Id-specific TCR-transgenic SCID mice are resistant against subcutaneous challenge with MOPC315 and F9 cells. The protection is Id-specific, $CD4^+$ T cell–mediated and does not require the presence of B cells and $CD8^+$ T cells.[7,8] On cancer cell inoculation, Id-specific $CD4^+$ T cells first become activated in the draining lymph nodes where they acquire a T helper type 1 (Th1) phenotype. Id-specific Th1 cells then migrate to the incipient tumor site and secrete IFN-γ, resulting in local activation of macrophages which become tumoricidal and eradicate the cancer cells.[9,10]

Id-specific TCR-transgenic SCID and control nontransgenic SCID mice were inoculated with cancer cells and treated daily with fingolimod or vehicle only. Fingolimod efficiently blocked rejection of both MOPC315 myeloma and F9 B-cell lymphoma by TCR-transgenic mice (Figure 1A-B). Fingolimod had no effect on the survival of control nontransgenic SCID mice inoculated with MOPC315 (Figure 1C). Fingolimod did not block activation of tumor-specific $CD4^+$ T cells in the draining lymph nodes, as defined by up-regulation of CD69 (Figure 1D). In contrast, fingolimod strongly inhibited migration of tumor-specific $CD4^+$ T cells to the incipient tumor site (Figure 1E). Furthermore, fingolimod prevented Th1-mediated activation of tumor-infiltrating macrophages, as measured by up-regulation of surface major histocompatibility (MHC) class II molecules. In fact, in TCR-transgenic mice treated with fingolimod, tumor-infiltrating macrophages had surface MHC class II levels that were as low as those of macrophages from T cell–deficient SCID mice (Figure 1F). Thus, the data strongly suggest that fingolimod blocks immunosurveillance of B-cell cancers by suppressing migration of tumor-specific Th1 cells from lymph nodes to the incipient tumor site, thereby preventing Th1-mediated activation of tumoricidal macrophages.

In summary, fingolimod is a strong immunosuppressive drug which blocks immunosurveillance of myeloma and B-cell lymphoma by $CD4^+$ T cells in experimental mouse models, resulting in cancer development. Further studies are required to determine the effect of fingolimod on other immune cells involved in cancer immunosurveillance such as $CD8^+$ T cells and NK cells.[4] In our experiments, mice were treated with rather high doses of fingolimod (1-2 mg/kg bodyweight/d) to efficiently block sphingosine-1-phosphate receptors. Patients receive much lower doses (0.5-1.25 mg/d) but for longer time periods (years). Therefore, our findings cannot be directly translated to patients. However, the data suggest that long-term treatment with fingolimod may potentially lead to increased risk of cancer in humans.

Kristina Berg Lorvik
Centre for Immune Regulation, Department of Immunology, Oslo University Hospital Rikshospitalet and University of Oslo, Oslo, Norway

Bjarne Bogen
Centre for Immune Regulation, Department of Immunology, University of Oslo and Oslo University Hospital Rikshospitalet, Oslo, Norway

Alexandre Corthay
Center for Immune Regulation, Department of Immunology, Oslo University Hospital Rikshospitalet and University of Oslo, Oslo, Norway

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Correspondence: Kristina Berg Lorvik or Alexandre Corthay, Department of Immunology, Oslo University Hospital Rikshospitalet, PO Box 4950 Nydalen, 0424 Oslo, Norway; e-mail: k.b.lorvik@medisin.uio.no or alexandre.corthay@medisin.uio.no.

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To the editor:

CEBPE activation in PML-RARA cells by arsenic

In a recent perspective, Ablain and de The challenged the classic model of acute promyelocytic leukemia (APL), whereby differentiation-impairment in lineage-committed progenitors causes self-renewal, and instead proposed that APL arises from deregulation of stem cell self-renewal pathways in leukemia initiating cells, with the curative potential of arsenic (As_2O_3) relating to abrogation of these pathways.[1] However, differentiation syndrome occurs at a similar rate with As_2O_3 as with all-trans retinoic acid (ATRA), and furthermore, disseminated intravascular coagulation, the potentially fatal complication frequently triggered by cytotoxic treatment, is not typical.[2] Hence, clinical experience suggests that important actions of As_2O_3 include terminating APL proliferation by differentiation. To evaluate this further, the APL cell line NB4 was treated with low concentrations of As_2O_3 which do not induce early apoptosis[3] (Figure 1A; supplemental Figure 1, available on the *Blood* Web site; see the Supplemental Materials link at the top

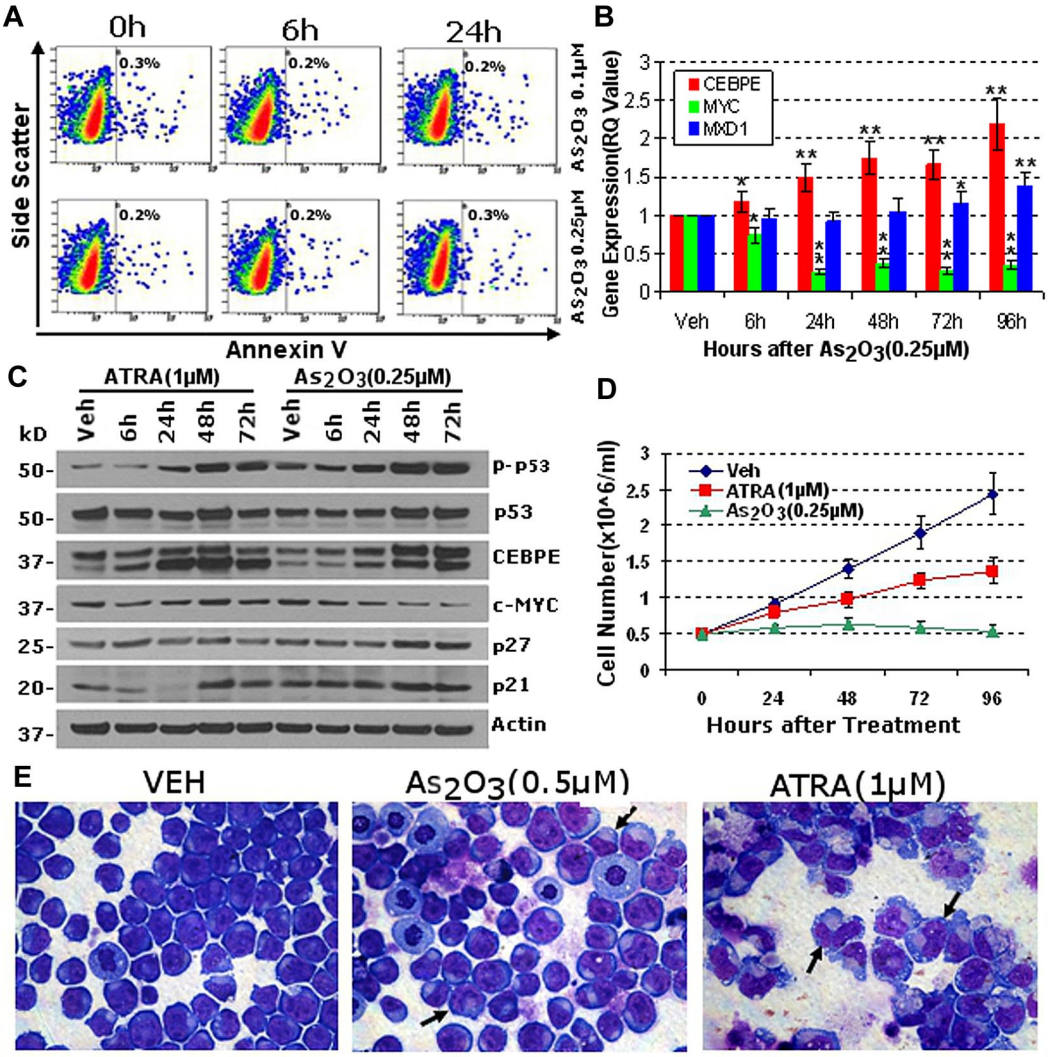

Figure 1. Concentrations of As_2O_3 that do not cause apoptosis activate key late-differentiation genes (*CEBPE*, *MXD1*) that repress *MYC*. (A) Concentrations of As_2O_3 that do not cause early apoptosis in NB4 cells were identified. Apoptosis measured by annexin-staining and flow cytometry. (B) As_2O_3 0.25μM activated the myeloid late-differentiation genes *CEBPE* and *MXD1*, and repressed *MYC*. mRNA levels measured by QRT-PCR. $^*P < .05$, $^{**}P < .01$ compared with baseline (*t* test). (C) Time-course changes in protein expression reiterated changes in mRNA expression. Protein expression measured by Western blot after As_2O_3 0.25μM or ATRA 1μM. Antibodies for MXD1 did not work. (D) As_2O_3 was more anti-proliferative than ATRA. Cumulative cell counts by automated counter. Error bars = SD, 3 experiments. (E) Both As_2O_3 and ATRA induced morphologic differentiation, although this was much more extensive with ATRA. Giemsa-stained cytospins 96 hours after addition of As_2O_3 0.5μm or ATRA 1μm. Black arrows indicate cells with maturation changes.

of the online article). These concentrations rapidly activated *CEBPE*, a key myeloid late-differentiation driver which is repressed by PML-RARA[4] and which directly represses *MYC*[5] (supplemental Figure 2; MYC is an oncogene product that drives myeloid progenitor proliferation[6]). As expected therefore, *CEBPE* activation was accompanied by *MYC* repression (Figure 1B). *MXD1*, a down-stream target of CEBPE[7] and MYC-antagonist, was also activated, but subsequent to activation of *CEBPE* (Figure 1B). CEBPA, a key lineage-specifying transcription factor that drives *CEBPE* expression,[8] is expressed at high levels in NB4 and primary APL cells (supplemental Figure 1B), providing a potential explanation for rapid activation of *CEBPE* after repressive actions of PML-RARA are inhibited by As_2O_3. Time-course changes in CEBPE and MYC protein levels reiterated the time-course changes in expression of mRNA (Figure 1C). The cyclin-dependent kinase inhibitor p27/CDKN1B mediates cell cycle exit by differentiation and was up-regulated by As_2O_3 at late-time points (Figure 1C); p21/CDKN1A mediates cell cycle exit by differentiation or apoptosis, and was also up-regulated (Figure 1C). Phosphorylation and up-regulation of the master regulator of apoptosis p53 was also observed, but as a late event that occurred subsequent to up-regulation of CEBPE and down-regulation of MYC (Figure 1C). Both ATRA and As_2O_3 were anti-proliferative, with the greater effect from As_2O_3 (Figure 1D). Others have shown that As_2O_3 0.1-0.5μM induces morphologic differentiation in primary APL and NB4 cells,[3] but not to the same extent as ATRA,[3] an observation we recapitulated (Figure 1E).

In normal hematopoiesis, MYC-mediated proliferation in progenitors is self-limited by progressive maturation that activates key late-differentiation genes such as *CEBPE* and *MXD1*[4-7] (supplemental Figure 2). Thus, aberrant epigenetic repression of *CEBPE* and other key late-differentiation genes by PML-RARA[4] (supplemental Figure 2), and as a consequence of cooperating genetic abnormalities (eg, *UTX* deletion), can potentially contribute to MYC-related self-renewal (proliferation at the same level of differentiation) in progenitors,[4,9,10] is consistent with the MYC up-regulation and amplification that is characteristic of APL,[11] and as shown here, is a molecular pathway targeted by As_2O_3 to potentially explain the

anti–self-renewal effects of this drug. The curative potential of As_2O_3 compared with ATRA could reflect superior pharmacodynamics, that is, more effective degradation of oncogenic PML-RARA.[3,12] Hence, in important aspects, As_2O_3 could be a differentiation-therapy drug for APL, with a mechanism of action that supports the classic model of this disease, even if it does not recapitulate the full-spectrum morphologic differentiation that is produced by the more physiologic interactions of ATRA with the RARA moiety in PML-RARA.

Zhenbo Hu
Taussig Cancer Institute, Cleveland Clinic, Cleveland, OH

Yogen Saunthararajah
Taussig Cancer Institute, Cleveland Clinic, Cleveland, OH

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Correspondence: Yogen Saunthararajah, Taussig Cancer Institute, Cleveland Clinic, 9500 Euclid Ave, MC R40, Cleveland, OH 44195; e-mail: saunthy@ccf.org.

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