10. Shen WF, Montgomery JC, Rozenfeld S, et al. AbdB-like Hox proteins stabilize DNA binding by the Meis1 homeodomain proteins. Mol Cell Biol. 1997;17(11):6448-6458.
11. Huang Y, Sitwala K, Bronstein J, et al. Identification and characterization of Hoxa9 binding sites in hematopoietic cells. Blood. 2012;119(2):388-398.
12. Garcia-Cuellar MP, Steger J, Füller E, et al. Pbx3 and Meis1 cooperate through multiple mechanisms to support Hox-induced murine leukemia. Haematologica. 2015;100(7):905-913.
13. Aulisa L, Forraz N, McGuckin C, Hartgerink JD. Inhibition of cancer cell proliferation by designed peptide amphiphiles. Acta Biomater. 2009;5(3):842-853.
14. Carroll SB. Homeotic genes and the evolution of arthropods and chordates. Nature. 1995;376(6540):479-485.
15. Argiropoulos B, Humphries RK. Hox genes in hematopoiesis and leukemogenesis. Oncogene. 2007;26(47):6766-6776.
16. Pineault N, Helgason CD, Lawrence HJ, Humphries RK. Differential expression of Hox, Meis1, and Pbx1 genes in primitive cells throughout murine hematopoietic ontogeny. Exp Hematol. 2002;30(1):49-57.
17. Shen WF, Krishnan K, Lawrence HJ, Largman C. The HOX homeodomain proteins block CBP histone acetyltransferase activity. Mol Cell Biol. 2001;21(21):7509-7522.
18. Ohno Y, Yasunaga S, Janmohamed S, et al. Hoxa9 transduction induces hematopoietic stem and progenitor cell activity through direct down-regulation of geminin protein. PLoS One. 2013;8(1):e53161.
19. Pinsonneault J, Florence B, Vaessin H, McGinnis W. A model for extradenticle function as a switch that changes HOX proteins from repressors to activators. EMBO J.1997;16(8):2032-2042.
20. Shen WF, Rozenfeld S, Kwong A, Köm ves LG, Lawrence HJ, Largman C. HOXA9 forms triple complexes with PBX2 and MEIS1 in myeloid cells. Mol Cell Biol. 1999;19(4):3051-3061.
21. Schnabel CA, Jacobs Y, Cleary ML. HoxA9-mediated immortalization of myeloid progenitors requires functional interactions with TALE cofactors Pbx and Meis. Oncogene. 2000;19(5):608-616.
22. Moskow JJ, Bullrich F, Huebner K, Daar IO, Buchberg AM. Meis1, a PBX1-related homeobox gene involved in myeloid leukemia in BXH-2 mice. Mol Cell Biol. 1995;15(10):5434-5443.
23. DiMartino JF, Selleri L, Traver D, et al. The Hox cofactor and proto-oncogene Pbx1 is required for maintenance of definitive hematopoiesis in the fetal liver. Blood. 2001;98(3):618-626.
24. Dickson GJ, Liberante FG, Kettyle LM, et al. HOXA/PBX3 knockdown impairs growth and sensitizes cytogenetically normal acute myeloid leukemia cells to chemotherapy. Haematologica. 2013;98(8):1216-1225.

Rituximab maintenance therapy in diffuse large B-cell lymphoma: is XY the most important variable?

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In B-cell non-Hodgkin lymphomas (NHL) rituximab has extended the disease-free intervals of hundreds of thousands of patients. At the inception of rituximab a considerable amount of academic vigor was invested in finding the appropriate dose and frequency during induction therapy. This was followed by consideration of rituximab maintenance or extended dosing strategies. However, if maintenance rituximab does not significantly improve treatment outcomes it only represents expensive plasma. An integral step in harnessing the excitement for maintenance rituximab is to look for patients' characteristics that can help to tailor or risk-adapt rituximab dose and/or duration of use with the goal of providing benefit to all. The primary endpoint of interest, improvement in overall survival, has only been seen in meta-analyses, leaving surrogate markers of benefit, such as event-free survival and progression-free survival in trials, to be debated at podiums and in patients' examination rooms without a clear consensus being reached.[1]

The original report that triggered the spark of enthusiasm for maintenance rituximab was published by Dr. Ghielmini and colleagues and concerned patients with follicular lymphoma (FL) in whom prolonged rituximab treatment extended the duration of remission.[2] The use of rituximab in FL subsequently expanded as results of randomized trials emerged showing remission prolongation with maintenance rituximab after single agent rituximab and combined rituximab-chemotherapy and then similar results in mantle cell lymphoma.[3-7] A theme began to develop: rituximab maintenance was most useful in B-cell NHL subtypes in which the majority of patients do not have durable remissions. However, in diffuse large B-cell lymphoma (DLBCL), the most common NHL, in which the majority of patients who achieve a complete remission after rituximab-chemotherapy are cured, maintenance rituximab therapy has not been felt to be efficacious.

Nevertheless, Huang and colleagues reported a randomized trial of maintenance rituximab in patients with an objective response after six cycles of R-CHOP-14 (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone). The maintenance rituximab was administered monthly during the first 12 months and once every 3 months during the second year.[8] Patients who received maintenance rituximab had a progression-free survival rate at 5 years of 45% compared to 34% in the patients who were observed (P=0.006). The overall survival rate at 5 years was 62% with maintenance rituximab and 49% with observation (P=0.03). Maintenance rituximab improved the progression-free and overall survival of patients in all International Prognostic Index groupings. The lower progression-free and overall survival rates might be expected and were probably related to the fact that all patients who had an objective response (i.e., not just complete remissions) were included in the analysis. In this study, the results were not reported by gender, so it is not possible to determine whether the observed benefit was greater in males than in females.

In a subsequent, larger randomized trial carried out by the Eastern Cooperative Oncology Group (EGOG; ECOG 4494) in the USA, patients over 60 years of age with DLBCL were randomly assigned to receive R-CHOP or CHOP; there was then a second randomization to maintenance rituximab or no maintenance rituximab.[9] Thus, this was a four-arm study including patients who received R-CHOP and no maintenance rituximab, R-CHOP with maintenance rituximab, CHOP with maintenance rituximab, and CHOP without maintenance rituximab. The results were comparable within the three groups who

received rituximab during induction, as maintenance, or both, but distinctly inferior in the patients who never received rituximab. The fact that maintenance rituximab did not add to the outcome when administered after rituximab during induction has been taken as strong evidence that maintenance rituximab was superfluous in patients who achieved a remission with R-CHOP. Interestingly, a subsequent analysis of this study demonstrated that men did less well than woman in those arms given any rituximab (3 arms), but there was no sex difference in the patients who received only CHOP (1 arm).[10]

In 2012, the German High Grade non-Hodgkin Lymphoma Study Group (GHLSG) reported that men >60 years old had more rapid rituximab clearance than women.[11] They proposed that the resultant higher plasma rituximab levels in women might be the explanation for the observed superior treatment outcome with R-CHOP in women than in men. The same group tested this hypothesis in a pharmacokinetic study in which eight doses of rituximab were administered on specific days in combination with CHOP-14 in the SMARTE–R-CHOP-14 study. They found that this seemed to eliminate the poor outcome in men that had been seen previously without the intensified rituximab therapy.[12]

More recently the GHLSG tested a higher dose of rituximab in men in the SEXIE-R-CHOP-14 study.[13] This study investigated six cycles of R-CHOP-14 dosed at 500 mg/m^2 in men, but the dose of rituximab in women was the standard 375 mg/m^2. Pharmacokinetic assays were included within the study and showed that men achieved higher peak levels of rituximab, but their total exposure to the drug was approximately the same as that of women because of more rapid drug clearance. The 3-year progression-free survival in men was 74% *versus* 68% in women (*P*=NS); the overall survival was also not significantly different.

So how do the above data integrate with those of the NHL13 trial reported by Jaeger *et al.* in this issue of Haematologica?[14] First of all, it is important to consider the specifics of the NHL13 trial. This was an industry-supported trial that included not only patients with DLBCL, but also those with follicular FL grade IIIb. Only a small fraction of patients had central review of the initial biopsy, but the International Prognostic Index score was available for everyone. Furthermore, it appears that some patients with FL grade III, especially FL grade IIIb have aggressive lymphoma that responds to therapy similarly to DLBCL. Nevertheless, the pathological grading of FL is not precise.[15] Some patients with FL grade IIIa will have a course similar to that seen in patients with grade 1-2 FL. In those patients, maintenance rituximab would be expected to prolong remission duration on average but most would eventually relapse. However, since only about 3% of patients in the study by Jaeger *et al*. had FL grade IIIb, it is unlikely that this greatly affected the results.

What the authors did discover in a prospective fashion is that maintenance rituximab appeared to improve the outcome in men but not women, with no obvious impact of age on the outcome - something that was not tested in prior trials. The authors concluded that maintenance rituximab eliminated the previously found poorer outcome in men with DLBCL. This study also found that rituximab improved the treatment outcome for patients who had bone marrow involvement by NHL. We cannot tell from the paper how many of these patients had large cell lymphoma in the marrow rather than only small cell involvement. Whether this is a statistical "quirk" because of the large number of analyses done, or a real finding is not clear. Approximately 10% of the patients in the study had bone marrow involvement.

How do we interpret and apply all this information? There is increasing evidence that sex may play a part in DLBCL outcomes: in particular, men >60 years of age are relatively under-dosed with the "standard" dose of rituximab compared to women. They might, therefore, benefit from a higher dose or longer duration of treatment with rituximab. Does this mean that we should consider maintenance rituximab in men with DLBCL or only in those >60 years old? From a practical point of view, giving a higher dose of rituximab, such as the 500 mg/m^2 administered by the GHLSG as part of R-CHOP, would be easier and more cost-effective (i.e., less total rituximab and fewer treatment visits) if the results were comparable, but this has been tested only in R-CHOP-14 and not in R-CHOP-21, which is more commonly used. This begs the question, based on the available data, should we change practice immediately? Although there are controversial aspects of this information, we believe both men and women should be made aware of the data and that older men should be offered a higher dose of rituximab or maintenance. Women on the other hand should not be offered a higher dose or rituximab maintenance after treatment for DLBCL. Whether payers will buy into this approach is unknown, but as the data now stand we believe it is a reasonable approach supported by facts not anecdotes.

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References

1. Wang Y, Shen Y, Yang F, et al. Rituximab maintenance therapy in B-cell lympoma: a meta-analysis. J Clin Oncol 2015;22 (15s):462s. Abstract 8851.
2. Ghielmini M, Schmitz SF, Cogliatti SB, Pichert G, Hummerjohann J, Waltzer U, et al. Prolonged treatment with rituximab in patients with follicular lymphoma significantly increases event-free survival and response duration compared with the standard weekly x 4 schedule. Blood. 2004;103(12):4416-4423.
3. Ardeshna KM, Qian W, Smith P, et al. Rituximab versus a watch-and-wait approach in patients with advanced-stage, asymptomatic, non-bulky follicular lymphoma: an open-label randomised phase 3 trial. Lancet Oncol. 2014;15(4):424-435.
4. Hochster H, Weller E, Gascoyne RD, et al. Maintenance rituximab after cyclophosphamide, vincristine, and prednisone prolongs progression-free survival in advanced indolent lymphoma: results of the randomized phase III ECOG1496 Study. J Clin Oncol. 2009;27(10):1607-1614.
5. Salles G, Seymour JF, Offner F, et al. Rituximab maintenance for 2 years in patients with high tumour burden follicular lymphoma responding to rituximab plus chemotherapy (PRIMA): a phase 3, randomised controlled trial. Lancet. 2011;377(9759):42-51.
6. Vitolo U, Ladetto M, Boccomini C, et al. Rituximab maintenance compared with observation after brief first-line R-FND chemoimmunotherapy with rituximab consolidation in patients age older than 60 years with advanced follicular lymphoma: a phase III randomized study by the Fondazione Italiana Linfomi. J Clin Oncol. 2013;31(27):3351-3359.

7. Kluin-Nelemans HC, Hoster E, Hermine O, et al. Treatment of older patients with mantle-cell lymphoma. N Engl J Med. 2012;9;367(6):520-531.
8. Huang BT, Zeng QC, Yu J, et al. How to determine post-RCHOP therapy for risk-tailored adult patients with diffuse large B-cell lymphoma, addition of maintenance rituximab or observation: multicenter experience. J Cancer Res Clin Oncol. 2012;138(1):125-132.
9. Habermann TM, Weller EA, Morrison VA, et al. Rituximab-CHOP versus CHOP alone or with maintenance rituximab in older patients with diffuse large B-cell lymphoma. J Clin Oncol. 2006;24(19):3121-3127.
10. Habermann TM HF, Morrison V, et al. Differences in outcomes in males and females with diffuse large B-cell lymphoma with induction rituximab and follicular lymphoma treated with maintenance rituximab. Blood (ASH Annual Meeting Abstracts) 2012 120: Abstract 3705.
11. Muller C, Murawski N, Wiesen MH, et al. The role of sex and weight on rituximab clearance and serum elimination half-life in elderly patients with DLBCL. Blood. 2012;119(14):3276-3284.
12. Pfreundschuh M, Poeschel V, Zeynalova S, et al. Optimization of rituximab for the treatment of diffuse large B-cell lymphoma (II): extended rituximab exposure time in the SMARTE-R-CHOP-14 trial of the German High-Grade non-Hodgkin Lymphoma Study Group. J Clin Oncol. 2014;32(36):4127-4133.
13. Pfeundschuh M, Held G, Zeynalova S, et al. Increased rituximab (R) doses and effect on risk of elderly male patients with aggressive CD20+ B-cell lymphomas: results of the SEXIE-R-CHOP-14 trial of the DSHNHL. J Clin Oncol. 32:5s, 2014 (suppl; abstr 8501).
14. Jaeger U, Trneny M, Melzer H, et al. Rituximab maintenance for patients with aggressive B-cell lymphoma in first remission: results of the randomized NHL13 trial. Haematologica. 2015;100(7):955-963.
15. A clinical evaluation of the International Lymphoma Study Group classification of non-Hodgkin's lymphoma. The Non-Hodgkin's Lymphoma Classification Project. Blood. 1997;89(11):3909-3918.

Personalized medicine in adult acute lymphoblastic leukemia

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Knowledge concerning acute lymphoblastic leukemia (ALL) has increased greatly,[1] and personalized medicine has become a reality. More sophisticated diagnostic procedures, including immunophenotyping, cytogenetics, molecular genetics, and new genomics, have allowed the definition of new ALL sub-entities which, in some cases, has translated into specific therapies. A great achievement is the possibility of evaluating minimal residual disease (MRD), which can now be done in about 95% of ALL patients. MRD is the most important prognostic factor and thus a major component of a personalized treatment algorithm. Progress has also come from targeted therapies, extending the existing backbones of chemotherapy and stem cell transplantation (SCT). Targeted therapy in Philadelphia chromosome-positive ALL (Ph^+ ALL) with tyrosine kinase inhibitors (TKI) and immunotherapy with monoclonal antibodies targeting surface antigens expressed on leukemic blast cells have extended the armamentarium. A new promising approach is the activation of patients' T cells directed against their own leukemic blast cells either through a bispecific antibody, or chimeric antigen receptor modified T cells.

Diagnostics

Immunophenotyping is still the most important diagnostic feature, separating B-lineage ALL (~75%) from T-lineage ALL (~25%), and their subtypes according to the stage of maturation/differentiation (Table 1).

Other diagnostic techniques are standard cytogenetics, fluorescence *in situ* hybridization, and reverse transcriptase polymerase chain reaction. These methods allow the detection of Ph^+ ALL, with the chromosomal translocation t(9;22)(q34;q11), and the detection of the corresponding *BCR-ABL1* gene rearrangement. Further ALL entities that have been identified are t(4;11)(q21;q23)/*MLL-AFA4*, abn11q23/*MLL*, and t(1;19) (q23;p13)/*PBX-E2A*.

Gene expression profiling, single nucleotide polymorphism array analysis, array-comparative genomic hybridization, and next generation sequencing recognize newly defined ALL entities with poor prognosis: Ph-like ALL, and early T precursor ALL.

Ph-like ALL, also called *BCR-ABL1*-like ALL, is characterized by genetic lesions similar to Ph^+ ALL, associated with *IKZF1* deletion, *CLRF2* overexpression and tyrosine kinase activating rearrangements involving *ABL1*, *JAK2*, *PDGFRB* and several other genes.[2,3] The frequency is 10% in children and 25-30% in young adults, but does not increase further with age.[4] Treatment could be directed at the underlying genetic pattern with BCR-ABL inhibitors (e.g. dasatinib) or JAK2 inhibitors (e.g. ruxolitinib).[5]

Early T precursor ALL is characterized by lack of CD1a and CD8, weak CD5 expression, at least one myeloid/stem cell marker, a specific transcriptional profile and the possible involvement of several critical genes.[6] No new treatment approaches are currently available for this subtype, and thus SCT in first complete remission is the preferred option.

Minimal residual disease

MRD is the detection of residual leukemic cells, not detectable by light microscopy.

Methods for determining MRD are based on the detection of leukemia-specific aberrant immunophenotypes by flow cytometry, the evaluation of leukemia-specific rearranged immunoglobulin or T-cell receptor sequences by real-time quantitative polymerase chain reaction, or the detection of fusion genes associated with chromosomal abnormalities (e.g., *BCR-ABL*, *MLL-AF4*). The detection limit with these methods is 10^{-3}-10^{-5} (0.1%– 0.001%). The phenotypic aberrations are unique to each patient with ALL and can be detected in up to 95% of individuals.

Methods for MRD evaluation and standardization of MRD quantification have been extensively described.[7,8]

Minimal residual disease response and terminology

Molecular response can be evaluated only for patients in