DNA sequencing determines lymphoma origin, prognosis

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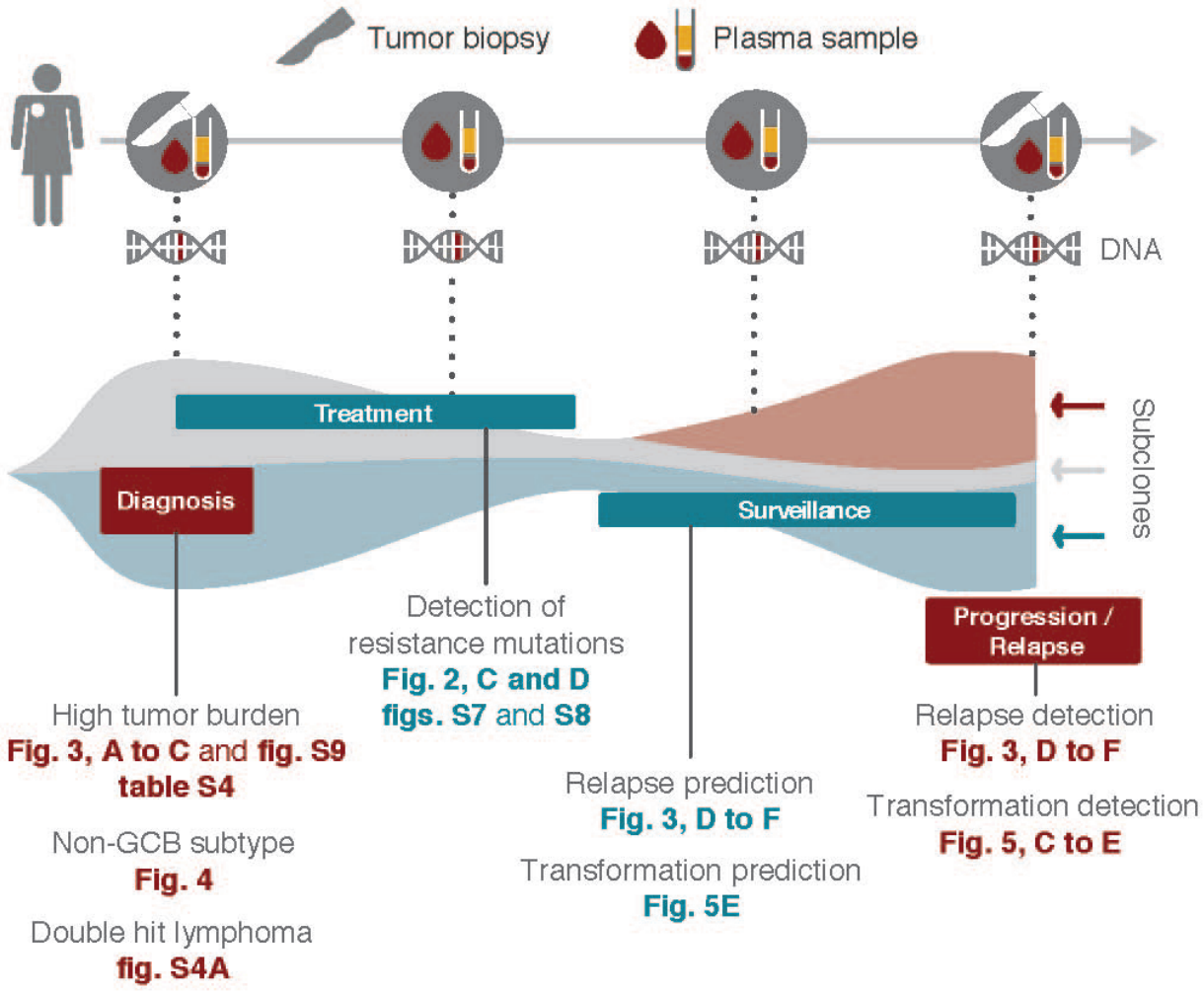

Framework for noninvasive identification of diffuse large B cell lymphoma (DLBCL) poor-risk groups at different disease milestones. Credit: F. Scherer et al., *Science Translational Medicine* (2016)

Sequencing tiny bits of DNA circulating in the blood of patients with lymphoma can accurately identify the cancer subtype and pinpoint mutations that might cause drug resistance, according to researchers at the Stanford University School of Medicine.

This knowledge could help personalize cancer treatment by revealing which patients are likely to be treated successfully and those who may have a poorer prognosis.

Tracking sequence changes over time could also provide a kind of early warning system to identify the emergence of an aggressive form of the cancer by providing a real-time window into tumor evolution. The findings bolster the growing notion that noninvasive, blood-based biopsies of what's known as circulating tumor DNA are likely to transform cancer care.

"Now we can identify the subtype of the tumor, watch how it changes over time and begin to tailor our chemotherapy choices based on the presence or absence of specific mutations," said assistant professor of medicine Ash Alizadeh, MD, PhD. "We've moved beyond just measuring disease burden based on the amount of tumor DNA in the blood."

Alizadeh and assistant professor of radiation oncology Maximilian Diehn, MD, PhD, share senior authorship of the study, which will be published Nov. 9 in *Science Translational Medicine*. Postdoctoral scholars Florian Scherer, MD, and David Kurtz, MD, and instructor Aaron Newman, PhD, are the lead authors.

Longitudinal study of 92 patients

The researchers conducted a study of 92 prospectively enrolled patients with diffuse large B-cell lymphoma. DLBCL is the most common type

of non-Hodgkin lymphoma and is highly biologically variable. As a result, patients vary widely in their response to treatment. About one-third of seemingly successfully treated patients eventually relapse, or their tumors become resistant to treatment. Additionally, a form of indolent B cell lymphoma, which progresses slowly with only mild symptoms, can transform without warning into an aggressive form of the disease.

"This transformation is very difficult to detect, and usually requires an invasive biopsy to diagnose," said Diehn. "Our approach will allow us to monitor patients over time with a simple blood test, and may help us identify transformation much earlier."

The researchers used an enhanced version of a technique they developed called CAPP-Seq to isolate and sequence circulating tumor DNA, or ctDNA, from blood samples from the patients. Unlike previous studies, which tracked lymphoma progression by monitoring the sequence of just one cancer-associated protein, CAPP-Seq can identify a much larger range of mutations in the tumor genome.

They then compared the ctDNA sequences obtained from the patients' stored blood samples with those of the tumor cells from invasive biopsies, and paired the information with what was known about the course of the patient's disease and eventual outcome. They found that low levels of ctDNA after diagnosis but before treatment correlated strongly with progression-free survival in the patients. Those with higher levels of ctDNA faired more poorly overall. Furthermore, they were able to detect the presence of ctDNA in the blood of relapsing patients on average six months before any clinical symptoms appeared and as long as 2.5 years before clinical signs of relapse.

Determining cancer's cell of origin

Perhaps even more importantly, however, the researchers found they could use CAPP-Seq to determine the type of B cell from which the cancer originated and predict prognosis. About two-thirds of people with the germinal center subtype live for five years or more after diagnosis, while those with activated B-cell-like tumors have a poorer prognosis with current treatment regimes. These subtypes are known to predict differential responses to emerging targeted therapies, but they are cumbersome to measure accurately and require biopsies.

Finally, the researchers were able to predict from the ctDNA sequences those patients whose disease was transforming into a much more aggressive form prior to the emergence of clinical symptoms, and even to identify and track specific mutations known to inhibit the response to the targeted therapy with a drug known as ibrutinib.

"In this study we've shown five distinct ways—by quantifying tumor burden, identifying disease subtype, cataloging mutations, predicting transformation and providing early warnings of recurrence—that circulating tumor DNA can yield potentially clinically useful information," said Diehn. "Now we're eager to conduct prospective studies in recently diagnosed patients to learn how we can best improve patient care."

Alizadeh and Diehn are both investigators at the Ludwig Center for Cancer Stem Cell Research and Medicine at Stanford.

The team's work is an example of Stanford Medicine's focus on precision health, the goal of which is to anticipate and prevent disease in the healthy and precisely diagnose and treat disease in the ill.

More information: "Distinct biological subtypes and patterns of genome evolution in lymphoma revealed by circulating tumor DNA," *Science Translational Medicine*, stm.sciencemag.org/lookup/doi/ …

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