factors can confound the assessment. If patient clinical care is changed based on MRD knowledge outside of the protocol-directed approach, then the potential surrogacy relationship is uninterpretable. Further complicating the use of MRD as a surrogate is the fact that MRD dynamics vary incredibly by type of treatment. For example, compared with chemoimmunotherapy, novel biologic therapies such as ibrutinib have a much slower response time and often do not result in undetectable MRD status, yet they may improve PFS. This introduces uncertainty for timing of MRD assessment for various therapies in the quest toward establishing surrogacy. Establishment of surrogacy for one type of therapy does not necessarily support a claim of surrogacy for another therapeutic agent. Surrogacy has to be constantly verified with new therapeutic agents.

Importantly, surrogacy is not required for immediate incorporation of MRD assessment into clinical trials designed to improve clinical outcome for the individual patient and to address important individual and public health concerns. As novel therapies for CLL involve chronic administration of expensive medicines, trials focused on duration, intensification, or deintensification of therapy can provide precision in determining who will benefit from different treatment strategies. Provided such studies measure the true clinical benefit end points, they will also provide the necessary data to evaluate claims for potential surrogacy of MRD.

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Comment on Zhang et al, page 2774

Metformin: treating the cause of Fanconi anemia?

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In this issue of Blood, Zhang et al have uncovered that metformin, a first-line treatment for type 2 diabetes, can improve hematopoietic stem cell (HSC) function and reduce cancer risk in a mouse model of Fanconi anemia (FA).1

FA is a rare genetic disease that leads to bone marrow failure and an extreme predisposition to cancer. Cells from these patients are unable to repair a particular kind of DNA damage, and this defect is thought to be the basis of the observed pathologies. At the molecular level, 21 gene products act in a common pathway to repair damage caused by DNA interstrand crosslinking agents (eg, cisplatinum). Although we understand quite a considerable amount about how these proteins cooperate to repair damaged DNA, we have very little understanding of why this should lead to bone marrow failure or why cancer predisposition is worse in certain tissues. One explanation could be that certain tissues have greater exposure to damage, but until recently, we had very little idea of the physiological sources of DNA damage that precipitate FA. A recently identified source of damage may be simple aldehydes.2-4 Alternatively, it has been proposed that when HSCs leave their quiescent state, they accumulate DNA damage, necessitating FA-mediated repair.4 Building on these foundations, Zhang et al reveal that metformin may be the first agent that targets the source of DNA damage in FA.

Mice deficient in the key Fanconi protein FANCD2 were fed a diet supplemented with metformin. In FA-deficient mice, this treatment attenuated the blood cytopenias, and improved, but did not fully correct, the reduced frequency of HSCs and restored them to a quiescent state. Finally, treatment with metformin resulted in a small but significant reduction in the tumor predisposition of Fancd2+/−p53+/− mice. The magnitudes of these effects are relatively small but this may in part be due to the fact that the HSC loss in FA begins during embryonic development, however the metformin treatment was only initiated in adults (see figure).5

Despite this, metformin is the first example of a pharmacological intervention that both improves hematopoietic function and suppresses tumor predisposition. As the effect of metformin is restricted to Fanconi-deficient mice, it is plausible that metformin could be attenuating the source of damage that drives the FA phenotype.

The mechanism(s) of metformin’s effect remains to be fully uncovered. The authors go some way to address this by using a poly(I:C) treatment that mimics viral infection and induces a type I interferon response. This treatment has been shown to drive HSCs to...
cycle and cause bone marrow failure in a mouse model of FA, but there is no evidence of increased cancer predisposition. The mechanism by which the treatment with poly(I:C) causes aplastic anemia in FA is unclear. However, it has been shown that poly(I:C) treatment of wild-type mice leads to increased production of reactive oxygen species (ROS) and an accumulation of 8-oxo-dG, a base adduct of guanine caused by ROS. The increase in ROS production could be due to the increased metabolic demands of HSCs as they exit a quiescent state and begin to cycle, but it is unknown how this could lead to bone marrow failure in FA-deficient mice, as a functional Fanconi pathway is not required to repair DNA damage caused by agents that induce 8-oxo-dG (eg, H$_2$O$_2$). It is possible that requirement for a FA pathway upon poly(I:C) treatment is because cells spend more time in S phase, the period when the FA pathway is active. Zhang et al report that metformin is able to prevent the anemia and HSC loss caused by exposure to poly(I:C). Metformin is known to alter the metabolic activities of cells in a range of ways, notably by activating adenosine 5'-monophosphate–activated protein kinase. Metformin may attenuate the metabolic response of HSCs as they enter the cell cycle.

Alternatively, the source of DNA damage that drives FA may be reactive aldehydes. It has been shown that disruption of aldehyde detoxification in Fanconi-deficient mice leads to a phenotype that is very similar to human patients with increased cancer predisposition and spontaneous bone marrow failure. In addition, cells require a functional Fanconi pathway in order to resist the toxic effects of these aldehydes. In this report, Zhang et al propose that metformin may act by reacting with aldehydes, thereby rendering them inert. Fanconi patient cells treated with inhibitors of the enzyme that detoxifies the simplest aldehyde, formaldehyde, accumulate chromosomal breaks that metformin is capable of suppressing. They also show that aminoguanidine, structurally related to metformin, is able to increase the resistance of Fanconi patient cells to formaldehyde exposure. Taken together, this suggests that the mechanism of action of metformin may be through scavenging aldehydes. This hypothesis is attractive as reactive aldehydes are capable of driving both the cancer predisposition and the HSC loss—phenotypes that can be suppressed by metformin. It is plausible that in an analogous fashion to ROS, there may be a burst of aldehyde production when HSCs leave the quiescent state and enter the cell cycle, a hypothesis that should be tested. If true, this may mean that the HSC stress response and aldehyde toxicity are in fact a singular source of DNA damage in FA (see figure). Although it is clear that reactive aldehydes can drive the main phenotypes of FA in mice, it remains unclear how this occurs. It is supposed that aldehydes cause DNA damage that is repaired by the Fanconi pathway, but there is little or no evidence that aldehydes cause DNA damage in vivo. If DNA damage does occur, how does that lead to HSC loss? Finally, in the absence of FA repair, how is DNA mis-repaired leading to the chromosomal instability observed in FA patients?

Many questions remain about the mechanism of how metformin can suppress 2 of the key features of FA. However, it is plausible that metformin is the first example of a drug targeting the cause of FA. Given the exceptional
Comment on Todd et al, page 2785

Macrophage precursors PLASTed INTO alveolar space

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In this issue of \textit{Blood}, Todd et al demonstrate that L-plastin (LPL) is crucial for the neonatal transmigration and engraftment of macrophage precursors into the alveolar space.\(^1\)

Most tissue-resident macrophages derive from embryonic precursors that seed the various organs before birth and then self-maintain throughout life. Alveolar macrophages develop primarily from fetal liver monocytes that colonize the embryonic lungs 1 week before birth, differentiate into prealveolar macrophages shortly before birth, and then transmigrate into the alveolar space and further develop into terminally differentiated alveolar macrophages within the first week of life.\(^2\) This developmental pathway is driven by granulocyte-macrophage colony-stimulating factor (GM-CSF), which induces the expression of the critically required transcription factor peroxisome proliferator-activated receptor-γ (PPAR-γ).\(^3\) The mechanisms involved in colonization of the alveolar macrophage niche by embryonic precursors remain poorly defined. Todd et al studied the role of the actin-bundling protein LPL in alveolar macrophages. LPL is part of the α-actinin family and is widely expressed in immune cells (see www.immgen.org), playing an important role in T-cell motility,\(^4\) neutrophil activation,\(^5\) and marginal zone B-cell development.\(^6\) To study the function of LPL in alveolar macrophages, the authors crossed CD11c\(^-\)/CRE mice onto LPL-floxed mice, yielding mice with a dramatic reduction in the number of alveolar macrophages.

Although LPL RNA is highly expressed throughout the distinct stages of alveolar macrophage development pathway (M.G., unpublished results), the fact that CD11c\(^-\) CRE \times LPL-floxed mice have an important alveolar macrophage defect suggests that LPL is required after the prealveolar macrophage stage, the stage at which CD11c is first expressed.\(^2\) Moreover, equal numbers of fetal monocytes and pre–alveolar macrophages were found in wild-type and LPL\(^-/-\) mice. In the first week of life, only a twofold reduction in alveolar macrophages is observed in LPL\(^-/-\) mice as compared with wild-type mice, but in adults there is a 10-fold reduction. The authors investigated multiple potential mechanisms that might explain this loss of alveolar macrophages in LPL\(^-/-\) mice.

LPL\(^-/-\) macrophages had lower PPAR-γ expression. The lower PPAR-γ expression was not due to impaired production of GM-CSF by the lung epithelial cells, because the levels of GM-CSF found in the lungs of LPL\(^-/-\) mice were not decreased compared with wild-type mice and were even slightly higher, which may suggest diminished consumption of GM-CSF. The lower PPAR-γ expression was also not due to impaired expression of the granulocyte-macrophage colony-stimulating factor receptor (GM-CSFR) or to deficiency of GM-CSFR signaling, as prealveolar macrophages and macrophages from wild-type and LPL\(^-/-\) mice displayed similar GM-CSFR signaling (measured by STAT-5 phosphorylation). Moreover, treatment of LPL\(^-/-\) alveolar macrophages with GM-CSF ex vivo induced normal PPAR-γ expression. Because the GM-CSFR and PPAR-γ responsiveness seemed normal, the authors next wondered whether monocyte localization in the lungs was compromised in the absence of LPL. The authors hypothesized that alveolar macrophage precursors need to localize in close contact with the lung epithelial cells that are the main source of GM-CSF in the lungs to undergo alveolar macrophage development and therefore evaluated whether LPL was required for monocyte trafficking and engraftment into the alveolar space. First, they demonstrated that IV-transferred LPL\(^-/-\) monocytes failed to transmigrate into the alveolar space upon intratracheal instillation of CCL2. Next, they demonstrated that in LPL\(^-/-\) mice there was a lower percentage of CD11c\(^+\) cells found within the alveolar space as compared with wild-type mice, supporting a role for LPL in transmigration into the alveolar space (see figure). In addition, they also found that when LPL\(^-/-\) and wild-type monocytes were transferred into the lungs of neonatal mice, LPL\(^-/-\) monocytes had a much lower capacity to engraft into the lungs. A similar effect was observed when LPL\(^-/-\) and wild-type alveolar macrophages were transferred, demonstrating that LPL remains important for retention of alveolar...
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