PRECISION ONCOLOGY NEWS

# Transcriptomics Plus 'Immune Tolerance' of Normal Tissue Shows Promise in Lung Cancer Prognosis

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NEW YORK – Researchers involved in the Worldwide Innovative Networking (WIN) Consortium have shown, for the first time, that biomarkers detected in normal lung tissue can be valuable for predicting lung cancer patients' likelihood of post-surgery recurrence.

With several exceptions — namely germline testing — most biomarker insights in oncology rely on analysis of tissue or cells from the cancerous tumor itself. This is especially true in lung cancer, where somatic mutations or expression of genes in tumor tissue are routinely used to guide treatment decisions. Even most blood-based biomarker tests are tumor-centric, focusing on pieces of DNA shed from the tumor tissue circulating in blood.

Now, for the first time, researchers have demonstrated that the normal, healthy tissue in a patient's lung — that is, non-tumor lung tissue — can be rich in biomarkers that shed light on a patient's likelihood of relapse after surgery, information that oncologists can use to guide adjuvant therapy choices.

In a paper published last week in *JCO Precision Oncology*, WIN researchers, including Vladimir Lazar, the consortium's founder and chief scientific and operating officer, demonstrated that the so-called "immune tolerance profile" of normal lung tissue, when paired with a tumor-normal transcriptomics-based score, may allow oncologists better insights into patients' prognoses and the treatments most likely to benefit them.

"The problem is that today, there is no biomarker for predicting recurrence," Lazar said. "The recurrence risk, today, is guided by stage and histology."

In the retrospective analysis described in the *JCO Precision Oncology* paper, Lazar and colleagues analyzed data from the observational CHEMORES study, which followed 123 early-stage non-small cell lung cancer patients who underwent surgical resection. Following surgery, based on their treating oncologists' decisions, 61 of these patients received adjuvant chemotherapy, while the other 62 did not. The observational study followed these patients for 92 months.

Researchers in the CHEMORES study performed DNA sequencing and gene expression analysis of resected tumor and normal lung tissue samples obtained during the patients' surgeries. These data were available to WIN researchers who aimed to test their hypothesis in silico that the differential gene expression between the tumor and normal lung tissues, together with the immune competent versus immune tolerant status of the normal lung tissue, could explain patients' risk of recurrence.

**Validating DDPP**

For the gene expression analysis, Lazar and colleagues used DDPP, short for Digital Display Precision Predictor. The tool is fundamentally an algorithm that allows researchers to easily relate any differential expression between normal and cancerous tissue with patients' clinical outcomes.

The transcriptomics-based algorithm is designed to be applicable in virtually any cancer type, with any drug or outcome of interest. DDPP combs through tumor-normal differences in gene expression and homes in on those that correlate best with disease-free survival outcomes. Full clinical and transcriptomic data were available for 120 CHEMORES study participants, whom the researchers organized into six groups: patients with adenocarcinoma who

did and did not receive adjuvant chemo; patients with squamous cell carcinoma who did and did not receive adjuvant chemo; and patients with large-cell carcinoma who did and did not receive the adjuvant chemo.

Using the DDPP, the WIN researchers homed in on six distinct gene expression signatures that correlated with patients' disease-free survival outcomes after surgery. Using a clustering method to select a cutoff, they delineated 75 patients with a DDPP high score and 45 patients with a low score. Those with a high score were more likely to have a longer disease-free survival than the DDPP-low patients. The DDPP-low patients derived a greater benefit from adjuvant chemotherapy, whereas the DDPP-high patients did not benefit as much.

The findings further validated the biomarker approach, which Lazar and colleagues had been testing out using data from patients treated with various immunotherapies in the WINTHER trial.

**Key insights in normal tissue**

While this additional validation is a necessary and important part of advancing any new biomarker approach, the most notable insights from the *JCO Precision Oncology* paper, in Lazar's view, come from the analysis pairing DDPP with normal lung tissue immune analysis.

In this part of the study, researchers considered expression of immune checkpoint genes PD-L1, CTLA-4, and ICOS, either alone or in combination, in patients' normal lung tissue and observed how they correlated with patients' disease-free survival outcomes. For instance, if the normal tissue expressed low CTLA-4 but high ICOS, the median disease-free survival time was 53 months, whereas patients whose normal lung tissue expressed high levels of CTLA-4 and high levels of ICOS had a median disease-free survival of just 25 months.

Meanwhile, patients with high CTLA-4, PD-L1, and ICOS expression in their normal lung tissue had the shortest median disease-free survival time, at 22.5 months, whereas patients with low CTLA-4, high PD-L1, and high ICOS expression lived for a median of 53 months without recurrence.

Combining gene expression levels in normal lung tissue — an approach that the researchers called immune-tolerant profiling — with the DDPP score led to the strongest prediction of recurrence.

"This is the first time the importance of studying not only the tumor but also the host environment of the normal tissue has been demonstrated," Lazar said. "If you take normal tissue immune-tolerance alone, or DDPP status alone, we can predict the outcomes in some way, but if you combine both of them … you obtain a phenomenal separation of the curves."

Indeed, patients with low DDPP scores and immune-tolerant status lived for a median of 10.5 months without recurrence, whereas patients with high DDPP scores and immune-competent status lived for a median of 60 months without recurrence. All of the other patients with various iterations of DDPP scores and immune-tolerant status were grouped together and had a median disease-free survival of 39 months.

WIN researchers believe that this combination biomarker approach has the potential to become a powerful method for predicting cancer recurrence and guiding adjuvant treatment. But before that can happen, their approach needs prospective validation. According to Lazar, this is already in the works.

"Future prospective studies could examine the utility of adjuvant chemotherapy or chemoimmunotherapy in patients predicted to be at intermediate or high risk of recurrence," wrote Lazar and colleagues, likening the approach to the way that the TAILORx trial was used to validate Exact Sciences' OncotypeDx recurrence score as a tool for determining early-stage breast cancer patients' prognosis and whether they'd benefit from adjuvant chemotherapy. In fact, Lazar noted that the WIN team is also validating DDPP plus immune-tolerance profiling as a tool for breast cancer patients.

**Not all that complicated**

Although the DDPP algorithm and normal tissue immune profiling may seem more complex than, say, immunohistochemistry-based single-gene expression testing, Lazar maintained that this new approach can be easily implemented as part of routine cancer treatment.

"This is potentially very easy to translate into the clinic because all it requires is the tumor versus normal comparison of gene expression," he said.

Although tumor-normal genomic analysis is currently not standard practice among oncologists for patients with lung or other types of cancers, Lazar pointed out that most major hospitals have the necessary tools, such as microarray technologies or RNA sequencing, to implement the DDPP algorithm, which the WIN Consortium is "happy to share" with those interested.

The process for patients would remain unchanged from other biomarker analyses. Already, surgeons remove a big chunk of normal tissue surrounding the tumor during resection, and cells from this sample could be used for the normal tissue analysis. "There is no supplementary biopsy and no added risk for the patient because they're in the surgery anyway," Lazar said.

One key difference would be that transcriptomic analysis uses fresh, frozen tissue rather than formalin-fixed, paraffin-embedded (FFPE) tissue, and the latter is far more routine. Historically, switching from FFPE to fresh, frozen tissue collection has been a hurdle for researchers, but Lazar believes it is a "problem that should not exist," since collecting and freezing tissue is no more labor- or resource-intensive. Freezing requires dry ice and different fixing agents, whereas FFPE requires formalin and paraffin. "It is very easy to switch from one to the other [and] the quality of the analysis is much better [with fresh, frozen tissue]," he said.

As for commercializing the scores as a marketed test, Lazar said that industry players have expressed interest. Even though research labs within cancer centers could implement this biomarker testing in-house, he said a commercial partner could standardize the process. "If this is for academic purposes, we can share the codes and have interaction with the site, but the best would be for industry to make it a standard product that is available to everyone," he said.

Although Lazar declined to disclose which industry players have shown interest, he pointed out that the WIN Consortium comprises not just a global network of physician-scientists but also industry players, such as AstraZeneca, Pfizer, Merck KGaA, HTG Molecular, Illumina, and InterVenn. Researchers from AstraZeneca and Pfizer are authors on the *JCO Precision Oncology* paper.

As the WIN Consortium researchers continue prospective validation of their biomarker approach and explore commercialization paths, Lazar is spreading the message throughout the oncology community that this type of analysis is not all that complicated.

"The pathologist dissects the tumor, … you perform DNA and RNA extraction, and then you analyze these data," he said, pointing out that this part of the workflow is "always the same" in any molecular test. "What is most important is doctors' knowledge that their patients can benefit from this analysis," he added, explaining that oncologists' lack of buy-in and understanding of the value of this type of biomarker approach can be more of a hindrance than the technology and infrastructure needs.

This is no small problem. There is data showing inconsistent adoption among community oncologists in the US and Europe when it comes to NGS sequencing panels, even though these panels are readily available through multiple commercial labs and even when their use is backed by practice guidelines in certain settings. According to a recent survey from the European Society for Medical Oncology, for instance, the availability of comprehensive biomarker tests varies tremendously throughout 53 European countries, and many health systems only use multi-gene biomarker tests in research settings as opposed to routine patient care.

Lazar recognizes that these implementation barriers exist, as much as he argues they shouldn't. But he's also optimistic about the years ahead. "Analyzing both tumor and normal tissue from the patient is new and will take some time to see if people may be willing to accept it or not," he said. "But remember, even tumor biopsy is not so old. There is a lapse of time required for new approaches to become reality in clinical care."

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