055 METHYLATION PROFILING IN 158 CASES OF PREVIOUSLY UNTREATED FOLLICULAR LYMPHOMA (FL)
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Introduction: Disruption of DNA methylation is a hallmark of cancer, is frequently associated with changes in gene expression and is a potential therapeutic target in haematological malignancies. The methylation patterns of FL are not yet well characterised. We investigated gene methylation in 158 diagnostic cases of FL using a high-throughput Illumina® GoldenGate™ system.

Materials and Methods: The methylation status of 1505 CpG loci representing 807 genes was assessed in DNA from 158 FL samples, human tonsil and pooled mononuclear cells (MNC). Results are expressed as a β value between 0 and 1 as a ratio of methylated to methylated & unmethylated alleles. In our subsequent analysis samples were categorised according to β value as hypermethylated (β >0.6), intermediate (0.4 ≤ β ≤ 0.6), unmethylated (β < 0.4). Comparisons were made between FL group (n=158), tonsil (n=17) and MNC control group (mean <0.25). Comparison between the FL group and this control group revealed a tumour-specific methylation profile for 101 loci, (7.4%) uniformly hypermethylated in all FL samples, 54 (3.6%) showing heterogeneous methylation patterns (cluster analysis showed differentiation of tumour (n=158) from non-tumour samples (n=7) in all but two tumour cases. Fifty-four per cent of CpG loci (n=820) were unmethylated in the tonsil and MNC control group (mean <0.25). Contrast between the FL group and the control group revealed a tumour-specific methylation profile for 101 loci, corresponding to 73 genes, with mean hypermethylated values in the FL group. Included among the 101 hypermethylated loci were genes known (DAK, MYOD1) and not known (CEBPAP, CDH1, CDH3, EGRBP1, WNT) to be hypermethylated in lymphoma. In contrast, 559 loci were unmethylated (mean <0.25) in both FL and control groups. Among the unmethylated loci were a number of potential genes of lymphoma. In contrast, 559 loci were unmethylated (mean <0.25) in both FL and control groups.

Results: The methylation profile in 158 FL samples showed 111 of the 1505 CpG loci (7.4%) uniformly hypermethylated in all FL samples, 142 (9.4%) unmethylated while the remaining loci (83.2%) showed heterogeneous methylation patterns. Cluster analysis allowed discrimination of tumour (n=158) from non-tumour samples (n=7) in all 2 tumour cases. Results are expressed as a β value between 0 and 1 as a ratio of methylated to methylated & unmethylated alleles. In our subsequent analysis samples were categorised according to β value as hypermethylated (β >0.6), intermediate (0.4 ≤ β ≤ 0.6), unmethylated (β < 0.4). Comparisons were made between FL group (n=158), tonsil (n=17) and MNC control group (mean <0.25). Contrast between the FL group and this control group revealed a tumour-specific methylation profile for 101 loci, (7.4%) uniformly hypermethylated in all FL samples, 54 (3.6%) showing heterogeneous methylation patterns (cluster analysis showed differentiation of tumour (n=158) from non-tumour samples (n=7) in all 2 tumour cases. Fifty-four per cent of CpG loci (n=820) were unmethylated in the tonsil and MNC control group (mean <0.25). Contrast between the FL group and the control group revealed a tumour-specific methylation profile for 101 loci, corresponding to 73 genes, with mean hypermethylated values in the FL group. Included among the 101 hypermethylated loci were genes known (DAK, MYOD1) and not known (CEBPAP, CDH1, CDH3, EGRBP1, WNT) to be hypermethylated in lymphoma. In contrast, 559 loci were unmethylated (mean <0.25) in both FL and control groups. Among the unmethylated loci were a number of potential genes of lymphoma. Studies are in progress to correlate methylation of these genes with clinical outcome within this diagnostic series.

Conclusion: Epigenetic deregulation is a common feature of FL. We identified a novel group of hypermethylated genes in FL. Studies are in progress to correlate methylation status with gene expression profile and clinical outcome within this diagnostic series.

056 FOLLICULAR LYMPHOMA DERIVED B CELLS ARE SUFFICIENT TO CONVERT CD4+ T CELLS INTO CD4+ CD25+ FOXP3+ REGULATORY T CELLS VIA CELL-CELL CONTACT WITHOUT STIMULATION OF T CELL RECEPTOR
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Background: MyVax® personalized immunotherapy consists of the tumour-specific Id protein, produced using molecular methods, conjugated to keyhole limpet hemocyanin (KLH) and administered in a series of SC immunizations with GM-CSF.

Methods: This is a multi-center, randomized, blinded, controlled trial examining the safety and efficacy of MyVax compared to a control treatment (pts) with previously untreated NHL. Pts received 8 cycles of CVF followed by a 6-month rest period. Pts who maintained at least a PR for 6 months post CVF were randomized to receive MyVax or control immunotherapy in a 2:1 ratio. All pts received GM-CSF at each immunization and the next 3 days. Pts received a series of 7 immunizations over 24 wks. Sera for Id- and KLH-specific humoral immune response (IR) assays were collected prior to, during and for 1 yr following immunization.

Results: 287 pts were randomized and 278 pts received at least one immunization. Anti-Id IRs were observed in 41.0% of evaluable pts. No statistical difference in PFS and time to subsequent anti-lymphoma therapy (SALT) was seen in the pts who received MyVax compared to those who received the control immunotherapy. Both arms of the study have a plateau of PFS >30% at 3yrs. A highly statistically significant improvement in PFS (p=0.0017) was seen in pts mounting an anti-Id immune response (IR+) vs IR- patients with >2-fold increase (59.7 vs 18.1 months) in PFS. The IR+ pts show a statistically significant improvement in PFS over the control immunotherapy pts while there is not a statistically significant difference in PFS between the IR+ pts and the control immunotherapy pts. High risk FLIPI patients did as well as the other pts in both PFS and SALT.

Conclusions: As previously reported in phase 2 trials, this large prospective randomized controlled trial shows that NHL pts mounting anti-Id IRs have a significantly improved clinical outcome.

057 F2 PROGNOSTIC INDEX
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Introduction: So far prognostic models developed for follicular lymphoma (FL) have been based on retrospective analysis of archive data. The F2-study was designed as a complement of the International Follicular Lymphoma Prognostic Factors Project with the aim of validating FLIPI and verifying whether a prognostic collection of data would allow the development of a more accurate prognostic index.

Patients and Methods: Patients were registered in the study regardless their planned treatment. Study sample was calculated on the following statistical considerations: i) each risk factor has a prevalence of at least 10%; ii) the 5-yr survival of the remaining subjects is 70%; iii) the odds ratio is 2 for death with the risk factor compared to that without. A sample size of 900 assessable patients was planned. The primary focus of the study was Survival (OS) (specifically, at 5 years). Subsequently the study Executive Committee decided to consider Progression Free Survival (PFS) as an additional primary outcome measure. Variables to be used for score definition were selected by means of a bootstrap resampling procedures (N=250) on Cox proportional hazard regression analysis with backward elimination. The best variables to select the model minimizing the misclassification error were performed, and proportionality of the risks, overfitting and calibration of the model were also checked.

Results: Between January 2003 and May 2005 1,093 patients were registered by 69 European and American Institutions, 1,057 fitted inclusion criteria; 942 received anti-lymphoma therapy, and 931 were assessable for FLIPI. After a median follow-up of 38 months, 292 events for PFS evaluation were recorded. In addition to FLIPI, univariate analysis 11 variables significantly influencing PFS were found. Multivariate
Conclusions: The F2 study demonstrates that a web-based world-wide collection of data is feasible, allows the opportunity to analyze a relevant and quite complete set of significant data, and is undoubtedly a powerful instrument for investigating the prognosis of FL. The F2 prognostic index seems a promising new tool for the identification of patients at different risk of disease progression.

059 STRIKING DIFFERENCES IN THE IG REPERTOIRE BETWEEN CLL AND MBL: IMPLICATIONS FOR THE PATHOGENESIS OF THE DISEASE

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Introduction: The term Monoclonal B Lymphocytosis (MBL) defines the presence of Monoclonal B Lymphocytes circulating in the blood of healthy aging individuals. This phenomenon attracted the attention of the investigators as the cell phenotype closely resembles that of Chronic Lymphocytic Leukemia (CLL) cells and it is more frequent among relatives of CLL-affected patients. All these evidences suggest that MBL is a precursor state for CLL, as MGUS for Multiple Myeloma. That notwithstanding, few MBL may express CLL “stereotyped receptors”, indicating that the potential transformation into a leukemia exists within MBL cases, though at a low frequency, and it depends on a precise selection mechanisms based on the molecular features, if not the antigen specificity, of the B cell receptor.

Results: We show that the most frequently used IGHV gene in MBL is IGHV4-59/61 which is rather uncommon in CLL, while MBL cells completely lack the expression of the most frequent and characteristic genes in CLL (IGHV1-69 and IGHV4-34). Nevertheless, the analysis of the HCDR3 sequences of the IG allowed us to identify two MBL cases carrying a sequence similar to previously described CLL cases (“stereotyped receptors”).

Conclusions: We demonstrate for the first time that the overall IG repertoire expressed by MBL does not show the typical CLL-related IGHV gene usage biases, suggesting the absence of a direct correlation between the presence of monoclonal B lymphocytes and the subsequent transformation. That notwithstanding, few MBL may express CLL “stereotyped receptors”, indicating that the potential transformation into a leukemia exists within MBL cases, though at a low frequency, and it depends on a precise selection mechanisms based on the molecular features, if not the antigen specificity, of the B cell receptor.

060 A PROSPECTIVE MULTICENTER TRIAL ON NONMYELOABLATIVE ALLOGENEIC STEM CELL TRANSPLANTATION (NST) FOR POOR-RISK CHRONIC LYMPHOCYTIC LEUKEMIA (CLL): FINAL RESULTS OF THE GCLLSG CLL3X STUDY

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The purpose of this study was to investigate prospectively feasibility, toxicity and efficacy of NST in patients with poor-risk CLL based on guidance of immunomodulating therapy by prospective monitoring of minimal residual disease (MRD).

Methods: Patients <65 years eligible if they had aggressive disease in the presence of an unfavorable VH status, were fludarabine refractory, or had relapse after auto-SCT. Conditioning was based on fludarabine and cyclophosphamide with standard GVHD prophylaxis. Prospective longitudinal MRD monitoring was done by MRD-flow or RQ-PCR. Donor lymphocyte infusions were administered after immunosuppression withdrawal in case of incomplete chimerism or MRD.

Results: Between June 2001 and March 2007, 113 patients were accrued. For the purposes of this abstract, data of 100 patients was available. Of these, 12 had to be excluded due to ineligibility. The 88 patients remaining had received 4 (1-11) regimens. 44/69 (64%) had an unfavourable FISH karyotype or fludarabine resistance on relapse. Survival was significantly influenced by disease status at NST. Allografts were obtained from related (40%) or unrelated donors (28%). 40/69 (64%) had an unfavourable FISH karyotype, but only 22% had uncontrolled disease at NST. Allografts were obtained from related (40%) or unrelated donors (60%). With a median follow-up of 12 (1-70) months, 2-year treatment-related mortality, relapse incidence, and overall survival were 9%, 36%, and 77%, respectively. Univariate analysis showed no adverse impact of unfavourable FISH karyotype or fludarabine resistance on relapse. Survival was significantly influenced by disease status at NST. Of 58 patients with MRD data available, 37 became MRD negative 1-27 months post NST. Only one relapse was observed in 23 patients who reached MRD clearance by month +12 or later.

Conclusions: NST as used here is a safe and effective treatment for patients with poor-risk CLL including those with fludarabine resistance or an adverse FISH karyotype. An analysis of the full patient set will be presented.