Carli King, PhD: Thank you all for joining us today for our 2024 GI ASCO Highlights Webinar. My name is Carli King, and I am the current research advocacy project manager here at Fight Colorectal Cancer. We're going to go ahead and start today, but first I'm going to start with a quick disclaimer. The information and services provided by Fight Colorectal Cancer are for general informational purposes only. The information that's provided here today are not intended to be a substitute for professional medical advice, diagnosis, or treatment. Fight Colorectal Cancer never recommends or endorses any specific physician's products or treatments for any condition.

Just to take care of a few quick housekeeping items, we'll have a question and answer session at the end of the webinar today. Feel free to use the question and answer button on the right side of your screen to ask questions, and we'll cover them at the end. We'll do our best to address all the questions that we have at the end, but the ones that we don't get to, I'll do my best to address them via email after the webinar has ended. There will be a recording of this webinar available on our website within the next few days, and if you're registered for this webinar, you'll get an email with a direct link to the recap. Lastly, feel free to follow along on Twitter or X using the hashtag #colorectalcancercrcwebinar.

With that, I want to briefly introduce our presenters today. Joining us today, we have gastrointestinal medical oncologist, Dr. Van Morris and Fight Colorectal Cancer research advocate Lee Jones. I'm going to go ahead and allow Dr. Morris to start sharing his screen, but thank you everyone for taking the time out of your schedules to join us today. I'm going to go ahead and hand it off to Dr. Van Morris and Lee Jones. Thanks.

Van Morris, MD: Does that work?

Carli King, PhD: Yep. Good to go.

Van Morris, MD: Sorry, and thanks everybody for bearing with me through the technical difficulties there. My name is Van Morris. I'm an associate professor at MD Anderson in the Department of GI Medical Oncology. It really is a privilege to get to come today and participate in this webinar with Fight CRC, who of course is just doing amazing things, advocating for the well-being of our patients.

Lee and I have been tasked with the impossible job of summarizing all the exciting findings that were reported two weeks ago in San Francisco at the ASCO GI meeting. For the sake of a one-hour discussion, I chose two major topics that I wanted to focus on today with some of the updates with various levels of evidence, which I think is important to keep in mind as we're interpreting and figuring out what to make of the data which are presented. But I think all the data are exciting, will move the field forward, and I'm really excited to engage with everybody today in going over these results.

The first part of the webinar will be talking about some advances in novel uses of dual immunotherapy, PD-1 CTLA-4 blockade in patients with colorectal cancer, and then we'll talk in the last four studies here about new updates in use of circulating tumor DNA across different contexts of patients with colorectal cancer. To get right into things, the NEST-1 trial was a trial that was reported by Dr. Pashtoon Kasi at Cornell from New York City. This was a trial, it was a poster that was presented that I thought was really, really interesting, looking at use of botensilimab and balstilimab in patients with microsatellite stable localized mismatch repair, proficient and deficient colorectal cancer.

Botensilimab is a CTLA-4 antibody. Balstilimab is a PD-1 antibody, and what they did in this study was looked at, I think, a total of 12 patients with newly diagnosed colorectal cancer that was not metastatic. As you can see here, the patients got one dose of combined PD-1 CTLA-4, followed two weeks later by another dose of the PD-1 antibody balstilimab, and then the patients went to surgery. This was a trial that was similar to what had been done several years ago in the NICHE study. That was a trial that Dr. Chalabi had reported that looked at nivolumab with ipilimumab in patients with localized MSS and MSI-high colorectal cancer.

What the investigators here looked for was the rate of pathologic response when these patients went to surgery following treatment with this immunotherapy. We saw here that they defined, I think, a major pathologic response as about 50%. That means that when the patient went to surgery, they looked at the tumor under the microscope, it could see where that tumor had been, how much residual tumor was left versus how much tumor had died off, and if more than half of the tumor had died off following treatment with the immunotherapy, they classified this as a pathologic response. In the MSI-high patients as we, I think would expect, we saw major pathologic responses, near 100% in all of the patients. 100% in two, 98% reduction in the remaining patient. I think what's interesting is we saw major pathologic responses in about two thirds of the patients with over 50% reduction of tumor even after about a month of immunotherapy.

I think that these data were very interesting. There were also just some reports in this poster about how, when they looked at the surgical samples, increased rates of immune infiltration, which is what we could expect, knowing the mechanism of action of these drugs, was associated with a favorable treatment response as well. Again, this was a poster, so we didn't get as much information just from the poster. I do think that we'll see this in the subsequent manuscript that will come out, but we don't know for sure what the radiographic correlates were in these patients. I mean, for example, there was a patient who had 0% reduction. Did the tumor grow while that patient was on immunotherapy? These are the answers we still don't fully know yet, but I'm really eager when Dr. Kasi reports these findings in a manuscript, what were the radiographic correlates? What were the characteristics of these tumors when they came in? What percent were stage one, stage two, stage three? What percent were colon, what percent were rectal?

These are some of the other questions. I think the other question is, what was the toxicity? We did see here, I think very importantly, that all patients were able to make it to surgery in a timely fashion. It doesn't appear that the month of treatment was so toxic such that patients weren't able to make it to surgery. Again, I think given some of the findings associated with dual checkpoint blockade in patients with colorectal cancer, we have to be thinking about the added toxicities as we look at these in larger studies.

I think the other thing that's important to keep in mind as well, and this is going to be a theme of the rest of the webinar, is this idea of clearance of CtDNA. There were apparently on this report seven patients who at baseline before they started treatment, when that tumor is present, seven of the 12 patients had detectable circulating tumor DNA. Despite tumor being present in five of these patients, it doesn't appear that they were able to find that in all 12 of the patients who participated on this study. Again, as we think about how can we move in the future, CtDNA assays into neoadjuvant space in order to attract patients who hopefully are cured with various therapies, what are the characteristics of when we do and don't detect this in the assays that we're looking at?

My takeaways from these are, trials like these are really, really feasible to do. I think that Dr. Kasi has done a really nice proof of concept and showing that, but these are scientifically important. As we're thinking about how we can augment systemic therapies, perhaps move them earlier on into the treatment course with a goal of curing more patients. Studies like these are nice as well, because you really get to see what is the impact of these drugs on the tumor, without the confounding effects of prior chemotherapy or other treatments as well.

I think that trials like these are important. We like to call them windows of opportunity trial, because again, you're not in theory deferring these patients from getting them to a curative surgery. I think again, it's also critical to remember not all patients had detectable CT baseline. This is all very exploratory right now, but very interesting. But we should be careful in that setting about how we use CtDNA to fully inform on whether a patient is not cured. Following neoadjuvant therapy, it certainly and definitely is a tool, but it's one arrow in our quiver of against fighting colorectal cancer.

I think as well, the clinical signal here I think is really, really interesting with neoadjuvant PD-1 CTLA-4 is consistent with what we saw again in the NICHE study as well. I'm really excited to look and hear more data as this data matures, and more patients are put on prospective studies like this one. I'll stop here and see if Lee has any comments from our research advocacy side.

Lee Jones: Yeah, I also found this study to be very exciting because for most colorectal cancer patients, the treatment they get today is the same treatment that I had 20 years ago. Really, the FOLFOX, FOLFIRI, with or without bevacizumab has been pretty effective. Here I am alive 20 years later with stage four colon cancer, but we really need to see new treatments coming into the clinic. Even

though this is really preliminary, very small sample size, as you've mentioned, Dr. Morris, a lot of questions. It almost raises more questions than it answers, but I think it's a very promising direction and approach, particularly in the MSS space, which we've been trying to find a way to unlock the MSS tumors to the immune system. Is this something that will do that? Well, it looks like maybe it is.

The other good thing is it also does seem to be very effective for the MSI-high tumor, so it'd be one approach could deal with both sets of tumors, both MSI-high and MSI-stable. I think it's very promising. It's very interesting that Dr. Kasi both had this study, and then also a circulating tumor DNA study. He's looking at both things. It's really amazing the amount of territory he's looking at these days and very promising approaches. Anyway, that was my thinking on that, waiting to see more information on it, but from patient perspective, it's good news.

Van Morris, MD: We'll move on to more discussions about PD-1 and CTLA-4 in the management of patients with colorectal cancer, this time with the CheckMate 8HW study. This was a much larger study, was not an exploratory study, but this was a trial looking at the role of immunotherapy in patients in the frontline MSI-high metastatic colorectal cancer setting. Of course, we feel strongly that all patients, regardless of stage of CRC diagnosis, should be tested for MSI status given the implications for use of immunotherapy.

The KEYNOTE-177 was a trial reported several years ago, which showed that pembrolizumab, so a PD-1 agent alone, was better than chemotherapy, and really defined the role and led to an FDA approval in the United States for pembrolizumab as a frontline therapy for MSI-high colorectal cancer. It was interesting, and I think that the authors mentioned this here. When you look at the subset analysis from the KEYNOTE-177 trial, really all subsets that they looked at as they teased out the various patterns of response, seemed to benefit with immunotherapy relative to chemotherapy alone.

What I think, I'll be honest, I scratched my head out when that data first came out, was the fact that patients with KRAS or NRAS mutated MSI-high CRC did not seem to experience the same degree of benefit with immunotherapy relative to other molecularly annotated populations of patients with MSI-high colorectal cancer. The CheckMate 8HW trial was a trial that was looking at either in patients with untreated, newly diagnosed metastatic colorectal cancer in the frontline setting, either PD-1 therapy alone, anti-PD-1 therapy with nivolumab alone, PD-1, anti PD-1 therapy of nivolumab with anti-CTLA-4 therapy with ipilimumab for four doses followed by continuation of nivolumab or the investigator's choice of chemotherapy.

As you can see here, based on the randomization, patients had an 80% chance of getting randomized to the immunotherapy arm. The primary endpoints for this study were PFS in comparing dual checkpoint blockade versus chemo alone, and that was reported, and we'll talk about today. Another one is looking at two immunotherapy drugs, NIVO-IPI versus nivo alone. Again, treatment was

continued for a maximum of two years in patients who were benefiting to the immunotherapy.

Just a couple of things. 50% of patients in the KEYNOTE-177 were diagnosed with initial stage four disease at the time of registration, which is fairly similar to the numbers here. It's interesting to me though, that despite only less than half of patients being diagnosed with stage four disease at diagnosis, the vast majority of patients had had some type of surgery for the presenting cancer, which put them on the study. I think that the reviewer hat in me, if I were reading this, would ask for a little bit more clarification when these data get fully reported.

It's important to note that 200 patients were treated with NIVO-IPI versus 88 in the chemotherapy arm. Approximately one-fifth of the patients with NIVO-IPI stopped treatment on this study due to an adverse event related to the immunotherapy. Again, I just really want to drive home the point. We're of course, very excited about immunotherapy in all contexts of treatment of cancer, of course, but we do have to be mindful and we cannot be sweeping under a rug, the toxicities which are associated with this as oncologists talk to our patients and their families, and as patients and their families are doing research. Very exciting. The study did hit its primary end point, and as you can see here, quite an impressive separation of the curve. Median PFS of chemotherapy was about six months, was not reached in the NIVO-IPI arm, and 12 month PFS rates of 79% versus 21%, 72 versus 14% at the 24 month.

It's very important. I'll say that, just to put this into context, for pembro, it was 16 and a half months was the median PFS, and the 12 and 24 month rates of PFS survival here were 54% and 48%. With that said, I think one of the very important lessons from this trial is that we cannot be comparing the NIVO-IPI results with pembro head to head. These are different trials, different populations. We certainly are awaiting the results of this second analysis I had mentioned earlier, that is comparing NIVO-IPI versus nivo alone. I think we'll be able to make better conclusions when that data comes out, not only in terms of survival benefits and the PFS endpoints, but also the toxicity profiles as well.

I thought this was so exciting to see. Basically, no matter what category you fit into, with NIVO-IPI, you are more likely to benefit in terms of PFS than with chemotherapy. The numbers were greater in this study than KEYNOTE-177, which could also be some of the reason that we're seeing less heterogeneity, I would say, than was reported in the subgroup analysis of the pembro study, but again, I don't want to compare head-to-head.

Just very importantly though, I love the fact that there were so many patients over the age of 65 enrolled over this study. I think that we have to think about this idea of ageism, and the fact that the investigators were very inclusive in a population of patients where sometimes oncologists may be a little bit more worried about giving more toxic agents here in the version of a PD-1 CTLA-4, so I definitely applaud and appreciate what the investigators did in designing this in

trial in terms of inclusivity. But also, again, as you can see here, whether the tumor was BRAF mutated, whether it was KRAS or NRAS mutated, or if it was triple wild type, all of these populations of patients benefited from the NIVO-IPI relative to nivolumab alone.

I really just again want to highlight that of the 200 patients who were treated with NIVO-IPI, two of these patients passed away due to treatment-related death. One patient with myocarditis inflammation of their heart muscle, and another patient with pneumonitis or inflammation of the lungs. It's just so important with nivo, when patients are being treated with immunotherapy. While we're all very excited about these, we really try to counsel our patients. We don't like surprises as oncologists. If something doesn't feel right about your body, we need to know this ASAP, so that we can assess for the treatment complications known with immunotherapy, because, as was shown in this trial, the toxicities were very serious here.

Just again, to be mindful of, but again, I think we have great data here on that dual PD-1 -CTLA-4 blockade is now a proven effective therapy for patients with deficient mismatch repair, MSI-high metastatic colorectal cancer in the frontline setting. Regardless of the mutation status, this benefit was consistent. The toxicity is real. We have to be discussing this with our patients and empowering our patients with the knowledge about this if and when we're starting these therapies. Again, I just want to caution, it's too early to say whether this is superior to PD-1 blockade alone, but these results are highly awaited by me, and I think the oncology community. I'll stop there and see if there are any other ... Lee, thoughts here?

Lee Jones: Yeah, I also picked up on the toxicity, and the fact that there were two deaths among the immunotherapy arm. We pretty much know what to expect with chemotherapy. I had FOLFOX and bevacizumab 20 years ago, and although it wasn't pleasant, the side effects were not lingering, and I didn't approach death by any means. We know the side effects from chemotherapy. Some of these immunotherapy side effects are more challenging, I think. As a patient, you really have to think about them. Everyone thinks, "Oh, immunotherapy is great," but as you said, Doctor Morris, you've got to consider the fact that, yeah, it's great in terms of effectiveness, but not always great in terms of toxicity.

I did like the fact that they allowed people to cross over from the chemo arm to the immunotherapy arm. I think that is from a quality of life perspective or just effectiveness, it really would be malpractice, I think, if they didn't allow them to cross over. I do recall them saying that it's still in analysis, so it's not ready to be presented to the FDA anytime soon, but it certainly is promising, and I wondered why they compared it with chemotherapy, which for MSI-high population, is not the standard of care anymore. Why they didn't compare it with monotherapy pembro directly, because we can say, "Well, it looks like it's much more effective than monotherapy pembro," but I wonder why I didn't put them head to head and do the comparison that way. Certainly, we would be better than chemo, because pembro is better than chemo. Monotherapy is

better than chemo. Again, it's good progress. Let's hope it's a better, new standard of care, and it looks like it's going to be better for patients, but toxicity will be an issue.

Van Morris, MD: Thanks. Yeah, I agree on all points. We'll move next to the CtDNA study. We'll start with the DYNAMIC-Rectal trial. This is a trial that was led by Jeanne Tie and her team out of Australia, who really have been the leaders really in bringing CtDNA to become clinically relevant in colorectal cancer, and leading all of the studies really on how we utilize this in the management of patients with colorectal cancer.

Of course, the DYNAMIC study, which was first reported at ASCO two years ago in 2022, looked at use of CtDNA as a de-escalation approach in patients with stage two colon cancer, and showed that relative to standard treatment, that de-escalation with no adjuvant chemotherapy proceeding to observation in patients who are determined to be low risk according to a negative CtDNA result in the stage two post-operative setting, not only resulted in less chemotherapy use, but also no significant differences in survival relative to standard practice patterns.

This group has been absolutely just amazing in terms of the work that they're doing. The DYNAMIC-rectal study was a trial that was looking at bringing this technology into patients with localized rectal cancer. As you can see here, most of the patients on this study were all fairly clinically ... You had clinically advanced localized disease. Either a T3 or T4 primary tumor alone, or in combination with nodal positive status. The thing I think that's critical to remember about this study is that this trial was done prior to total neoadjuvant therapy. Again, if a patient comes today with localized rectal cancer, microsatellite stable rectal cancer, to MD Anderson, we would probably in a situation like this, under standard practices, start this patient on long course chemo radiation, followed by several months of doublet cytotoxic chemotherapy, and then if they don't have residual disease, watch and wait, because there are of course patients who can be cured in that setting, or surgery if there is persistent disease.

This trial was done before the total neoadjuvant studies, was designed before the total neoadjuvant studies came out, so patients here were getting long course chemo radiation followed by surgery, followed by chemotherapy. It's important to realize that the context of this study being done is a little bit obsolete, just given, fortunately, the advances we've had in treatment of localized rectal cancer over the past several years. Patients were randomized after long course chemo, radiation, and surgery to either standard management, which was adjuvant therapy at the discretion of the clinician, and hopefully the patient as well, or two-thirds of patients to a CtDNA informed management, where, if they are CtDNA positive such that there's reason to believe that residual microscopic disease is still present, they got four months of adjuvant chemo, either doublet or single fluoropyrimidine at the discretion of the patient and clinician, or if they were CtDNA negative and low risk according

to a pathologic node negative status, these patients went to observation. If they were negative and had positive nodes, then it was the choice of whether to proceed with chemo or observation.

Again, it's important that to realize this trial did not hit its planned enrollment of 408 patients. It stopped analysis after 230 patients due to slow enrollment, not only due to the updated treatment standard of care as I referenced earlier, but also the COVID-18 pandemic. Overall, these patients were fairly balanced. Around 30% of patients had node positive status after neoadjuvant chemo radiation at the time of surgical resection. Similar rates of pathologic complete response, although the numbers are fairly low.

Sorry, let me just go back. As you can see here, overall in the CtDNA formed arm, there was less use of single agent chemotherapy in the CtDNA positive CtDNA informed arm. I think this is something that we had seen in the DYNAMIC study was that when you see a CtDNA positive result, clinicians and patients are more inclined to escalate therapy to a doublet when perhaps given the option. This was the secondary endpoint of the 36 month, three year PFS rate. It's interesting that the CtDNA informed patients, that the recurrence free survival was lower. Again, this was not statistically significant, so we can't definitively say that this was inferior, nor was this study powered with the number of patients analyzed to make that conclusion.

To me, this is an observation but doesn't necessarily slow the momentum. Well, it does not slow the momentum for other studies in the future, which look at CtDNA in informing the multi-modality treatment of rectal cancer. As we would expect in patients who were CtDNA positive after their surgery, these patients had a less favorable recurrence-free survival than those patients who were CtDNA negative. This is something we've seen consistently across colorectal studies, and I thought that this was just such a fascinating slide to me.

We know that when a patient has completed a definitive therapy and is CtDNA positive, the cancer is likely microscopically somewhere, but we don't know really where that means. Is it localized still within the pelvis? Has it metastasized out? What, again, we're continuing to see, and I think with more and more data, and I think this study adds a nice additional sample of patients to further drive this point home, many of the CtDNA positive cases where you can't see residual disease on imaging studies, we'll predict the development of liver metastases. A high suspicion for micrometastatic spread when we see the CtDNA positive results.

Just going back here, sorry, there are some patients who are CtDNA negative, yet still recur. Who are these patients? The theme that we're starting to see here is that you're not seeing really liver patients who are CtDNA negative who recur, but the majority of patients are these lung-only or lung-involved disease for the most part. I think that this is helping to parse out the implications of what these results mean when patients do recur, how they recur according to their CtDNA status. I think that that's really interesting.

I think this was overall a really important study. Again, it's important to note that this was done with a non-TNT regimen, so it's a little bit obsolete in the treatment approach, given the advances that we've had. I think it's still a great trial though. I think that redoing this with a TNT approach is something that will be happening, and I think that all of the neoadjuvant TNT trials are, of course, incorporating CtDNA into their translational science plans, so we'll ... Certainly a lot more to learn in the coming years as this.

Again, I don't want to draw conclusions based on the lower three-year recurrence free survival rate for the CtDNA informed relative to standard of care, due to the limitations that we've talked about earlier. Again, I just think, as I mentioned earlier, what does the CtDNA positive result mean? We're getting more and more confident about early identification of these liver mets in patients who are CtDNA positive. I'll stop there and see if ... Lee, any other comments?

Lee Jones: Yeah, some of my comments really refer to all the CtDNA studies, which we have several of them. But I have a question for you, Dr. Morris. The circulating tumor DNA positive, we know pretty much how to deal with that, but it's the negative ones that show 23% end up getting chemotherapy. One of the things that was mentioned during the symposium was that, well, a couple of things. First, you have to be aware of biology. Some tumors are non-shedding, and will not shed circulating tumor DNA, so it can't be identified. Also, they said, "Don't do a circulating tumor DNA test if you don't know what you're going to do with the results when you get them back." If someone comes in negative, how would you decide with them, in shared decision making, how would you decide those 23% who got chemotherapy when they were negative? How would you help make that determination?

Van Morris, MD: We use it as a tool. We don't use it to make or break decisions in patients with localized rectal cancer yet. Again, most of these patients are getting upfront chemotherapy prior to their surgery now, so really when that patient starts their treatment, we're committing to a treatment plan. I'm not sure that I would de-escalate and not offer neo-adjuvant chemotherapy after long course if the CtDNA were to clear. We're really more in the process now of committing to a treatment plan upfront, collecting and observing the CtDNA patterns over time, and then learning from that about how we could use that more prospectively.

Lee Jones: The idea is that, with any luck, someday it'll be much more predictive in terms of treatment outcomes and would help guide the treatment than it is today, which is still experimental, essentially, in clinical trials.

Van Morris, MD: For the sake of time, I'm going to kind of go through quickly these last three, because I think that they all come together about how can we use CtDNA to inform on its clearance with adjuvant chemotherapy. The BESPOKE trial was a tumor observational study that was looking at patients in the United States with stages two or three colon or colorectal cancer. It says here to inform on adjuvant treatment, but I don't think the study was designed to do that. It was

basically, patients sign up to participate in the study. They got the treatment as agreed on between them and their oncologist, and then blood was collected at these various time points.

The investigator should be commended, did an amazing job of collecting many time points to really understand the serial kinetics of circulating tumor DNA across many patients. I can't praise the study team enough for that effort. Around 700 patients were reported in this study. The MRD window was immediately in the post-operative setting. The surveillance window was what happened after completion of all plan definitive therapies. As we would expect, patients who were CtDNA positive at the time of their post-operative check, initial post-op check, fared more unfavorably than patients who were CtDNA negative. We saw this across stages as well.

They did look at patients who received adjuvant chemo among patients who were CtDNA positive in the post-operative setting. They compared use of adjuvant chemotherapy with no adjuvant chemotherapy. The authors said here that the benefit of adjuvant chemotherapy was observed in this setting. I think we have to be very careful about drawing that conclusion just because number one, it's only eight patients in the observation arm. We don't know fully who these patients were. But again, I think an interesting signal.

Again, I think to me, and what really excites me, and this study did a great job of that, are the patients who remain serially negative are near 100. What a beautiful curve here. Near 100% likelihood of their cancer not recurring. I think more and more oncologists are utilizing this as a tool, for now, in conjunction with standard CT imaging studies. I think the question was asked at the beginning of, should we be replacing CTs with this? I'm not sure that we're fully there yet, but I think that the power of this tool and accurately identifying patients who are not going to recur once serially negative is really high.

I think it's interesting though that again, there's still a fraction of patients who remain CtDNA positive, yet don't recur even two years out. I'm very interested in who these patients are. I'll talk about this in the next slide. I get a little bit nervous about this notion and this term transient clearance, and what we make about that, but I'm going to reserve that for the Galaxy study coming next. The authors were able to, I think, conclude that identification of patients who were CtDNA positive allowed maybe closer observation and surveillance of patients for more earlier identification of metastatic disease when it did show up. As you can see here, patients were treated with a variety of localized therapies.

The final point I just want to make is that I think just some interesting findings, really that patients reported that CtDNA results reduced anxiety about their cancer recurrence in 73% of cases. I would assume those are all patients who are CtDNA, whereas the other 27% most likely are patients who are CT DNA positive. Probably, among patients who are CT DNA negative, there's a near 100% rate of hopefully reducing anxiety about cancer recurrence. 87% of patients felt they were receiving the right treatment after receiving their results.

Again, I'm not sure how exactly the CtDNA result was used to inform the treatment, and I think that will come out in the manuscript, but again, we're starting to see some kind of patient-reported outcomes with use of CtDNA.

I also just want to say, this is with the caveat of people who willingly signed up for a study to participate and have their CtDNA collected and returned to them. I still think that there are just some oncologists and patients perhaps, as well, who worry a little bit about this. I'm happy to talk offline about why I think that could be, and what we observed in our COBRA study that supports that maybe not all patients are ready to get their results, but for the sake of time, we'll keep moving on. The conclusions here, it's a large study, I think really well done with a large number of time points. Identification of a CtDNA-positive status may help identify these patients with localized metastatic disease that are more amenable to localized therapies like radiation, surgery, ablations, these kinds of things. We don't know yet whether that actually translates to an improvement in survival, but I think that will be analyzed in the future as well. Again, really nice patient-reported outcomes, I think, from the study as well.

GALAXY was a similar study that was set out of Japan in a larger setting. They looked at this similar surrogate windows, the MRD window is what they call it here, and the surveillance window, 10 plus weeks out after surgery. Again, patients who were CtDNA-positive after surgery had less favorable outcomes than those who were CtDNA-negative. I thought that this was really an interesting point here, which was of the patients who were CtDNA-positive, over half of the patients were treated with adjuvant chemotherapy. About a quarter or so of those never cleared their circulating tumor DNA, but about approximately a third or so of the patients did clear their circulating tumor DNA, at least at one point. Among those who it kept, it maintained, clearance occurred in about half of those patients, but still 60 of these patients, so one-fourth of all the patients treated with adjuvant chemotherapy, greater than one third of patients who had some clearance.

A large fraction of these patients had this idea, this transient clearance, and while there was some improvement in the disease-free survival in the transient clearance versus the no clearance, really by 24 months, you can see these curves converging. I still think we have a lot of questions about what transient clearance means. Is this a distinct biologic entity? Is this a group who has just a lower VAF, a variant allele fraction that's able to clear? I don't think we know, but I think the concerning thing again here is that if a patient experiences transient clearance, we're talking about patients in whom we're going for cure, 98% of these patients, unfortunately, their cancer still came back. I really will be careful about using this terminology with my patients until I understand it better.

For the sake of time, I'm just going to keep moving on. Again, this is another large study that I think confirmed what we've been seeing in the BESPOKE. Again, sustained clearance of CtDNA after adjuvant chemo really prognosticates a very favorable curative outcome. Again, really not a lot of difference between

the transient and no clearance patients at 24 months. The bottom line is, the cancer comes back in both cases. It's really important that we refine this definition before we ... Apologize for the spelling error, but incorporate this into our clinical discussions with patients.

Then finally, the COBRA study. This was a prospective trial looking at, can we use CtDNA to inform on identifying patients who benefit from adjuvant chemotherapy? Patients with, it was low-risk stage 2a colon cancer, were randomized either ... These are patients who were low-risk, would not get adjuvant chemo based on the fact that according to standard practice patterns, they didn't need it, according to how we're used to treating patients with stage 2a colon cancer. They were either randomized to observation, which is the standard of care, or to prospective testing for circulating tumor DNA, using a tumor uninformed tissue agnostic assay, the Guardant Reveal assay. If patients were CtDNA positive, they were treated with six months of adjuvant chemotherapy.

These patients who were CtDNA positive, prospectively identified, the six-month clearance time point was collected just before their last dose of chemotherapy. Again, the assay that was used here was a little bit different than the ones which had been used in the prior studies I mentioned, but it incorporated both genomic calling and colon cancer methylation biomarkers calls for identification of circulating tumor DNA.

The primary objective was to compare the rates of clearance at six months between patients who were CtDNA positive at baseline and treated with chemotherapy, versus who were observed. Basically, what we saw at the futility analysis was that for the 16 patients who were CtDNA positive at baseline, among the seven who had gone onto surveillance versus the nine who were randomized for treatment with chemotherapy, there was no improvement in clearance with chemotherapy relative to surveillance.

Unfortunately, because of that, the trial was stopped early by protocol, due to not satisfying the futility rules that we had set out when we designed the study. There wasn't really any improvement in CtDNA clearance in this prospective randomized controlled trial. However, we accrued really well thanks to our Canadian colleagues as well, and we thought that we had a really strong scientific hypothesis. It was not confirmed with this prospective randomized controlled trial, but again, I think that it's critical that we not assume hypotheses, but really test hypotheses in order to bring the best and safest treatments to our patients.

Even though this was a negative study, this was the first trial that the NCI did in any solid tumor type to use CtDNA as a biomarker for study entry. This was a first of its kind clinical trial, and I think lessons learned from this are going to be every bit as important about, than the actual results themselves as we seek to do better in the future. Certainly, given the rapid evolution of CtDNA technologies, given the fact that we know these technologies are here to stay,

we have to incorporate how we're going to account for this in the future in order to stay up to date as we're reporting out trials. I will stop there. I'll let Lee take over, and I'm happy to answer any other questions afterwards.

Lee Jones: Yeah, thanks Dr. Morris. I just wanted to point out and applaud the BESPOKE researchers for including patient-reported outcomes. As you may have noticed, the only one of these studies both circulating tumor DNA and the treatment studies that had patient-reported outcomes. In terms of how patients would respond to getting the CtDNA testing, remember that if you don't get that, the standard of care is scans, and they're so anxiety-producing, there's a term for them, "scanxiety". The fact that we're all nervous waiting on the results of those tests, whether it's a scan or a blood test, is not surprising.

What I know now, as a patient, I would rather be able to rely on circulating tumor DNA rather than the CAT scans that I got way too many of when I was going through my treatment. Anyway, these are all amazing studies, and it was an amazing conference. So many things that we didn't talk about here that were presented at ASCO GI, that it's too bad we didn't have three or four hours to talk about everything that we learned and was presented there, both during the sessions and off campus, and some of the evening sessions that were also amazing. I'll stop there.

Carli King, PhD: Thank you both for your comments, and for presenting this data to us. We'll go ahead and go over to the question and answer session. We have a few question and answers right now, so I'll start reading those and I'll allow everyone to have a little bit of time to ask questions. If you have any questions, please feel free to ask that in the question and answer section. But to start, how difficult in the United States is it to enroll patients to CtDNA trials, as there are two commercial tests available?

Van Morris, MD: Yeah, I think that I didn't have ... Sorry. I think it's still very feasible. We talked about this. We didn't anticipate this when we set out to design our study, but we still saw continued enrollment that was steady, even in the first half of 2023 as the study was soon to close. I think when we talk to a lot of oncologists in the community, when we talk to patients as well, there are many people out there who say, "I don't know what to do exactly with this result, and really, if given a trial option, this is where I feel is the best place to evaluate this." We saw steady enrollment despite the two commercial assays.

Certainly, there are early adopters who, and I think a lot of it depends on, you're willing to accept the level of evidence which is out there. Again, the BESPOKE and GALAXY studies are very informative. These are observational studies, and we have to account for heterogeneity of treatments in patients, and these kinds of things before we generalize these. Again, I think it depends on these factors, but I think it is still very feasible.

Carli King, PhD: Next, referring to the COBRA trial, is it likely the issue was false positives with the methylation-based test?

Van Morris, MD: I think that we're still trying to learn with that. As soon as we saw the results from the primary objective, we halted the study. I think that we're in discussions with the NCI for analyzing the survival outcomes. As time goes on, that data will mature, and we look forward to reporting that in the future, an then trying to understand how the circulating tumor DNA correlates to that, both in the patient's own observation and who got chemotherapy.

Carli King, PhD: Referring to the NEST-1 trial, they asked if the results are specific to, you think, BOT-BAL, or could this potentially be mimicked with another anti-CTLA-4 and anti-PD-1 combination?

Van Morris, MD: Well, we saw similar results in the NICHE study. That trial defined a major pathologic response of 90%, versus 50% in the NEST trial. Again, I think around a third of the patients, if I remember correctly, had major pathologic responses, again in a small number, a fairly small cohort of patients. My suspicion is that it's more class of drugs than specific drugs, so I think generalizing promising signals seen from PD-1 CTLA-4 in early studies and multiple efforts.

Carli King, PhD: Referring to the CheckMate 8HW trial, do we know if there's any specific subgroup that had more significant toxicity to others?

Van Morris, MD: That was not reported. It's a really good question, but that was not reported.

Carli King, PhD: For those no evidence of disease after surgery, chemo, radiation, about how many negative CtDNA tests would seem fairly conclusive? At one point, it was theorized three tests.

Van Morris, MD: Also a really good question. Yeah, I think that that's not really been defined yet. There was another poster that was presented, and I'm sorry, I thought I had updated it. I had added a slide this morning, but it didn't show up. It was presented by Dr. Stacey Cohen out of Seattle, looking at a tumor-informed assay among patients who got total neoadjuvant therapy, and either went to watch and wait, or went to surgery. Again, with that, we saw similar outcomes as you would expect among patients who were CTDN negative, whether they received total neoadjuvant therapy, went to watch and wait, and were CtDNA negative over time, whether they had total neoadjuvant therapy, went to surgery and were CtDNA negative, or even if they got chemotherapy alone, or a prospect style approach, I presume, or chemo radiation alone. We saw similar favorable outcomes among patients who were CtDNA negative. The numbers were small, and I think that they'll update this in the future, but more and more data coming out about this. Very interesting question you're asking.

Carli King, PhD: I think we have time for one more question, so I'll read this one. It says, "It seems that complaints about CtDNA false negatives don't consider the accuracy of the best current surveillance, which is scans. I'm not suggesting replacing scans, but seeing accuracy of scans in general would be nice in the context of understanding the additional value of CtDNA. I know that comparisons exist in

earlier trials, but I've never seen a summary of the reliability of scans. Is there such a thing?"

Van Morris, MD: That's a great question. I'm not aware of ... I can't quote the data on that, but we have a multidisciplinary conference for patients with colorectal cancer at MD Anderson every week, and I mean, every week we have a case of that small nodule in the lung, or that single pesky lymph node. What do we think that is? These questions are hard, even with CT scans, especially when you're talking about these smaller lesions. A PET scan may not be sensitive enough to pick up a four millimeter lung metastasis. These kinds of questions are real with regards to the limitations of radiographic performance. Maybe this is a place where AI will come in to help improve the process in the years to come. We'll see.

Carli King, PhD: I was muted. Sorry, we only have about two minutes left, so I wanted to open the floor to both you, Lee, and you, Dr. Morris, if you have any closing remarks. Lee, if you have anything to say as the patient advocate that attended GI ASCO, I would love to open the floor for you guys. Then we can finish up.

Lee Jones: Just wanted to thank Fight Colorectal Cancer for inviting me to speak at this webinar, and also for making it possible for me to attend ASCO GI Symposium. As I have mentioned before, it was just amazing the number of things that were discussed, all the metastatic, artificial intelligence, early onset, FIT versus colonoscopy, things we didn't have a chance to talk about here, just a number of things that are happening that are all going to potentially benefit patients in the future. I think it's a very good trend and interesting. Thank you.

Carli King, PhD: Great. Well, thank you so much. Dr. Morris, did you have anything to add at the end?

Van Morris, MD: Nope. Thank you for letting us come and talk today. Always happy to.

Carli King, PhD: Of course. Thank you guys so much, and thank you everyone that took the time out of their day to attend.

Van Morris, MD: Thank you.

Lee Jones: Okay, thanks. Bye.