employed in this study, proximity ligation assays. The new opportunities linked to all these studies include unprecedented insight into the architecture of the tumor microenvironment and into spatial relationships of different cell types at single-cell resolution. These relationships have relevance not only to HL but also to other lymphoma entities that rely on a complex cellular ecosystem of malignant cells with reactive immune cells. However, the molecular underpinnings (the "interior design") in the form of somatic gene mutations, receptor-ligand interactions, and involved soluble factors (cytokines/chemokines) are still only partially understood and are rarely combined with the characteristic morphology features ("architecture") of HL. Thus, the present study can be seen as a paradigm for an accelerating process deciphering the relatedness of "architecture" and "interior design."

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MYELOID NEOPLASIA

Comment on Dzama et al, page 2442

MLL-menin and FLT3 inhibitors team up for AML

Alexander E. Perl | University of Pennsylvania

In this issue of *Blood*,[1] Dzama and colleagues demonstrate that inhibiting MLL binding to menin synergizes with FLT3 inhibition in preclinical models of NPM1c and *KMT2A* (*MLL*)-rearranged AML, thus identifying a novel, rational combination for clinical development.

Potent, selective *Fms*-like tyrosine kinase 3 (FLT3) inhibitors improve remission rate and survival compared with standard chemotherapy in patients with relapsed or refractory *FLT3*-mutated acute myeloid leukemia (AML).[2] However, single-agent FLT3 inhibitors are not curative, prompting studies of drug resistance. These data identified clonal selection for cells bearing new mutations in Ras or related signaling proteins downstream of FLT3 as well as on-target FLT3 kinase domain resistance mutations that impair drug binding or facilitate activation.[3,4] In addition to combining FLT3 inhibitors with cytotoxic agents and moving these drugs earlier in therapy, there is a need to develop rational combinations of FLT3 inhibitors with other biologically targeted drugs. Recent data suggest nucleophosmin (*NPM1*) mutations, and *KMT2A* (*MLL*) translocations might identify promising populations for this approach, due to shared biologic features and cooperativity with *FLT3* in leukemogenesis.

NPM1 encodes a phosphoprotein that shuttles between nucleolus and cytoplasm and contributes to histone chaperoning, ribosome trafficking, DNA damage response, and centrosome duplication. NPM1 is commonly mutated in AML by 4 base-pair insertions in exon 12 that cause frameshift, truncation, and loss of a C-terminal nucleolar localizing segment. Consequently, mutated *NPM1* localizes exclusively cytoplasmic (NPM1c), which perturbs multiple cellular functions and strongly promotes leukemogenesis. Although *NPM1* mutations are thought to initiate AML, the full leukemic phenotype requires comutations. *FLT3* mutations are found in approximately half of cases and can be associated with high rates of treatment failure and generally poor survival, especially with comutation of DNMT3A and/or high *FLT3*-internal tandem duplication (ITD) allele burden.[5]

NPM1c is mutually exclusive with other balanced translocations that initiate AML, such as *RUNX1-RUNX1T1*, *CBFB-MYH11*, *PML-RARA*, and lysine methyltransferase 2A (*KMT2A*) rearrangements (formerly mixed-lineage leukemia, *MLL*). This suggests overlapping functions of NPM1c and these gene fusions. Gene expression profiling highlights overlapping patterns in NPM1c and *KMT2A*-rearranged AML, specifically overexpression of homeobox (*HOX*) A cluster genes, which resemble hematopoietic stem cells' expression pattern.[6] In particular, *HOXA9* and its cofactor *MEIS1* overexpression are thought to be critical for enhanced self-renewal in both of these AML subtypes.

KMT2A, the human homolog of the drosophila gene *trithorax*, is essential for both fetal and adult hematopoiesis. *KMT2A* is a member of a large multiprotein complex that interacts with chromatin and normally acts as a histone H3 lysine 4 (H3K4) methyltransferase, a

function catalyzed by its SET domain. Balanced translocations of *KMT2A* with >60 partner genes occur in ~3% of adult AML. These gene fusions create novel oncoproteins that initiate leukemia. Cases of AML with *KMT2A* rearrangements have among the lowest mutation burden of any described cancer, and these translocations alone may be sufficient to generate the full leukemic phenotype. Through translocation, *KMT2A* always loses its SET domain and associated H3K4 methylation activity. However, fusion oncoproteins frequently gain novel epigenetic activity via interaction with disruptor of telomeric silencing 1-like (DOT1L), an H3K79 histone methyltransferase. In addition, the translocation partner gene, canonically a transcription factor, becomes functionally deregulated to promote a stereotypical pattern of novel target gene expression, including overexpression of *FLT3*, *HOXA9*, and *MEIS1*. Transcription of the latter 2 genes promotes self-renewal and is critically dependent on the *KMT2A*-fusion protein binding to menin.

Small molecules that disrupt the protein-protein interaction between *MLL* and menin show substantial antileukemic efficacy in cellular and murine models of *KMT2A*-rearranged AML.[7,8] In addition, using a CRISPR/cas9 negative selection screen, Kühn and Armstrong confirmed wild-type *MLL* binding to menin was critical for expression of *HOXA9*, *MEIS1*, and *FLT3* in *NPM1*-mutated AML cells.[9] Cellular consequences of MLL-menin inhibition included growth arrest and induction of hematopoietic differentiation. Subsequently, this class of drugs was studied in murine models of *NPM1/FLT3* mutated AML and shown to reduce leukemic burden, eliminate established leukemia, and improve survival.[10] Two drugs that inhibit MLL-menin binding have recently entered clinical testing as single agents for AML (K0539, NCT04067336 and SNDX-5613, NCT04065399); after safety and dose optimization are established, efficacy testing will follow in patients with *MLL*-rearrangements or *NPM1* mutations. Thus, MLL-menin inhibitors could emerge as important targeted therapies for NPM1c and *KMT2A*-rearranged AML and might open considerable therapeutic options.

Dzama and colleagues now take the logical step to combine MLL-menin targeted therapy with FLT3 inhibitors in preclinical models of *FLT3*-ITD$^+$ AML and NPM1c or *KMT2A* rearrangements. As noted above, small molecule inhibition of MLL-menin interaction alone clearly induces differentiation of the leukemic clone. Here, MLL-menin inhibitors or a menin knockdown by short hairpin downregulated and dephosphorylated FLT3, potentiating kinase inhibitor antileukemic effects. A marked leftward shift in the 50% inhibitory concentration for combinations over the individual drugs was observed, indicating in vitro synergy. Encouragingly, the combination of MLL-menin inhibition and FLT3 inhibitor appeared to overcome clinically relevant, drug-resistant tyrosine kinase domain mutations in *FLT3*.[3,4] Adding to these in vitro data, a murine model of *KMT2A*-rearranged, *FLT3*-ITD$^+$ AML showed improved survival with the combination therapy.

These data are encouraging for eventual clinical translation, although several important questions remain unanswered. First, given known roles of FLT3 in regulating stem and progenitor populations, there is potential for significant myelosuppression from overlapping toxicity, which might not become manifest until human testing. Second, resistance to FLT3 inhibitors can develop by selection for cells whose growth is not dependent upon FLT3, including *FLT3*-wild-type clones.[3] Further work is needed to characterize whether such cells' growth requires MLL binding menin. In addition, because clinical use of FLT3 inhibitors induces differentiation, functional redundancy with MLL-menin inhibitors might fail to recapitulate the in vitro synergy suggested by preclinical studies. MLL-menin inhibitors also might more reasonably be combined with drugs that induce apoptosis and eliminate *NPM1* mutated cells, such as BH3 mimetics or traditional cytotoxic chemotherapy. Last, the clinical translation of these results to *KMT2A*-rearranged patients may be limited by the low incidence of *FLT3* comutations.

Overall, however, these are encouraging data with a clear translational application. Results of single-agent MLL-menin inhibitor trials in NPM1c and *KMT2A*-rearranged AML are eagerly awaited. With these data, Dzama and colleagues have cleared a path to show the next step for dual-novel targeted agent regimens for these patients.

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