5-AZA-2′-Deoxycytidine in Cancer Immunotherapy: A Mouse to Man Story

To the Editor:

We read with great interest the article by Guo et al. (1) reporting that the DNA hypomethylating agent 5-aza-2′-deoxycytidine (5-AZA-CdR) induced a persistent expression of the murine cancer/testis antigen (CTA) P1A in different cultured murine tumors. Systemic administration of 5-AZA-CdR to BALB/c mice grafted with 4T1 mammary adenocarcinoma was also reported to induce a *de novo* expression of P1A on neoplastic cells that significantly reduced their metastatic potential to the lung; this therapeutic effect of 5-AZA-CdR on lung metastasization was synergized by the adoptive transfer of P1A-specific CTL. Based on their findings in the murine system, the authors agreeably concluded that 5-AZA-CdR represents a useful drug to design novel strategies of combined chemoimmunotherapy of cancer.

We have recently drawn similar conclusions in humans, based on *in vitro* data we (2, 3) and others (4–6) had previously generated, and on our *in vivo* evidences obtained in different solid and hemopoietic malignancies (7, 8). Among the major findings, 5-AZA-CdR was shown to (*a*) persistently induce and up-regulate the concomitant expression of multiple members of different CTA families in cultured neoplastic cells, which became efficiently recognized by anti-CTA CTL (2); to (*b*) revert the constitutive intratumor heterogeneity of CTA expression, allowing a homogeneous recognition of transformed cells by anti-CTA CTL (3); to (*c*) induce and up-regulate the expression of several CTA in human melanomas grafted into BALB/c *nu/nu* mice (7); and to (*d*) induce the expression of different CTA in circulating neoplastic cells of patients affected by acute myeloid leukemias or myelodysplastic syndromes after a single i.v. administration of the drug (8).

In addition to these evidences, using human melanoma as a "model disease," we have now gained additional information on the immunomodulatory properties of 5-AZA-CdR *in vivo*, which additionally help to define its prospective clinical potential in patients with cancer. Quantitative real-time reverse transcriptase-PCR analysis showed a strong *de novo* expression of NY-ESO-1 (9×10^{-4} NY-ESO-1 molecules/β-actin molecules) in Mel 313 melanoma xenografts excised from BALB/c *nu/nu* mice 4 days after the last administration of 5-AZA-CdR. Although it progressively decreased with time, NY-ESO-1 expression was still detectable (8×10^{-5} NY-ESO-1 molecules/β-actin molecules) in xenografts excised 30 days following the last treatment. This persistent expression of NY-ESO-1 *in vivo* was further confirmed at the protein level by the staining of melanoma xenografts with the anti–NY-ESO-1 monoclonal antibody B9.8 (kindly provided by Dr. Giulio C. Spagnoli, Department of Surgery, Division of Research, University of Basel, Basel, Switzerland). Consistent with these data, treatment with 5-AZA-CdR also induced a long-lasting up-regulation of the constitutive expression of MAGE-3 in Mel 313 melanoma xenografts, both at the molecular and protein level (data not shown).

The ability of 5-AZA-CdR to persistently induce and up-regulate *in vivo* the expression of NY-ESO-1 and MAGE-3, which are the most utilized therapeutic CTA at present (2), provides further strong support to its immunotherapeutic potential in the clinical setting. In fact, the long-lasting modulation of the expression of therapeutic CTA on neoplastic cells, along with the complex of available data on the immunomodulatory activity of 5-AZA-CdR in human tumors and with the recent evidences provided by Guo et al. in the murine system (1), provides a strong scientific rationale to implement novel immunotherapeutic approaches combining active and/or adoptive CTA-based immunotherapy with systemic administration of 5-AZA-CdR in the clinical setting.

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Acknowledgments

Grant support: This work was supported in part by a grant from the Associazione Italiana per la Ricerca sul Cancro (M. Maio).

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doi:10.1158/0008-5472.CAN-06-2986

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Cancer Res 2007;67:2900.