Hodgkin lymphoma prognosis, biology tracked with circulating tumor DNA

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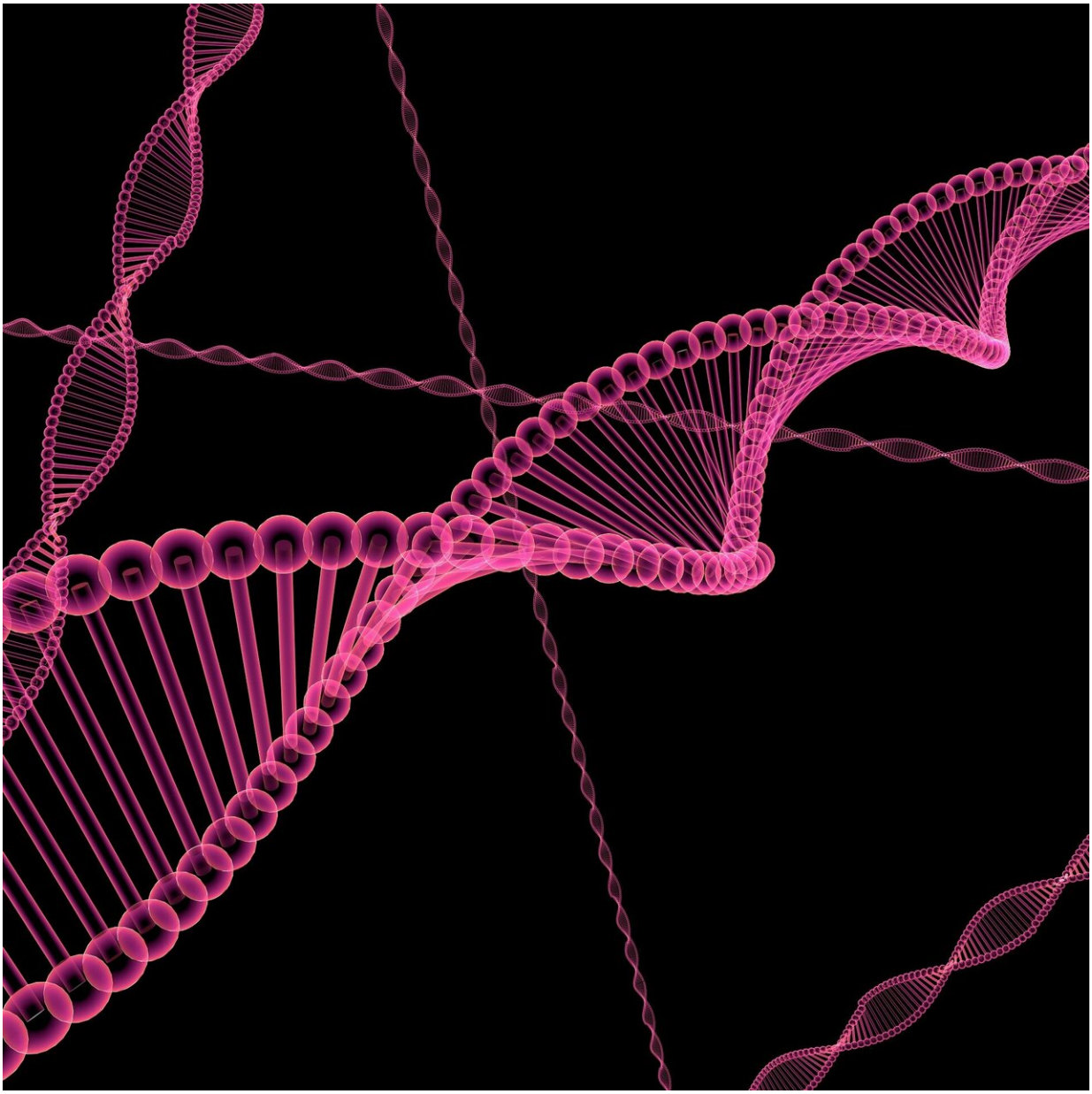

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A Stanford Medicine-led, international study of hundreds of samples from patients with Hodgkin lymphoma has shown that levels of tumor DNA circulating in their blood can identify who is responding well to treatment and others who are likely to experience a disease recurrence—potentially letting some patients who are predicted to have favorable outcomes forgo lengthy treatment.

Surprisingly, the study also revealed that Hodgkin lymphoma, a cancer of the lymph nodes, can be divided into two groups, each with distinct genetic changes and slightly different prognoses. These changes hint at weaknesses in the cancer's growth mechanisms that could be targeted by new, less toxic therapies.

The idea of establishing molecular profiles of tumors is not new. But unlike other cancers, Hodgkin lymphoma has resisted these types of analyses. That's because Hodgkin lymphoma cells are relatively scarce—even within a large tumor.

"This approach offers our first significant look at the genetics of classical Hodgkin lymphoma," said professor of medicine Ash Alizadeh, MD, Ph.D. "Compared with other cancers, finding Hodgkin lymphoma cancer cells or cancer DNA to study is like searching for a needle in a haystack. A patient can have a football-sized tumor in their chest, but only about 1% of the cells in the mass are cancer cells, with the remainder representing an inflammatory response to the tumor. This has made it very difficult to find the smoking guns that drive the disease."

Alizadeh, who is the Moghadam Family Professor, and Maximilian Diehn, MD, Ph.D., professor of radiation oncology and the Jack, Lulu,

and Sam Willson Professor, are the senior authors of the research, which was published in *Nature*. Former postdoctoral scholar Stefan Alig, MD; instructor of medicine Mohammad Shahrokh Esfahani, Ph.D.; and graduate student Andrea Garofalo are the lead authors, as is graduate student Michael Yu Li at British Columbia Cancer.

About 8,500 people are diagnosed with Hodgkin lymphoma each year in the United States. The disease primarily affects people between the ages of 15 and 35, and people older than 55.

Stanford Medicine's role

Just over 60 years ago, Stanford radiologist Henry Kaplan, MD pioneered the use of targeted radiation to treat Hodgkin lymphoma.

The new therapy, delivered by a high-energy linear accelerator Kaplan developed in the 1950s for medical use, was the first step in a Stanford-driven effort to turn the once fatal cancer of the lymph nodes into one that is now highly curable.

Soon thereafter, Kaplan was joined by medical oncologist Saul Rosenberg, MD, and the two worked out ways to combine radiation therapy with chemotherapy regimens, including one known simply as the Stanford 5 (named because it was the fifth in a series of gradually less toxic treatments).

During the subsequent decades, however, the genetic changes that cause the cancer have remained mysterious. That's because, unlike many other cancers, Hodgkin lymphoma tumors are made up primarily of immune cells that have infiltrated the cancer, making it difficult to isolate the diseased cells for study. Today, patients are treated with chemotherapy, radiation or a combination of both; about 89% of patients survive five years or more after their initial diagnosis.

Alizadeh, Diehn and their colleagues used an optimized DNA sequencing technique called PhasED-Seq, or phased variant enrichment and detection sequencing, they developed at Stanford Medicine in 2021 to home in on vanishingly rare bits of DNA in a patient's bloodstream to identify genetic changes that drive the growth of Hodgkin lymphoma.

PhasED-Seq builds upon a technique called CAPP-Seq, or cancer personalized profiling by deep sequencing, developed in 2014 by Alizadeh and Diehn to assess lung cancer levels and response to treatment. But PhasED-Seq is much more sensitive.

"CAPP-Seq could detect as few as one cancer DNA sequence in 10,000 non-cancer DNA sequences," Diehn said. "But PhasED-Seq can detect less than one cancer DNA sequence in 1 million non-cancer DNA sequences."

Their goal was to learn more about what drives the cancer and how to make successful treatments even easier for patients.

"We typically can cure most patients with one line of therapy," Alizadeh said. "But we are always trying to figure out less toxic chemotherapeutic agents that are gentler to the bone marrow, lungs and other organs, and ways to more precisely target radiation therapy. And a small minority of patients experience a recurrence that can be challenging to treat successfully."

The researchers used CAPP-Seq and PhasED-Seq to analyze blood samples from 366 people treated for Hodgkin lymphoma at three medical centers including Stanford Medicine. The technique was remarkably sensitive.

"Surprisingly, we detected more cancer DNA in the blood than in the cancer tissue itself," Alizadeh said. "That seemed hard to believe until

we had analyzed enough samples to show that it was reproducible."

Two paths

The researchers used machine learning techniques to categorize the different types of genetic changes present in the cancer cells. They found that patients could be separated into two groups: one that predominantly has mutations in several cancer-associated genes implicated in cellular survival, growth and inflammation, and another with a type of genetic change called copy number alterations that affects larger swathes of the genome, subbing in or excising regions of DNA that influence cell growth and cancer.

"We adapted a method from natural language processing to find these two Hodgkin subtypes, and then used a variety of methods to identify key biological and clinical features and to confirm that the subtypes are also seen in other groups of patients," Esfahani said.

The first group, which makes up about one-half to two-thirds of patients, occurs primarily in younger patients and has a comparatively more favorable outcome. About 85–90% of these people survive for three years without disease recurrence.

The second, which makes up about one-half to one-third of the total, occurs in both younger and older patients and has a less favorable, although still good outcome. About 75% of these people live for at least three years without recurrence.

Critically, a subset of both groups contained a unique mutation in a gene for the receptor for cellular signaling proteins called interleukin 4 and interleukin 13.

"We discovered a new class of mutations in the interleukin 4 receptor

gene that enhance a key pathway characteristic to Hodgkin lymphoma," Alig said. "These mutations may be indicative of unique vulnerabilities of the tumor that can be exploited therapeutically."

The researchers also showed that patients who had no detectable circulating tumor DNA in their blood shortly after starting treatment were much less likely to have disease recurrence than those who had even small amounts of residual circulating cancer DNA at the same time point—a distinction researchers had hoped to see, but were unsure about being able to detect even with PhasED-Seq.

"I was surprised that we could predict which patients would recur," Diehn said. "Even with our ultrasensitive assay there was a significant chance that the signal from the cancer DNA could become undetectable after treatment, even in patients who eventually recurred. But this didn't happen."

The researchers seeking to understand more about the biology of Hodgkin lymphoma have one key goal: the improvement of care for patients.

"The number of people who experience recurrence is small, but, like Henry Kaplan and Saul Rosenberg, we want to save every one of them," Diehn said. "They would have been amazed and gratified by these findings, which build upon their important work from decades ago. We look forward to an era in which we can cure every patient with no toxicity."

Researchers from British Columbia Cancer, University Hospital François Mitterand, St. Jude Children's Research Hospital, the Oncology Institute of Southern Switzerland, KU Leuven, the University of Strasbourg, Emory University, the Fred Hutchinson Cancer Research Center, the Hospices Civils de Lyon, and the Université Catholique de

Louvain contributed to the work.

More information: Stefan K. Alig et al, Distinct Hodgkin lymphoma subtypes defined by noninvasive genomic profiling, *Nature* (2023). DOI: 10.1038/s41586-023-06903-x , doi.org/10.1038/s41586-023-06903-x