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Biomarker of sensitivity to PR-104 in leukemia

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In this issue of *Blood*, the paper by Moradi Manesh et al[1] reports that PR-104 represents a potential novel treatment of relapsed/refractory T-lineage acute lymphoblastic leukemia (T-ALL) and that aldo-keto reductase 1C3 (AKR1C3) expression could be used as a biomarker to select patients who may respond to PR-104 in prospective clinical trials.

Acute lymphoblastic leukemia (ALL) represents the most common childhood malignancy.[2] Because patients with T-ALL are more likely to experience induction therapy failure and early relapse compared with the B-cell precursor subtype (BCP-ALL), there is a high medical need to develop novel treatment strategies for these patients. PR-104, a pre-prodrug currently tested in adult early clinical trials for the treatment of cancer, is converted in vivo to the nitrogen mustard prodrug PR-104A and subsequently activated via hypoxia-dependent reductases or independently of hypoxia by AKR1C3.[3,4] Because AKR1C3 is frequently overexpressed in human cancers, including leukemia, PR-104 is considered a promising targeted drug for cancers with high AKR1C3 expression. Testing of PR-104 in a panel of primary pediatric cancer xenografts in immune-deficient mice has previously revealed high in vivo efficacy in childhood leukemia, in particular against T-ALL compared with BCP-ALL, when tested at doses providing plasma pharmacokinetics achievable in humans.[5,6] However, the underlying mechanism for this differential sensitivity of ALL subtypes remained elusive.

Against this background, the aim of this study was to test whether AKR1C3 is a predictive biomarker of in vivo PR-104 sensitivity. To investigate whether PR-104 exhibits lineage-specific in vivo efficacy against T-ALL, Moradi Manesh et al extended the evaluation of PR-104 to a panel of patient-derived pediatric ALL xenografts.[1] PR-104 exerted significantly greater antileukemic efficacy against T-ALL xenografts than BCP-ALL.[1] Comparison of PR-104 with an induction-type regimen consisting of vincristine, dexamethasone, and L-asparaginase revealed that single-agent PR-104 was more efficacious against T-ALL xenografts than this standard-of-care regimen.[1] To identify a biomarker for PR-104 sensitivity the authors performed gene expression profiling comparing PR-104A–sensitive and PR-104A–resistant xenografts.[1] This analysis revealed AKR1C3 as one of the top 2 differentially expressed genes, and AKR1C3 expression correlated with PR-104/PR-104A sensitivity in vivo and in vitro.[1] A causal relationship between AKR1C3 expression and sensitivity to PR-104 was then demonstrated by overexpression of AKR1C3 in a resistant BCP-ALL xenograft, which led to increased sensitivity to PR-104 in vivo.[1] To validate their results, the authors went on to test ex vivo sensitivity to PR-104A against patient-derived leukemic blasts.[1] Primary T-ALL cells proved to be more sensitive than BCP-ALL to PR-104A in vitro, and this sensitivity correlated with AKR1C3 expression.[1] Together, these findings indicate that PR-104 represents a promising novel therapeutic for refractory and relapsed T-ALL and that AKR1C3 expression could serve as a biomarker to select patients who will most likely benefit from treatment with PR-104 in prospective clinical trials.

This study has important clinical implications. In line with the present study showing the safety of PR-104 in preclinical mouse xenograft models with no toxicity-related events,[1] PR-104 proved to be well tolerated in early-phase clinical trials in adult solid tumors and hematologic malignancies.[7,8] PR-104 may be of particular interest for refractory and relapsed cases of T-ALL, as PR-104 has been shown to specifically target hypoxic regions of leukemia infiltration (eg, in the bone marrow),[6] which are considered as important contributors to chemoresistance and relapse in ALL.

Nevertheless, there are also a number of open questions. Although in the study by Moradi Manesh et al, AKR1C3 expression levels proved to be a strong predictor of response to PR-104 independently of hypoxia in ALL,[1] AKR1C3 expression levels did not significantly correlate with overall tumor responsiveness to PR-104 across a panel of solid and hematologic pediatric cancers in another study.[5] Furthermore, ex vivo testing of primary T-ALL blasts in the present study revealed 1 case with low AKR1C3 expression but high sensitivity to PR-104.[1] Although the molecular basis of this outlier is currently not well understood, this observation suggests that AKR1C3 may not universally serve as a suitable biomarker to select patients who may benefit from PR-104. Moreover, the underlying mechanisms responsible for the reported increased expression levels of AKR1C3 in T-ALL compared with BCP-ALL remain subject to further investigation. Also clinically relevant is the question as to whether or not PR-104 is active against biologically distinct subgroups of T-ALL at high risk of treatment resistance and relapse that have recently been identified, such as early T-cell precursor ALL.[9] Another issue relates to PR-104–based drug combinations to exploit synergistic drug interactions or to overcome resistance to PR-104. Because DNA interstrand cross-link repair proficiency, in addition to hypoxia and reductase activity, has been reported to determine sensitivity to the alkylating agent PR-104,[10] evaluation of PR-104 together with DNA repair inhibitors might be of special interest.

In sum, the present study supports the further investigation of PR-104 for refractory or relapsed T-ALL patients that are selected by high AKR1C3 expression in their leukemic blasts as a biomarker.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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Expanding the peptidome for immunotherapy

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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 step, the application of (humanized) antibodies against CD20, became clinical practice. Since the realization, some 25 years ago, that MHC class I presents intracellular antigens in the form of peptides, these have been used for vaccination against tumors. The recent development of checkpoint antibodies blocking PD-1/PD-1L and/or CTLA-4 proteins further illustrated the power of cytotoxic T lymphocytes (CTLs) in the control of tumors. These checkpoint antibodies allowed the activation of various CTLs present in tumor tissue where they were kept dormant, with stunning clinical effects in a significant percentage of cancer patients. The best responding tumors are melanoma and lung cancer, but effects in many other tumor types have been reported.[2-4] Because melanoma and lung cancer are characterized by a high mutation rate, the theory predicting clinical effects of checkpoint antibodies became "the more mutations, the more cure." This makes sense, as mutated antigens yield altered peptides that are considered by T cells as "nonself" followed by tumor elimination. Indeed, T cells responding to mutated antigens have been identified by first sequencing the genome of cancer cells. Subsequently, peptides presented by the patient's MHC class I alleles are predicted from these mutated genes. Then, peptides are chemically synthesized and loaded onto corresponding MHC class I tetramers. These tetramers are subsequently used to detect corresponding T cells in cancer patients.[5,6] This pipeline for detecting tumor immune responses by definition only detects mutated peptides, 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.[1] 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,[7] 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