055 METHYLATION PROFILING IN 158 CASES OF PREVIOUSLY UNTREATED FOLLICULAR LYMPHOMA (FL)

C.O'Riain[1], D. O'Shea[1], Y. Yang[1], J. Gribben[1], J. Afari[1], C.A. Mein[1], T. Crook[2], P. Smith[2], A. Rosenwald[1], E. Campo[1], R. Roberts[1], L.M. Rimsza[1], E.B. Smeland[1], R. Braziel[1], W.C. Chan[1], N. Johnson[1], R.D. Gascoyne[1], L.M. Staudt[1], T.A. Lister[1], J. Fitzgibbon[1]

[1]Centre for Medical Oncology, Barts and the London School of Medicine, London, United Kingdom, On Behalf of the Lymphoma/Leukemia Molecular Profiling Project, [2]Cancer Genetics and Epigenetics, Institute for Cancer Research, London, United Kingdom

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 were assayed in DNA from 158 FL samples, human tonsil and pooled mononuclear cells (MNC). Results are expressed as a ß value between 0 and 1 as a ratio of methylated to methylated & unmethylated alleles. In our subsequent analysis samples were categorised according to ß value as hypermethylated (>0.6), intermediate (0.6-0.25) or unmethylated (<0.25).

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 heterogenous methylation patterns. Cluster analysis allowed discrimination of tumour (n=158) from non-tumour samples (n=7) in all but 2 tumour cases. Fifty-four per cent of CpG loci (n=820) were unmethylated in the tonsil 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, corresponding to 73 genes, with mean hypermethylated values in the FL group. Included among the 101 hypermethylated loci were genes known (*DAPK, MYOD1)* and not known (*CEBPA*, *CDH1*, *CDH13*, *IGFBP3*, *WT1)* 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 interest; genes known to be mutated in lymphoma (*CDKN2A*, *PTEN*), genes involved in drug response (*GSTP1*, *MGMT*), apoptosis (*BMP6*) and cell cycle regulation (*CCND1*) suggesting methylation of these genes may not play as significant a role in the pathogenesis of FL as previously suspected. Also included in this group were genes involved in catalyzing DNA methylation (*DNMT1, DNMT3b).*

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

W. Ai[1], J. Hou[2], R. Zeiser [3], D. Czerwinski[4], R. Negrin[4], R. Levy[4]

[1]Medicine, University of California, San Francisco, San Francisco, United States, [2]Medicine, University of Pittsburg, Pittsburg, United States, [3]Medicine, Albert Ludwig University, Freiburg, Germany, [4]Medicine, Stanford University, Stanford, United States

Background: $Foxp3^+CD4^+CD25^+$ regulatory T cells (Treg) control T cell homeostasis in healthy individuals by suppressing self-reactive cells. In the context of cancer, there has been accumulating evidence that Treg are highly concentrated in tumors, thereby fostering an immune-privileged microenvironment. Studies have shown that T cell receptor (TCR) stimulation via antiCD3 in combination with antiCD28 costimulation can convert conventional T cells (Tconv) isolated from either peripheral blood (PBMC) or from follicular lymphoma (FL) involved lymph nodes into Treg. Furthermore, FL derived B cells can enhance the conversion of tumor infiltrating T cells to Treg. However, it is still not full understood how FL derived B cells cells interact with its microenvironment in promoting immune escape.

Materials and Methods: We investigated the impact of malignant B cells derived from human FL tissue as compared to B cells from normal PBMC on conversion of Treg from $CD4^+CD25^-$T cells (Tconv).

Results: We found that tumor B cells could independently induce Tconv to express FoxP3 and to acquire regulatory function without TCR stimulation. In contrast to their malignant counterpart, normal B cells did not result in Treg conversion without TCR stimulation. The Treg conversion was independent of the T cell background, since Tconv isolated from FL or from PBMC were converted to the same extend.

Conclusion: Our study provided the first evidence for a tumor-specific mechanism by which tumor B cells promote immune escape in FL.

057 RESULTS OF A PHASE 3 TRIAL EVALUATING SAFETY AND EFFICACY OF SPECIFIC IMMUNOTHERAPY, RECOMBINANT IDIOTYPE (ID) CONJUGATED TO KLH (ID-KLH) WITH GM-CSF, COMPARED TO NON-SPECIFIC IMMUNOTHERAPY, KLH WITH GM-CSF, IN PATIENTS WITH FOLLICULAR NON-HODGKINS LYMPHOMA (FNHL)

R. Levy[1], M. Robertson[2], J. Leonard[3], J. Vose[4], D. Denney[5]

[1]Med Onc, Stanford U Medical Center (MC), Stanford, United States, On Behalf of Study 2000-03 Investigators, [2]Hem/Onc, Indiana U MC, Indianapolis, United States, [3]Lymphoma/Myeloma, Weill Medical College of Cornell U, New York, United States, [4]Hem/Onc, U of NE MC, Omaha, United States, [5]CEO, Genitope Corp, Fremont, United States

Background: MyVax® personalized immunotherapy consists of the tumor-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 immunotherapy in patients (pts) with previously untreated fNHL. Pts received 8 cycles of CVP followed by a 6-month rest period. Pts who maintained at least a PR for 6 months post CVP 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 5yrs. 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 (39.7 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 fNHL pts mounting anti-Id IRs have a significantly improved clinical outcome.

058 F2 PROGNOSTIC INDEX

M. Federico[1], M. Bellei[1], L. Marcheselli[1], S. Luminari[1], A. Lopez-Guillermo[1], U. Vitolo[1], B. Pro[1], M. Martelli[1], P. Soubeyran[1], S. Cortelazzo[1], G. Martinelli[1], S. Pileri[1], P. McLaughlin[1], P. Solal-Céligny[1].

[1]on behalf of the IFLPFP, F2-study Group, Modena Trial Office, Italy

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 set at 0.10. Analyses 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, in univariate analysis 11 variables significantly influencing PFS were found. Multivariate

analysis showed that Beta2-microglobulin > ULN, Hemoglobin < 12 g/dL, Age > 60 years, Maximum diameter of the largest involved node > 6 cm and bone marrow involvement were factors independently predictive for PFS. Using these 5 variables, a prognostic model was devised to identify 3 groups at different risk. The 3-yr PFS rate was 92%, 70% and 50% for patients at low, intermediate and high risk respectively (Logrank=65.9, p<0.00001). The model was also predictive in the group of patients treated with (p<0.0001) or without (p<0.0001) Rituximab. The 3-yr survival rate was 99%, 96% and 84% for patients at low, intermediate and high risk respectively (p<0.00001).

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

P. Ghia[1], A. Dagklis[1], C. Fazi[1], V. Cantarelli[1], D. Toniolo[1], R. Massacane[1], K. Stamatopoulos[1], F. Caligaris-Cappio[1].

[1]*Department of Oncology, Universita` Vita-Salute San Raffaele, Milano, Italy*

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, one has to consider that MBL is 100 times more frequent than CLL and the appearance of monoclonality in the immune repertoire is considered part of the normal immune-senescence process.

Materials and Methods: We studied by cytofluorograph analysis the blood of healthy individuals older than 18 years; we amplified by PCR and direct sequenced the Immunoglobulin (IG)HV-D-J rearrangements expressed by 47 MBL cases. Sequence data were analyzed on the IMGT database, extracting IGHV-D-J gene usage, percentage of identity to germline, and HCDR3 features. The MBL sequences were aligned to a comprehensive panel of IG sequences from CLL cases and normal or pathological B lymphocytes.

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 leukaemia 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

P. Dreger[1,2], S. Stilgenbauer[2], S. Böttcher[2], M. Ritgen[2], R. Busch[2], D. Bunjes[2], D. Beelen[2], S. Cohen[2], U. Hegenbart[1], J. Schubert[2], B. Hertenstein[2], M. Stadler[2], B. Glass[2], A. Burchert[2], L. Uharek[2], N. Basara[2], A. Humpe[2], U. Dührsen[2], M. Hallek[2], N. Schmitz[2], M. Kneba[2], H. Döhner[2].

[1]*Medicine V, University of Heidelberg, Heidelberg, Germany,* [2]*German CLL Study Group, Cologne, Germany*

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 were 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 (del 11q22 (36%) or del 17p13 (28%)). 40/73 (55%) were fludarabine refractory, 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.