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.\(^5\)\(^,\)\(^10\) 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.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

REFERENCES

\begin{center}
\textbf{HEMATOPOIESIS AND STEM CELLS}
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\textbf{Metformin: treating the cause of Fanconi anemia?}
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In this issue of \textit{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.\(^2\)\(^,\)\(^3\)

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 FANC D2 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 \textit{Fancd2}\(^−/−\)\(\times\)\(p53^{\text{R248Q}}\) 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

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\textbf{DOI 10.1182/blood-2016-11-745323}
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A treatment with metformin partially suppresses cancer predisposition and rescues HSC defects. Under conditions of stress, HSCs are recruited into active cell cycling, leading to altered metabolism with the production of additional ROS and potentially an increase in aldehyde production. Metformin may have a metabolic effect on HSCs to reduce the production of these toxic molecules. Metformin can also react with aldehydes, directly rendering them inert and acting as a scavenger for excess aldehydes. Metformin leads to decreased levels of DNA damage circumventing the need for an intact Fanconi DNA repair pathway.

Treatment of a Fanconi mouse model with metformin partially suppresses cancer predisposition and rescues HSC defects. Under conditions of stress, HSCs are recruited into active cell cycling, leading to altered metabolism with the production of additional ROS and potentially an increase in aldehyde production. Metformin may have a metabolic effect on HSCs to reduce the production of these toxic molecules. Metformin can also react with aldehydes, directly rendering them inert and acting as a scavenger for excess aldehydes. Metformin leads to decreased levels of DNA damage circumventing the need for an intact Fanconi DNA repair pathway.

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 *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 CD11cΔ CRE mice onto LPL-floxed mice, yielding mice with alveolar macrophages in 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 macrophages.

References


Metformin: treating the cause of Fanconi anemia?

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