Immunotherapy is an exciting advance in tumor treatment and identifying relevant peptides presented by major histocompatibility complex (MHC) class I on tumors is critical for this process. In this issue of Blood, Walz et al describe a massive mass spectrometry experiment to identify relevant peptides presented by multiple myeloma (MM) cells and show that these represent just normal antigens. This expands the tumor-relevant peptidome beyond mutated antigens, implying that even tumors that are not highly mutated can be amenable to T-cell–based immunotherapies.1

Tumor immunotherapy started almost a century ago with the first experiments with bacillus Calmette-Guérin in bladder cancer. It took another 70 years before the next major advance was made by vaccinating against melanoma with bacillus Calmette-Guérin in bladder cancer. Since then, the number of antigens that can be used to develop immunotherapies has grown exponentially. Indeed, T cells responding to mutated antigens have been identified in many tumor types, and these are frequently observed in the blood of immunotherapy–responding patients (see figure). The interpretation was simple: mutations make new peptides that are recognized by patient’s T cells responding to immune system activation by the checkpoint antibodies. These T cells then eliminate the corresponding tumor cells. And the result: successful immunotherapy of patients! At least, so goes the interpretation, when the tumor is specified by a high mutational load, for example, as a result of smoking (lung, head and neck, bladder, and other tumors) or sunlight (melanoma).

Although this concept fulfills the general dogma related to T-cell selection and antigen presentation developed over the last 20 years, it also implies that tumors with few if any mutations would fail immunotherapy. But is this correct? Walz et al report in this issue a massive mass spectrometry analysis of the MHC class I– and MHC class II–associated peptidome of MM cells of 29 patients by subtracting the normal B-cell peptidome. They identified over 58 MM-specific peptides that were all derived from normal unmutated proteins. They then performed experiments similar to those described earlier, they generated MHC class I tetramers with identified peptides to show that T cells against (some of) these original peptide–MHC class I combinations could be detected in the circulation of MM patients. The T cells are there; they only require a wake-up call. This procedure does not imply that mutated antigens are entirely absent in the original peptidome of MM cells. The procedure to identify peptides may simply have ignored altered peptides when these could not be mapped on the reference human protein sequences. Incorporating the most common mutated peptides in cancer cells (including those identified through the genome sequencing approach) in these procedures may identify the mutated peptidome. Yet, this does not change a major conclusion from this elegant work: even normal antigens can yield tumor antigens! Every tumor may then in principle be a target of tumor immunotherapy, not only melanoma and other cancers specified by high mutation rates.

How then do normal peptides induce a tumor-specific immune response? This is only conceivable when such peptides have...
been missed in the negative selection steps in the thymus, thus allowing specific CTLs to progress into fully functional entities. CTLs with low-affinity T-cell receptors (TCRs) against self-antigens may escape negative selection. When tumors express these antigens in large quantities, a resulting high MHC-peptide concentration will allow low-affinity TCR to build up a sufficient signal for CTL activation, then following the laws of mass action. Also, CTLs can be made inactive by so-called peripheral tolerance and respond to tumor antigens, especially for antigens more or less selectively and highly expressed in tumor tissue, when activated by checkpoint antibodies. Of note, many patients undergo chemotherapy and/or radiotherapy as first-line treatment of their malignancies. These treatments have marked effects on the peptidome. Many drugs used in chemotherapy of cancer also affect the epigenome and thus the transcriptome, the proteome, and, ultimately, the MHC class I-associated peptidome. Radiotherapy activates the mammalian target of rapamycin pathway, which controls translation of a subset of transcripts and thus alters the proteome and the MHC class I peptidome as well. These and possibly many other options can affect the MHC class I peptidome in tumor cells. These nonmutated peptides can then be recognized by CTLs that have escaped negative selection and allow successful immunotherapy of tumors with low mutation rates, especially when checkpoint control is removed. Mass spectrometry analysis of the tumor-specific peptidome in combination with tetramer analyses identified these peptides for MM. But the study by Walz et al also implies that the full spectrum from tumors with many mutations to tumors with few or no mutations can in fact respond to immunotherapy with checkpoint antibodies and other immunotherapy approaches under development. Feeding the DNA sequencing activities into the programs for identifying the oncopeptidome associated with clinical responses and ultimately be applied in personalized tumor vaccination programs.
may further expand the oncopeptidome for personal vaccination options of cancer patients. The recent developments in tumor immunotherapy may then have clinical consequences for more cancer types than currently appreciated. And this is good news for all cancer patients.

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REFERENCES


Comment on Tawana et al, page 1214

Getting a handle on hereditary CEBPA mutations

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In this issue of Blood, Tawana et al describe 24 patients with acute myeloid leukemia (AML) within 10 families with germline, ie, hereditary, mutations in the CCAAT/enhancer binding protein α (CEBPA) gene. Distinct biology and clinical outcomes, including unique patterns of “relapse,” are identified.1

CEBPα is a key hematopoietic transcription factor involved in lineage-specific myeloid differentiation. Somatic mutations in the gene encoding CEBPα (CEBPA) occur in ~10% of AMLs and contribute to leukemic transformation through impaired myeloid differentiation.2

Pathogenic CEBPA mutations occur primarily within 2 discrete regions (see figure). N-terminal mutations are characteristically frameshift insertions or deletions, leading to forced translation of an alternate 30-kDa protein from within an internal start site, which exerts dominant-negative effects on the full-length CEBPA protein. C-terminal in-frame insertions/deletions occur within the DNA-binding or leucine zipper domains and disrupt DNA binding and dimerization.3

In 2004, the first family with a germline CEBPA mutation was described, when 3 family members diagnosed with AML demonstrated an identical N-terminal CEBPA deletion in all diagnostic tumors and normal/remission samples.4 This autosomal dominant syndrome is now formally designated “familial AML with mutated CEBPA”.5 Although several CEBPA-mutated families have been previously reported, genetic events underlying subsequent AML development and the clinical course of these patients remain ill defined.

This collaborative multicenter study details the clinicopathologic features of 24 AML patients from 10 families with germline CEBPA mutations and sheds more light on this unique inherited syndrome. First, Tawana et al confirm that germline CEBPA mutations occur primarily within the N-terminal domain, with secondary C-terminal CEBPA mutations acquired at AML diagnosis. In 18 diagnostic tumors tested, C-terminal CEBPA mutations leading to double-mutant CEBPA (dmCEBPA) AML were universally identified. In addition, 5 of 9 exome-sequenced tumors acquired a GATA2 mutation, reinforcing a previously described and still poorly understood connection between dmCEBPA and mutated GATA2.6

Furthermore, Tawana et al establish the highly penetrant nature of germline CEBPA mutations leading to subsequent AML development. AML developed at a median age of 24 years (range, 1.8-46 years) in affected individuals. Compared with other inherited leukemia syndromes such as RUNX1-mutated familial platelet disorder/propensity to acute leukemia with ~35% lifetime risk of developing hematologic malignancy,7 the sparse available literature on hereditary CEBPA suggests 100% AML penetrance. In this study, 3 young asymptomatic carriers (ages 19, 24, and 41 years) are described; all other affected individuals have developed AML.

An important discovery comes from molecular sequencing of diagnostic and relapsed AML samples from 5 patients. In these well-annotated tumors, the relapse was clonally distinct from the diagnostic AML, including different acquired CEBPA mutations in all cases, indicating the recurrences are second primary leukemias rather than relapsed disease. This highlights again the preferential specificity of acquired CEBPA mutations leading to dmCEBPA at the time of AML and helps explain the excellent outcomes observed even in the relapsed setting. With a >90% remission rate and a >80% cumulative incidence of relapse, sustained remissions after the third and even fourth relapses were observed. Of 7 deaths occurring after AML recurrence, 5 occurred in remission due to treatment or transplant-related toxicity. In this maturing era of individualized cancer therapy, attention to this striking chemosensitivity (with 1 patient obtaining a 45-year remission with steroids and mercaptopurine alone) is noteworthy and suggests treatment-related toxicity as the primary cause of mortality in familial CEBPA-mutated AML patients.
Expanding the peptidome for immunotherapy

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