Elaine S. Jaffe

The Microscope as a Tool for Disease Discovery—A Personal Voyage

Elaine S. Jaffe

Hematopathology Section, Laboratory of Pathology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892;
email: elainejaffe@nih.gov

Annu. Rev. Pathol. Mech. Dis. 2017. 12:1–24

First published online as a Review in Advance on December 5, 2016

The *Annual Review of Pathology: Mechanisms of Disease* is online at pathol.annualreviews.org

This article's doi:
10.1146/annurev-pathol-052016-100351

Keywords

malignant lymphoma, B cells, T cells, molecular diagnosis, molecular oncology, cell of origin

Abstract

This review reflects the trajectory of my career in hematopathology, and my personal reflections on scientific advances in the field. During the course of more than 40 years, the approach to classification of hematological malignancies has evolved from descriptive approaches, based on either cytological or clinical features, to a modern approach, which incorporates cutting-edge technologies. My philosophy has focused on defining individual diseases, an approach that can best lead to an understanding of molecular pathogenesis. To quote Carolus Linnaeus (1, p. 19), the father of taxonomy, "The first step in wisdom is to know the things themselves; this notion consists in having a true idea of the objects; objects are distinguished and known by classifying them methodically and giving them appropriate names. Therefore, classification and name-giving will be the foundation of our science."

EARLY ROOTS

My parents were both immigrants to the United States, born in shtetls (villages) in what is now the Ukraine. My father was born in Steblov, and my mother in Ryzhanovka. My father's father, who was a cantor, came to the United States in 1914 with his two oldest children, and he expected the rest of the family to follow once he was settled. Cousins who already lived in New York facilitated their arrival. However, World War I delayed the departure of the rest of the family, which included my father, his three younger siblings, and his mother. For the next seven years, my father was the man of the house. He was a strong figure, and being the head of the household probably helped to mold his personality. My father arrived in the United States at age 16 and immediately went to work as an apprentice in the diamond trade. He worked his way up in the business, eventually establishing his own firm in New York's diamond district on 47th Street. His company manufactured diamond jewelry, which was sold to jewelry stores around the United States by salesmen in five territories. My father started a second business as a diamond trader, dealing in loose stones. As a child, I was fascinated to see him open little tissue paper packets, often containing dozens of glimmering stones. My father's two brothers became employees of his firm, and when two of his sisters married, their husbands came to work for him as well, which provides evidence of my father's forceful leadership skills.

My father never attended school in the United States, and he never lost his eastern European accent. He had a sharp mind and was a shrewd business man but always felt he had something to prove. He was helped in the business by my mother. My mother arrived in the United States at age 9 and was placed in first grade. She did not know a word of English, but she rapidly progressed. Her uncle, who was born here, tutored her in English. She strived to lose every trace of accent. My mother finished high school with high marks, but this was the early 1930s, during the Depression, and college was not an option. She got a job in the Equitable Life Insurance Company. According to my mother, they did not hire Jews at the time, but with her bright red hair, green eyes, and last name (Shane), she convinced them she was Irish. She stayed at Equitable until she married my father in 1935. For the next 52 years, she was his right-hand "man," playing a key role in managing the business. After my father died in 1987, she obtained membership in the New York Diamond Dealers Club in her own right, one of only a few women who were members. She was very proud of her membership card and her ability to wheel and deal with the men.

My parents were typical of many immigrant families in their drive to succeed in their new homeland. Our house was always the center for extended family events and gatherings. Political events and intense debate dominated conversations. My older sister, Louise, eight years ahead of me in school, headed off to Cornell where she majored in government, graduating Phi Beta Kappa in 1957. However, she married soon after and did not return to work until some years later. I eagerly followed my sister to attend Cornell, but I was determined that I would follow a different career path. I registered as a premedical student and selected zoology as my major.

EARLY STEPS

Although it would be convenient to say that I always knew that hematopathology would be my life's work, my career has been characterized by serendipity and being in the right place at the right time. Nevertheless, I cannot imagine another career path in medicine besides pathology. As second-year medical students at Cornell University Medical College, we had a year-long course in pathology. It formed the basis for the subsequent clinical years, and I feel that I acquired most of my knowledge of clinical medicine in that course. In fact, I have some objective proof for that statement. When we were rising third-year medical students, a group of us were asked to take Part 3 of the National Board Examination (now the USMLE 3). The National Board of

Medical Examiners wanted to compare the knowledge base of second-year students with medical school graduates at the end of their first postgraduate year (PGY-I). I confess that I performed better on the exam at the end of my second year than I did at the completion of my PGY-I in pathology—although I did readily pass the exam both times.

I saw pathology as laying the groundwork for the understanding of all disease states. Looking at a microscopic slide of diseased tissue provides a wealth of information regarding the pathogenesis and pathophysiology of disease. Pathologists are very visual in their approach, and many pursue art or photography as hobbies. Most are adept at recognizing faces or images as well as discerning the varied microscopic patterns that inform us about the diagnosis and the underlying pathobiology. An astute pathologist can detect many facets of the patient's history and course from a single slide, such as the age, sex, clinical symptoms, sites of disease, and patterns of spread. To me, the power of the visual microscopic image is probably greater than the power of a gene expression microarray reporting on the activity of thousands of genes. In pathology, the disease process came alive for me—providing insight and also raising questions for future discovery. In my subsequent research career, most of my investigations have begun with a diagnostic case that presented enigmas that needed to be answered.

At Cornell, we had the usual series of lectures, which took us through all of the various organ systems. Most of these were not very memorable. However, I had the good fortune to be in a lab group that was directed by two eager pathology residents who were destined to pursue careers in academic medicine and were excited about teaching. Dr. Janet Mouradian completed her pathology residency at Cornell and stayed on as a staff surgical pathologist, ultimately becoming Director of Surgical Pathology at New York Hospital. Dr. Daniel Alonso also stayed on at Cornell, eventually becoming Dean of Students. He continued his career in administration, becoming the founding Dean of Cornell Medicine in Qatar, the first American medical school to be set up overseas.

I left Cornell at the end of my second year, in 1967, and transferred to the University of Pennsylvania, School of Medicine. I had met my future husband, Michael Evan Jaffe, when he was a law student at Columbia University in New York, across town from Cornell. He was accepted for a federal clerkship with the Federal District Court of Delaware, clerking for the Honorable Caleb Layton. The clerkship was an important stepping stone in my husband's career, and it also offered him an important deferment of military service during the Vietnam War. We married in July 1967, and I continued my studies in Philadelphia.

As a married student, I lived off campus. I went through all of the usual clinical rotations, working mostly with students in my alphabet group, last names beginning with J, K, and L. There were six women in my class at the University of Pennsylvania; there had been five of us at Cornell. Especially at Cornell, we women bonded as a peer group, offering support, friendship, and encouragement. At Penn, the administration was not always sure how to manage us as women. During my third-year internal medicine rotation, students were expected to take call in the hospital every third night. However, they did not think it proper for women to sleep in the same on-call room as men slept, so, usually around 11 PM, they sent me home to my apartment off campus and asked me to return in the morning for blood-drawing rounds.

Blood-drawing rounds provided another pivotal experience. Third-year students made the rounds of the inpatients each morning, drawing blood for subsequent laboratory studies. I recall vividly one elderly woman with thin, delicate veins, who was a particular challenge for me. When I would enter her room, she would exclaim, "Oh no, not you again; get me a real doctor." Truthfully, she had a right to complain. I was not very good at drawing blood or in dealing with patients. I began to realize that my strengths did not lie at the bedside.

As my fourth year approached, my husband's clerkship was coming to an end. He had secured a second clerkship with the Honorable Collins J. Seitz, in which he would divide his time between

the Federal District Court and the US Court of Appeals for the Third Circuit. Again, this was a superb professional opportunity, but unfortunately, a deferment was not part of the package. My husband had several choices: (*a*) enlist as an officer in the Navy with a four-year commitment, (*b*) take his chances with the draft, or (*c*) select Door Number Three by obtaining a 3A deferment as a father. We took Door Number Three, and on September 11, 1968, I gave birth to Gregory Merrill Jaffe. (Our second son, Caleb Adam Jaffe, was born during my residency in 1972.) I returned to my clinical rotations as a fourth-year student 3.5 weeks later. As a fourth-year, I had great flexibility in my rotations; nearly all of my rotations were medical subspecialties. I particularly liked hematology and endocrinology; however, by this time I knew that I wanted to do pathology.

As a two-career family, we had various options to consider. My husband was from Texas; he was a graduate of Rice University and very much enjoyed his time in Houston. I grew up in New York, but New York City was not to my husband's liking. Michael interviewed for jobs in Houston and Washington, DC, and secured offers from major law firms in both cities. In the end, he knew I was not partial to living in Texas, and he accepted a position as an associate with Arent, Fox, Kintner, Plotkin, and Kahn. I considered the various options for pathology training in Washington, DC, and accepted a position as a PGY-I pathology resident at Georgetown University Hospital. The pathology department at Georgetown had a strong clinical service but was not a major research department. Dr. Abner Golden headed this department, and he had a strong commitment to pathology education. Dr. B.K. (Byungkyu) Chun, a generalist of the old school who always had a smile on his face, was the head of surgical pathology. At this point, I had not thought about a research career and was focused on developing my diagnostic skills. However, a cousin of my husband was doing a clinical research fellowship at the National Institutes of Health (NIH) in the Public Health Service. This was the era of the yellow berets, when many eligible, newly minted physicians could fulfill their military service requirements by obtaining an NIH fellowship. This cousin serendipitously mentioned that the NIH also had a residency program in pathology and that I should consider applying.

Following his advice, I applied and was selected for one of the NIH's three slots in the Anatomic Pathology residency program. Dr. Louis B. Thomas had recently taken over leadership of the department, following Dr. Harold Stewart, who was a leader in the field of chemical carcinogenesis. Dr. Stewart was an icon in pathology circles. During his career, he was president of six medical societies, including the United States and Canadian Academy of Pathology (USCAP), the American Association for Cancer Research, the American Society for Experimental Pathology, and the International Academy of Pathology. He helped found the American Society of Clinical Pathologists, and in 1978, he received the Gold-Headed Cane from the American Association of Pathologists and Bacteriologists (later renamed the American Society for Investigative Pathology). Dr. Stewart's department was a gathering place for visiting pathologists from around the world. A steady stream of visiting lecturers and pathology dignitaries provided an exciting intellectual environment. Under Dr. Stewart, and later under Dr. Thomas, the chairman's office was an informal lunchroom, where faculty and visitors would gather to discuss research and the events of the day. Dr. Stewart was a great raconteur, aside from his abilities as a scientist.

When I entered the pathology program at the NIH, I succeeded Dr. Deborah Powell, who was to become one of the great leaders of pathology and education. Debbie had been a resident for one year at Georgetown prior to coming to the NIH, and she returned to Georgetown as a faculty member. For most of my tenure as a resident, Dr. Thomas would refer to me as "Debbie," which I regarded as a compliment. Dr. Powell went on to become Chair of Pathology at the University of Kentucky, Dean of the University of Kansas School of Medicine and Vice Chancellor for Clinical Affairs, and Dean of the University of Minnesota Medical School, in addition to the leadership positions she held at the Association of American Medical Colleges. We had a strong resident class at the NIH (**Figure 1**). My fellow residents included Ron DeLellis (Chair

Figure 1

National Institutes of Health, Laboratory of Pathology resident class of 1970–1971. (*Shown left to right, front row*) Gerhard Krueger, Elaine Jaffe (author), and Hugh Bonner. *(Back row)* Joel Roth, L. Maximilian Buja, and Ronald DeLellis.

of Pathology, Brown University), Max Buja (Chairman of Pathology and Dean, University of Texas at Houston), Joseph Davie (head of the Department of Microbiology and Immunology, Washington University), Gerhard Krueger (Chair, University of Cologne, Germany), and Hugh Bonner (professor of Pathology and fellow hematopathologist, University of Pennsylvania).

The NIH residency program placed an emphasis on initiative and research. As a resident, I was drawn to liver pathology and hematopathology, both of which require close collaboration between the pathologist and clinician. In hematopathology, the clinical findings are integral to making the correct diagnosis, and the hematopathologist is part of the clinical team in many other respects. Exemplifying the collaborative nature of patient care, clinicians often seek my opinion about the clinical management for rare conditions that they may encounter in their practice.

As a resident and fellow at the NIH, I benefitted from wonderful mentors, and I have since found facilitating the careers of young scientists greatly rewarding. Costan Berard, my immediate mentor in hematopathology, was unusually generous. He, too, was a leader in pathology circles, later becoming President of the USCAP. He facilitated my collaborations with immunologists that were critical to our success, and early in my career gave me every opportunity to present my work at national and international meetings.

EARLY SUCCESS

I entered the field of lymphoma research at a unique time—when all of the planets were aligned, creating great opportunities. It was an era when dramatic advances were being made in

chemotherapy at the National Cancer Institute (NCI), led by Vincent DeVita, Paul Carbone, George Canellos, Bruce Chabner, and many others. The advances in therapy made it more important and relevant to sort out the different diseases among the many different types of lymphoma. At the same time, advances in immunology and molecular biology enabled us to take hematopathology beyond pure morphology. However, it is the integration of all of these disciplines that has really changed the way we diagnose lymphomas.

In the 1960s, two discoveries revolutionized the understanding of the immune system and its neoplasms. These were (*a*) the potential of lymphocytes—which had been thought to be end-stage, terminally differentiated cells—to transform into proliferative blastoid cells in response to mitogens or antigens (2) and (*b*) the existence of multiple, distinct lymphocytic lineages [T, B, and natural killer (NK)] that were morphologically similar but had different functions and physiology (3, 4). When I began my fellowship in hematopathology in 1972, immunologists were just beginning to explore ways to distinguish these cell types in the laboratory.

When I was a new fellow, Costan Berard introduced me to Ira Green, Michael Frank, and Ethan Shevach, all of whom were working in the immunology branch of the National Institute of Allergy and Infectious Disease. Ira Green was a good friend and former colleague of Victor Nussenzweig at New York University, and Nussenzweig's lab was investigating surface markers expressed on normal immune cells. Lay et al. (5) had shown in 1971 that there was spontaneous binding of sheep red blood cells (SRBCs) to a large population of human lymphocytes. The binding occurred in the cold and depended on temperature, but the nature of the lymphocyte population was unknown. Shortly thereafter, Jondal et al. (6) showed that the binding occurred with thymocytes, that the cells binding the SRBC lacked surface immunoglobulin (Ig) determinants, and that lymphocytes from three patients with chronic lymphocytic leukemia (CLL) failed to bind. They suggested that the erythrocyte rosetting (E rosette) could be used as a T cell marker. Lay & Nussenzweig (7) had previously identified receptors for complement on the surface of lymphocytes. Subsequent studies showed that lymphocytes bearing complement receptors were B cells and that binding did not require the cold conditions of the E rosette technique (8). These were termed EAC rosettes, as the erythrocytes (E) were coated with IgM antibody (A) and complement (C). At the NIH, we knew we could form these rosettes with human lymphocytes in the test tube, but it was my task as a fellow to see if we could get the technique to work on tissue sections so that we could visualize in the intact tissue the distribution of the various cell types.

I set to work on what we would come to call the hanging drop technique (**Figure 2**). A frozen section was mounted on a glass slide. The well in a microculture glass slide was filled with either E or EAC rosettes. The frozen section was placed over the well, the two slides inverted, then incubated for 30 minutes either at 4°C (for E rosettes) or at room temperature (for EAC rosettes). After incubation, the slide was gently tapped to remove unbound red cells; the sections were gently washed, fixed, and examined by dark field microscopy. Adherent rosettes were refractile and readily visualized on the section. We first employed this technique on frozen sections of normal lymph nodes and spleen, and we showed that we could observe the expected binding to T cell and B cell areas. Our next step was to study what was known at the time as nodular lymphoma. Henry Rappaport (9), in his classification published in 1966, had questioned whether nodular lymphomas were related to the lymphoid follicle, a view disputed by Karl Lennert (10). Lennert thought that the cytological features of the neoplastic cells resembled those of normal germinal center cells, which he initially termed germinocytes and germinoblasts and later termed centrocytes and centroblasts to avoid confusion with germ cell neoplasms. Our study, published in the *New England Journal of Medicine*, showed that the cells in the lymphoma expressed the same receptors as normal germinal center B cells (11) and provided critical evidence that linked nodular lymphoma to the normal lymphoid follicle (**Figure 3**). The paper subsequently became a *Citation*

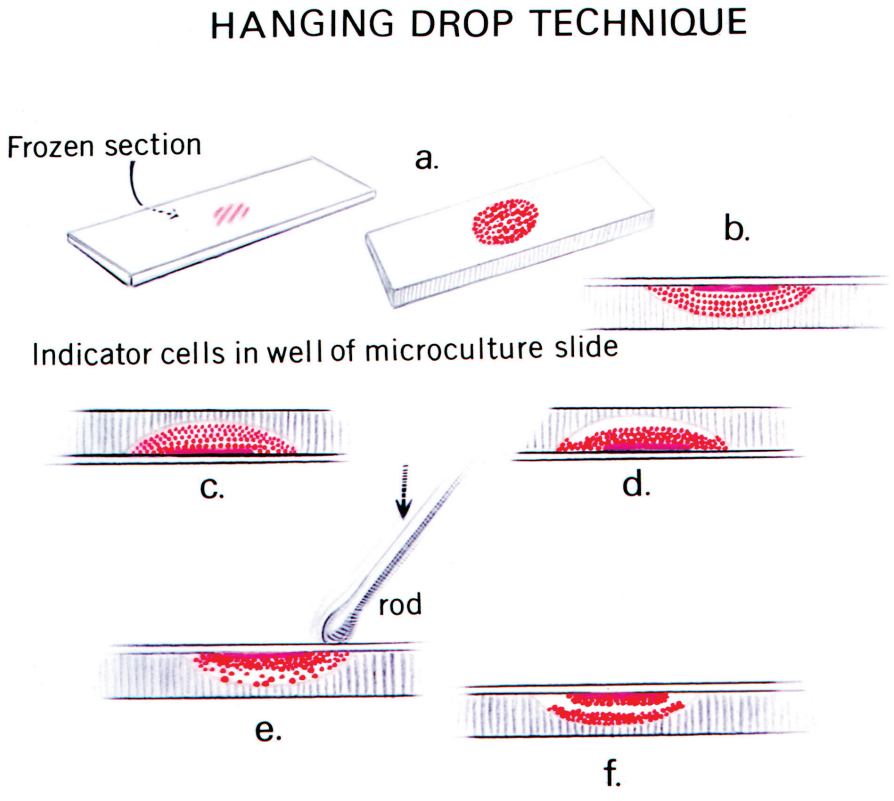

Figure 2

Hanging drop technique. This method was used to visualize sheep erythrocyte rosettes (E rosettes) and EAC rosettes (erythrocytes coated with immunoglobulin M antibody and complement) on frozen sections mounted on glass slides, respectively identifying T cells or B cells in situ.

Classic and provided some of the first evidence that neoplastic lymphoid cells recapitulated the functional properties of their predecessors.

At about the same time, Smith et al. (12) characterized a single case of what was known as mediastinal Sternberg sarcoma as being of T cell or thymic origin, based on the formation of E rosettes by 86% of the cells in a cell suspension. We now recognize this disease as T cell lymphoblastic leukemia/lymphoma. Notably, Carl Sternberg (13), working in Austria in 1916, had proposed a thymic origin for this tumor based on clinical and pathological observations. Studies of Sézary syndrome followed suit (14), and in 1975, we identified a case of nodal lymphoma as being of T cell origin in tissue sections (15). The immunological revolution was under way.

In the 1970s, pathologists published several proposals which attempted to link this new immunological information to the classification of lymphomas. The first and most significant of these efforts was led by Karl Lennert, in Kiel, Germany, who had first recognized that many lymphomas resembled germinal center cells and had used ultrastructural studies to identify follicular dendritic cells in normal and neoplastic lymph nodes. The Kiel classification was published in German in 1974 (16) and in English shortly thereafter (17, 18). Lymphomas were classified according to a hypothetical scheme of lymphocyte differentiation, and the nomenclature reflected the putative normal counterpart of the neoplastic cells. Although the majority of the neoplasms described

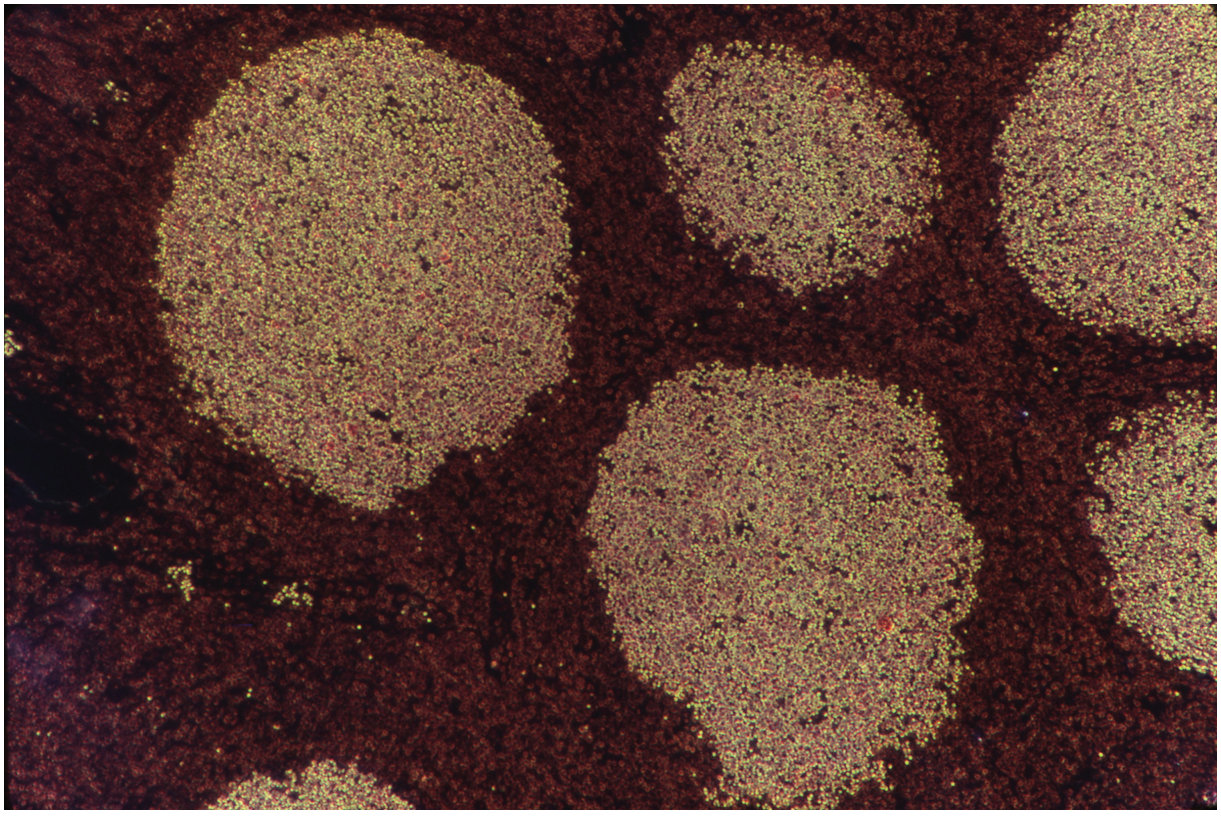

Figure 3

Follicular lymphoma that exhibits binding of EAC rosettes (erythrocytes coated with immunoglobulin M antibody and complement) to a frozen section. Neoplastic follicles bind the complement-coated red blood cells, visualized by dark field microscopy.

were of B cell lineage, several well-defined types of T cell lymphomas were included, later to be expanded on through collaborations with Suchi (19) in Japan. The neoplasms were grouped according to histological features into low-grade malignancy (predominance of small cells or "-cytes") and high-grade malignancy (predominance of "-blasts"). This classification became widely used in Europe but never supplanted the Rappaport classification, popular among clinicians, in the United States.

By the mid-1970s, six different classification systems had been published, although only those proposed by Rappaport and Lennert were widely used. However, the use of different classification systems in clinical studies made it difficult to compare published results from different centers. Several meetings were held to try to break the deadlock, the last of which was held at Airlie House in Warrenton, Virginia, in 1975 (20). The inability of the pathologists to develop consensus and agree on a common approach led to an NCI-directed study to evaluate the six published schemes. Led by my mentor, Cos Berard, this study was based on a retrospective review of hematoxylin- and eosin-stained sections from 1,175 cases of non-Hodgkin's lymphoma. None of the schemes proved clearly superior in predicting survival, which led to the publication of the Working Formulation (WF) for the non-Hodgkin's lymphomas. The WF was proposed as a common language to translate among the six classifications (21). It abandoned efforts to relate tumors to the normal immune system and instead proposed a purely descriptive approach based on cytological features and patterns. In

truth, it was a step backward, largely proposed for political motives. It recapitulated the Rappaport classification but substituted some words borrowed from the Lukes-Collins system.

THE NEW TECHNOLOGIES

In 1975, Kohler & Milstein (22) created the hybridoma technology that led to the development of monoclonal antibodies. In 1979, the first monoclonal antibody against a human lymphocyte differentiation antigen was generated against an antigen expressed on normal thymocytes (23). The antigen recognized by NA1/34 was later designated as cluster of differentiation (CD)1a in the Human Leukocyte Differentiation Antigen system (24). Today more than 350 groups of CDs have been identified, with many more monoclonal antibodies recognizing nuclear antigens, which lack a CD designation.

In the early years, monoclonal antibodies were applied to cells in suspension, or, somewhat later, to cryostat tissue sections. However, a paradigm shift occurred when David Mason and others (25) not only adapted the techniques to routine formalin-fixed, paraffin-embedded (FFPE) sections but also changed the strategy for screening monoclonals to look for those antibodies that would have optimal reactivity in FFPE sections. These advances had a dramatic impact on the field. First, one did not have to ensure that tissues were snap frozen or made into cell suspension to perform immunophenotypic studies. Second, the better morphology of paraffin sections improved the pathologist's ability to characterize positive and negative cells. This technology was rapidly adopted by pathologists throughout the practice community.

In parallel, and equally dramatic, an understanding of the genetics of lymphoid malignancies progressed. Recurrent cytogenetic abnormalities were identified, which followed the early observations of recurring cytogenetic alterations in myeloid leukemias (26). The first to be recognized were the t(8;14)(q24;q32) of Burkitt lymphoma and the t(14;18)(q32;q21) of follicular lymphoma (FL) (27–29). Subsequent studies led to the cloning of the genes involved in these translocations, *MYC* and *BCL2* (30–32). Other similar discoveries soon followed, such as the identification of *CCND1* as the key gene in the BCL1 breakpoint of what came to be known as mantle cell lymphoma (33–35).

Studies in the laboratory of Tom Waldmann (36) at the NCI capitalized on the known rearrangement of the Ig and T cell receptor genes during normal lymphoid cell development and exploited the technology to use rearrangement of the antigen receptor genes as markers of both lineage and clonality in lymphoid neoplasms. It was later shown that B cells that have transited the germinal center exhibit evidence of somatic hypermutation of the *IGH* variable region genes (37). Thus, this knowledge could be exploited to show not only lineage but also stage of differentiation, at least within the B cell system (38, 39).

The development of polymerase chain reaction (PCR) methods further facilitated the introduction of gene rearrangement techniques to the routine clinical laboratory (40). Additionally, genetic probes could be hybridized to paraffin sections by fluorescence in situ hybridization (FISH)–based strategies, which enable the recognition of key translocations in routine clinical samples for diagnostic purposes (41).

Whereas translocations involving oncogenes played a key role in the pathogenesis of many lymphomas, other studies identified the important role of lymphotropic viruses in many lymphoid malignancies. Epstein-Barr virus (EBV) was shown to be especially promiscuous and capable of transforming B cells, T cells, and NK cells (42–45). Uchiyama et al. (46) had described a high-grade T cell malignancy, adult T cell leukemia/lymphoma (ATLL), which was endemic in southwestern Japan. A viral etiology was suspected, and subsequently, Gallo and colleagues (47) identified human T-lymphotropic virus type I (HTLV-I) as the first retrovirus shown to cause a human

malignancy. Working with Gallo, we showed that the disease also existed in the Western Hemisphere, with some prevalence in the Caribbean basin, Central and South America, and in immigrants to the United States (48, 49). The pathological spectrum of ATLL was broad, but both serological studies for antibodies to the virus and molecular detection of viral sequences facilitated diagnosis (50). HHV-8/KSHV was soon added to the list of oncogenic viruses and associated with not only Kaposi sarcoma but also primary effusion lymphoma (51) and multicentric Castleman disease (52–54).

The next decades brought a rapid succession of new, mainly high-throughput methodologies to further interrogate the nature of lymphoid neoplasms. These included gene expression profiling, methylation profiling, array-based comparative genomic hybridization (CGH), and next-generation sequencing. This wealth of tools can be utilized to produce a strategic vision for further discovery of key pathogenetic events in the lymphomas (55).

This era has also seen an expansion of team science, with large multidisciplinary groups, such as the Lymphoma/Leukemia Molecular Profiling Project (LLMPP), collaborating to generate vast amounts of data (56–58). These studies produced many new insights, among them (*a*) the discovery that diffuse large B cell lymphomas comprised two major subtypes derived from germinal center type B cells (GCB) and activated B cells (ABC), and (*b*) clarification of the molecular heterogeneity of peripheral T cell lymphomas (57, 58). The prognostic relevance of the proliferation signature in mantle cell lymphoma was shown (59), which was something that had been suggested in earlier studies using immunohistochemical detection of Ki-67 (MIB-1) (60, 61). Beyond studies of the tumor cells themselves, the data shed light on the significance of the tumor cell microenvironment, with crosstalk among tumor cells, nonneoplastic immune cells, and stromal elements (62–64).

The success of the LLMPP has provided a model for this type of collaborative effort, which includes participation from pathologists, clinicians, geneticists, molecular biologists, and bioinformaticians. The paradigm features dynamic interplay among the participants. For example, in the LLMPP, the pathology panel reviews and validates all cases prior to study. However, we may return to the biopsies to investigate immunohistochemical surrogates of key molecules identified by gene expression profiling, which facilitates the translation of discoveries to the general practice setting (65–67). These data also are beginning to impact therapy, for example, informing the effective use of *BTK* inhibition in the ABC type of diffuse large B cell lymphoma but not in the GCB subtype (68). Most recently we have seen exciting inroads in the use of checkpoint inhibitors, which target the tumor microenvironment (69).

ACHIEVING INTERNATIONAL CONSENSUS

A major consequence of the new technologies in the 1990s was the rapid accumulation of knowledge about the immunophenotypic and genetic properties of normal and neoplastic lymphoid cells. Pathologists in the United States, Europe, and Asia were rapidly coming to the realization that they spoke a common language and agreed on major principles regarding the types of lymphoma they recognized in daily practice. They appreciated that neither the WF nor the Kiel classification entirely met their needs and that consensus could be reached on many issues.

This fact was appreciated by Peter Isaacson in the United Kingdom and Harald Stein in Germany, who undertook the organization of the International Lymphoma Study Group (ILSG) in 1990. The ILSG was founded to facilitate collaboration among hematopathologists around the world and break down the barriers that had been created by the controversy over the competing classification proposals. Each of the classification systems proposed in the 1970s was primarily the work of one or two experts, with the three principle schemes proposed by Rappaport, Lennert, and Lukes and Collins. The authors were deeply invested in the success of their own scheme and

unwilling to compromise or collaborate to achieve a single, comprehensive scheme. The pathologists who were invited to attend the first meeting of the ILSG in London were members of the next generation, more or less liberated from the work of their mentors. Initial consensus statements for the diagnosis of mantle cell lymphoma and nodular lymphocyte predominant Hodgkin's lymphoma were first steps in achieving international harmony (70, 71). The now 25 or so members have been meeting annually since that first meeting in London to present their work, share ideas, and discuss controversial or evolving issues. The membership has continued to evolve but has stayed small enough to facilitate discussion and open exchange of ideas. For me, ILSG has led to important professional friendships and collaborations. Additionally, I think the group has advanced the level of research in the field.

The founding of the ILSG led to the publication of the Revised European-American Classification of Lymphoid Neoplasms (REAL) classification of lymphomas in 1994 (72) and subsequently the World Health Organization (WHO) Classification of Tumours of the Hematopoietic and Lymphoid Tissues in 2001 (73). A preliminary draft of the diseases that could be recognized with current techniques was circulated among the group—consistent with the collaborative aspects of the ILSG—prior to the third ILSG meeting, held in Berlin in 1993 (**Figure 4**). Two members of the group were delegated to present the data related to each of the topics, which was followed by discussion to reach consensus. The subsequent REAL classification, published in *Blood*, quickly became the international standard (72). It became the most highly cited paper in all of clinical medicine over a period of 10 years and now has been cited nearly 7,000 times.

Even broader international participation was sought for the publication of the WHO classification. The first clinical advisory committee meeting was held in 1997, with participation from 44 hematologists, oncologists, and radiation therapists (**Figure 5**) (74). Seventy-five pathologists contributed as authors to the WHO monograph, the scope of which was expanded to include all hematological neoplasms of lymphoid, myeloid, histiocytic, and dendritic cell origins. The WHO classification created an international standard, with a single system embraced on a worldwide basis for the first time in history (73). Since the publication of the third edition, updates have been published in 2008 and in 2016, with the fully revised monograph to appear in 2017 (**Figure 6**) (75–77).

THE MICROSCOPE AS A TOOL FOR DISEASE DISCOVERY

Many of my seminal publications have had their inception in a clinical diagnostic case. My clinical and investigational studies have been intertwined to enhance our understanding of disease. I believe that disease discovery and disease definition are critical first steps in elucidating pathogenesis. Most insights into the molecular pathogenesis of lymphomas have followed on the heels of a precise description based on clinical, pathological, or immunophenotypic grounds. The discovery of the disease nearly always precedes the discovery of the gene. However, the process is iterative, such that knowledge of the genetic pathogenesis provides new diagnostic tools, which help define the disease and its borderlands.

This sequence is exemplified by anaplastic large cell lymphoma (ALCL), which was recognized on the basis of its characteristic morphology and immunophenotype, with strong staining for CD30 (79). This in turn led to the discovery of translocations involving *ALK* and *NPM* or other partner genes in ALCL, and the generation of monoclonal antibodies to the ALK protein, which could be used in routine diagnosis (80, 81). The subsequent morphological spectrum of ALCL was considerably broadened, beyond what was originally conceived as ALCL, now recognizing a small cell variant. However, some cases initially suspected as being part of ALCL, so-called Hodgkin-like ALCL, were excluded with the additional genetic information (82, 83).

Figure 4

Meeting of the International Lymphoma Study Group held in Berlin, Germany, in 1993. At this meeting, the group developed the consensus classification that came to be known as the Revised European-American Classification of Lymphoid Neoplasms (REAL) classification. (*Left to right*, *front row*) Harald Stein, Elaine Jaffe (author), Chris de Wolf Peeters, Peter Isaacson. (*Second row*) Miguel Piris, Nancy Lee Harris, Elisabeth Ralfkiaer. (*Third row*) Roger Warnke, Georges Delsol, Dan Knowles, Peter Banks. (*Fourth row*) Brunangelo Falini, John K.C. Chan, Hans Konrad Muller-Hermelink. (*Fifth row*) David Mason, Stefano Pileri, Kevin Gatter, Michael Cleary, Thomas Grogan.

Significantly, this knowledge has direct bearing on the clinical outcome and response to therapy, in comparison with other T cell lymphomas (79).

We know that Denis Burkitt (84, 85) recognized the key clinical features of endemic Burkitt lymphoma while riding around Africa in a jeep. Subsequently, pathologists were able describe its distinctive pathological features, leading to the recognition of Burkitt lymphoma in nonendemic areas (86, 87). Discovery of the association of Burkitt lymphoma with *MYC* may not have been possible without this accurate pathological and clinical description. ATLL was described as a clinical syndrome before discovery of the HTLV-I virus (88). Mantle cell lymphoma was recognized by pathologists, initially under different names (centrocytic lymphoma, lymphocytic lymphoma of intermediate differentiation), and proposed to be related to the lymphocyte cuff or mantle (89–91). Only later was the disease linked to *CCND1*/BCL1 (92). Each of these discoveries was facilitated by an accurate disease definition.

Figure 5

Clinical Advisory Committee meeting, Airlie, Virginia, November 3–5, 1997. A joint meeting was held for the discussion of lymphoid and myeloid neoplasms, including lymphomas and leukemia, in preparation for the publication of the World Health Organization monograph, published in 2001. Participants are named in the report of this meeting (74).

This scenario has been repeated over and over again and differs from the standard hypothesis-driven approach to research. It can be considered hypothesis-generating rather than hypothesis-driven. To investigate disease pathogenesis, you need to first accurately identify the subject of your investigation, or, as Carolus Linnaeus (1, p. 19), the father of taxonomy, noted, "The first step in wisdom is to know the things themselves; this notion consists in having a true idea of the objects; objects are distinguished and known by classifying them methodically and giving them appropriate names. Therefore, classification and name-giving will be the foundation of our science."

In the text that follows, I give a few additional examples from my own work, which illustrate how clinical and diagnostic observations led to further discovery. These anecdotes were included in the Maude Abbott Lecture I delivered at the one-hundredth meeting of the USCAP in 2011.

Follicular Lymphoma in Situ

The idea of malignant transformation of cells through a multistep process of accumulating genetic and molecular events is well accepted and recognized in solid tumors (93). However, in hematopathology, there are no designated benign lymphomas. Importantly, lymphocytes are not sessile cells. Lymphocytes are cells that naturally traffic and home throughout the lymphoid

Figure 6

Clinical Advisory Committee meeting, Chicago, Illinois, March 31–April 1, 2014, held in preparation for the revised fourth edition of the World Health Organization classification. Concurrent meetings were held to discuss (*a*) mature lymphoid neoplasms (participants named in 76) and (*b*) myeloid neoplasms and acute leukemias (participants named in 78).

system. A benign lymphoid neoplasm does not remain localized to be surgically excised, but rather it disseminates based on patterns of lymphocyte homing. This concept prompted me to propose, some years ago, that FL could be considered a benign lymphoma (94). Cytologically and immunophenotypically, the cells resembled the cells of the B cell follicle and spread to occupy normal germinal centers but did not invade privileged sites. However, the natural history was characterized by histological transformation to a high-grade process, with secondary and tertiary

genetic events. Our studies in recent years have focused on early lesions, both FL in situ (FLIS) and, more recently, mantle cell in situ.

We first described FLIS more than decade ago (95), but its clinical significance remained uncertain. The study began with a diagnostic case. In 1992, we received a lymph node biopsy in consultation. The patient was a 23-year-old female with an isolated inguinal lymph node. The pathologist who received the case thought it looked reactive, but in an overabundance of caution, he had sent it for cytogenetic testing, which disclosed a small population of cells with t(14;18)(q32;q21), which is characteristic of FL. He still did not see features of FL, and so the case arrived on my desk for a second review. We had recently received a monoclonal antibody to BCL2 prepared by David Mason and colleagues at Oxford (96), and we were beginning to explore its use in a series of cases. We decided to test it on this perplexing case. We observed just a single follicle filled with centrocytes strongly positive for the BCL2 protein, while the remainder of the germinal centers was appropriately BCL2-negative. We did not change our diagnosis of atypical hyperplasia, but made note of this unusual finding. By way of follow-up, the patient never developed clinical evidence of FL, most recently assessed in 2006.

We now know that the cells in FLIS, now termed in situ follicular neoplasia in the latest iteration of the WHO classification (76), are clonal and carry the *BCL2*/JH translocation (95). The lesion is seen in 2–3% of routine lymph node biopsies (97), but the risk of subsequent lymphoma is very low, on the order of 5% or less (98, 99). Interestingly, FLIS is more often seen in lymph nodes that are involved by another B cell lymphoma, but this may be a fortuitous finding, being the factor that prompts the lymph node biopsy (98, 100). Cells carrying the *BCL2* translocation also are found, using many rounds of sensitive PCR-based amplification, in normal peripheral blood in up to 70% of adults over the age of 50 (101, 102). These cells were termed FL-like B cells. Their numbers in blood increase with age and with pesticide or herbicide use in agricultural workers (103). The cells are memory B cells that have encountered the germinal center reaction and are prone to intense trafficking among lymph nodes (104). Histologically, FLIS is always encountered in a reactive lymph node with hyperplastic germinal centers; thus, some sort of immune stimulation may induce the circulation and homing of the cells to the germinal center environment. It is known that in patients with hepatitis C infection, the level of FL-like B cells rises in the blood with disease activity and is lowered with treatment of the underlying viral infection.

We and others have examined the genetic load of mutations in FLIS, in comparison with overt FL, and duodenal-type FL (105, 106). Notably, by array-based CGH, the cells of FLIS carry very few aberrations beyond the t(14;18). Among the most notable alterations, we observed and validated deletions of 1p36 and gains of the 7p and 12q chromosomes and related oncogenes, which include some of the most recurrent oncogenic alterations in overt FL (*TNFRSF14*; *EZH2*; and *MLL2*, currently known as *KMT2B*) (105).

Interestingly, duodenal-type FL had a similar low level of genetic complexity (105). It too has a low risk of progression and usually remains confined to the small intestine, without invasion or dissemination (107, 108). Patients can be managed conservatively, without need for aggressive therapy. Significantly, proper recognition of both duodenal-type FL and FLIS avoids unnecessary treatment and patient anxiety.

Lineage Plasticity in Human Lymphoid Neoplasms

Current models of hematopoietic cell differentiation suggest that as cells differentiate they become lineage-committed. However, some clinical data have shown that two hematopoietic populations in the same patient may share identical genetic changes, which raises the possibility that tumors expressing the phenotype of one hematopoietic lineage might transdifferentiate into

a genetically similar but phenotypically distinct tumor of a different lineage. We first reported examples of patients with lymphoblastic leukemias or lymphomas who also had histiocytic or dendritic cell (H/DC) tumors and demonstrated that the lymphoblastic neoplasm and the H/DC tumor from each patient were clonally related (109, 110). However, tumors of precursor cells display more lineage plasticity than mature lymphoid tumors. Reprogramming of mature human B cells into cells of a different lineage had not previously been shown, although this had been accomplished in murine models (111, 112).

We showed for the first time that human B cells of mature B cell neoplasms in both FL and CLL could be reprogrammed into H/DC neoplasms, probably through downregulation of the B cell transcription factor PAX5 (113, 114). In addition to loss of PAX5 in the H/DC tumors, they acquired expression of CEBP-beta and PU.1, which are transcription factors known to be expressed in cells of the histiocytic lineages. The subsequent H/DC tumors were shown to be clonally related to the underlying FL or CLL and carrying identical Ig heavy chain gene rearrangements and genetic aberrations. For example, the H/DC tumors arising in patients with FL had the t(14;18), as shown by FISH. Most of the H/DC tumors occurred some years after primary diagnosis, although treatment did not appear to play a role. In one case, the FL and H/DC tumors were diagnosed concordantly. Our study was published as a plenary paper in *Blood* and provided insight into the capacity for lineage plasticity in human cells (113). The work has implications beyond the hematopoietic system, with relevance for the generation of human stem cells. Again, this was a story that began with a single diagnostic case. When we saw the first histiocytic tumor in a patient with FL, we did not understand if the two tumors were related or whether this was purely coincidental. This was one of the cases that I called fascinoma, which I put aside for future study, and indeed, we encountered additional cases as time went by.

Classical Hodgkin's Lymphoma, the Elusive Reed-Sternberg Cell, and Mediastinal Gray Zone Lymphomas

Although Hodgkin's disease was the first of the malignant lymphomas to be described as a specific disease process, this unique cancer has been the source of many persistent questions over the past 180 years (115). One enigma has been the paucity of the Hodgkin and Reed-Sternberg (HRS) cells and their variants (thought to be the malignant cells in this disease) in comparison to the large numbers of normal inflammatory cells. This apparent imbalance raised questions about the pathogenesis of the disease process. Was it infectious or neoplastic? A second question concerned the nature of the Reed-Sternberg cell. The characterization of these cells had been frustrated by their scarcity in the tumor tissue (<1–2% of the total cell population) and the resultant difficulty in obtaining them in pure populations. Many theories had been put forth that included an origin from T cells, B cells, histiocytes, granulocytes, and interdigitating reticulum cells. In a series of papers in the early 1990s, we noted that classical Hodgkin's lymphoma often occurred sequentially or as composite lymphoma with a variety of other non-Hodgkin's lymphomas, nearly all of which were of B cell lineage (116–119). These studies provided key, early evidence for the B cell origin of Hodgkin's lymphoma. Definitive evidence came some years later when single-cell microdissection techniques were combined with PCR for Ig gene rearrangement to show that the HRS cells had clonal Ig rearrangement and evidence of somatic hypermutation (39, 120–123).

Subsequently, our studies focused on the association of nodular sclerosis classical Hodgkin's lymphoma (NSCHL) with primary mediastinal large B cell lymphoma (PMBL) (124). These tumors share many clinical and biological features such as increased frequency in females, mediastinal involvement, and genetic overlap (125, 126). There were also isolated instances in which both diagnoses were encountered in the same patient. In 1985, a 29-year-old male was

treated at the NCI for NSCHL with MOPP chemotherapy. The patient achieved remission, but one year later, he developed a new mediastinal mass. Was this a recurrence or a new malignancy, perhaps secondary to chemotherapy? A biopsy was performed and showed PMBL. Moreover, subsequent genetic studies showed that the HRS cells in the original NSCHL were clonally related to the cells of PMBL, which provided evidence that this was a manifestation of the original tumor (124). At about the same time, we encountered tumors that were challenging to categorize and had overlapping features of NSCHL and PMBL. We termed them mediastinal gray zone lymphoma and characterized them using a variety of immunophenotypic, epigenetic, and genomic approaches, which provided evidence for a common cell of origin and overlapping pathogenesis (127, 128). These studies showed evidence for common pathogenetic links between NSCHL and PMBL and provided a rationale for new therapeutic approaches, which were subsequently explored in clinical protocols at the NCI (129).

Natural Killer Cell Enteropathy

Sometimes a slide crosses your microscope stage and reveals something you have never seen before. This happened one day in 2002, when I received a colon biopsy from a 32-year-old male with superficial hemorrhagic lesions in the colon and terminal ileum. The submitting pathologist favored a diagnosis of NK-cell lymphoma, but the cells were negative for EBV, which argued against that diagnosis. Still, the case had been circulated to another consultant who made a diagnosis of NK-cell malignancy and recommended aggressive chemotherapy followed by autologous bone marrow transplantation. The patient also questioned the diagnosis. He had undergone routine colonoscopy for a family history of colon cancer and was asymptomatic. An active runner, his exercise routine was unchanged. Examining the slides, I saw an atypical NK-cell infiltrate largely confined to the lamina propria. Although the CD56-positive cells were highly atypical, there was no invasion, and I was reluctant to make a diagnosis of malignancy. The patient called me to discuss the diagnosis and ask my opinion about the appropriate course of action. I told him that although I could not provide a definite diagnosis, I recommended against therapy, and suggested that careful follow-up would soon determine if a more aggressive approach was warranted. Treatment was deferred; lesions have waxed and waned over a period of more than 10 years, without progression. The case was subsequently published and proposed to be an atypical NK-cell lymphocytosis (130). Over the next few years, we saw a number of similar cases, and published a larger series, in which we recommended the term NK-cell enteropathy (131). Interestingly, at the same time, pathologists in Japan saw a very similar condition that affected the stomach, which they termed lymphomatoid gastropathy (132). The etiology of this condition is unknown, although we suspect it is an unusual response to an unknown antigenic stimulus (133). The condition can affect the entire gastrointestinal tract. Some of the cases we saw were submitted from patients who had received chemotherapy but in whom the lesions recurred. Thus, although optimal management is not known, we recommend a conservative approach.

CONCLUSION

My career in pathology has been a voyage of discovery. Every day, I look forward to coming to work, not knowing what I might encounter. I still achieve great fulfillment from tackling a difficult diagnostic problem that has eluded others and reaching a diagnosis that will lead to appropriate therapy, if indicated, and a positive clinical outcome. Pathology is a lot like detective work—you have to put together all of the clues to reach the correct diagnosis. When you achieve that aha moment, it can be a lot of fun. Even more intriguing are the cases that do not seem to have an easy

answer but raise questions about underlying biology. These may prompt further studies, using the expanding array of tools available to the investigative pathologist.

I never lose sight of the importance of our work to the patient. From time to time, I receive a call from a patient or family member, telling me how much my diagnosis has altered the outcome of their case. Recently, I received a call from a physician whose son was initially diagnosed with a high-grade lymphoma some 10 years ago. They were ready to begin a Burkitt lymphoma regimen when I got the slides and realized that the diagnosis was incorrect—it was a pediatric-type FL, which could be treated with simple excision and follow-up. I made a last-minute call on a Friday night, just in time. The physician telephoned me a few months ago to report that her son, still well, had just graduated from college and that each day she was grateful for my intervention.

DISCLOSURE STATEMENT

The author is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

I would like to acknowledge my long-time collaborators at the National Cancer Institute, Drs. Stefania Pittaluga and Mark Raffeld, both of whom have been integral to my scientific accomplishments. I also would like to thank the members of the International Lymphoma Study Group; I have benefitted from their friendship, stimulating discussions, and frequent collaborations.

LITERATURE CITED

1. Linnaeus C. 1964 (1735). *Systema naturae, sive regna tria naturae systematice proposita per classes, ordines, genera, and species*, transl. MSJ Engel-Ledeboer, H Engel. Nieuwkopp, Neth.: Hes & De Graaf
2. Nowell PC. 1960. Phytohemagglutinin: an initiator of mitosis in cultures of normal human leukocytes. *Cancer Res.* 20:462–66
3. Cooper MD, Raymond DA, Peterson RD, South MA, Good RA. 1966. The functions of the thymus system and the bursa system in the chicken. *J. Exp. Med.* 123:75–102
4. Papermaster BW, Good RA. 1962. Relative contributions of the thymus and the bursa of Fabricius to the maturation of the lymphoreticur system and immunological potential in the chicken. *Nature* 196:838–40
5. Lay WH, Mendes NF, Bianco C, Nussenzweig V. 1971. Binding of sheep red blood cells to a large population of human lymphocytes. *Nature* 230:531–32
6. Jondal M, Holm G, Wigzell H. 1972. Surface markers on human T and B lymphocytes. I. A large population of lymphocytes forming nonimmune rosettes with sheep red blood cells. *J. Exp. Med.* 136:207–15
7. Lay WH, Nussenzweig V. 1968. Receptors for complement of leukocytes. *J. Exp. Med.* 128:991–1009
8. Shevach EM, Jaffe ES, Green I. 1973. Receptors for complement and immunoglobulin on human and animal lymphoid cells. *Transplant. Rev.* 16:3–28
9. Rappaport H. 1966. *Tumors of the Hematopoietic System.* Natl. Res. Counc. Comm. on Pathol. Atlas Tumor Pathol., sect. 3, fasc. 8. Washington, DC: Armed Forces Inst. Pathol.
10. Lennert K. 1969. Germinal centers and germinal center neoplasia. *Acta Haematol. Jpn.* 32:495–500
11. Jaffe ES, Shevach EM, Frank MM, Berard CW, Green I. 1974. Nodular lymphoma—evidence for origin from follicular B lymphocytes. *N. Engl. J. Med.* 290:813–19
12. Smith JL, Clein GP, Barker CR, Collins RD. 1973. Characterisation of malignant mediastinal lymphoid neoplasm (Sternberg sarcoma) as thymic in origin. *Lancet* 301:74–77
13. Sternberg C. 1916. Leukosarcomatose and myeloblasten leukamie. *Beitr. Anat. Pathol.* 61:75
14. Broome JD, Zucker-Franklin D, Weiner MS, Bianco C, Nussenzweig V. 1973. Leukemic cells with membrane properties of thymus-derived (T) lymphocytes in a case of Sézary's syndrome: morphologic and immunologic studies. *Clin. Immunol. Immunopathol.* 1:319–29

15. Mann RB, Jaffe ES, Braylan RC, Eggleston JC, Ransom L, et al. 1975. Immunologic and morphologic studies of T cell lymphoma. *Am. J. Med.* 58:307–13
16. Lennert K, Mohri N. 1974. Histologische Klassifizierung and Vorkommen des M. Hodgkin. *Internist* 15:57–65
17. Lennert K, Stein H, Kaiserling E. 1975. Cytological and functional criteria for the classification of malignant lymphomata. *Br. J. Cancer Suppl.* 2:29–43
18. Lennert K. 1974. Letter: origin of malignant lymphomas. *Lancet* 2:586
19. Suchi T, Lennert K, Tu L-Y. 1987. Histopathology and immunohistochemistry of peripheral T cell lymphomas: a proposal for their classification. *J. Clin. Pathol.* 40:995–1015
20. Trumper LH, Brittinger G, Diehl V, Harris NL. 2004. Non-Hodgkin's lymphoma: a history of classification and clinical observations. In *Non-Hodgkin's Lymphomas*, ed. PM Mauch, JO Armitage, B Coiffier, R Dalla-Favera, NL Harris, pp. 3–19. Philadelphia: Lippincott, Williams and Wilkins
21. International Non-Hodgkin's Lymphoma Prognostic Factors Project. 1993. A predictive model for aggressive non-Hodgkin's lymphoma. *N. Engl. J. Med.* 329:987–94
22. Kohler G, Milstein C. 1975. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* 256:495–97
23. McMichael AJ, Pilch JR, Galfre G, Mason DY, Fabre JW, Milstein C. 1979. A human thymocyte antigen defined by a hybrid myeloma monoclonal antibody. *Eur. J. Immunol.* 9:205–10
24. Bernard A, Boumsell L. 1984. The clusters of differentiation (CD) defined by the First International Workshop on Human Leucocyte Differentiation Antigens. *Hum. Immunol.* 11:1–10
25. Warnke RA, Gatter KC, Falini B, Hildreth P, Woolston RE, et al. 1983. Diagnosis of human lymphoma with monoclonal antileukocyte antibodies. *N. Engl. J. Med.* 309:1275–81
26. Nowell PC, Hungerford DA. 1961. Chromosome studies in human leukemia. II. Chronic granulocytic leukemia. *J. Natl. Cancer Inst.* 27:1013–35
27. Zech L, Haglund U, Nilsson K, Klein G. 1976. Characteristic chromosomal abnormalities in biopsies and lymphoid-cell lines from patients with Burkitt and non-Burkitt lymphomas. *Int. J. Cancer* 17:47–56
28. Fukuhara S, Rowley JD, Variakojis D, Golomb HM. 1979. Chromosome abnormalities in poorly differentiated lymphocytic lymphoma. *Cancer Res.* 39:3119–28
29. Yunis JJ, Oken MM, Kaplan ME, Ensrud KM, Howe RR, Theologides A. 1982. Distinctive chromosomal abnormalities in histologic subtypes of non-Hodgkin's lymphoma. *N. Engl. J. Med.* 307:1231–36
30. Taub R, Kirsch I, Morton C, Lenoir G, Swan D, et al. 1982. Translocation of the c-*myc* gene into the immunoglobulin heavy chain locus in human Burkitt lymphoma and murine plasmacytoma cells. *PNAS* 79:7837–41
31. Taub R, Moulding C, Battey J, Murphy W, Vasicek T, et al. 1984. Activation and somatic mutation of the translocated c-*myc* gene in Burkitt lymphoma cells. *Cell* 36:339–48
32. Tsujimoto Y, Cossman J, Jaffe E, Croce CM. 1985. Involvement of the bcl-2 gene in human follicular lymphoma. *Science* 228:1440–43
33. Tsujimoto Y, Jaffe E, Cossman J, Gorham J, Nowell PC, Croce CM. 1985. Clustering of breakpoints on chromosome 11 in human B-cell neoplasms with the t(11;14) chromosome translocation. *Nature* 315:340–43
34. Rosenberg C, Wong E, Petty E, Bale A, Tsujimoto Y, et al. 1991. *PRAD1*, a candidate *BCL1* oncogene: mapping and expression in centrocytic lymphoma. *PNAS* 88:9638–42
35. Raffeld M, Jaffe ES. 1991. bcl-1, t(11;14), and mantle cell-derived lymphomas. *Blood* 78:259–63
36. Arnold A, Cossman J, Bakhshi A, Jaffe ES, Waldmann TA, Korsmeyer SJ. 1983. Immunoglobulin-gene rearrangements as unique clonal markers in human lymphoid neoplasms. *N. Engl. J. Med.* 309:1593–99
37. Kuppers R, Zhao M, Hansmann ML, Rajewsky K. 1993. Tracing B cell development in human germinal centres by molecular analysis of single cells picked from histological sections. *EMBO J.* 12:4955–67
38. Kuppers R, Klein U, Hansmann ML, Rajewsky K. 1999. Cellular origin of human B-cell lymphomas. *N. Engl. J. Med.* 341:1520–29
39. Tamaru J, Hummel M, Zemlin M, Kalvelage B, Stein H. 1994. Hodgkin's disease with a B-cell phenotype often shows a VDJ rearrangement and somatic mutations in the VH genes. *Blood* 84:708–15
40. Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, et al. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 239:487–91

41. Haralambieva E, Kleiverda K, Mason DY, Schuuring E, Kluin PM. 2002. Detection of three common translocation breakpoints in non-Hodgkin's lymphomas by fluorescence in situ hybridization on routine paraffin-embedded tissue sections. *J. Pathol.* 198:163–70
42. Epstein MA, Achong BG, Barr YM. 1964. Virus particles in cultured lymphoblasts from Burkitt's lymphoma. *Lancet* 283:702–3
43. Jaffe ES, Chan JKC, Su IJ, Frizzera G, Mori S, et al. 1996. Report of the workshop on nasal and related extranodal angiocentric T/NK cell lymphomas: definitions, differential diagnosis, and epidemiology. *Am. J. Surg. Pathol.* 20:103–11
44. Delecluse HJ, Anagnostopoulos I, Dallenbach F, Hummel M, Marafioti T, et al. 1997. Plasmablastic lymphomas of the oral cavity: a new entity associated with the human immunodeficiency virus infection. *Blood* 89:1413–20
45. Quintanilla-Martinez L, Kumar S, Fend F, Reyes E, Teruya-Feldstein J, et al. 2000. Fulminant EBV^+ T-cell lymphoproliferative disorder following acute/chronic EBV infection: a distinct clinicopathologic syndrome. *Blood* 96:443–51
46. Uchiyama T, Yodoi J, Sagawa K, Takatsuki K, Uchino H. 1977. Adult T-cell leukemia: clinical and hematologic features of 16 cases. *Blood* 50:481–92
47. Poiesz B, Ruscetti F, Gazdar A, Bunn PA, Minna JD, Gallo RC. 1980. Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. *PNAS* 77:7415–19
48. Blattner WA, Blayney DW, Robert-Guroff M, Sarngadharan MG, Kalyanaraman VS, et al. 1983. Epidemiology of human T-cell leukemia/lymphoma virus. *J. Infect. Dis.* 147:406–16
49. Blayney DW, Blattner WA, Robert-Guroff M, Jaffe ES, Fisher RI, et al. 1983. The human T-cell leukemia-lymphoma virus in the southeastern United States. *JAMA* 250:1048–52
50. Jaffe ES, Blattner WA, Blayney DW, Bunn PA Jr., Cossman J, et al. 1984. The pathologic spectrum of adult T-cell leukemia/lymphoma in the United States. Human T-cell leukemia/lymphoma virus-associated lymphoid malignancies. *Am. J. Surg. Pathol.* 8:263–75
51. Cesarman E, Chang Y, Moore PS, Said JW, Knowles DM. 1995. Kaposi's sarcoma–associated herpesvirus-like DNA sequences in AIDS-related body-cavity-based lymphomas. *N. Engl. J. Med.* 332:1186–91
52. Oksenhendler E, Boulanger E, Galicier L, Du MQ, Dupin N, et al. 2002. High incidence of Kaposi sarcoma–associated herpesvirus-related non-Hodgkin lymphoma in patients with HIV infection and multicentric Castleman disease. *Blood* 99:2331–36
53. Dupin N, Fisher C, Kellam P, Ariad S, Tulliez M, et al. 1999. Distribution of human herpesvirus-8 latently infected cells in Kaposi's sarcoma, multicentric Castleman's disease, and primary effusion lymphoma. *PNAS* 96:4546–51
54. Du MQ, Diss TC, Liu H, Ye H, Hamoudi RA, et al. 2002. KSHV- and EBV-associated germinotropic lymphoproliferative disorder. *Blood* 100:3415–18
55. Weinstock DM, Dalla-Favera R, Gascoyne RD, Leonard JP, Levy R, et al. 2015. A roadmap for discovery and translation in lymphoma. *Blood* 125:2175–77
56. Dave SS, Fu K, Wright GW, Lam LT, Kluin P, et al. 2006. Molecular diagnosis of Burkitt's lymphoma. *N. Engl. J. Med.* 354:2431–42
57. Lenz G, Wright GW, Emre NC, Kohlhammer H, Dave SS, et al. 2008. Molecular subtypes of diffuse large B-cell lymphoma arise by distinct genetic pathways. *PNAS* 105:13520–25
58. Iqbal J, Wright G, Wang C, Rosenwald A, Gascoyne RD, et al. 2014. Gene expression signatures delineate biological and prognostic subgroups in peripheral T-cell lymphoma. *Blood* 123:2915–23
59. Rosenwald A, Wright G, Wiestner A, Chan WC, Connors JM, et al. 2003. The proliferation gene expression signature is a quantitative integrator of oncogenic events that predicts survival in mantle cell lymphoma. *Cancer Cell* 3:185–97
60. Lardelli P, Bookman MA, Sundeen J, Longo DL, Jaffe ES. 1990. Lymphocytic lymphoma of intermediate differentiation. Morphologic and immunophenotypic spectrum and clinical correlations. *Am. J. Surg. Pathol.* 14:752–63

61. Tiemann M, Schrader C, Klapper W, Dreyling MH, Campo E, et al. 2005. Histopathology, cell proliferation indices and clinical outcome in 304 patients with mantle cell lymphoma (MCL): a clinicopathological study from the European MCL Network. *Br. J. Haematol.* 131:29–38
62. Dave SS, Wright G, Tan B, Rosenwald A, Gascoyne RD, et al. 2004. Prediction of survival in follicular lymphoma based on molecular features of tumor-infiltrating immune cells. *N. Engl. J. Med.* 351:2159–69
63. Steidl C, Lee T, Shah SP, Farinha P, Han G, et al. 2010. Tumor-associated macrophages and survival in classic Hodgkin's lymphoma. *N. Engl. J. Med.* 362:875–85
64. Nicolae A, Abdullah S, Davies-Hill T, Pittaluga S, Jaffe ES. 2014. EBV^+ B cell lymphomas (BCL) in young patients without immunodeficiency. *Proc. 17th Meet. Eur. Assoc. Haematopathol.*, pp. 84–85. Istanbul: Eur. Assoc. Haematopathol.
65. Hans CP, Weisenburger DD, Greiner TC, Gascoyne RD, Delabie J, et al. 2004. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. *Blood* 103:275–82
66. Mozos A, Royo C, Hartmann E, De Jong D, Baro C, et al. 2009. SOX11 expression is highly specific for mantle cell lymphoma and identifies the cyclin D1-negative subtype. *Haematologica* 94:1555–62
67. Soldini D, Valera A, Sole C, Palomero J, Amador V, et al. 2014. Assessment of SOX11 expression in routine lymphoma tissue sections: characterization of new monoclonal antibodies for diagnosis of mantle cell lymphoma. *Am. J. Surg. Pathol.* 38:86–93
68. Wilson WH, Young RM, Schmitz R, Yang Y, Pittaluga S, et al. 2015. Targeting B cell receptor signaling with ibrutinib in diffuse large B cell lymphoma. *Nat. Med.* 21:922–26
69. Ansell SM, Lesokhin AM, Borrello I, Halwani A, Scott EC, et al. 2015. PD-1 blockade with nivolumab in relapsed or refractory Hodgkin's lymphoma. *N. Engl. J. Med.* 372:311–19
70. Banks P, Chan J, Cleary M, Delson G, De Wolf-Peeters C, et al. 1992. Mantle cell lymphoma: a proposal for unification of morphologic, immunologic, and molecular data. *Am. J. Surg. Pathol.* 16:637–40
71. Mason D, Banks P, Chan J, Cleary M, Delsol G, et al. 1994. Nodular lymphocyte predominance Hodgkin's disease: a distinct clinico-pathological entity. *Am. J. Surg. Pathol.* 18:528–30
72. Harris NL, Jaffe ES, Stein H, Banks PM, Chan JK, et al. 1994. A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. *Blood* 84:1361–92
73. Jaffe ES, Harris NL, Stein H, Vardiman J. 2001. *Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, Fr. IARC Press
74. Harris NL, Jaffe ES, Diebold J, Flandrin G, Muller-Hermelink HK, et al. 1999. World Health Organization classification of neoplastic diseases of the hematopoietic and lymphoid tissues: report of the Clinical Advisory Committee meeting, Airlie House, Virginia, November 1997. *J. Clin. Oncol.* 17:3835–49
75. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, et al. 2008. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, Fr.: Int. Agency Res. Cancer
76. Swerdlow SH, Campo E, Pileri SA, Harris NL, Stein H, et al. 2016. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. *Blood* 127:2375–90
77. Campo E, Swerdlow SH, Harris NL, Pileri S, Stein H, Jaffe ES. 2011. The 2008 WHO classification of lymphoid neoplasms and beyond: evolving concepts and practical applications. *Blood* 117:5019–32
78. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, et al. 2016. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood* 127:2391–405
79. Jaffe ES. 2001. Anaplastic large cell lymphoma: the shifting sands of diagnostic hematopathology. *Mod. Pathol.* 14:219–28
80. Morris SW, Kirstein MN, Valentine MB, Dittmer KG, Shapiro DN, et al. 1994. Fusion of a kinase gene, *ALK*, to a nucleolar protein gene, *NPM*, in non-Hodgkin's lymphoma. *Science* 263:1281–84
81. Pulford K, Lamant L, Morris SW, Butler LH, Wood KM, et al. 1997. Detection of anaplastic lymphoma kinase (ALK) and nucleolar protein nucleophosmin (NPM)–ALK proteins in normal and neoplastic cells with the monoclonal antibody ALK1. *Blood* 89:1394–404
82. Leoncini L, Del Vecchio M, Kraft R, Megha T, Barbini P, et al. 1990. Hodgkin's disease and CD30-positive anaplastic large cell lymphomas—a continuous spectrum of malignant disorders. *Am. J. Pathol.* 137:1047–57

83. Zinzani PL, Martelli M, Magagnoli M, Zaccaria A, Ronconi F, et al. 1998. Anaplastic large cell lymphoma Hodgkin's-like: a randomized trial of ABVD versus MACOP-B with and without radiation therapy. *Blood* 92:790–94
84. Burkitt D. 1958. A sarcoma involving the jaws in African children. *Br. J. Surg.* 46:218–23
85. Burkitt DP. 1983. The discovery of Burkitt's lymphoma. *Cancer* 51:1777–86
86. Wright DH. 1963. Cytology and histochemistry of the Burkitt lymphoma. *Br. J. Cancer* 17:50–55
87. Mann RB, Jaffe ES, Braylan RC, Nanba K, Frank MM, et al. 1976. Non-endemic Burkitts's lymphoma. A B-cell tumor related to germinal centers. *N. Engl. J. Med.* 295:685–91
88. Uchiyama T, Yodoi J, Sagawa K, Takatsuki K, Uchino H. 1977. Adult T-cell leukemia: clinical and hematologic features of 16 cases. *Blood* 50:481–92
89. Jaffe ES, Braylan RC, Nanba K, Frank MM, Berard CW. 1977. Functional markers: a new perspective on malignant lymphomas. *Cancer Treat. Rep.* 61:953–62
90. Jaffe ES, Bookman MA, Longo DL. 1987. Lymphocytic lymphoma of intermediate differentiation–mantle zone lymphoma: a distinct subtype of B-cell lymphoma. *Hum. Pathol.* 18:877–80
91. Swerdlow S, Habeshaw J, Murray L, Dhaliwal H, Lister T, Stansfeld A. 1983. Centrocytic lymphoma: a distinct clinicopathologic and immunologic entity. *Am. J. Pathol.* 113:181–97
92. Campo E, Raffeld M, Jaffe ES. 1999. Mantle-cell lymphoma. *Semin. Hematol.* 36:115–27
93. Cho KR, Vogelstein B. 1992. Genetic alterations in the adenoma-carcinoma sequence. *Cancer* 70:1727–31
94. Jaffe ES. 1983. Follicular lymphomas: possibility that they are benign tumors of the lymphoid system. *J. Natl. Cancer Inst.* 70:401–3
95. Cong P, Raffeld M, Teruya-Feldstein J, Sorbara L, Pittaluga S, Jaffe ES. 2002. In situ localization of follicular lymphoma: description and analysis by laser capture microdissection. *Blood* 99:3376–82
96. Gaulard P, d'Agay M, Peuchmaur M, Brousse N, Gisselbrecht C, et al. 1992. Expression of the *bcl-2* gene product in follicular lymphoma. *Am. J. Pathol.* 140:1089–95
97. Henopp T, Quintanilla-Martinez L, Fend F, Adam P. 2011. Prevalence of follicular lymphoma in situ in consecutively analysed reactive lymph nodes. *Histopathology* 59:139–42
98. Jegalian AG, Eberle FC, Pack SD, Mirvis M, Raffeld M, et al. 2011. Follicular lymphoma in situ: clinical implications and comparisons with partial involvement by follicular lymphoma. *Blood* 118:2976–84
99. Bermudez G, Gonzalez de Villambrosia S, Martinez-Lopez A, Batlle A, Revert-Arce JB, et al. 2016. Incidental and isolated follicular lymphoma in situ and mantle cell lymphoma in situ lack clinical significance. *Am. J. Surg. Pathol.* 40:943–49
100. Montes-Moreno S, Castro Y, Rodriguez-Pinilla SM, Garcia JF, Mollejo M, et al. 2010. Intrafollicular neoplasia/in situ follicular lymphoma: review of a series of 13 cases. *Histopathology* 56:658–62
101. Mamessier E, Broussais-Guillaumot F, Chetaille B, Bouabdallah R, Xerri L, et al. 2014. Nature and importance of follicular lymphoma precursors. *Haematologica* 99:802–10
102. Roulland S, Lebailly P, Lecluse Y, Heutte N, Nadel B, Gauduchon P. 2006. Long-term clonal persistence and evolution of t(14;18)-bearing B cells in healthy individuals. *Leukemia* 20:158–62
103. Agopian J, Navarro JM, Gac AC, Lecluse Y, Briand M, et al. 2009. Agricultural pesticide exposure and the molecular connection to lymphomagenesis. *J. Exp. Med.* 206:1473–83
104. Roulland S, Sungalee JS, Morgado E, Mamessier E, Gregoire E, et al. 2012. Iterative germinal center re-entries of memory B-cells with t(14;18) translocation and early steps of follicular lymphoma progression. *Blood* 120:150
105. Mamessier E, Song JY, Eberle FC, Pack S, Drevet C, et al. 2014. Early lesions of follicular lymphoma: a genetic perspective. *Haematologica* 99:481–88
106. Schmidt J, Salaverria I, Haake A, Bonzheim I, Adam P, et al. 2014. Increasing genomic and epigenomic complexity in the clonal evolution from in situ to manifest t(14;18)-positive follicular lymphoma. *Leukemia* 28:1103–12
107. Ganapathi KA, Pittaluga S, Odejide OO, Freedman AS, Jaffe ES. 2014. Early lymphoid lesions: conceptual, diagnostic and clinical challenges. *Haematologica* 99:1421–32
108. Schmatz AI, Streubel B, Kretschmer-Chott E, Puspok A, Jager U, et al. 2011. Primary follicular lymphoma of the duodenum is a distinct mucosal/submucosal variant of follicular lymphoma: a retrospective study of 63 cases. *J. Clin. Oncol.* 29:1445–51

109. Feldman AL, Berthold F, Arceci RJ, Abramowsky C, Shehata BM, et al. 2005. Clonal relationship between precursor T-lymphoblastic leukaemia/lymphoma and Langerhans-cell histiocytosis. *Lancet Oncol.* 6:435–37
110. Feldman AL, Minniti C, Santi M, Downing JR, Raffeld M, Jaffe ES. 2004. Histiocytic sarcoma after acute lymphoblastic leukaemia: a common clonal origin. *Lancet Oncol.* 5:248–50
111. Xie H, Ye M, Feng R, Graf T. 2004. Stepwise reprogramming of B cells into macrophages. *Cell* 117:663–76
112. Cobaleda C, Jochum W, Busslinger M. 2007. Conversion of mature B cells into T cells by dedifferentiation to uncommitted progenitors. *Nature* 449:473–77
113. Feldman AL, Arber DA, Pittaluga S, Martinez A, Burke JS, et al. 2008. Clonally related follicular lymphomas and histiocytic/dendritic cell sarcomas: evidence for transdifferentiation of the follicular lymphoma clone. *Blood* 111:5433–39
114. Shao H, Xi L, Raffeld M, Feldman AL, Ketterling RP, et al. 2011. Clonally related histiocytic/dendritic cell sarcoma and chronic lymphocytic leukemia/small lymphocytic lymphoma: a study of seven cases. *Mod. Pathol.* 24:1421–32
115. Hodgkin T. 1832. On some morbid experiences of the absorbent glands and spleen. *Medico-Chir. Trans.* 17:68–97
116. Jaffe ES, Zarate-Osorno A, Medeiros LJ. 1992. The interrelationship of Hodgkin's disease and non-Hodgkin's lymphomas—lessons learned from composite and sequential malignancies. *Semin. Diagn. Pathol.* 9:297–303
117. Gonzalez CL, Medeiros LJ, Jaffe ES. 1991. Composite lymphoma: a clinicopathologic analysis of nine patients with Hodgkin's disease and B-cell non-Hodgkin's lymphoma. *Am. J. Clin. Pathol.* 96:81–89
118. Zarate-Osorno A, Medeiros LJ, Longo DL, Jaffe ES. 1992. Non-Hodgkin's lymphomas arising in patients successfully treated for Hodgkin's disease. A clinical, histologic, and immunophenotypic study of 14 cases. *Am. J. Surg. Pathol.* 16:885–95
119. Zarate-Osorno A, Medeiros LJ, Kingma DW, Longo DL, Jaffe ES. 1993. Hodgkin's disease following non-Hodgkin's lymphoma. A clinicopathologic and immunophenotypic study of nine cases. *Am. J. Surg. Pathol.* 17:123–32
120. Brauninger A, Hansmann ML, Strickler JG, Dummer R, Burg G, et al. 1999. Identification of common germinal-center B-cell precursors in two patients with both Hodgkin's disease and non-Hodgkin's lymphoma. *N. Engl. J. Med.* 340:1239–47
121. Kuppers R, Zhao M, Rajewsky K, Hansmann ML. 1993. Detection of clonal B cell populations in paraffin-embedded tissues by polymerase chain reaction. *Am. J. Pathol.* 143:230–39
122. Kuppers R, Rajewsky K, Zhao M, Simons G, Laumann R, et al. 1994. Hodgkin disease: Hodgkin and Reed-Sternberg cells picked from histological sections show clonal immunoglobulin gene rearrangements and appear to be derived from B cells at various stages of development. *PNAS* 91:10962–66
123. Kanzler H, Kuppers R, Hansmann ML, Rajewsky K. 1996. Hodgkin and Reed-Sternberg cells in Hodgkin's disease represent the outgrowth of a dominant tumor clone derived from (crippled) germinal center B cells. *J. Exp. Med.* 184:1495–505
124. Traverse-Glehen A, Pittaluga S, Gaulard P, Sorbara L, Alonso MA, et al. 2005. Mediastinal gray zone lymphoma: the missing link between classic Hodgkin's lymphoma and mediastinal large B-cell lymphoma. *Am. J. Surg. Pathol.* 29:1411–21
125. Joos S, Otano-Joos MI, Ziegler S, Bruderlein S, du Manoir S, et al. 1996. Primary mediastinal (thymic) B-cell lymphoma is characterized by gains of chromosomal material including 9p and amplification of the *REL* gene. *Blood* 87:1571–78
126. Steidl C, Gascoyne RD. 2011. The molecular pathogenesis of primary mediastinal large B-cell lymphoma. *Blood* 118:2659–69
127. Eberle FC, Rodriguez-Canales J, Wei L, Hanson JC, Killian JK, et al. 2011. Methylation profiling of mediastinal gray zone lymphoma reveals a distinctive signature with elements shared by classical Hodgkin's lymphoma and primary mediastinal large B-cell lymphoma. *Haematologica* 96:558–66
128. Eberle FC, Salaverria I, Steidl C, Summers TA Jr., Pittaluga S, et al. 2011. Gray zone lymphoma: chromosomal aberrations with immunophenotypic and clinical correlations. *Mod. Pathol.* 24:1586–97

129. Wilson WH, Pittaluga S, Nicolae A, Camphausen K, Shovlin M, et al. 2014. A prospective study of mediastinal gray-zone lymphoma. *Blood* 124:1563–69
130. Vega F, Chang CC, Schwartz MR, Preti HA, Younes M, et al. 2006. Atypical NK-cell proliferation of the gastrointestinal tract in a patient with antigliadin antibodies but not celiac disease. *Am. J. Surg. Pathol.* 30:539–44
131. Mansoor A, Pittaluga S, Beck PL, Wilson WH, Ferry JA, Jaffe ES. 2011. NK-cell enteropathy: a benign NK-cell lymphoproliferative disease mimicking intestinal lymphoma: clinicopathologic features and follow-up in a unique case series. *Blood* 117:1447–52
132. Takeuchi K, Yokoyama M, Ishizawa S, Terui Y, Nomura K, et al. 2010. Lymphomatoid gastropathy: a distinct clinicopathologic entity of self-limited pseudomalignant NK-cell proliferation. *Blood* 116:5631–37
133. Bennett MS, Round JL, Leung DT. 2015. Innate-like lymphocytes in intestinal infections. *Curr. Opin. Infect. Dis.* 28:457–63