently went on to relapse, and they also had a very high risk of death from their relapse.

[00:05:30] In this study, our lead time was about eight months, so median lead time. Then illustrate here with one lady, it actually had a pathological complete response with HER2-positive breast cancer. We were unable to detect ctDNA immediately post-surgery, presumably because there was very little residual disease, but she then subsequently had [inaudible 00:05:43] a year from detecting ctDNA to her relapse.

[00:06:00] What about other tumor types? Now, Max has shown you this before in his study, so we've got from Bert Vogelstein's collaborating with an Australian group in colon cancer, again showing very similar characteristics to what we see in breast cancer, a very high risk of relapse for those who had post-operative ctDNA using an amplicon-based assay for circulating tumor DNA. Then, this is the data that Max showed you earlier from his group, using a hybrid capture, error corrected ctDNA analysis in patients predominately with Non-small cell lung cancer.

[00:06:30] We've really got a number of all relatively small studies that have shown this, but the common features are coming out quite clearly of using circulating tumor DNA assays, that there appears to be a very high positive predictive value. Those patients who have circulating tumor DNA detected appear to have a very high risk of going on to get future relapse.

[00:07:00] There appears to be a lower negative predictive value, at least where you look for just single post-operative samples, so we have incomplete sensitivity for detecting very low levels of residual disease with our current generation of assays.

Potentially, serial sampling improves that, but as Max showed as his talk, this is clearly a need to go on and develop more sensitive assays than our current generation of assays. I think this, of course, is important where you have curative
[00:07:30] adjuvant therapy, that perhaps the current generation of assays aren't there to start taking away curative therapy from patients.

Now, let's just move on to looking at how we can use ctDNA to guide further therapy. I just want to touch on a few of the challenges of taking this forward into clinical application, to then coming on to clinical studies. There's clonal
[00:08:00] selection from primary to the metastasis. Sanctuary sites, where it's hard to pick up circulating tumor DNA, clonal hematopoiesis and then on to the clinical studies.

In our Proof of Principle study, when we detected ctDNA with digital PCR, we used high-depth hybrid capture sequencing to interrogate the genetics of the circulating tumor DNA. Why we were keen to do this, is we were keen to
[00:08:30] understand if the residual disease matched the recurrence for tumors that look like this. This is just one anecdote I'm going to show you, which is the mutations found in the tumor primary and then in the metastasis. In this individual patient, clearly there are substantial differences between the primary and the metastasis.

This illustrates one of the challenges, if you're just following a few mutations. That if you're following a PIK3CA mutation, you're going to be able to pick up
[00:09:00] relapse, but if you're following these mutations that are lost in the metastasis, clearly there's a challenge, and clearly there's a need to potentially follow either known drivers, or multiple mutations to account for this.

How about the changes in genetics that we see? Patients have chemotherapy, and if you sequence the tumor at surgery, there's clearly reproducible changes, but not those that match the metastasis. Sequencing the circulating tumor DNA
[00:09:30] eight months before the patient relapsed, you can then begin to robustly pick up the changes in the genetics and, in particular, that this lady had picked up an activating mutation in an NGF receptor gene that potentially was driving metastasis, and that's just confirming the findings. Potentially, you can then look in the MRD at which drivers are present in the MRD as a way of optimally selecting targeted therapy for those patients.

[00:10:00] Challenges. So far in our series, there are some patients who we were not able to pick up circulating tumor DNA before or at recurrence. Interestingly, three of these patients had solitary brain-only metastases. We know from the work, looking at GBM's, that the blood-brain barrier, to a certain extent, blocks release of circulating tumor DNA, so we know that we will have some challenges picking
[00:10:30] up relapses in the brain using these techniques.

Then, the final thing to be aware of is the recent discovery of quite how frequently clonal hematopoiesis is in patients, especially as going up with increasing age. This was identified from G-Wire studies, which found that although, of course, you would expect snips to have an allele fraction of 50%, there's a shoulder here that have got a much lower frequency in the G-Wires.
[00:11:00] This was subsequently discovered these are clonal expansions in the bone marrow that are essentially normal clonal expansions, because patients very rarely go on to develop myeloid malignancies, but with increasing age, it gets very common. These mutations can also be found in circulating tumor DNA. Clearly, the optimal strategies to ensure that these mutations don't present false
[00:11:30] positives have not really been defined, but sequencing the buffy coat and tracking multiple sematic mutations are clearly a way to take this forward.

I think before we get extremely excited about the potential of these techniques, we do need to pause and question whether this is definitely evidence of clinical utility, or just validity. What I mean by that is we've clearly got two very different settings for treating cancers. We've got adjuvant settings, where we treat
[00:12:00] micrometastatic disease that are potentially curable, especially in cancer types like breast cancer. We cure substantial numbers of ladies in this setting.

In the metastatic setting, where we've got frank macro metastatic disease, patients incurable. We've clearly got a spectrum here. At this time, we don't know for certain where patients who are ctDNA positive lie on this spectrum.
[00:12:30] We, of course, hope they're at this end, but we don't know for certain. Clearly, what we need before we think about implementing this in clinical practice is studies to assess whether we can use circulating tumor DNA residual disease detection to improve patient outcome.

The study that we're running at the Marsden that we're just about to get open relatively soon, I hope in November, is one of the Proof of Principle studies,
[00:13:00] where we're going to assess whether we can use residual disease detection to guide therapy. We're going to take 150 patients with triple negative breast cancer who've completed their standard treatment, many of whom will be cured. We'll then screen them for circulating tumor DNA three monthly in their first year of followup, blinded ctDNA surveillance, and then randomize them to be intervention and observation. The intervention we're going to use here is the
[00:13:30] PD-1 antibody pembrolizumab. The primary endpoint of this study is ctDNA clearance. Can we clear circulating tumor DNA with intervention?

Now in this study, we're going to do blinded ctDNA surveillance, and the randomization will also be blind. The reason for this is we feel that there's an ethical issue randomizing patients to observation when their ctDNA is positive, because if you've got an assay that predicts with high accuracy for recurrence,
[00:14:00] and ultimately, of course, unfortunately, death, it's practically impossible to tell ladies they're ctDNA positive and ask them just to do nothing. Of course, in this study we need to validate that ctDNA positive patients do relapse.

[00:14:30] That also suggests another paradigm going forwards. Do we really need to randomize to observation in these types of studies? Can we just use ctDNA clearance as a surrogate endpoint, as is mentioned in the previous study, to assess efficacy and if you've got an assay that truly is predictive for future occurrence? If we're going to use a ctDNA clearance as an endpoint, at what time point do we assess ctDNA clearance? I don't know the answer to this, but it's something we need to work on resolving very rapidly as a surrogate endpoint.

[00:15:00] The reason for that is is that we know that as tumors ... this is a patient in a metastatic setting on a chemotherapy Taxol. We know that circulating tumor DNA drops very rapidly as tumors respond to treatment and then subsequently, in the metastatic setting, they obviously progress. If we assess circulating tumor DNA very early, we might over-call the efficacy of treatments, and potentially [00:15:30] late assessment will much more robustly predict the potential to predict DFS with a surrogate endpoint.

[00:16:00] Now what about, just in my last few slides, the challenges of Phase 3 design? If we're going to use circulating tumor DNA to guide further therapy in patients in the adjuvant setting. Clearly, one potential is to take triple negative breast cancers, as we were just discussing and then randomize them to standard followup, which is no treatment for these ladies until relapse, or randomize them to active ctDNA surveillance, and when they're positive, come in with an intervention.

[00:16:30] There are two problems with this relatively simple design. One is it's incredibly inefficient and requires sample sizes potentially larger than the studies I've just shown you. Also, there's a bias against the intervention arm. These patients here don't know they're at risk of relapse when they're ctDNA positive, but these ladies do, and that's going to clearly change behavior of physicians and patients to do more scans and earlier scans. Clearly, that's a potential problem for what might be one of the initial most obvious designs to use ctDNA in this setting.

[00:17:00] Clearly, what may be a potentially better design is to use open ctDNA surveillance and then randomize patients who are ctDNA positive to interventional followup. This certainly is much more statistically efficient, but potentially requires a novel endpoint of time from ctDNA detection to relapse.

[00:17:30] There's no bias against intervention. Both patient groups know they're ctDNA positive, but really, of course, it's probably ethically unacceptable to randomize to no treatment. Potentially you could randomize to placebo, but of course, if adverse events un-blind randomization, you have the same issue. Probably the best designs are those that have standard treatment with placebo, or intervention to assess in those have ctDNA positivity, which is, of course, an appropriate design, provided that standard therapy exists.

[00:18:00] Just to conclude, ctDNA analysis has identified patients at high risk of relapse through detecting residual disease, now in multiple tumor types. Really, the question is no longer, "Does it work?" But, "How can we use it to guide therapy?"

[00:18:30] CtDNA analysis can potentially be used to identify the target of all genetic events in a residual disease that might differ from the original primary, but really working out whether ctDNA has utility in this setting is going to require prospective trials to show improved patient outcome using assays. I'll just finish by acknowledging some of the people who contributed to my own work that I've shown. Thank you. [inaudible 00:18:42]

Carlos Arteaga: Thank you, Nick. I'd like to invite Dr. Hartman and Dr. Landgren to the podium so we can have our Q&A.

[00:00:30] Before we go on, I'd like to just make an announcement communicated to me by Drs. Sobara and Srivastava of the NCR Division of Cancer Prevention. There's a request for applications - you may want to write this down - CA17-029. It's a pre-competitive collaboration on liquid biopsy for early cancer assessment with application dates of October 24, two weeks from now - I assume that's a letter of intent - followed by a final application on January 23 of next year. RFACA17-029.

[00:01:00] So I'm told by the organizers that I'm supposed to start with a few questions to get the blood pressures up, and then I can open the floor for questions for everybody. I start with Dr. Landgren. I wonder whether ... I'm sorry, no, Dr. Hartman first. And then Dr. Landgren.

[00:01:30] I wonder if you can walk us through the process of the selection of breast cancer for your Proof of Principle study in patients with no cancer. I ask this because this is a disease where, if you have stage 1, 2, 3 breast cancer in the develop world, your chances of cure extremely high, as highlighted by Dr. Turner. Now, we diagnose cancer pretty well already, and so I don't know whether the emphasis is improving survival - which may be very hard to show - or alternatively, as you alluded, sparing negative biopsies, right?

Anne-Renee Hartman: Yeah.

Carlos Arteaga: [00:02:00] You quoted a rate of negative biopsies that, I find it extremely high, of 90%. I've never heard that before, but I believe you. Is that the impetus of this, because as I said, changing the natural history of disease that is doing so well, I find that extremely challenging. But, please walk us through your thinking here.

Anne-Renee Hartman: Thank you. That's a great question. So-

Carlos Arteaga: Is that on?

Anne-Renee Hartman: [00:02:30] Okay. Yep. That is a great question. So, as you pointed out and as Nick had pointed out as well, that we do a pretty good job of, I mean probably too good of a job, of diagnosing breast cancer through screening mammography. As I mentioned, there's two areas where we think we can improve upon existing screening paradigms. So, we do know that women develop cancer in between their screenings. And as we move towards biannual screenings, so every two years - and in the U.K., for example, it's every three years and in other countries,

[00:03:00] there's not even any screening for breast cancer - we know that we will find these interval cancers that develop. And they're higher-stage and they're higher-grade. Part of that is linked to breast density, so there's a masking effect with women that have extremely dense breasts. You just can't see the cancer that is actually there. And some of it is just really fast-growing cancers that are clinically not detectable, you know, by mammography.

[00:03:30] But I think to your point, importantly, there are a huge number of women that go on to a diagnostic work-up. Not all of them are getting biopsies. So to be fair, 10% of women will have what we call an incomplete mammogram, so that's a BI-RAD zero. And they have to go on to additional imaging. That could be diagnostic mammogram and then you stop, it could be an MRI, an ultrasound, and ultimately a biopsy. So we believe that we can do a much better job in collaboration, you know, or being complimentary to mammography in reducing the number of women. And when you look at that on a population scale, there's [00:04:00] 35 million women in the United States that are getting breast cancer screening at any given time. So that's a huge number of women that go on to this, you know, diagnostic work-up with many of them ending up with a biopsy that do not have cancer. So those are really the two areas that we think we can be helpful and improve.

[00:04:30] But also to your point, there's a question of, you know, are we over-diagnosing some breast cancers? So we know, again I mentioned "Modeling Work" has suggested by Welch et al, that we are potentially over- diagnosing a lot of breast cancers with mammography. And we don't know that and it's really challenging to prove. But we believe that, you know, with careful follow-up of our cohorts over time we can begin to understand which cancers are highly lethal and which cancers are perhaps more indolent and may not require an intervention.

Carlos Arteaga: [00:05:00] And is your study designed in such a way that, if you find abnormalities, well I guess, will you be able to tell whether a tumor is ER-positive or ER-negative? You know, for ER-positive we do have a prevention approach. Thinking forward, I mean, is that something that you'll ... I guess you won't be able to pick that up on your [essay 00:05:12], huh?

Anne-Renee Hartman: [00:05:30] I mean, I certainly hope we can do that, yes. I mean, we're looking really broadly across those platforms, and we'll be building out those classifiers from our CCGA study. But we also will be looking at, you know, different subtypes of cancers, different age groups, you know, where are we good at finding evidence of these cancers. It might not be broadly across all breast cancers. We might not be able to find some of the very early stage small hormone-positive breast cancers. I think that will be challenging.

Carlos Arteaga: Okay.

Anne-Renee Hartman: But the rapidly growing breast cancers we think will be very possible.

Carlos Arteaga: Okay. Thank you.

[00:06:00] Dr. Landgren, I saw in your presentation a lot things that we solid tumor doctors can learn from the advance that you have done in multiple myeloma. I was happily jealous to see all your data, actually. So, is the myeloma field at a point where MRD, again, it is an almost perfect surrogate for survival?

Ola Landgren: You ask me if MRD is the perfect surrogate for overall survival?

Carlos Arteaga: Yes.

Ola Landgren: Well, I showed you the data from the MAT analysis. That was the second MAT analysis, also showing the same thing. So, I think those two studies clearly point

[00:06:30] in that direction. I do think, however, as we keep on going forward, it's going to be harder and harder to show overall survival patterns for early interventions. There are so many drugs, so we can just treat patients over and over again.

Carlos Arteaga: Yeah.

Ola Landgren: So to me, almost, I feel like if there is a study showing overall survival, it almost sends a signal to me that this study was done in a part of the world where access to drug was not the same as if it is in the United State. That's a very, kind of, bold statement. But I feel like overall survival is almost on its way out for these

[00:07:00] chronic diseases where we have so many drugs available.

Carlos Arteaga: Yep.

Ola Landgren: How can we keep on using that?

Carlos Arteaga: We have a similar good problem in breast cancer where it's very hard to show differences in overall survival with drugs that are combinations that are very effective. And I don't know, and this is a question of ignorance, whether there is any precedent of approvals of drugs based on MRD negativity, and whether that is a surrogate that regulators should consider.

[00:07:30]
Carlos Arteaga: Gideon, do you have a-

Gideon Blumenthal: [inaudible 00:07:33] able to that MRD and CML, that would be one precedent. BC are able a major molecular response at 12 months.

Carlos Arteaga: Okay, so there is a precedent for using this type of approach indepen- Okay, great. Okay. Well, thank you. And in your ... I mean, clearly some tumors, some myelomas, are still breaking through after 15, 20 years. How different are they

[00:08:00] from the original tumor? Do we know that? I assume they are going through some type of Darwinian block so I assume they have to evolve.

Ola Landgren: There is actually not a whole lot of detailed data. If you look at available information out there from smaller series, there are indications that at presentation the average patient presents with five to ten subclones of myeloma the first time you set the diagnosis. And if you quantify the different subclones as
[00:08:30] proportions of the total tumor burden, as you put pressure on the disease you can shrink it down to complete response for MRD negativity. If it comes back in the relapse setting, the distribution between these different subsets now is skewed. And there are some of them that don't seem to reappear, so maybe we actually can cure some of these subtypes. But, eventually, based on small series,
[00:09:00] the subclones that seem to be the lethal subclones from these small studies I'm referring to, they were actually less than 1% of the total tumor burden to begin with. So that probably is a major clonal selection. And I'm sure that the puzzle is probably much more complicated as we start looking into more and more patients. These are very small series.

Carlos Arteaga: Okay, thank you. Thank you. And Dr. Turner, that was a very elegant discussion of all the caveats and the difficulties of doing clinical trials in this setting, patients
[00:09:30] with MRD. And I like the point that you sensitizing how difficult it is to randomize a patient that is MRD positive to no treatment. That'd be very difficult. But you also showed a slide where patients post-triple negative breast cancer need [inaudible 00:09:48] therapy and surgery, I presume, that they would be randomized to follow up with a plasma tumor ctDNA and they'd be randomized
[00:10:00] to [inaudible 00:09:59] versus observation. But-

Carlos Arteaga: [inaudible 00:10:01] observation. But I assume that in the observation patients, you would have to follow them with some periodicity for clinical relapse. What type of follow-up you do on those? Because you're worried about them recurring clinically at any time.

Nick Turner: So I [inaudible 00:10:21] have standard follow-up, which, as per all international guidelines, is standard clinical follow-up without imaging.

Carlos Arteaga: Without-

[00:10:30]
Nick Turner: Yes, so, obviously, within the study individual investigators can do imaging if they wish, but they have standard follow-up in that individual study.

Carlos Arteaga: Gee, wouldn't that be complicated. Because I assume that the average physician is going to just get up, Pet CT every three to four months. Some patients don't, so-

[00:11:00]
Nick Turner: I don't think that's a problem, from a perspective of our study. In the observation arm we're just keen to reconfirm in a perspective study, that the ctDNA detection goes on to relapse and how individual people follow them up in that arm. Is not really a great problem, because we know once disease is detectable on a CT PET, unfortunately that lady is no longer curable.

Carlos Arteaga: Correct.

[00:11:30]
Nick Turner: So what we're keen, though, is do we need to have done that randomization? What we hope to demonstrate, if genuinely all ctDNA positive patients go on to relapse, and they don't spontaneously clear their ctDNA. Actually the phase two paradigm in this setting is to offer the treatment to the patient, and show you can clear ctDNA and ultimately, of course, the-

Carlos Arteaga: I think that endpoint is very clear. It's very clear that you would achieve it, but if
[00:12:00] you also want to answer the questions "What's your lead time between MRD detection and clinical detection?" That randomness of clinical follow-up may not allow you to answer that question. But I presume that would be a different trial. And the other thing is that you showed a patient that had a pathologic complete response to [inaudible 00:12:20] therapy. I tend not to worry too much about those patients, because they complete [inaudible 00:12:26] and usually I tell
[00:12:30] them that the odds of cure are very high. But you show that even that patient had a molecular relapse. I guess that's a very rare occurrence, I presume.

Nick Turner: Yeah, so obviously there's a negative association between path CR and the ability to detect ctDNA as for relapse. But of course path CR is just a relative improvement in outcome, not an absolute cure. And so, just [inaudible 00:12:56]
[00:13:00] with one example, if you like, that actually some of these ladies do go on a relapse, and you can't pick that up with ctDNA. It's just you don't want them in your initial studies, because the event rates too low-

Carlos Arteaga: Correct.

Nick Turner: But potentially, in the fullness of time, that really might be where the real indication for this is. Patients who actually have done really very well on their standard therapy, and you want to identify those who need further adjuvant therapy, who otherwise, you're just not going to have any realistic possibility of
[00:13:30] treating them with further treatment.

Carlos Arteaga: And have you had any opportunity to biopsy tumors? Clinical recurrences, that are CTDNA negative? What could be going on ... I know there are few of those, but what could be going on in those? They are driven by things that are not somatic alterations, so they are just not leaking what's going on?

Nick Turner: So some of it could be, especially when you're only following a few mutations, as
[00:14:00] I've discussed, that you've just lost those mutations in the recurrence. And so far we've had only a few. We've only had two where we've got biopsies and they have got the mutations we're tracking. They're just not releasing sufficient circulating tumor DNA to pick up with the technique we're using. And of course, what you would hope is, as you move to the kind of techniques where you're
[00:14:30] following very large numbers of mutations, you then would be able to pick up them where techniques when you're following only a few you can't.

Carlos Arteaga: Also you detect ... You made a point that one gets more information from serial sampling that only one time spot. One time look. Does that mean that some tumors become negative? Some patients become negative, and some others retain the positivity?

Nick Turner: So without a change in treatment, we so far have not seen any patients become negative-

[00:15:00]
Carlos Arteaga: Negative.

Nick Turner: Once they've been positive.

Carlos Arteaga: Once they're positive.

Nick Turner: With the current generation of assays, once you pick it up, I think what you're doing is you're picking up proliferating tumor cells, and they unfortunately just keep proliferating.

Carlos Arteaga: They don't go on holiday, guess?

Nick Turner: Yeah.

Carlos Arteaga: Okay. All right, well thanks a lot. Questions from the audience, please? All the mics are open, one, two, and three and four.

Speaker 1:
[00:15:30] Thanks. Thanks for the really beautiful presentations. I think one question that I have, in thinking about MRD detections. So after a patient has had cancer, is the personalized detection assay versus a all-comers targeted panel sequencing assay that was described earlier, and would love both your thoughts, Dr. Turner and then obviously Grail is taking sort of a broad approach as well, doing genome level sequencing as well as targeted panel. So would love both your thoughts on the utility of those.

[00:16:00]
Nick Turner: So it depends on the tumor type, and it depends on mutation load in your tumor type. So if you're treating lung cancer, there with a very high mutational load, actually you can get quite a large number of mutations to follow with relatively small panels, which are then cost-efficient to go and sequence. But if you treat breast cancer with a much lower mutational load, then in some tumors pretty
[00:16:30] low, actually using a generic panel to identify enough mutations is really tough. And so, clearly, it depends. Different tumor types, different mutational loads, match a bespoke panel or a generic panel. That's what I think we just need to learn where the cutoff is, to whether you need a generic or bespoke panel.

Speaker 1:
[00:17:00] I think that makes sense. And your thoughts on the scalability of personalized MRD detection, going forward?

Nick Turner: I think if you can use generic panels as Max has shown in lung cancer, potentially it's incredibly scalable. I think it's not insurmountable, but there's more challenges if you need patient specific panels. But I don't see that's insurmountable, if they're really impactful for outcome.

Speaker 2: [00:17:30] Dr. Haber? I have another question, to follow up on next points, which is, if you imagine, from a conceptual point of view, we don't cure metastatic cancer because there are just too many cells. You have billions of cells, so resistance to targeted or chemotherapy is preordained, if you will. We cure patients with adjuvant breast cancer, because there are fewer cells. So your example of using [inaudible 00:17:48] immunotherapy doesn't quite fit that as well. But if you were to go for targeted or chemotherapy, could you cure the patients when they have the earlier sign of recurrence by MRD? So my question is have you ever [00:18:00] modeled the numbers of cells? How many log fewer cells do you have when you first see MRD, and is there a chance then that either targeted or chemotherapy could be curative at that early setting?

Nick Turner: [00:18:30] So we're also going to do a study in ER positive breast cancer with targeted therapies, which, perhaps, Daniel gets at your first criticism. Although, potentially, even for immunotherapy, tumor bulk is important, and actually it's reasonably clear in metastatic setting that latter lined therapy, the IO agents have less activity than front line. So I'm not sure I would absolutely accept that trying to bring the IO agents further forward in their case, is not potentially beneficial. I think it's really hard to model. We just don't know, in reality, what the proliferative rate of tumor cells is. To really confidently model back the [00:19:00] number of cells that were there at the point you detected CTDNA. But I agree with you, I think it's the key issue. Are you really identifying tumor at the point where it's still potentially curable with CTDNA, as I brought up in my talk. And that's, of course, why you have to have clinical trials showing patient outcome before you would think of implementing this in the clinic. Because we know protein tumor markers don't work, we know that doing scans doesn't work in [00:19:30] picking up disease early enough at the point where you can cure it. And what we hope is with CTDNA assays you can, but that's what we have to demonstrate.

Carlos Arteaga: Number one, please.

Speaker 3: [00:20:00] Yes. Well I hope you don't disagree with me here, but this MRD studies, every publication I've seen has got a very short follow-up. In other words, all these recurrences that they're documenting, it doesn't matter whether it's

Speaker 3: [00:20:30] Dr. Turner's study. The recurrences which are occurring predominantly within the first year. This is not typical recurrent patterns. I would even question if they staged this patients aggressively with pet scans where they're really stage 4 to begin with. So this is not our usual biologically recurrent disease. So it really is a question, since we all know the patients who recur early do very poorly with systemic therapy. Dr. T pointed out in her rectal studies that those patients with

residual selfrie DNA of the completion of chemo and radiation therapy, chemotherapy did not make a difference.

[00:21:00] Now I like Dr. Turner's study because he's not going to try chemotherapy because I doubt chemotherapy will have an impact on these patients and maybe hemotherapy will. I would like to make a comment that keeps coming up about the surveillance. Clearly surveil intensified surveillance does not increase cure rate. This was just reported to the ESM. Oh a randomized study of [inaudible

[00:21:30] 00:21:22] race to CT scans and in fact, the intensively surveilled patients did worse. The reason is simple. It's a length/time bias. Those extra cancers you pick up are biologically where we're picking up with this MRD. They're a different beast and you don't necessarily cure them. You may resect them but in fact you may be doing the patient worse because you're resecting more [inaudible 00:21:48] than are biologically should be resected.

I'm sorry to hog the microphone.

Carlos Arteaga: Do you have a question?

Speaker 3: What?

Carlos Arteaga: Do you have a question?

[00:22:00]

Speaker 3: Well I'm curious what Dr. Turner's response is to the MIT.

Nick Turner: I think to a certain extent just saying what I said, just in a different way. Which is we don't know if you're picking up disease that you can materially effect. That's why I say you need to have therapy to trials to assess that. Without I, you can't have certainty video identifying. So I agree with your point because I think you're

[00:22:30] just expressing the same point in a different way.

Speaker 3: Can I ask Dr. Hartman, when you showed us that concordance, was that only the breast cancer subsid of your collaboration with Memorial?

Anne-Renee Hartman: Yep. That's correct. We had a total of 124 patients but that also included those with long prostate and breast cancer. But what I showed you is the 39 patients with breast cancer in that cohort.

[00:23:00]

Speaker 3: Predominantly, what stage were they?

Anne-Renee Hartman: They were all advanced stage. So they were either de novo metastatic or progressed on therapy.

Speaker 3: Am I correct in saying your sensitivity for advanced stage breast cancer with predominantly clonal mutations, not sub clonal, is really no better performance

[00:23:30] than we have seen many of the commercial companies. It wasn't in the 70%-80% positive agreement?

Anne-Renee Hartman: Yeah so of all of the variants, so it was roughly 74% but if we're just talking about detection, we found 97% or 38 of the patients. We found at least one variant that was derived from the tumor.

Carlos Arteaga: Number four please.

Speaker 4: [00:24:00] Yes. I'd like to come back to your question a bit around the personalized diagnostic versus larger panels. Well first of all, there's the whole regulatory pathway to take into account. My question is around the fact that here we're talking about serial sampling and so there's initially, particularly with using these large panels if that's the only practical way for the cost. So how do you decide how are we going to deal with this ultimately in terms of the burden to the [00:24:30] health systems with earlier and earlier detection and keeping them on essentially falling patients. So what's your opinion on these tools which are great and phenomenal but then ultimately there's a burden in terms of when you're looking at these large panels right now, the cost that they are going to be at from different companies.

Nick Turner: [00:25:00] I'm slightly worried. I'm not sure I have much more to say than I did to the previous question on this topic. I wonder if there's other experts like Max in the field who've thought about this a lot. If something's truly impactful and it helps you cure patients, it can be incredibly cost effective. So I'm not sure at this point we should be that concerned about that because curing patients of cancer is incredibly cost effective. It's all about how impactful it is. Max, go.

[00:25:30]

Max Diehn: Yep. So I very much agree with Nick's answer. I think mostly what Nick is talking about with the question is MRD detection which could be theoretically at one time point post treatment and maybe that that's enough. I think if we're thinking about doing surveillance we will have to do studies to actually prove that A it's beneficial, has quite a utility and B is cost effective. I think it could be too expensive but as Nick says if it's clinically useful, then we will do that calculation. I would also encourage research in the area to get that answer and not to just [00:26:00] say it's too expensive and therefore we shouldn't.

Anne-Renee Hartman: I would also just point out that moving towards and early detection test in a population scale level, the cost has to be relatively low right? Similar to those screening tests such as mammography or LDCT that are currently in use. AT least that's the way Grail is viewing that approach.

Speaker 4: Thank you.

Carlos Arteaga: Nick, please.

[00:26:30]

Nick Turner: Could I just ask a question? So I'm a breast cancer doctor. I'm delighted that Grail is focusing on breast cancer but when I think of the chemotypes where early diagnosis really make a difference it's lung cancer and pancreas and upper GI. So if it's just a question really, how is Grail going to demonstrate and address those points as well as breast cancer?

Anne-Renee Hartman: [00:27:00] So that's a great question. So of course we're interested in lung cancer. It's the number one cancer killer world wide. So we are looking at that as well. Also pancreatic cancer. You have a challenge with pancreatic cancer that we could discuss in that even if you find individuals with early stage disease, you have lead time bias such that you don't know if you're going to cure them. They might've already gone through this sort of metastatic event even though you're finding them at early stages. I think the key for pancreatic cancer is finding them when they're receptable. So with pancreatic cancer, if you find them at stage one I [00:27:30] think your five year survival, depending actually on the size, can range anywhere from 20% to 70% but it's very size dependent. So it's a significant challenge.

So Grail is looking across all tumor types and one of the interesting things about the strive cohort is that it's a general women's cohort. We're using mammography as a ... we are building out a test for breast cancer but [00:28:00] mammography is an attractive way to catch women at their point of screening and to be able to enroll them in a quick way. So that study though is going to individuals and that study will develop 650 none breast cancer events a year. Lung will be one of the highest ones. Colon cancer and then pancreatic cancer is going to require a different special effort and we're looking into collaborating with the NCI and others on that right now.

Carlos Arteaga: Number one.

[00:28:30]

Speaker 5: Yeah I have a question for Dr. Hartman. So I congratulate Grail on their goal of having their early detection. The deep sequencing makes sense with the approved bioformatics. Not easy but it makes sense. You're still limited by the fact that you can't go below one molecule of CTDNA. So do you have any ways to sort of enrich increasing the blood volume or other ways to find very very low levels of CTDNA?

[00:29:00]

Anne-Renee Hartman: Yeah so that's a great question. We are again as I mentioned looking across different platforms. We're leveraging the deep sequencing to look for low Leo frequencies but we are hitting a limit there with the sequencing depth beyond 60,000. So that's where the other sort of platforms we think can be added in in terms of prediction so that's methylation and then of course copy number alterations. I think your point is well taken. It's easy in the metastatic disease as [00:29:30] we showed where there's more copies of the genome that are present coming from tumors but it's more of a challenge in early stage disease. That is where these additional platforms are going to improve our sensitivity.

Carlos Arteaga: Number four.

Speaker 6: [00:30:00] [inaudible 00:29:50] This a question for you. Just a followup of what Nick pointed out. So when you have 120,000 cohort and you had bought 1 and 2 and then the other arm was none bio something?

Anne-Renee Hartman: Non bi-

Speaker 3: The other arm was non-bi something?

Anne-Renee Hartman: Non-bired zero, but it's essentially non-bireds.

Speaker 3: Did it include three, four, and beyond?

Anne-Renee Hartman: Yes, yes.

Speaker 3: Okay. The question is that, then you also reported some other cancers in your bi-red one and two?

Anne-Renee Hartman: Yes.

Speaker 3: What were they? What type of cancer were they?

Anne-Renee Hartman: [00:30:30] So, just as we were discussing, so we, based on SEER data, we've modeled that we're gonna obtain 600 women are gonna get ... Over the course of one year, there'll be 650 non-breast cancers, invasive, that present. So, again, lung, colon cancer, ovarian cancer, uterine cancer, renal cancer, so we'll be tracking all of those events on those individuals and we'll also be getting the second blood draw that I discussed so we can look at the CFNA kinetics.

Speaker 3: [00:31:00] [00:31:30] [00:32:00] Great, so are there ... It's a comment and I would like to hear anybody who can help me respond to the question. Any time you hear about [inaudible 00:30:59] you hear so called improved survival. In many ways it's misleading because there's a lead time bias. And mostly my friends who are expert at randomized trials, they'll ask me, well you haven't shown me any benefit, benefit in a sense that mortality deduction. So, I usually say that all it detects [inaudible 00:31:24] would be expanded to include any device or biomarker that can also reduce number one; over-diagnosis, number two; unnecessary biopsy, and also number three, which can also solve which of the lesions are likely to progress, progressive versus non progressive. And all of these have embedded cost benefit. Not really mortality benefit, but cost benefit unless we randomize [inaudible 00:31:53] we won't be able to say. This is what I struggle to defend [inaudible 00:31:58] because we haven't done any biomarkers or genetic biomarkers, randomized to show the deduction, mortality deduction. So I'd like

to hear anyone else that can help me respond in a better way of what all the detection means.

Anne-Renee Hartman: I'd might be able to add ... One, I think you're making some really great points and that you do need to do these randomized control trials to look for cancer specific mortality. I think looking at cancers that are currently screened, where

[00:32:30] there's an existing paradigm that has shown a difference in cancer specific mortality with the intervention, or the paradigm, is a good way to see how we can add value, not only to reducing all of the people that go on to additional biopsy but find cancers that are currently missed.

One of the things I didn't talk about in this drive study is that there are ... A lot of

[00:33:00] women going on to get additional work up right now, outside of screening mammography, because they either have dense breasts or they have a lifetime risk of greater than 20% so we're using MRIs and all of these expensive interventions to try to look for cancer. So we're also going to be looking and quantifying that in our study. What is the use of additional imaging modalities outside of mammogram for these women and how many of these women end up having cancer diagnosed with these modalities versus not.

Speaker 3: Thank you.

[00:33:30]
Carlos Arteaga: Thank you. Do you have a question Ola?

Ola Landgren: No. Actually I would just like to add another perspective on these aspects that were brought up, just crossed my mind. I do think ... I see a lot of patients as part of my weekly job and I do think that with all these new assets that we're developing, I do think that the focus of what we use as benchmark for success and not, I.E, the endpoints of studies, are also going to change very much in the

[00:34:00] future. Patients don't like to have complications. In a lot of diseases that actually result in complications when the disease comes back. Myeloma, that I talked about, happens in 30,000 patients every year but because people live longer and longer, there are 120,000 people living with it. We can not cure it, and that number goes up every year. There are very many patients living with this disease and they continue to relapse over and over again.

[00:34:30] What happens when you relapse is that you can develop acute kidney failure. You can go into dialysis. You can have structural damage of your spine that leads to very bad pain. You can have pathological fractures. You can have all types of horrible outcomes with cardiac failure, etcetera. So if you can pick up the disease early on and initiate pretreatment before those things happen, that's something that we don't capture, we don't talk about it, if you talk about overall survival and progression free survival. Patients don't care about statistics, they care about these things, so I think that's a very, very important area to focus studies

[00:35:00] on also in parallel with all paradigm. I just want to bring that up.

Carlos Arteaga: No, no, this is great. Thanks for that look to the future, which is impressive probably. I have a question for you all actually, going back to this issue of the lead time bias ... The lead time, not the bias, the lead time we may gain in a solid tumor like breast cancer, for example, by detecting molecular relapse versus
[00:35:30] clinical. Can we learn ... From what you have done with myeloma, if you go back 30 years you are probably not doing many more residual disease, you are using other clinical markers of recurrence, that were probably very gross and primitive by today's standards. What lead time did the implementation of this molecular
[00:36:00] essays give you, what lead time? That lead time ... God knows how many populations doubling and this is when you lose the battle ... I just want to know what can we learn from the experience of myeloma where you're already seeing a big impact of MRD on survival.

Ola Landgren: I think those are great questions. I don't have the numbers obviously too ...

Carlos Arteaga: What's your sense?

Ola Landgren: Well I think that the lead time probably goes on both sides of the spectrum. I
[00:36:30] think we go after the disease earlier so when we capture success rates for therapies, we treat patients earlier so we don't formally reach progressive disease the way it used to be. Patients have to be sick before they were retreated. I actually think also for the initial diagnosis, just looking at the diagnostic criteria, myeloma used to be a disease defined by symptoms. You need to be in severe pain or you need to have a cute kidney failure, you need to have fractures, all those things.

Now you can have myeloma without knowing it. A lot of patients that come to
[00:37:00] me were patients that were doing a new paper work for life insurance, so the insurance company took the protein from the blood test and one thing leads to the other and there is a high protein. They come see me and I say "You have a diagnosis of multiple myeloma." They say "What's that?" Those are patients we have and when you work these patients up, they may have 60% of the bone marrow filled with myeloma cells. If you had just waited another 6 or 9 months,
[00:37:30] they'd be the guys in the emergency room with kidney failures and fractures and all of those things.

We picked these diseases up much earlier ... We have done sequencing on early disease. What we see, if we have done whole exome sequencing, is that the mutational burden is about the same. The non-synonymous mutational burden ballpark is around 50 mutations per patient, or 50 genes being mutated. If you look at the pattern of the mutational landscape it is not the same genes that are
[00:38:00] being mutated, at least that we can pick up with the [inaudible 00:38:02]. The top 15 genes that are the ones that we see over and over again in the myeloma patients, those we don't see as often in earlier cases. There is very little known about it, but it may be that the disease could be more treatment susceptible earlier on, so that's another thought. That's kind of something that we have not really talked about. Maybe we could use the same treatment paradigm and just
[00:38:30]

go earlier, instead of just throwing disease therapy at the disease the way we do it now and look if it's left. Maybe we should do both.

Carlos Arteaga: Okay, thank you. Dr. Hartman, can you tell us about anything with viral cancers and your approach, how can you use that to detect those type of cancers?

Anne-Renee Hartman: Thanks for that question. We are definitely interested in looking at viral cancers because there is a higher likely hood of technical success, and we know that breast cancer is challenging. Grail is recently merged with a company that was

[00:39:00] founded by Dennis Lowe and they just published a paper in the New England Journal looking at the EBV PCR based tests for early detection of nasopharyngeal carcinoma, that is definitely something of interest that Grail is pursuing. There are other viral cancers. HPV driven cancers. We know oral cancers that are associated and driven by HPV where the likely hood of technical success is higher

[00:39:30] because there are more copies of the genome in the blood and the cell-free compartment to detect. We believe that we can build out tests that will be highly sensitive and have a very high positive predictive value for those cancers.

Carlos Arteaga: Thank you. Well I don't see anymore questions and it's noon so I think it's time for lunch. I want to thank the speakers for being on time and for these very

[00:40:00] enlightening discussions and also for you, for the generosity of your questions. I will reconvene at 1:00. Thank you for being here.