session 4 – diffuse large B-cell lymphoma

050 GENE EXPRESSION SUBTYPES OF DIFFUSE LARGE B CELL LYMPHOMA ARISE BY DISTINCT GENETIC PATHWAYS

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Introduction and methods: Gene expression profiling has been used to define three molecular subtypes of diffuse large B cell lymphoma (DLBCL), termed germinal center B-cell-like (GCB) DLBCL, activated B-cell-like (ABC) DLBCL, and primary mediastinal B-cell lymphoma (PMBL). To investigate whether these DLBCL subtypes arise by distinct pathogenetic mechanisms, we analyzed 203 DLBCL biopsy samples by high resolution, genome-wide copy number analysis (aCGH) coupled with gene expression profiling. We defined minimal common regions (MCRs) that were recurrently altered in copy number and identified those that dysregulated the expression of their constituent genes. Statistical differences in MCR frequency between DLBCL subtypes were corrected for multiple hypothesis testing using a false discovery rate (FDR) calculation.

Results: Of 258 MCRs that occurred in more than 5% of any DLBCL subtype, 31 were utilized differentially by the DLBCL subtypes ($p<0.005$, false discovery rate <0.05). Amplification of the oncogenic mir-17-92 microRNA cluster and deletion of the tumor suppressor *PTEN* were recurrent in GCB DLBCL but did not occur in ABC DLBCL. Conversely, deletion of the *INK4a/ARF* tumor suppressor locus, gain of chromosome arm 18q, and trisomy 3 occurred almost exclusively in ABC DLBCLs and were associated with inferior outcome within this subtype. *BCL2* and *NFATC1* are candidate oncogenes affected by the chromosome 18 aberrations. An amplicon on chromosome 19 was detected in 20% of ABC DLBCLs but in only 3% of GCB DLBCLs and PMBLs. The gene most upregulated by this amplicon was *SPIB*, which encodes an ETS family transcription factor. Knockdown of *SPIB* by RNA interference was toxic to ABC DLBCL cell lines, but not to GCB DLBCL, PMBL or myeloma cell lines, strongly implicating *SPIB* as an oncogene involved in the pathogenesis of ABC DLBCL.

Conclusions: In summary, aCGH revealed novel copy number abnormalities in DLBCL that had significant different frequencies in the three DLBCL subtypes. These data provide genetic evidence that the molecular DLBCL subtypes are distinct diseases that utilize different oncogenic pathways.

051 P21, BCL-2, AND THE IPI, BUT NOT BCL-6, PREDICT CLINICAL OUTCOME IN DLBCL TREATED WITH RITUXIMAB(R)-CHOP: LONG-TERM FOLLOWUP FROM E4494

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Background: Bcl-6 immunoreactivity predicted clinical outcome in older patients with DLBCL treated with CHOP but not R-CHOP on E4494. Now, with a median followup of 6.85 years, we update the analysis of this prospective correlative study of prognostic markers including p21 and p53 in addition to Bcl-2 and Bcl-6.

Material/Methods: ECOG or SWOG patients enrolled in the phase III US Intergroup trial comparing CHOP to R-CHOP with or without maintenance R (MR) were eligible if sufficient pathologic material was available. Protein expression was quantified by immunohistochemical staining of DLBCL paraffin-embedded biopsy specimens and scored by an expert panel. Cases in which more than 50% of lymphoma cells stained with anti-Bcl-2 or anti-p53 were called positive. For Bcl-6 and p21, any definitive immunostaining of large, neoplastic cells was considered positive.

Results: In this trial, R had differential effects on outcomes for p21+ vs p21- and Bcl-6+ vs Bcl-6- patients when analyzed according to induction arm (removing the effect of MR). For p21+ cases (n=108), but not p21- cases (n=89), the addition of R to CHOP improved 5 year failure-free survival (FFS; p21+: 61% vs. 24%, p=0.01; p21-: 38% vs 37%, p=0.99) significantly and overall survival (OS; p21+: 64% vs 47%, p=0.11; p21-:47% vs 52%, p=0.70) marginally. Consistent with our earlier report, the addition of R to CHOP improved outcomes in Bcl-6- but not Bcl-6+ cases. In Bcl-6- patients (n=45), FFS and overall survival OS were prolonged for those treated with R-CHOP alone compared to CHOP alone (5-yr FFS: 71% vs 0%, $p<0.0001$; 5 yr OS: 79% vs 9%, $p<0.0001$). In contrast, no differences in FFS and OS were detected between treatment arms for Bcl-6+ cases (n=154; p= 0.85 and 0.26). There was no association between Bcl-6 and p21 immunostaining (p=0.72). The prognostic profile differed according to treatment arm. For patients treated with R-CHOP, the independent prognostic indicators were p21+ (FFS: RR 0.3, p=0.002; OS: RR 0.4, p=0.003), Bcl-2+ (FFS: RR=2.3, p=0.01; OS: RR=3.6, p=0.002), and the IPI (HI/high; FFS: RR=4.5, p=0.002; OS: RR=5.6, p=0.001). Only Bcl-6 immunoreactivity (FFS: RR=0.2; $p<0.0001$; OS: RR=0.2; $p<0.0001$) predicted outcome in CHOP treated patients. Among patients treated with R-CHOP, Bcl-2-/p21+ cases had a better FFS and OS compared to all other patients (p=0.02, p=0.02), whereas Bcl-2+/p21- cases did less well (p=0.02, p=0.06). P53 immunoreactivity did not predict for outcome in uni- or multivariate analyses ($p>0.22$).

Conclusions: The addition of R to CHOP improves outcomes in identifiable biologic subsets of DLBCL and thereby alters its prognostic profile. P21, Bcl-2 and the IPI remain powerful predictors of outcome in the R-CHOP era, providing clues to underlying survival pathways and to putative targets for drug development.

052 LIMITED-STAGE DLBCL PATIENTS WITH A NEGATIVE PET SCAN FOLLOWING THREE CYCLES OF R-CHOP HAVE AN EXCELLENT OUTCOME FOLLOWING ABBREVIATED IMMUNO-CHEMOTHERAPY ALONE

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One standard management of limited-stage DLBCL combines 3 cycles of chemotherapy and involved-field radiation therapy (IFRT). Recently, four cycles of CHOP chemotherapy alone has been shown to be sufficient for low-risk elderly patients. FDG-PET scanning is an effective response assessment tool that may identify chemo-sensitive patients (regardless of clinical risk factors) who can be treated with abbreviated immuno-chemotherapy alone.

Patients: Since 2005, we have recommended that all patients with limited-stage DLBCL (stage I/II, no B-symptoms, mass < 10 cm) treated in British Columbia (BC) undergo a PET scan following 3 cycles of R-CHOP; PET-negative patients are then offered one additional cycle of R-CHOP, while PET-positive patients receive IFRT. To date, 65 patients have been treated according to this algorithm with the following clinical characteristics: median age 67 y (range 31-88); 58% male; 58% stage I, 42% stage II; 5% PS>1; 8% elevated LDH; 57% at least 1 extranodal site. Stage-modified IPI risk factors (Miller et al): 21% 0; 71% 1-2; 8% 3-4. Median follow-up is 17 mos (range 3-30).

Results: 49 patients (75%) were PET-negative and 16 patients (25%) were PET-positive after 3 cycles of R-CHOP. No clinical factors were found to be predictive of PET status. Of the 49 PET-negative patients, 47 completed treatment with one additional cycle of chemotherapy, 1 received IFRT due to poor chemotherapy tolerance and 1 died of toxicity before receiving any more treatment. Only 1/49 PET-negative patients has relapsed (alive with lymphoma after salvage therapy). All 16 PET-positive patients received IFRT, with 3 relapses (all out-of-field) and 2 deaths from lymphoma to date. The 2-year estimated progression-free survival is 93% overall (97% and 83% for PET-negative and PET-positive patients, respectively, p=0.04). The 2-year overall survival is 97% for PET-negative and 76% for PET-positive patients, p=0.12.

Conclusion: Patients with limited-stage DLBCL who are PET-negative after 3 cycles of R-CHOP have an excellent outcome following 4 cycles of R-CHOP alone. Mid-treatment PET scanning can be used to identify patients who can be spared the long-term toxicity of radiation.

053 IMPROVED OUTCOME OF ELDERLY PATIENTS WITH POOR-PROGNOSIS DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) AFTER DOSE-DENSE RITUXIMAB: RESULTS OF THE DENSE-R-CHOP-14 TRIAL OF THE GERMAN HIGH-GRADE NON-HODGKIN LYMPHOMA STUDY GROUP (DSHNHL).

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Background: While 6 to 8 cycles of CHOP in combination with rituximab (R) are widely accepted as standard regimen of care for aggressive lymphomas, the optimal dose and number of R application have not yet been determined. In a previous pharmacokinetic study we had shown that the concomitant application of CHOP and R does not achieve a plateau of R trough levels until cycle 5 (*Reiser, Blood 108, 778a,*

2006). In order to achieve high R levels early, we increased the number of R applications.

Methods: 100 elderly pts. with aggressive CD20$^+$ B-cell lymphoma received 6 cycles of biweekly CHOP-14 combined with 12x R (375 mg/m^2) on days 0, 1, 4, 8, 15, 22, 29, 43, 57, 71, 85, and 99. Radiotherapy was planned to sites of initial bulk and/or extranodal involvement. The primary endpoint was event-free survival (EFS). 306 pts. treated within the RICOVER-60 trial (*Pfreundschuh et al., Blood 64a, 2006*) with 6xCHOP-14 and 8 applications of R served as control.

Results: 97/100 pts are evaluable for response. Plateau trough serum levels were achieved by day 1 of the first chemotherapy cycle and higher R levels were maintained throughout the treatment compared to 8 bi-weekly applications. Because 3 therapy-associated deaths were observed among the first 20 pts treated, prophylaxis with levofloxacin, acyclovir and cotrimoxazol became mandatory for the following pts. Despite a less favorable study population DENSE-R-CHOP-14 resulted in a somewhat higher CR (83% vs. 78%) and lower progression under therapy rate (5% vs. 7%) rate, but event free and overall survival were not different compared to 8 biweekly applications of R. However, a subgroup analysis of pts according to IPI risk group showed that DENSER-R-CHOP-14 resulted in a higher CR-rate of pts with poor-prognosis (IPI:3-5) disease (81% vs. 68%) and in a better 1-year EFS-rate (74% vs. 65%) of these pts.

Conclusion: Densification of R results in higher serum levels and in higher CR and EFS-rates in elderly pts with poor-prognosis DLBCL. An update of the results of the completed trial with 125 pts will be presented.
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054 SEQUENTIAL RCHOP AND YTTRIUM-90 IBRITUMOMAB TIUXETAN (RIT) IS A HIGHLY EFFECTIVE REGIMEN FOR HIGH RISK ELDERLY PATIENTS WITH UNTREATED DLBCL

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Introduction: Elderly patients (pts) ≥60 years with aaIPI high-intermediate (HI) and high risk (H) disease have 5 yr survival (OS) rates of 37%, and 21%, respectively. Rituximab's addition to CHOP has improved outcomes, but >50% of high risk pts still relapse. We hypothesized consolidation of RCHOP with ^{90}Y ibritumomab tiuxetan radioimmunotherapy (RIT) would improve outcomes by eradicating minimal residual disease.

Methods: Untreated ASCT-ineligible pts ≥60 yrs with aaIPI HI or H risk DLBCL are eligible. Induction: standard RCHOP q 21 days x 6 with peg/G-CSF and darbepoetin alfa support. Consolidation: pts with ≥stable disease post RCHOP are eligible for RIT 6-9 weeks later at approved doses and schedule. A total of 65 pts are planned.

Results: 60 pts have been enrolled as of 1/18/08 (MSKCC 50, MDACC 10). Median age is 75 years (range 62-86; M:F 19:41); KPS <80% in 60% (median 70%); LDH > nl: 90%; Stage III/IV: 25%/72 %; Extranodal sites >1 in 43%; B symptoms in 49%; aaIPI HI/H = 53% / 47%. Moderate or high impact comorbidity is present in 87%. 38/60 pts have received RIT; of 22 pts with no RIT: 3 too early, 3 excluded by pre-RIT marrow evaluation, 10 toxicity on RCHOP, 5 POD, 1 withdrew. Median nadir counts during RIT occur at week 6-8: ANC=1.2K cells/mm^3, Hgb= 10.9 g/dl, plt = 33K cells/mm^3; CTC 3.0 Grade 3/4 ANC 26%/37%, Hgb 17%/0%, Plt 34%/34%; 6 pts had delayed count recovery >12wks. Non-Heme serious toxicity during RIT included: 2 grade 5 events (CNS bleed, CHF), 1 tMDS. OS and PFS by intent to treat (58 evaluable pts) are 67% and 59%, with 15 months median followup. For RIT patients (n=38), OS and PFS are 88% and 80%, with 23 months median follow-up (range 4.3-51). Median PFS for pts who did not receive RIT is 3.6 months vs. not reached in the RIT group. OS and PFS by aaIPI HI/H are 84%/49% and 75%/42%, respectively. Responses improved post RIT in 11 pts (CRu->CR 7, PR->CRu/CR 4).

Conclusions: In a high risk, elderly patient population with significant comorbidities, sequential RCHOP followed by RIT results in excellent OS and PFS. This approach is now the focus of an international Phase III study.

054bis FIRST RESULTS OF AN INTERNATIONAL STUDY TO ESTABLISH A NEW CLINICO-BIOLOGICAL PROGNOSTIC INDEX FOR DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL)

The Lunenburg Lymphoma Biomarker Consortium (LLBC)

Background: Due to lack of sufficient validation, prognostic biomarkers in DLBCL have not gained a broad acceptance and thus far the International Prognostic Index (IPI) remains the only standard for practical clinical use. Clinical factors of the IPI however represent surrogates for the underlying biology. The LLBC aims to move risk stratification beyond the IPI by performing systematic biomarkers validation with DLBCL patients (pts) treated in European and North-American clinical trials.

Methods: Based on the availability of pathology material suitable for tissue microarrays (TMA) and a minimum 3-year follow-up, cases from 13 prospective studies for DLBCL pts were selected from 7 LLBC collaborative study groups. Disease, treatment and outcome characteristics were collected in a single LLBC database from studies incorporating CHOP (or CHOP-like) chemotherapy regimen as the standard arm (n=1117) or more recently comparing CHOP (n=620) vs Rituximab (R)-CHOP (n=620). TMAs were generated, centrally stained and circulated for triple independent scoring of 8 immunohistochemical markers using criteria previously defined and validated (de Jong et al, JCO 2007). Optimal cut-points for overall survival (OS) prediction were first assessed based on the earlier CHOP studies and their prognostic significance was evaluated on randomized R-CHOP studies.

Results: Analyzed cases covered the complete distribution of risk and age groups both for R- and non-R- treated pts. With a 5-year median follow-up, median OS was 7.6 years, and the IPI was found to be predictive both for CHOP and R-CHOP pts (p<0.0001). Biomarker data were available for 1424 cases with all 8 markers evaluable in 756 cases. Cut-points for OS prediction in CHOP pts were defined as ≤75 vs >75% for Bcl-2 (Hazard Ratio=1.79; P<10-4) and MUM1 (HR=1.33; P=.029), no staining vs staining for Bcl-6 (HR=0.57; P=.0003) and CD5 (HR=1.53; P=.0027) and negative vs positive for HLA-DR (HR=0.59; P=.0008), respectively. No optimal cut-point could be determined for Ki67 and CD10 as single markers predictive of outcome. Bcl-2, Bcl-6 and MUM1, but not CD5 or HLA-DR cut-points were validated in the independent series of randomized CHOP pts. In R-CHOP treated pts, only Bcl-2 (HR=1.53; P=.032) and CD5 (HR=1.85; P=.0072) were found to predict patient outcome.

Conclusion: Using a validated approach on a large series of pts, this study 1) establishes optimal non-arbitrary cut-points for candidate prognostic biomarkers in DLBCL, 2) provides a rational basis for their combination and the design of biologically relevant algorithms and 3) enables one to assess their influence in the rituximab era. Data detailing the design of a new biological IPI (BIPI) for DLBCL patients will be discussed.