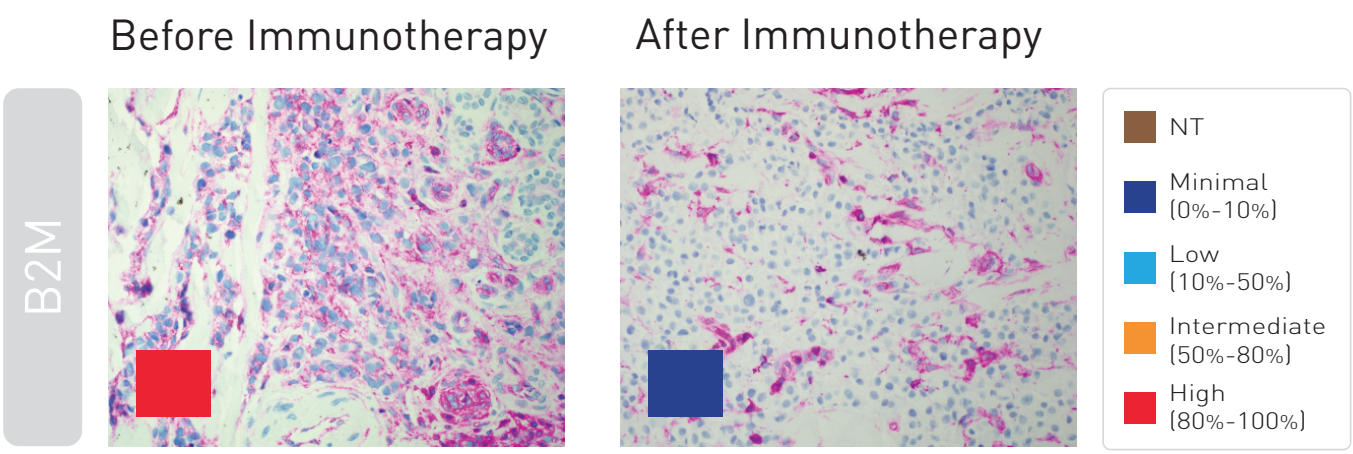
MASSACHUSETTS GENERAL HOSPITAL
CANCER CENTER

ADVANCES AT **MASS GENERAL CANCER CENTER** MARCH 2018

Beating Resistance to Immunotherapy in Metastatic Melanoma

Can defects in the B2M gene that drive resistance be overcome by epigenetic or natural killer cell therapies?



B2M Reduction After Immune Checkpoint Blockade Therapy

These images show beta-2-microglobulin (B2M) protein levels dramatically reduced in tumor cells of a patient who progressed on checkpoint blockade therapy, making the tumor resistant to T-mediated immunity.

Immune checkpoint blockade (CPB) therapy has a high success rate at killing metastatic melanoma compared with other therapies. CPB therapy works by stimulating the body's T-cells to identify and destroy tumor cells that present cancer-specific antigens. But resistance to CPB—both primary and acquired—remains a major cause of mortality.

To understand the mechanisms underlying resistance and identify biomarkers that might predict poor prognosis, Nir Hacohen, PhD, director of the Center for Cancer Immunology at the Massachusetts General Hospital Cancer Center, and his colleagues at Mass General Cancer Center and the Broad Institute of Harvard and MIT searched for genetic mutations associated with that resistance.

They found that 29.4% of patients in a Mass General Cancer Center cohort of 17 patients (with 49 longitudinal tumor samples) with resistant or relapsed tumors had aberrations in the beta-2-microglobulin gene (B2M). These aberrations included loss of an entire copy of the gene and surrounding parts of the chromosome (also known as loss of heterozygosity, or LOH),

multiple frameshift mutations caused by insertions or deletions of nucleotides in the DNA, and a drop in tumor-specific B2M expression levels. No B2M defects were found among those who responded well to the CPB therapy. Their results were published in *Nature Communications* in October 2017.[1]

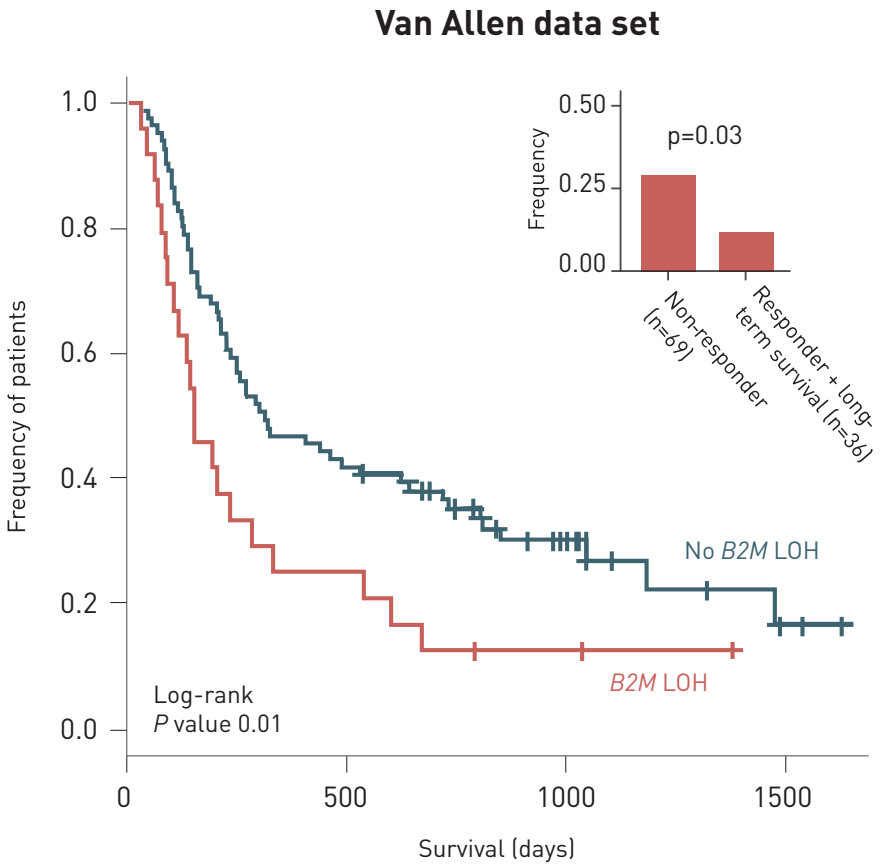
To validate their findings, they examined biopsies of two independent cohorts of 110 and 38 patients. They found that about 30% of patients whose tumors did not respond to therapy had an LOH mutation in the B2M gene, compared with 10% of patients who responded to the therapy. In pretreatment samples, B2M mutations in both copies of the gene were seen only in patients who did not respond to CPB therapy.

PRIMARY AND ACQUIRED RESISTANCE

Because T-cells can detect tumor cells only if they present antigens, and because tumor cells cannot present antigens without functional B2M protein, the researchers were not surprised that the B2M gene plays an important role in resistance.

"The surprise was that we didn't realize that up to a third of patients may already have lost a copy of the B2M gene before therapy," says Dr. Hacohen. "Furthermore, when you get the therapy, you can get an additional mutation in this gene that would make the tumor even more resistant."

The researchers noted in their study that an LOH mutation in the B2M gene could be the initial event leading to complete loss of the B2M gene, followed by antigen presentation loss. They believe that B2M mutations may produce resistance in other cancers, as well.



The Clinical Relevance of B2M Aberrations in an Independent Cohort

To validate the clinical importance of B2M aberrations, biopsies were examined from 110 patients, but five with low tumor content were filtered out. This graph shows survival curves for 105 patients with (red) and without (black) B2M LOH. Inset shows the frequency of patients with B2M LOH in non-responders vs. responders and long-term survivors.

Indeed, it has been shown that high concentrations of LOH B2M mutations are found in 53% of breast cancers, 44% of bladder cancers and 35% of MSS colon cancers.

A TWO-PRONGED APPROACH

These findings lay the groundwork for two potential therapies. The immune

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system's own natural killer (NK) cells are understudied as potential vehicles for immunotherapy, says Dr. Hacohen, and they are very good at destroying cells that lack antigen presentation. To test whether NK cells would attack tumor cells that lack B2M (and therefore the ability to present antigens), Moshe Sade-Feldman, a postdoctoral fellow in Dr. Hacohen's lab, deleted B2M in a melanoma cell line, using CRISPR-Cas9 gene editing tools. He combined these cells with the original melanoma cells and transplanted this mixture into mice with differing levels of NK cells.

Mice lacking NK cells saw an increase in the proportion of tumor cells without B2M. The researchers took this to mean that NK cells may help overcome resistance driven by a lack of B2M. "What we need to do now is develop a therapy that stimulates the NK cells in the people who have B2M loss," says Dr. Hacohen.

Another avenue of future research is to study what drives loss of the B2M protein when only one copy of the B2M gene is missing—which should only occur when both copies of the gene are defective. Dr. Hacohen notes that if epigenetic changes to the genome are involved, they may be reversible with therapy.

"If just one copy is lost, it's possible we could design a drug that would turn the gene and protein expression back on epigenetically," he says. "If both copies are lost, we could deliver a natural killer cell therapy." Either way, Dr. Hacohen and his team see viable directions to move forward with patients who become resistant to CPB.

1 Sade-Feldman M, Jiao Y, Chen J, et al. Resistance to checkpoint blockade therapy through inactivation of antigen presentation. ***Nature Communications***. 2017 Oct; 1136.

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