

identify increased numbers of these cells including GrzB expression in FL compared with nonmalignant counterpart reactive lymph node tissue. The authors then used 3-D image reconstructions to allow, for the first time, the in situ visualization of CTL lytic immune synapse structures with FL tumor cells. Interestingly, these effector cells did not enter the follicle but were mostly located within the interfollicular space around nodules. The co-staining of activated caspase-3 on FL cells that formed cell conjugates with CTLs provides strong evidence for cytotoxic function. This was verified by performing functional cytotoxicity assays using expanded tumor-infiltrated CD8⁺ T cells (TILs) from FL patients against autologous FL cells pulsed with superantigen (in vitro immunologic assay that stimulates a large proportion of T cells triggering antigen-induced T-cell receptor signaling and effector function). Of interest, this study also reveals that high GrzB expression in CTLs (high GrzB score patient group) correlates with prolonged PFS in FL patients treated with R-chemo compared with the low GrzB score patient cohort.

To date, the results of many IHC studies investigating the role of the immune microenvironment in FL have been contradictory with technical scoring variations between laboratories acknowledged as a potential issue. Laurent et al have overcome this challenge by combining IHC with confocal microscopy, 3-D reconstructed image analysis, and functional cytotoxicity assays. This immunologic approach has identified strong GrzB expression as a biomarker for activated CTLs capable of forming lytic immune synapses and mounting cytotoxic function against autologous tumor cells. There are, however, some important considerations and future study is required to identify biomarkers and targets for enhancing the clinical potential of immunotherapy. Laurent and colleagues show that autologous CTLs exhibiting a strong GrzB content did not enter the FL nodule. It is possible that a cancer-associated T-cell motility defect or tumor “barrier” may prevent CTLs from infiltrating the intrafollicular tumor site in FL. Moreover, work using purified TILs, confocal imaging, and quantitative image analysis has identified that it is not only expression levels of biomarkers that is important, but also critical whether these proteins can traffic and polarize to the synapse effector site. FL tumor cells were shown to rapidly induce failure of re-

cruitment of cytoskeletal signaling proteins including cytotoxic machinery (Rab27A) to the synapse site in previously healthy CTLs.⁵ This profound tumor-induced T-cell immune synapse defect in FL could be repaired by the immunomodulatory drug lenalidomide. The high-quality confocal images presented by Laurent et al suggest that TILs may exhibit nonpolarized GrzB trafficking defects to the synapse contact site compared with nontumor-exposed autologous peripheral blood T cells from FL patients. Clearly, translational researchers must consider identifying strategies to repair tumor-induced CTL cell defects to address the unmet clinical needs in FL and maximize the anti-tumor activity of immunotherapy regimens such as R-chemo. This has been highlighted in CLL with strikingly impressive clinical results after infusion of genetically modified autologous T cells targeting tumor cells.⁶ The challenge now is, can CTL activity be enhanced or re-engineered by immunomodulatory strategies to tilt the balance away from immunosuppression in the microenvironment and toward potent anti-tumor ac-

tivity (see figure) and maximizing the kiss of death in FL?

Conflict-of-interest disclosure: A.G.R. declares no competing financial interests. J.G.G. has received honoraria from Roche, Celgene, and Merck for work on advisory boards. ■

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● ● ● MYELOID NEOPLASIA

Comment on Shen et al, page 5593

Gene mutation and AML pathogenesis

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In their large study of 1185 patients with acute myeloid leukemia (AML), Shen and colleagues have dissected the overlapping incidences and prognostic significances of mutations of the 12 genes most frequently mutated in AML, including *FLT3*, *NPM1*, *CEBPA*, *KIT*, *N-RAS*, *MLL*, *WT1*, *IDH1/2*, *TET2*, *DNMT3A*, and *ASXL1*.¹

They preferentially studied 176 patients with core binding factor (CBF) AML (mostly AML carrying the 8;21 translocation), 390 patients with acute promyelocytic leukemia (APL), and 605 patients with cytogenetically normal (CN)-AML or AML carrying 11q2.3 abnormality. While they confirmed that *FLT3* and *KIT* are the most frequently mutated genes in APL and CBF-AML, respectively,^{2,3} their analysis of the largest cohort of CN-AML reported to date is of particular interest. In this subgroup of patients, the highest mutation incidences were observed for the *NPM1* (20.9%), *MLL* (14%), *TET2* (12.7%), and *DNMT3A* (12.3%) genes. Surprisingly, *FLT3* mutation incidence was only 10.8%, while the incidence of *CEBPA* muta-

tions was as high as 22%. In this subgroup of 605 patients, because of the poor prognosis associated with *DNMT3A* mutations^{4,5} and the significant association observed between *NPM1* and *DNMT3A* mutations, 2 distinct prognostic groups now emerge among patients with AML carrying the so-called “favorable” *NPM1* mutation, according to *DNMT3A* mutation status. In multivariate analysis, *DNMT3A* mutations and *MLL* rearrangements were identified as bad prognostic factors, while bi-allelic *CEBPA* mutations and only the *NPM1*_{m+} / *DNMT3A*_{m-} pattern were associated with a better outcome.

Interestingly, *DNMT3A* mutations and *MLL* abnormalities seem to share more than a poor prognostic value. Both deregulate gene

Table 1. Gene mutations in AML

	Class I	Class II	Class III?	Other
Pathways:	Signal Transduction	Differentiation	Epigenetic regulation	Tumor suppression
Genes	<i>FLT3</i>	<i>RUNX1 (AML1)</i>	<i>TET2</i>	<i>WT1</i>
	<i>KIT</i>	<i>CBFβ</i>	<i>IDH1, IDH2</i>	<i>TP53</i>
	<i>NRAS, KRAS</i>	<i>CEBPA</i>	<i>DNMT3A</i>	
	<i>JAK2</i>	<i>NPM1</i>	<i>ASXL1</i>	
	<i>PTPN11</i>	<i>PU1</i>	<i>EZH2</i>	
		<i>MLL</i>		
		<i>RARA</i>		

promoter methylation. Both are responsible for an up-regulation of *HOXA7*, *HOXA9*, and *HOXA10* gene expression. Both are associated with the myelomonocytic or monocytic AML subsets of the French-American-British classification.⁶ Actually, a third class of genes encoding epigenetic modifiers, including *DNMT3A*, *IDH1*, *IDH2*, *TET2*, *ASXL1*, and *EZH2*, appears to play a major role in AML pathogenesis.⁷ This new class should be distinguished from the already proposed class I and class II genetic abnormalities affecting genes involved in signal transduction and differentiation pathways, respectively (see table). Very interestingly, most of the abnormalities belonging to this new class III seem to be associated with a worse patient outcome and more frequently observed in older patients with the disease. They may thus provide a genetic explanation for the poorer treatment effects observed in older as opposed to younger patients, even in patients with favorable cytogenetic or genetic features, as defined by the European LeukemiaNet classification.⁸ Large studies, taking into account the incidence and prognostic impact of these newly described genetic events in older patients specifically, are thus needed.

Awaiting efficient targeted therapies, the open issue is how gene mutation patterns may help physicians to guide the management of patients with AML in the daily practice. Given the large number of mutations already described and the fact that they often partially overlap, the definition of a standardized and well-accepted prognostic algorithm based on mutation patterns will not be an easy task. Other tools, such as gene expression profiling (GEP) or minimal residual disease (MRD) monitoring, might also be useful, for integrating multidimensional information in a single signature or measure. Monitoring MRD may offer the additional advantage to also integrate personalized information on anti-leukemic drug metabolism. However, all these approaches, including mutation patterns, GEPs,

or MRD monitoring, remain based on AML bulk disease examination. Thus, they do not take into account the level of genetic instability and potential for clonal evolution of leukemic stem cells or AML cell subpopulations with stem cell features that could give birth to AML relapse, as recently brilliantly demonstrated in acute lymphoblastic leukemia.^{9,10}

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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● ● ● **VASCULAR BIOLOGY**

Comment on Singh et al, page 5701

Angiogenesis: the HETE is on

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In this issue of *Blood*, Singh and colleagues identify HMG-CoA reductase-dependent farnesylation of Rac-1 as critical for 15(S)-HETE-induced angiogenesis.¹ These findings establish a novel link between eicosanoid and cholesterol metabolism with important biologic and therapeutic implications for angiogenesis.

Angiogenesis plays an important role in embryonic development as well as in numerous pathologic states including malignancy, atherosclerosis, and ocular diseases.² The discovery that angiogenesis is a key contributor to multiple disease states provided the essential goad for vigorous efforts to identify key molecular pathways mediating this process. Such efforts have not only revealed many

biologic insights but have also led to the development of novel therapies directed at major pro-angiogenic stimuli such as vascular endothelial growth factor (VEGF). However, initial enthusiasm has been somewhat muted by the recognition that drugs targeting VEGF are of limited efficacy, underscoring the fact that angiogenesis is mediated by myriad of subcellular systems and that a complex interplay