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The role of monoclonal antibodies in the therapeutic strategy of chronic lymphocytic leukemia

Monoclonal antibodies (MoAb) have led to a profound shift in the therapeutic scenario of CLL. Alemtuzumab and rituximab are the most active MoAbs to date, and their single-agent activity has been established both in previously untreated and in relapsed patients with CLL. MoAbs act through unique mechanisms distinct from conventional cytotoxic chemotherapy: engagement of restricted cell-surface antigens, activation of intracellular pro-apoptotic signaling pathways, and activation of effector functions, including components of the complement system and antibody-dependent cellular cytotoxicity through the activation of natural killer cells and macrophages through their IgG fragment C receptors.[1] The highest response rates and the better quality of response thanks to eradication of minimal residual disease (MRD), but not yet cure, have been reported with chemoimmunotherapy combinations, namely fludarabine-cyclophosphamide-rituximab (FCR) and fludarabine-campath (FluCam).[2] However, randomized studies of chemoimmunotherapy versus chemotherapy alone are needed to further substantiate the superiority of MoAb-containing associations. Rituximab is a highly affinity chimeric mouse anti-CD20 MoAb, currently approved for the treatment of B-cell non-Hodgkin lymphomas. Clinical single-agent efficacy of rituximab has been shown in CLL and small lymphocytic lymphoma (SLL), even though with a substancially lower response rate compared to other low-grade non-Hodgkin lymphomas. In the pivotal study in relapsed low grade B-cell lymphomas, patients with SLL achieved only 13% overall response rate (ORR) with rituximab monotherapy, whereas in patients with follicular lymphoma the ORR was 58%.[3] Further studies showed limited activity of rituximab as single-agent in previously treated CLL patients, with a partial response (PR) rate of 10 to 15% at conventional doses.[4] This was probably only partially due to the low expression of CD20 antigen on the surface of CLL cells, the more plausible reason being instead the inability of rituximab at standard dose to saturate CD20 molecules present on large tumor masses, but also in blood as a soluble antigen.[5] Intensified dose regimens were in fact able to increase the efficacy of rituximab: Byrd e*t al.*[6] reported an improved ORR of 52% in untreated CLL patients, giving the conventional dose of 375 mg/m^2 three times a week rather than once a week. Moreover, a 75% response rate was observed by O'Brien and coworkers in patients receiving a

dose as high as six times the standard dose of rituximab (2250 mg/m^2).[7]

In vitro studies demonstrated synergy between rituximab and fludarabine in B-cell lines resistant to the cytotoxic activity of either drug alone. Moreover, fludarabine was able to down-regulate the expression of the complement protection proteins CD55 and CD59, thus sensitizing malignant lymphocytes to the rituximab-mediated complement lysis.[2,8] Following to these findings, also clinical trials in low grade lymphomas showed high efficacy of chemo-immunotherapy, providing the rationale for the combination of fludarabine and rituximab also in CLL.

The experience of the Cancer and Leukemia Group B (CALGB), confirmed that the addition of rituximab to fludarabine regimen (FR) improves the CR rate in CLL patients. Furthermore, in this randomized study it appeared also clear that the two drugs guarantee higher response rate when concurrently administrated, compared to a sequential regimen (OR 90%, CR 47% *vs.* OR 77%, CR 28%).[9] Recently however, the same authors pointed out that high-risk CLL patients characterized by unmutated IgVH genes or high-risk interphase cytogenetics, including either del(17p) or del(11q), appear to have a significantly shorter PFS and OS with this chemoimmunotherapy and that this finding justifies a risk-adapted therapy.[10]

Taking into account that fludarabine and cyclophosphamide are more effective than fludarabine alone[11] (a result very recently confirmed by a UK randomized trial[12]), and considering the aforementioned synergy between fludarabine and rituximab, the combined used of FC and rituximab was soon assessed in different phase II studies, both in treated and untreated CLL patients.

In untreated CLL patients, fludarabine was given at 25 mg/m^2 and cyclophosphamide at 250 mg/m^2, both intravenously, daily for 3 days. Rituximab was administered on day 1 of each cycle at 375 mg/m^2 intravenously during cycle 1 and then at 500 mg/m^2 intravenously during subsequent cycles, up to a total of 6 cycles. 224 previously untreated patients were enrolled in this MD Anderson trial, achieving an ORR of 95% and a complete response rate of 70%. The trial also evaluated the quality of response in terms of molecular remission: 78% of patients in CR achieved a MRD-negative remission in the marrow, assessed by flow cytometry (<1% CD5/CD19 positive marrow lymphocytes). Remission were durable, with no evidence of disease progression at 5 years in 68% of patients.[2,13]

The same regimen was tested in previously treated patients relapsed o refractory mainly to fludarabine treatment.[14] Among 177 patients, 73% responded to treatment and 25% achieved complete response. Interestingly, the majority of patients in CR had negative flow cytometry for MRD on marrow aspirate. Remissions were durable with a median duration of 28 months.

The combination FCR seems to be well tolerated, rituximab not adding to the toxicity of chemotherapy, with the exception of a higher rate of neutropenia.

Nevertheless, the occurrence of unexplained delayed peripheral blood cytopenia, mainly neutropenia, after rituximab have been recently reported.[15] This finding and its relationship with a possible rituximab-induced myelodysplasia need to be further evaluated.

Compared to the modest activity of rituximab monotherapy, better efficacy in CLL was shown for the humanized monoclonal antibody alemtuzumab or campath-1H, which binds specifically to the CD52 antigen. CD52 is abundantly expressed on the surface of normal and abnormal B- and T-lymphocytes, monocytes and macrophages and on a small percentage (<5%) of granulocytes, but not on erythrocytes, platelets, or bone marrow stem cells.[16]

It the 1999 pivotal trial, Keating *et al.* recruited 93 CLL-patients who had been heavily pretreated with different chemotherapy regimens, including fludarabine (CAM 211 trial). Campath was applied at a dose of 30 mg intravenously 3 times a week, for a maximum of 12 weeks. In addition, premedication with diphenhydramine and acetaminophen and an infection prophylaxis against herpes viruses and *Pneumocystis carinii* were administered. An overall response rate of 33% including 2% CRs was observed. The median duration of response was of 8.7 months, while median time to progression was of 4.7 months for all patients, and of 9.5 months for responders. The median survival time for all patients was 16 months and 32 for responders. The main toxicities were mild infusion-related side effects such as rigor, fever, nausea, vomiting, rash and fatigue, which occurred predominantly during the first week of therapy. About half of the patients developed transient thrombocytopenia, neutropenia or infections, which were severe (grade 3 and 4) in around 20% of patients.[17] Peculiar adverse effects were a severe and protracted T-cell lymphopenia and a relatively frequent CMV reactivation. A similar safety profile was described for pretreated CLL patients in the supportive studies CAM 005 and 009, respectively. Grade 3/4 infections were experienced by 25% of the 76 patients and were shown to correlate with the stage of the disease. Severe infections were much less frequent in patients with low tumor burden (13% in patients with Rai 0-II, compared to 31% of patients with Rai stage III-IV).[16] Österborg and coworkers, who treated 29 chemotherapy refractory CLL patients, reported partial remissions in 38%, and complete remissions in 4% of patients. As in aforementioned trials responses to campath varied significantly between different body compartments. CLL cells were rapidly eliminated from blood in almost all cases, complete resolution of disease was achieved in bone marrow, small lymph nodes and spleen in roughly 1/3 of patients, whereas massive lymphadenopathy was normalized in only a minority of them.[18]

Mutations or deletions of the p53 gene, and in particular the poor-risk 17p- chromosomal aberration, are associated with a blunted or absent response to conventional chemotherapy, which require an intact p53 pathway for inducing cell death. Campath activity is largely p53-independent, enabling this MoAb to be effective also in p53-dysfunctional CLL. This peculiarity of campath activity has been clinically confirmed in several studies.[16] Lozanski *et al.* enrolled 36 patients with fludarabine-refractory CLL (42% of whom had p53 dysfunctions) in a trial designed to evaluate whether campath was effective in patients with mutations or deletions of the p53 gene. After a treatment with campath accordig to the *classical* i.v. schedule, the overall response rate among the patients with p53 mutations or deletions was 40%, with a median duration of response of 8 months. There were no significant differences in response according to age, disease stage, number of prior therapies or chromosomal aberration.[19] The German CLL Study Group (GCLLSG) designed a trial to assess the relationship between genetic abnormalities and response to subcutaneous campath (30 mg t.i.w) in 30 fludarabine-refractory CLL patients. The overall response rate was 36% (including 2% complete response), median progression-free survival was 9.7 months and median overall survival 13.1 months. Again campath was effective in terms of both ORR and OS across all biologic risk groups, including those considered to be very poor-risk, that is in patients with unmutated IgVH genes, 17p- and/or 11q-.[20]

Twenty-eight refractory CLL patients enrolled by Osuji and coworkers had p53 deletions and six of these cases showed p53-dysfunction in >20% cells. The overall response

rate to i.v. campath was 53.6% (CR 18%, PR 36%). Two out of the six p53-dysfunctional patients completely cleared pathologic lymphocytes from both the peripheral blood and the bone marrow, although they did not fulfill remission criteria solely because of cytopenias.[21] In a series of 40 pre-treated patients, the presence of the 17p- clonal aberration did not significantly impaired the ability of patients to respond to low-dose subcutaneous campath (10 mg t.i.w.), administered for a prolonged time (18 weeks).[22] These data support the use of campath as initial therapy for CLL patients with del(17p13.1), particularly for those who are either elderly or with disease predominately in the blood, marrow, and spleen.

The subcutaneous route of campath administration was pioneered by researchers at the Karolinska Institute in Sweden, being soon after the almost universally accepted way of administration of this drug due to the dramatic reduction in the number and severity of first-dose side effects, the preserved clinical efficacy, and the possibility offered to patients to self-administer the MoAb at home.[16]

An important step forward, i.e. towards an easier use of campath and a reduction of the fear of its adverse effects, came in fact from the study by Lundin *et al.* designed to investigate the efficacy and safety of subcutaneous administration of first-line campath, with prolonged treatment duration of 18 weeks. In 38 valuable patients the overall response rate was 87% (complete response 19%, partial response 68%), with a time to treatment failure in responders of 35+ months. An immunophenotypic response was observed in 45% of patients, verified by negative 3-colour flow-cytometry. Noteworthy, high response rates were seen in patients >65 years and even in those older than 70 years of age (ORR 90%). Of interest, the best bone marrow response was achieved only after 18 weeks of treatment, but it was documented in more than 50% of patients.[23]

The number of adverse events, including infectious complications, was far lower than in previous studies, apart from easy manageable local injection site reactions, which disappeared during continued treatment, usually within two weeks. Also fever was generally less severe and *flu*-like symptoms were greatly reduced. Earlier treatment with campath also appeared to improve lymphnode response, and prolonged treatment duration resulted in high-quality, meaningful bone marrow remissions.[23]

First-line campath therapy was very recently compared with chlorambucil in the phase III, open-label, international CAM 307 study, having as primary endpoint the progression free survival. In this trial, enrolling previously untreated CLL patients with progressive disease requiring treatment, 297 patients were randomized to receive either campath 30 mg i.v. three times weekly for a maximum of 12 weeks, or chlorambucil, at 40 mg/m^2, administered orally once every 28 days, for up to 12 cycles. Important conclusions of this trial concern both efficacy and safety aspects. Infections rate was similar in both arms (alemtuzumab 44,9 vs chlorambucil 51%), with the exclusion of CMV. No CMV-related deaths however were documented. First-dose infusion reactions were reported in up to 65% of alemtuzumab-treated patients, but these were mild to moderate in the vast majority of cases (>90%). For what response rate is concered, campath appeared clearly superior to chlorambucil as front line therapy of CLL (OR 83.2% with campath compared to 56.1% with chlorambucil, and CR 24% *vs.* 2%). Responses to 12 weeks of i.v. campath were comparable to those previously observed with s.c. administration (87% OR; 29% CR). In CAM 307 study, an ORR three times higher (64% vs 20%) was obtained in patients with del 17p treated in the alemtuzumab arm, nevertheless, due to the small patient population, this trend did not

reach statistical significance.[24]

According to the NCI-WG criteria, a complete response is still defined in CLL as less than 30% of CLL persisting in the bone marrow, whereas MRD is defined as the presence of CLL cells in the peripheral blood or bone marrow in patients who have achieved a complete response according to NCI-WG criteria, that cannot be detected using conventional assays. It is well known however, that patients with persistance of residual disease, after achieving a NCI-WG complete remission, are at greater risk of relapse than MRD negative ones. On the other hand, modern chemoimmunotherapy enables patients to obtain a high quality of response and in a high proportion of cases, at least after first line treatment. As a consequence, the complete eradication of detectable CLL cells, or MRD-negativity, has become an endpoint of several investigational trials.[25]

The effect of conventional i.v. campath on MRD was assessed by Moreton *et al.* in a trial enrolling 91 relapsing/refractory CLL patients. About on half of patients responded to campath therapy (OR 53%), moreover and more importantly, one fifth obtained an MRD-negative complete response as assessed by 4-colour flow cytometry (MRD-ve CR 20%). An MRD-positive complete response was observed in 15% and a partial response in 19% of the patients. In addition, patients who achieved an MRD-negative complete response (refractory to fludarabine therapy and expected to have poor prognosis and limited survival) had significantly longer treatment-free survival and overall survival compared with other response groups.[26]

Because of the lack of a curative approach for CLL, stem-cell transplantation (SCT) is being increasingly performed in these patients. The available evidence indicates that autologous SCT may prolong survival in highly selected patients, but does not result in cure. Conversely, allogeneic SCT may cure a proportion of patients, including those who are refractory to purine-analog-based therapy or with other unfavorable risk parameters, mostly due to its GVL potential, but at the cost of high morbidity and transplant-related mortality. Non-myeloablative transplant with reduced-intensity conditioning regimens appear to lower toxic deaths while preserving the antileukemic effect of the graft, at least in patients with chemosensitive disease. If the curative potential of allogeneic stem-cell transplants relies on graft-versus-leukemia effect, then a better understanding of this complex process could help refining post-transplant immunomodulation in CLL.[27]

Autologous transplants may prolong survival in patients with chemosensitive disease, having obtained a good response to front-line therapy and transplanted earlier in the course of the disease: there is therefore the need to obtain the best quality of response to first-line therapy in patients candidate to this procedure. The experience by Montillo *et al.* successfully demonstrated that low doses of alemtuzumab can be safely applied as consolidation to CLL patients candidates to a hematopoietic stem cell autograft, realizing an *in vivo* purging with this MoAb and improving quality of response to previous therapy.[28]

While single-agent rituximab seems not to have a role in todays' treatment of CLL, mostly because of the very high doses needed to obtain an adequate response rate in this disease, campath surely does. The majority of clinical trials with this MoAb in fact utilized campath alone, including the pivotal trials in refractory CLL, the Karolinska study demonstrating its activity and safety when admistered subcutaneously, and more recently the prospective comparison with chlorambucil as an upfront approach to untreated patients. Therefore the bulk of evidence on the efficacy of campath in CLL relies its activity as single

agent.[29] However a number of experiences, mostly on small series of patients, have been gained during the last years on alemtuzumab-containing combinations in the treatment of relapsing/refractory patients. Campath has in fact been associated to chemotherapy (i.e. with fludarabine: flucam; or with fludarabine and cyclophosphamide: FCC), to rituximab or to both classes of agents (i.e. AR and CFAR combinations, respectively) or even to corticosteroids.[30,31] Not only the real potential of campath-containing regimens is largely unexplored, but also the more effective and safe dosage of this MoAb to be employed in the various clinical setting of CLL needs to be ascertained. The main factor affecting bio-distribution and pharmacokinetics of campath appeared to be tumour burden. The *classical* schedule (thirty mg i.v., three times a week per twelve weeks) implies the administration of a total of 1080 mg of antibody, but even substancially lower doses are probably equally effective. In addition as low as 10 mg per dose have been shown to be efficacious in heavily pre-treated patients, with a favourable toxicity profile.[32]

The development of campath-containing chemoimmunoterapic combinations has been delayed with respect to rituximab combinations pricipally because of lack of a *history of effectiveness and efficacy* of campath in other non-Hodgkin lymphomas, and because first-dose reactions and infectious complications following campath administration have been probably overemphasized. Certainly, the availability of the subcutaneous route of administration greatly aided in promoting the evaluation of such campath-containing combinations.

Major advances in the knowledge of both the efficacy and toxicity profile of campath came from: a) the introduction of mandatory anti-infective prophylaxis in patients treated with this MoAb, b) the routinary use of CMV reactivation monitoring, coupled with the pre-emptive or therapeutic use of (val)ganciclovir), c) the shift to the subcutaneous route of administration, d) the demonstration of its higher and more durable activity when administered early in the course of disease, e) the favourable results of the prospective comparison with chlorambucil as first line treatment of CLL, f) the high response rate of refractory CLL patients to the Flucam combination, and finally from g) the demonstration that achieving MRD-negativity with campath leads to significant improvements in event-free and overall survival, offering a important advance in the treatment of CLL, and that a consolidation/ maintenance with campath can subtancially improve the quality of responses obtained by previous chemoterapy, thus increasing the percentage of MRD-negative CRs and significantly prolonging the patient progression-free survival.

Taken togheter, all these pieces of information made the clinicians more confident in the usage of campath, opening in the meantime new perspectives for a wider and safer use of this MoAb in the field of CLL treatment not only alone but also combined with other agents.

As already mentioned, very positive results have been obtained in the field of chemoimmune combinations by Elter *et al.* treating relapsing CLL patients with four cycles of the association of fludarabine and campath (Flucam; F 30 mg/m^2 i.v. and A 30 mg i.v. /daily on days 1-3). Of 34 evaluable patients, 85% responded, with a CR rate of 29%. MRD negativity was demonstrated on peripheral-blood in 44% of patients. Flucam also enabled the resolution of pre-existing autoimmune hemolytic anemia in several patients and was accompanied by few infectious complications. This encouraging experience of Flucam has led to an ongoing randomized phase III trial of this combination versus fludarabine alone in CLL relapse.[33]

The group at MDACC has investigated the safety and efficacy of adding campath to FCR (CFAR); FCR schedule with the addiction of alemtuzumab 30 mg intravenously on days 1, 3, and 5). Cycles were repeated every 4 weeks for a total of six cycles. Among 21 evaluable CLL patients to date, the OR rate was 52%, including 14% of patients who achieved CR.[30]

Several experiences have been gained over the last few years on the association between MoAbs. The results of these exploratory studies confirm the feasibility of combining rituximab and campath, with acceptable and non-cumulative toxicity.[30]

Many antigens take place on the surface of CLL lymphocytes and are potential targets for adoptive immunotherapy. A number of new MoAbs are now available, enriching the potential armamentarium of CLL therapy. Several Moabs, namely ocrelizumab and hA20 (IMMU106) both humanized, TRU-015 humanized and truncated, ofatumumab (HuMax CD20) human with enhanced CDC, and GA101 humanized with enhanced induction of ADCC and apoptosis, are directed against the CD20 antigen (frequently targeting distinct epitopes from rituximab) and are at various phases of clinical development.[34] The reasons for developing such new anti-CD20 MoAbs are that human and humanized structures can reduce the potential for HAMA and HAHA induction, and may result in better pharmacokinetics and increased efficacy via the enhancement of specific citotoxicity patways. A decreased immunogenicity could also decrease infusion-related adverse events.[34–36] Other new MoAbs target antigens different from CD20, such as CD23 (Lumiliximab) or CD40 (Chiron 12.12, fully human). The chimeric antibody lumiliximab is in a quite advanced stage of clinical development as its activity has already been tested in combination with the chemoimmune association FCR.[2] Our knowledge of antigen expression on the surface of lymphoma cells and has also led to the development of other antibodies possibly suitable for CLL therapy such those against CD22 (unconjugated epratuzumab and calicheamicin conjugated CMC-544 [inotuzumab ozogamicin]), a B-cell restricted marker expressed only at the mature stages of differentiation with >90% expression on follicular lymphomas and diffuse large B-cell lymphomas and CD80 (primatised galiximab) a membrane-bound costimulatory molecule involved in regulating T-cell activation.[35] With the aim of overcoming the ability of cancer cells to become resistant to a MoAb therapy, by activating alternative pathways to escape programmed cell death, combinations of *new and old* MoAbs such as epratuzumab plus rituximab (ER) or ER plus standard CHOP chemotherapy (ER-CHOP), inotuzumab ozogamicin plus rituximab, campath plus CHOP (CHOP-C) are under evaluation in the field of non-Hodgkin lymphoma therapy. Other potentially active combinations, to be explored in the near future are bevacizumab (vascular endothelial growth factor inhibitor) plus rituximab, rApo2L/TRAIL (agonist to TRAIL [tumour necrosis factor related apoptosis-inducing ligand]; mapatumumab) plus rituximab.[37] Also the associaton between molecules highly active as single agents in CLL, such as lenalinomide and flavopiridol, and already established treatments of CLL reprent an area of future investigation. Rituximab has been combined with the radioisotopes of yttrium (^{90}Y) and iodine (^{131}I), to form the radioimmuno-conjugates ^{90}Y-ibritumomab tiuxetan and ^{131}I-tositumomab, respectively, with the goal of potentially improving Rituximab therapeutic efficacy. Treatment with this radioimmunotherapy has shown a very good therapeutic benefit in patients with refractory low-grade or transformed B lymphomas, but still there are no data on CLL patients.[34]

A research agenda, aimed to delineate the

right place of MoAbs in the context of the todays' complex therapeutic strategy of CLL, must take into account some issues:

1. The clinical course of CLL is extremely variable so that there are patients whose life expectancy is not different from that of the general population, while others have a rapidly downhill course. Therefore treatment decisions in patients with CLL cannot be made without taking their prognosis into consideration.

2. Since nowadays more than 75% of CLL cases are diagnosed in early clinical stages, there is a need to better identify cellular and molecular markers that may help to refine outcome prediction for these patients. Issues related to the clinical use of these markers includes the problem of whether a single marker may represent a valid surrogate of the others, or if there a rationale way to put them in a hierarchical model.

3. This would be of paramount importance for selecting patients with high risk features, who need to be treated early, perhaps even they do not fullfill the commonly accepted criteria for starting cytotoxic therapy. It is presently unclear, for example, whether patients with Binet stage A whose leukemic B cells express unmutated VH genes or high CD38 and Zap-70 cell expression or unfavourable chromosomal abnormalities such as 11q and 17p deletions, would benefit from early treatment. These principles are at the basis of the ongoing CLL7 German study and the Cam-CLL1 Italian study, evaluating if early treatment with more effective therapies (a rituximab- and a campath-containing regimen, respectively) are able to induce a significant prolongation of the EFS in Binet stage A patients at high risk for disease progression. The question of whether patients with early-stage disease and poor risk markers ought to be treated before indicated by NCI guidelines is also being addressed by a CALGB study (F plus rituximab) and by an UK trial (campath monotherapy). There is no evidence to support treating such patients outside a clinical trial.

4. CLL is incurable with conventional chemotherapy, and this had limited to palliation of symptoms and to prolongation of PFS and OS the goals of therapy. New and more effective therapies are shifting the focus of CLL treatment from pure palliation to a curative attempt.

5. Chemoimmunotherapy produces higher number and a better-quality of responses than chemotherapy alone, therefore randomized trials of chemoimmunotherapy versus chemotherapy alone are needed to confirm and further substantiate the advantage of adding MoAbs to chemo.

6. One major determinant for prolonged progression free survival is the quality of the remission. The eradication of the leukemic clone is therefore increasingly becoming an important endpoint of therapy, aimed to prevent relapse and the occurrence of resistant disease. MoAbs are playing an increasing role in consolidation and maintenance of response, but to fully exploit the potential of MoAb therapy optimal combinations to maintain or even ameliorate the quality of response, timing and duration of maintenance, and impact on progression-free and overall survival need to be carefully explored. Moreover, is relapse at MRD level an indication for re-treatment?

7. Antibody-based therapies are appealing, since they target tumour cells while potentially sparing normal cells. There is a need to develop fully humanised antibodies in order to minimise both infusion reactions and the development of human antibodies against the drug. The mechanisms of action and the potential additive or synergistic effects of combining already available MoAbs with new ones, or with conventinal chemotherapy, or with targeted therapies must be explored and elucidated.

8. A *Monoclonal antibody option* could be an answer to many open issues in the field of stem cell transplantation in CLL: finding the best pre-transplant consolidation, conditioning and immunosuppressive or immunomodulating regimens in the setting of allogeneic transplants, identifying the role of MoAbs, as pre-transplant purging as well as post-transplant maintenance, to reach and maintain a MRD-negative status in the setting of autologous transplants.

9. Alternative strategies should be pursued in older or frail patients with a low likelihood of benefit from newer and aggressive chemoimmunoterapeutic regimens.

10. The introduction of new technologies (i.e. FISH, Multi-parameter flow cytometry, PCR, CT scan, PET) raises the question as to whether the guidelines by the NCI Working Group on CLL, more than ten years old, are still adequate for staging a patient, planning therapy and assessing response. Criteria for response assessment need to be reviewed to incorporate MRD status.

References

1. Maloney DG, Smith B, Rose A. Rituximab Mechanism of action and resistance, Semin Oncol 2002;29:2–9.
2. Tam C S, Keating MJ. Chemoimmunotherapy of chronic lymphocytic leukemia, Best Pract Res Clin Haematol 2007;20:479–8.
3. McLaughlin P, Grillo-Lopez AJ, Link BK, et al. Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent: Half of patients respond to a four-dose treatment program. J Clin Oncol 1998;16: 2825–33.
4. Huhn D, von Schilling C, Wihelm M, et al. Rituximab therapy of patients with B-cell Chronic lymphocytic leukemia, Blood 2001;98:1326–31.
5. Manshouri T, Do KA, Wang X et al. Circulating CD20 is detectable in the plasma of patients with chronic lymphocytic leukemia and is of prognostic significance. Blood 2003;101:2507–13.
6. Byrd JC, Murphy T, Howard RS, et al. Rituximab using a thrice weekly dosing schedule in B-cell chronic lymphocytic leukaemia and small lymphocytic lymphoma demonstrates clinical activity and acceptable toxicity. J Clin Oncol 2001;19:2153–64.
7. O'Brien SM, Kantarjian H, Thomas DA, et al. Rituximab dose-escalation trial in chronic lymphocytic leukemia. J Clin Oncol 2001;19:2165–70.
8. Di Gaetano N, Xiao Y, Erba E, et al. Synergism between fludarabine and rituximab revealed in a follicular lymphoma cell line resistant to the cytotoxic activity of either drug alone. Br J Haematol 2001;11:800–9.
9. Byrd JC, Peterson BL, Morrison VA, et al. Randomized phase 2 study of fludarabine with concurrente versus sequential treatment with rituximab in symptomatic, untreated patients with B-cell chronic lymphocytic leukaemia: results from Cancer and Leukaemia Group B 9712 (CALGB 9712). Blood 2003;101:6–14.
10. Byrd JC, Gribben JG, Peterson BL, et al. Select high-risk genetic features predict earlier progression following chemoimmunotherapy with fludarabine and rituximab in chronic lymphocytic leukemia: Justification for risk-adapted therapy. J Clin Oncol 2006;24:437–43.
11. O'Brien SM, Kantarjian HM, Cortes J, et al. Results of the fludarabine and cyclophosphamide combination regimen in chronic lymphocytic leukemia. J Clin Oncol 2001;19:1414–20.
12. Catovsky D, Richards S, Matutes E, et al. NCRI Chronic Lymphocytic Leukaemia Working Group. Assessment of fludarabine plus cyclophosphamide for patients with chronic lymphocytic leukaemia (the LRF CLL4 Trial): A randomised controlled trial. Lancet 2007;370:230–9.
13. Keating MJ, O'Brien S, Albitar M, et al. Early result of a chemoimmunotherapy regimen of fludarabine, cyclosphamide, and rituximab as initial therapy for chronic lymphocytic leukemia. J Clin Oncol 2005;23: 4079–88.
14. Wierda W, O'Brien S, Wen S, et al. Chemoimmunotherapy with fludarabine, cyclophosphamide and rituximab for relpased and refractory chronic lymphocytic leukemia. J Clin Oncol 2005;23:4070–8.
15. Cattaneo C, Spedini P, Casari S, et al. Delayed-onset peripheral blood cytopenia after rituximab: frequency and risk factor assessment in a consecutive series of 77 treatments. Leuk Lymphoma 2006;47:1013–7.
16. Alinari L, Lapalombella R, Andritsos L, et al. Alemtuzumab (Campath-1H) in the treatment of chronic lymphocytic leucemia. Oncogene 2007;26;3644–53.
17. Keating MJ, Flinn I, Jain V, et al. Therapeutic role of alemtuzumab (Campath-1H) in patients who have failed fludarabine: results of a large international study. Blood 2002;99:3554–61.
18. Osterborg A, Dyer MJ, Bunjes D, et al. Phase II multicenter study of human CD52 antibody in previously treated chronic lymphocytic leukemia. European Study Group of CAMPATH-1H Treatment in Chronic Lymphocytic Leukemia. J Clin Oncol 1997;15:1567–74.
19. Lozanski G, Heerema NA, Flinn IW, et al. Alemtuzumab is an effective therapy for chronic lymphocytic leukemia with p53 mutations and deletions. Blood 2004;103: 3278–81.
20. Stilgenbauer S, Dohner H. Campath-1H-induced complete remission of chronic lymphocytic leukemia despite p53 gene mutation and resistance to chemotherapy. N Engl J Med 2002;347:452–3.
21. Osuji N, Del Giudice I, Matutes E, et al. Alemtuzumab for chronic lymphocytic leukemia with and without p53 deletions. [abstract]. Blood 2004;104: A2510.
22. Cortelezzi A, Pasquini MC, Barcellini W, et al. Low-dose subcutaneous Campath is higly effective and safe in advanced and chemotherapy-refractory B CLL: results of a prospectic and multicenter phase II study. Haematologica 2007;92(s3):22.
23. Lundin J, Kimby E, Bjorkholm M, et al. Phase II trial of subcutaneous anti-CD52 monoclonal antibody alemtuzumab (Campath-1H) as first-line treatment for patients with B-cell chronic lymphocytic leukemia (B-CLL). Blood 2002;100:768–73.
24. Hillmen P, Skotnicki A, Robak T, et al. Preliminary phase

III efficacy and safety of alemtuzumab vs chlorambucil as front-line therapy for patients with progressive B-cell chronic lymphocytic leukemia (BCLL). J Clin Oncol 2006;24: (abstract 6511), 339.
25. Eichhorst B, Hallek M. Revision of the guidelines for diagnosis and therapy of chronic lymphocytic leukemia (CLL). Best Pract Res Clin Haematol 2007;20 469–77.
26. Moreton P, Kennedy B, Lucas G, et al. Eradication of minimal residual disease in B-cell chronic lymphocytic leukemia after alemtuzumab therapy is associated with prolonged survival. J Clin Oncol 2005;23:2971–9.
27. Gribben JG. Salvage therapy for CLL and the role of stem cell transplantation. Hematology 2005;282–98.
28. Montillo M, Tedeschi A, Miqueleiz S, et al. Alemtuzumab as consolidation after a response to fludarabine is effective in purging residual disease in patients with chronic lymphocytic leukemia. J Clin Oncol 2006;24: 2337–42.
29. Fraser G, Smith CA, Imrie K, et al. and the Hematology Disease Site Group of Cancer Care Ontario's Program in Evidence-Based Care. Alemtuzumab in chronic lymphocytic leukemia. Curr Oncol 2007;14:96–109.
30. Faderl S, O'Brien S, Keating MJ. Monoclonal antibody combinations in CLL: evolving strategies. Best Pract Res Clin Haematol 2007;20:781–93.
31. Kasamon YL, Flinn IW. Management of symptomatic, untreated chronic lymphocytic leukemia. Blood Rev 2007;21:143–56.
32. Cortelezzi A, Pasquini MC, Sarina B, et al. A pilot study of low-dose subcutaneous alemtuzumab therapy for patients with hemotherapy-refractory chronic lymphocytic leukemia. Haematologica 2005;90: 410–2.
33. Elter T, Borchmann P, Schulz H, et al. Fludarabine in combination with alemtuzumab is effective and feasible in patients with relapsed or refractory B-cell chronic lymphocytic leukemia: results of a phase II trial. J Clin Oncol 2005;23:7024–31.
34. Fanale MA, Younes A. Monoclonal antibodies in the treatment of non-Hodgkin's lymphoma. Drugs 2007;67: 333–50.
35. Czuczman MS, Thall A, Witzig TE, et al. Phase I/II study of galiximab, an anti-CD80 antibody, for relapsed or refractory follicular lymphoma. J Clin Oncol 2005;23: 4390-8.
36. Teeling JL, Mackus WJ, Wiegman LJ, et al. The biological activity of human CD20 monoclonal antibodies is linked to unique epitopes on CD20. J Immunol 2006; 177:362–71.
37. Czuczman MS, Maddipatia S, Knight J, et al. In vitro synergistic anti-tumor activity by targeting TRAIL-R1 and CD20 antigen by combining HGS-ETR1 (agonistic human monoclonal antibody to TRAIL receptor 1) and rituximab monoclonal antibody against non-Hodgkin's lymphoma cells (NHL) [abstract no. 1475]. Blood 2006;106:425a.