



Immunotherapy Response Prediction Improved by Baseline, On-Treatment Biomarker Combo

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NEW YORK – New research has begun to make a case for both multi-biomarker approaches and multi-timepoint testing as a way to identify more cancer patients who benefit from immunotherapies while saving others who do not from wasted treatment.

In a study [published last week in *Cell*](#), investigators from Stanford and Memorial Sloan Kettering collaborated to explore a novel combination biomarker approach for immunotherapy response prediction in non-small cell lung cancer.

The strategy incorporates both tumor and immune-related factors, including circulating immune cells, blood-based tumor mutational burden, PD-L1 status, and levels of mutated circulating DNA. But unlike some other multi-modal approaches, investigators also added cell-free DNA testing after patients had started treatment, looking for changes in the earliest weeks of therapy that would indicate benefit where pre-treatment markers alone could not.

According to the authors, a pre-treatment composite model combining tumor PD-L1 expression with ctDNA for blood-based mutational burden calculation and circulating immune cell profiling — which they called "DIREct-Pre" — was promising on its own, outperforming some previously described integrative approaches.

But adding an early on-treatment measurement of circulating tumor DNA improved prediction even further, as well as obviated the need for PD-L1 status. The resulting method, called DIREct-On, outperformed any individual feature used alone, the authors wrote. Statistical analyses showed that each factor, including on-treatment ctDNA changes, was required for optimal classification of responders versus non-responders. And the method also did better than what has been seen with on-treatment monitoring only.

Overall, it appeared that the combination was additive in both directions. On-treatment ctDNA changes help capture responders that pre-treatment biomarkers, even combinations, miss. But the reverse is also true.

"There have been several papers looking at ctDNA response as a way of identifying responders prior to when you can do it radiographically," said Maximillian Diehn, a clinical research lab leader at Stanford and one of the papers lead authors. "That can work, but it doesn't catch everybody at the earliest time point ... and if you're going to use a response-based biomarker, it makes sense that you want to be able to read that out as soon as possible, the earlier the better."

"We show in our paper that if you look really early, after a single cycle of immunotherapy, [lower ctDNA] does identify people who are likely to respond. But there's a group of patients who haven't really changed much after that one cycle, but who ultimately will have a response or durable benefit," he added. "We showed that together, [our combination] outperforms any of the individual components, including ctDNA changes alone."

According to Diehn, the need for better immunotherapy biomarkers has been an ongoing concern for oncologists, especially in lung cancer where immune checkpoint inhibitors are now commonly used.

Although ICI treatment can produce durable responses in NSCLC, many patients progress despite the therapy. According to the study authors, current practice in the US is to use the ICI pembrolizumab (Merck's Keytruda), with or without chemotherapy, based on a patient's PD-L1 status. Low PD-L1 supports adding chemo, while high PD-L1 indicates immunotherapy alone has a good chance of working just as well.

But trials have shown that a significant subset of patients whose tumors have low PD-L1 staining can still respond well to immunotherapy alone, demonstrating that the biomarker is not doing the best job of identifying all potential responders. The same has been seen with tumor mutational burden, which recently made the jump from an investigational marker to an approved companion diagnostic, with the US Food and Drug Administration's [approval this July](#) of a second tissue-agnostic indication for pembrolizumab, using tumor mutational burden as a biomarker to guide which refractory solid tumor patients can receive the immunotherapy.

In trying to overcome the limitations of individual features like PD-L1 expression or TMB, more and more studies are now exploring [combination strategies](#) that amass these and/or other factors like immune or microenvironmental gene-expression signatures, into an algorithmic predictive score.

"This area has shown itself more complicated than, let's say, targeted therapies that are mutation driven, where the tumor mutation status really defines the patients very likely to respond," Diehn said. "With immunotherapy, because you're asking two cell types in the body to interact — the tumor and the immune system — it's more complicated," he argued. "There seem to be factors on the tumor side as well as on the immune system side, but we don't have good enough pure predictive biomarkers."

Separately, researchers have also been exploring whether changes in certain biomarkers as patients go through their first weeks or months of treatment — especially circulating tumor DNA — might presage what will turn out to be a robust or failed response.

In clinical practice patients are already monitored during immunotherapy using imaging so the principle of an on-treatment response marker isn't a hard sell. But unfortunately, radiographic scans take a relatively long time to resolve in one way or another. And, like pre-treatment biomarkers, they can miss or mistake some of what turn out to be powerful responses, according to the study authors.

Encouragingly, circulating tumor DNA has shown promise as a more sensitive and rapid indicator of the same therapy effects that doctors hope to glean from scans. Evidence using [patient-specific mutation panels](#), for example, has demonstrated that changes in ctDNA levels after six weeks of treatment could predict whether patients would benefit from immunotherapy.

And new research presented last month by University of California, San Diego researchers at the European Society for Medical Oncology's Virtual Congress has added more evidence, demonstrating that even a more blunt measurement of changes in the variant allele frequency (VAF) of circulating cell-free DNA can provide significant prediction of patients' likelihood of a long-term response across a variety of solid tumors.

Investigators in that study followed 80 patients with a range of different tumor types given anti-PD-1 or PD-L1 treatment. Authors, headed by Razelle Kurzrock, director of the UCSD Center for Personalized Cancer Therapy, reported that patients in a small cohort with notable drops in their cfDNA VAF after one or two immunotherapy treatments had significantly better progression-free and overall survival compared to those who didn't show a significant drop.

With their own endeavor, Diehn and his collaborators hoped to outperform both the multi-modal approaches that have been shared previously, and what has been achieved with on-treatment cell-free DNA monitoring alone.

"We think this is a potential strategy going forward where you combine some of the pretreatment factors that are each predictive, just weakly predictive ... with early on-treatment response assessment. You don't have to know exactly what the mechanism is, but you can still have a very strong predictor of who's ultimately going to get durable benefit."

Using a cohort of about 100 patients that they divided into investigation and validation sets, the group first developed and evaluated the DIREct-Pre combination, and then the multi-timepoint DIREct-On. Instead of measuring overall and progression-free survival, the researchers chose to calculate a binary endpoint of durable complete benefit or non-durable benefit.

According to the authors, DIREct-On clearly added value over the pre-treatment combination alone and showed strong performance in various subsets of patients such as those on different combination treatments or with squamous versus non-squamous histology.

Although they admitted that making cross-study comparisons can be problematic, the authors said that DIREct-On seemed to outperform previously described integrative models relying on tumor RNA and/or DNA sequencing. It also performed significantly better than looking at ctDNA dynamics alone in their own sample set.

Biologically, it would make sense that there might be independent and additive value for the various components and pathways represented by the different aspects of both DIREct-Pre and DIREct-On, the group added. Pre-treatment ctDNA, for example, reflects total-body tumor burden, something that is known to correlate with poorer response to ICIs, while tumor mutation burden, as a surrogate for tumor neoantigens, represents increased or decreased immunogenicity, which is also recognized as a factor in immunotherapy success or failure.

Similarly, changes in the quantity and landscape of immune cells mark biological processes affecting outcome. And finally, early ctDNA dynamics likely mirror changes in disease burden in response to treatment: a direct reflection of tumor response or resistance.

According to Diehn and colleagues, its completely possible other additions, like deeper immunophenotyping, could improve either the pre-treatment DIREct-Pre, or the more complex Direct-On even further.

Kurzrock, who spearheaded the UCSD poster presentation on ctDNA dynamics last month at ESMO, said in an email that she sees composite biomarkers as crucial in the push to personalized cancer immunotherapy.

"The bottom line is that the baseline evaluations narrow down the patients most likely to respond, but then serial readouts give an early glimpse into response and resistance and may also help differentiate pseudoprogression (which ultimately may turn into response) from real progression," she wrote.

Her team is also looking at multiple baseline markers to predict response, including TMB, as well as other specific genomic signatures and measures of adaptive immunity, trying to capture both how immunogenic the neoantigens produced by a cancer may be, as well as "how well the cancer cell presents the neoantigens for recognition (and hence eradication) by the [immune system.]"

Kurzrock added that a hope for strategies like this would also be that genomic and other factors, whether baseline, or dynamic, could help oncologists decide not just if a particular immunotherapy is going to work,

but also which combinations of targeted, immune, or systemic treatments should be given to each individual patient.

Dynamic changes of circulating tumor DNA in the blood could also provide information about the reasons for resistance and the paths to overcome it.

Authors of the new *Cell* study wrote that the most important next steps for taking their approach forward will be conducting additional validation — potentially exploring predictive power in other cancer types — and doing prospective studies to prove the clinical utility suggested by the data thus far.

"It's complicated now because of the recent approvals outside of lung cancer, whether this should be done just in lung cancer or beyond lung cancer, but a prospective clinical trial where you would make a decision based on this information is something we're definitely interested in," said Ash Alizadeh, a Stanford oncologist and professor, and Diehn's co-author.

According to Diehn and Alizadeh, one potential strategy that could be tested in prospective trials would be to treat all NSCLC patients with single-agent immunotherapy for one cycle, regardless of their PD-L1 status, and then to personalize subsequent cycles based on DIREct-On measured a few weeks after the start of treatment. Patients with high DIREct-On scores could remain on single-agent treatment, and patients with low scores would be treated with other options such as the addition of chemotherapy to PD-L1 blockade, adding other immunotherapies like CTLA-4 blockers for a dual immunotherapy approach, or changing completely to another regimen.

"It's quite a sizable undertaking if we wanted to do a true predictive biomarker exercise ... where you would take away something from people who are not responding or adding something to those who aren't responding," Alizadeh said.

Diehn added that he has already had discussions with the study's third co-author, MSK's Matt Helman, about potential prospective follow-up. "There's nothing launching imminently, but we are discussing it," he said.

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