Discussion of 2021-1756

IMPACT OF CO-MUTATION OF *RAS* AND *TP53* ON POSTOPERATIVE ctDNA DETECTION AND EARLY RECURRENCE AFTER HEPATECTOMY FOR COLORECTAL LIVER METASTASES

**DR REBEKAH R WHITE** (LaJolla, CA): Circulating tumor DNA (ctDNA) has become a household abbreviation, and yet, there are still many questions about how we should use this technology clinically. I would like to congratulate the authors on putting together what will be the largest study specifically looking at ctDNA as a prognostic factor after resection of colorectal liver metastases. They have taken advantage of the large clinical volume at MD Anderson to identify a homogeneous population of patients who underwent state‑of‑the‑art treatment, tumor profiling, and ctDNA profiling. It is important to have both tumor and ctDNA profiling, because ctDNA is, by definition, circulating DNA with mutations. Therefore, patients with mutations are going to be more likely to have circulating mutant DNA. This makes it hard to separate the effects of having the mutations themselves vs the effects of the release of ctDNA into the circulation.

 You recognized this, and you have accounted for the *KRAS/TP53* co‑mutation, which was an independent prognostic factor, but what about the other 70% of patients who did not have the co‑mutation? For example, patients who may have had *KRAS* or *TP53* alone? You have the data. I think it is important to consider the tumor profiles more carefully to determine whether the ctDNA is adding prognostic value to the tumor profiles themselves. However, your data that having positive ctDNA puts you at very high risk for early recurrence are compelling. As you also highlighted, the specificity was much better than the sensitivity.

 The tests you used in your study are the clinically available ones that rely on rationally selected panels of genes and do not require knowledge of the tumor profiles. It makes sense if you do not have tumor profiles, but, since you do, have you considered using tumor-informed tissue tests, such as Signatera? A related question is, how much do these tests cost? You have already addressed that you might like to try using them longitudinally. Who would pay for it?

 Finally, your overall survival outcomes are excellent: 80 months. So, I was just wondering whether you looked at survival in patients who underwent ctDNA testing vs those who did not, because I wonder if there is some selection bias based on socioeconomic status or the sophistication of the treating oncologist.

**DR JOHN S BOLTON** (New Orleans, LA): There is a lot of excitement about the use of liquid biopsy to assess disease status after curative intent cancer resection. For many years, clinical examination and imaging studies have been the backbone of our follow‑up for most patients who have undergone resection. In the absence of findings on these modalities, these patients were said to be "NED," no evidence of disease. Unfortunately, many NED patients ultimately recur, and by the time recurrence is evident, clinically or radiographically, our therapeutic options are limited, and almost never curative.

 To me, the most compelling finding in Dr Newhook's study was that, among patients who were clinically and radiographically NED postoperatively but who had a positive ctDNA, all recurred early. The 2 not within the first year recurred within the next couple of months after that.

 In essence, have we not changed the definition of NED by adding a molecular dimension? The implication for adjuvant therapy is profound, especially for cancer in which good therapeutic options remain. Unfortunately, for the cohort of ctDNA-positive patients in this study who were largely in the *KRAS/TP53* co‑mutant group and heavily pretreated with chemotherapy, we do not currently have much to offer. Nonetheless, the demonstration of the high positive predictive value for minimal residual disease with postoperative ctDNA assay will be enormously useful for patients in whom good additional therapeutic options exist, and hopefully, one day, when effective targeted therapy for cancer driven by mutant *KRAS* will exist for this population as well.

 How did you select the patients with the ctDNA assay, since they comprise only a small number of your overall hepatic resections? Were these consecutive patients?

 In the ctDNA-negative patients who had a recurrence, and there were a lot of them, with only about 30% relapse‑free survival at 36 months, do you think it is a problem with the sensitivity of the assay, the lack of sequential testing, or both?

 There are other molecular platforms available that might be more robust, such as circulating micro‑RNA signatures in cell‑free or exosomal form. Do you have any experience with them, and can you comment on them?

 Finally, how will the use of the ctDNA assay change your clinical practice in the future?

**DR LEONIDAS KONIARIS** (Indianapolis, IN): Could you briefly describe how you corrected or adjusted for the confounder of either chemotherapy or immunotherapy? Specifically in the subset of the ACCs, if you included those who had immunotherapy, how did that seem to change the prognosis?

**DR PERRY SHEN** (Winston Salem, NC): You are really on the cutting edge, in profiling or prognostic assessment of patients undergoing resection of colorectal liver metastasis, and sure to generate a lot of interest. You mentioned in your analysis that the ctDNA-negative patients still had a 50% rate of early recurrence, and you alluded to more sensitive testing in the future. I wanted to expand on that question in your current clinical trial. What are you doing in treating those patients who are ctDNA-negative? Have you changed the testing platform or what are you doing to assess these people, if your initial study found that half of them have early recurrence?

**DR VIKAS DUDEJA** (Birmingham, AL): Most patients at MD Anderson receive perioperative chemotherapy; what was the effect of chemotherapy on ctDNA? Did you measure ctDNA before the operation, and did any patients who received chemotherapy become ctDNA-negative?

 Why was the time point of 180 days chosen? Many of these companies recommend doing it 6 weeks postoperatively. Were there some early time points done, and did some patients have positive ctNA early on after operation, or did they become negative afterward?

**DR TIMOTHY E NEWHOOK** (Houston, TX): Regarding the analysis of mutations other than just the co‑mutation of *RAS* and *TP53,* as well as the potential for other undetected mutations that could render patients ctDNA-positive or -negative, we did focus this study primarily on adverse somatic mutations that we commonly look for, including *RAS, TP53, FBXW7, PIK3CA, ERAS*, and the only ones that were associated with outcomes were *RAS* alone or *RAS* with co‑mutation of *TP53*. The co‑mutation had a significantly higher hazard ratio in a much more refined prediction method for the outcomes for these patients. There is obviously the possibility, because we are not using a tissue‑informed personalized approach, that the fixed platform, which usually has a selection of mutations associated with colorectal cancer, may miss patients, and have false-positives and false-negatives. Dr White touched upon the use of the Signatera assay, which, for those who are not familiar, is a tissue‑informed personalized approach, based upon creation of an individualized ctDNA test from tumor‑derived tissue at the time of resection. It promises to potentially be a much more sensitive, and, as I said, individualized approach for these patients. We do have experience with the Signatera.

 The patients in this study were obviously preliminary, from 2013 to 2020, and in the last year, there has been a flurry of activity, with the integration of other platforms in the care of our patients. Signatera is forming the backbone going forward of our minimal residual disease initiative and is being used in our preoperative trials. I do agree that, if possible, a tissue‑informed approach is best, much more sensitive, and individualized, but as you mentioned in your questions, obviously it poses a problem for patients who potentially do not have tissue available or for use for prognostication and treatment response in the neoadjuvant setting.

 To the question regarding a comparison of the 105 patients who had ctDNA available in the study compared with the roughly 800 patients who, across the time period did not have ctDNA available, it is an excellent question. It would be great to answer that in a subsequent study, potentially comparing some of our clinical factors for recurrence, such carcinoembryonic antigen (CEA), compared with our ability to prognosticate with ctDNA.

 The last question was, “Who would pay for ctDNA analyses in the arena such as surveillance?” Currently, the use of the Signatera platform is FDA-approved in the surveillance setting for patients with stage 1‑3 colorectal cancer, and soon to be approved for stage 4. In fact, we have been sending it for patients with stage 4 disease and will be integrating it into every 3 to 4‑month postoperative setting going forward. We have not had any problems with that. So, that is the cutting edge for the Signatera at our institution.

 Regarding Dr Bolton's questions about the selection of patients for ctDNA; this was a retrospective preliminary early analysis of non‑consecutive patients. The small end is a reflection of the introduction of this technology in our experience in our practice, but in the last year or so has become almost routine, and we expect to begin reporting our more recent experience in the coming year.

 This cohort, because of the time restriction, is a relatively restricted cohort compared with all the patients who undergo resection at MD Anderson. We wanted to focus on patients who underwent curative intent hepatectomy and potentially left the operating room NED. So, for example, we frequently perform hepatectomy on patients who have small and controlled lung metastases after preoperative chemotherapy. Without disease progression, they may still harbor clinically evident disease, and therefore would confound our measurement of minimally residual disease. As to the problem of assay sensitivity, sequential testing, or both, for patients who were ctDNA-negative, the answer is both. These platforms are relatively early in this study vs some of the commercially available standard-of-care tests that we discussed, such as the Signatera, and they have since been improved with more sensitive detection methods, more stringent calls of what is a positive or a negative, and the addition of other markers, such as epigenetic methylation markers, to refine sensitivity. Also, there was not a standardized testing time in this project, as it is a retrospective analysis. That may have reflected some false negatives if they were tested too close to surgery. It has been well described that testing too close to surgery decreases the proportion of ctDNA, compared with the cell-free DNA compartment, and results in a dilution of the positive marker we are trying to measure.

 We do not have personal experience with other molecular platforms at our institution, such as MicroRNA signatures, however, we do know it has been well reported that there are other markers of minimal residual disease, such as MicroRNA, both in the cell-free compartment, and also in exosomal structures. It has also been well reported that the proportion of those is much higher in the cell-free compartment compared with ctDNA. Maybe the best step going forward is a combination of the 2, and I think we found another research project in that question.

 How will this change our practice? We are integrating ctDNA in the perioperative setting, both pre‑chemotherapy, post‑ chemotherapy, postoperatively, and in the surveillance setting, as we feel it is a much more sensitive marker compared with clinical markers we used earlier. It is also leading us to stratify patients for intensity of therapy, as I will address in response to another question.

 We did not correct for the confounder of chemotherapy in the study, as almost 90% of patients underwent preoperative chemotherapy. There were no hepatocarcinoma patients in the study, as it was restricted to colorectal liver metastases, and no patients in the study received immunotherapy.

 It is obviously a problem that patients who are ctDNA-negative after operation still recur, and that is probably a result of the early platform, but definitely something that will improve as platforms become much more sensitive. Hopefully, as I said, we can decrease the proportion of these patients.

 In the context of the clinical trial we recently opened, we are using the Signatera assay for this test, and the sensitivity of the Signatera is much better than what was reported in the early analysis here. What we do know from another study which is currently under consideration for publication, is that in patients who are preoperatively ctDNA-positive, there is no association with oncologic outcomes in the population of patients who underwent perioperative chemotherapy, but postoperative positivity or negativity is what determines outcomes. Patients who are ctDNA-negative preoperatively remain ctDNA-negative postoperatively, and they have the best outcomes. But there is a cohort of patients who are ctDNA-positive preoperatively and are rendered ctDNA-negative after curative intent hepatectomy. Those patients have outcomes like those who are negative the whole time, identifying patients who have the best surgical outcomes.

 With an extremely sensitive test such as the Signatera, we found that these are the patients who are unlikely to benefit from intensive adjuvant chemotherapy. It would obviously improve our recurrence‑free survival in this population compared with this preliminary analysis, and, therefore, spare patients potentially unwanted adverse effects of ongoing intensive chemotherapy and reserve lines of chemotherapy for recurrence when and if they do recur.

 In this study, we reported our postoperative ctDNA-positive or -negative results. We did not include any pre‑chemotherapy ctDNA results in this study. But there is a paper under consideration for publication from our group detailing this, using a different platform in a prospective manner.