DECEMBER 2020 # 71

the Pathologist®

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Hematology and Hematopathology SPECIAL TAKEOVER!

In the Blood

Dive into the intriguing world of hematology-oncology and hematopathology

10 – 42

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References: 1. PD-L1 IHC 22C3 pharmDx [Instructions for Use]. Carpinteria, CA: Agilent Technologies, Inc.; 2020.

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Magic

Join us on a journey through hematopathology and hematology–oncology

Sanam Loghavi is Assistant Professor of Hematopathology, Department of Hematopathology, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA.

Kamran Mirza is Associate Professor and Vice Chair of Education in the Department of Pathology and Laboratory Medicine, Loyola University Chicago Stritch School of Medicine, Maywood, Illinois, USA.

Magic. There is no other word to describe it. We clearly remember the wide-eyed wonder with which we looked up to our hematopathology mentors as they converted bits and pieces of complex data into comprehensive hematopathology reports. Interpretation of subtle morphologic findings and unusual (often unheard-of) cytogenetics and molecular genetics – these were all "simple" tricks of their trade. We watched in awe as they spoke with their hematology-oncology colleagues, assuring them with mutual respect. Even now, as practicing hematopathologists ourselves, we marvel at our good fortune in having stumbled across this subspecialty and the incredible ancillary toolbox it brings with it.

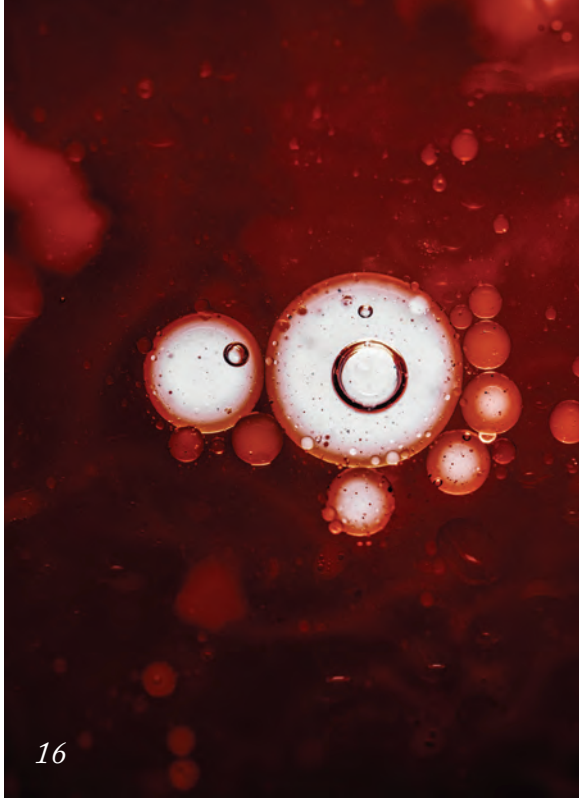
It's difficult to believe that it was only around 50 years ago that Janet Rowley discovered the chromosomal translocation underlying chronic myeloid leukemia and changed our understanding of cancer biology forever. The field of hematopathology grew exponentially thanks to significant changes to the way we approach and classify hematopoietic disorders. Within the pathology subspecialties, hematology led the way in using phenotypic, cytogenetic, and molecular genetic classification schemes –continually improving our ability to care for our patients.

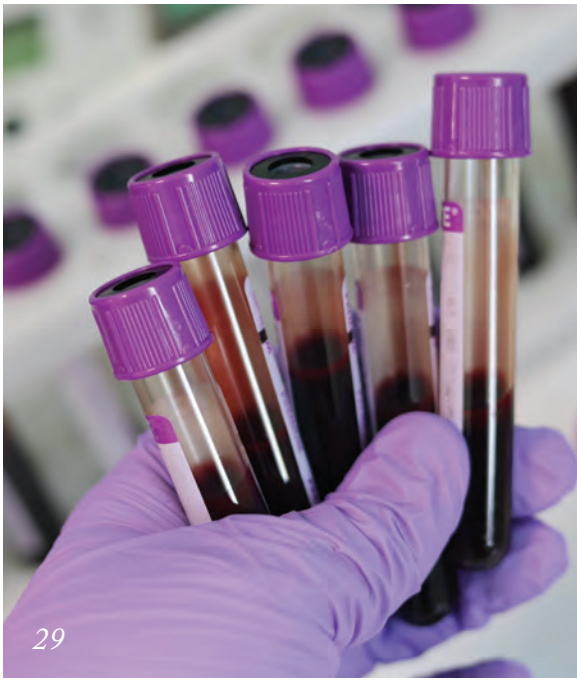
As hematopathologists, one of our most important responsibilities is to apply literature-based, objective criteria when establishing a diagnosis and communicating our findings with our hematologist-oncologist colleagues so that they can offer the best possible care to our patients. Precise subclassification is particularly essential for accurate risk stratification and appropriate treatment selection. The WHO classification scheme for hematopoietic neoplasms is an incredible resource that has rendered this task feasible for the vast majority of patients we encounter in daily practice – but, even so, it is essential to realize that no classification system is perfect.

We are excited to present this "hematology/hematopathology takeover" issue of The Pathologist. In it, some of the world's most renowned experts in our field summarize the historical context of hematopathology classification and discuss contemporary issues in diagnosing and treating hematopoietic disorders. We have selected cases that present challenges in the categorization and therapeutic approach of bone marrow and lymph node malignancies – and it's our hope that they will help guide you in understanding how to approach these diseases from diagnostic and therapeutic angles. We hope that you find these cases interesting, educational, and inspiring, and that you leave with a sense of renewed wonder and intrigue about disorders of the hematopoietic system.

Sanam Loghavi
Kamran M. Mirza

Contents





On The Cover

Takeover curators Sanam Loghavi and Kamran M. Mirza in action.

Upfront

From The ASCP

47


51

58

Feature

Reports

Sitting Down With

the Pathologist

ISSUE 71 - DECEMBER 2020

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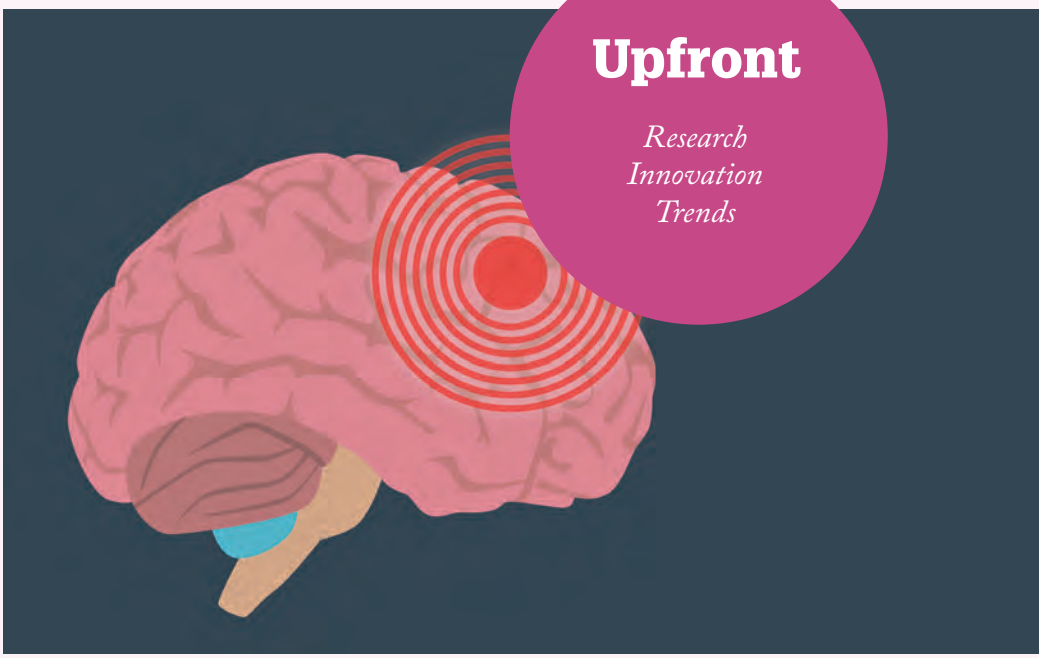


Looking Beyond the Brain for Tumor Biomarkers

Three breakthrough studies may improve diagnosis and monitoring of brain tumors

Although diagnosing brain tumors early is critical for better outcomes, accessing biomarkers in the brain presents unique challenges – a lack of minimally invasive methods, a complex location, and the natural blockade created by the blood-brain barrier, to name a few. Medulloblastomas and gliomas are the most common brain tumors in children and adults, respectively. Genomic characterization of medulloblastomas may lead to more precise molecular diagnoses – but biopsies are risky and may not accurately represent the tumor.

Analyzing circulating tumor DNA (ctDNA) in cerebrospinal fluid (CSF) may offer a route to accurate characterization and diagnosis of pediatric brain tumors. In a proof-of-concept study, researchers at the Vall d'Hebron Institute of Oncology showed that CSF ctDNA holds valuable information about a tumor's mutations and provides valuable information about prognosis and intratumoral genomic heterogeneity (1).

Researchers at the University of Michigan had a similar idea for children with high-grade gliomas. Collecting CSF is not currently part of standard care, so they applied nanopore genetic sequencing technology through a handheld device to investigate actionable alterations in patient CSF samples, which they confirmed with well-established methods (2). "This approach suggests we can rapidly and reliably detect key tumor-driving mutations in high-grade gliomas with very small samples – overcoming some of the barriers that were preventing the use of spinal cord fluid in diagnosing and monitoring these patients," said principal investigator Carl Koschmann (3).

In a third study, researchers at Massachusetts General Hospital investigated TERT mutations – common promoters of tumor growth not previously detected in circulating free DNA of gliomas. A new digital droplet polymerase chain reaction (ddPCR) blood test compared glioma patients' blood samples with their tumor biopsy tissue (4). The test accurately detected two TERT gene mutations with a 62.5 percent sensitivity rate – significantly higher than other assays that detect TERT mutations in the blood.

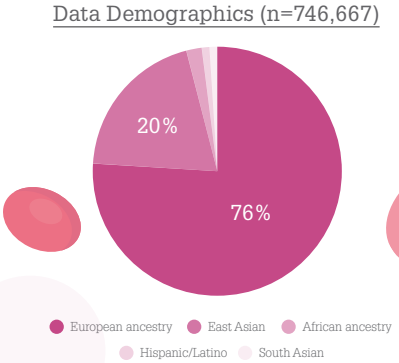
Please see references online at: tp.txp.to/brainmarkers

INFOGRAPHIC

The Wider Picture

Meta-analyses of hematological traits highlight the need for global representation in genetic studies

Meta-analyses show how genetic studies that examine only a single ethnicity or ancestry may overlook important hematological traits



QUICK HITS

We round up some of the latest research news in pathology and laboratory medicine

Detect, Diagnose, Delay

A new test has been developed to diagnose amyotrophic lateral sclerosis and frontotemporal dementia. The test detects the presence of biomarker TDP-43 even in the earliest disease stages when levels are low (1). Early diagnosis may enable drug development to stop or delay progression of disease.

Redefining Antibody Testing

SARS-CoV-2 antibody testing has so far lacked specificity, but scientists believe a new two-step definition – dual-antibody positivity – will improve screening. Under this definition, antibodies are measured as a dual-positive response against the receptor binding domain and nucleocapsid proteins of SARS-CoV-2 (2).

Peripheral Predictors

Up to half of patients with rheumatoid arthritis prove unresponsive to disease-modifying antirheumatic drugs – although the reasons are unknown. Now, researchers have measured blood samples from responsive and unresponsive patients and found that levels of peripheral blood specialized pro-resolving mediators may predict treatment response (3).

Turn Up the Heat

Researchers have developed a new test to diagnose sickle cell disease in one minute with high sensitivity and precision (4). The Acousto Thermal Shift Assay uses ultrasound to heat protein samples and measure the rate of disintegration to distinguish between sickle cell and normal proteins.

Autoantibody Hierarchy

The TEDDY study has discovered risk factors that predict which children may develop type 1 diabetes (T1D) following their first autoantibody appearance (5). Risk factors include age, family history of T1D, emergence of a second autoantibody, and appearance of a second antibody within one year of the first.

References

1. *C Scialò et al., Brain Commun, 14, fcaa142 (2020). PMID: 33094285.*
2. *M Hippich et al., Med, [Online ahead of print] (2020). PMID: 33163984.*
3. *EA Gomez et al., Nat Commun, 11, 5420 (2020). PMID: 33110080.*
4. *Y Ding et al., Small, 16, e20003506 (2020). PMID: 32893496.*
5. *K Vehik et al., Diabetes Care, 43, 2066 (2020). PMID: 32641373.*

Skin Deep

Testing skin samples may lead to early, reliable diagnosis of Parkinson's disease

We're told that "beauty is only skin deep" – but is that true for Parkinson's disease? It's one of the few diseases that can only be confirmed post-mortem, relying on clinical signs and symptoms until then. Because of this, diagnostic accuracy is poor, leading to frequent misdiagnoses.

But a new study shows potential for early diagnosis (1). Researchers at Iowa State University optimized a real-time, quaking-induced conversion assay for detecting misfolded proteins in humans – testing 25 skin samples from Parkinson's disease patients and 25 samples from people without neurological disease. The assay correctly detected clumping of alpha-synuclein proteins in 24/25 Parkinson's disease patients and 1/25 controls.

With clinical trials hampered by misdiagnosed patients, the team hope that improving diagnostic accuracy through skin tissue testing will lead to better treatments for Parkinson's disease.

Reference

1. *S Manne et al., Mov Disord, [Online ahead of print] (2020). PMID: 32960470.*

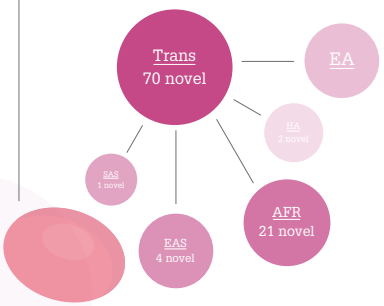
Trans-ethnic meta-analysis

5,552 trans-ethnic trait-variant associations, including

- *71* novel associations in non-EA populations
- *128* loci with allelic heterogeneity across populations

Ancestry-specific meta-analysis

28 novel trait-variant associations not found in EA populations



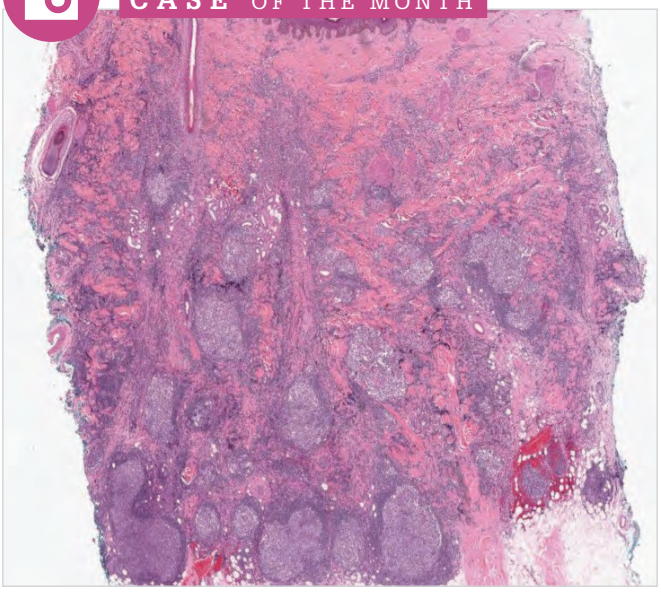
A need for diversity

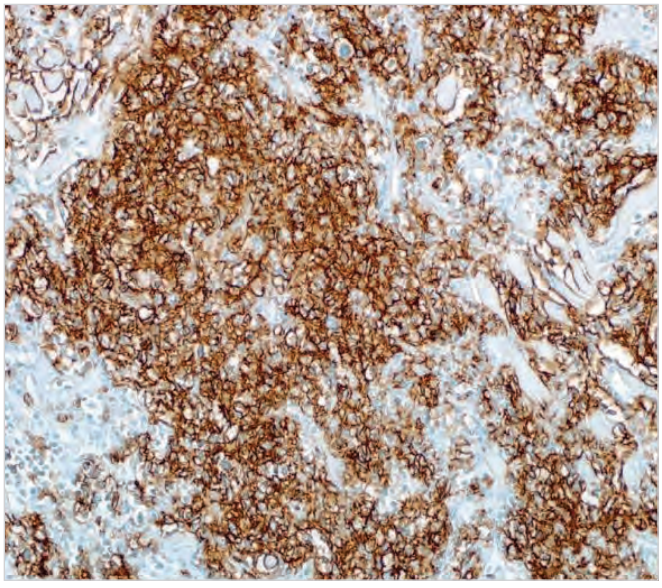
- Non-EA populations highlight important heterogeneous traits.
- Diversity and inclusion are required for a complete genetic basis of human traits.

Reference

1. *MH Chen et al., Cell, 182, 1198 (2020). PMID: 32888493.*

CASE OF THE MONTH





A 70-year-old woman presented with an erythematous papule, neither pruritic nor painful, on the right side of her chest. A biopsy of the lesion shows the following histopathology (Figures 1-6).

What is the most likely diagnosis?

a) Cutaneous B cell pseudolymphoma
b) Primary cutaneous marginal zone lymphoma
c) Primary cutaneous diffuse large B cell lymphoma, leg type
d) Primary cutaneous follicle center lymphoma
e) CD4+ small- to medium-sized pleomorphic T cell lymphoma

Answer to last issue's Case of the Month…

c) CD3

Blastic plasmacytoid dendritic cell neoplasm is a rare, clinically aggressive hematologic malignancy characterized by clonal expansion of precursors to plasmacytoid dendritic cells and with frequent cutaneous involvement whose pattern may include isolated areas with a purplish nodule or bruise-like papule or more disseminated purplish nodules/papules/macules. Bone marrow involvement with associated cytopenias is also common and leukemic dissemination may be present at diagnosis (1). Lymph nodes are involved in 40–50 percent of cases.

A diffuse, monotonous proliferation of medium-sized, blast-like cells with relatively scant, greyish-blue cytoplasm is appreciated. The nuclei have irregular contours with fine chromatin and small nucleoli. There is typically extensive involvement of the dermis with extension into the subcutaneous fat. Lymph node involvement tends to be interfollicular with sparing of the B-cell nodules. Bone marrow frequently shows myelodysplastic features (2).

Courtesy of PathologyOutlines.com. Case by Gabriel Lerner (Boston University School of Medicine, Boston, Massachusetts, USA), Lija Joseph, Bethany Tierno, Rebecca Shore, and Murat Anamur (Lowell General Hospital, Lowell, Massachusetts, USA); discussion by Genevieve M. Crane, Cleveland Clinic, Cleveland, Ohio, USA.

References

1. *F Facchetti et al., Mod Pathol, 29, 98 (2016). PMID: 26743477.*
2. *K Alayed et al., Am J Hematol, 88, 1055 (2013). PMID: 23940084.*

To register your guess, please go to http://tp.txp.to/1220/case-of-the-month
We will reveal the answer in next month's issue!

Case of the Month is curated by Anamarija M. Perry, University of Michigan, USA.

The Power of Collaboration

Working together to forge our post-pandemic future

By E. Blair Holladay

We are about to turn the page on a new year. The next 12 months are as yet unwritten, but we all have hope that they will be far less tumultuous than the previous ones have been. Despite the upheaval of 2020, we can't forget that it was also full of opportunity – for learning, for change, and for understanding. Of the many lessons we can take away from this past year, I think one of the most important ones is the power that lies in collaboration.

When COVID-19 started spreading wildly across the US, as a nation, we were ill-prepared. We knew nothing about SARS-CoV-2 and we had to learn while simultaneously dealing with daily issues like dwindling supplies and decreased staff as testing ramped up across the country. The spring of 2020 was not an easy time for laboratories – and the fall proved even more challenging as another wave of the virus took hold.

From the beginning, as pathologists and medical laboratory scientists worked behind the scenes to flatten the curve, they also stepped into the spotlight to break down myths and misconceptions about COVID-19. Colleagues from one side of the country to the other shared insight and research. Pathologists and medical laboratory scientists worked closely with clinicians to help patients understand why precautionary measures are so important. For patients who became severely ill, the laboratory partnered with clinicians to trial new processes to ameliorate symptoms and help people recover faster – safely.

At ASCP we, too, embraced collaboration to help our peers and members navigate the ever-changing pandemic environment. Over the past year, we've worked with experts in pathology and laboratory medicine to develop content and education that benefits practice today and will provide guidance for tomorrow. We've partnered with multiple organizations, including the American Medical Association, the Council of Medical Specialty Societies, AABB, the Association of Public Health Laboratories, and others to advance elements of our national testing strategy, enhance the blood supply, and promote the right test at the right time. We've fostered strong bidirectional communication with the White House as well as government agencies like Health and Human Services, the Centers for Medicare & Medicaid, the Centers for Disease Control and Prevention, the National Institutes of Health, and now the Biden transition team to ensure patients get the care they need throughout the pandemic. We have launched new ways for pathologists and medical laboratory scientists to engage with the latest research and resources, from live Town Hall events featuring subject matter experts to educational modules that help our colleagues better understand best practices when dealing with surging cases of COVID-19.

We've all been asked to do a lot over the past year, whether in the form of additional job duties, leadership tasks, or simply managing the day-to-day pressures of living and working in a high-stress environment. As pathology and medical laboratory professionals, we have gone above and beyond what has been asked of us – but we haven't done it alone. We've come together, we've supported each other, and we've set ourselves up for success that will sustain us through the end of this pandemic and into new beginnings.

IN THE BLOOD

Curious about hematology and hematopathology? Looking to test your skills in the diagnosis of myeloid and lymphoid neoplasms? Welcome to our special takeover, in which hematopathologists Sanam Loghavi and Kamran M. Mirza present eight cases to both challenge and educate, with clinical perspectives provided by a range of experts in the field.

MYELOID NEOPLASMS

An introduction and brief historical perspective

By Attilio Orazi

The 2001 WHO Classification of Tumors of Hematopoietic and Lymphoid Tissues reflected a paradigm shift in our approach to myeloid neoplasms. For the first time, genetic information was incorporated into the diagnostic algorithms. The revised fourth edition integrates clinical features, morphology, immunophenotyping, cytogenetics, and molecular genetics to define disease entities of clinical significance and explicitly acknowledges that recurrent genetic abnormalities not only provide objective criteria for recognition of specific entities, but are also vital to identify abnormal gene products and pathways that can be used as therapeutic targets. Several disease subgroups and sets of defining criteria now include the presence of gene mutations with or without a cytogenetic correlate. However, the importance of careful clinical, morphological, and immunophenotypic characterization of every myeloid neoplasm – and correlation with genetic findings – cannot be overemphasized.

MYELOPROLIFERATIVE NEOPLASMS (MPN)

Since the 1980s, chronic myeloid leukemia (CML) has been recognized as a molecularly defined entity based on the presence of the *BCR-ABL1* gene fusion. The discoveries of activating *JAK2* mutations and mutations in *CALR*, *MPL*, and *CSF3R* have revolutionized the diagnostic approach to myeloproliferative neoplasms (11–16). However, these mutations are not specific to any single clinical or morphological MPN phenotype and some are also reported in certain cases of myelodysplastic syndromes (MDS), MDS/MPN, and acute myeloid leukemia (AML). Therefore, we need an integrated multimodality for the classification of these myeloid neoplasms. Early MPN can be difficult to identify. Polycythemia vera, for instance, is often missed by relying only on CBC data; bone marrow morphology represents a critical criterion for diagnosis. Essential thrombocythemia must be distinguished from prefibrotic or early primary myelofibrosis (pre-PMF) – achieved by applying standardized morphologic criteria (10).

MYELODYSPLASTIC/ MYELOPROLIFERATIVE NEOPLASMS (MDS/MPN) AND MYELOID/LYMPHOID NEOPLASMS WITH EOSINOPHILIA

The MDS/MPN neoplasms category was introduced in 2002 to include myeloid neoplasms with clinical, laboratory, and morphologic features that overlap between MDS and MPN. Chronic myelomonocytic leukemia (CMML) became a separate entity within this new disease group. In 2016, based on accumulated scientific evidence, MDS/MPN with ring sideroblasts and thrombocytosis was moved from provisional to full status (9,10). The approach for diagnosing MDS/MPN is strictly multiparametric. An important point is the separation of CMML from PMF with monocytosis in patients with *JAK2* V617F mutation, which requires careful consideration of clinical and genetic results (17). The presence of specific gene rearrangements is key to the classification of myeloid/lymphoid neoplasms with eosinophilia, a group of truly molecularly defined diseases.

MYELODYSPLASTIC SYNDROMES (MDS)

Persistent cytopenia is required for diagnosing a myelodysplastic syndrome (MDS) (19). MDS classification still incorporates morphologic elements of the FAB classification originally proposed in 1982 (3), with cytogenetics added in 2001 and expanded in 2008. Targeted sequencing of myeloid neoplasm-associated genes can detect mutations in a vast majority of MDS patients (20,21) and selected gene mutations are integrated into several diagnostic algorithms. SF3B1 mutation is now a defining criterion for MDS with single or multilineage dysplasia and ring sideroblasts in cases with less than 15 percent ring sideroblasts. Evaluation for *TP53* mutation, a negative prognostic marker, is particularly relevant in cases of MDS with isolated del(5q), which identifies a clinically adverse subgroup. Importantly, acquired clonal mutations identical to those seen in MDS can occur in the hematopoietic cells of apparently healthy older individuals (22,23) – so-called "clonal hematopoiesis of indeterminate potential" (CHIP). Although a minority of patients with CHIP subsequently develop MDS, the presence of MDS-associated somatic mutations alone is not considered diagnostic of MDS.

"Persistent cytopenia is required for diagnosing a myelodysplastic syndrome (MDS)."

ACUTE MYELOID LEUKEMIAS (AML)

The WHO defines specific AML disease entities by focusing on significant cytogenetic and molecular genetic subgroups. Many recurring, balanced cytogenetic abnormalities are recognized in AML; most that are not formally recognized by the classification are rare (9,10,24), often occurring in pediatric patients, and do not represent separate disease categories. The realization that the improved prognosis seen in AML with mutated *CEBPA* is only associated with the presence of a biallelic mutation of the gene has modified the disease definition. Additionally, the presence of *NPM1* or biallelic *CEBPA* mutation now supersedes the presence of multilineage dysplasia in the classification. Finally, a provisional category of AML with mutated *RUNX1* has been added for cases of de novo AML with *RAS* mutation that are not associated with MDS-related cytogenetic abnormalities.

MYELOID NEOPLASMS WITH GERMLINE PREDISPOSITION

Cases of MDS or acute leukemia can be associated with germline mutations. The 2016 revision of the WHO classification added a section on myeloid neoplasms with germline predisposition, which includes cases of MDS, MDS/ MPN, and AML that occur on the background of those mutations (9,10). The presence of germline genetic aberrations indicates a need to screen family members for these aberrations; healthy family members diagnosed with a leukemia predisposition syndrome should be counseled regarding appropriate cancer surveillance (25).

The development of a globally adopted classification system crafted both by hematopathologists and clinicians has been of great benefit to the field of hematologic malignancies in general and of myeloid neoplasm in particular. Its flexible approach allows for the seamless integration of new data into a sound diagnostic scaffold. It remains an effective instrument for daily therapeutic decision-making and clinical trial design and will inform any future classification scheme.

Attilio Orazi is Professor and Chair in the Department of Pathology, Paul L. Foster School of Medicine, Texas Tech University Health Sciences Center, El Paso, Texas, USA.

Please see references online at: http://tp.txp.to/mintro

CLONAL CYTOPENIA OF UNCERTAIN SIGNIFICANCE OR MYELODYSPLASTIC SYNDROME?

The first case in our series on myeloid neoplasms

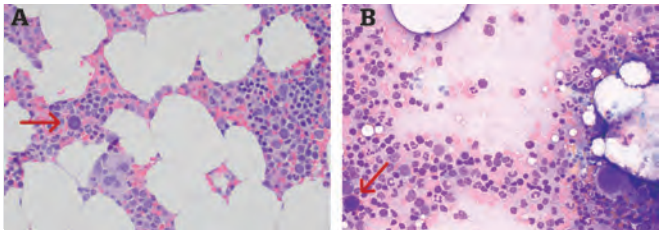
CLINICAL HISTORY

51-year-old man with progressive fatigue and exertional dyspnea.

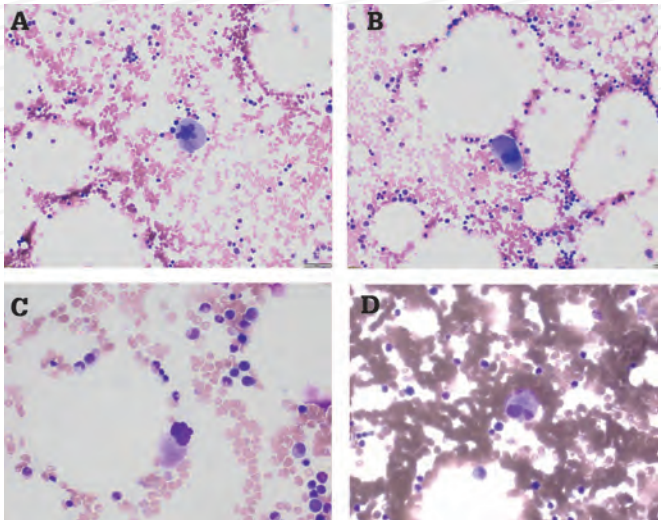
COMPLETE BLOOD COUNT AND DIFFERENTIAL (REFERENCE RANGE)

WBC	5.8 x10^9/L (4.0–11.0)
Hgb	9.9 gm/dL (14.0–18.0)
MCV	101 fL (82–98)
Platelets	247 x10^9/L (140–440)
Neutrophils	66.5% (42.0–66.0)
Monocytes	11.9% (2.0–7.0)
Absolute neutrophils	3.85 x10^9/L (1.70–7.30)
Absolute monocytes	0.69 x10^9/L (0.08–0.70)

BONE MARROW MORPHOLOGY



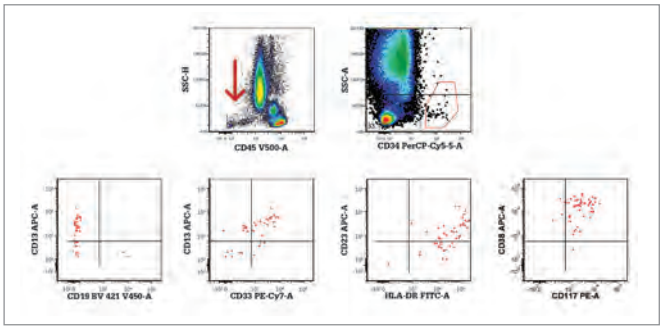
Bone marrow is hypercellular for age. Megakaryocytes are variably distributed and include occasional small, hyperchromatic forms (arrow) and other morphologically unremarkable forms (A; H&E 400x). Granulocytes are largely unremarkable in morphology; erythroid precursors show megaloblastoid maturation but no other features of dysplasia; megakaryocytes include occasional small, hyperchromatic forms (B; Giemsa 1000x).



Bone marrow aspirate smears showed numerous morphologically unremarkable megakaryocytes (A–B; Giemsa 1000x), as well as occasional small and variably hyperchromatic forms (C–D; Giemsa 1000x).

FLOW CYTOMETRY

Mildly decreased side scatter of granulocytes; myeloid progenitors with unremarkable phenotype.



Flow cytometric immunophenotyping of the bone marrow aspirate shows granulocytes with decreased side scatter and a small number of CD34+ myeloid progenitors with unremarkable immunophenotype.

KARYOTYPE

46,XY[20]

NEXT-GENERATION SEQUENCING

Next-generation sequencing studies showed the following mutations:

Gene	HGVS	VAF (%)
SRSF2	NM_003016.4(SRSF2):c.281_283dupGCC p.R94dup	14
DNMT3A	NM_022552.4(DNMT3A):c.1376del p.K459fs*192	6
SETBP1	NM_015559.2(SETBP1):c.2608G>A p.G870S	2
SETBP1	NM_015559.2(SETBP1):c.2602G>A p.D868N	1
ASXL1	NM_015338.5(ASXL1):c.1283_1284del p.Q428fs*9	22

VAF: variant allele frequency.

FINAL DIAGNOSIS

Variably cellular (40–60 percent) bone marrow showing trilineage hematopoiesis with left-shifted erythropoiesis and granulopoiesis, mild dysmegakaryopoiesis, mild eosinophilia, and mild polytypic plasmacytosis.

CASE DISCUSSION

The pathologist's view

When evaluation of a patient with cytopenias demonstrates ineffective hematopoiesis, the pathologist's challenge is to differentiate a clonal myeloid malignancy that meets criteria for myelodysplastic syndrome (MDS) from other causes. MDS encompasses a heterogeneous group of clonal hematopoietic stem cell disorders, but morphologic dysplasia in one or more of the myeloid lineages is a near-constant phenotypic manifestation. Therefore, despite major advances in the understanding of the genetic changes associated with MDS, morphologic examination of hematopoietic precursors continues to be a cornerstone in its evaluation. The World Health Organization (WHO) classification (1) continues to require the presence of quantifiable morphologic dysplasia in >10 percent of any of the myeloid lineages, with or without genetic evidence of clonal disease, to unequivocally establish a diagnosis of MDS. The current WHO classification scheme recognizes certain recurrent chromosomal alterations as presumptive evidence of MDS in the absence of quantifiable morphologic dysplasia; however, they rightfully argue against using somatic gene mutations as diagnostic evidence of MDS in the absence of unequivocal morphologic dysplasia, given the occurrence of these changes in healthy older individuals as age-related clonal hematopoiesis (1,2). Herein lies the conundrum of clonal cytopenia(s) of undetermined significance (CCUS) (3), which often demonstrates only mild dysplasia not meeting the quantitative cutoff for MDS. Any experienced pathologist can attest to the significant interobserver variability of discerning morphologic dysplasia, particularly in cases of low-risk MDS. In this example, subtle morphologic changes – including bone marrow hypercellularity and mild megakaryocytic dysplasia – are not sufficient to warrant a "WHO-sanctioned" MDS diagnosis and myeloid precursors exhibit an essentially normal immunophenotype by flow cytometry, further limiting our ability to establish an unequivocal diagnosis of MDS. Nevertheless, given the presence of multiple gene mutations (some with relatively high allelic frequency), macrocytic anemia, and subtle morphologic changes, it is conceivable to predict that this patient's disease will evolve into MDS with time. Our task is to alert the oncologist and recommend close observation.

The hematologist's view

By David Steensma

Hematologists are frequently asked to assess patients with persistent cytopenias. Often, despite careful evaluation including marrow aspiration and biopsy, diagnosis remains unclear. Historically, such patients were described as having "idiopathic cytopenia(s) of undetermined significance" (ICUS) (4) – "idiopathic" highlighting unclear etiology, and "undetermined significance" underscoring a variable and unpredictable natural history.

The recognition that most patients with MDS and other myeloid neoplasms have somatic genetic mutations in hematopoietic cells that can be detected with next-generation sequencing (NGS) promised to improve diagnostic testing and offer some clarity in ambiguous cases (5). Detection of SF3B1 mutation, for instance, defines a subtype of MDS with a strong association with ring sideroblast morphology and usually indolent natural history, and can help rule out an exclusively reactive cause for sideroblastic anemia (6).

At the same time, we have learned that clonal hematopoiesis associated with somatic mutations in hematopoietic cells is common with aging, and that the most frequent mutations seen in aging-associated clonal hematopoiesis (e.g., *DNMT3A*, *TET2*, *ASXL1*, *TP53*, *JAK2*) are also highly recurrent in myeloid neoplasms (3). Therefore, simply finding an MDS-associated mutation in a patient with cytopenias and indeterminate morphology is not enough to classify the patient as having MDS. When a clonal mutation is present in a patient with cytopenias, it is referred to as "clonal cytopenia(s) of undetermined significance" (CCUS) (7).

Malcovati and colleagues showed a few years ago that patients with CCUS are much more likely than those with ICUS to progress to MDS or another neoplasm meeting World Health Organization (WHO) diagnostic criteria (8). Patients with multiple mutations and "larger" clones – variant allele frequency (VAF) >10–20 percent in blood-derived DNA – were more likely to progress than those with single mutations and small clones. Similarly, Abelson and colleagues found that a broad red cell distribution width, specific mutations such as splicing factor

mutations, and higher VAF indicating greater clonal expansion predicted acute myeloid leukemia (AML) in healthy people with clonal hematopoiesis (9). Effectively, higher-risk forms of CCUS have a natural history comparable to lower-risk MDS and can be thought of as "MDS without dysplasia." The WHO allows certain clones detected by conventional metaphase karyotyping (e.g., del(5q) or monosomy 7) to support a diagnosis of MDS in the absence of cellular dysmorphology.

This information about the relative risk of disease evolution in people with ICUS versus CCUS can be helpful in counseling patients, but identifying which allelic patterns actually cause cytopenias can be a challenge. The patient described here, who presented with macrocytic anemia without other cytopenias, exhibits some worrisome features. Five clonal mutations were detected, two at VAF >10 percent. *SRSF2*, *ASXL1*, and *SETBP1* are all enriched in MDS/myeloproliferative neoplasm (MPN) overlap syndromes compared with MDS without MPN features (10,11), so although splenomegaly is not mentioned and there is no leukocytosis or thrombocytosis, his condition may, with time, evolve in that direction.

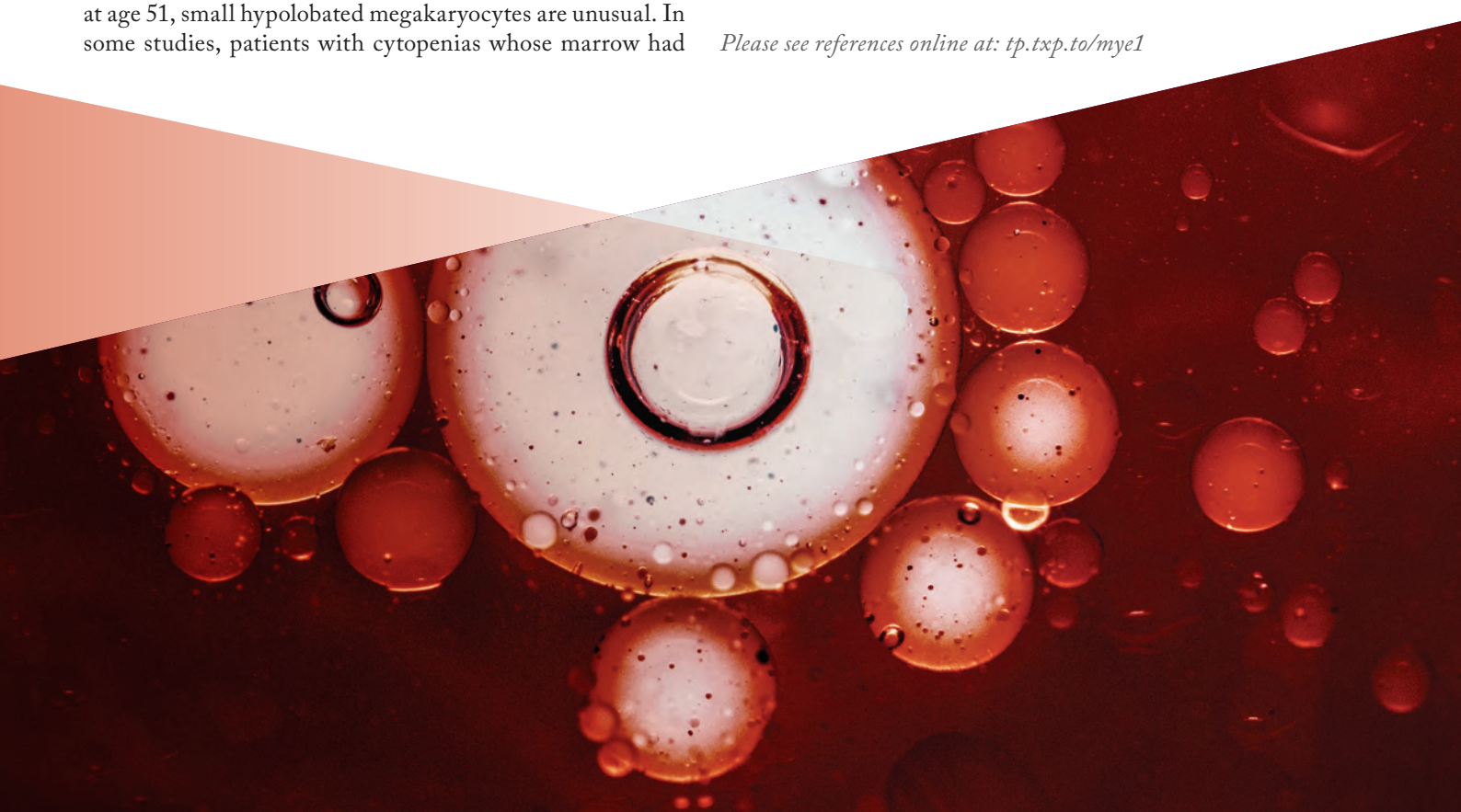
The patient's marrow is also not "stone-cold normal." It is hypercellular for age and there are a few dysplastic cells on the aspirate film. We regularly observe a few atypical or dysplastic cells in the marrow of healthy older people (12) but, at age 51, small hypolobated megakaryocytes are unusual. In some studies, patients with cytopenias whose marrow had some dysplasia, but not enough to meet WHO criteria for MDS, were more likely to progress to incontrovertible MDS than those with normal marrow.

Might some morphologists have called this case MDS rather than CCUS? Inter- and intra-observer reproducibility is not as high as we would like in MDS diagnosis, especially in lower-risk MDS where morphologic changes can be subtle (13,14). In cooperative group trials, historically at least one in five enrolled patients have not had the diagnosis confirmed on central review, either because dysplasia was subtle or the marrow sample provided to the reviewing hematopathologist was inadequate.

I suspect this man with CCUS will eventually develop an overt myeloid neoplasm, either MDS or MDS/MPN. For now, monitoring with serial blood counts and supportive care (possibly with an erythropoiesis-stimulating agent) would be appropriate. But he bears close watching and will likely need disease-modifying therapy and allogeneic hematopoietic cell transplant in the future.

David Steensma is Edward P. Evans Chair in MDS Research and Director, Center for Prevention of Progression (CPOP) of Hematological Malignancies, Division of Hematologic Malignancies, Department of Medical Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts, USA.

Please see references online at: tp.txp.to/mye1



SF3B1 -MUTANT CHRONIC MYELOMONOCYTIC LEUKEMIA

The second case in our series on myeloid neoplasms

CLINICAL HISTORY

82-year-old man with progressive exertional dyspnea for one year.

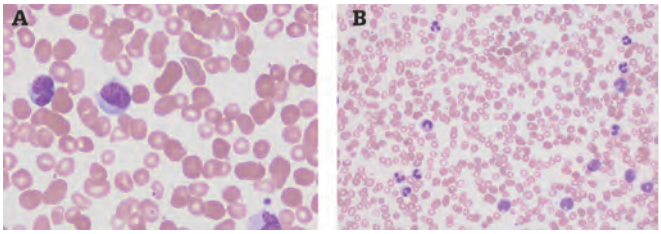
PERTINENT PHYSICAL EXAM

No hepatosplenomegaly.

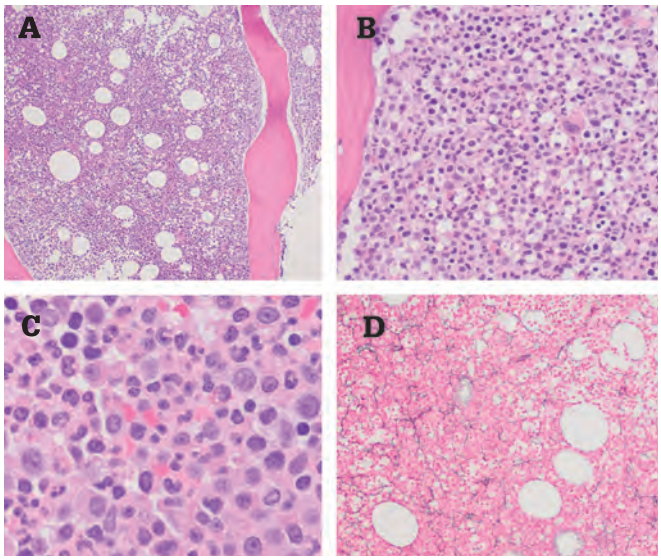
COMPLETE BLOOD COUNT AND DIFFERENTIAL (REFERENCE RANGE)

WBC	12.5 x10^9/L (4.0–11.0)
Hgb	8.7 gm/dL (14.0–18.0)
MCV	104 fL (82–98)
Platelets	315 x10^9/L (140–440)
Neutrophils	65.0% (42.0–66.0)
Lymphocytes	14.0% (24.0–44.0)
Monocytes	18.0% (2.0–7.0)
Eosinophils	1.0% (1.0–4.0)
Basophils	1.0% (0.0–1.0)
Metamyelocytes	1.0% (<=0.0)
Absolute neutrophils	8.13 x10^9/L (1.70–7.30)
Absolute lymphocytes	1.75 x10^9/L (1.00–4.80)
Absolute monocytes	2.25 x10^9/L (0.08–0.70)

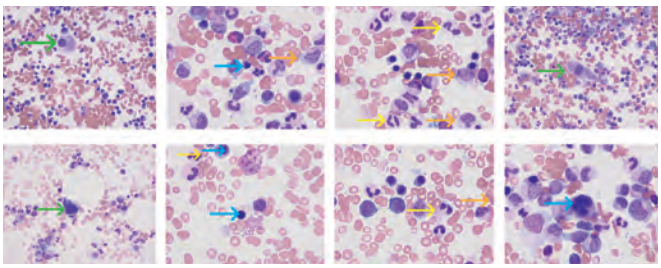
BONE MARROW MORPHOLOGY



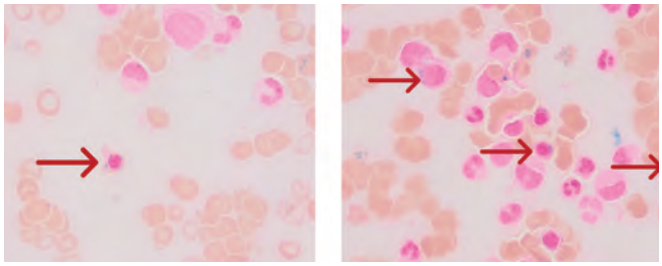
Peripheral blood smear shows macrocytic anemia with leukocytosis and absolute monocytosis. Monocytes are morphologically atypical and include increased immature forms (promonocytes) (Giemsa; A 200x, B 400x).



The bone marrow core biopsy is hypercellular for age (A; H&E 100x). There is myelomonocytic hyperplasia; megakaryocytes are small and dysplastic (B; H&E 200x). Erythroid precursors include increased left-shifted forms (C; H&E 400x). A reticulin stain shows mild reticulin fibrosis (D; 100x).



The bone marrow aspirate smears show trilineage dysplasia. Erythroid dysplasia (blue arrows) is characterized by nuclear contour irregularities, basophilic stippling of cytoplasm, and asynchronous maturation of cytoplasm and nucleus. Dysmegakaryopoiesis (green arrows) is manifested by increased small, hypolobated forms. Granulocytes (yellow arrows) are also dysplastic with increased hypolobated and hypogranular forms. Monocytes and monocytic precursors (orange arrows) are increased.



An iron stain performed on the bone marrow aspirate smear shows increased ring sideroblasts (red arrows) (Prussian blue 1000x).

KARYOTYPE

Routine cytogenetic studies show an abnormal male karyotype – 46,XY,del(12)(p12)[19]/46,XY[1]

NEXT-GENERATION SEQUENCING

Next-generation sequencing studies showed the following mutations:

Gene	HGVS	VAF (%)
CSF3R	NM_156039.3(CSF3R):c.1853C>T p.T618I	2
SETBP1	NM_015559.2(SETBP1):c.2602G>A p.D868N	4
RUNX1	NM_001754.4(RUNX1):c.964_965del p.S322fs*277	22
ASXL1	NM_015338.5(ASXL1):c.1934dupG p.G646fs*11	32
SRSF2	NM_003016.4(SRSF2):c.284C>T p.P95L	34
SF3B1	NM_012433.2(SF3B1):c.1998G>C p.K666N	40

VAF: variant allele frequency.

FINAL DIAGNOSIS

SF3B1-mutant chronic myelomonocytic leukemia.

CASE DISCUSSION

The pathologist's view

Though the diagnosis of myelodysplastic syndrome (MDS)/myeloproliferative neoplasm (MPN) is unequivocal in this case, further subclassification is challenging, particularly if one attempts to stay within the current WHO classification scheme's confines. When strictly applying the current WHO1 criteria (1), given the presence of dysplastic morphologic features and relative and absolute monocytosis, this case is best classified as chronic myelomonocytic leukemia (CMML), dysplastic subtype (WBC <13,000/μL). However, the presence of numerous ring sideroblasts is unusual and a direct manifestation of this neoplasm's genetic profile, which harbors a dominant *SF3B1* mutation. In our opinion, this case represents an example of a potentially distinct biologic entity, the so-called *SF3B1*-mutant CMML (2), which is not currently accounted for in routinely used classification schemes. Cases of *SF3B1*-mutant CMML typically present with predominant MDS-like features, distinct molecular profiles, and superior prognosis; therefore, appropriate classification and risk stratification directly impact patient management strategies.

The hematologist's view

By David Steensma

From a management perspective, CMML is challenging, thanks to its clinical and molecular heterogeneity. By demonstrating overlapping features of MDS and MPN, it necessitates an individualized treatment approach (3). This particular patient presented with macrocytic anemia, leukocytosis, and monocytosis with a normal platelet count. His bone marrow biopsy showed trilineage dysplasia with ring sideroblasts (RS). His karyotype showed 12p deletion (intermediate risk) and next-generation sequencing identified mutations involving *SF3B1* (40%), *SRSF2* (34%), *ASXL1* (32%), *RUNX1* (22%), *SETBP1* (4%) and *CSF3R* (2%). Among these, truncating *ASXL1* mutations are universally detrimental, predicting shorter overall and acute leukemia-free survival (4). *SF3B1* and *SRSF2* mutations both impact pre-mRNA splicing, with *SRSF2* mutations most common in CMML (5). *SF3B1* mutations strongly correlate with the presence of BM RS, are seen in 5 percent or fewer of CMML patients, and associate with a predominantly dysplastic CMML subtype, with preserved platelet counts and a superior acute leukemia-free survival (2). The variant allele frequency burden of both splicing mutations is unusual and makes it difficult to predict the dominant clone. Splicing

mutations are thought to be mutually exclusive, but can coexist (6). Finally, *RUNX1* and *SETBP1* mutations are seen in about 15 percent of CMML and confer an adverse prognostic impact (4,7). *CSF3R* T618I mutations are exceedingly uncommon in CMML and represent a known oncogenic driver for chronic neutrophilic leukemia (8); the significance of this subclonal mutation (VAF 2%) in the context of the patient's disease is unknown.

For this patient, despite his dysplastic CMML subtype and favorable *SF3B1* mutations, the *ASXL1*, *RUNX1*, and *SETBP1* mutations are concerning for higher-risk disease. Given his age, he is not a stem cell transplant candidate. Hence, for the management of his anemia, I would first check his endogenous erythropoietin level. If <500, he would be a good candidate for recombinant human erythropoietin therapy. Although luspatercept, a first in class TGF-β modulator, has shown excellent activity in MDS-RS, it has not been approved for the management of anemia in CMML-RS (9). If his endogenous EPO is >500, or if he does not respond to erythropoietin, he would be a reasonable candidate for low-dose- hypomethylating agent therapy (10). Low doses of azacitidine and decitabine have been associated with epigenetic restoration of hematopoiesis and improved anemia. At our institute, we also strongly recommend that all CMML patients consider enrollment in clinical trials – because, other than stem cell transplant, there are currently no curative therapies.

David Steensma is Edward P. Evans Chair in MDS Research and Director, Center for Prevention of Progression (CPOP) of Hematological Malignancies, Division of Hematologic Malignancies, Department of Medical Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts, USA.

Please see references online at: tp.txp.to/mye2

MYELODYSPLASTIC SYNDROME WITH EXCESS BLASTS AND FIBROSIS

The third case in our series on myeloid neoplasms

CLINICAL HISTORY

68-year-old woman with progressive fatigue and exertional dyspnea.

PERTINENT PHYSICAL EXAM

Mild splenomegaly.

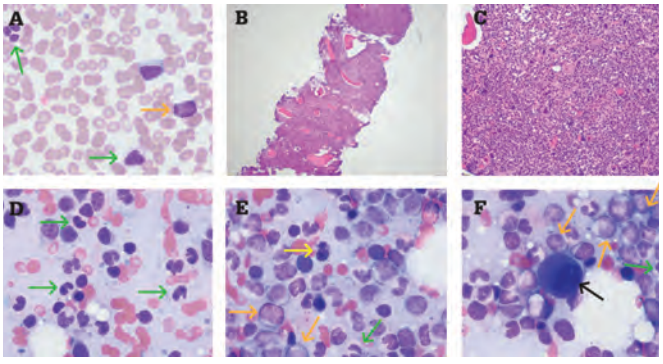
COMPLETE BLOOD COUNT AND DIFFERENTIAL (REFERENCE RANGE)

WBC	7.4 x10^9/L (4.0–11.0)
Hgb	7.3 gm/dL (14.0–18.0)
Platelets	52 x10^9/L (140–440)
Absolute neutrophils	4.22 x10^9/L (1.70–7.30)
MCV	98 fL (82–98)
Absolute monocytes	2.25 x10^9/L (0.08–0.70)

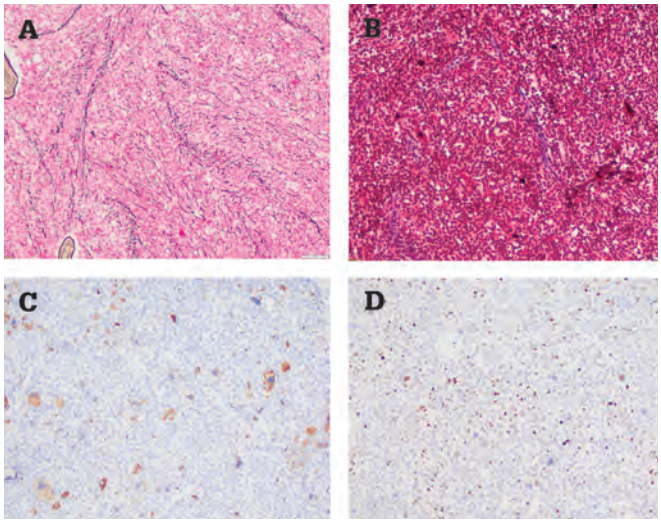
SERUM LACTATE DEHYDROGENASE

497 U/L (135–214)

PERIPHERAL BLOOD AND BONE MARROW MORPHOLOGY



Peripheral blood smear shows anemia, thrombocytopenia, dysplastic granulocytes (green), and occasional circulating blasts (orange) (A; Giemsa 1000x). Bone marrow core biopsy is markedly hypercellular (B; H&E 20x). Megakaryocytes are increased and variably distributed without significant clustering. They are frequently dysplastic, characterized by small size, nuclear hypolobation, and hyperchromasia (C; H&E 200x). Bone marrow aspirate smears show dysplastic (hypogranular, hypolobated) granulocytes; dysplastic erythroid precursors with nuclear budding (yellow); small, dysplastic megakaryocytes (black); and increased blasts (D–F; Giemsa 1000x).



The bone marrow core biopsy shows dense reticulin fibrosis forming intersections (A; reticulin stain 200x) and scattered fine bundles of collagen (B; Masson Trichrome 200x). A CD61 stain highlights increased dysplastic megakaryocytes and a p53 stain shows aberrant overexpression with moderate-to-bright nuclear staining in a subset of cells, including some megakaryocytes (C and D, respectively; immunohistochemistry with hematoxylin counterstain 200x).

KARYOTYPE

46,XX,der(3)del(3)(p21p23)inv(3)(p21q27),del(5)(q13q33),del(11)(q22q24)[15]/46,XX[5]

NEXT-GENERATION SEQUENCING

Next-generation sequencing studies showed the following mutations:

Gene	HGVS	VAF (%)
TET2	NM_001127208.2(TET2):c.1270del p.S424fs*3	45
TP53	NM_000546.5(TP53):c.578A>T p.H193L	37
JAK2	NM_004972.3(JAK2):c.1849G>T p.V617F	6
ASXL1	NM_015338.5(ASXL1):c.3076G>A p.G1026R	4

VAF: variant allele frequency.

FINAL DIAGNOSIS

Myelodysplastic syndrome with excess blasts-2 and fibrosis.

CASE DISCUSSION

The pathologist's view

A variety of bone marrow (BM) disorders, both benign and malignant, may be associated with a pathologic increase in stromal fibrosis – but, within the broad spectrum of chronic myeloid neoplasms, reticulin fibrosis is most often associated with myeloproliferative neoplasms, in particular with primary myelofibrosis (PMF).

The diagnostic approach to myeloid neoplasms begins with careful review of peripheral blood findings, including trends in the hemogram. Typically, cytopenias and morphologic dysplasia are diagnostic clues to myelodysplastic syndromes (MDS), cytoses in the absence of frank dysplasia points towards MPNs, and those with mixed features fall into the MDS/MPN overlap category. In most cases, distinction between these entities is possible with careful assessment of a good quality BM sample, including an adequately cellular aspirate smear to allow for evaluation of dysplasia in all three hematopoietic lineages and an adequate trephine biopsy to allow for assessment of megakaryocytic distribution and morphology, stromal fibrosis, and osteosclerosis. However, several situations can lead to challenges in accurate subclassification. This case is one such example.

Given the presence of splenomegaly, BM hypercellularity, megakaryocytic hyperplasia, increased reticulin fibrosis, and the presence of *JAK2* V617F, a differential diagnosis of PMF is not unreasonable. However, there are several clues here that point toward a diagnosis of MDS. These include bicytopenia in the absence of cytosis, megakaryocytes without significant clustering; predominantly small, monolobated, hyperchromatic megakaryocytes (unlike PMF, which typically shows a wide range of large, atypical, hyperlobulated megakaryotyes and some smaller, hyperchromatic forms), and prominent granulocytic and erythroid dysplasia. The karyotypic abnormalities involving chromosomes 3 and 5 and the presence of a dominant clone with a *TP53* mutation that has a significantly higher VAF than the *JAK2* mutation also suggests a diagnosis of MDS. An alternative differential diagnosis would be acute panmyelosis with myelofibrosis; however, the chronic nature of this patient's presentation, lack of bone pain and fever, and blast count in this case argue against this diagnosis.

MDS with excess blasts and fibrosis (MDS-EB-F) is recognized as a distinct form of MDS in the newest iteration of the WHO classification (1). The presence of significant fibrosis is an independent prognostic predictor and should be specifically noted in the pathology report. If the bone marrow aspirate smear is insufficient for a 500-cell differential count, CD34 immunohistochemistry should be considered for accurate assessment of the blast count. The prevalence of *TP53* alterations

is significantly higher in cases of MDS-F compared with other variants of MDS. In most cases, aberrant p53 overexpression by immunohistochemistry correlates well with *TP53* mutation status and serves as a reasonable crude surrogate marker for assessment of *TP53* alterations in MDS-F (2). However, more recently, the implications of *TP53* allelic state have been further elucidated in MDS (3). A *TP53* multi-hit state predicts an increased risk of death and leukemic transformation independently of the Revised International Prognostic Scoring System (IPSS-R). It is therefore critical to carefully assess the allelic state of *TP53* using a wide array of ancillary techniques including mutation analysis, routine karyotype, FISH, and array-CGH for complete assessment and prognostication.

The hematologist's view

By Mikkael Sekeres

A 68-year-old woman comes to my office with progressive fatigue and dyspnea.

But that isn't what she tells me.

Rather, she recounts a period of months during which, having just retired from her job as an office manager, she devotes herself to the upkeep of her garden and two-acre property and finds that she has to rest more and more. She even resorts to taking an hour-long nap each afternoon before preparing dinner for her and her husband. She hands over the responsibility of doing laundry to him when it becomes a chore to slog up and down the stairs to the basement to reach the washer and dryer (she stops halfway to catch her breath). She now sleeps 11 or 12 hours each night, but wakes in the morning no more refreshed than when her head first hit the pillow.

With two cytopenias, she buys herself a bone marrow biopsy that shows multilineage dysplasia, MF2 fibrosis, blasts, karyotypic abnormalities, and molecular abnormalities. For her diagnosis of MDS, we use the IPSS-R, for which she earns a number of points: 1.5 for having a hemoglobin < 8 g/dL, 0.5 for a platelet count between 50,000 and 90,000/mL, 3 for a blast percentage >10, and 3 for poor risk cytogenetics – a total of 8 points, a risk category of "very high," and a predicted survival of about nine months. Though the IPSS-R does not formally include molecular abnormalities, adding them to our prediction (4) makes little difference; it's hard to make dismal even worse.

After impressing on our patient the seriousness of her diagnosis, we make two treatment recommendations: the hypomethylating agent azacitidine and hematopoietic cell transplantation.

Azacitidine is given for seven days of a 28-day cycle and can only be administered in clinic, so represents a time commitment on the part of frequently older patients. It should be given for at least four to six cycles before response can be accurately assessed. Indeed, premature dose lowering, truncated cycles, or discontinuation contributes mightily to treatment "failure." Other hypomethylating agents are available, too, though neither decitabine nor the newly approved oral cedazuridine/decitabine have demonstrated survival advantages in clinical trials.

Hematopoietic cell transplantation is the only known cure for MDS and, for higher-risk patients, should be encouraged at diagnosis. Azacitidine is often given as a bridge to those opting for a transplant, both to cytoreduce the MDS tumor burden pre-transplant and to avoid treatment delays should transplant turn out not to be an option. Unfortunately, except at highly specialized MDS and transplant centers, this curative therapy is undertaken by a small minority of patients, whether because of comorbidities that make transplant too risky, poorly matched or unavailable donors, or – most commonly – patient preference.

Do combination chemotherapy approaches work? Yes, but often no better than azacitidine monotherapy in randomized trials. One study combined azacitidine and the drug eprenetapopt, a small molecule drug that reactivates mutant and inactivated *TP53* by restoring its confirmation and function. Investigators reported an overall response rate of 87 percent, more than double what would be expected for azacitidine monotherapy (5). This combination is being studied in a randomized trial that has completed accrual.

Patients with higher-risk MDS are caught between a rock and a hard place. Should they choose treatment with hypomethylating agents, sacrificing many days each month to receive their shots? Transplantation, with its attendant complications? Both? Or neither and opt instead for palliative options? It's our job to help guide our patients on this unwanted and often treacherous journey, and ensure their choices meet their goals for the often limited amount of time they have left.

Mikkael Sekeres is Chief of the Division of Hematology at Sylvestr Comprehensive Cancer Center, University of Miami, Miami, Florida, USA.

Please see references online at: tp.txp.to/mye3

ELEVATING THE TREATMENT STANDARD IN OLDER PATIENTS WITH AML

The final case in our series on myeloid neoplasms

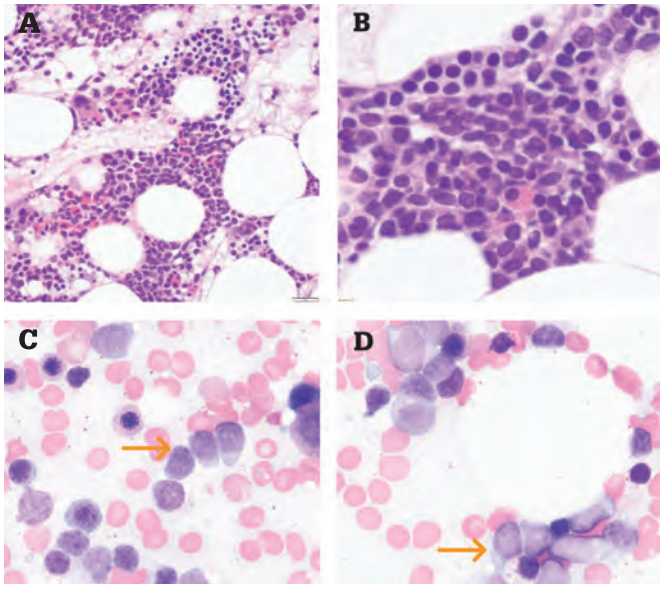
CLINICAL HISTORY

72-year-old woman with a history of chronic renal disease and coronary artery disease, referred to our institution with newly diagnosed acute myeloid leukemia.

COMPLETE BLOOD COUNT AND DIFFERENTIAL (REFERENCE RANGE)

WBC	1.5 x10^9/L (4.0–11.0)
Hgb	12 gm/dL (14.0–18.0)
MCV	102 fL (82–98)
Platelets	155 x10^9/L (140–440)
Absolute neutrophils	0.69 x10^9/L (1.70–7.30)

BONE MARROW MORPHOLOGY



The bone marrow core biopsy is hypercellular for age (A; H&E 200x) and shows clusters of immature-appearing mononuclear cells (B; H&E 1000x). The bone marrow touch imprint shows increased blasts. Blasts are small to intermediate in size with predominantly smooth nuclear contours, finely dispersed chromatin, and variably conspicuous nucleoli (orange arrows) (C,D; Giemsa 1000x).

CYTOCHEMICAL STAINS

Blasts were negative for myeloperoxidase.

FLOW CYTOMETRY IMMUNOPHENOTYPING

Aberrant myeloid blasts (8%) positive for CD13, CD33, CD34, CD38, CD117, and CD123; negative for cytoCD3, CD7, CD19, HLA-DR, and MPO.

KARYOTYPE

Routine cytogenetic studies show an abnormal female karyotype – 47,XX,+8[6]/46,XX[14]

NEXT-GENERATION SEQUENCING

Next-generation sequencing studies showed the following mutations:

Gene	HGVS	VAF (%)
RUNX1	NM_001754.4(RUNX1):c.1036dupC p.R346fs*254	2
IDH2	NM_002168.2(IDH2):c.515G>A p.R172K	3

VAF: variant allele frequency.

FINAL DIAGNOSIS

Acute myeloid leukemia with minimal differentiation.

CASE DISCUSSION

The pathologist's view

In cases of acute myeloid leukemia (AML), the role of the pathologist doesn't stop at the identification of 20 percent blasts. Gone are the days when morphology and cytochemistry to differentiate myeloid from lymphoid leukemia was considered state-of-the-art diagnostics. An ever-increasing understanding of the pathogenesis of AML, with evolution of therapeutics, requires us to go beyond morphology and phenotype and play a critical role in risk stratification and treatment decisions.

From the diagnostic perspective, before deeming an AML "not otherwise specified," a panel of phenotypic, cytogenetic, and genetic abnormalities must be queried. Once a diagnosis of acute leukemia is established, we must test for recurrent cytogenetic and molecular abnormalities that inform prognosis and therapeutic

strategies. The identification of specific gene mutations – including those involving *FLT3*, *IDH1/2*, or *TP53* – now have clear therapeutic implications and inform selection of targeted therapies, and the list of such mutations continues to grow.

Because morphology, phenotype, and genetics play key roles in the prognostic and therapeutic decision tree, sampling is crucial. In this case, although an adequate differential cell count was performed on the bone marrow touch imprint, a poor-quality aspirate smear was responsible for the underestimation of blast percentage by flow cytometry and of mutation VAFs by next-generation sequencing. As pathologists, our duty to the patient does not stop at diagnosis; it includes incorporation of the results of ancillary studies and rectifying potential discrepancies to enable our clinical colleagues to offer patients the best possible options.

The hematologist's view

By Curtis Lachowiez and Courtney D. DiNardo

Recent approvals of new and effective targeted AML therapies provide renewed optimism – and present clinicians with new management challenges.

The initial approach in any patient with newly diagnosed AML requires ascertaining the acuity of the disease and ruling out acute promyelocytic leukemia. Is a proliferative leukocytosis or leukostasis present, necessitating urgent cytoreduction? Is there evidence of an underlying coagulopathy or systemic infection? Outside of these emergent scenarios, data suggest that treatment delays to complete a diagnostic workup are safe (1).

Our 72-year-old patient presented with relatively asymptomatic pancytopenia. After confirming the diagnosis of AML and ruling out the need for urgent intervention, attention should be directed at risk stratification (2,3), selecting induction therapy, and considering consolidative allogeneic stem cell transplantation. Intrinsic factors, such as karyotype and mutations (4), and extrinsic factors, such as comorbidities and performance status (5), inform treatment decisions. Adding to these complexities is the sobering fact that the median age of AML diagnosis is 68, and older patients with even the most favorable-risk disease have inferior outcomes with standard induction.

Due to a history of chronic renal disease and coronary artery disease, the patient was considered ineligible for intensive chemotherapy. Initiation with azacitidine in combination with venetoclax was recommended.

The reduced efficacy (CR rates of 30–45 percent) and increased mortality observed in older patients treated with standard induction is partially attributable to increases in relapse (6,7),

In advanced ovarian cancer,

If you're not testing for HRD, you're not seeing the whole picture

1 in 2 women with HRD-positive tumors do not have a *BRCA1/2* mutation[1-4]

Homologous recombination repair deficiency (HRD) testing identifies tumor characteristics—beyond *BRCA1/2* mutation—that make it sensitive to PARP inhibition.[1,5]

Personalized medicine begins with personalized pathology. Discuss establishing a testing protocol for HRD in ovarian cancer with the multidisciplinary team at your institution.[6-8]

Learn more at **testforHRD.com**

BRCA, breast cancer susceptibility gene; PARP, poly ADP-ribose polymerase.

References: 1. Frey MK et al. *Gynecol Oncol Res Pract.* 2017;4:4. **2.** Pennington KP et al. *Clin Cancer Res.* 2013;20(3):764-775. **3.** Konstantinopoulos PA et al. *Cancer Discov.* 2015;5(11):1137-1154. **4.** Ledermann JA et al. *Eur J Cancer.* 2016;60:49-58. **5.** Watkins JA et al. *Breast Cancer Res.* 2014;16(3):211. **6.** Cheema PK et al. *J Oncol Pract.* 2017;13(2):e130-e138. **7.** Hoskins PJ et al. *CA Cancer J Clin.* 2017;67(6):493-506. **8.** Sundin T. *Med Lab Manag.* 2019;8(11):6.

Precision Medicine & Nurse Education

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frequency of adverse-risk disease, and medical comorbidities (5,8). In adults over 70, intensive chemotherapy resulted in dismal overall survival (5,8). Those deemed ineligible for curative intensive therapy with or without transplantation are often offered single-agent HMA or LDAC therapy with comparatively modest expectations (9,10).

Fortunately, recent therapeutic advancements are changing the natural history of AML. Treatment incorporating the targeted BCL2 inhibitor venetoclax in combination with HMAs (HMA+VEN) has emerged as a safe and strikingly effective therapy for older AML patients across all spectra of disease (11,12).

In the original phase Ib dose escalation and expansion study of HMA+VEN (11) and the recently reported confirmatory international phase III VIALE-A trial comparing HMA+VEN to azacitidine (12), the median age of study subjects was 76 years, with an early mortality rate of 7 percent, CR/CRi rate of 66 percent, and median overall survival of 15 months. Responses to HMA+VEN were rapid, with a median time to CRc and best response of 1.2–1.3 and 2.1 months, respectively. These increased and early responses resulted in more patients achieving durable transfusion independence compared to single-agent treatment (12). Patients with MRD-negative CR (as determined using standardized multiparameter flow cytometry) fare remarkably well, with a two-year survival rate of 73 percent.

HMA+VEN is generally well-tolerated. Infectious complications, particularly febrile neutropenia, are increased, though 30-day mortality does not differ significantly compared to HMA monotherapy (12).

Our patient's *IDH2* mutation is predictive of a response to HMA+VEN. The patient obtained a complete remission with negative measurable residual disease and remains in remission four years from diagnosis.

Unlike other targeted therapies, venetoclax is not dependent on a specific mutation (13). Mutations in *IDH1/2* impart leukemic cell dependency on BCL2, increasing susceptibility to apoptosis upon exposure to VEN (14). Patients with *IDH1/2* mutations in VIALE-A demonstrated favorable CR rates of 75 percent and a HR for death of 0.34. Other mutations, such as NPM1 and splicing factor mutations, may also provide VEN sensitivity (13,15,16).

Despite improved responses in patients with *TP53* mutations treated with HMA+VEN over azacitidine (CRc: 55 percent vs. 0), this group remains at high risk for relapse. Mutations in intracellular signaling genes are additionally recognized as adaptive resistance mechanisms to HMA+VEN, warranting close molecular surveillance on treatment. Mutations in *RUNX1* in the absence of co-occurring mutations in *NPM1* denote adverse-risk disease (2); however, as our case highlights, certain molecular groups have yet to be validated in large cohorts treated with HMA+VEN. Although *RUNX1* mutations may predict resistance to VEN-based therapy, co-mutations in IDH2 appear to abrogate this negative prognostic impact.

The results of VIALE-A usher in a long-awaited treatment standard for older patients with AML and highlight exciting opportunities to harness our understanding of AML biology to exploit molecular vulnerabilities and individualize treatment. Ongoing investigation is needed to define the differential impact of co-occurring mutations on outcomes to HMA+VEN, assess the safety and efficacy of additional targeted agents in combination with HMA+VEN, and develop effective therapies for relapsed patients or those with persistent MRD positivity. In the face of these challenges, VIALE-A confirms that HMA+VEN represents a new foundation of care upon which the future of AML therapy for older patients will be built.

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Please see references online at: tp.txp.to/mye4

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LYMPHOID NEOPLASMS

An introduction and brief historical perspective

By L. Jeffrey Medeiros

In 1832, Thomas Hodgkin described seven patients with similar disease findings involving lymph nodes and spleen. Hodgkin made his observations on autopsy patient specimens and never used a microscope. Three decades later, Sir Samuel Wilks reported 15 similar cases, recognized the earlier work of Hodgkin, and coined the eponym "Hodgkin's disease" (now Hodgkin lymphoma). It subsequently became apparent that there are many types of lymphoma in addition to Hodgkin lymphoma, and these neoplasms became known as non-Hodgkin lymphomas.

Technological advances, in large part, facilitated insights into lymphomas. For almost 150 years after the study by Hodgkin, light microscopic evaluation was the principal technology employed. Pathologists compared lymphomas to normal lymph nodes and began to recognize entities. Patterns and cellular features were correlated with clinical features. We recognized that lymphomas composed of large cells were associated with aggressive clinical behavior; most composed of small cells were clinically indolent. Cases with a nodular pattern resembled germinal centers of lymphoid follicles. Various morphologic classification systems were proposed, most of which separated Hodgkin from non-Hodgkin lymphomas and then further subclassified these groups.

New technologies led to seminal discoveries that changed our understanding of lymphocytes and lymphoma classification:

- Lymphocyte lineages were discovered. This began with Glick and colleagues' recognition of B cells, followed by Miller and others' identification of T lymphocytes and Klein and others' discovery of NK cells.
- Lymphocytes – previously thought to be terminally differentiated cells – were shown to respond to antigens or mitogens by transforming into larger proliferating cells.
- Lymphocytes were discovered to have surface antigens we can exploit to identify normal and neoplastic cells – indicating their utility in diagnosing and classifying lymphomas. Simultaneous technological advances in immunophenotyping methods facilitated the characterization of lymphoid cells. Kohler and Milstein's discovery of hybridoma technology led to widespread availability of monoclonal antibodies.

These immunologic insights led to the proposal of two lymphoma classifications in 1974: the Kiel classification and the Lukes-Collins classification. For the remainder of the decade, multiple immunology-based and morphology-based lymphoma classifications competed. The situation, confusing for clinicians and pathologists alike, led to a National Cancer Institute-sponsored study in 1982 that compared all six classifications using morphology and outcomes. All systems rendered diagnostic categories that could broadly divide non-Hodgkin lymphomas into prognostic groups: low-grade indolent neoplasms (indolent), intermediate-grade neoplasms (aggressive), and high-grade neoplasms (very aggressive). A Working Formulation was proposed to serve as a common language for translating between systems. Although it became popular in the United States and functioned as a de facto classification, Europe and many other nations continued to use the Kiel system. A stalemate of sorts set in.

Alongside advances in immunology, our ability to study human chromosomes blossomed. Fluorescent dyes that facilitated chromosome banding allowed improved recognition of chromosomal abnormalities in lymphomas. Chromosome 8q24 translocations were linked to Burkitt lymphoma, t(14;18)(q32;q21) to follicular lymphoma, t(11;14)(q13;q32) to mantle cell lymphoma, and t(2;5)(q23;q35) to anaplastic large cell lymphoma.

The field of genomics began to emerge with Nathans' 1971 discovery of restriction endonucleases, which allowed researchers to cut and manipulate DNA fragments. Subsequent application of molecular methods to the study of lymphomas yielded many insights. These methods enabled the cloning and characterization of oncogenes and tumor suppressor genes involved in various chromosome abnormalities. For example, *MYC* was identified at chromosome 8q24, *BCL2* at chromosome 18q21, *CCND1* at chromosome 11q13, and *NPM1-ALK* was shown to be a fusion gene created by t(2;5). This type of information greatly enhanced our understanding of lymphomas, and it didn't take long for the findings to be incorporated into lymphoma diagnosis, patient risk stratification, and prognostication. However, they were not incorporated in a consensus manner… and the stage was set for a new attempt at lymphoma classification.

In 1994, Nancy Harris and colleagues proposed the Revised European-American Lymphoma (REAL) classification, which used a multiparametric approach to lymphoma diagnosis by employing clinical data, morphology, immunophenotype, genetic information, and presumed cell of origin. The REAL

"Hodgkin made his observations on autopsy patient specimens and never used a microscope."

classification was the foundation of the third edition of the WHO classification (2001), and the subsequent fourth edition (2008) and revision (2017). As part of the WHO classification effort, pathologists and clinicians worked collaboratively to develop a consensus classification of lymphomas, each more detailed and granular than the last. The current WHO classification is accepted internationally as a consensus classification for lymphoma diagnosis and as a tool to facilitate lymphoma discovery.

The completed human genome heralded the advent of high-throughput genomic testing to interrogate these genes in various cancers. In lymphomas, gene expression profiling was used for both discovery and classification. The results provided possible targets for therapy and showed the heterogeneity of a number of disease categories defined in the WHO classification. Then came next-generation sequencing of lymphomas. Targeted sequencing using gene panels has shown their molecular landscapes at the DNA and RNA level as never seen before. Molecular pathways critical for lymphomagenesis have been recognized and drugs that specifically target molecular abnormalities are being developed or are in clinical trials. Some novel agents are already approved, and many more will follow. Our understanding of lymphomas has never been greater, and the prospects for future targeted therapy that is more effective and less toxic have never been brighter.

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Please see references online at: tp.txp.to/lintro

CHRONIC LYMPHOCYTIC LEUKEMIA/SMALL LYMPHOCYTIC LYMPHOCYTIC LYMPHOMA – ACCELERATED PHASE

The first case in our series on lymphoid neoplasms

CLINICAL HISTORY

67-year-old woman presented with bilateral neck swelling.

IMAGING

Multicompartmental lymphadenopathy above and below the diaphragm.

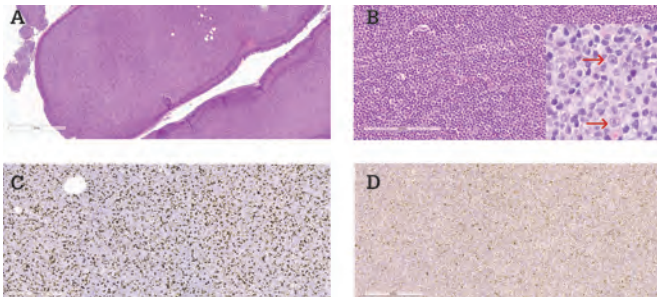
COMPLETE BLOOD COUNT AND DIFFERENTIAL (REFERENCE RANGE)

WBC	9.3 x10^9/L (4.0–11.0)
Hgb	13.3 gm/dL (14.0–18.0)
MCV	101 fL (82–98)
Platelets	247 x10^9/L (140–440)
Absolute lymphocytes	6.32 x10^9/L (1.00–4.80)
Absolute neutrophils	2.23 x10^9/L (1.70–7.30)

ADDITIONAL LABORATORY RESULTS

Serum lactate dehydrogenase	358 U/L (135–214)
Serum beta 2 microglobulin	2.6 U/L (0.8–2.3)

LYMPH NODE MORPHOLOGY



Histologic examination of a left cervical lymph node shows near-total replacement of the normal architecture by lymphoma. At low power (A; H&E 10x), large, vague, pale nodules are identified, consistent with proliferation centers. In some areas, these proliferation centers appear to be beginning to fuse with one another. On higher-power examination (B; H&E 200x, inset 1000x), there are increased paraimmunoblasts (red arrows) and prolymphocytes; however, there are no sheets of large cells to indicate histologic transformation to diffuse large B cell lymphoma. The Ki-67 proliferation index is increased; there is weak expression of p53 protein in the small lymphoid cells and stronger (aberrant) nuclear staining in the larger neoplastic cells (C and D, respectively, immunohistochemistry with hematoxylin counterstain).

IMMUNOHISTOCHEMICAL CHARACTERIZATION OF NEOPLASTIC LYMPHOID CELLS

Gene	HGVS	VAF (%)
NOTCH1	NM_017617.3(NOTCH1):c.7541_7542del p.P2514fs*4	20
BIRC3	NM_001165.4(BIRC3):c.1654del p.Q552fs*16	4

VAF: variant allele frequency.

Examination of bone marrow showed extensive involvement by a small B cell neoplasm. Aberrant B cells represented 63 percent of all cells by flow cytometry and were positive for CD5, CD23, and CD200.

FLUORESCENCE IN SITU HYBRIDIZATION PERFORMED ON BONE MARROW

Positive for +12 and del(13q) in 41 percent and 2.2 percent of analyzed interphases, respectively. The *TP53* locus was intact.

DNA SEQUENCING

DNA sequencing studies performed on a bone marrow sample showed unmutated *IGHV*.

NEXT-GENERATION SEQUENCING

Next-generation sequencing performed on a bone marrow sample showed mutation involving *NOTCH1* and *BIRC3*. *TP53* was wild-type.

CD20	Positive
CD23	Positive
CD5	Positive
Ki-67	Positive in ~25% of cells
p53	Weakly positive in ~50% of cells
Cyclin D1	Negative

FINAL DIAGNOSIS

Chronic lymphocytic leukemia/small lymphocytic lymphoma with increased large cells and proliferation rate (25 percent) suggestive of accelerated phase.

CASE DISCUSSION

The pathologist's view

Routine cases of chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) often present little diagnostic challenge. The histopathologic features of most CLL/SLL are typical: a proliferation of small, mature B-cells with irregularly condensed chromatin, dim CD20 expression, CD5 co-expression, light chain restriction, and no evidence of t(11;14). A histologic hallmark of CLL/SLL, particularly when it involves lymph nodes, is the formation of proliferation centers (PCs) characterized by nodular expansions of prolymphocytes and paraimmunoblasts admixed with small lymphocytes.

Richter transformation (RT) is often used synonomously with Richter syndrome, a term used to describe a setting in which a patient with CLL/SLL also develops diffuse large B cell lymphoma (DLBCL), most commonly as a result of histologic transformation of the underlying CLL/SLL to DLBCL. RT is also applicable when CLL/SLL transforms to aggressive lymphoid neoplasms such as Hodgkin lymphoma, plasmablastic lymphoma, or B lymphoblastic leukemia/lymphoma. Though histologic transformation occurs in patients with other types of low-grade B cell neoplasms, the term RT is used solely in the context of CLL/SLL (1).

Clinically, RT is suspected when patients with CLL/SLL develop sudden-onset B symptoms and present with rapid lymph node enlargement (often with radiologic evidence of increased metabolic uptake). From the pathologist's perspective, clear-cut CLL/SLL and DLBCL pose little diagnostic challenge – but a grey zone, known as accelerated CLL/SLL or "CLL with expanded proliferation centers," exists between the two. It is histologically described by the presence of proliferation centers (PC) broader than a 20x field (or 0.95 mm2) and a high proliferation rate (Ki-67 >40% or >2.4 mitoses/PC) (2). This phenomenon is histologically distinct from RT, which requires the presence of confluent sheets of large B cells in DLBCL-type transformation or Reed-Sternberg cells in Hodgkin-type transformation. The current case represents an example of CLL/SLL with expanded PCs, as shown by expanded, pale, nodular areas on low-power magnification and increased numbers of paraimmunoblasts within these areas on high-power magnification; however, confluent sheets of large cells are lacking.

Genotypically, a subset of CLL/SLL cases with confluent PCs have been shown to be associated with 17p- and +12 (3). The larger cells in these expanded PCs may express cyclin D1 or overexpress p53 and/or MYC without the associated gene alterations. In this context, cyclin D1 expression does not imply a diagnosis of mantle cell lymphoma (4,5). Nevertheless, performing these in addition to Ki-67 may help highlight the expanded PCs, as is seen in the current case (6). Weak nuclear p53 positivity in a large number of cells in this case is not a manifestation of *TP53* alteration.

It is important to recognize and document accelerated-phase CLL/SLL because of the correlation with more aggressive clinical course. Despite application of criteria for accelerated phase, it is often difficult to decide where a case lies on the pathologic spectrum of CLL/SLL to DLBCL. Given the need to see a broad architectural landscape, tissue sampling is extremely important. Smaller core biopsies may miss areas of increased large cells, high mitotic rate, or even overt DLBCL, making excisional biopsies (when possible) the preferred sample type when RT is a clinical consideration.

The hematologist's view

By Amy Wang and Sonali M. Smith

It is increasingly clear that many hematologic malignancies present along a spectrum reflecting progression from premalignant to malignant to accelerated/aggressive states. This is illustrated by chronic lymphocytic leukemia or small lymphocytic lymphoma (CLL/SLL), where the spectrum begins with a monoclonal B cell lymphocytosis at one end and Richter transformation at the other. Despite attempts

to "draw the line" with strict diagnostic criteria, there will always be grey areas that challenge an algorithmic approach to diagnosis and treatment.

Clinically, accelerated CLL/SLL is difficult to identify or predict because patients with standard versus accelerated CLL/SLL often present similarly with regard to B symptoms, disease bulk, functional status, or clinical stage. Only LDH and ZAP-70 levels are noted to be higher in accelerated CLL/SLL (2). In contrast, patients with Richter transformation are more symptomatic and have elevated LDH, lower performance status, and high uptake on PET scans.

Accelerated CLL/SLL with expanded or highly active proliferation centers behaves more aggressively than standard CLL/SLL and is associated with inferior survival (3). Although there is limited data, one report showed that median survival from the time of lymph node biopsy was 4.3, 34, and 76 months for Richter transformation, accelerated CLL/SLL, and non-accelerated CLL/SLL, respectively (2). However, this dataset precedes the current era of biologic agents' use in CLL/SLL, so the survival discrepancies may not be accurate today.

The relative prognostic impact of cytogenetic and molecular factors in accelerated CLL/SLL is also unclear. This patient has trisomy 12 (a more favorable cytogenetic abnormality) but, more importantly, she does not have a *TP53* mutation or 17p deletion. Sequencing revealed a NOTCH1 clonal mutation, found in about 20 percent of CLL/SLL patients, that predicts shorter overall survival (7); allelic frequency can potentially be tracked over time to determine the patient's response to therapy. *NOTCH1* mutations are associated with more aggressive disease and RT. The subclonal *BIRC3* mutation has no clear prognostic value in her case.

In the era of novel targeted agents and immunotherapy, we have seen major paradigm shifts in CLL/SLL management over the past five years, with chemoimmunotherapy largely replaced by bruton tyrosine kinase (BTK) inhibitors, BCL2 inhibitors, and potent monoclonal antibodies against CD20. Are these agents and regimens equally effective in accelerated CLL? Should accelerated CLL be treated like RT or standard CLL? Given the poor prognosis relative to standard CLL/SLL, should treatment be initiated when patients are asymptomatic? Unfortunately, prospective studies on management of accelerated CLL/SLL are lacking, so it is often treated like standard CLL/SLL by default.

For this patient with a confirmed diagnosis of accelerated CLL/SLL, we recommend treatment initiation given the new adenopathy, elevated LDH, and overall high tumor burden with extensive marrow involvement. However, a period of very close observation is also reasonable, especially because we do not know whether early treatment impacts survival. Our preferred option is a limited-duration regimen of venetoclax plus obinutuzumab, which is associated with an 85 percent overall response rate (8) and 82 percent three-year progression free survival in standard CLL/SLL (7). Reflecting the lack of directly comparative data, continuous use of a BTK inhibitor is also reasonable. There is an ongoing study evaluating the role of a limited-duration triplet regimen (ibrutinib, venetoclax, obinutuzumab, NCT03701282), but it will be several years before results are available. Overall, this patient has a much more aggressive disease than standard CLL/SLL, but it is not RT, and our personal experience is that novel agents offer excellent disease control. Going forward, clearly defined parameters and treatment data for accelerated disease will be essential.

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Please see references online at: tp.txp.to/lym1

LYMPHOMA-DRIVEN HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS

The second case in our series on lymphoid neoplasms

CLINICAL HISTORY

21-year-old man with a two-month history of worsening fatigue and fever. He was transferred to our hospital from an outside institution for acute-onset renal failure and suspected tumor lysis syndrome.

PERTINENT PHYSICAL EXAM

Temperature: 103.2°F; hepatosplenomegaly.

COMPLETE BLOOD COUNT AND DIFFERENTIAL (REFERENCE RANGE)

WBC	1.9 x10^9/L (4.0–11.0)
Hgb	7.5 gm/dL (14.0–18.0)
Hct	22.4% (40.0–54.0)
MCV	79 fL (82–98)
MCH	26.4 pg (27.0–31.0)
Platelets	71 x10^9/L (140–440)
Absolute lymphocytes	0.1 x10^9/L (1.00–4.80)
Absolute neutrophils	1.71 x10^9/L (1.70–7.30)

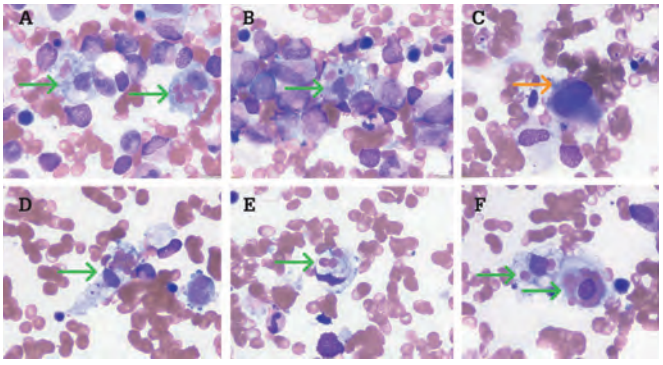
ADDITIONAL LABORATORY RESULTS

ALT	108 U/L (≤41)
AST	93 U/L (≤40)
Ferritin	11,484 ng/mL (30–400)
LDH	292 U/L (135–225)
Creatinine	4.88 mg/dL (0.67–1.17)
Triglycerides	205 mg/dL (≤149)

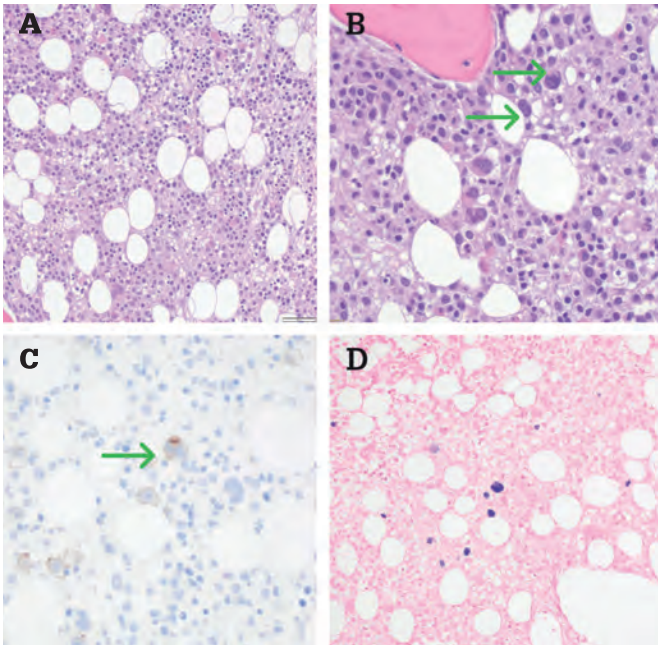
PET-CT

FDG-avid multicompartmental lymphadenopathy above and below the diaphragm. Heterogeneously increased activity within the spleen and several foci of activity within the skeleton.

BONE MARROW MORPHOLOGY

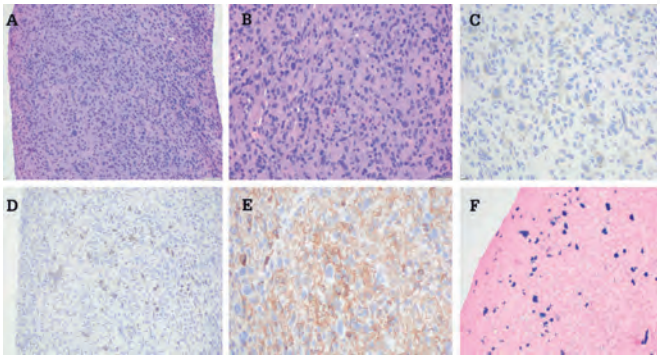


Bone marrow aspirate smears show numerous histiocytes exhibiting hemophagocytosis (green arrows). Megakaryocytes include increased small, hypolobated forms; an example is highlighted by the orange arrow. Granulocytes show left-shifted maturation; erythroid precursors were unremarkable in morphology (A–F; Giemsa 1000x).



The bone marrow core biopsy shows an extensive infiltrate of histiocytes and a few scattered larger cells (green arrows). The differential morphologic consideration for the larger cells includes dysplastic megakaryocytes and possibly Hodgkin cells. Of note, the typical stromal reaction and inflammatory infiltrate usually associated with Hodgkin lymphoma is lacking. (A–B; H&E 100x and 200x). A CD30 immunohistochemical stain is weakly positive in a few plasma cells as well as a large, atypical cell (green arrow) (C; immunohistochemistry with hematoxylin counterstain). In situ hybridization for EBV-encoded RNA highlights a few EBER positive cells (D).

LYMPH NODE BIOPSY



Core biopsy of the pelvic lymph node shows complete replacement of the nodal architecture by a proliferation of large atypical cells (A; H&E 100x) on a background of histiocytes and rare eosinophils (B; H&E 400x). The large cells are weakly positive for CD30 and PAX5 and negative for CD45/LCA (C–E, respectively; immunohistochemistry with hematoxylin counterstain) and positive for EBV-encoded RNA (EBER) by in situ hybridization (F).

FINAL DIAGNOSIS

Acute myeloid leukemia with minimal differentiation.

CASE DISCUSSION

The pathologist's view

A young patient presenting with constitutional symptoms, hepatic and renal failure, and diffuse lymphadenopathies with no overt diagnosis presents a high-stakes situation in which time is of the essence. In this case, the initial specimens received were bone marrow (BM) aspiration and biopsy that showed hypercellularity; scattered large, atypical cells; and a striking number of hemophagocytic histiocytes.

An increase in hemophagocytic histiocytes has several possible causes – most importantly hemophagocytic lymphohistiocytosis (HLH). HLH is a severe, potentially fatal hyperinflammatory syndrome induced by aberrantly activated macrophages and cytotoxic T cells. HLH is divided into primary and secondary forms. The primary form typically manifests in children and is associated with genetic abnormalities affecting T and NK cell regulation. The secondary and far more common form typically occurs in adults, often in the setting of an associated condition, such as malignancy, infection, or autoimmune disorders. Though infections typically trigger the onset of HLH in pediatric patients, malignancy is the most common cause of HLH in adults (Mal-HLH) (1). Among all HLH subtypes, Mal-HLH has the worst prognosis, and its risk increases with age. Lymphoma is the most common underlying malignancy, with Mal-HLH observed in up to 3 percent of patients with lymphoma.

Timely HLH diagnosis is critical, especially because symptoms can be nonspecific. The diagnostic criteria for HLH include several clinical, laboratory, and biopsy findings; increased hemophagocytic activity is only one criterion and not required for diagnosis (2).

In this case, the most time-sensitive task was to alert the clinical team to the presence of marked hemophagocytosis and confirm their clinical impression of HLH. Of equal importance is a meticulous search to identify an associated underlying pathologic disease to guide therapeutic decisions. The first specimen we saw was the bone marrow. A number of scattered large, atypical cells were present, so we performed a battery of immunohistochemical stains that highlighted the presence of large CD30+, EBV+ neoplastic cells suggestive of classic Hodgkin lymphoma. The typical stromal reaction and inflammatory infiltrate usually associated with Hodgkin lymphoma was lacking – possibly a manifestation of an underlying immunocompromised status.

Fortunately, not long after reviewing the BM specimen, a retroperitoneal lymph node biopsy was performed. In this case, the biopsy consisted of scant, poorly preserved tissue. In the better-preserved areas, one could appreciate a proliferation of large, atypical cells associated with histiocytes and few eosinophils. Though CD30 expression was weak (possibly due to poor fixation), the expression of PAX5, lack of CD45/LCA, and positivity for EBER were consistent with EBV+ Hodgkin and Reed-Sternberg cells of classic Hodgkin lymphoma.

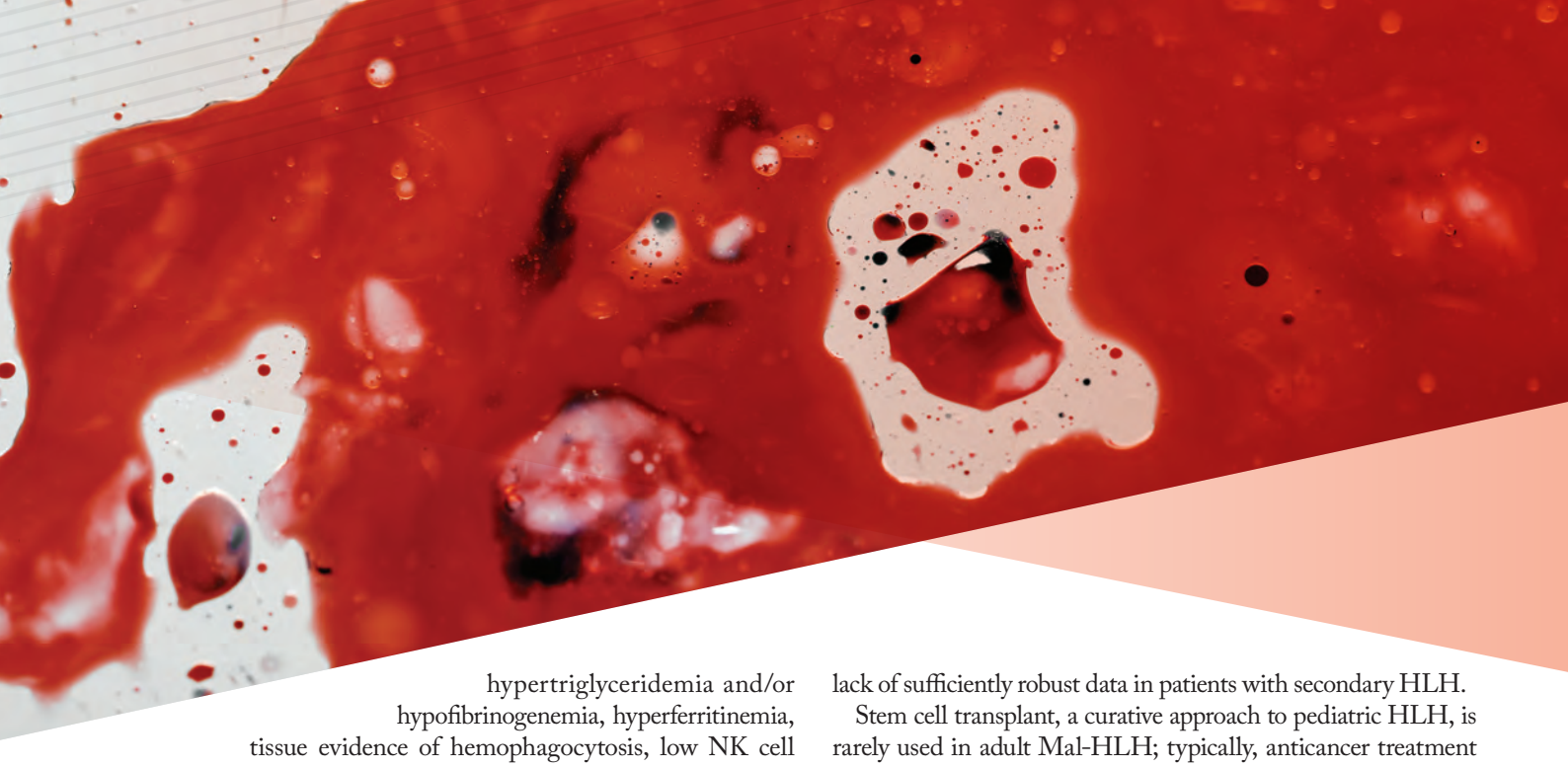
This case highlights the importance of a holistic approach and the need for adequate, well-fixed tissue in cases of malignancy-associated HLH. Classic Hodgkin lymphoma is a diagnosis rendered when architecture and phenotype are assessed together. Limited tissue sampling hampers accurate diagnosis and subclassification. In this case, identifying the underlying malignancy was critical to proper diagnosis and management.

The hematologist's view

By Paolo Strati

My threshold for suspecting HLH is low in patients with lymphoma, particularly those with NK/T cell lymphoma or EBV+ lymphoma, the two subtypes more likely to induce Mal-HLH.

The diagnosis of HLH is currently based on the HLH-2004 criteria (3). Five of eight criteria must be met to confirm HLH and prompt treatment initiation: fever, splenomegaly, cytopenia,



hypertriglyceridemia and/or hypofibrinogenemia, hyperferritinemia, tissue evidence of hemophagocytosis, low NK cell activity, and high soluble IL-2 receptor. However, in the adult population, particularly when Mal-HLH is suspected, these criteria can be quite nonspecific. Thus, I recommend also using the H-score, (4) a web-based tool developed on adult cases, when considering HLH in older patients with underlying malignancy.

Although lymphoma-directed therapy is the preferred strategy in patients with lymphoma-triggered HLH, a definitive histological diagnosis is often not immediately available. In these cases, waiting for a final diagnosis is not recommended; mortality can be quite high during the first days and HLH-directed therapy needs to be quickly initiated.

The regimen currently used is derived from the HLH-1994 trial (5) and includes corticosteroids, cyclosporine, intrathecal chemotherapy, and parenteral etoposide to suppress NK/T cell function and cytokine production. However, its use can be limited in adult patients, for reasons including comorbid health conditions and HLH-induced organ failure. Additionally, etoposide may be needed as part of a lymphoma-directed therapy; as such, its use while awaiting a final diagnosis may hinder effective anti-lymphoma treatment, because excessive etoposide use may result in prolonged and potentially life-threatening myelosuppression. In these cases, it is acceptable to start using corticosteroids only; however, if no improvement is observed within two to three days, consider a subsequent line of therapy. I recommend corticosteroids in combination with tocilizumab (based on IL-6's role in HLH biology), rather than etoposide, for patients with lymphoma-triggered HLH awaiting initiation of anti-lymphoma treatment.

IFN-γ also plays a crucial role in the etiology of HLH. The US Food and Drug Administration (FDA) recently approved emapalumab, an anti-IFN-γ antibody, for the treatment of patients with refractory, recurrent, or progressive disease or intolerance to conventional HLH therapy (6). Albeit a fascinating treatment option for patients in whom etoposide is contraindicated, emapalumab's use is currently limited by extremely high costs and lack of sufficiently robust data in patients with secondary HLH.

Stem cell transplant, a curative approach to pediatric HLH, is rarely used in adult Mal-HLH; typically, anticancer treatment resolves the condition and relapses are very infrequent. However, in young adult patients with HLH induced by EBV+ lymphoma, a genetic basis should be suspected. I recommend genetic counseling and evaluation for stem cell transplant in young patients with EBV-associated lymphoma to avoid risk of future recurrences.

Hodgkin lymphoma (HL) is responsible for about 6 percent of Mal-HLH cases – but exponentially more in the presence of EBV-associated HL. Advanced HL presenting with HLH has a high internal prognostic score (IPS) by definition and, as such, may benefit from aggressive treatment. Unfortunately, these patients typically present with organ dysfunction – including renal and hepatic impairment – making use of the standard HL regimen virtually impossible. In cases with organ dysfunction, I recommend initially treating patients with hyperfractionated cyclophosphamide, which is safe in patients with multi-organ failure and may result in significant lymphoma and HLH control. Once clinical stability is achieved, typically starting with cycle 2, more appropriate regimens can be instituted.

In conclusion, we recommend a low threshold of suspicion for HLH in patients with NK/T cell or EBV-associated lymphomas. If anti-lymphoma treatment cannot be immediately initiated, chemotherapy-free approaches, including corticosteroids and tocilizumab, should be considered. Once a final lymphoma diagnosis is obtained, hyperfractionated cyclophosphamide is a reasonable frontline option, whereas more standard regimens can be initiated with subsequent cycles. A better understanding of HLH biology in the adult population will hopefully spur the development of safer and more effective treatment strategies for these patients.

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Please see references online at: tp.txp.to/lym2

HIGH-GRADE B CELL LYMPHOMA LYMPHOMA WITH LEUKEMIC PRESENTATION

The third case in our series on lymphoid neoplasms

CLINICAL HISTORY

42-year-old man with no prior medical history had emergent neurosurgical decompression of a cervical spinal tumor after acute onset of paraplegia. At presentation, he was noted to have marked leukocytosis and was transferred to our institution for treatment of suspected acute leukemia.

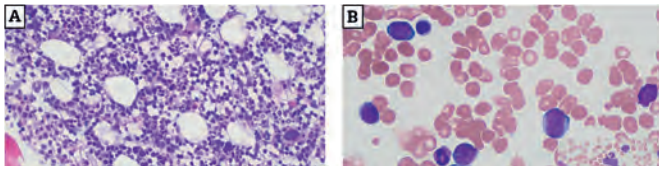
IMAGING

No lymphadenopathy or focal PET+ lesions other than the cervical spine lesion.

COMPLETE BLOOD COUNT AND DIFFERENTIAL (REFERENCE RANGE)

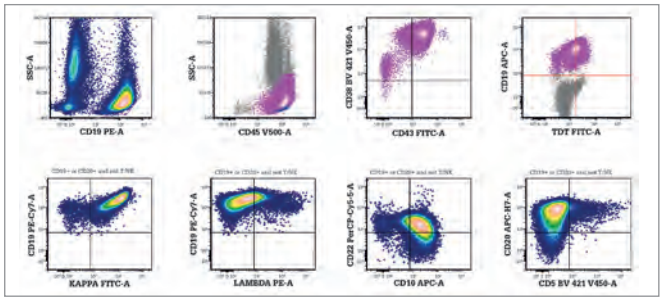
WBC	287.4 x10^9/L (4.0–11.0)
Hgb	7.5 gm/dL (14.0–18.0)
MCV	90 fL (82–98)
Platelets	67 x10^9/L (140–440)
"Blasts"	94.0% (<=0.0)
Lymphocytes	2% (24.0–44.0)
Neutrophils	3.0% (42.0–66.0)
Metamyelocytes	1.0% (<=0.0)

PERIPHERAL BLOOD AND BONE MARROW MORPHOLOGY



Bone marrow trephine biopsy shows extensive replacement of normal hematopoietic elements by an infiltrate of immature-appearing mononuclear cells with an interstitial pattern of involvement. Apoptotic cells are readily identifiable (A; H&E 400x). The bone marrow aspirate smear shows numerous large, atypical mononuclear cells with scant basophilic cytoplasm, finely dispersed nuclear chromatin, and prominent nucleoli (B; Giemsa 1000x). Examination of the peripheral blood smear shows numerous circulating cells similar to those observed in the bone marrow aspirate smear (B, inset; Giemsa 1000x). Images courtesy of Chi Young Ok, MD Anderson Cancer Center.

FLOW CYTOMETRY

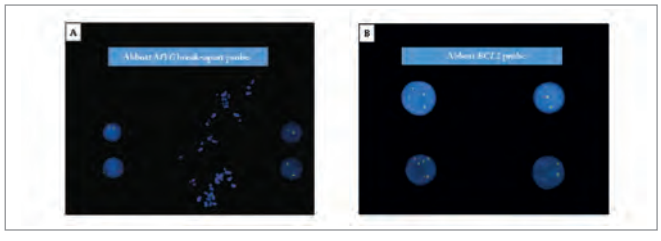


Flow cytometric immunophenotyping of peripheral blood samples shows an expanded population of CD19+ cells. Backgating the CD19+ population (pink gate) shows the CD19+ events with bright CD45 expression. The CD19+ cells show bright CD38 and dim TdT expression (top panel). The population of interest shows monotypic expression of surface kappa light chain, bright expression of CD20, dim expression of CD22, and is positive for CD10 and negative for CD5, supporting germinal center B cell derivation (bottom panel).

IMMUNOHISTOCHEMICAL CHARACTERIZATION OF NEOPLASTIC LYMPHOID CELLS

CD20	Positive
BCL2	Positive
MYC	Positive for strong overexpression (3+ staining in >90% of cells)
p53	Aberrantly overexpressed
TdT	Negative

FLUORESCENCE IN SITU HYBRIDIZATION PERFORMED ON BONE MARROW



Fluorescence in situ hybridization performed on the bone marrow aspirate sample showed rearrangement of *MYC* and *BCL2* in 83 and 90 percent of analyzed interphases, respectively. The *MYC* probe (A) hybridizes to band 8q24.2 (5′ *MYC* is centromeric and labeled in spectrum orange; 3′ *MYC* is telomeric and labeled in spectrum green). The *BCL2* probe (B) hybridizes to band 18q21 (3′ *BCL2* is centromeric and labeled in spectrum green; 5′ *BCL2* is telomeric and labeled in spectrum orange).

Cytologic and flow cytometric examination of cerebrospinal fluid showed involvement by B cell neoplasm.

FINAL DIAGNOSIS

High-grade B cell lymphoma with *MYC* and *BCL2* rearrangements with leukemic presentation and central nervous system involvement.

CASE DISCUSSION

The pathologist's view

B cell neoplasms with blastoid morphologic features are further subclassified into B lymphoblastic leukemia/lymphoma, high-grade B cell lymphoma, NOS, and double-hit B cell lymphoma, according to the 2016 WHO classification (1). Using the World Health Organization (WHO)-recommended diagnostic algorithm, the distinction between B lymphoblastic leukemia/lymphoma and the latter two entities is made by assessing the presence or absence of terminal deoxynucleotidyl transferase (TdT) expression.

This patient presents with primarily leukemic, non-nodal disease with numerous circulating blastoid neoplastic cells. The major differential diagnosis in this case is de novo B lymphoblastic leukemia/lymphoma. This morphologic conundrum is not new; before the age of routine phenotyping confirmed that circulating Burkitt lymphoma cells are mature B cells, they were considered "L3 blasts." For the pathologist, differentiating mature from immature lymphoid cells is a significant first step in the diagnosis, for which ancillary studies are essential. In this case, initial flow cytometry (FCM) immunophenotypic studies demonstrated markers that support a mature lymphoma cell phenotype: bright CD45 and presence of uniformly bright surface CD20 expression with surface light chain restriction are features not typical of B-ALL. However, FCM also showed dim TdT expression. Given FCM support for a more mature immunophenotype, we performed additional FISH studies that confirmed the presence of concurrent *MYC* and *BCL2* rearrangements and established a diagnosis of high-grade B cell lymphoma with *MYC* and *BCL2* rearrangements (previously "double-hit" lymphoma). Notably, these ancillary FISH studies are not routinely recommended in patients with de novo B-ALL and therefore classification as B lymphoblastic leukemia/lymphoma, NOS based on TdT expression may have led to omission of these studies from the diagnostic workup. In the example presented here, the discrepancy between TdT expression as detected by FCM versus immunohistochemistry is likely attributable to the higher sensitivity of FCM.

There is little guidance on whether TdT expression trumps maturity markers for classification of B cell neoplasms; however, TdT expression has been reported in de novo high-grade B cell lymphomas with *MYC*, *BCL2*, and/or *BCL6* rearrangements, in "transformed" aggressive B cell lymphomas in patients with known follicular lymphoma, and in other mature B cell lymphomas that acquired TdT expression at relapse (2,3). Awareness of this phenomenon and appropriate disease classification is clinically relevant because it has direct prognostic and management implications (2).

The hematologist's view

By Hua-Jay Cherng and Jason Westin

The management of patients with high-grade B cell lymphomas with translocations involving *MYC* and *BCL2* or *BCL6*, colloquially referred to as "double-hit lymphomas" (DHL), is doubly challenging because of their aggressive clinical presentation and poor long-term prognosis after standard immunochemotherapy.

This case is particularly unique because of the one-two punch of a leukemic presentation and central nervous system (CNS) involvement at time of diagnosis. Though bone marrow involvement is relatively common in DHL, leukemic phase presentation with circulating lymphoma in the peripheral blood occurs in only 12 percent of patients and is associated with inferior overall survival (4). Double-hit status and leukemic presentation are likely both associated with risk of secondary CNS involvement (5), found in approximately 10 percent of DHL at diagnosis and also associated with inferior overall survival (4).

How should this critically ill patient be managed in the acute setting? They should be admitted to an intensive care unit

for close monitoring. Risk for spontaneous tumor lysis syndrome is high; telemetry to watch for arrythmias, allopurinol, and brisk continuous intravenous fluids as tolerated are important baseline interventions. Rasburicase is likely needed to rapidly lower uric acid levels. Renal replacement therapy may also be required to support the patient through the immediate crisis. Symptomatic leukostasis is typically associated with acute leukemias secondary to large circulating leukemic blasts plugging microvasculature. It is unclear whether this phenomenon can happen with circulating lymphoma cells but, with such significant hyperleukocytosis, we would consider leukapheresis, particularly if the patient has respiratory, cardiac, or nervous system symptoms. Prephase intravenous corticosteroids can be used for cytoreduction while finalizing a definitive treatment plan.

After stabilizing this patient, which systemic therapy regimen should be used? Retrospective (4,6,7) and single-arm prospective (8) studies suggest the superiority of intensive immunochemotherapy regimens such as dose-adjusted rituximab, etoposide, prednisone, vincristine, and cyclophosphamide (DA-R-EPOCH) over standard therapy (R-CHOP). DA-R-EPOCH is an appropriate choice for most DH-L (6), but does not contain agents that adequately penetrate the CNS. Although CNS prophylaxis may include the addition of intrathecal (IT) or high-dose systemic methotrexate depending on the treating institution (9), this patient presented with CNS involvement at diagnosis and requires a strategy to rapidly clear disease in his bone marrow and cerebrospinal fluid (CSF).

The combination of rituximab, fractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone alternating with intravenous methotrexate and cytarabine (R-hyperCVAD) is commonly used in the treatment of B cell lymphoid neoplasms that carry a high risk of CNS involvement or relapse (10). This intensive regimen can be difficult for older patients to tolerate, but comes with the benefit of adequate CNS penetration by the inclusion of systemic methotrexate and cytarabine on "even" cycles. If this patient has adequate performance status, we recommend eight cycles of R-hyperCVAD, adding IT chemotherapy with each cycle. For a less fit patient, six cycles of DA-R-EPOCH with IT chemotherapy and mid-cycle systemic methotrexate (11) would be another effective choice.

How should this patient be monitored on therapy? Response assessment during frontline immunochemotherapy in DLBCL is done with a PET/CT scan at baseline, at an interim timepoint during treatment, and at end of treatment to assess for reduction in systemic disease (12). This alone would not be adequate for this patient because he has disease sites not easily detectable by PET/CT. He should have magnetic resonance imaging of the CNS and flow cytometric and cytopathologic assessment of CSF with each dose of IT chemotherapy. He should also undergo bone marrow assessment during and at the end of treatment to ensure marrow involvement has cleared. Flow cytometry on peripheral blood can also be used for disease monitoring. The goal of therapy would be to achieve a complete response (CR) on imaging studies, in the CSF, and in the bone marrow at end of treatment.

Should this patient receive consolidative therapy if he is able to achieve a CR? Generally, patients with DHL who achieve a first CR after intensive immunochemotherapy have good outcomes and do not benefit from consolidative high dose chemotherapy (HDC) and autologous stem cell transplant (ASCT). (13). However, this patient has secondary CNS lymphoma. Outcomes of patients with systemic lymphoma who suffer primary CNS lymphoma (14) or CNS relapse (15) and are consolidated with HDC and ASCT after induction therapy are encouraging. Notably, these studies and others favor the inclusion of thiotepa in the ASCT conditioning regimen for better CNS penetrance. We recommend this patient receive an ASCT with thiotepa-based conditioning in his first CR to maximize chances of long-term remission.

This patient faces numerous hurdles in the short- and long-term that could easily prevent him from achieving a cure. Treatment-refractory or relapsed disease would mean a dismal prognosis. Additional treatment strategies and novel agents are needed for DHL management, and we eagerly await the results of clinical trials in progress.

Hua-Jay Cherng is a hematology-oncology fellow in the Division of Cancer Medicine, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA

Jason Westin is Director of Lymphoma Clinical Research, Section Chief of Aggressive Lymphoma, and Associate Professor in the Department of Lymphoma and Myeloma, Division of Cancer Medicine, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA

Please see references online at: tp.txp.to/lym3

FOLLICULAR LYMPHOMA

The final case in our series on lymphoid neoplasms

CLINICAL HISTORY

73-year-old with a history of follicular lymphoma, presenting with palpable inguinal lymphadenopathy.

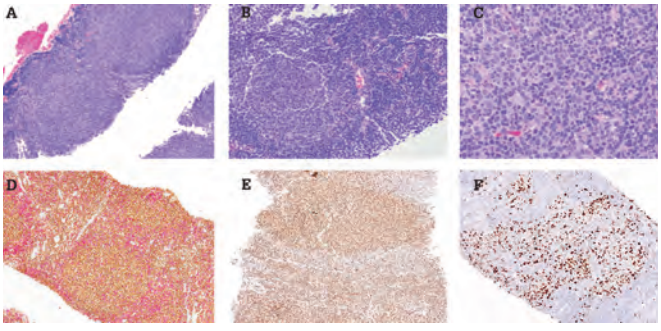
COMPLETE BLOOD COUNT AND DIFFERENTIAL (REFERENCE RANGE)

CD10	Positive
PAX5	Positive
BCL6	Positive
Ki-67	Positive in ~40% of cells

SERUM LACTATE DEHYDROGENASE

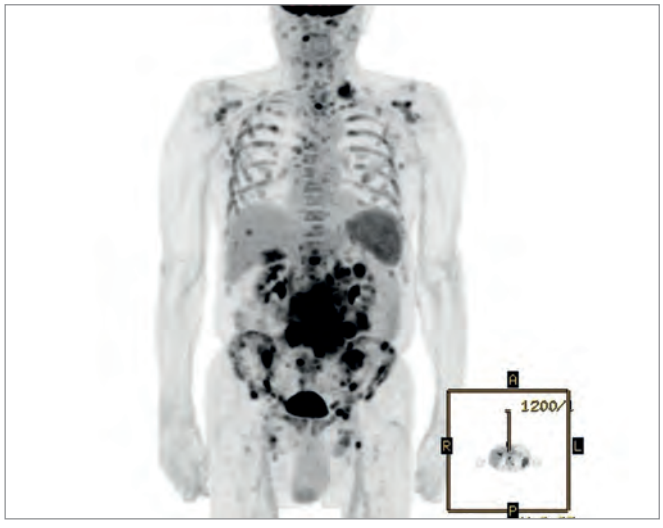
272 (135–214 U/L)

LYMPH NODE MORPHOLOGY



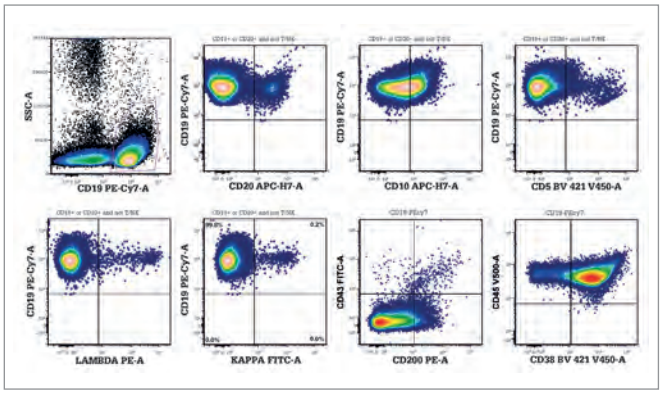
Core biopsy of an abdominal mass shows fragments of lymphoid tissue involved by lymphoma. The neoplasm has a follicular/nodular pattern of growth (A and B; H&E 40x and 200x, respectively). Cytologically, the neoplastic follicles are composed predominantly of centrocytes with few admixed centroblasts in the range of histologic grade 2 (C; H&E 400x). Dual-stain immunohistochemistry shows that the lymphoma cells are positive for PAX5 (brown); residual small, admixed CD5+ T cells are seen in the interfollicular regions (red) (C). The neoplastic cells are positive for LMO2, supporting germinal center derivation (D). The Ki-67 proliferation index is ~40 percent (E). Photomicrographs courtesy of Francisco Vega-Vasquez, MD Anderson Cancer Center.

PET/CT



A PET/CT study showed hypermetabolic adenopathy above and below the diaphragm and widespread osseous lesions.

FLOW CYTOMETRY



Flow cytometric immunophenotyping of a concurrent fine needle aspirate sample showed an expanded population of CD19+ B cells that were positive for CD10 (dim), predominantly negative for CD20, negative for CD5, and lacked surface light chain expression. These cells lacked expression of CD43 and CD200 and showed moderate/dim expression of CD38 and bright expression of CD45.

LYMPH NODE MORPHOLOGY

WBC	3.9 x10^9/L (4.0–11.0)
Hgb	12.1 gm/dL (14.0–18.0)
MCV	96 fL (82–98)
Platelets	114 x10^9/L (140–440)

FINAL DIAGNOSIS

Follicular lymphoma, grade 2, with high Ki-67 proliferation index.

CASE DISCUSSION

The pathologist's view

Follicular lymphoma (FL) is a mature B cell lymphoma of germinal center cell origin, usually easy to diagnose with adequate tissue and typical morphology, phenotype, and cytogenetic findings. Common FL presents with a nodular expansion of malignant lymphoid follicles. Underlying the clonal expansion is constitutive overexpression of the anti-apoptotic protein BCL2, most often as a result of the presence of t(14;18)*/IGH-BCL2*. The resultant monoclonal B cells typically show CD10/BCL6 expression, aberrant expression of BCL2 by immunohistochemistry, and surface immunoglobulin light chain restriction by flow cytometry.

An important prognostic indicator of FL is grading of malignant follicles wherein the number of centroblasts per high power field (hpf) distinguishes low-grade FL (grade 1–2, <15 centroblasts/hpf) from high-grade FL (grade 3, >15 centroblasts/hpf). The WHO classification scheme for lymphoid neoplasms recommends evaluating 10 follicles and calculating average centroblasts/hpf (1). In reality, most tissue biopsies we evaluate in our practice are small core needle biopsies; we rarely have 10 neoplastic follicles available for evaluation. Nevertheless, distinction between low- and high-grade FL is often critical for treatment decisions because grade 3B FL is biologically and clinically distinct from other forms and more akin to diffuse large B cell lymphoma (2,3).

In this patient with a previous history of follicular lymphoma, with elevated serum LDH and marked fatigue, tissue biopsy was pursued to evaluate for possible transformed disease. However, morphologically the neoplasm is still in the low-grade spectrum (grade 2), with a predominance of small centrocytes and occasional admixed centroblasts. Of note, the Ki-67 proliferation index is higher than expected for typical low-grade FL.

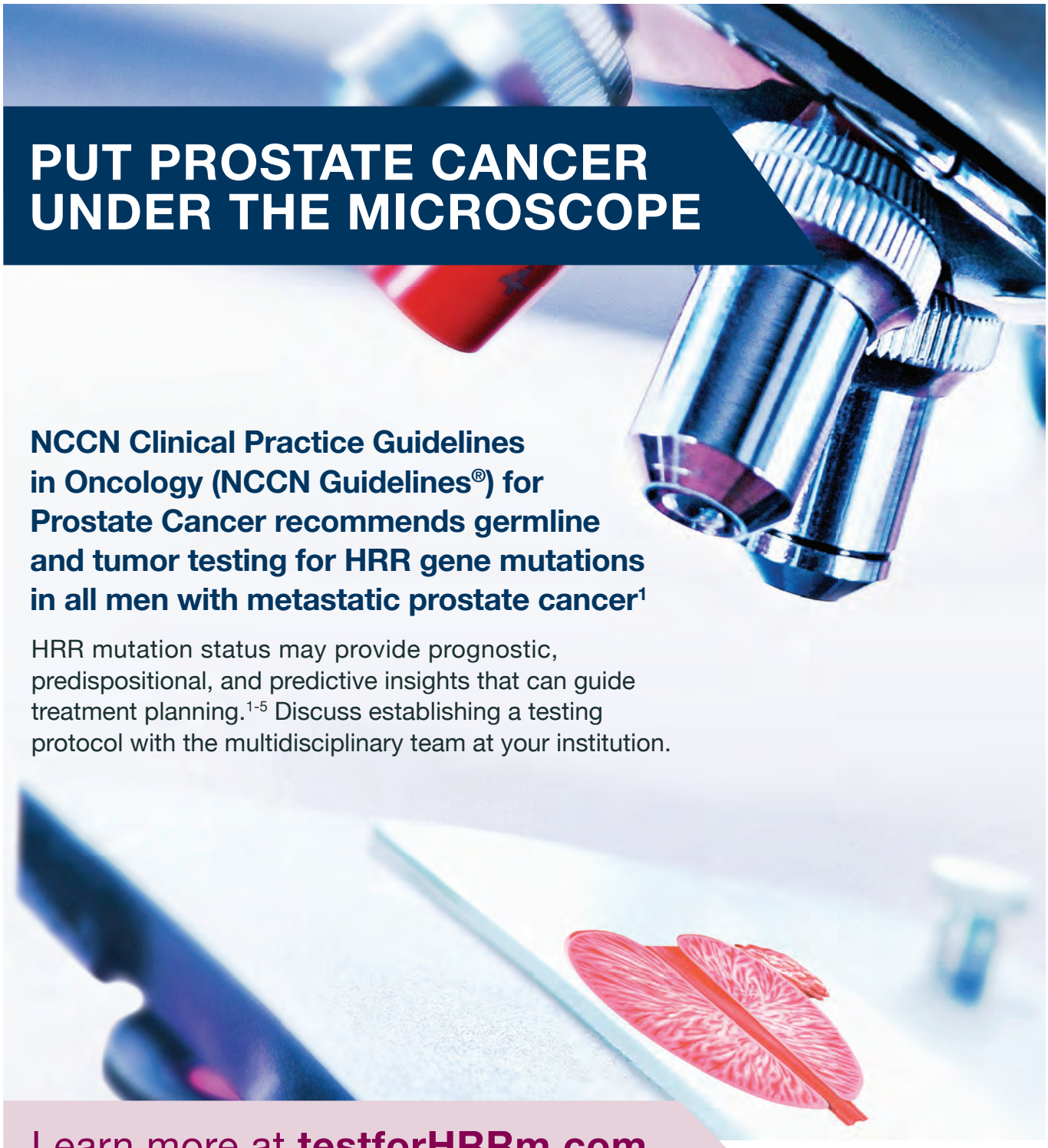
Along with morphologic grading, the Ki-67 proliferation index (PI) serves as an additional prognostic biomarker (4). In most cases, histologic grade and Ki-67 index are concordant; however, in a subset of cases, there is morphologic evidence of low-grade FL, but the neoplastic follicles demonstrate a high Ki-67 (>30%). Low-grade FL with high PI appears to be a subgroup of FL with clinical behavior more akin to grade 3 FL – and some investigators believe these neoplasms should be treated as such (5). Although Ki-67 PI evaluation is not formally required by the WHO, they advocate for its inclusion in the FL diagnostic workup. We believe there is sufficient evidence available to support specific inclusion of routine Ki-67 assessment in the pathology report.

The hematologist's view

By Loretta J. Nastoupil

Management of follicular lymphoma (FL) has evolved rapidly in the past few years, with many more treatment options available, but a lack of predictive biomarkers to inform selection. Navigating this expanding landscape without a map can be challenging.

This 73-year-old man presents with palpable lymphadenopathy. His history dates to 2002, when he was first diagnosed with low-grade follicular lymphoma with lymphadenopathy above and below the diaphragm and bone



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HRR, homologous recombination repair; NCCN, National Comprehensive Cancer Network.

References: 1. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Prostate Cancer V.2.2020. © National Comprehensive Cancer Network, Inc. 2020. All rights reserved. Accessed August 26, 2020. To view the most recent and complete version of the guideline, go online to NCCN.org. NCCN makes no warranties of any kind whatsoever regarding their content, use or application and disclaims any responsibility for their application or use in any way. **2.** Friedlander TW, et al. *American Society of Clinical Oncology Educational Book*. Vol. 37;2017:358-369. **3.** Pritchard CC, et al. *N Engl J Med*. 2016;375(5):443-453. **4.** Abida W, et al. *JCO Precis Oncol*. 2017. doi:10.1200/PO.17.00029. **5.** Na R, et al. *Eur Urol*. 2017;71(5):740-747.

Precision Medicine & Nurse Education

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marrow involvement indicating stage IV disease. Serum lactate dehydrogenase (LDH) was elevated; hemoglobin and beta-2 microglobulin were within normal limits. He had some poor prognostic features: a high-risk follicular lymphoma international prognostic index (6) and high tumor burden (7). He was treated with six cycles of chemoimmunotherapy (rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone), achieving a complete response lasting six years. With his first relapse, he received rituximab monotherapy and achieved complete remission lasting several years. In 2014, he relapsed once again with bulky disease and was treated with R-bendamustine. He achieved remission, but with persistent, mild cytopenias. Now, he has significant fatigue, but still excellent performance status. He has no fever or significant weight loss. Labs are notable for elevated LDH and mild leukopenia and thrombocytopenia. Biopsy showed recurrent follicular lymphoma, grade 2. How do I approach a fit 73-year-old with relapsed FL who has had meaningful responses to three prior lines of therapy?

My first approach is to risk-stratify the patient. Based on observational data, he should anticipate a normal life expectancy despite his FL as a result of durable remission (>24 months) following frontline chemoimmunotherapy (8). Therefore, toxicity and impact on quality of life should factor into the treatment decision. Historically, outcomes generally decline dramatically after two prior lines of therapy (9), so efficacy is also important. Should we repeat any of the prior successful treatments, such as bendamustine, in combination with a CD20 antibody? The GADOLIN study reported favorable outcomes with obinutuzumab – a type II, fully humanized anti-CD20 antibody – in combination with bendamustine (10). The potential concern would be the risk for worsening cytopenias, given the underlying mild cytopenias already present.

Lenalidomide, an oral immune modulator, is approved in combination with rituximab for relapsed FL based on the AUGMENT study, which demonstrated a significant improvement in PFS over rituximab monotherapy (11). How confident am I that a non-chemotherapy approach will adequately address the disease burden in this case? High-tumor-burden patients were just as likely to respond to lenalidomide as those with low tumor burden. The potential advantage to this approach is a fixed duration of therapy. The favorable efficacy and manageable toxicity profile make lenalidomide and rituximab an attractive option for the patient's treatment strategy.

Three phosphatidylinositol-3-kinase (PI3K) inhibitors are also available for relapsed FL. They differ in their selectivity against the four isoforms and potentially, as a result, in their toxicity profiles. Idelalisib is an oral PI3Kd inhibitor approved based on a single-arm, phase II study with an objective response rate (ORR) of 57 percent and median PFS of 11 months in patients with refractory disease (12). Copanlisib is an intravenous pan-PI3K inhibitor with most of its clinical activity against the a and d isoforms. The ORR in a slightly less refractory patient population was 60.6 percent, with a median PFS of 14.1 months (13). Duvelisib, an oral g-d PI3K inhibitor had an ORR of 47.3 percent and median PFS of 9.5 months (14). The toxicity profiles of all three, though manageable, can lead to intolerance over time and can be reserved for higher-risk situations. Nonetheless, the efficacy of PI3K inhibitors warrants consideration and may be more attractive in earlier lines of therapy if toxicity can be mitigated with intermittent dosing.

Tazemetostat, an oral EZH2 inhibitor, is approved for relapsed FL patients with an *EZH2* mutation (approximately 10-20 percent) (15) after two prior lines of therapy, or for those without an acceptable standard of care in which the mutation is not present or unknown. The ORR of tazemetostat was 69 percent in the *EZH2* mutant cohort and 35 percent in the wild-type cohort (16). Interestingly, the median PFS was 13.8 months in the *EZH2* mutant cohort and 11.1 months in the wild-type cohort. With its efficacy and favorable toxicity profile, tamezostat is a reasonable consideration even in the absence of the *EZH2* mutation.

Additional therapies are on the horizon, including mosunetuzumab (a bispecific antibody targeting tumor antigens and T cell engagement) (17) and chimeric antigen receptor (CAR) T cell therapies (18,19). As more therapies emerge, so too will questions as to the most effective sequencing of therapy – but, either way, new developments will provide additional options for patients.

What would I advise for this patient? At this point, I would pursue lenalidomide and rituximab based on the fixed duration of treatment, efficacy, and manageable safety profile. I would also explore *EZH2* mutation status to inform my next treatment approach, recognizing that the number of therapies will likely be even more expansive by that time.

Loretta J. Nastoupil is Associate Professor, Director of the Lymphoma Outcomes Database, and Section Chief of New Drug Development in the Department of Lymphoma and Myeloma, Division of Cancer Medicine, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA.

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An interview with Alain Mita

Tell us about your background in precision oncology…

I am a medical oncologist who treats mainly lung, head, neck, and thyroid cancers. For lung and thyroid cancers, molecular tumor profiling is becoming increasingly important. In fact, at this point, there's no way you can treat a lung cancer patient without a molecular profile – and you need it as early as possible to help make treatment decisions.

I'm also Co-Director of the Experimental Therapeutics Program. Many new drugs in the pipeline are molecularly targeted based on genetic and molecular findings in tumors and blood. And, finally, I also co-chair our molecular tumor board, in which we discuss prospective cases with molecular pathologists and other specialists to help us determine the right treatment approach for patients with complex genetic findings. So I clearly have a vested interested in precision oncology from many angles!

How is precision oncology conducted at your institution – and what inspired that approach?

For many years, medical oncologists have understood that we're moving away from purely histology-based treatment toward molecular-based treatment. In other words, the tissue or organ origin of the cancer is no longer as relevant as its molecular profile. We have seen that different types of cancer can share the same driver mutations – for instance, *BRAF* mutations are found not only in melanoma, but also in lung and thyroid cancer. We know that a tumor's molecular profile and driver mutations are critical, so we think that treating patients based on these data is the way to go. We try to characterize as many of our patients from a molecular standpoint as possible and make treatment decisions based on the results.

We also have a molecular tumor board at Cedars-Sinai because not all medical oncologists are familiar with cancer genetics – and some cases are complicated even for those who are. The molecular tumor board is the perfect venue to discuss complex cases and make treatment decisions.

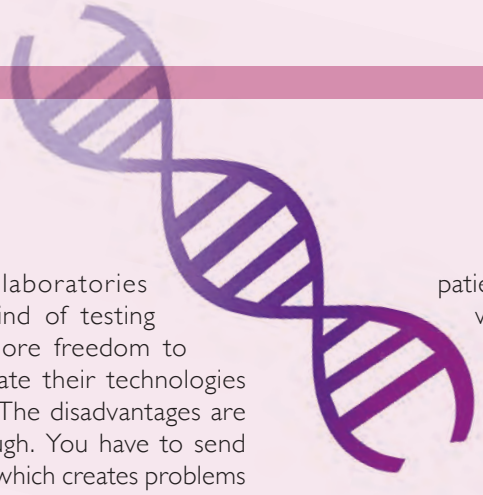
Finally, we're conducting an investigative trial here in which we prospectively analyze the outcomes of patients who are treated based on their molecular profiles versus those who are treated without that information. Similar studies have been conducted retrospectively – ours, which should yield results in about a year, is the first to take a prospective view.

"Our relationship with our colleagues in the laboratory is invaluable; in-house testing allows us to develop it to the best possible advantage – for us and for our patients."

What's your opinion on in-house versus centralized testing?

For many years, our molecular testing was exclusively in-house. Now, we have both in-house and outsourced testing – a mixed model.

I think there are pros and cons to each method. For in-house testing, the pros are shorter turnaround times and the availability of local molecular pathologists to discuss complex cases with oncologists and drive a more personalized approach. The disadvantage is that it's more expensive; the technology evolves very quickly and you have to make sure that your institution stays up-to-date. When we began, we used a 50-gene panel – but, soon after we implemented it, we moved to a 150-gene panel. Now, we even use a 500-gene panel in some cases! It's not always easy for institutions to keep up and the cost can be prohibitive.



Centralized laboratories who do this kind of testing in bulk have more freedom to continually update their technologies and databases. The disadvantages are numerous, though. You have to send your tissue out, which creates problems – did you send out the right tissue? the right amount? high enough quality to yield reliable results? The timelines also present a challenge; it takes about two weeks to get a result back from a central laboratory, whereas in-house testing can return results within days. And it lacks a personal touch; you get the reports, but not the direct contact with your laboratory colleagues.

So what are the biggest benefits of in-house genetic profiling?

Turnaround time is one of the big advantages. I recently had an elderly patient with lung cancer who was not a candidate for chemotherapy, so we needed to decide between immunotherapy and targeted therapy. We didn't want to make the wrong decision, because the sequence of treatment matters; the risk of side effects from targeted therapy is much higher after immunotherapy. The decision had to be made quickly, so we did an in-house panel and chose a treatment right away. I don't know what would have happened if we had waited three weeks for results from a central lab.

"At this point, there's no way you can treat a lung cancer patient without a molecular profile – and you need it as early as possible to help make treatment decisions."

Sometimes, samples are even lost in transit – and, when that happens, the consequences for patients are very serious. Because the information is so vital, we usually repeat the biopsy if the original sample is lost. That is neither pleasant for patients nor devoid of risks, so the less often we send samples to central laboratories, the better.

I also think in-house testing is better for tissue preservation. Pathologists know exactly what they can do with a given amount of tissue and how much sample is needed for each test. In-house, we can perform bespoke testing, rather than simply sending the tissue to a central lab for pre-selected genetic tests. The molecular pathologists also help us decide between (or combine) treatments when tests reveal multiple actionable targets.

Should everyone be testing with large panels – is bigger always better?

This is a tough question. Doctors need quick answers, which is why sequential testing isn't a good option. In an ideal world, the more genes you test, the better. The problem is that, when you test a large number of genes, most are not relevant or actionable. It can be valuable to know that a patient has a specific mutation – but, if there isn't a treatment to target it, the additional knowledge doesn't translate directly into better treatments or outcomes.

Sometimes, having a huge amount of information that you can't really apply becomes counterproductive – and that's where the balance becomes difficult. I don't think there's one right answer, but "bigger" is not necessarily "better." Information curation is a critical part of testing, too. Whether you have a little information or a lot, the key lies in asking the right questions – and that's where teamwork between molecular pathologists and oncologists can help, especially if the testing is conducted in-house.

How do you work with your colleagues in pathology?

At our institution, molecular data is an integral part of the electronic medical record (unlike results received from central labs). That helps us not only with patient care, but also with clinical research – if we can link the tests to treatments and outcomes, we can learn more about how (and why) our treatments work.

We also have strong communication between pathologists and clinicians – personalized according to our preferences. Some receive phone calls, some emails, and some, like me, prefer texts. That way, we get results conveniently and in real time, allowing us to act fast. Our relationship with our colleagues in the laboratory is invaluable; in-house testing allows us to develop it to the best possible advantage – for us and for our patients.

Alain Mita is a medical oncologist and Co-Director of the Experimental Therapeutics Program at the Samuel Oschin Comprehensive Cancer Institute, Cedars-Sinai, Los Angeles, California, USA.

the Pathologist

THE INNOVATORS

SHOWCASING THE PRODUCTS AND COMPANIES MAKING A DIFFERENCE IN 2020

GETTING MORE DATA OUT OF THE DAY WITH THE ZULU RT®

Accelerating real-time PCR results with accuracy and sensitivity

The Zulu RT® is a rapid, modular qPCR instrument capable of performing up to four six-channel, 40-cycle qPCR reactions in less than 20 minutes. The modular design is a defining feature of the tool – it enables four independent qPCR analyzers in one chassis to perform these independent reactions simultaneously. The fast processing time of the Zulu RT is achieved through its unique thermal design, with rapid ramp rates for heating (15˚C per second) and cooling (12˚C per second), thin-walled PCR tubes, and precise temperature control. The innovative tube design allows rapid heat transfer to the entire PCR reaction. This reduces hold times at the set temperatures while assuring complete PCR.

The Zulu RT was designed by researchers, for researchers, to overcome the limitations of available instruments – with a key motivator of enabling laboratory professionals to perform multiple rapid PCR reactions without sacrificing accuracy or sensitivity. By allowing several users to work simultaneously and perform four reactions at once, the Zulu RT significantly increases the efficiency of the laboratory workflow.

But it's not just pathologists who will benefit from this innovation – its rapid turnaround times will allow assay developers and researchers to quickly determine the optimal reaction conditions for their tests and publish their results faster. Developers will be able to bring their tests to market sooner, which will help the ultimate end user – the patient.

With the Zulu RT, Streck believes it has developed a unique solution that will redefine the PCR testing landscape and address key challenges faced by labs around the world. By enabling labs to get more data out of their day through a streamlined workflow, tasks from assay development to processing sample backlogs will no longer be limited by daily run restrictions.

MAGNUS: ACHIEVING AUTOMATION THROUGH ONE-STEP TISSUE PROCESSING AND AUTO-EMBEDDING

Milestone's technology offers a groundbreaking solution to increase productivity, efficiency, and patient safety

Milestone's game-changing innovation, MAGNUS, is a rapid, automatic tissue processor. The goal? Enhanced throughput, which it achieves with dual robotic arms that simultaneously perform two crucial steps in biospecimen histological preparation: tissue processing and embedding. Empowered by Milestone's Synergy, an auto-embedding rack system with dedicated molds and pads, batches of small biopsies can be processed and embedded in as little as 90 minutes. MAGNUS automatically detects when a Synergy rack is loaded into one of the two retorts and selects the auto-embedding processing programs. As the cassette rack automatically moves to the wax retort, a new rack can be loaded in the first retort and a new program started. With continuous, rapid processing cycles, MAGNUS works within lean laboratory principles.

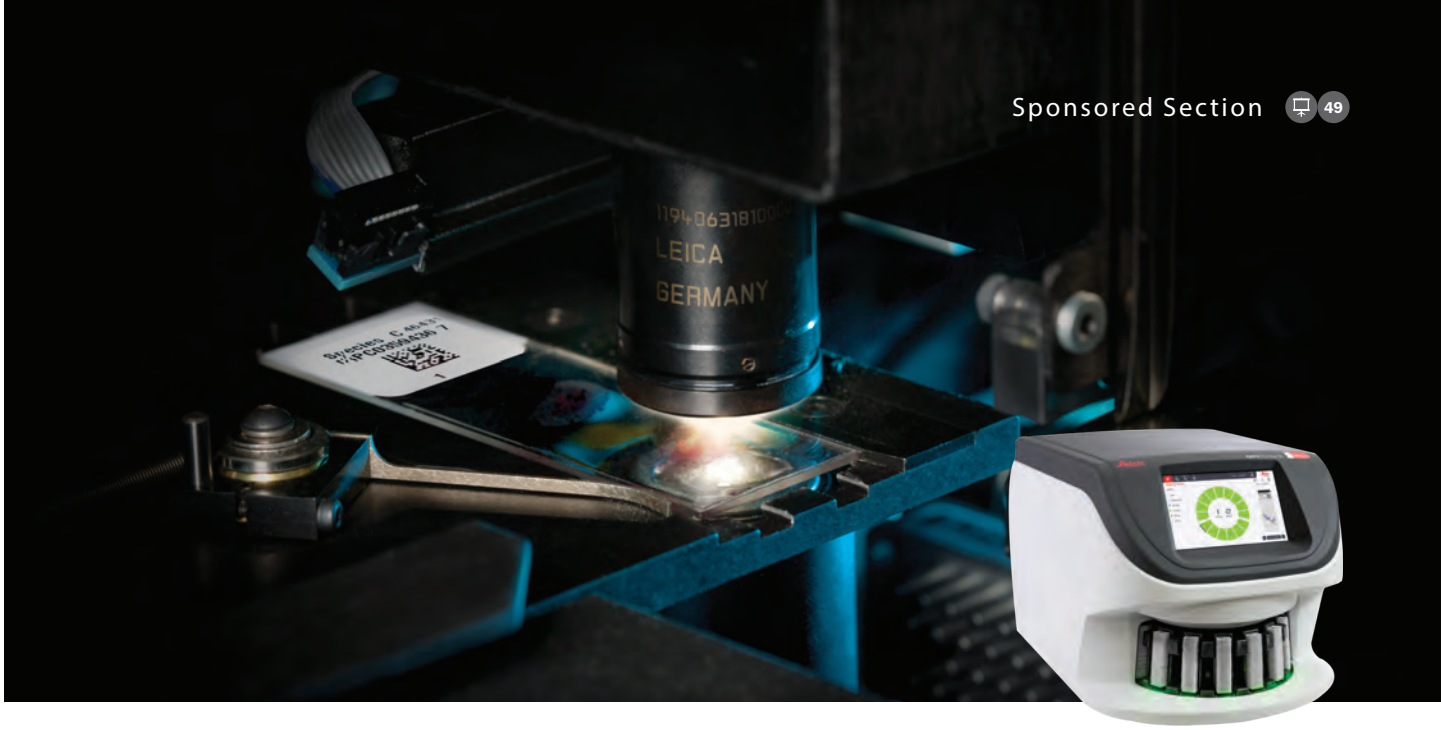
MAGNUS' built-in auto-embedding system reduces the potential for errors during manual embedding, which eliminates the need for time-consuming re-embedding, facilitating a more streamlined workflow that benefits everyone from pathologist to patient.

The MAGNUS tissue processor is not only an effective solution for increasing productivity, efficiency, and patient safety – it also dramatically reduces costs by reducing reagent consumption and avoiding the need for cleaning cycles. MAGNUS is lean and safe by design; processing does not require paraffin transfers, saving time and potential blockages, and xylene-free operation allows long paraffin reuse. Additionally, its integral reagent sensors check alcohol purity before every step.

By combining MAGNUS with Milestone's auto-embedding method, Synergy, they fulfill the need to optimize the laboratory workflow, offering flexibility, speed, and high throughput – crucially, without compromising on quality or safety.

This is true automation – simple, immediate, and stress-free.

www.milestonemed.com



REAL-TIME FOCUSING: A LEICA BIOSYSTEMS BREAKTHROUGH

The solution to scaling up pathology operations – no matter the laboratory size

Over the last several years, digital pathology engineers at Leica Biosystems have tried many technologies to help achieve high-speed line scanning at 40x* magnification – with some proving more promising than others. But when they took a novel approach to real-time focusing (RTF), they hit upon a breakthrough – and a successful patent application (USPTO patent #9,841,590). RTF has been put to good use – joining an extensive portfolio of Leica Biosystems patents in its next-generation digital pathology scanner: the Aperio GT 450 DX.

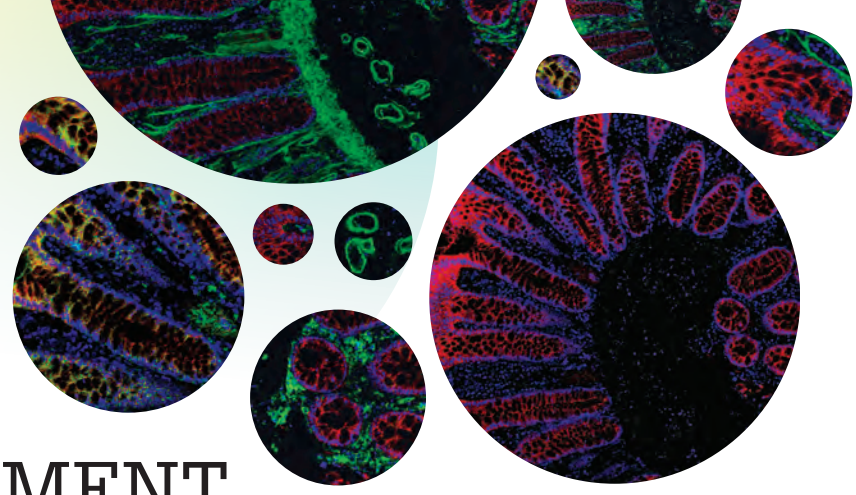
RTF uses an imaging line sensor and a focusing line sensor to capture digital images of a slide. While scanning, the focusing line sensor receives focusing data from the tissue; meanwhile, the proprietary algorithms determine the best-focus value in a graphics processing unit in real time. Through a control loop, each best-focus value is fed to the objective position on the fly – allowing continuous image capture at the best possible focus.

By visiting pathologists in high-volume anatomic pathology laboratories around the world, the Leica Biosystems product development team saw firsthand the barriers they faced in scaling up digital pathology operations. A common theme quickly emerged: current technologies' scan speeds were not fast enough at 40x magnification to keep up with high-volume (over 120,000 slides per year) scanning. If high-volume labs wanted to increase workflow efficiency through digital pathology, they were limited to adopting digital for only one or two organs – for instance, breast or prostate samples. RTF offers the solution to this growing problem – the potential to scale up digital pathology operations even in high-volume labs.

By employing Leica Biosystems RTF, labs can achieve scan speeds at 40x* while maintaining excellent focus on the tissue sample. With continuous loading, no-touch operation, and 32-second scan time at 40x*, the benefit of this innovation is clear: the Aperio GT 450 DX with RTF delivers improved throughputs, reduced turnaround time, and a high-quality image viewing experience to enable healthcare organizations to significantly scale up digital pathology operations – no matter the size of lab or workload – so they can meet ever-increasing demands without sacrificing quality.

**15 mm by 15 mm tissue area*

For in vitro diagnostic use. The clinical use claims described for the products in the information supplied have not been cleared or approved by the U.S. FDA or are not available in the United States.



EXPLORING THE TISSUE MICROENVIRONMENT TO UNCOVER SPATIAL BIOLOGY SECRETS

Fluidigm systems enable in-depth characterization of tumor microenvironments and offer key pathology research insights

Changing the course of how disease is treated or cured requires a comprehensive understanding of complex cellular phenotypes, their functional states, and their spatial relationships to each other and to other structures in the tissue or tumor microenvironment (TME). Fluidigm's new AccuLift™ Laser Capture Microdissection (LCM) system – along with the growing use of Imaging Mass Cytometry™ (IMC™) – enables a better understanding of these characteristics based on the pathology and immune response of the TME and the molecular underpinnings of disease.

The AccuLift LCM system integrates slide digitization and a unique cloud collaboration feature to offer laboratories a comprehensive digital research approach. The system also combines patent-pending laser positioning with a streamlined user interface – so, with just a few clicks, the pathologist can precisely capture cells and regions of interest from limited tissue to achieve a better understanding of the heterogeneous TME. The solution even includes a complete reagent portfolio that improves downstream molecular analyses of small samples.

When it comes to IMC, the high-dimensional spatial visualization of the TME provided by the Hyperion™ Imaging System enables research pathologists and laboratory professionals to gain deep single-cell insights to better understand the cell phenotypes and functions present in their samples.

Highly-multiplexed IMC has already been demonstrated to provide an exceptional approach to: i) identifying single-cell signatures in breast cancer that correlate with clinical outcomes (1), ii) gaining novel insights that may aid in the development of new biomarkers and combination treatment strategies to immune-checkpoint targeting in Hodgkin lymphoma (2), and iii) shedding light on lung pathology at the single-cell level through interrogation of the interplay between infected cells and the immune system in COVID-19 (3).

Fluidigm's AccuLift LCM system and Hyperion Imaging System have become essential tools for clinical researchers who want to reveal how immunotherapy responses and immune cell composition within the TME can be exploited to develop diagnostics, prognostics, and treatments in the future.

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2. *T Aoki et al., Cancer Discov, 10, 406 (2020). PMID: 31857391.*
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SHAPING THE PRESENT AND FUTURE OF LYME DISEASE DIAGNOSIS

Gold Standard Diagnostics is leading the way with its Lyme disease portfolio

Lyme disease – a multifaceted disease of the spirochete *Borrelia burgdorferi* – was first brought to national awareness in the 1980s. Although clinical symptoms continue to be the primary basis for diagnosis, the distinct stages of progression can mimic other diseases – leading to misdiagnosis and failed treatment.

Early diagnostic innovations capitalized on ELISA serological testing to detect and measure immune responses to *B. burgdorferi* infections. By the 1990s, it became clear that the sensitivity and specificity of laboratory testing needed to improve. Innovative technology rose up once again, with academic laboratories developing the Western blot method to directly observe both specific and nonspecific immune responses. Commercial developers quickly followed suit to create standardized testing and develop the two-tier diagnostic algorithm we still use today

In more recent years, Gold Standard Diagnostics has been developing innovative technology that is once again shaping the future of testing for Lyme disease. Although the Western blot has served the community well for decades, scientists have capitalized on the development of molecular recombinant techniques to produce and purify the highly specific *B. burgdorferi* antigens needed for a brand-new approach. Gold Standard Diagnostics is a part of that movement; its Lyme Line Blot offers significant improvements over the Western blot, with increased sensitivity and specificity for consistent, reliable laboratory testing. Next, the company further developed the technology to create Lyme Immunoblot, which also provides easy-to-interpret results. Gold Standard Diagnostics continues to develop both ELISA and Line Blot assays to meet the ever-evolving need for accurate, innovative diagnostics for Lyme and related diseases.

Confirmatory testing for Lyme disease has also continued to evolve; recognizing the need for easier confirmation, the CDC introduced the modified two-tier testing (MTTT) algorithm as an alternative to classic methods. In response, Gold Standard Diagnostics has developed the testing needed for the MTTT with industry-leading proprietary antigens.

In the USA, cases of vector-borne diseases continue to rise and Gold Standard Diagnostics is driven to develop new diagnostic methods to tackle this major challenge. As a market leader for both ELISA and immunoblot assays, Gold Standard Diagnostics will always aim to meet the evolving need for accurate diagnosis of Lyme and other tick-borne diseases.

HALO® SOFTWARE SUITE: FROM BENCH TO BEDSIDE

Indica Labs facilitates bench-to-bedside transition of quantitative and deep learning assays in digital pathology

Indica Labs' HALO® image analysis platform uses computer vision algorithms and HALO AI™ deep-learning networks to enable rapid, quantitative tissue evaluation. Designed around the needs of organizations performing tissue-based research, HALO is the industry standard for biomarker expression profiling, particularly in immuno-oncology. And it has been cited in multiple studies that identify novel spatial immune signatures associated with patient prognosis and immunotherapeutic response (1–4).

Beyond immuno-oncology, HALO AI has been used to develop deep-learning tools for routine pathology – including tissue classifiers to detect lung cancer lymph node metastases and to differentiate between common gastric injury types (5,6). The potential benefits are clear: improved clinical decision-making, streamlined workflows, improved practice efficiency, and reduced costs.

Whereas research and discovery image analysis workflows are flexible and open, clinical assays must be locked down and delivered in an intuitive, standardized format. For assay developers, HALO AP® allows seamless transition of quantitative image analysis and deep-learning assays from bench to bedside. HALO AP's flexible Assay Builder and intuitive interface enables the user to import novel algorithms built in HALO or HALO AI. The guided, stepwise workflow is designed around these algorithms to make the assay easy to follow and deploy, ensuring accurate and standardized results – even for the most complex assays. After validation, the assays are signed and locked to prevent tampering.

The Clinical Trials module lets the user take full control of their assay during clinical trials and multi-site validation – inviting collaborators, monitoring status, and sharing results with stakeholders. As cases arrive, the workflow guides collaborators through the review process and manages all image analysis and algorithm settings.

For CLIA laboratories wishing to deploy novel quantitative assays, HALO AP can integrate directly with existing laboratory information or image management systems or function as a standalone browser-based image management system with end-to-end case management – from image import to report generation.

References

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3. *A Rasmusson et al., Am J Pathol, 190, 1309 (2020). PMID: 32194048.*
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BREAKING DOWN BARRIERS TO DIGITAL PATHOLOGY

How Corista's image management platform – DP3 – can help improve clinical review and research workflows

Developed in collaboration with a team of pathologists, DP3 aims to address the complex, competing demands of the modern pathologist. The imaging management platform provides pathologists with a 360° view of their cases and the anatomic pathology workflow by consolidating data and native images into a single dashboard – whether it's within a single laboratory or across a network of hospitals, laboratory information systems, or whole-slide imaging scanners. Segregating data and repositories between facilities is an integral part of the easy-to-use interface, and pathologists can quickly check and manage their workloads while accepting, reviewing, and transferring cases to their colleagues – regardless of patient or location.

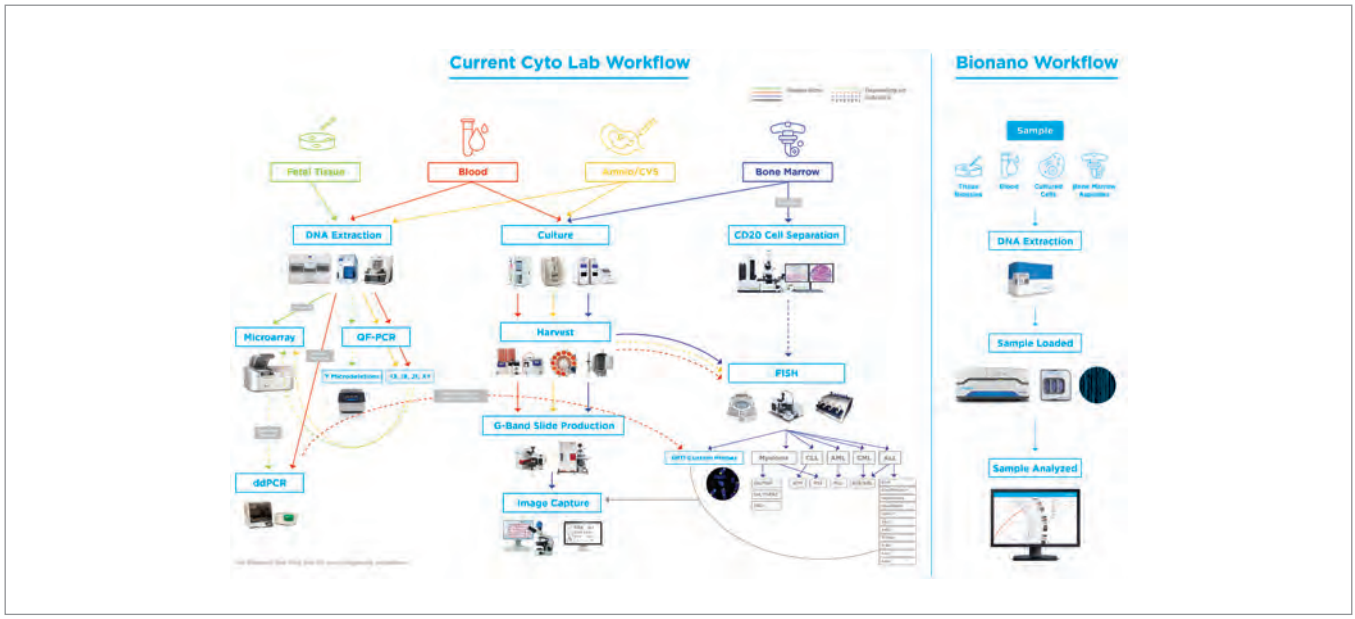
Device interface ergonomics have been a key obstacle to the acceptance of digital pathology. Many viewing methods rely on standard devices – such as a mouse or trackpad – to navigate on-screen slides, which requires highly repetitive hand movements and can lead to rapid fatigue. Understanding this challenge, Corista patented the Virtual Slide Stage (VSS) to eliminate the mouse altogether. VSS requires only one hand to manipulate and view a slide on the platform, which significantly improves the ergonomic efficiency of digital slide viewing to yield the same productivity as standard microscopy.

With a commitment to advancing the adoption and utility of digital pathology, Corista recognized the importance of integration with leading image analytics providers. Today, Corista provides direct access to industry-leading image analysis within DP3, which enables pathologists to receive results from each analysis directly in the case. And that allows pathologists to manage cases quickly and efficiently while ensuring information is securely stored and accessed in one place.

In short, DP3 optimizes the image management workflow – whether the pathologist is consulting on cases, collaborating on research projects, presenting at a conference, or managing quality assurance activities. The scalable, secure platform offers medical centers the flexibility to create a unified digital working environment that promotes collaboration, communication, teaching, and reporting. Corista continues to push the digital pathology market forward, creating innovative solutions for the modern pathologist.

ACCELERATE TIME TO ACTIONABLE RESULTS WITH SAPHYR®

Next-generation cytogenomics consolidate traditional cytogenetic assays into a single workflow



Despite a sequencing technology-inspired revolution in genomics research and diagnostics, cytogenomic labs' approach to structural variants has hardly changed. Though next-generation sequencing (NGS) identifies single-nucleotide variants and small (<150 bp) insertions and deletions, it fails to identify most large insertions, deletions, and copy number variations in repetitive regions of the genome. It also does not reliably detect balanced structural variants, such as inversions and translocations. NGS relies on short-read sequences that are mapped to a reference human genome – introducing bias when calling structural variants. In addition, the cost of long-read sequencing is still too high and the throughput too low for routine use.

Current cytogenetic methods, such as karyotyping, fluorescence in situ hybridization, and array comparative genomic hybridization, cannot address complex cases alone because of their technical limitations and need to be combined with molecular methods – such as MLPA, qPCR, or RNAseq – to provide a complete therapeutic and prognostic assessment of the tumor genome.

The Saphyr® system from Bionano Genomics was designed to address these challenges through optical mapping of megabase-sized DNA molecules and studies around the world are confirming its performance. A US study by leading cytogeneticists of 100 patients with acute myeloid leukemia (AML) found 100 percent concordance with traditional clinical cytogenetics analysis. Moreover, Saphyr identified clinically relevant structural variants in 11 percent of cases that had been missed by routine testing and, in 13 percent of cases, it refined the underlying genomic structure reported by traditional cytogenetic methods. In 6 percent of cases with normal karyotypes, optical genome mapping detected cryptic translocations involving gene fusions. Based on their results, they recommend Saphyr to be considered as the first-line test for the detection and identification of clinically relevant structural variants in AML.

Saphyr is the only technology available that consolidates traditional cytogenetic assays into a single workflow. By providing a complete, unambiguous picture of the genome structure, Saphyr can identify prognostic markers that are not currently monitored and enable complete characterization of the cancer or patient genome in a single test.

SOLVING PROBLEMS SAVING LIVES TOGETHER

2021 ASCP MEMBERSHIP

ASCP remains committed to serving you and helping you achieve your professional goals, while you continue to lead from the laboratory to solve critical problems and save countless lives. The laboratory is the cornerstone of healthcare, and pathologists and medical laboratory professionals are critical to successful patient care.

As we move forward together, we can accomplish so many great achievements, even in extraordinary times. ASCP is dedicated to you. We're solving problems. We're saving lives. And we're doing it together.

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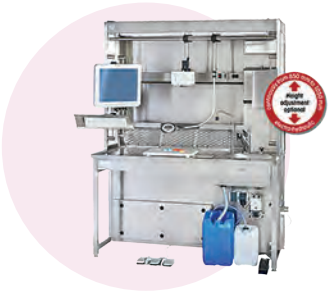
Spotlight on...

Technology

Pathology Workflow: Challenged by Increasing Workload?

Pathology labs are striving to improve turnaround times and provide fast results to patients while managing increasing test volumes and complex workflows. Agilent's workflow consultancy model is designed to help pathology labs optimize workflows and efficiency, enabling you to complete more patient cases sooner.

Visit the web page below to learn
tp.txp.to/Agilent/Consultancy



Professional grossing tables perfectly suited to your lab

GrossPath – the special worktable that ensures a pollution-free environment for histological slide preparations. Buy it, set it up, and power it on: thanks to its generously proportioned active-carbon air recirculation system, it is ready for immediate use! Hazardous vapors are vacuumed downward; formalin concentrations are significantly below maximum allowable concentrations throughout the extraction zone.
tp.txp.to/Kugel/GrossPath

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These labels withstand extended immersion in solvents such as xylene, toluene, and acetone, as well as in safer substitutes such as EZ Prep and Clearify™. Ideal for identifying glass microscope slides, these labels do not acquire colors from reagents and can be used in many histological protocols (routine and special).
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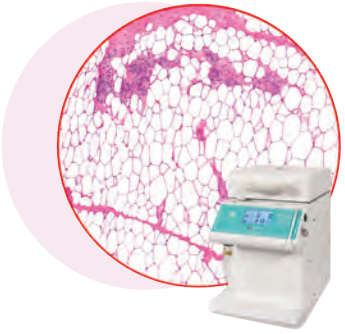

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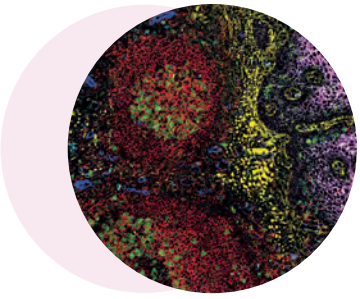

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Researcher at Heart

Sitting Down With… Charles Clevenger, Professor and Chair of Pathology at Virginia Commonwealth University School of Medicine, Richmond, Virginia, USA

What prompted your interest in medicine?

It started when I was in high school. I became broadly interested in research and, as that evolved, I realized that what really excited me was human research with a translational angle. I've been involved in research ever since I was 16 years old.

I chose pathology because it seemed like the best marriage of research and the clinic. As I finished my residency and fellowship, I found myself gravitating toward breast cancer research. At the time, I ran a DNA flow cytometry lab that principally worked on breast cancer, and my research postdoc focused on prolactin and the immune system. I told my mentor I wanted to port that research into the breast, so I could have congruence between my research and my clinical practice – and he consented.

What has been the most unexpected moment of your career?

I would say there have been two.

One is our discovery that both prolactin and its receptor can enter – and function in – the nucleus. The notion that peptide hormones could enter the nucleus was heretical at the time. People thought steroid hormones worked in the nucleus and peptide hormones worked at the cell surface – and ne'er the twain shall meet. Now, of course, we know that's not true.

The other involved shedding new light on an old discovery. There are seven prolactin receptor isoforms; we cloned four, including an intermediate that is missing about half of its intracellular domain. We had originally shelved it; after all, why use something that only works half as well as the complete protein? But, three years ago, Schreiber and colleagues performed a *STAT1* knockout mouse study in which all of the female mice got breast cancer. Their tumor DNA had one thing in common: a mutant, truncated form of the mouse prolactin receptor that strongly resembled the intermediate form of the human receptor. They also showed that it didn't have any special properties by itself – but, when co-expressed with the wild-type receptor, it was profoundly transforming in mouse fibroblasts. I immediately recognized that this form of the receptor was very similar to the human intermediate form and wondered, "Could it be playing a similar role in the human breast?" The results of our research in that area are forthcoming!

What are your other meaningful accomplishments to date?

I think I'm most proud of my accomplishments in the realm of mentoring. When I assumed the chair position at Virginia Commonwealth University seven years ago, I was tasked with rebuilding our research division. I recruited three junior faculty into the department – and, since then, they have all published and they have all received major grant support. And that's hugely gratifying. I've had the opportunity to mentor young scientists to a stage where their career is now set – not easy in the current funding environment.

I view the role of a mentor as a service – not something to micromanage. Some scientists believe that chairs and mentors should be in every aspect of people's business, which I don't think is successful. The model my chairs and mentors used was not to interfere with what I was doing. They gave me free rein, but they were always there when I had a problem or hit a brick wall. I view my role as chair in the same way – not as someone who directs every movement, but as someone who helps when there's a real problem.

What do you think lies in pathology's near future?

I think we'll increasingly see digital pathology make inroads into our practice. Digital pathology offers us a permanent record of every slide we make; it allows us to be quantitative in ways that we haven't been before; and – particularly relevant in today's pandemic environment – it allows us to readily share images with colleagues around the world.

Unlike radiology, pathology can never be truly digital. We'll always have to make a slide and scan it to obtain a digital image. But I don't see that as a downside; if anything, it facilitates the transition, because if you have any reservations about the technology, you can still go back to the paraffin block. You can still practice pathology the "old" way.

Do you have any advice for pathologists at the start of their careers?

Find a niche. Don't leap in and start trying to cure cancer right away; try to find a subdiscipline that you can become an expert in before you move on to bigger things.

Ask yourself constantly, "Am I still being challenged? Am I still having fun?" And, if the answer is no, do something about it. Moves are a great time-waster if you don't have a good reason – but you'll know when you do have a good reason. If you can't achieve what you want in your current position, it's time to consider a move.

If you hadn't become a pathologist, what would you have become?

My great loves are playing the piano and cooking. In fact, my wife and I are both pretty good cooks – so it's a great hobby to share. If I had to pick a specialty, I would say it's baking bread – an increasingly popular pandemic hobby, but one I have pursued for many years. And I have to say, my bread is pretty darn good… So to answer your question: a baker!

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- *Bacteroides fragilis*
- *Enterobacterales*
  - *Enterobacter cloacae* complex
  - *Escherichia coli*
  - *Klebsiella aerogenes*
  - *Klebsiella oxytoca*
  - *Klebsiella pneumoniae* group
  - *Proteus*
  - *Salmonella*
  - *Serratia marcescens*
- *Haemophilus influenzae*
- *Neisseria meningitidis*
- *Pseudomonas aeruginosa*
- *Stenotrophomonas maltophilia*

GRAM-POSITIVE BACTERIA
- *Enterococcus faecalis*
- *Enterococcus faecium*
- *Listeria monocytogenes*
- *Staphylococcus*
  - *Staphylococcus aureus*
  - *Staphylococcus epidermidis*
  - *Staphylococcus lugdunensis*
- *Streptococcus*
  - *Streptococcus agalactiae*
  - *Streptococcus pneumoniae*
  - *Streptococcus pyogenes*

YEAST
- *Candida albicans*
- *Candida auris*
- *Candida glabrata*
- *Candida krusei*
- *Candida parapsilosis*
- *Candida tropicalis*
- *Cryptococcus neoformans/gattii*

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