PROCEEDINGS

Basic Immunology-Humoral

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Humoral immunity is accepted as a part of the adaptive immune system and includes B cells and antibody responses. The maturation of B lymphocytes occurs mainly in the bone marrow and continue in the peripheral lymphoid organs including spleen, lymph nodes, tonsils, Peyer patches, and mucosal tissues. Functionally, the humoral immune system protects the host form infections by neutralize and eliminate extracellular microbes and microbial toxins.

The activation of B lymphocytes results in the proliferation of antigen-specific cells, leading to clonal expansion, and in their differentiation into plasma cells, which actively secrete antibodies. In general, there are two types of antibody responses: 1) T-dependent and 2) T-independent. The classification of these responses is based on the requirement for T cell help during the process. In T-dependent immune responses, class-switched memory B cells and plasma cells were developed in germinal centers. The antibodies, which are produced in response to T-dependent antigens show more isotype switching and affinity maturation than antibodies against T-independent antigens. As a result, the most specific and longtime effective antibody productions are generated under the effect of helper T cells, whereas T-independent responses are comparatively naïve and includes predominantly IgM response with less affinity to antigens. Mutations affecting components of the class-switch and somatic hypermutation machinery prevent the formation of memory B and plasma cells expressing IgA, IgG, and IgE.

Currently, it is well known that defects in B-cell development, selection, and function lead to autoimmunity, malignancy, immunodeficiencies and allergy. The most common proportion of the primary immune deficiencies were consisted by humoral immune deficiencies including agammaglobulinemia and common variable immune deficiency (CVID). These diseases are characterized by early onset and predominantly susceptibility to bacterial infections. Up to 90% of patients who receive diagnoses of early-onset hypogammaglobulinemia carry mutations in the Bruton tyrosine kinase (BTK) gene located on the X chromosome, so called as X-linked agammaglobulinemia. The lack of peripheral blood B cells leads to severely reduced antibody titers of all isotypes. The CVID disorder is characterized by hypogammaglobulinemia, low class switched B cells and defective antibody responses. The long term favorable outcomes of these diseases are related to the early diagnosis and treatment.

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Regulatory Issues and Standards for CAR-T Cell Administration

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CAR-T cells are human immune effectors that are equipped with a chimeric antigen receptor (CAR) targeting a membrane antigen regardless of HLA restriction. Although, evidences of in vivo reprogramming through the use of nanospheres have been published, all CAR-T cells that are currently approved or in development are manufactured in vivo, and mostly through viral transfer of the sequence coding for the CAR. As such they qualify as Gene Therapy Medicinal Products, a sub-category of Advanced Therapy Medicinal Products in the European regulatory framework. Although a small number of cases treated with off-the-shelf allogeneic CAR-T cells have been published, most available information describe the administration of autologous CAR-T cells that are paradigmatic of personalized medicines. The two approved products: tisgenlecleucel (Kymriah, Novartis) and axicabtagene ciloleucel (Yescarta, Kite Pharma/ Gilead) are autologous in nature, and uniquely manufactured for the intended recipient, through a complex supply chain where autologous mononuclear cells are collected by apheresis, shipped to a central manufacturing facility, and returned as a cryopreserved cell suspension following lymphocyte immuno-selection, genetic engineering and expansion. Hospitals or blood banks that provide the starting material act as critical suppliers to the marketing authorization holder (MAH). While rationally designed, CAR-T cells are also examples of targeted therapies that are associated with very significant and potentially lethal side effects. This unique combination of logistical and clinical issues is the reason why MAH must qualify hospitals before they are allowed to deliver the treatment to patients. In this context, there is a need for harmonization of the qualification process across various countries, hospitals and pharmaceutical companies. The Immune Effector Cells standards that have been developed by FACT in the USA, and are now adapted by JACIE in Europe represent a minimal set of essential requirements designed to ensure the quality of the drug product and the safety of the treated patient.

Cell procurement for CAR-T cell manufacturing.

Peripheral blood mononuclear cells (MNC) represent the starting material for autologous or allogeneic CAR-T Cell manufacturing. Allogeneic MNC are obtained from healthy donors, using a procedure that is very similar to the one used for the procurement of "donor lymphocyte infusions" (DLI), administered prophylactically or curatively after allogeneic hematopoietic stem cell transplantation. Since the two approved CAR-T cells are autologous, we will focus on procedural aspects in this situation. Autologous MNC are obtained through apheresis, using the same cell processors and same settings as the ones used in the context of hematopoietic cell transplants. Patients are screened for transmissible diseases in a similar manner to patients undergoing cell collection for autologous transplantation to support high-dose chemotherapy. Most patients are lymphopenic as the result of previous chemotherapy; however the collection and manufacturing process are robust enough that even severely lymphopenic patients can be collected and the DP can be engineered. However, since the Marketing Authorization Holder (MAH) must comply with good manufacturing practices, this includes evaluating and auditing the facilities that procure the staring material: a robust quality management system must thus be in place. The collected cell product is then shipped to the manufacturing facility, according to the MAH instructions; these in some instances include cryopreservation of autologous MNC before shipping. Cross-border shipment will usually require to comply with specific regulatory requirements, as defined by national and international health agencies.

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Treatment with CARTs in Malignant Hemopathies

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The immune system defends the body against diseases. T cells are one of the key soldiers, eliminating infected or abnormal cells. Cancer cells can block those defenses. Now we are genetically modifying the patient's own T cells to make them more intelligent and powerful and to seek and destroy cancer. One version of these genetically modified T cells is called CART T cell therapy, or CART. The "CAR" procedure consists of four phases that can be summarized in a simplified manner: a) preparation of the CAR and integration in a viral vector, b) by leucoapheresis, mononuclear cells are extracted to the patient, b) the T lymphocytes are selected, activated and then they are cultivated with the CAR-viruses, and these cells are expanded ex vivo, c) the patient is administered a conditioning chemotherapy (in general, fludarabine and cyclophosphamide), before reinfusing the genetically modified T lymphocytes. Once CARs are administered, severe immunological reactions are triggered in 20-30% of cases. The success of the treatment with CARs is monitored by the analysis of the residual disease and the detection of blood CARs lymphocytes.

There is a marker, a target for these CARTs cells, called CD19. The CD19 molecule is expressed on the surface of the vast majority of lymphoid B cells, from the most immature stage to the intermediate stage and the mature stage. CD19 is only lost at the end of differentiation of B cells (plasma cells). Therefore, virtually all B lymphoid cells are CD19 +. CART cells are very effective against those diseases that express CD19 on the surface of malignant cells: the malignant transformation of Pro B or Pre B cells (acute lymphoblastic leukemia, or the malignant transformation of mature or activated B cells (diffuse cell lymphoma large, chronic lymphatic leukemia, mantle lymphoma, or follicular lymphoma). Thus, several American and Chinese groups have shown impressive results with the therapy of CART cells directed against CD19 in acute relapsed or refractory B-cell lymphoblastic leukemia (ALL), non-Hodgkin's lymphoma (NHL), and in chronic lymphocytic leukemia. It was not a surprise to anyone that the US Food and Drug Administration (FDA) and the European Agency for Medicines (EMA) approved very quickly the use of these anti CD19 CART in pediatric patients and young adults with ALL R / R and in patients with NHL R / R. Other CARTs, which target BCMA, a molecule expressed almost exclusively in plasma cells, are also showing excellent results in patients with multiple myeloma (MM) previously treated with many anti-MM regimens. The use of a CART targeting the CD30 antigen in patients with Hodgkin's lymphoma and anaplastic lymphomas or T-lymphomas expressing this molecule, and the use of a CARTCD123 in acute myeloid leukemias is also being tested. The effectiveness of this treatment raises the question of whether CARTs will replace hematopoietic stem cell transplantation in a few years or CARTs will be used as a bridge treatment to achieve an excellent remission of the disease and thus bring the patient to optimal conditions. In order to answer this question, a longer follow-up of the clinical evolution of patients treated with CART is needed. Clinical trials are also lacking to see how CARTs behave in earlier phases of these diseases. Clinical trials are already being evaluated in this regard, and the results will be available in the near future. This procedure is marketed in some European countries. The cost of this treatment per patient also raises the concern that it is affordable for all European patients who need it. The production and distribution of academic CARTs could be part of the solution to this economic problem. Unfortunately, less than 10% of academic CARTs around the world are produced in Europe. In this regard, at the Hospital Clínic de Barcelona we have prepared a CAR-CD19 (ARI001) that, after the preclinical development and the authorization of the Spanish Agency for Medicines and Health Products, is already being administered to patients. The clinical trial began in July 2017, and 30 patients with ALL R / R or NHL R / R have already received the ARI001. The CART19 of the Clinical Hospital is showing efficacy and toxicity

results very similar to those published with commercial CARTs. In the coming months we will start a national multicenter clinical trial with a CARTBCMA for patients with MM who have already received at least the 3 families of most effective drugs in this disease.

Conclusions

1- The effectiveness of CARTs in ALL and NHL in relapse or resistant to treatment is extraordinary.
2- The preliminary clinical results of a BCMA CAR in multiple myeloma and a CARCD30 in Hodgkin's lymphoma are very encouraging.
3- It is possible to develop academic CART environments for clinical use in our environment.
4- The CAR CD19 (ARI001) produced at the Hospital Clínic seems safe and very effective.
5- More patients are needed and a longer follow-up to define the role of CARTs in early phases of the disease treatment with CARTs in malignant hemopathies.

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The Emerging Role of MRD in AML

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Prognostic factors determined at diagnosis are predictive for outcome while achievement of morphological complete remission (CR) is still an important endpoint during treatment. Residual disease after therapy may reflect the sum of all diagnosis and post-diagnosis resistance mechanisms/factors, its measurement could hypothetically be very instrumental for guiding treatment. The possibility of defining residual disease (measurable residual disease:MRD) far below the level of 5% blast cells is changing the landscape of risk classification.1,2 Currently the two methods mostly used are flowcytometry based immune MRD(MPFC) and molecular MRD assessed by RT-qPCR. Both have advantages and disadvantages. MPFC can be applied in most cases of AML but is less sensitive then RT-qPCR which can however only be applied in 40% of cases. Also new technologies are emerging like next generation sequencing and digital droplet PCR.

Although the concept of MRD negativity as an indicator for the quality of treatment response is the same in AML and other hematological diseases such as chronic myeloid leukemia (CML), multiple myeloma (MM), and acute lymphoblastic leukemia (ALL), application of MRD assessment in AML has lagged behind. Retrospective single center studies already demonstrated that MRD detection by MPFC provides strong prognostic information in AML after both induction and consolidation therapy. A couple of studies have now also been performed prospectively in a multicenter setting showing the independent predictive value mainly determined after 2 cycles of chemotherapy.2,3 An example indicative for the usage of molecular MRD was recently published by Ivey et al who showed in a large study by NCRI that the presence of MRD, assessed by Q-PCR of NPM1-mutated transcripts, provided powerful prognostic information independent of other risk factors. Persistence of NPM1-mutated transcripts in blood was present in 15% of the patients after the second chemotherapy cycle and was associated with a greater risk of relapse after 3 years of follow-up than was an absence of such transcripts (82% vs. 30%; hazard ratio, 4.80) and a lower rate of survival (24% vs. 75%; hazard ratio for death, 4.38. 4 Collectively, these studies showed that low levels of MRD were associated with improved survival and lower risk of relapse superior to other well-defined prognostic factors such as AML type, age, WBC count at diagnosis, and classification of cytogenetic risk.

Evidence is accumulating that the presence of MRD assessed by multi-color flow cytometry immediately prior to allogeneic HCT is a strong, independent predictor of post-transplant outcomes in AML.5 In a recent update, Araki et al showed that in 359 adults, the 3-year relapse rate was 67% in MRD positive patients, compared to 22% in MRD negative patients, resulting in OS of 26% vs 73%, respectively.6 Depth of response prior to transplant, as measured by level of MRD, has emerged as one of the most important predictors of transplant outcome. Randomized trials are warranted to determine if MRD-guided pre-emptive therapy is associated with improved outcome. MRD assessment in AML could be used

1) to provide an objective methodology to establish a deeper remission status, 2) to refine outcome prediction and inform post-remission treatment, 3) to identify impending relapse and enable early intervention, 4) to allow more robust posttransplant surveillance, and 5) to use as a surrogate endpoint to accelerate drug testing and approval.

Various major AML trial groups now use MRD status to guide further treatment. The question whether MRD could be used as a surrogate endpoint for survival which would be very helpful for faster dug approval is still unsolved. It is important that MRD assessment should be part of every clinical trial in order to achieve this important goal.

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Choosing First, and Second Line Therapies in the Era of Generic TKI

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Imatinib has become a mainstay of therapy in patients with CML. It induces high cumulative rates of complete cytogenetic responses (CCyR) and improved overall survival (OS), as it was demonstrated in the International Randomized Study of Interferon vs STI571 (imatinib) (the IRIS trial), the German CML-IV study as well as in other independent retrospective analysis performed on patients outside clinical trials. Patients who achieve and maintain CCyR for at least two years have the similar OS to that of a control population without leukemia. In contrary patients who do not achieve optimal cytogenetic or molecular responses to imatinib at defined time points have a worse outcome, characterized by an increased risk of relapse, of progression and of death. Based on this principle the European Leukemia Net (ELN) panel experts and members of the National Comprehensive Cancer Network (NCCN) have established treatment recommendations and the milestones to be achieved during CML therapy with TKIs to match the criteria for optimal response. It has been demonstrated that the achievement of CCyR is associated with the highest probability of long-term survival for CML patients. According to some reports further reduction of BCR-ABL IS level to ≤ 0.1% (MMR) did not improve OS relative to achieve CCyR without MMR, nevertheless a 4-year landmark analysis performed within the context of the German CML-study IV indicates that the achievement of a stable MR4.5 after 4 years is associated with a statistically significant better survival at 8 years with respect to those patients who have achieved CCyR only (without MMR). Stable MR4 or MR4.5 (Deep Molecular response; DMR) seems to be a new, attractive treatment goal for those CML patients who intend to stop TKI therapy. It has been shown in several discontinuation trials that the achievement of durable DMR is needed to obtain a long lasting TFR. The high cost of imatinib and second-generation TKI (2GTKI) therapy is an important concern for healthcare payers, not only in countries with restricted resources. Generic imatinib is already available in several countries. Poland was the first European country where generic drugs entered the market after the reimbursement of branded imatinib was stopped (on 1st July 2014). Some concerns about its efficacy and safety have been raised, causing anxiety among many patients in Poland. Therefore, the Polish Adult Leukemia Group (PALG) Imatinib Generics Registry was established in 2014 to provide clinical data on efficacy and safety in a large cohort of patients, who commenced CML therapy with generic drugs right after the diagnosis or were switched from branded imatinib to generic drug. The results of three years observation suggest that molecular and cytogenetic response rates and side – effect rates on imatinib generics and branded imatinib are alike when used in the upfront setting, as well as when used subsequently. The analysis of patients in the intention–to-treat population of the IRIS trial indicated, that after 10 years of follow-up the percentage of patients achieving DMR accounts for 23.3%, and 21,6% after 6 years of imatinib treatment within another study. Approximately 50% of those who achieve DMR and fulfil the criteria for imatinib discontinuation could achieve long-lasting treatment-free remission (TFR) as demonstrated by many discontinuation trials. The chance for durable TFR have therefore about 10% of patients treated initially and continuously with imatinib or imatinib generic. Patients receiving second generation TKIs (2GTKIs) as initial treatment achieve faster a cytogenetic and molecular responses, have a higher chance to reduce BCR-ABL transcript level to ≤10% at 3 months, and in higher rates achieve DMR. As demonstrated by ENESTnd and ENESTfreedom trials the rates of patients who met the criteria for attempting TFR during therapy with Nilotinib 300 mg BID, Nilotinib 400 mg BID were almost doubled when compared to Imatinib 400 mg QD (37.9%, 34.2% and 21.6%, respectively). The additional benefits for patients treated initially with 2GTKIs include a lower rate of transformation to more advanced phases of CML. It should be emphasized that approximately 15-20% of patients treated initially with imatinib are at high risk of progression

and death in a short time. Nevertheless, some reported long-term toxicity effects, like a higher rate of cardiovascular events, could raise concerns for second- and third-generation TKIs use, particularly in some categories of patients. The comorbidities (such as atherosclerosis and its complications, disorders in lipid and glucose metabolism, congestive heart failure, hypertension, etc.) appear in group of CML patients with increasing with age and with prolongation of TKI therapy incidences. Since many years some efforts are done to characterize and describe the risk profile of CML patients at diagnosis. Recently developed Eutos Long-Term Survival Score (ELTS) was established based on analysis of patients treated with TKIs. Italian observational study reported on ASH 2018 covered 1051 patients newly diagnosed with CML and treated between January 2012 and June 2016 in 66 Italian centers. Five hundred-ten (48.5%) and 541 (51.4%) patients were treated in first-line with imatinib (IMA) and with 2GTKI. The 2GTKI were able to obtain a higher, earlier and deeper molecular response at 3th, 6thand 12thmonth than Imatinib, additionally in the intermediate risk group of patients according to ELTS, administration of 2GTKI was associated with significantly better 4-year OS when compared to imatinib (99% vs 90,5%; p=0,0030). In another analysis presented during last ASH–Conference it was demonstrated that the ELTS can predict achievement of MR3 and MR4 and leukemia-free survival better than Sokal score in elderly (aged > 65 years) patients and is therefore recommended to assess the baseline disease-risk in this group of patients and to select candidates for frontline therapy with 2GTKI minimizing the risk for overtreatment. The prognostic significance of early monitoring of cytogenetics and of BCR-ABL transcript level decline have been suggested in many studies. The depth of cytogenetic and molecular responses within the first year of therapy represent very important prognostic parameters being the strongest predictors not only for OS, progression- free survival (PFS) or event- free survival (EFS), but also defining the chance to achieve deeper molecular responses required for attempting the trial of TKI discontinuation. The reduction of the BCR-ABL transcript level below 10% IS at 3 months is associated with a high statistically significant difference in OS and PFS therefore it represents the most clinically significant early target to be achieved during TKI therapy. The optimal choice of initial and subsequent TKI therapy should take into account the initial risk profile including the phase of CML, safety and tolerability of drug of choice, patient characteristics, particularly age and comorbidities, and last but not least the dynamics of initial and early response to TKI. The special attention should be paid on appropriate, and timely follow-up with cytogenetic and molecular methods, which should be performed in certified, reliable laboratories issuing the results of Real-Time Quantitative PCR (RQ PCR) using the international scale (IS).

Clinical Applications of Prognostic Markers in Chronic Lymphocytic Leukemia

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CLL has an extremely variable natural history with survival from diagnosis ranging from months to decades. Some individuals require little or no therapeutic intervention and enjoy a normal life expectancy. Others require multiple courses of treatment and ultimately die from the disease. The desire to identify prognostic markers in CLL was originally sought to risk stratify these different groups of patients. More recently, prognostic markers have also been used to determine treatment choices and predict response. With the increasing number of new therapies available for CLL, including B-cell receptor (BCR) inhibitors such as Ibrutinib and Idelalisib, Bcl-2 antagonists such as Venetoclax and monoclonal antibodies such as Obinutuzumab, the overall clinical application of prognostic markers has broadened.

The range of prognostic markers is extremely extensive and some of the most traditional also remain the most robust. The clinical staging systems of Rai1 and Binet2 remain useful in predicting whether a patient is likely to progress and can be performed on any patient at time of presentation. However, for patients diagnosed with Rai Stage 0/ Binet Stage A disease, they are not capable of predicting progression: with increasing numbers diagnosed in this group (a result of early diagnosis through increased prevalence of blood testing) their utility is fairly limited for many. Another traditional measure, the lymphocyte doubling time (LDT- where a LDT of >12 months represents an excellent prognosis with <12 months indicating a poorer one), remains clinically robust and simple to perform, although by definition it cannot be measured at a single time point. LDT may actually be represented via serum markers such as beta2 microglobulin3, a marker of cell bulk/turnover, and a test that is readily measurable from blood testing. Flow cytometry based assays, for example measurement of CD38 and ZAP-70 on circulating peripheral blood CLL cells, have been extensively investigated and reported in clinical trials and retrospective series. Their clinical application, however, is limited by lack of standardisation in how these assays are performed and what cut off to use.

As genomic analyses have become more widely available and costs reduced, these have come to the fore. Chromosomal additions and deletions are common, including trisomy 12 and deletions of 6q, 11q, 13q14, and 17p. The pivotal study of Dohner defined specific genomic aberrations that determined prognosis in CLL. This led to standard FISH panels being used, with a hierarchical model for genomic aberration danger existing. Patients with del(17p) have the worse overall prognosis with the hierarchy then being del(11q) > tri(12) > del)13q4. Metaphase cytogenetics is difficult to perform in CLL and often fails, but the advent of array based technologies may supersede FISH allowing for more comprehensive analyses of gross genomic abnormalities in the disease5. There is increasing interest in the significance of the presence of a complex karyotype (usually defined as ≧3 abnormalities) and whether this should determine therapy choices especially after failure of BCR inhibitor therapy6. Importantly, however, no standard definition for complex karyotype exists and different platform usage makes comparisons between series difficult. Gene sequencing is becoming more common, and it is now becoming mandated to sequence the TP53 gene prior to initiation of therapy (especially in the absence of 17p deletion) as this is of vital importance in choosing appropriate licenced therapies: those patients with TP53 disruption (defined as the presence of TP53 mutation or 17p deletion) should not receive chemo-immunotherapy due to poor responses7,8 but be treated with newer agents9. Whole exome and whole genome studies are indicating a huge breadth of abnormalities present in CLL but are not yet suitable for routine clinical application.

The mutational status of the immunoglobulin heavy chain gene (IGHV) was reported twenty years ago as defining two groups

of CLL patients with different prognoses10,11: those patients with unmutated IGHV (as defined by 98% homology or more to the closest germline gene sequence) have significantly poorer outcomes than those with mutated genes. The ability to perform these assays has become easier and recent data suggests that this marker may also be used to guide choice of therapy for patients. Patients with mutated IGHV genes appear to respond extremely well to chemo-immunotherapy, with many obtaining long and durable remissions especially when minimal residual disease negativity is achieved12,13, whereas those patients with unmutated IGHV appear to universally relapse earlier and particularly benefit from newer therapies that target the B cell receptor pathway and anti-apoptotic machinery.

Combining prognostic factors has allowed streamlining of which ones to test for and provides useful clinical information. For example, the combined measurement of IGHV status, chromosomal losses and specific gene mutations allows for prediction of which patients require therapy within 3 years of presentation14. More recently, a CLL-IPI has been proposed using a 10 point weighted grading system assessing TP53 disruption (4), IGHV status (2), Beta2microglobulin (2), Clinical Stage and Age (1)15. This predicts at 5 years whether patients are likely to be alive or dead, will have received treatment or not, and it has also been proposed to be used for treatment recommendations.

In summary, prognostic marker assessment has been a major area of research in CLL for the past few decades. Indeed, many insights into the underlying biology of the disease have been identified through their study, for example linking CD38 expression with the disease microenvironment16. Their clinical application has, until recently, mainly been the ability to predict those progressing patients with overall poorer prognosis. With the advent of newer therapies, including the licensing of Ibrutinib, Idelalisib, Venetoclax and Obinutuzumab, their clinical application may become much more important in determining which therapies to use and what response to expect. Prognostic marker assessment in CLL will be part of the personalized medicine of the future.

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The Management of Chronic Lymphocyctic Leukaemia (CLL) is Undergoing Rapid Change

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In the last decade, chemo-immunotherapy has been the standard of care therapy for treatment naïve patients. Improvements in progression free survival (PFS) and overall survival (OS) of fit patients with CLL without comorbidities were first achieved by the addition of rituximab to the chemotherapy backbone combination consisting of fludarabine and cyclophosphamide (FCR) (1,2,3). Subsequently, it was shown that although bendamustine and rituximab (BR) was inferior to FCR with regards to PFS, it had a more favourable safety profile making it an attractive combination therapy for patients over the age of 65 (4). The German CLL11 study (5,6) and the UK COMPLEMENT study (7) demonstrated a significant PFS advantage in frail patients using chlorambucil & obinutuzumab or chlorambucil & ofatumumab, respectively, compared to chlorambucil alone. Patients treated with the chlorambucil & obinutuzumab combination also showed improved OS compared to those treated with chlorambucil alone.

Following on from the significant step change seen in the management of relapsed CLL using the small molecule B-cell receptor inhibitors (BCRi) ibrutinib and idelalisib or the BCL2 inhibitor venetoclax, these targeted agents are now being tested in the frontline setting.

Burger et al published the results of the RESONATE 2 trial (8) that directly compared chlorambucil against ibrutinib in frail patient with standard risk CLL. Not unexpectedly, this study showed clear superiority of the BCRi, however the study was critiqued for not having the right comparator.

At the 2018 American Society of Hematology meeting, three investigator-led trial provided further evidence for the superiority of ibrutinib with or without anti-CD20 antibody with respect to PFS and safety. These studies directly compared ibrutinib against either chlorambucil & obinutuzumab (9), BR (10) or FCR (11). Importantly, OS of patients treated with Ibrutinib and rituximab was also improved compared to FCR treated patients.

Long-term follow-up of these studies is awaited, especially to decide whether certain subgroups like patients with hypermutation of the immunoglobulin heavy chain still benefit preferentially from FCR treatment. It is likely that ibrutinib will become the new standard of care therapy for patients with therapy-naïve CLL.

However, many outstanding questions still remain. Ibrutinib does not induce minimal residual disease (MRD) negativity and does not cure. Second generation BCRi and combination therapies with antibodies and/or venetoclax and are under evaluation. The available data does not show any advantage of adding anti-CD20 antibodies to ibrutinib. The second question is that of fixed duration therapy or "drug holidays" that may be guided by MRD negativity. Finally, the significant improvements in outcomes of treatment-naïve patients with CLL will inevitably have knock-on effects on the management and long-term outcome of patients with relapsed CLL.

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Treatment of Relapsed Chronic Lymphocytic Leukemia

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Introduction

The development of targeted therapies has radically changed the management of CLL patients. These new agents such as ibrutinib, the first inhibitor of the Bruton tyrosine kinase (BTK), idelalisib, a PI3k delta inhibitor and more recently venetoclax, a bcl2 inhibitor have shown impressive results in the relapse setting. The place of chemoimmunotherapy (CIT) is sharply decreasing. Recent studies demonstrated interesting results with ibrutinib in frontline treatment of CLL, and some authors consider that ibrutinib should represent the standard of care for treatment naive patients, even in the absence of TP53 disruption [1–3]. Nevertheless, at the present time, most patients who relapse have received CIT as first line of treatment. Defining the best strategy in the relapse setting for these patients may differ from those treated with ibrutinib in front line, making the decision even more complicated. The aim of this lecture is to discuss the key questions concerning relapsed CLL.

Relpased CLL after CIT

Most of the early clinical trials of novel agents only included patients relapsing after CIT. The 5-years follow-up of the phase 1b-2 of ibrutinib in relapsed CLL has recently been published, with an overall response rate (ORR) of 89% and a median progression free survival of 51 months [4]. Even if the comparator is questionable, the phase 3 RESONATE study confirmed these results with a 3-years PFS of 59% in the ibrutinib arm, with substantial benefit in all subgroups [5]. The combination of idelalisib and rituximab also demonstrated high efficacy on relapsed CLL, but the toxicity profile of idelalisib has restricted its use [6,7]. Thus, unless limiting comorbidities or comedications, ibrutinib now represents the standard of care in CLL patients relapsing after CIT. Patients with low-risk CLL (ie IGHV-M without unfavorable cytogenetics) relapsing after a prolonged response to CIT could represent an exception. For this particular patients, both CIT and novel agents can be discussed [8,9].

The MURANO trial may challenge the primacy of ibrutinib as first novel agent in this setting. In this phase 3 randomized trial comparing 6 cycles of bendamustine + rituximab (BR) to a 24 months fixed-duration of venetoclax associated with 6 cycles of rituximab (VR), most patients had received one previous line of CIT [10]. With a median follow-up of 36 months, 3-years PFS was 71.4% in the VR arm vs 15.2% in the BR arm (HR 0.16 [95 CI, 0.12 to à.23], p<.001). Moreover, 64% of the patient who had completed the two years of venetoclax had blood minimal residual disease (MRD) < 10^{-4}. Even if the follow-up is still limited, for responding patients, 1-year PFS after venetoclax discontinuation was 87% [10]. This combination has been approved by the European Medicines Agency (EMA) and is an alternative to ibrutinib in patients relapsing after CIT. Medical history, comorbidities and concomitant therapies may be helpful to choose between these two options.

Relapsed CLL after BCR inhibitors

BCR inhibitors (BCRi) discontinuation is mainly due to three reasons: toxicity, Richter syndrome and CLL progression. Discontinuation of treatment because of toxicity often occurs early, during the first year of treatment, and CLL progressions usually occur much later [11,12]. Only very few data on the efficacy of ICT after BCR inhibitors are available, and two option seem valuable : alternative BCRi or venetoclax. Two retrospective studies demonstrated that the reason for first BCRi discontinuation is of great importance. The use of an alternative BCRi is a reasonable option only in case of intolerance to first BCRi. In case of CLL progression, the alternate KI seems unlikely to induce long-term control of CLL [13,14]. In the phase 2 M14-032 trial evaluating the efficacy of venetoclax for CLL patients relapsing after BCRi, median PFS was 24.7 months in the ibrutinib arm. The results of this trial led to venetoclax approval (FDA and EMA) for patients having received at least one BCRi. Nevertheless, relapses after both BCRi and venetoclax represent an unmet need.

Current indication of cellular therapies

In 2007, the European society for Blood and Marrow Transplantation (EBMT) proposed a definition of "high-risk" CLL to identify situations where allogenic stem cell transplantation (allo-SCT) might be a indicated for CLL patients. Patients with i) non-response or early relapse (within 12 months) after purine analogues, (ii) relapse within 24 months after having achieved a response with purine-analogue-based combination therapy, and (iii) patients with p53 abnormalities requiring treatment were considered as "high-risk" [15]. In CLL, because of the emergence of novel therapies, allo-SCT indications have decreased, and less than 20 allo-SCT have been performed in this indication in France in 2018. Moreover, the increasing availability of CAR-T cells may also give alternative options for "high risk" patients [16–18]. Both the EBMT and European Research Initiative on CLL (ERIC) recently proposed new definitions for "high-risk" CLL, along with indications for cellular therapies (ie, allo-SCT or CAR-T cells). CLL "high-risk I" (CIT-resistant) is defined by clinically CIT-resistant disease with TP53 aberrations, but fully responsive to novel agents. For these patients, cellular therapy remains an option only in selected patients with low individual procedure-related risk. For CLL "high-risk II" (CIT- and novel agent-resistant), characterized by increasing exhaustion of pharmacological treatment possibilities, cellular therapies must be considered in all eligible patients [19].

Conclusion and perspectives

Current sequencing of relapse therapies is primarily a consequence of their historical availability rather than an optimal prescription based on biological properties of the drugs. Moreover, the use of these molecules as single agents seems to facilitate the development of resistances [20,21]. Fixed-duration combinations of novel agents, with the goal of deep remission are currently being tested, but defining the best combinations is still challenging. Similarly, identifying the best populations who could profit from these new strategies seems mandatory.

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First-Line Treatment of Hodgkin Lymphoma

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Introduction

Hodgkin lymphoma has become one of the best curable cancers today[1]. Many patients affected with this disease are young and the median age at diagnosis is 34 years. On the other hand, patients aged over 50 years have a poor prognosis, which is even worse in those aged over 65 years. In part, this has to do with the poorer tolerability of aggressive chemotherapy such as BEACOPP escalated or high-dose chemotherapy in this setting. On the other hand, a substantial number of Hodgkin lymphoma patients who are cured from their original disease develop late side effects including secondary neoplasia, organ damage and others such as infertility or fatigue.

The German Hodgkin Study Group (GHSG) divides first-line diagnosed Hodgkin lymphoma patients into early favorable, early unfavorable and advanced stages.

Early favorable HL

Particularly in early favorable Hodgkin lymphoma, i.e. those patients in stages I-II without risk factors, are being treated with 2 cycles of ABVD followed by 20 Gy involved field radiotherapy (IFRT)[2]. Attempts to further reduce toxicity in early favorable Hodgkin lymphoma patients failed with poorer progression free survival (PFS) in those patients who had bleomycin, darcarbizine or both deleted from the original ABVD backbone[3].The more recent GHSG trial in early favorable stages demonstrated that 2 cycles of ABVD only result in significantly poorer outcome as compared to the standard of care, 2 x ABVD + 20 Gy IFRT. The difference in tumor control was 10% at 5 years[4].

Early unfavorable HL

In early unfavorable classical Hodgkin lymphoma (cHL), the HD11 trial by our group showed that there was little difference between 4 cycles of ABVD or 4 cycles of BEACOPP baseline[5]. However, patients receiving only 20 Gy involved field radiotherapy had a significantly poorer outcome as compared to those receiving 30 Gy IFRT after 4 cycles of ABVD and there was no improvement when patients were treated with 4 cycles of BEACOPP baseline. In our follow-up trial (HD14), we then introduced BEACOPP escalated into the treatment of early unfavorable Hodgkin lymphoma so that 2 cycles of BEACOPP escalated were followed by 2 cycles of ABVD plus additional radiotherapy[6]. Other groups in this setting such as the UK NCRI rapid trial showed similar results, i.e. those patients receiving combined modality treatment had significantly better outcomes as those who had ABVD chemotherapy only. The EORTC/GELA/IIL H10 trial showed that those patients in early unfavorable HL receiving 2 cycles of ABVD did much better if they were switched to BEACOPP escalated if PET-positive. Those who were PET-negative after 2 cycles of ABVD just needed 2 additional cycles of ABVD in early favorable and 4 cycles of ABVD in early unfavorable settings.

Advanced stages

For decades, the standard of care in advanced stage Hodgkin lymphoma was MOPP-ABVD or, more recently, 6-8 cycles of ABVD. With this treatment, a tumor control of about 60% at 5 years and an overall survival of 75% can be achieved.[7] This was the rationale by the GHSG to improve the outcome using COPP-ABVD as standard which was compared with 8 cycles of BEACOPP baseline and 8 cycles of BEACOPP escalated. The outcome was substantially improved with the use of 8 cycles of BEACOPP escalated giving an 80% difference between 8 cycles of BEACOPP escalated or 4 double-cycles of COPP-ABVD[8,9]. Follow-up trials then demonstrated that the reduction to 6 cycles of BEACOPP escalated gave significantly better outcomes as compared to 8 cycles of BEACOPP escalated or 8 cycles of BEACOPP baseline[10]. This was demonstrated by the GHSG HD15 trial that also included PET-driven radiotherapy for those patients who were PET-negative irrespective of the size of residual disease. More recently,

the GHSG HD18 trial demonstrated that Hodgkin lymphoma patients in advanced stages who were PET-negative after 2 cycles just needed 2 more cycles of BEACOPP escalated resulting in excellent outcomes of 94.8% at 3 years as compared to 92.3 % for those receiving 6 or 8 cycles of BEACOPP escalated[11]. Even more impressive was the overall survival for these patients with 98.7% of those receiving 4 cycles of BEACOPP escalated only as compared to 95.9% for those receiving 6 or 8 cycles of BEACOPP escalated. Thus, the PET-driven approach in advanced stage Hodgkin lymphoma has become standard of care, which was also demonstrated by our French colleagues[12]. Another trial by the UK RATHL group deleted bleomycin from the ABVD backbone after two cycles of ABVD demonstrating that bleomycin can safely be deleted from the ABVD regimen sparing substantial toxicity for most of these patients[13].

Perspectives

The antibody drug conjugate brentuximab vedotin targeting CD30 has been registered for relapsed and refractory Hodgkin lymphoma patients as well as those who are at high risk after second-line high-dose chemotherapy followed by autologous stem cell transplant[14]. More recently, a combination of brentuximab vedotin plus AVD was compared in a large prospectively randomized trial against 6 cycles of ABVD[15]. The GHSG is also currently evaluating a modified BEACOPP variant incorporating brentuximab vedotin in this setting. More interesting combinations include checkpoint inhibitors that had shown impressive activity in multiple relapsed Hodgkin lymphoma patients[16]. Here, a smaller phase II study with a total of 51 advanced stage Hodgkin lymphoma patients received a combination of AVD plus nivolumab[17]. A similar trial was conducted by the GHSG in a randomized fashion comparing AVD plus nivolumab with a modified regimen that includes 4 cycles of nivolumab alone followed a combination of AVD plus nivolumab (NIVAHL).

Particularly elderly patients with Hodgkin lymphoma have a rather poor prognosis so that new approaches for this very high-risk group are badly needed.

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Nodular Lymphocyte-Predominant Hodgkin Lymphoma

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Introduction

Nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL) is a rare lymphoma entity accounting for approximately 5% of all Hodgkin lymphoma (HL) cases. The disease is characterized by distinct pathological and clinical features. Of note, the malignant lymphocyte predominant cells consistently express CD20 but lack CD30. Clinically, NLPHL mostly has a rather indolent course and patients are usually diagnosed in early stages [1].

First-line treatment of NLPHL

Similarly to classical HL (cHL), treatment of NLPHL is stage-adapted. However, patients with stage IA NLPHL appear to be treated sufficiently with less aggressive approaches than their counterparts with cHL. A larger retrospective study from the German Hodgkin Study Group (GHSG) included patients with stage IA NLPHL who had received involved-field radiotherapy (IF-RT) alone (n=108), extended-field radiotherapy (EF-RT) alone (n=49) or combined-modality treatment (CMT) (n=72). The 8-year progression-free survival (PFS) rates for patients receiving IF-RT, EF-RT and CMT were 91.9%, 84.3% and 88.5% and did thus not differ. The excellent PFS rates translated into 8-year overall survival (OS) rates close to 100% (IF-RT: 99.0%; EF-RT: 95.7%; CMT: 98.6%) [2]. Based on these data and results from additional studies conducted by other groups, limited-field radiotherapy (limited-field RT) alone represents the accepted standard of care for patients with stage IA NLPHL.

The treatment of NLPHL in early stages other than stage IA and intermediate stages usually consists of CMT and is thus very similar to cHL. An analysis from the GHSG included 271 patients with early-stage NLPHL who had received ABVD (doxorubicin, bleomycin, vinblastine, dacarbazine) or ABVD-like protocols followed by limited-field RT within the HD7, HD10 and HD13 trials. At 8 years, PFS and OS rates after two or four cycles of chemotherapy plus RT were 83.2% and 95.1%, respectively [3]. A study using the British Columbia Cancer Agency (BCCA) database compared the outcome of early-stage patients treated with radiotherapy (RT) alone (n=32) and patients treated with two cycles of ABVD or ABVD-like chemotherapy followed by RT or ABVD chemotherapy alone (n=56). After a median follow-up of 6.4 years, the 10-year PFS estimate was significantly better for patients receiving chemotherapy-containing treatment than for patients treated with RT alone (91% vs 65%). The OS did not differ between the patient groups [4]. Thus, two cycles of ABVD followed by limited-field RT is the standard approach for early-stage NLPHL at most institutions.

Data on the treatment of intermediate-stage NLPHL are scarce. However, treatment with four cycles of ABVD or ABVD-like chemotherapy followed by limited-field RT results in excellent outcomes and should therefore be considered in this patient group [3].

In advanced NLPHL, cHL approaches such as ABVD or BEACOPP (bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisone) and the B-cell non-Hodgkin lymphoma protocol R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone) have been evaluated. A matched-control analysis using the BCCA database included 42 patients with advanced NLPHL and 84 patients with cHL. Treatment consisted of chemotherapy with ABVD or ABVD-like regimens. The 10-year freedom from treatment failure (FFTF) and OS rates were comparable for both patient groups. However, the definition of FFTF did not include cases of lymphoma recurrence with histological transformation. Those were taken into account in the time to progression (TTP) definition. As a result, the TTP was significantly impaired in

patients initially diagnosed with NLPHL histology as they had a cumulative 15-year transformation rate of 24% [5]. The more aggressive BEACOPP regimen resulted in better treatment results than ABVD. An analysis from the GHSG including 144 patients with advanced NLPHL who had received therapy within the HD9, HD12 and HD15 trials revealed 8-year PFS and OS rates of 76.2% and 87.4% [3]. The largest report on the use of the R-CHOP protocol so far came from the MD Anderson Cancer Center. Fourteen patients with advanced NLPHL who had been treated with R-CHOP optionally followed by RT were included in a retrospective analysis. All patients had responded to treatment. The 5-year PFS rate was 85.7% [6]. Taken together, BEACOPP and R-CHOP result in favorable outcomes in advanced NLPHL. However, no standard of care for this patient group has been established to date.

Treatment of relapsed NLPHL

Different approaches ranging from single-agent anti-CD20 antibody treatment to high-dose chemotherapy followed by autologous stem cell transplantation (ASCT) represent options in relapsed NLPHL.

A phase II study by the Stanford group included 39 patients (18 patients with relapsed NLPHL and 21 patients with previously untreated NLPHL). Patients received four weekly doses of rituximab at 375 mg/m^2 either alone or followed by rituximab maintenance every six months for two years. All patients responded to treatment. After a median follow-up of 9.8 years for patients treated with rituximab alone and 5.0 years for patients receiving rituximab induction followed by rituximab maintenance, 5-year PFS estimates for previously treated patients were 36.4% and 71.4% whereas the 5-year OS estimates were 90.9% and 71.4% after rituximab alone and rituximab induction followed by rituximab maintenance, respectively [7]. More recently, the second-generation anti-CD20 antibody ofatumumab was evaluated in a prospective study including 28 patients with relapsed NLPHL. The overall response rate was 96%. After a median follow-up of 26 months, the 2-year PFS and OS estimates were 80% and 100%, respectively [8]. Thus, single-agent anti-CD20 antibody treatment results in high response rates and durable remissions in a relevant proportion of patients with relapsed NLPHL.

However, patients with NLPHL recurrence who present with high-risk features such as a short time interval between first-line treatment and the diagnosis of relapse are candidates for more aggressive salvage approaches, e.g. high-dose chemotherapy followed by ASCT. The largest analysis evaluating this treatment modality came from the European Society for Blood and Marrow Transplantation. A total of 60 patients were included. The patients had a median of two prior lines of therapy, the median time interval between NLPHL diagnosis and ASCT was 21 months. After a median follow-up of 56 months, the 5-year PFS and OS rates were 66% and 87%, respectively [9].

Risk factors

A prognostic score including the risk factors low serum albumin, male gender and variant NLPHL histology was developed using data from 413 NLPHL patients treated within nine prospective GHSG studies. On the basis of this score, three distinct risk groups with significant differences in terms of PFS and OS could be defined. 5-year PFS rates ranged between 68.7% and 95.2% and OS rates ranged between 88.3% and 98.7% [10].

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Follicular Lymphoma – Focus on Therapy

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Follicular lymphoma (FL) is a heterogenous disease both molecularly and clinically. The outcome has improved continuously during the last two decades, mainly due to the development of combinations based on rituximab (R) (Tan D, et al, 2013; Junlen HR, et al, 2014; Karmali R , et al, 2018). With immunochemotherapy most patients with disseminated disease achieve long-lasting remissions and have an excellent overall survival (OS). However, still most patients, also young ones, have a shorter life expectancy than that of an age-matched healthy population.

Diagnosis

Diagnostic tumor biopsies are required for an adequate diagnosis, preferentially an excisional lymphnode, but also core biopsies are used. Less-invasive sampling techniques, including small-caliber core biopsies or fine-needle aspiration (FNA) are mostly not diagnostic. FL is composed of centroblasts and centrocytes, both CD20+, growing in the follicles of lymph nodes, and is according to the World Health Organization (WHO) classification graded according to the number of centroblasts counted in 10 random neoplastic follicles. FL 1-3a is considered clinically indolent as opposed to grade 3b, which is an aggressive lymphoma. Most FLs harbor the t(14;18) translocation leading to over-expression of the anti-apoptotic gene BCL-2.

Staging

Initial staging includes physical examination; standard laboratory assessments including blood counts with differential, beta-2 microglobulin and LDH; computed tomography (CT) scans of the neck, chest, abdomen, and pelvis; and bone marrow biopsy.

PET-CT is required as a standard component of both the staging and response assessment of FDG-avid lymphoma histologies like FL according to the Lugano Classification, (Cheson BD et al, 2014).

Prognosis and predictive markers

Several risk classifications of FL have been used; the Follicular Lymphoma International Prognostic Index (FLIPI), the FLIPI 2 and PRIMA-PI. FLIPI was elaborated in the pre-rituximab era, and is well-established for predicting OS (Solal-Celigny P, et al, 2004). The FLIPI2 was developed for prediction of failure-free survival in mainly rituximab-treated patients (Federico M, et al, 2009). The computation of nodal areas, used in the FLIPI, is cumbersome and prone to error and the FLIPI2 is easier to calculate, but it is still not used as often as the FLIPI. The PRIMA-PI is based solely on two parameters, bone marrow involvement and serum beta2 microglobulin (ß2m), and is proposed for patients treated with immunochemotherapy (Bachy et al., 2018). The prognostic impact of the total metabolic tumor volume (TMTV) measured at baseline with [18F]fluorodeoxyglucose ([18F]FDG/PET-CT) scans has been shown to add value to FLIPI, especially in patients with high-tumor-burden FL (Meignan M et al, 2016).

More recently a "clinicogenetic" risk model, the m7-FLIPI, based on seven gene mutations, integrated into clinical risk models, has been used to stratify FL patients into "low-risk" and "high-risk" with respect to 5-year failure-free survival after first-line immunochemotherapy (R-CHOP or R-CVP) (Pastore A, et al, 2017). The prognostic significance of copy-number aberrations (CNAs) and copy-neutral loss of heterozygosity (cnLOH) identified by chromosome genomic-Array testing (CGAT) at FL diagnosis has been demonstrated in an US clinical trial. The TP53 and CDKN2A/B deletion was validated in the m-7-FLIPI cohort (Qu X, et al, 2019).

Identifying patients with high risk of progression to a specific therapy, before initial therapy, is unsatisfactory with the above prognostic models. Re-assessment of patient status 12 - 24 months after diagnosis/start of treatment, is another potential prognostic tool. With immunochemotherapy, R-CHOP, around

20% of patients showed progression of disease within 24 months (POD24) and this was related to a short OS (Casulo C et al, 2015). These observations were confirmed and extended in a study including a large dataset from FL patients (Maurer MJ, et al, 2017). Development of an event within or after 12/24 months after initial treatment (EFS12/EFS24) was evaluated. In patients treated initially with R-chemo, an event before 24 months was predictive for short OS. FL patients managed with different treatment strategies, also "wait and watch", and free of events at 12months (EFS 12) following initial management, showed survival rates comparable to rates in an age and sex matched general population.

Reliable predictive tools for patient stratification also before starting therapy are needed for avoiding overtreatment of low-risk patients and prioritizing alternative approaches in high-risk patients.

Efficacy endpoints

Response is valuated according to the Lugano criteria (Cheson BD et al, 2014). The term "indeterminate response" was introduced in the context of immunomodulatory therapy, to identify "unspecific" lesions until these were confirmed as flare/pseudo-progression or true progressive disease, by either biopsy or subsequent imaging (Cheson BD, et al 2016).

Progression-free survival (PFS) is often a primary efficacy endpoint in clinical trials, and is especially adequate if supported by objectively assessed improvement of life quality. Studies on life quality is however sparse.

Several surrogate endpoints have been proposed: One is "maintaining" complete response at 30 months (CCR30), which is an "intermediate" endpoint response (Shi Q et al, 2015). Another is end-of-induction PET (Trottman J, et al, 2018).

These surrogate endpoints have been used mainly for first line FL therapy in clinical trialds, and are not yet widely used in the clinic.

Treatment first-line

Early therapy with chlorambucil has not shown to prolong survival for asymptomatic low-burden patients (Ardeshna KM, et al, 2009). However, rituximab monotherapy in these patients results in delayed time to new treatment with minimum toxicity, but still without a survival benefit (Ardeshna KM, et al, 2014). This is why a "watch and wait" strategy" is still an option. The optimal timing, sequence and choice of therapy still remain matters of debate.

For symptomatic patients with advanced disease and in need of treatment, the combination of rituximab and chemotherapy, often followed by rituximab maintenance, has become standard (Salles G., et al, 2011; Rummel M , et al 2013), but for patients presenting with a slow progression of disease after an initial period of "watch and wait", rituximab single therapy remains a good choice. Results of the clinical studies by the Swiss Group for Clinical Cancer Research (SAKK) and the Nordic Lymphoma Group (NLG) were both positive (Martinello G, et al, 2010; Kimby E, et al 2015). The long-term follow-up of the FL patients from the two NLG trials, with rituximab monotherapy or rituximab in combination with interferon (IFN)-α2a, showed a 72% 10-years survival with no major safety issues and 38% of the patients had not needed any later chemotherapy (Lockmer S, et al. 2018).

Lenalidomide is an immunomodulary drug (IMiD), boosting natural killer cell and monocyte-mediated antibody-dependent cellular cytotoxicity, thereby enhancing the activity of rituximab against CD20+ tumor cells in vivo (Wu L, et al, 2008). This synergism has been supported by promising results in clinical trials (Fowler N, et al, 2014; Leonard JP, et al, 2015). In the SAKK/3510 trial previously untreated patients were randomized to either R monotherapy (375 mg/m2 intravenously at day 1 of weeks 1 - 4, repeated in responding patients at day 1 of weeks 12-15) or to rituximab (given at the same schedule) in combination with lenalidomide (15 mg daily orally, starting 14 days before the first R administration and continuously given until 14 days after the last, up to a total 18 weeks). Results after a 3-year folow-up is promising (Kimby E. et al, 2017). In the large randomized Relevance trial the efficacy of rituximab plus lenalidomide was similar to that of rituximab plus chemotherapy; but differences were seen in safety profiles, with a higher incidence of grade 3/4 neutropenia and febrile neutropenia of any grade with rituximab plus chemotherapy and a higher incidence of grade 3/4 cutaneous reactions with rituximab plus lenalidomide (Morschauser F. et al, 2018).

Responses in most FL-trials with rituximab + lenalidomide were mostly irrespective of tumor bulk, stage and symptoms, why chemo-free approaches may be applied to both low and high tumor burden patients. However, for patients in need of rapid reduction of tumor burden, chemoimmmunotherapy, mostly R-CHOP, is still considered the best option. For the elderly bendamustine in combination with rituximab is an often used therapy (StiL study, Rummel M, et al 2014). A 5-year update of the BRIGHT study confirmed the findings of StiL, with a better PFS and a similar 5-year OS in BR versus R-CHOP/R-CVP groups (Flinn I, et al, 2014). CHOP or bendamustine in combination with the second generation anti-CD20 antibody obinotuzumab both in induction and maintenance, has favored the new antibody (Marcus R, et al, 2017).

Also with immunochemotherapy, like R-CHOP, around 20% of patients will show early progression of disease with short OS (Casulo C, et al, 2015).

Treatment att progress/relapse

Choice of treatment is often based on duration of response and type of prior therapies, and patient comorbidities. Rituximab monotherapy is a treatment option in patients with a previous response to rituximab, as frequent responses can occur with rituximab retreatment. The first approved indication for single-agent rituximab was for FL patients refractory to chemotherapy (Mc Laughlin P, et al, 1997). The immunomodulatory agent lenalidomide can increase the activity of rituximab (see above). In a phase III, multicenter, randomized trial (AUGMENT), lenalidomide plus rituximab was found more effective than placebo plus rituximab (Leonard et al, 2019). Rituximab was administered once per week for 4 weeks in cycle1 and then day1 of cycles 2 to 5 and lenalidomide or placebo was given orally for 12 cycles. The main endpoint, PFS, was significantly improved with lenalidomide plus rituximab versus placebo plus rituximab with a median duration of 39.4 months versus 14.1 months. Infections ocurred more often with the lenalidomide combination (63% v 49%) and also neutropenia and cutaneous reactions were more common.

In rituximab refractory patients phosphoinositide 3-kinase (PI3K) inhibitors have emerged as effective drugs. Both idelalsib and the newer agent copanlisib are now approved in this setting. These two agents differ in terms of specificity for PI3K isoforms and also in adverse effect profiles. Moreover, while idelalisib is an oral drug, copanlisib is administered intravenously.

Radioimmunotherapy with Zevalin® remains a therapy available for specialized centres. Fondazione Italiana Linfomi (FIL) has designed a response-adapted treatment based on an end-of-treatment PET scan by adding consolidation therapy with 90Y-ibritumomab tiuxetan in the PET-positive group and, at the same time, they study the effect of minimal residual disease in the PET-negative group.

The duration of response has been shown to shortens after each relapse, also after first-line immuno-chemotherapy (Rivas-Delgado A, et al, 2019).

Maintenance therapy

It was shown early that a prolonged treatment with rituximab significantly increases response duration and event-free survival(Ghielmini M, et al. 2004). Later, it was shown that 2-years rituximab maintenance lead to improvements in remission status and duration in both treatment-naïve and relapsed/refractory patients (Salles G, et al, 2011; von Oers, et al 2014). Maintenance also improved OS after a successful induction with R-CVP or R-CHOP, when compared with observation in a meta-analysis (Vidal L. et al 2017). If the good effect of maintenance is true for patients having received rituximab also in first induction is still unknown. Maintenance with binotuzumab has shown a positive effect both after first-line immunochemotherapy (Marcus R, et al, 2017) and in rituximab refractory patients (Sehn L et al, 2017). The effect of maintenance after bendamustine-rituximab/obinotuzumab induction is however questionable due to toxicity and should be further explored. Rituximab in combination with lenalidomide has been used as maintenance and will be further explored (Morschhauser F, et al 2018).

For low-tumour burden patients re-treatment with rituximab seems preferable to an extended schedule (Kahl BS, et al , 2014).

New Therapies

The BTK inhibitor, ibrutinib, and the bcl2 inhibitor venetoclax, have not been promising as single agents, in FL, but studies on combinations are ongoing, also with epigenetic modifiers and checkpoint inhibitors. Each drug is associated with unique, often surprising toxicity profiles. Recently, results from a trial with a triplet combination of rituximab, lenalidomide, and ibrutinib for untreated FL patients were reported. A high incidence of cutaneous toxicity was noticed and the efficacy was not superior to that of rituximab in combination with lenalidomide , why this regimen was eliminated from further study.

EZH2 is a mutation found in 25% of FL and can be targeted by tazemetostat. In relapsed or refractory FL single agent tazemetostat has resulted in an ORR of 82% in patients with EZH2-mutated and 35% in wild-type EZH2 FL (Morschhauser F , et al, 2018).

A new antibody Hu5F9-G4 is blocking the CD47 antigen, a "non-eat me" signal on monocytes/ macrophages, and has shown promising results in combination with rituximab (Advani R, et al, 2018).

Antibody–drug conjugates provide specific delivery of potent microtubule inhibitors or DNA-damaging agents to FL cells, while minimizing the systemic toxicities. Inotuzumab ozogamicin, an anti-CD22 IgG4 antibody linked to calicheamicin, has shown an ORR of 71% in refractory FL-patients. Polatuzumab vedotin, an anti-CD79b IgG conjugated to monomethyl auristatin E, a microtubule inhibitor, is evaluated in clinical trials (Palanca-Wessels MC, et al, 2015; Sehn L, et al, 2018).

Betalutin® is a first-in-class antibody radionuclide (Lutetium-177) conjugate which targets CD37, which is expressed on CD20+/ CD22+ B-cell subset. A cross-fire effect is seen, so that also tumor cells with less antigens or non-accessible tumor cells, are hit by the cytotoxic radiation. Good clinical results have been shown in early trials including rituximab refractory FL pts with ≥ 2 prior lines (ORR 62%, CR 19%) (Holte H, et a l, ASH 2018, abstract 2871).

Bi-specific T-cell engaging antibodies, like blinatumomab, joins CD19-positive B-cells to CD3ε-positive T-cells, resulting in

T-cell-mediated B-cell lysis along with T-cell activation. For FL several such bispecific antibodies are under development.

Therapy with chimeric antigen receptor (CAR) T-cells, autologous T-lymphocytes with engineered receptors for antigen-recognition moieties and T-cell signaling domains, has shown activity in DLBCL and transformed FL. Anti-CD19 CAR T-cells is also an appealing concept in the treatment of refractory FL.

Transformation to aggressive disease

During the course of the disease the lymphoma acquires additional genetic aberrations and may transform to a highly malignant lymphoma, most often diffuse large B-cell lymphoma (DLBCL). These transformed lymphomas tend to have a worse prognosis than their de novo counterparts. The risk of histological transformation as a first event can be significantly reduced by the use of rituximab (Link et al, 2015; Federico M, et al, 2018)

At any clinical suspicion of transformation a PET scan should be performed and if high SUV, especially if localized or a destructive bony lesion, a biopsy is required.

In summary

FL is a heterogenous disease both biologicaly and clinically, but mostly with a long survival. Rituximab in combination with lenalidomide is one alternative to standard immunochemotherapy as first or second line therapy and more toxic immunochemotherapy can be deferred in many patients without compromising outcomes. All therapies, both the type of 1 st-line induction and maintenance, as well as relapse treatment, are of importance for disease-free survival, long-term toxicity and quality of life. Early events following immunochemotherapy identifies a subset of patients who are at high risk for early mortality. There is a great need of new concepts for curative treatment.

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Cellular Therapy for Follicular Lymphoma

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Despite major advances in the treatment of follicular lymphoma, the disease generally remains incurable. Novel targeted agents often require prolonged or continuous administration with issues of cost, compliance and cumulative toxicity. Autologous stem cell transplantation results in very prolonged remissions and cure in up to 50% of patients with chemotherapy-sensitive recurrence. Recent data indicate that salvage autologous transplantation leads to improved survival for patients with early treatment failure, i.e. recurrence within 2 years after appropriate initial treatment. It may be the preferred treatment for such patients. Autologous transplantation has also been extensively investigated in the consolidation treatment of younger patients with high-risk features, but has largely been abandoned in that setting because of concerns over late therapy-related MDS/AML. Purging techniques to reduce graft contamination have been associated with decreased rates of disease recurrence after autologous transplantation, as has post-transplant rituximab maintenance. Allogeneic transplantation has low rates of disease recurrence but a higher rate of complications, despite widespread use of reduced-intensity conditioning. Haplo-transplant, umbilical cord blood transplant or haplo-cord transplants are excellent graft sources for those lacking HLA-identical donors. For transformed lymphoma, autologous transplant improves survival. Novel cellular therapies including CAR T therapy is playing an increasing role in the management of transformed B-cell lymphoma and will likely also find a role follicular lymphoma.

Autologous Transplantation

Follicular lymphoma is an exquisitely chemosensitive disorder, with a high response rate, but upon treatment with conventional chemotherapy also a very high recurrence rate. Dose intensification with autologous stem cell rescue was one of the earliest methods available to overcome inherent resistance of residual lymphoma cells and has proven remarkably effective. With a length of follow up of a minimum of 12 years, 48% of patients with follicular lymphoma, transplanted in second or subsequent remissions, were free from disease progression and 54% were alive at 10 years.[1] But these initial studies also found a high rate of therapy related MDS. The risk for MDS persisted for up to ten years after transplant and its cumulative rate was approximately 10% may have been in part related to use of TBI and, in some patients, to prior chemotherapy exposure. Efforts to further improve on the efficacy of autologous transplant have mostly relied on intensification of the conditioning regimen, but have largely failed. Radio-immunotherapy using tositumomab (Bexxar ®) or ibritumomab (Zevalin®) in combination with BEAM has been extensively tested in recurrent B cell lymphoma, including follicular lymphoma and were not superior to Rituximab BEAM [2,3] Autologous stem cell transplantation has been extensively studied as consolidation of first remission, with most studies conducted in the pre-rituximab era and all comparing high dose chemotherapy to CHOP-like regimens. Most studies found an improvement in progression-free survival but due to transplant associated toxicities, no definitive overall survival advantage was ever established.[4-9] The lack of convincing survival data has led to a consensus statement by the EBMT-Lymphoma working party supporting autologous transplant after relapse, but not as consolidation of first remission [10]. Most recently however, the Spanish group found – with a median follow up of 12 years (interquartile range 8-15 years) - a projected 12 year PFS of 74% for patients transplanted in first remission. They argue that previous studies lacked sufficient follow-up,and that autologous transplant remains a superior treatment. Several recent report provide strong evidence for a benefit of autologous transplantation in patients with unfavorable features, i.e. early treatment failure after appropriate initial therapy.[11]

Allogeneic Transplantation

Allogeneic transplantation was initially investigated as a treatment of last resort in patients with very advanced low grade lymphoma [12]. The rates of disease recurrence after allogeneic transplantation have been remarkably low, establishing it as a highly curative therapy that can often be effective in patients with considerable amounts of residual disease. The advantage of an assuredly lymphoma free graft – exemplified by the low recurrence rates after syngeneic transplant[13]- acting synergistically with graft versus lymphoma (GVL) effect – demonstrated through observations of disease regression after donor lymphocyte infusion [14,15] all contribute to these low rates of disease recurrence.

CAR-T Cells

CAR- T cell technology (Chimeric antigen receptor) has revolutionized the management of B-cell ALL and of refractory diffuse large B cell lymphoma. CAR-T cells are patient-derived-lymphocytes that are transduced in-vitro with a chimeric receptor, part antibody, part co-signaling domain, part T-cell signaling domain. Anti CD19 CAR T have resulted in impressive and durable responses in patients with refractory ALL and large cell lymphoma [16] The CAR T cell field is developing rapidly with studies of modified CARs, and new targets being reported daily. Most of the studies have been conducted in aggressive and transformed lymphoma, where approximately 50% of treated patients obtain durable remissions- a rate of response that is unheard of with other therapies. Experience in untransformed follicular lymphoma remains at present limited although some of the initial observations were made in this setting [17] The toxicity of CAR-T cell therapy is considerable and includes severe cytokine release syndrome and neurological toxicity [18] Commercial products have only recently been approved and the use of CAR T cells is – for now- restricted to experienced centers [19]

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