Follicular lymphomas: a tapestry of common and contrasting threads

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The vast majority of human B-cell lymphomas are derived from germinal center B cells. These include most diffuse large B-cell lymphomas (DLBCL), Burkitt's lymphoma, and an expanding spectrum of neoplasms with a follicular growth pattern included under the broad heading of follicular lymphoma (FL). Germinal centers form within pre-existent primary follicles in response to exogenous antigen.[1] The ensuing response involves marked B-cell proliferation which, coupled with somatic hypermutation (SHM) and class-switch recombination (CSR) mediated by activation-induced cytidine deaminase (AID), creates a potential for genomic instability and chromosomal translocations. Aberrant SHM can also lead to mutations in proto-oncogenes.[1] The targets of these genetic accidents are most often genes involved in B-cell development and function, such as *BCL6*, that may be subject to SHM physiologically.[2]

In contrast, the *BCL2/IGH@* translocation, which is the hallmark of classical FL, is thought to occur very early in B-cell development as an error in V(D)J recombination in a bone marrow derived pre-B cell.[3] This initiating event occurs outside the germinal center compartment, and does not involve either SHM or AID. However, the mature progeny home to germinal centers throughout the lymphoid system, and in early stages are often largely confined to the germinal centers in lymph nodes and extranodal sites.[4]

Follicular lymphoma is defined as a neoplasm composed of germinal center B cells that recapitulates the cellular composition and architecture of the normal lymphoid follicle.[5] Histologically, the neoplastic follicles are composed not only of clonal germinal center B cells, e.g. centrocytes and centroblasts, but other essential elements including follicular dendritic cells, follicular T-helper cells and macrophages. More than 85% of FLs are associated with the *BCL2/IGH@* translocation and occur almost exclusively in adults over 18 years of age. They are unified by similarities in clinical presentation and course, despite some variations in the cytological grade. Nearly all cases show overexpression of the BCL2 protein and express high levels of CD10. Bone marrow involvement is common at presentation, and long-term eradication of disease is very difficult to achieve, despite a generally indolent clinical course.

In recent years, the histological and clinical spectrum of germinal center derived B-cell neoplasms with a follicular growth pattern has expanded, leading to the conclusion that FL is more than a single disease entity (Table 1). This heterogeneity first became apparent with the analysis of cases of FL, classified as Grade 3B, and composed exclusively of centroblasts. These tumors were often associated with areas of DLBCL in the same nodal mass. Early studies showed the rarity of the *BCL2/IGH@* translocation but the role of BCL6 translocations has been less clear.[6,7] A somewhat similar story was encountered in 'high-grade' FL negative for CD10. These cases were nearly always negative for *BCL2/IGH@*, showed high expression of MUM1/IRF4, and often had amplifications of *BCL6*.[8] Karube *et al.* reported that the CD10-/MUM1+ FL were more often diagnosed in the elderly.[9] The basis for the activated B-cell phenotype in these cases is not clear, but aberrations in *IRF4* have not been demonstrated. IRF4 is physiologically up-regulated in late centrocytes within the germinal center.[10]

Strong expression of MUM1/IRF4 also was reported in another subset of B-cell lymphoma, but in this instance linked to translocations involving the *IRF4* gene.[11] Salaverria *et al.* reported this genetic anomaly in both DLBCL and FL, all of which were Grade 3, with most of the cases occurring in children and young adults. They noted a predilection for the head and neck, including Waldeyer's ring. The propensity for involvement of Waldeyer's ring was confirmed by Liu *et al.* who reported 7 cases of pediatric FL with strong expression of MUM1/IRF4, 6 of whom presented in tonsil, with breaks in *IRF4* confirmed in 3 cases.[12]

Conventional FL is exceedingly rare in children, despite the frequent occurrence of florid follicular hyperplasia in this age group. However, the WHO classification recognized a pediatric variant of FL with distinctive features.[13] Most often localized, pediatric FL can show involvement of extranodal sites, including the gastrointestinal tract and testis.[14,15] Most cases are cytologically high grade, with a component of DLBCL reported in some cases. Chromosomal translocations involving *BCL2* are absent, and the protein is generally also negative.[16] Most reported cases originated from pediatric trials either in the United State or Europe, and patients had been treated aggressively on standard protocols.[14,16] Outcome was highly favorable with only rare recurrences.

The clinical profile of the pediatric variant of FL was expanded by two recent reports, largely based on cases submitted in consultation and not entered on clinical trials.[12,17] In these two series, nearly all of the patients with pediatric-type FL presented with localized lymph node involvement, most often involving lymph nodes of the head and neck region. A marked male predominance was seen. The treatment was heterogeneous, but a number of patients had been treated with simple surgical excision without recurrence.

Cytologically, the vast majority were Grade 3 with a high proliferation rate. Both series reported large expansile follicles, often with a serpiginous growth pattern. Liu *et al.* noted that the cytological composition was atypical for FL, and that the cells were neither classical centrocytes nor centroblasts, but more medium-sized blastoid cells.[12] The cases of pediatric-type FL were readily distinguished from occasional cases of 'usual FL' occurring in patients under the age of 30 years.[12] The 'usual FL' had a higher median age (24 *vs.* 14) with no case under the age of 18, a slight predominance in females, and expression of BCL2 in more than 80%. Louissaint *et al.* reported that they encountered occasional cases of pediatric-type FL in older adults, and that the disease was not restricted to the pediatric age group.[17] Thus, this pediatric-type of FL was emerging as a clonal B-cell disorder of uncertain malignant potential, and both groups suggested a conservative therapeutic approach for these cases.

Knowledge of the molecular pathogenesis of pediatric-type FL has been very limited. Aside from the cases with *IRF4* translocations, studies of the usual suspects, such as *BCL2*, *BCL6*, and *MYC* generally have been negative.[12,16,17] In the cur-

Table 1. Germinal center derived B-cell neoplasms with a follicular growth pattern.

Variant	Clinical Features	Immunophenotype	Common Genomic Features
Follicular lymphoma (prototype)	Adults, M=F, Advanced Stage	BCL2+, CD10+, MUM1-, BCL6+	*BCL2/IGH@* + *BCL6* R frequent *TNFRSF14* mutation/deletion
Follicular lymphoma, 3B (may contain DLBCL)	Adults, M=F, often Advanced Stage	BCL2 and CD10 often – MUM1 often + BCL6+	*BCL6* gains, *BCL6R* (subset) *MYC* R occasional
FL, pediatric type, nodal	Children, young adults M >> F Often localized	BCL2-, CD10+, MUM1-, BCL6+	*TNFRSF14* mutation/deletion (subset) (*current report*)
FL, pediatric type, testicular	Children (M) Stage I	BCL2-, CD10+, MUM1, BCL6+	*BCL6* R (subset)
FL, pediatric type, tonsillar	Children, young adults; M=F	BCL2+, CD10+, MUM1+, BCL6+	*IRF4/IGH@* R *BCL6* split in a subset *BCL2* R negative
Primary cutaneous follicle center lymphoma	M=F, Adults ; Skin, mainly head, neck and upper trunk	BCL2-, BCL6+, CD10+/-, MUM1-	*BCL2* R negative No recurrent aberrations

M: male; F: female; R: gene rearrangement.

rent report from Martin-Guerro *et al.*, further insights into the underlying genetic lesions of at least a subset of these cases begin to emerge.[18] The authors used two strategies to comprehensively screen for recurrent genetic aberrations in pediatric follicular lymphomas: array comparative genomic hybridization (array-CGH) and molecular inversion probe (MIP) analysis. The MIP assay allows the interrogation of thousands of single nucleotide polymorphisms (SNPs) in a high throughput fashion. Potential point mutations detected by the MIP assay were also analyzed by direct sequencing. Finally, multiple FISH probes were utilized to investigate potential gene fusions or breaks. The authors excluded from this series cases of pediatric FL with known *IRF4* aberrations that had been the subject of an earlier report.[11]

Based on this survey of 18 cases (not all of whom could be examined by the full complement of techniques) the authors identified two groups of cases. The FL component in nearly all of the cases in both groups was classified as Grade 3. One group of 11 cases contained one or more genomic aberrations, whereas 7 cases lacked any detectable genetic abnormality. The cases without aberrations were more likely to be localized, all Stage I or II, and consistently lacked any component of DLBCL. Approximately one-third of the cases with genomic aberrations had areas of DLBCL and were more likely to have advanced stage disease. Therapy was instituted independent of genomic status, but nearly all patients had a good outcome; the only patient who suffered a relapse had a *BCL6* split, with *IGH@* being the likely partner. Given the clinical differences in the two groups, a potential strategy for the future would be to reserve aggressive therapy for those cases with aberrations, most of whom had more advanced disease, and to employ a 'watch and wait' strategy for most Stage I pediatric-type FL, following surgical excision. Notably, 4 of the 18 cases were treated with only surgical excision (3 cases) or rituximab (1 case), and all remain in complete remission.

Importantly, the most common genetic aberration identified involved loss of heterozygosity at 1p36 associated with mutations in *TNFRSF14* identified in 7 of 11 cases with aberrations. Loss of 1p36 is one of the most frequent secondary genetic events in FL of the usual type, with a high incidence of mutations affecting the *TNFRSF14* gene in two recent reports.[19,20] *TNFRSF14* is a member of the tumor necrosis factor receptor superfamily, but how its loss might contribute to FL pathogenesis has not been fully clarified. It may function as a tumor suppressor gene. There are conflicting data regarding the clinical significance of these alterations in adult FL,[19,20] but, based on the current report, mutations do not appear to confer an adverse prognosis in pediatric FL. Interestingly, deletions of 1p36 also were identified with high frequency in another rare variant of t(14;18)-negative FL that has a diffuse growth pattern and presents as localized bulky, most commonly inguinal, lymph node masses.[21] Thus, this aberration links pediatric-type FL with at least two other clinical and histological forms of FL.

EZH2 mutations were seen in 2 cases, both of whom had a component of DLBCL, where the mutation has been most often encountered in prior studies.[22] These and other data suggest that FL with a component of DLBCL, and pure FL (either pediatric type or Grade 3B) should be distinguished, and may in fact differ from inception, rather than the component of DLBCL representing histological transformation.[6]

The current report expands our understanding of the pediatric variant of FL and provides data linking it to other germinal center-derived neoplasms. What remains to be determined is how these patients should be managed, especially those with localized Stage I disease. It may be that pediatric-type FL lacking genomic aberrations should be considered a clonal B-cell proliferation of undetermined clinical significance, analogous to follicular lymphoma *in situ*, monoclonal B lymphocytosis, and monoclonal gammopathy of undetermined significance.[23]

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References

1. Klein U, Dalla-Favera R. Germinal centres: role in B-cell physiology and malignancy. Nat Rev Immunol. 2008;8(1):22-33.
2. Pasqualucci L, Migliazza A, Fracchiolla N, William C, Neri A, Baldini L, et al. BCL-6 mutations in normal germinal center B cells: evidence of somatic hypermutation acting outside Ig loci. Proc Natl Acad Sci USA. 1998;95(20):11816-21.
3. Roulland S, Faroudi M, Mamessier E, Sungalee S, Salles G, Nadel B. Early steps of follicular lymphoma pathogenesis. Adv Immunol. 2011;111:1-46.
4. Jegalian AG, Eberle FC, Pack SD, Mirvis M, Raffeld M, Pittaluga S, et al. Follicular lymphoma in situ: clinical implications and comparisons with partial involvement by follicular lymphoma. Blood. 2011;118(11):2976-84.
5. Jaffe ES, Harris NL, Stein H, Isaacson PG. Classification of lymphoid neoplasms: the microscope as a tool for disease discovery. Blood. 2008;112(12):4384-99.
6. Horn H, Schmelter C, Leich E, Salaverria I, Katzenberger T, Ott MM, et al. Follicular lymphoma grade 3B is a distinct neoplasm according to cytogenetic and immunohistochemical profiles. Haematologica. 2011;96(9):1327-34.
7. Harris NL, Kluin P. Follicular lymphoma grade 3B: is it a real disease? Haematologica. 2011;96(9):1244-6.
8. Karube K, Ying G, Tagawa H, Niino D, Aoki R, Kimura Y, et al. BCL6 gene amplification/3q27 gain is associated with unique clinicopathological characteristics among follicular lymphoma without BCL2 gene translocation. Mod Pathol. 2008;21(8):973-8.
9. Karube K, Guo Y, Suzumiya J, Sugita Y, Nomura Y, Yamamoto K, et al. CD10-MUM1+ follicular lymphoma lacks BCL2 gene translocation and shows characteristic biologic and clinical features. Blood. 2007;109(7):3076-9.
10. Falini B, Fizzotti M, Pucciarini A, Bigerna B, Marafioti T, Gambacorta M, et al. A monoclonal antibody (MUM1p) detects expression of the MUM1/IRF4 protein in a subset of germinal center B cells, plasma cells, and activated T cells. Blood. 2000;95(6):2084-92.
11. Salaverria I, Philipp C, Oschlies I, Kohler CW, Kreuz M, Szczepanowski M, et al. Translocations activating IRF4 identify a subtype of germinal-center-derived B-cell lymphoma affecting predominantly children and young adults. Blood. 2011;118(1):139-47.
12. Liu Q, Salaverria I, Pittaluga S, Jegalian AG, Xi L, Siebert R, et al. Follicular lymphomas in children and young adults: a comparison of the pediatric variant with usual follicular lymphoma. Am J Surg Pathol. 2013;37(3):333-43.
13. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al. WHO classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th ed. Lyon, France: International Agency for Research on Cancer, 2008.
14. Lorsbach RB, Shay-Seymore D, Moore J, Banks PM, Hasserjian RP, Sandlund JT, et al. Clinicopathologic analysis of follicular lymphoma occurring in children. Blood. 2002;99(6):1959-64.
15. Finn LS, Viswanatha DS, Belasco JB, Snyder H, Huebner D, Sorbara L, et al. Primary follicular lymphoma of the testis in childhood. Cancer. 1999;85(7):1626-35.
16. Oschlies I, Salaverria I, Mahn F, Meinhardt A, Zimmermann M, Woessmann W, et al. Pediatric follicular lymphoma--a clinico-pathological study of a population-based series of patients treated within the Non-Hodgkin's Lymphoma--Berlin-Frankfurt-Munster (NHL-BFM) multicenter trials. Haematologica. 2010;95(2):253-9.
17. Louissaint A Jr, Ackerman AM, Dias-Santagata D, Ferry JA, Hochberg EP, Huang MS, et al. Pediatric-type nodal follicular lymphoma: an indolent clonal proliferation in children and adults with high proliferation index and no BCL2 rearrangement. Blood. 2012;120(12):2395-404.
18. Martin-Guerrero I, Salaverria I, Burkhardt B, Szczepanowski M, Baudis M, Bens S, et al. Recurrent loss of heterozygosity in 1p36 associated with TNFRSF14 mutations in IRF4 translocation negative pediatric follicular lymphomas. Haematologica. 2013 Feb 26. [Epub ahead of print]
19. Cheung KJ, Johnson NA, Affleck JG, Severson T, Steidl C, Ben-Neriah S, et al. Acquired TNFRSF14 mutations in follicular lymphoma are associated with worse prognosis. Cancer Res. 2010;70(22):9166-74.
20. Launay E, Pangault C, Bertrand P, Jardin F, Lamy T, Tilly H, et al. High rate of TNFRSF14 gene alterations related to 1p36 region in de novo follicular lymphoma and impact on prognosis. Leukemia. 2012;26(3):559-62.
21. Katzenberger T, Kalla J, Leich E, Stocklein H, Hartmann E, Barnickel S, et al. A distinctive subtype of t(14;18)-negative nodal follicular non-Hodgkin lymphoma characterized by a predominantly diffuse growth pattern and deletions in the chromosomal region 1p36. Blood. 2009;113(5):1053-61.
22. Kridel R, Sehn LH, Gascoyne RD. Pathogenesis of follicular lymphoma. J Clin Invest. 2012;122(10):3424-31.
23. Campo E, Swerdlow SH, Harris NL, Pileri S, Stein H, Jaffe ES. The 2008 WHO classification of lymphoid neoplasms and beyond: evolving concepts and practical applications. Blood. 2011;117(19):5019-32.

CD30-positive malignant lymphomas: time for a change of management?

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In this issue of Haematologica, Zinzani *et al.* describe their experience with the anti-CD30 antibody-drug conjugate brentuximab vedotin in patients with relapsed/refractory Hodgkin's lymphoma. A total of 65 heavily pretreated patients who had been treated in a named patient program in nine Italian centers were analyzed.[1] Overall, 70.7% of these patients responded with 21.5% achieving complete remission. The progression-free survival rate at 20 months was 24.2% while the overall survival rate was 73.8%. Given the extensive pre-treatment with a median of four prior chemotherapy regimens, autologous or allogeneic stem cell transplantation in 92.3% of patients, and more than 50% refractory to their most recent therapy, these results are encouraging and in line with similar reports by other groups. Importantly, the treatment was also well tolerated. There is an additional short report in this issue in which Sabattini *et al.* describe CD30 expression in peripheral T-cell lymphomas.[2] They report that 43.2% (83/192) of the cases investigated expressed CD30. These data contribute to the growing body of evidence on a changing landscape in the treatment of CD30-positive malignant lymphomas.

Since the initial description of monoclonal antibodies against Hodgkin and Sternberg-Reed (HRS) cells in Hodgkin's lymphoma,[3,4] the CD30 antigen has attracted substantial scientific interest. Initially termed Ki-1, this antigen was clustered as CD30 showing a very strong expression on the malignant cells in Hodgkin's lymphoma. Importantly, only a few activated lymphocytes and eosinophils physiologically express this antigen and there is very little cross-reactivity with vital organs.[5,6] Shortly thereafter, CD30 was also found on the malignant cells of anaplastic large cell lymphoma (ALCL) and other malignant lymphomas. ALCL is an aggressive T-cell lymphoma representing about 1% of all lymphatic neoplasias.[7] Whereas in tissue samples from patients with Hodgkin's