drug candidates, with implications for antibiotic development in general. Lee *et al.*[3] inferred that efflux was a crucial obstacle on the development path for spectinomycin and that chemical modification circumvented it, although they did not directly show this. Mass spectrometry affords a direct, definitive approach to the assessment of compound uptake and retention by bacterial pathogens and should become a standard assay in the analysis of antibiotic structure-activity relationships[10]. Pharmacokinetic assessment of TB drug candidates should no longer rest with measuring compound concentrations in blood, pulmonary alveolar epithelial lining fluid or lung homogenates. Mass spectroscopic positional imaging underscores that TB drug candidates should be tested for their ability to accumulate in heterogeneous TB lesions, such as pneumonitic infiltrates, granulomas and cavities[11].

Finally, it is important to find anti-infectives that kill diverse nonreplicating populations of bacterial pathogens. It is not known whether Mtb forced into nonreplication by oxygen deprivation *in vitro* is an adequate model for the diverse conditions that Mtb encounters in culture. It will be particularly challenging to test TB drug candidates for their ability to kill Mtb bacilli that have entered a nonreplicating state from which they are unable to emerge when plated on agar—so-called 'viable but nonculturable' forms[12]. To my knowledge, results of such a test have not yet been reported for a TB drug.

Antibiotic development poses a daunting array of obstacles. It is difficult even to approach the goal of introducing a safe and effective new therapy. When we get close enough to take a shot, most shots are blocked. The best shots on goal—like that taken by Lee *et al.*[3]—give subsequent shots a better chance.

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Engaging a senescent response to cure leukemia

Véronique Bourdeau & Gerardo Ferbeyre

Retinoic acid and arsenic trioxide cure individuals with acute promyelocytic leukemia (APL). In mouse models, these drugs eradicate tumor cells by activating the tumor suppressors p53 and PML to induce senescence of cancer cells (pages 167–174).

APL has been of great interest for oncologists despite its low frequency because the disease can be definitively cured with retinoic acid (RA), arsenic or their combination[1,2]. At the molecular level, it was shown that most cases of APL are associated with chromosomal translocations between the genes coding for the retinoic acid receptor α (RARA) and promyelocytic leukemia (PML), a protein capable of forming nuclear bodies, and whose function still remains unclear[3]. The resulting fusion protein PML-RARA impairs the ability of PML to form nuclear bodies, but these are restored after treatment with RA, which induces PML-RARA degradation[1].

The interest in PML was broadened by the discovery of its implication in p53 activation and in cellular senescence[4–6], which is a general tumor suppression mechanism. In particular, senescence is a programmed response triggered by short telomeres or oncogenic stimuli leading to stable cell cycle arrest and the secretion of a variety of proinflammatory factors capable of recruiting immune effector cells that can ultimately eliminate senescent cells[7,8]. PML-RARA not only represses differentiation genes but also disrupts formation of nuclear bodies by normal PML, potentially inactivating the senescence checkpoint; however, most investigators believed that cell differentiation through transcriptional reactivation of PML-RARA–repressed genes explained the antitumor efficacy of RA.

Now Ablain *et al.*[9] used mouse models of APL to show that upon therapy-induced PML-RARA degradation, re-formation of PML nuclear bodies, p53 activation and cellular senescence are the driving forces explaining the cure of APL by RA and arsenic **(Fig. 1)**. They used two transgenic mouse models that develop APL owing to expression of either PML-RARA or PLZF-RARA (a fusion protein that causes an RA-resistant APL) and measured survival and transplantability of the leukemia cells to follow disease remission after treatment with low, medium or high doses of RA. Although previous work on the effects of RA on APL cells and individuals with APL implicated transcriptional activation and cell differentiation as the primary mechanism of RA action, Ablain *et al.*[9] found no correlation between disease remission (which only occurs at high doses of RA) and markers of granulocyte differentiation of APL cells (which occurred at all RA doses) in their PML-RARA mouse model.

Transcriptome analysis of PML-RARA APL cells treated with medium or high doses of RA also revealed that cell differentiation markers were common to all RA doses used, irrespective of disease remission; however, disease-curing doses (the higher doses) were specifically associated with downregulation of E2F target genes and induction of genes linked to the p53 transcription factor or the senescence-associated secretory phenotype[7]. This triple-gene signature is characteristic of cells that senesce in response to oncogenes or overexpression of PML[10], suggesting the possibility that high doses of RA activate PML and p53, leading to APL cell senescence.

To further investigate the mechanism for APL cure, the authors wanted first to evaluate the possibility of apoptosis given that both PML and p53 proteins can trigger this process in other models[1,11]. Strikingly, whereas inhibition of caspase-dependent apoptosis improved the therapy with RA, genetic ablation of p53 or PML markedly decreased the curative response to this drug. These results

Véronique Bourdeau and Gerardo Ferbeyre are at the Département de Biochimie et Médecine Moléculaire, Université de Montréal, Montréal, Québec, Canada.
e-mail: g.ferbeyre@umontreal.ca

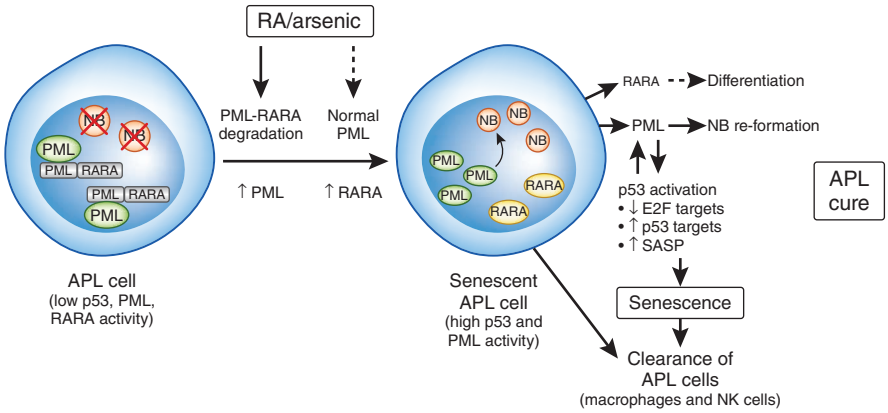

Figure 1 A senescence mechanism for the antileukemia effect of RA and arsenic. The two drugs degrade the oncogenic fusion protein PML-RARA, allowing wild-type PML to re-form nuclear bodies, activate p53 and repress E2F target genes via the retinoblastoma tumor suppressor, inducing cellular senescence. As the gene encoding PML is also a p53 target gene[11], this mechanism is reinforced via positive feedback. NB, nuclear body; NK, natural killer; SASP, senescence-associated secretory phenotype.

strongly point at the cell cycle and senescent functions of the PML-p53 axis as the mechanism driving the curative response to RA in APL. Ablain *et al.*[9] further confirmed the essential role of senescence in APL elimination by showing that treatment with high doses of RA induced the senescence regulators Pml and serpine 1 (also known as Pai1) and decreased the levels of lamin B1, whose loss is also observed when senescence is induced. Moreover, arsenic trioxide, a compound that acts synergistically with RA to cure APL, reinforced the re-formation of nuclear bodies and further activated the p53 targets induced by RA. Perhaps more dramatic was the phagocytosis of APL cells in the liver of PML-RARA transgenic mice treated with high doses of RA. This is consistent with the concept of senescent cell clearance first proposed by Lowe and colleagues in liver tumor cells where p53 expression was reactivated[8].

Although the case for PML body reformation and senescence demonstrated by Ablain *et al.*[9] is compelling, the relative contribution to APL cure of the other different senescent markers and effectors identified remains to be investigated. Key questions include the role of serpine 1 and other p53 target gene products induced by RA and arsenic in APL cells, the role of the repression of E2F target genes and the mechanism leading to recognition and elimination of senescent APL cells by liver macrophages. How treatment with high doses of RA activates p53 also deserves further investigation. One possibility is that reformation of PML bodies after degradation of PML-RARA may be the trigger for p53 activation in APL cells as PML body formation correlates with p53 activation in oncogene-induced senescence[4–6] **(Fig. 1)**, but how these newly formed PML bodies activate p53 in the context of RA-treated APL remains unknown.

Taken together, the study by Ablain *et al.*[9] supports the idea that APL may constitute the first clinically relevant example of a successful cancer therapy based on induction of cellular senescence. Indeed, at the molecular level, both p53 and PML were required in their mouse model to observe a therapeutic response that correlated with the appearance of senescence markers in the leukemia cells, yet many human tumors contain mutations in the gene encoding p53 (ref. 12) and/or have very low levels of PML[10]. In addition, RA and arsenic are successful against APL cells via the degradation of the PML-RARA fusion protein that interferes with endogenous PML. These compounds cannot use the same mechanism in other tumor types. Given these constraints, perhaps the lesson to learn from APL is that forcing senescence in other malignancies may achieve their cure—to achieve this goal we must further understand the mechanisms of senescence and find drugs that can trigger the process specifically in tumor cells.

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