since significance (by Fisher's exact test) disappears if a single patient in the placebo group is artificially reassigned from an unfavorable outcome to a favorable outcome on the basis of the group data provided. In other words, the modest benefit observed in the sparsely available data from phase 2 trials of progesterone in patients with brain injury suggested low prestudy odds of a true relationship existing. Nevertheless, investigators and funders were convinced enough by these tantalizing data to initiate phase 3 trials. With favorable-outcome rates of more than 50% observed among the participants assigned to receive placebo, neither phase 3 trial had much opportunity to show benefit.

Slower-than-expected enrollment, overly optimistic effect sizes, better-than-expected performance in the placebo groups, and sample-size estimates that were based on one of multiple efficacy outcomes in small safety trials are commonly observed patterns in failed trials. The opportunity cost of these failed studies is high. If we are to break this cycle, we need a radical change in the culture of investigation and its funding. This includes the creation of collaborative research networks such as those recently implemented by the National Institute of Neurological Disorders and Stroke, more rigorous reporting and pooling of preclinical data, coordinated and sequential exploratory phase 2 trials that use standardized outcomes to replicate potential findings, and phase 3 trials designed to test well-vetted hypotheses. These changes may result in more extensive and costlier early-phase work and fewer reported false positive findings owing to more early failures of replication, but eventually greater numbers of successful phase 3 trials. Negative trial results, when they do occur, will contribute more to medical progress because they will have definitively answered a question that was worth asking in the first place.

As a neuroscience community, we must emulate Homer’s crew of the Odyssey by plugging our ears with beeswax and steeling ourselves against the temptations of the Sirens’ song if we wish to navigate past the obstacles in our path, emerge victorious, and proceed onward to new adventures.

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From the Department of Neurology, TeleStroke and Acute Stroke Services, and Institute for Heart, Vascular, and Stroke Care, Massachusetts General Hospital, and the Department of Neurology, Harvard Medical School — both in Boston.

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Clone Wars — The Emergence of Neoplastic Blood-Cell Clones with Aging
Janis L. Abkowitz, M.D.

Blood cells originate from hematopoietic stem cells (HSCs). HSCs are infrequent (<2 per 10⁸ bone marrow cells, or about 11,000 to 22,000 per person) and rarely divide.¹² Because mutations occur with DNA replication during cell division, HSC quiescence maximizes genomic stability and thus safety.

Mutations, however, can and do occur. Most...
are innocuous and do not substantially alter protein quantities or structure. Some make subtle changes that contribute to cellular diversity and allow HSCs or their offspring to react efficiently to differing environmental stresses. However, if a mutation speeds HSC self-renewal divisions or slows apoptosis, this results in more replicate HSCs, a larger clone size, and a disproportionate contribution by the mutant HSC to blood-cell production.

Clonal expansion of an HSC or early multipotent progenitor cell is also a necessary step for hematologic cancers, and growth-promoting mutations in specific genes are common cancer-initiating events. Cooperating mutations ensure a malignant phenotype, often by impeding the differentiation of progeny cells.⁴⁻⁶ A minimum of two or three driver (i.e., initiating and cooperating) mutations are needed for cancer to develop.⁴⁻⁶ How mutations that occur during normal hematopoiesis relate to mutations that initiate cancer is unknown.

Two groups now report in the Journal⁶,⁷ the results of their investigations of this question by repurposing whole-exome sequencing information from large population-based studies involving persons not known to have hematologic disorders. Using different methods and data sets, they report similar results: somatic (i.e., acquired) mutations leading to clonal hematopoiesis are rare in persons younger than 50 years of age but increase in frequency with age and occur in at least 10% of persons older than 65 years of age. More remarkably, most mutations driving clonal expansion occur in three genes previously associated with the myelodysplastic syndrome, myeloproliferative disorders, and acute myeloid leukemia—ASXL1, encoding additional sex combs–like transcriptional regulator 1, which modifies chromatin, and DNMT3A and TET2, respectively encoding DNA (cytosine-5) methyltransferase 3 alpha and tet methylcytosine dioxygenase 2, which influence DNA methylation.

Jaiswal et al.⁸ studied 17,182 persons from three consortia interested in determining genomic risk factors for cardiovascular morbidity and mortality and assessed the incidence and abundance (variant allele fraction) of specific mutations within 160 genes known to be associated with myeloid and lymphoid cancers. They detected mutations in 746 persons (4.3%), affecting 73 genes. Clonal hematopoiesis generally involved ASXL1, DNMT3A, or TET2 mutation and was observed in 5.6% of persons 60 to 69 years of age, 9.5% of persons 70 to 79 years of age, 11.7% of persons 80 to 89 years of age, and 18.4% of persons 90 years of age or older. Notably, 93% of the 746 persons with mutations had only one hematologic-cancer–associated gene mutation, a finding that suggests that this marks an initiating event, and in half the persons with mutations, mutant clones contributed to less than 18% of nucleated blood-cell production (median variant allele fraction, 0.09). In 13 persons from whom additional samples were available over a period of 4 to 8 years, the clones persisted, which provides indirect evidence that they originated from very early cells, probably HSCs. As a caveat, because whole blood was the source of DNA for whole-exome sequencing, it is technically possible that some clonal hematopoiesis reflects expanded lymphocyte clones; T cells normally comprise 7 to 24% of blood leukocytes and can be long-lived.

Genovese et al.⁷ analyzed data from whole-exome sequencing of DNA in blood samples from 12,380 Swedish persons, including 6135 persons with psychiatric disorders and 6245 healthy controls. By cataloguing mutations that occurred at a high variant allele fraction but considerably less than 0.50 (to exclude heterozygous germ-line mutations), they identified similar frequencies of age-dependent clonal hematopoiesis and predominant mutations in the same three genes. Frequent mutations were also identified in a fourth potential driver gene, PPM1D (not included in the Jaiswal panel). A third study had similar findings.⁸

The two Journal articles also provide new insights into the connection between aging and cancer. Hematologic cancer was more likely to occur in persons with clonal hematopoiesis (hazard ratios, 11.1 and 12.9; 95% confidence intervals, 3.9 to 32.6 and 5.8 to 28.7). In addition, in the study by Genovese et al., a cause–effect relationship was suggested by data from a few informative persons. However, proving this will require longitudinal, prospective studies. Clonal hematopoiesis was also associated with all-cause mortality, coronary heart disease, and ischemic stroke (hazard ratios, 1.4 to 2.6),⁶,⁷ suggesting that it might be a surrogate for failing health with aging as well as a specific risk factor for hematologic cancers.
There are several reasons why normal aging might confer a predisposition to clonal hematopoiesis — processes that maintain metabolic integrity age; telomere length decreases; and the numbers of mutations per cell division increase. Also, HSCs functionally decline, exhaust (die), or both. With less competition, it is more likely that cells acquiring ASXL1, DNMT3A, or TET2 mutations would expand sufficiently to be considered clonal hematopoiesis. One might predict that clonal hematopoiesis would also be common in aplastic anemia and after HSC transplantation, both states of relative HSC depletion, and preliminary data suggest that this is correct.9

What does this mean for the clinician? Because clonal hematopoiesis is common with aging and its prevalence greatly exceeds the age-specific incidence of leukemias,10 caution is needed when predicting clinical consequences from a cancer-associated gene mutation in healthy persons. At present, we should consider clonal hematopoiesis as we do monoclonal gammopathy of undetermined significance, note that clonally expanded plasma cells are prevalent in elderly persons but are stable and rarely progress to myeloma, and rely on other factors when making patient-care decisions.

More information is needed about how and when clonal insurgency will result in clonal dominance before these observations can be translated into effective clinical strategies. The data are exciting and provocative, show the power of genomic information for analyzing complex issues of pathogenesis, and will clearly guide future investigations.

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From the Department of Medicine, Division of Hematology, University of Washington, Seattle.

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