A high proportion of heterogeneous neoantigens is associated with a poor response to checkpoint blockade in patients with non–small cell lung cancer (NSCLC). A previous report used whole-exome sequencing to characterize the genomic intratumor heterogeneity in 11 patients with NSCLC, and found that high levels of genomic heterogeneity were associated with an increased risk of disease relapse. Reuben and colleagues characterized the T-cell repertoire in the same patient cohort to determine how intratumor heterogeneity in the T-cell landscape correlates with the genomic landscape and with patient outcome in NSCLC. T-cell receptor (TCR) profiling of 45 tumor regions using ImmunoSEQ revealed a high level of intratumor heterogeneity in T-cell infiltration, clonality, and repertoire. Assessment of T-cell clones from different regions of individual tumors showed substantial intratumor heterogeneity, with most clones restricted to individual tumor regions. However, the most abundant T-cell clones in each patient were detectable throughout the tumor. The tumor-infiltrating lymphocytes were predominantly CD4-positive across patients. An increased degree of TCR intratumor heterogeneity correlated with neoantigen heterogeneity and was associated with disease relapse and reduced disease-free survival. Taken together, these findings suggest a relationship between genomic and immune intratumor heterogeneity and disease relapse in patients with NSCLC.

See article, p. 1088.

Breast cancer progresses from ductal carcinoma in situ (DCIS) lesions, in which the intact basement membrane and myoepithelial cell layer separate cancer cells from the immune microenvironment, to invasive ductal carcinomas (IDC), in which cancer cells are exposed to immune cells in the stroma. However, the changes in the immune landscape during this transition and the mechanisms that underlie immune escape in breast cancer remain unclear, prompting Gil Del Alcazar and colleagues to characterize the composition and activation status of leukocytes in normal breast tissue, DCIS, and HER2\(^+\) and triple-negative IDCs. IDCs contained relatively more tumor-infiltrating leukocytes but fewer activated T cells compared with DCIS. Of note, however, DCIS samples showed enrichment for cytotoxic T-cell gene signatures, more activated CD8\(^+\) T cells, and a higher T-cell receptor clonotype diversity compared with IDCs, which were instead enriched for regulatory T-cell gene sets, suggestive of a switch to a more immunosuppressive microenvironment in IDCs. Expression of the immune checkpoint protein TIGIT was more common in DCIS T cells, whereas the gene encoding PD-L1 was preferentially amplified and overexpressed in a subset of triple-negative IDCs. Furthermore, coamplification of a chemokine gene cluster on chromosome 17q12 with ERBB2 in HER2\(^+\)ER\(^+\) tumors negatively correlated with the presence of activated T cells, indicative of coevolution of cancer cells and leukocytes. These results identify the in situ to invasive breast carcinoma transition as an important step for immune escape and tumor progression and suggest that immunotherapies may be more effective in early-stage disease.

See article, p. 1098.
A superenhancer was identified at the retinoic acid receptor enhancer subtypes based on projection to public datasets. Differences in overall survival were observed between the superdefined subgroups independent of mutational status. Differing superenhancer maps stratified AML into 6 epigenetic states.

807 superenhancers were identified per sample, and the progenitor cell samples, and 6 monocyte samples. A median of 807 superenhancers were identified per sample, and the superenhancer maps stratified AML into 6 epigenetic state defined subgroups independent of mutational status. Differences in overall survival were observed between the superenhancer subtypes based on projection to public datasets. A superenhancer was identified at the retinoic acid receptor alpha (RARA; encoding RARα) locus in a subset of patients, including 2 patients with RARA fusions, and it was associated with high expression of RARA, suggesting a potentially critical cell state role for RARα. Accordingly, in patients lacking the acute promyelocytic leukemia (APL) RARA translocation, the RARA superenhancer was associated with increased sensitivity to the selective RARα agonist SY-1425 in vivo. In vitro, RARA-high AML patient-derived xenografts (PDX) responded to SY-1425, but RARA-low PDXs did not. In RARA-high AML cells, SY-1425 induced differentiation, suppressed proliferation, and induced expression of retinoic acid response genes, a response similar to that observed in APL treated with retinooids. In addition to identifying AML subtypes characterized by superenhancers, these findings suggest that patients with AML with a superenhancer at RARA may respond to RARα agonists. These findings support further investigation of SY-1425 in biomarker-selected patients.

See article, p. 1116.
In Hodgkin lymphoma an immunosuppressive tumor microenvironment (TME), including tumor associated macrophages (TAM), limits the efficacy of immunotherapies such as chimeric antigen receptor T cells (CART) targeting antigens expressed on Hodgkin lymphoma cells. To potentially enhance the efficacy of CART therapy, Ruella, Klichinsky, and colleagues aimed to develop CART that would target both the tumor cells and the TME in Hodgkin lymphoma. In tumor samples from patients with Hodgkin lymphoma, the IL3 receptor CD123 was highly expressed on both tumor cells and TAMs, nominating it as a potential target. CART directed against CD123 (CART123) had previously been developed for the potential treatment of acute myeloid leukemia, and CART123 was also able to kill Hodgkin lymphoma cells in vitro and in vivo in Hodgkin lymphoma xenografts. Further, when CART123-treated immunodeficient mice who entered remission were rechallenged with Hodgkin lymphoma cells, the tumor was rejected, indicating that CART123 established long-term immunologic memory. The presence of Hodgkin lymphoma cells polarized macrophages to an M2-like immunosuppressive phenotype, thereby suppressing T-cell proliferation, but CART123 was also able to kill TAMs to overcome immunosuppression. Taken together, these findings suggest that CART cell therapies, such as CART123, that target both the tumor cells and TAMs may overcome immunosuppression to improve their efficacy, and warrant further investigation for the treatment of patients with Hodgkin lymphoma.

See article, p. 1154.

Loss of MutL Promotes Intrinsic Endocrine Therapy Resistance

Although mechanisms of acquired resistance to endocrine therapy have been identified in estrogen receptor–positive (ER+) breast cancer, the causes of intrinsic resistance, present at the time of diagnosis, are less well understood. Haricharan and colleagues linked loss of the MutL mismatch repair (MMR) complex to intrinsic resistance to endocrine therapy. MMR has two damage-sensing complexes: MutS and MutL. In patients with ER+ breast cancer, endocrine therapy resistance was associated with dysregulation of MutL but not MutS. In ER+ breast cancer cell lines, silencing of MutL complex genes promoted resistance to endocrine therapy (including estrogen deprivation, a surrogate for aromatase inhibitor exposure, fulvestrant, and tamoxifen) by preventing CHK2-mediated inhibition of CDK4/6, resulting in deregulated CDK4/6 activity that drove endocrine therapy resistance. Further, mutations that dysregulated MutL promoted resistance to endocrine therapy in patient-derived xenograft models. Response to endocrine therapy required MutL-mediated activation of ATM/CHK2 and inhibition of CDK4. Thus, MutL-deficient ER+ breast cancer cells exhibited CDK4 upregulation and were sensitive to CDK4/6 inhibitors. Moreover, MutL-deficient xenografts regressed when treated with estrogen deprivation in combination with CDK4/6 inhibition, but not with estrogen deprivation alone. In patients with ER+HER2− breast cancer treated with an aromatase inhibitor, MutL deficiency was associated with failure to fully suppress Ki67, but the addition of the CDK4/6 inhibitor palbociclib increased Ki67 inhibition and the incidence of complete cell-cycle arrest. Collectively, these findings show that MutL dysregulation may be a mechanism of intrinsic resistance to endocrine therapy, and predict that, for patients with MutL-deficient ER+ breast cancer, adjuvant treatment with CDK4/6 inhibitors may be beneficial.

See article, p. 1168.
Pancreatic ductal adenocarcinoma (PDAC) is characterized by a desmoplastic stromal response, which promotes tumor development and prevents drug delivery; however, the role of the local tumor microenvironment in PDAC metastasis remains incompletely understood. To identify potential factors that drive PDAC metastasis, Chiou and colleagues added an HMGA2-GFP reporter to the \( \text{Kras}^{\text{LSL-G12D/}}; \text{Trp53}^{\text{LSL-R172H/}} \) (KPC) genetically engineered PDAC mouse model. GFP\(^+\) PDAC cells were highly metastatic, gave rise to metastases with heterogeneous GFP expression, and had a transcriptional signature that was enriched for genes induced by hypoxia/HIF that was predictive of poor survival for patients with PDAC. Significant gene expression changes were observed between GFP\(^+\) and GFP\(^-\) PDAC cells, and knockdown of genes upregulated in GFP\(^+\) PDAC cells revealed that the transcription factor BLIMP1 drives metastatic ability. Hypoxia induced BLIMP1 expression in a partially HIF1\(\alpha\)-dependent manner in human and mouse PDAC cell lines, and hypoxia-response elements upstream of \( \text{Blimp1} \) were bound by HIF1\(\alpha\). BLIMP1 was required for both the upregulation and suppression of hypoxia-regulated genes, including prometastatic factors and cell-cycle arrest genes, respectively. Knockdown of the BLIMP1-dependent prometastatic gene \( \text{Hilpda} \) reduced metastasis \textit{in vivo}. Further, a BLIMP1-dependent hypoxia-induced gene signature correlated with BLIMP1 expression and worse prognosis in human PDAC. Together, these results provide insights into the role of the tumor microenvironment in promoting PDAC metastasis and the development of potential therapeutic strategies.

\[\text{See article, p. 1184.}\]