Using genetic sequencing, researchers are identifying the total number of mutations in a tumor, and developing a biomarker called the tumor mutational burden, or TMB. Ten or more mutations within a tumor per megabase is considered a high TMB. Three years ago, a trial done across tumor types showed that patients with a high TMB are more likely to respond to immunotherapy (Marabelle et al. 2020). That study led the FDA to approve TMB as a biomarker for such treatments.

But what if the genetic tests that measure TMB are misleading or incorrect for some patients? If a patient's TMB is wrongly scored, what are the possible consequences? These questions are addressed in a recent eye-opening study by Amin Nassar, MD, clinical fellow at Yale Cancer Center.

"The problem with that trial," said Dr. Nassar, "is that more than 95 percent of the patients were white. We need to step back and ask, number one, how does this biomarker perform in patients who are non-white; and number two, are there technical issues with the way this biomarker has been studied?"

The most accurate way to determine TMB is to genetically sequence samples from both the patient's tumor and the patient's normal tissue, a process called tumor-normal sequencing. Genetic analysis can then differentiate mutations specific to the tumor from germline mutations inherited by patients. For an accurate TMB, inherited mutations must be excluded from the count.

The trial that the FDA relied upon to approve TMB as a biomarker used a testing platform that was tumor-only, meaning that the patients' normal tissue wasn't sequenced. "So, we can't tell if the mutations used to determine the TMB are coming only from the tumor or if the samples also include mutations the patient was born with," Dr. Nassar explained.

To estimate a patient's TMB, tumor-only platforms try to filter out germline mutations by comparing the genetic data from the patient's tumor with genetic information drawn from large public databases, not from the same patient's normal tissue. But Dr. Nassar points out that 80 to 85 percent of the samples in those databases are from white patients, a population whose typical inherited mutations have been characterized far more comprehensively than those of non-white populations. "As a scientific community, we've done a better job at filtering out germline mutations in white patients," he said, "because we have invested more in knowing more about white patients."

Dr. Nassar wondered if the TMBs of the few non-white patients in the trial included false positives from germline mutations, inflating their TMB scores. To test this, he and his colleagues used databases that included both tumor-only and tumor-normal samples for patients of both European and non-European ancestry. They measured TMB in two ways, using tumor-only sequencing and tumor-normal sequencing that filtered out an individual patient's germline mutations.

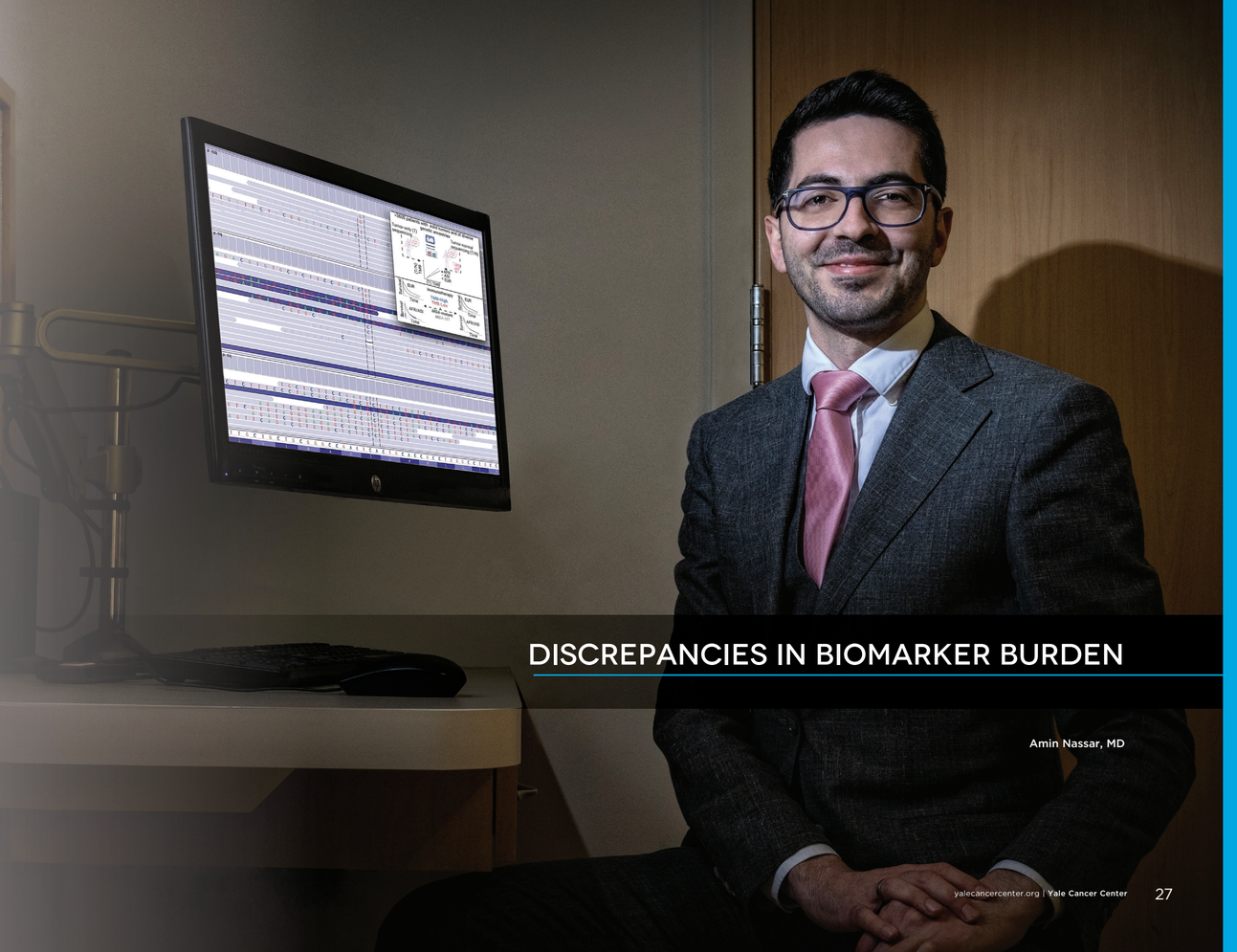
The discrepancy between the scores was striking. "The biggest surprise was the extent of the misclassification. Using tumor-only sequencing, more than 35 percent of Blacks and Asians were misclassified as TMB-high, when in fact they were TMB-low. There was some miscalculation in the white population as well, but way less," he said. "If you look at 100 patients, 21 whites would be misclassified as high when they are actually low, 37 Asians would be misclassified, and 44 Blacks. That's a big number, especially since we know that this biomarker is used to guide cancer management across certain cancer types treated with immunotherapies."

The false positives from the germline could be eliminated by sequencing samples from normal tissue as well as the tumor. "We haven't characterized the normal mutation landscape very well in Blacks and Asians, so the databases do not perform as well in filtering out the false positives from tumor-only estimates of TMB. This is causing us to overestimate TMB more so in Blacks and Asians than in whites."

Dr. Nassar and his colleagues developed an algorithm that recalibrates tumor-only estimates of TMB by considering ancestry and cancer type. Tests across two large patient-groups confirmed that the algorithm more accurately classified tumor-only data. The recalibration tool will be valuable where tumor-only sequencing is still the standard. Yale uses the gold standard, said Dr. Nassar, sequencing both the tumor and normal tissue for every patient, which removes the necessity for recalibration. As the cost of sequencing drops, Dr. Nassar expects more cancer centers to follow Yale's example.

Miscalculation of TMB can have serious consequences. If a physician treats a patient with immunotherapy based on a falsely high TMB, the patient is not only less likely to benefit but may suffer severe side effects. The therapy is not only less effective, but also very expensive.

Dr. Nassar's group also made another surprising finding. "For Asians and Blacks, unlike for whites, TMB didn't pan out as a useful biomarker," he said. That doesn't mean TMB should be discarded for non-whites, he continued. Rather, it needs to be refined by better data—first, by sequencing every patient's normal tissue; second, by placing greater focus on sequencing the human genome for Blacks and Asians; and third, by including a greater diversity of genetic backgrounds in clinical trials.



DISCREPANCIES IN BIOMARKER BURDEN

Amin Nassar, MD