'Hard to lose' mutations in tumors may predict response to immunotherapy

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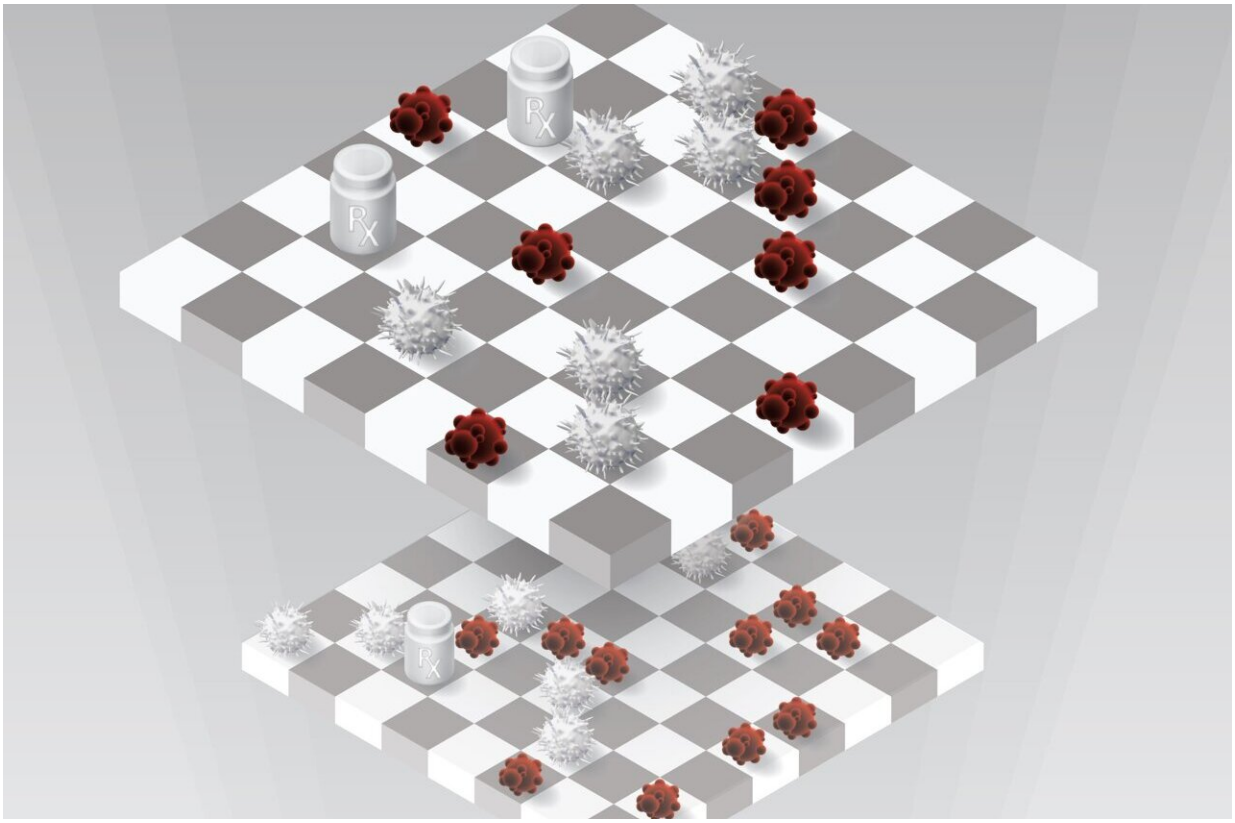

The figure shows the interactions between immune cells (white) and tumor cells harboring persistent mutations (red) in the context of immunotherapy (white) as a chess match captured in 5 snapshots. The piece placement is pulled from a Kasparov vs Deep Blue game. Credit: Christina Kostandi and Valsamo Anagnostou

Cancer experts have tried, sometimes unsuccessfully, to use the total number of mutations in a tumor, called the tumor mutation burden (TMB), to predict a patient's response to immunotherapy. Now, investigators at the Johns Hopkins Kimmel Cancer Center and its Bloomberg~Kimmel Institute for Cancer Immunotherapy have found that a subset of mutations within the overall TMB, termed "persistent mutations," are less likely to be edited out as cancer evolves, rendering tumors continuously visible to the immune system and predisposing them to respond to immunotherapy.

This persistent mutation load may help clinicians more accurately select patients for clinical trials of novel immunotherapies or predict a patient's clinical outcome with immune checkpoint blockade—a type of immunotherapy.

A description of the work was published January 26 in *Nature Medicine*.

"There's a lot of frustration in trying to use tumor mutation burden as a universal predictive biomarker of immunotherapy response across cancers," says senior study author Valsamo Anagnostou, M.D., Ph.D., an associate professor of oncology at Johns Hopkins, director of the thoracic oncology biorepository and co-leader of the Johns Hopkins Molecular Tumor Board and the Lung Cancer Precision Medicine Center of Excellence. "Therefore, it's imperative to identify the most biologically meaningful subset of mutations within the overall TMB. Our study showed that such mutations reside in aneuploid regions (regions with extra or missing genetic material) of the genome."

Immune checkpoints are the immune system's natural on and off switches used to ignite the immune response when it is needed and to turn it off when the job is done. Cancer cells exploit this mechanism, shutting down immune responses targeting cancer cells. Checkpoint blockade is a type of immunotherapy that uses a drug or combination of

drugs to block the off switch, releasing the breaks on immune cells so they can work against the cancer.

The investigators' working hypothesis was that not all mutations within the overall TMB carry the same weight, instead noting that a subset of mutations may be more important regarding keeping a tumor visible to the immune system and more likely to be key drivers of immunologic tumor control in the context of immunotherapy, Anagnostou says.

Generally, every cell in an organism contains two copies of each chromosome. But cancer genomes are aneuploid, meaning there may be one copy of some chromosomes or multiple copies of others in cancer cells. The investigators hypothesized that mutations residing in these genomic regions may be predominantly retained as cancer develops and evolves. In the case of genomic regions with one copy, eliminating that copy could be lethal to a cancer cell, and in case of mutations present in multiple copies, it is improbable that all could be removed by a single chromosomal deletion, Anagnostou explains.

"These 'stubborn,' or persistent, mutations are always there in cancer cells, and these mutations may render the cancer cells continuously visible to the immune system," she says. "If the cancer cell is seen by the immune system as something foreign, then there is an anti-tumor immune response. In the case of immunotherapy, this response is augmented, and the immune system continues to eliminate cancer cells harboring these persistent mutations over time."

Anagnostou and colleagues performed several investigations to arrive at these findings.

"We performed an analysis to determine the landscape of persistent mutations in more than 9,000 tumors across 31 tumor types from the Cancer Genome Atlas," says the study's first author, Noushin Niknafs,

Ph.D., a research associate at the Johns Hopkins Kimmel Cancer Center. "In looking at how different persistent mutation is compared to the overall TMB, we found re-classification rates of TMB-high/low to persistent mutation load-high/low tumors up to 53% in individual tumor types, and a median re-classification rate of 33% across tumor types."

The investigators assessed regions of the genome with a single copy per cell and with two copies per cell, and found that the rate of mutation losses was lower in the regions with a single copy than in those with two copies, supporting the idea that mutations in single copy regions would be difficult to eliminate. The distribution of persistent mutations also differed compared to the overall TMB, where a tumor's TMB was not always concordant with its persistent mutation load. Furthermore, in each tumor type, the team observed differential classification of tumors based on persistent mutations compared to the overall TMB.

The researchers also explored the relationship between persistent mutation load and TMB, using data from seven published cohorts of patients treated with immune-checkpoint blockade therapy across three tumor types: melanoma, nonsmall cell lung cancer and mesothelioma, totaling 485 patients. They also looked at these features in a newly sequenced cohort of 39 patients with human papillomavirus (HPV)-negative head and neck cancer who received this immunotherapy. Again, the team observed that overall TMB and persistent mutation burden were different across the cancers analyzed. There were tumors with a high overall TMB with a smaller subset of persistent mutations, and conversely, there were tumors with a low overall TMB but a higher fraction of persistent mutations.

In further analyses, the scientists evaluated whether a higher persistent mutation load (pTMB) was linked with clinical outcomes among patients with previously untreated tumors from the Cancer Genome Atlas. They found a significant association with prolonged overall survival for lung

squamous cell cancer, melanoma and uterine cancer but not for other cancer types studied.

The investigators hypothesized that tumors with a high pTMB would be most visible to the immune system and, therefore, would regress after exposure to immunotherapy. They evaluated the potential of pTMB, multicopy and single-copy mutations to predict response to immune checkpoint blockade among 542 patients with melanoma, nonsmall cell lung cancer, mesothelioma and head and neck cancer, discovering that tumors with a high pTMB attained higher rates of therapeutic responses to the immunotherapy, while TMB, the number of loss-prone mutations, or tumor aneuploidy, less optimally distinguished tumors that responded from those that did not respond. In addition, in comparing tumor samples prior to immunotherapy and at the time of acquired resistance, the team observed a more than 60-fold lower probability of loss for persistent mutations. Persistent mutation load showed promising performance in predicting immunotherapy response when pTMB was computed from targeted next-generation sequencing, which is the testing modality routinely used in clinical practice.

These findings further support the clinical utility of persistent mutations. Future steps include additional large-scale validation of the findings as well as prospective analyses to evaluate the role of persistent mutation load to select patients for cancer immunotherapy.

Study co-authors were Archana Balan, Xiaoshan Shao, Zineb Belcaid, Kristen Marrone, Joseph Murray, Kellie Smith, Benjamin Levy, Josephine Feliciano, Christine Hann, Vincent Lam, Drew Pardoll, Rachel Karchin, Tanguy Seiwert, Julie Brahmer, Patrick Forde and Victor Velculescu of Johns Hopkins, and Karlijn Hummelink and Kim Monkhorst of the Netherlands Cancer Institute in Amsterdam.

More information: Valsamo Anagnostou, Persistent mutation burden

drives sustained anti-tumor immune responses, *Nature Medicine* (2023). DOI: 10.1038/s41591-022-02163-w. www.nature.com/articles/s41591-022-02163-w

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