



From Bench to Bedside: The Vth Symposium on the Biology and Treatment of Chronic Lymphocytic Leukemia, January 2008, Tel Aviv

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The Vth symposium on the biology and treatment of chronic lymphocyte leukemia was held in Tel Aviv on 25 January 2008 with the participation of Israeli hematologists involved in the field of Hemato-Oncology. This meeting is the fifth symposium on the subject of CLL organized by Prof. Alain Berrebi, sponsored by the Israeli Society of Hematology and Blood Transfusion and the Israeli CLL Study Group. This year the focus was on the application of science to clinical CLL, and Israeli scientists from the Weizmann Institute were invited together with four speakers from abroad (Poland, France, Spain and Germany). The symposium was divided into two parts: the first session was devoted to biology and the second to treatment.

Biology

Ms. Inbal Binsky, a PhD student from the group of Prof. Idit Shachar of the Department of Immunology at Weizmann Institute, presented an original paper on the role of CD74 in the pathogenesis of B-CLL cells, which was the result of a fruitful collaboration with Dr. Michal Haran, a senior hematologist at the Hematology Institute of Kaplan Medical Center. Previous studies done in the Shachar lab have shown that CD74, a chaperon molecule of the MHCII system, has a significant role in the survival of mature B cells. CD74 is uniformly over-expressed on B-CLL cells obtained from patients at all the stages, both at the RNA level and the protein level, including a significant increase in surface expression as seen by flow cytometry. Activation of the cell surface CD74 leads to increased expression of BCL-2, and the resultant decreased apoptosis of the cells. It was previously shown that B-CLL cells express and secrete biologically active interleukin-8. In this study, IL-8 expression was shown to be up-regulated by CD74 stimulation in B-CLL cells and to lead to increased expression of BCL-2 resulting in decreased apoptosis. These findings suggest that: a) over-expression of CD74 is an early and important event involved in the abnormal survival of the cells, and b) blocking CD74 *in vitro* by the humanized monoclonal antibody hLL1 has a significant effect on this survival mechanism and suggests that hLL1 may be an attractive novel therapeutic agent in this disease.

CLL = chronic lymphocyte leukemia
IL = interleukin

Following this new and interesting finding, a phase I-II trial using a new anti-CD74 humanized monoclonal antibody, milatuzumab-hLL1, as a single agent in refractory chronic lymphocytic leukemia will be initiated very soon.

Prof. Florence Cymbalista from Hopital Avicenne Paris XIII-University gave a lecture on the significance of the relatively new prognostic factors in CLL, especially on the new parameter ZAP-70. In previous studies, this marker was found to correlate with the mutational status of B-CLL cells, i.e., a poor prognosis and high ZAP-70 expression in unmutated cases, a low expression of ZAP-70 and a good prognosis in mutated cases. Prof. Cymbalista emphasized the importance of this statement, especially regarding technical validation and standardization since different techniques lead to discordant values. The main problem to be addressed is: what is the minimal percentage of ZAP-positive cells in good-prognosis mutated cases, since different techniques showed a variation of 7% to 32%. For this reason, an International Standardization Workshop was established, including European countries, the United States and Canada. The conclusion was that the mean fluorescence intensity ratio is more accurate than the percentage of positive cells. Concerning other prognostic factors, Prof. Cymbalista emphasized the importance of increased soluble thymidine-kinase for the prediction of progression. CD38 values, which were supposed to correlate with expression of ZAP70, have been found inconstant, and finally, the cytogenetics abnormality, as tested by fluorescent *in situ* hybridization, was found to represent the best test for a prognostic factor, especially for the detection of 11q22 and the 17p deletions, which correlates with poor prognosis, and a poor response to the widely used agent fludarabine in the 17p deletion cases.

Mr. Assaf Lask, PhD student from the group of Prof. Yair Reisner, also from the Department of Immunology at the Weizmann Institute, presented a study on the eradication of B malignant cells by anti-third party cytotoxic T lymphocytes. This novel approach for the generation of human host non-reactive CTL is based on stimulation of donor CD-8 T cells against third-party stimulators under IL-2 deprivation. A major attribute of such anti-third party CTLs is their ability to eradicate pathological

CTL = cytotoxic T lymphocytes

B-CLL cells. This B-CLL killing was shown to be independent of T cell receptor recognition and was found to be mediated by both autologous and allogeneic anti-third party CTLs. Current studies have shown that these CTLs can also efficiently eradicate Mantle lymphoma cells and Burkitt cells, and killing of tumor cells was inhibited by blocking antibodies against lymphocyte function-associated antigen-1 or intercellular adhesion molecule-1, suggesting the importance of contact between CTLs and malignant cells. Daudi lymphoma cells lacking the MHC class I were not or poorly killed by CTLs. The conclusion of the study suggests that T cell receptor independent killing of B malignant cells by anti-third party CTL is mediated via rapid adhesion through ICAM1-LFA1 binding, followed by slow induction of apoptosis upon an interaction between CD8 and MHC class I molecule on the tumor cell.

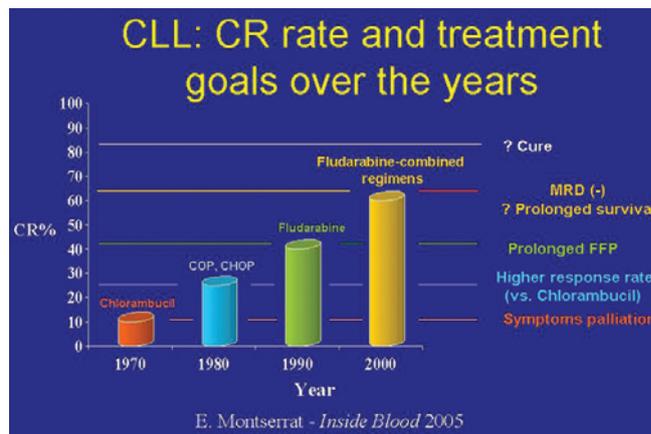
Treatment

Dr. Iwona Hus from the Department of Hemato-Oncology at the Medical University of Lublin, Poland, gave a lecture on the results of vaccinations of B-CLL patients with allo or autologous dendritic cells and their immunological and clinical response. Dendritic cells are a heterogeneous population of antigen-presenting cells found in tissues and peripheral blood (less than 1% of leukocytes). Their role in stimulation and regulation of immune response was established in the early 1980s. DCs play a pivotal role in T cell-mediated immunity and have been shown to induce strong anti-tumor response *in vitro* and *in vivo*. In B-CLL, dendritic cells were found to be deficient; Dr. Hus hypothesized that enhancement of DCs could be used as immunotherapy in B-CLL in an attempt to prolong the time to progression in untreated and treated patients. Normal allogeneic DCs and autologous DCs from CLL patients were generated from peripheral blood monocytes, then cultured with IL-4 and granulocyte macrophage-colony stimulating factor, and then matured with tumor necrosis factor. The enhanced DCs were used for vaccine to CLL patients, injected intradermally. Patients treated with allo-DCs demonstrated a marked prolonged treatment-free survival, compared with controls, in the early stages (51 versus 41 months, $P = 0.032$). Autologous DC vaccination resulted in a significant clinical response: hematological improvement in five patients, stable disease in five and no response in two. The percentage of CD3 T cells was significantly higher in the patients with clinical improvement. In conclusion, immunotherapy with allogeneic and autologous DCs stimulated with tumor cell lysates or apoptotic bodies was safe and well tolerated. During allogeneic and autologous DC vaccinations a transient clinical response as a decrease of CD19+/CD5+leukemic cells was observed in some patients. Treatment-free survival of the patients treated with allo-DCs was significantly longer as compared to the untreated patients. Increase in the counts, as well as in the function of T cells (stronger Th1 response), suggest enhance-

ICAM = intercellular adhesion molecule

LFA = lymphocyte function-associated antigen

DC = dendritic cells



ment of anti-tumor response (immunocompetence) during DC immunotherapy.

Prof. Emili Montserrat from Barcelona, Spain, one of the pioneers in the management of chronic lymphocytic leukemia, summarized the treatments of CLL adapted to age and prognostic factors. Patients who need treatment are those with symptoms related to the disease and that reflect disease activity or progression, with the addition of poor prognostic factors, including 11q22 and 17p cytogenetic deletions by FISH, detection of unmutated cases, or the surrogate marker ZAP70 whose expression correlates with the severity of disease. The factors that have to be considered are life expectancy, co-morbidity, association of autoimmune disorders, and detection of the 17p deletion which shows resistance to fludarabine. There is well-established evidence-based CLL therapy that includes fludarabine and cyclophosphamide combination and the addition of monoclonal antibody – either rituximab or alemtuzumab. These treatments are able to induce complete remission in more than 50% of the patients. The achievement of absence of minimal residual disease translates into longer progression-free survival and overall survival. Definitely, the combination of FCR (fludarabine, cyclophosphamide, rituximab) gave the best achievement: up to 72% complete response and 94% overall response, with progression-free survival of 4 years reaching 70%. Prof. Montserrat emphasized that although allogeneic stem cell transplantation has curative potential, due to the high transplant-related morbidity, this option should be reserved for young refractory patients. Prof. Montserrat concluded by underlining the significant progress made during the last 10 years [Figure 1] in the definition of prognostic factors and achievement of response by treatment that is individualized and risk adapted.

The last lecture was given by Prof. Peter Dreger, from Heidelberg University, Germany, on the role of stem cell transplant in CLL. There is a transplant consensus concerning allo-stem cell transplant in poor-risk CLL, which are: relapse less than 24 months after intensive treatment, the presence of p53 mutation (17p deletion), and poor response after purine analog-based therapy. When these indications are present, the appropriate conditioning – reduced intensity or myeloablative

FISH = fluorescent in situ hybridization

– should be adapted, depending on the individuals risk factors. According to previous studies performed by the German CLL Study Group, long-term disease control can be achieved with reduced intensity conditioning in nearly 60% of the patients, but there is no definite conclusion concerning the choice of reduced intensity versus myeloablative conditioning. Prior auto-stem cell transplant does not influence the event-free survival. There is also no evidence for inferiority of matched unrelated donor versus matched sibling donor, and no evidence for superiority of T cell-depleted transplant. In a survey on allo-stem cell transplant in 17p (p53) patients, 44% achieved a 3 year progression-free survival and a durable MRD negativity. Concerning autologous transplant, there is no evidence for cure, however Prof. Dreger cited a prospective multicenter randomized study comparing follow-up after fludarabine and cyclophosphamide versus autologous transplant. The study group comprises 223 patients and the results will be published in October 2009. There is a trend for the superiority of total

MRD = minimal residual disease

body irradiation, and promising results with *in vivo* purging while alemtuzumab seems to be effective for eradication of MRD before harvest.

Finally, there was a short roundtable discussion on the future of CLL research and treatment, particularly the importance of collaboration between clinicians and scientists. The focus was on how to gain a better understanding and improve management of the illness using the new prognostic factors efficiently, how and when to apply new research findings (for example, the enhancement of dendritic cells or CTLs, presented at this meeting), and finally, how to tailor and individualize patient treatment according to age and co-morbidity. Prof Montserrat stressed the significance of patient-centered care and of the overall well being of the patient as a result of a good selection of the new markers, the choice of an appropriate treatment and a supportive physician patient-relationship.

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