CLINICAL IMPLICATIONS OF BASIC RESEARCH

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RNA Splicing and Immune-Checkpoint Inhibition

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A wide spectrum of cancer-associated genetic alterations, including those that result in changes to the splicing of pre–messenger RNA (mRNA), can lead to the presentation of aberrant peptides as neoantigens on the surface of tumor cells. Subsequent attack by cytolytic T cells that may recognize such antigens has been a critical determinant of response to immune-checkpoint inhibition in immunotherapy for cancer. A recent study by Lu et al. underscores the value of mRNA splicing as a source of neoantigens that can elicit an antitumor immune response.[1]

Approximately a decade ago, mutations in the genes encoding splicing factors (notably, *U2AF1* [U2 small nuclear RNA auxiliary factor 1] and *SF3B1* [splicing factor 3b subunit 1]) were implicated in the etiology of acute myeloid leukemia and myelodysplastic syndromes. More recently, Seiler et al.[2] found 119 splice-related genes with elevated rates of nonsynonymous mutations (including insertions and deletions) in 33 different types of tumor. The messenger RNAs transcribed from some of these genes are aberrantly spliced in solid cancers; an example is *RBM10* (RNA binding motif protein 10), a regulator of splicing that occurs in adenocarcinoma of the lung and urothelial carcinoma of the bladder. In a similar study, Jayasinghe et al.[3] identified almost 2000 splice-creating mutations in more than 8500 tumors and reported that the neoantigens predicted by these mutations were likely to be more immunogenic than those encoded by other types of mutations. Delineation of the oncogenic mechanisms of specific splicing factors[4,5] has also fueled interest in abnormal splicing as a feature of tumor cells. Overall, scientific investigations of splicing biology have increasingly been steered by the desire to engineer alternative targets for immunotherapy applications in the clinic.

In their recent study, Lu et al.[1] tested the effect of disrupting splicing machinery in concert with checkpoint blockade in cancer treatment. They showed that the perturbation of splicing machinery — with the use of compounds such as indisulam, which degrades RBM39, and MS-023, an inhibitor of protein arginine methyltransferase [PRMT]) — when paired with an anti–programmed death 1 (PD-1) checkpoint inhibitor, suppressed the growth of melanoma and colorectal adenocarcinoma tumors (seeded by cell lines) in mouse models to a greater extent than either drug alone or anti–PD-1 alone (Fig. 1). RBM39 is a key regulator of alternative splicing, and the enzyme PRMT potentiates the RNA-binding proteins involved in splicing. Lu et al. also performed high-coverage RNA-sequencing analyses in which murine tumor-cell lines treated with indisulam or MS-023 were compared with those that were exposed to placebo to identify both treatment-induced, splicing-derived neoepitopes and the neoantigens presented by the major histocompatibility complex of the tumor cells. Finally, on injecting predicted, splicing-derived neoepitopes into mice, the investigators found that more than 40% of these neoepitopes triggered a CD8+ T-cell response. Further experiment showed that this response was dependent on the dose of peptide. These findings provide evidence that splicing aberrations are a source of immunogenic peptides that can elicit an antitumor response in animal models of melanoma and colorectal adenocarcinoma.

Lu et al. suggested that the most effective deployment of a "forced aberrant splicing" approach could be obtained through groups of peptides acting in concert. This proposition poses the familiar "combinatorial search" problem, which entails systematic evaluation of an unfea-

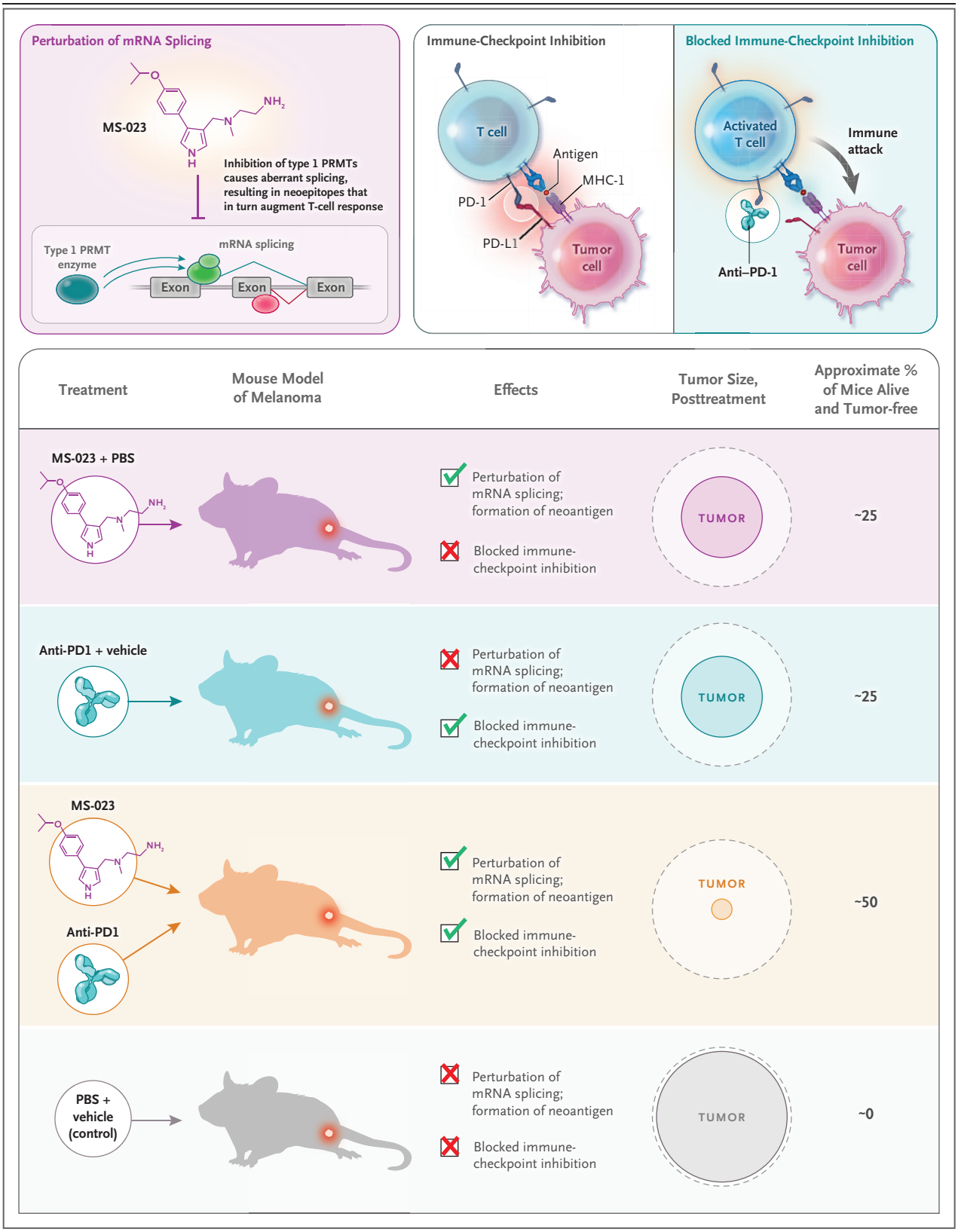

Figure 1 (facing page). Synergy between Aberrant Splicing and Immune-Checkpoint Inhibition.
Lu et al.[1] recently reported synergistic treatment effects between immune-checkpoint blockade and splicing modulation brought about by chemicals (MS-023, depicted here, and indisulam) that interfere with the regular splicing patterns of messenger RNA (mRNA). They observed that MS-023 and murine anti–programmed death 1 (PD-1) antibody had synergistic effects on the suppression of tumor growth and the life span of mice engrafted with a syngeneic melanoma cell line (as shown) as well as those engrafted with a syngeneic colorectal adenocarcinoma cell line. Lu et al. also reported increases in the levels of aberrantly spliced RNA in the nucleus and cytoplasm of mice receiving MS-023, the numbers of T cells in their tumors, and the numbers of predicted neoantigens. MHC denotes major-histocompatibility complex, and PRMT protein arginine methyltransferase.

sibly large set of possibilities. For example, given the 109 peptide candidates experimentally assessed by Lu et al., there would be approximately 6000 possible pairs and 210,000 possible trios of peptides to evaluate. Bioinformatic analyses that would prioritize — or perhaps rule out — certain combinations would be necessary before the more time-consuming experimental steps are taken. Future work would also benefit from the systematic integration of peptides (characterized by tandem mass spectrometry) with genomic and transcriptomic data for the purpose of building new databases. Targeted proteomic approaches could be of assistance in such endeavors. Systematic profiling of tumors with defined splicing deficiencies conducted with the use of bulk and single-cell RNA sequencing and coupled with mass spectrometry would provide data on the effect patterns of diverse splicing factors, enabling the selection of candidate proteins that, when targeted, would yield the strongest inhibition of cancer growth and progression.

In summary, the study described by Lu et al. provides support for the induction of aberrant mRNA splicing as a means to elicit a T-cell–mediated antitumor response, albeit in animal models of melanoma and colorectal adenocarcinoma. It thereby adds to a growing body of evidence supporting this approach as a strategy for augmenting immune-checkpoint inhibition in the treatment of cancer.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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