Lifting the differentiation embargo

A recent advance in understanding acute myeloid leukemia (AML) has high lighted its heterogeneous nature(Papaemmanuil et al., 2016). Despite the progress in elucidating the genetics of the disease, comparative leaps in AML therapeutics have been frustratingly few. Indeed, standard therapy still relies upon a 'one-size-fits-all' approach of conventional cytotoxics, and 5-year survival rates remain well below 50%(Burnett, 2012). It has long been recognized that a block in myeloid differentiation represents a cornerstone of myeloid leukemogenesis(Gilliland, 2002). However, the key to unlocking this block has proved elusive for the majority of cases, with the exception of a small genetically defined subset of patients with acute promyelocytic leukemia (APML). In this issue, Sykes et al. (Sykes et al., 2016) present one of the most exciting efforts to date to lift the differentiation block in AML (Figure 1.).

There are few unifying themes across AML subtypes, but key to the authors' success here was the knowledge that HoxA9 expression is a common feature of more than half of all AML cases. HoxA9 is a homeodomain-containing transcription factor that is normally repressed upon myeloid differentiation. Dysregulation of HoxA9 has been associated with various disparate drivers of AML, including *MLL*-translocations, *NUP98* fusions, *NPM1c* mutations and mutations seen in myelodysplastic syndromes, such as in *EZH2* and *ASXL1* (Collins & Hess, 2016). Sykes et al (2016) generated a model of conditional myeloid maturation arrest utilizing an estrogen-dependent form of HoxA9 (ER-HoxA9). The ER-Hox-A9 cells were propagated from murine bone marrow using GFP knocked into the lysozyme locus to track granule expression in differentiated cells, thus enabling culture of a cell line optimized for screening small molecules capable of promoting myeloid differentiation in the face of active HoxA9. Of 330,000 molecules screened, 12 compounds ultimately demonstrated reproducible myeloid differentiation. This list was then honed to 2 lead compounds selected for further study on the basis that they had documented activity in both murine and human AML models. A crucial next step was to identify the protein targets of these 2 lead compounds. For this purpose, the authors generated cell lines resistant to each drug and then elucidated the mechanism of drug resistance by analysis of commonly up-regulated genes across the two cell lines by RNA sequencing. It is worth noting that the ability to generate drug resistant cell lines infers a possible mechanism of escape for these agents as they progress towards clinical development. One of the eight commonly up-regulated genes encoded a critical enzyme in the intra-cellular *de novo* synthesis of pyrimidines: dihydroorotate dehydrogenase (DHODH). Both lead compounds were then confirmed as inhibitors of DHODH, and strikingly 11 out of the 12 compounds originally identified from the small molecule screen were credited as DHODH inhibitors, underscoring the vulnerability of the differentiation block to this target.

DHODH catalyzes an important step in the pyrimidine biosynthetic pathway fourth step of the nucleoside biosynthetic pathway. The authors used

saturating concentrations of pyrimidines to rescue the effects of the two lead compounds, verifying that myeloid differentiation was contingent on inhibition of pyrimidine synthesis. A derivative from one of the two lead compounds was shown to be efficacious in treating AML cells in vitro. However, its use in vivo was hampered by poor bioavailability. The authors therefore turned to brequinar sodium, a known potent inhibitor of DHODH, that has failed to demonstrate anti-tumor effects in solid tumors in early phase clinical trials(Munier-Lehmann et al., 2013), but notably has never been tested against leukemia. Impressively, brequinar sodium led to potent induction of myeloid differentiation and significant reduction in tumor burden in an array of AML models driven by varied genetic drivers including human cell line xenografts, patient derived xenografts and syngeneic mouse models. When brequinar sodium was tested against fully established primary human AML samples, the authors demonstrated delayed kinetics of leukemia evolution in primary and secondary transplantation.

In the era of precision medicine, the current standard blunderbuss approach of induction with cytarabine and daunorubicin is lacking in both precision and efficacy, evidenced by the unacceptably high relapse rates coupled with significant toxicity. The current work not only provided new insights into the biology of AML, but also identified a promising differentiation-promoting therapy. The finer details of the pro-differentiation mechanism, such as which protein modifications induced by DHODH inhibition are crucial to myeloid differentiation and what their pathogenic downstream targets might be, are intriguing areas worthy of further exploration. Future clinical trials of compounds targeting DHODH are clearly justified. Since the authors tested a limited number of patient derived xenografts, it remains to be seen whether DHODH inhibition alone will suffice to promote blast differentiation in the context of human AML with its extensive genetic and epigenetic complexity, or if other independent brakes on differentiation are imposed. Notably, while all-trans-retinoic acid has proven to be a spectacular advance for patients with acute promyelocytic leukemia, it is not curative as a single agent and the addition of arsenic trioxide has recently achieved 2-year event free survival rates of 97% with this chemotherapy-free regimen(Lo-Coco et al., 2013). Clearly rational combination partner selection with DHODH inhibition is merited and perhaps informed by further use of unbiased screens for synthetic lethality and/or by using the EC90 of brequinar to find targets that upon knockdown confer resistance.

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