BEST TREATMENT OPTIONS FOR HODGKIN´S DISEASE – RESULTS FROM TRIALS OF THE GERMAN HODGKIN´S LYMPHOMA TRIAL GROUP (GHSG)

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The GHSG is conducting clinical trials for patients suffering from Hodgkin´s Lymphoma (HL) since 1978. Currently, the fifth trial generation is ongoing, collaborating with more than 500 participants Europe. In accordance with international treatment groups, three subdivisions are used to tailor therapy according to initial risk at diagnosis: early stages (I-II without risk factors), intermediate stages (I-II with risk factors) and advanced stages (IIB with bulky disease and III-IV).

Early stages HL. With high cure rates achieved in treatment of HL, most current clinical trials in early stages aim at reducing long term toxicities while maintaining optimal tumor control. The HD10 trial (1998-2002) was designed to investigate the the optimal intensity of both, chemotherapy and radiotherapy. Patients (pts) were randomized to receive either 4 or 2 cycles ABVD followed by either 30 Gy or 20 Gy IF-radiotherapy. At the third interim analysis (2006), overall survival (SV) after a median observation time of 41 months were 94% and 93%, respectively there was no significant between the chemotherapy or the radiotherapy arms.

Intermediate stages HL. Compared to pts with advanced stages HL, results for intermediate stages appeared unsatisfactory in terms of efficacy. To ameliorate treatment outcome for pts in early unfavourable stages, the GHSG introduced the standard BEACOPP protocol in their HD11 trial (1998-2002). Pts. were randomized between 4 cycles of ABVD or 4 cycles of BEACOPP followed by either 30 Gy or 20 Gy IF-radiotherapy. At a median observation time of 44 months, the fifth interim analysis from 2006 showed FFTF rates of 87% (ABVD) and 88% (BEACOPP); and, 90% (30 Gy) and 87% (20 Gy), representing no significant differences with regard to chemotherapy or radiotherapy.

Advanced stages HL. The HD9 trial (1993-1998) achieved superior response rates for pts with advanced stages HL with the escalated BEACOPP protocol. The subsequently performed HD12 trial (1998-2002) was designed to maintain these superior results while deescalating chemotherapy by comparing 8 cycles escalated BEACOPP with 4 cycles escalated BEACOPP followed by 4 cycles standard BEACOPP with or without consolidatory radiation to sites of residual tumor or initial bulky disease. At a median observation time of 24 months, the FFTF for the total group was 89.2%, the overall survival was 94%. There were no differences for the arms with or without RT, for both, FFTF and SV. The fifth trial generation (HD13-15) is still ongoing and aims either at further reduction of treatment related toxicity, both acute and long-term toxicity (HD13 for early stages, HD15 for advanced stages), or exploring more effective chemotherapy regimen (HD14 for intermediate stages) in case of unsatisfying results so far. Future strategies will incorporate new diagnostic techniques such as PET imaging for tailoring treatment regimen. Furthermore, the value of modern drugs is tested in phase II trials, hopefully augmenting the spectrum of current therapeutic tools.

NOVEL THERAPY FOR HODGKIN LYMPHOMA

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The world health organization (WHO) classification of Hodgkin lymphoma (HL) distinguished between two major subtypes, classical HL and nodular lymphocyte predominant HL. Approximately 95% of patients with HL will have the classical HL histology, which is characterized by the presence of rare malignant Hodgkin and Reed Sternberg (HRS) cells among an overwhelming number of benign reactive cells. In recent years, new studies shed more light on the biologic and molecular features of HRS cells providing hopes that new targeted therapy may be developed to enhance the cure rate and to reduce treatment-related toxicity. Future treatment strategies based on our understanding of HL biology will be discussed. This will include novel monoclonal antibodies to CD30 and IL-13, and small molecule therapy targeting HDACs, Bcl-2 family, and mTOR.

SEIZING OF T CELLS BY HUMAN T CELL LEUKEMIA/LYMPHOMA VIRUS TYPE 1

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Human T cell leukemia/lymphoma virus type 1 (HTLV-1) causes neoplastic transformation of human T cells in a small number of infected individuals several years from infection. Several viral proteins act in concert to increase the responsiveness of T cells to extracellular stimulation, modulate pro-apoptotic and anti-apoptotic gene signals, enhance T cell survival, and avoid immune recognition of the infected T cells. The virus promotes T cell proliferation by usurping several signaling pathways central to immune T cell function. Viral proteins modulate the downstream effects of antigen stimulation and receptor-ligand interaction, suggesting that extracellular signals are important for HTLV-1 oncogenesis. Environmental factors such as chronic antigen stimulation are therefore important, as also suggested by epidemiological data. The ability of a given individual to respond to specific antigens is genetically determined. Thus, genetic and environmental factors together with the virus contribute to disease development. As in the case of other virus-associated cancers, HTLV-1-induced leukemia/lymphoma can be prevented by avoiding viral infection or by intervention during the asymptomatic phase with approaches able to interrupt the vicious cycle of virus-induced proliferation of a subset of T cells. Current knowledge of the mechanisms regulating HTLV-1 replication and the T cell pathways that are usurped by viral proteins to induce and maintain clonal proliferation of infected T cells *in vitro* will be reviewed. The relevance of these laboratory findings will be related to clonal T cell proliferation and adult T cell leukemia/lymphoma (ATLL) development *in vivo*.

MANAGEMENT OF HIV-RELATED LYMPHOMAS IN HAART ERA

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Systemic non-Hodgkin's lymphomas (NHL) are the main neoplasia-related cause of morbidity and mortality associated to HIV infection in the HAART era. In western countries, Hodgkin's disease (HD) is the non-AIDS defining tumor that shows the most remarkable and consistent increase. The most important epidemiological studies ran up to-day show that in the HIV-positive population the risk of developing HD is 8 to 16-times higher than that of the general population. The introduction of HAART has changed the natural history of HIV infection and it has allowed the exploration of potentially curative myeloablative therapy also in HIV-positive population.

However, so little is known about the possibility that the worsening of the immune system could be related to the consequent activation of HIV replication with clinical progression of the HIV infection itself and of lymphoma course. A high prevalence of chronic hepatitis C virus infection in patients with HIV-NHL has been reported. Even if HCV infection should not be taken as an absolute controindication to high dose chemotherapy (CT), it is well known that HCV-positive patients are at high risk of HCV reactivation and liver failure.

In HAART era the classical prognostic factors for NHL-HIV (i.e. previous AIDS, performance status, CD4 cell count) have been overcomed by those prognostic factors of NHL of the general population. In particular, the International Prognostic Index (IPI) seems to predict the outcome of Hiv patients with NHL similarly to that observed for HIV negative patients. Therefore, the prognostic role of the immunological deficit and the availability of HAART, make its association with CT mandatory.

Common metabolic pathways, mainly mediated by cytocrome P-450, or involving transmembrane carriers (multidrug-resistance related) have been described for protease inhibitors and many cytotoxic drugs. These provide a rational

background for possible in pharmacokinetic and pharmacodynamic interactions between antiretroviral and chemotherapeutic drugs. The evaluation of the feasibility of the association between CT and HAART and the study of pharmacokinetic and pharmacodynamic interactions between the two types of drugs are two of the main goals of the clinical trials in the HAART-era.

HCV AND NON-HODGKIN'S LYMPHOMA: EPIDEMIOLOGY, BIOLOGY AND THERAPY

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Hepatitis C virus (HCV) is associated with chronic liver disease as well as with lymphoproliferative disorders such as mixed cryoglobulinemia (MC) and non-Hodgkin's lymphomas (NHL). The association between HCV infection and B-cell lymphoma is controversial since it shows a strong regional variation. In fact, the prevalence of HCV infection in NHL shows a prevalence ranging between 7.4 and 37.0 %. However, the intimate pathogenetic mechanism involved in HCV-associated lymphomas remained unknown for years. Recently, it has been found that the major envelope protein of HCV (HCV-E2) binds with high affinity CD81, a tetraspanin expressed on several cell types, including B-cell. On this basis, we have shown that the engagement of CD81 on human B-cells by a combination of HCV-E2 and an anti-CD81 monoclonal antibodies triggers the JNK pathway and leads to the preferential proliferation of the naïve (CD27-) B cell subset. In parallel, we have found that B-lymphocytes from the great majority of chronic hepatitis C patients are activated, and that naïve cells display a higher level of activation markers than memory (CD27+) B lymphocytes. Moreover, the eradication of HCV infection by antiviral therapy is associated to normalization of the activation markers expression. These findings suggest that a polyclonal proliferation of naïve B lymphocytes (CD81-mediated) is a key initiating factor for the development of the HCV-associated B-lymphocyte disorders.

The management of HCV-associated NHL is similar to that of conventional lymphoma, although viral reactivation or the underlying chronic liver disease can complicate chemotherapy. Whether to treat low-grade HCV-related lymphomas with antiviral therapy was debatable for years, but some recent studies have shown that combination therapy with pegilated interferons and ribavirin is useful in some subtypes of NHL such as lymphoplasmacytic immunocytomas and splenic lymphomas with villous lymphocytes.

INFUSION OF CYTOKINE INDUCED KILLER CELLS IN PATIENTS RELAPSED AFTER ALLOGENEIC TRANSPLANTATION

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Cytokine Induced Killer (CIK) cells are naturally cytotoxic effectors with T/NK phenotype (CD3+CD8+CD56+) showing *in vitro* a potent antitumor activity. Previous Phase I studies have shown measurable antitumor activity when autologous CIK cells were given to patients with lymphomas and solid tumors. In experimental *in vivo* murine models allogeneic and xenogenic (human) CIK cells showed graft versus tumor (GVT) effects with reduced propensity for GVHD. Based on these preliminary observations, we planned to investigate the safety of repeated administrations of allogeneic CIK cells in patients relapsing after allogeneic transplantation. CIK cells were prepared in our GMP facility upon approval of the protocol by the national authority (Istituto Superiore di Sanità, Rome, Italy) and the local ethycal committee. CIK cells were expanded from donor lymphocyte aphereses exposed to clinical grade IFNγ (day 1) OKT-3 monoclonal antibody (day 2) and IL-2. After 21 days CIK cells were frozen until lot release criteria were met: negativity of microbiological analyses for bacteia, fungi, mycoplasma, endotoxin levels below 5 IU/Kg/hr, viability over 80%, presence of at least 40% CD8+/CD56+ cells and measurable cytotoxic activity against K562 cell line.

Ten patients with Acute Myelogenous Leukemia (n=4), Hodgkin Disease (n=3), Chronic Myelomonocytic Leukemia, (n=1), pre-B Acute Lymphoblastic Leukemia (n=1) and secondary myelodysplasia (n=1), all relapsed after sibling (5) or matched unrelated donor (5) HSCT, entered this study. Before CIK administration, 6 patients had received other salvage treatments including chemotherapy (5), radiotherapy (1) and unmanipulated donor lymphocytes (5) without any significant tumor response. The median number of CIK infusions was 2 (range 1-6) and the median number of total CIK cells was 13.5×10^6/kg (range 7.2-41.6). The infusion was well tolerated and no acute or late infusion-related reactions were registered. Acute GVHD (grade I and II) was observed in 3 patients 30 days after the last CIK infusion, which progressed into extensive chronic GVHD in one case. Disease progression and death occurred in 6 patients. Two stable diseases, one hematologic improvement and one complete response were observed. This study confirms the feasibility of preparing sufficient numbers of CIK cells under GMP conditions. More importantly, it shows for the first time that allogeneic CIK cells can be administered without significant toxicity to patients relapsing after hematopoietic stem cell transplantation. On the basis of these results we have used the same standardized 21-day expansion protocol to produce cytokine-induced killer (CIK) cells starting from very small amounts of nucleated cells (approximately 15×10^6 cells) isolated from a cord blood unit. Washouts of cord blood units bags (at the end of the infusion) may be sufficient to yield almost 500×10^6 CIK by the same expansion protocol. CIK cells show strong cytotoxic activity against a variety of tumor target cell lines including B and T lymphomas and myeloid leukemias. These observations open up the possibility of imaging a future clinical application of leukemia relapse following cord blood transplantation with CIK cells obtained from the same cord blood unit.

DEMONSTRATION OF DONOR ID SPECIFIC CD8 AND CD4 RESPONSES AFTER ALLOGENEIC TRANSPLANTATION – *IN VIVO* DEMONSTRATION OF GVL EFFECTOR CELLS

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Following allogeneic stem cell transplantation there is evidence of a graft versus leukemia effect (GVL) but it is not known if this is mediated by GVHD or might also be contributed to by response against specific tumor associated antigens (TAA). To investigate this we sought to determine if we could identify tumour antigen specific T cells allogeneic stem cell transplant (alloSCT) and as a model for this examined CD4 and CD8 responses against idiotype (Id) in 50 patients who had undergone reduced intensity conditioning allo-SCT for CLL. The Ig was sequenced and peptides identified from 26 patients that could bind to HLA-A*0101, HLA-A*0201 or HLA-A*0301 in serial PB and BM samples were available for analysis. Id specific cells could be detected by tetramer staining post transplant in 17 of these 26 patients (65%) at a median of 100 days (range 80-120), with a frequency ranging from 0.2 to 2.9% of CD8+ T cells. In four additional cases Id specific T cells were only detectable after subsequent DLI. In 9 of these cases CD4 responses against peptides that could bind to HLA-DRB1*0101, *0301, *0401 were also examined and Id specific CD4 cells detected in 5 of these cases post transplant and also in two additional cases after DLI. In all cases the antigen specific T cells were of donor origin. Of note an increased frequency of Id specific cells was often co-incident with subsequent development of chronic GVHD. In some cases, the Id specific cells remained detectable for up to one year post transplant, and in no cases did detectable cells persist. Id specific T cells could also be further amplified ex vivo using peptide pulsed antigen presenting cells and cytokines. In all cases we were able to demonstrate that the tetramer sorted T cells could kill the patients' primary CLL cells *in vitro*, but we have no direct evidence that this was occurring *in vivo*. Indeed it is unlikely that these Id specific T cell respons-