Loss of chromosome 11q21–23.1 and 17p and gain of chromosome 6p are independent prognostic indicators in B-cell non-Hodgkin’s lymphoma

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Summary Comparative genomic hybridization (CGH) was employed to study chromosomal aberrations in relation to cell proliferation, apoptosis, and patient survival in 94 cases of B-cell non-Hodgkin’s lymphoma diagnosed between 1983 and 1993. Eighty cases had aberrations by CGH. Chromosomal regions 1p21–31.1 (10%), 6cen-q24 (12%), 8p (11%), 9p21-ter (14%), 11q21–23.1 (11%), 13q13–21.1 (12%), and 17p (15%) were frequently lost. Gains were found at 3q21-ter (22%), 6p (11%), 7p (12%), 8q23-ter (13%), 12cen-q15 (17%), 17q24-ter (13%), and 18q13.3–21 (20%). A high number of aberrations (≥ 4, 33 cases) was associated (P < 0.001) with the mantle cell and diffuse large B-cell lymphoma subtypes, a high fraction of tumour cells in S phase, and short survival (RR (relative risk) = 3.7). Loss of 1p21–31.1, 8p, 9p21-ter, 11q21–23.1, and 13q13–21.1 were associated with mantle cell lymphoma (P < 0.03), while gain of 6p and 12cen-q15 were more frequent in diffuse large B-cell and small lymphocytic lymphoma, respectively (P = 0.04). Loss of 8p and 17p, and gain of 3qter-6p, 7p, and 8q23-ter were associated with a high S phase fraction (P ≤ 0.03), but none of the aberrations were associated with tumour apoptotic fraction (P ≥ 0.13). The most important prognostic CGH parameters (P < 0.001) were losses of 11q21–23.1 (RR = 3.8) and 17p (RR = 4.4), and gain of 6p (RR = 4.2). The latter parameters and IPI were the only ones with independent prognostic value (RR = 10, 5.0, 6.7, and 3.7, respectively; P < 0.001) when assessed together with lymphoma sub-type, primary versus relapse cases, treatment, B symptoms, S phase fraction, and presence of BCL1 and BCL2 translocations. A combined CGH/IPI binary parameter had high prognostic value for patients receiving different treatments, with various lymphoma sub-types, and for primary as well as relapse cases. © 2001 Cancer Research Campaign http://www.bjcancer.com

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Non-Hodgkin’s lymphoma (NHL) is clinically a heterogeneous group of malignancies for which the B-cell phenotype constitutes 85% of the cases. For the indolent non-localized lymphomas there is no curative treatment, but median overall survival is close to 10 years and differs markedly between patients. Approximately 40% of aggressive NHL are cured by combination chemotherapy (Fisher et al, 1993). Lymphoma sub-types (Harris et al, 1994) and prognostic factors like stage, age, serum LDH, WHO performance, and number of extranodal sites (The International Non-Hodgkin’s Lymphoma Prognostic Factors Project, 1993) are presently the tools for treatment selection and the background for informing the patient on life expectancy.

The development of B-cell NHL is, as for other neoplasias, accompanied by the sequential acquisition of monoclonal genetic aberrations. Typical aberrations in B-cell NHL include translocations involving the immunoglobulin loci and other chromosomal loci harbouring proto-oncogenes like c-MYC, BCL-1, BCL-2, and BCL-6 (Rabbits, 1994; Ong and Le Beau, 1998). These translocations activate inappropriate transcription of the proto-oncogene involved, and may, together with loss of tumour suppressor activities, lead to deregulated growth due to increased proliferation or decreased apoptosis. Losses and gains of tumour suppressor genes and proto-oncogenes or larger genomic regions containing such genes are common events in the genesis of most solid tumours. Classical cytogenetic studies have revealed that unbalanced translocations and gains and losses of whole chromosomes are frequent also in NHL (Offit, 1992; Mitelman et al, 1997; Ong and Le Beau, 1998).

Comparative genomic hybridization (CGH) is based on hybridization of tumour DNA from interphase cells to normal metaphases for the detection of gain or loss of specific chromosomal regions. CGH does not provide any information about the chromosomal organization of the tumour genome, but has several advantages compared to classical cytogenetics: Interphase cells can be analysed without the need for culturing to make metaphase spreads; The composition of complex marker chromosomes, which can be difficult to analyse by cytogenetics, is easily obtained by CGH. In agreement with the classical cytogenetic studies, CGH studies of B-cell NHL have shown genomic losses and gains to be frequent in follicle centre lymphomas (FCL, Bentz et al, 1996), small lymphocytic lymphomas/chronic lymphocytic leukaemias (SLL/CLL, Bentz et al, 1995), mantle cell lymphomas (MCL, Monni et al, 1998; Béa et al, 1999; Bentz et al, 2000), and diffuse large B cell lymphomas (DLBCL, Monni et al, 1996).

Specific sub-types of NHL are characterized by specific chromosomal translocations. However, it is less well known whether additional chromosomal changes are also confined to certain lymphoma sub-types, and whether these correlate with prognosis. The results of numerous reports strongly suggest that the presence...
of aberrations in the p53 pathway (i.e. 17p deletions, TP53 mutations, expression of wild-type p53) are adverse prognostic factors in NHL (Sander et al, 1993; Tilly et al, 1994; Dohner et al, 1995; Schoch et al, 1995; Hernandez et al, 1996; Panayiotidis and Kotsi, 1999; Dohner et al, 2000; Stokke et al, 2000; reviewed by Knutsen, 1998). Chromosomal aberrations are important prognostic factors and are used for treatment stratification in acute lymphoblastic leukaemia, including the indication for bone marrow transplantation in first remission (Pui and Evans, 1998).

We report here the findings of a CGH study of 94 cases of randomly selected B-cell NHL. The chromosomal gains and losses were compared with lymphoma subtype, S phase and apoptotic fraction, as well as prognosis.

**MATERIALS AND METHODS**

**Patients**

The 94 patients included in this study were randomly selected based on the availability of frozen cell suspensions (in DMSO) with more than 50% tumour cells (Ig-expressing cells by manual counting and flow cytometry). All patients were hospitalized at our institution during the period 1983–1993. Lymphoma subtyping was performed according to the REAL classification (Harris et al, 1994). Twelve of the 13 MCL cases in this study had cyclin D1 expression and t(11;14) (lacking in case 67/92), and 32 of the 36 FCL cases had t(14;18) (lacking in cases 577/90, 489/91, 533/91, and 300/92).

Clinical data were available for 92 of the patients (lacking in cases 159/90 and 244/91). The age range of these patients at the time of the relevant biopsy was 31–86 years, the median age was 57 years, and 41 of the patients were women (42 of the total of 94). For 62 patients, the biopsy was taken at diagnosis, for 15 patients at first relapse, and for 15 patients at second or later relapse/progression. No patients had received chemotherapy during the last month before the biopsies. The median follow-up of the 31 survivors was 59 months. Clinical stage was according to the Ann Arbor classification (Carbone et al, 1971) at the time of diagnosis. The patients were clinically examined and received standard blood testing, standard X-ray of the thorax, CT scan of the abdomen/pelvis and, if necessary, of the thorax, as well as bilateral bone marrow aspiration/biopsy.

Patients with DLBCL and BL were treated according to the following protocols: Eight courses of CHOP (cyclophosphamide 750 mg/m² i.v. day 1, doxorubicin 50 mg/m² i.v. day 1, vincristin 2 mg day 1, and prednisone 100 mg p.o. day 1–5) for stage III–IV, and 6 courses of CHOP followed by involved field radiotherapy for patients with stage I disease. If two neighbouring lymph node regions were involved, stage II DLBCL and BL patients were treated as stage I patients, otherwise, treatment was as for stage III–IV. For the SLL, MZL, FCL, and MCL lymphomas, the following treatment was given: When required, initial therapy to the patients consisted of radiotherapy to stage I–II disease, while patients with stage III–IV disease were given the CVP regimen (cyclophosphamide 750 mg/m² i.v. day 1, vincristin 2 mg day 1, and prednisone 100 mg p.o. day 1–5) during the early part of the study period, whereas chlorambucil 6 mg/m² p.o. and prednisone 5–10 mg p.o. daily for up to 6 months was given during the latter part of the period. Younger patients with an aggressive clinical course were given CHOP at the discretion of the responsible clinician. The CVP and CHOP regimens were repeated every 3 weeks for a total of 6–8 courses; the chemotherapy regimen was changed if at least a partial remission was not achieved within 2–3 months of therapy. Treatment at relapse or progression was given at the discretion of the responsible clinician. No patients had received chemotherapy during the last month before the biopsies were taken.

**Comparative genomic hybridization**

The isolation of genomic DNA from the (thawed) lymphoma samples was performed after a standard protocol based upon proteinase K and dodecyl sulphate as described by DeAngelis et al (1999). The DNA concentration was determined by Hoechst 33258 staining and measuring the fluorescence in a fluorimeter.

Genomic DNA was nick-translated and labelled with either Texas red-5-dUTP for normal sex-matched DNA or FITC-12-dUTP for tumor DNA (Kallioniemi et al, 1994a; DeAngelis et al, 1999).

The slides for CGH hybridization were prepared following routine procedures for PHA stimulated, methotrexate synchronized, peripheral blood lymphocytes. After dripping the cells onto the slides at 60–65% relative humidity, the slides were stored at −20°C in 100% ethanol or at 0°C in a nitrogen flushed container.

Denaturation, hybridization, and counterstaining with DAPI was essentially performed as outlined by Kallioniemi et al (1994a), and in more detail as described by DeAngelis et al (1999).

**Microscopy and data treatment**

DAPI fluorescence and probe signals were observed sequentially with a Zeiss Axioplan fluorescence microscope equipped with a triple-pass emission filter (blue, green and red), a corresponding beam splitter, and separate excitation filters (UV, 470–490 nm, 578 nm). All filters (‘Pinkel 1’ filter set) were obtained from Chroma, Brattleboro, VT. Images were captured and digitized in a cooled 16 bit CCD camera (Astromed, Cambridge, UK).

Segmentation and calculation of ratio profiles were performed with software that was kindly provided by D. Sudar. The software ran under the ‘Scilimage’ image analysis program (TNO, Delft, Netherlands). Hybridizations were repeated when the FITC or the Texas Red chromosomal fluorescence was low (signal to background less than ~2), or grainy, or when blocking of (labelled) probe hybridization to centromere regions and the p-arms ofacrocentrics was not satisfactory. Each tumour was satisfactory. Each tumour was hybridized 2.6 times on the average to get acceptable results. The average and standard deviation of several (>3) profiles of each chromosome were calculated, and more profiles were added until the averaged profile and standard deviation did not change after the addition of a new one. When using these criteria, ratios less than 0.85 and larger than 1.15 were never observed in normal versus normal hybridizations. Gains and losses were therefore scored if the ratio was above 1.15 and below 0.85, respectively (DeAngelis et al, 1999).

**DNA flow cytometry**

The flow cytometry procedure for determination of tumor-specific S-phase fraction and apoptosis has been described (Stokke et al, 1998a). Briefly, thawed cells were labelled with
phycoerythrin-labelled light chain antibodies for the tumour-characteristic light chain and washed. The cells were thereafter fixed with 1% paraformaldehyde followed by 100% methanol and labelled with FITC-dUTP after incorporation by terminal deoxynucleotidyl transferase for the detection of apoptotic cells, and Hoechst 33258 for assessment of DNA content. After gating away aggregates of cells and apoptotic cells, DNA histograms of the tumour cells and normal cells were obtained by gating on the light chain positive and negative cells, respectively. The fraction of tumour cells was assumed to be equal to the fraction of cells expressing the tumour-characteristic light chain. Ploidy, i.e. DNA index (DI), was calculated as the ratio between the $G_1$ peak positions of the tumour cells and the normal cells.

**Statistical analysis**

Relevant statistical tests were performed using the ‘SigmaStat’ (Jandel Scientific, Erkrath, Germany) and ‘SPSS’ (SPSS, Chicago, IL) software packages. Relative risks were calculated using Cox uni- and multi-variate regression analysis. Relative risk (RR) is the time-averaged ratio between the fractional survivals in the two groups defined by the assessed variable. $P$-values were calculated using Cox regression analysis and the log-rank test. $P$-values below 0.05 were considered to reflect statistical significance. Cancer-specific survival was determined from the time of the relevant biopsy. Death from any cancer or treatment complications defined an event. Other deaths were censored. The Kaplan-Meier method was used to generate survival curves.

**RESULTS**

**CGH aberrations in NHL**

Ninety-four cases of B-cell NHL were analysed for chromosomal aberrations by CGH. Table 1 and Figure 1 contain the complete results obtained by CGH, as well as the DNA index for all 94 lymphomas. Regions with a ratio above 1.5 are written in bold print in Table 1 and displayed in bold print in Figure 1. As an example, Figure 2 shows the results obtained for seven of the tumours with respect to chromosomes 1, 3, 9, and 18. Large-scale aberrations, i.e. aberrations detectable by CGH, were found in 80 of 94 tumours (85%), including a majority of the tumours that were found to be DNA diploid by flow cytometry (Table 1). The mean fraction of tumour cells was not different in the samples with and without CGH aberrations (75% for both groups; $P = 0.88$), indicating that the lack of observed aberrations cannot be attributed to a low fraction of tumour cells. The CGH aberrations were non-random, and some of them were local. For classification of an aberration as local, i.e. not encompassing the whole chromosome, we required that at least three tumours defined any border around the ‘consensus region’. The two arms of a chromosome were not assumed to be separate entities, and aberrations outside the consensus region(s) on a given
Table 1 Chromosomal aberrations in 94 cases of B-cell NHL

| Case | Lymphoma subtype (REAL) | Sex | CGH losses | CGH gains | DNA index | No. c-MYC alleles | No. RB1 alleles | No. TP53 alleles |
|------|--------------------------|-----|------------|-----------|-----------|------------------|----------------|----------------|
|      |                          |     |            |           |           |                  |                |                |
|      | Small lymphocytic lymphoma |     |            |           |           |                  |                |                |
| 140/83 | SLL                     | M   | None       |           | 1.00      | 2               | 1              | 2              |
| 086/85 | SLL                     | M   | 1q14–23.1  | None      | 1.00      | 2               | 1              | 2              |
| 320/88 | SLL                     | F   | X          | None      | 1.00      | 2               | 2              | 2              |
| 325/88 | SLL*                    | M   | None       | None      | 1.00      | 2               | 2              | 2              |
| 416/88 | SLL                     | M   | 17p        | 12        | 1.00      | 2               | 2              | 1              |
| 470/88 | SLL                     | M   | None       | 10cen-q21.1, 12cen-13, 12q22-ter | 1.02 | 2               | 2              | 2              |
| 191/89 | SLL                     | M   | None       | 12        | 1.02      | 2               | 2              | 2              |
| 329/89 | SLL*                    | F   | 8p11-ter, 11q14–23.1, 13q12.3-ter | 3p14-ter, 5p, 7p13-ter, 8q22-ter, 17q21.3-ter | 1.00 | 3               | 1              | 2              |
| 445/89 | SLL                     | M   | None       | 2p14–16, 3 | 1.02 | 2               |                |                |
| 452/89 | SLL                     | M   | 14q24-ter  | None      | 1.00      | 2               |                |                |
| 051/90 | SLL                     | M   | 1q41-ter, 17p | 1cen-q31, 3q, 18q12.3-ter, 22q13-ter | 1.05 | 2               | 2              | 1              |
| 159/90 | SLL                     | F   | None       | 12        | 1.04      | 2               |                |                |
| 571/90 | SLL                     | M   | 11q22–23.1 | None      | 1.00      | 2               | 2              | 2              |
| 156/91 | SLL                     | F   | 11q14–23.1, 15q14–21 | None      | 1.00      | 2               | 2              | 2              |
| 244/91 | SLL                     | M   | None       | None      | 1.00      | 2               | 2              | 2              |
| 462/91 | SLL*                    | F   | 17p        | 11q23-ter, 12pter-q15, 13q31-ter, 20p | 1.00 | 2               | 2              | 1              |
| 225/92 | SLL                     | M   | None       | 12        | 1.03      | 2               |                |                |
| 315/92 | SLL                     | M   | None       | 12, 15q25-ter | 1.00 | 2               | 1              | 2              |
| 538/92 | SLL                     | M   | 8cen-p21   | 3q21-ter, 8q23-ter | 1.00 |                |                |                |
|      | Marginal zone lymphoma   |     |            |           |           |                  |                |                |
| 315/88 | MZL                     | M   | None       | 12        | 1.00      | 2               | 1              | 2              |
| 237/91 | MZL*                    | M   | 11q14.3–23 | None      | 1.00      | 2               | 1              | 2              |
| 448/91 | MZL                     | M   | None       | 3, 18, 19 | 1.05      | 2               |                | 2              |
|      | Follicle centre lymphoma |     |            |           |           |                  |                |                |
| 377/83 | FCL II                  | M   | None       | 7pter-q12 | 1.00      | 2               | 2              | 2              |
| 364/86 | FCL II + SLL            | F   | 19p13.2-ter | 18pter-q21 | 1.02 |                |                |                |
| 369/86 | FCL I                   | F   | None       | 18pter-q21.1 | 1.00 |                |                |                |
| 021/87 | FCL III                 | M   | None       | None      | 1.00      | 2               | 2              | 2              |
| 241/87 | FCL III                 | F   | None       | None      | 1.02      | 2               | 2              | 2              |
| 345/87 | FCL II                  | F   | None       | 18q      | 1.00      | 2               | 2              | 2              |
| 018/88 | FCL II                  | F   | None       | 1p21–22, 13q21-ter | 1.00 | 2               | 2              | 2              |
| 041/88 | FCL II                  | F   | None       | 18q12.3-ter | 1.03 |                |                |                |
| 046/88 | FCL II                  | F   | None       | None      | 0.97      | 2               | 2              | 2              |
| 047/88 | FCL II*                 | M   | 6cen-q22, 13q13–21.1 | None      | 1.00      | 2               | 1              | 2              |
| 176/88 | FCL II                  | F   | None       | 7, 8q21.3-ter, 18q12–22 | 1.04 |                |                |                |
| 233/88 | FCL II                  | F   | 6q14-ter, 13cen-q22 | None      | 1.00      | 2               | 1              | 2              |
| 275/88 | FCL III                 | M   | None       | None      | 1.00      | 2               | 2              | 2              |
| 284/88 | FCL II                  | F   | None       | 20pter-q12, 22cen-q12 | 1.02 | 2               | 2              | 2              |
| 381/88 | FCL II                  | M   | None       | 18, X    | 1.03      | 2               |                |                |
| 399/88 | FCL III                 | M   | 6cen-q24, 17p | 1p34.3-ter, 8q24-ter, 17q, 22q | 1.00 | 2               | 2              | 1              |
| 468/88 | FCL I                   | M   | None       | 15q25-ter | 1.00      | 2               |                |                |
| 064/89 | FCL II*                 | F   | 1cen-p22.2 | 13q22-ter, 6pter-q15, 12pter-q21, 12q14, 13q31-ter, 13q32-ter, 16, 20q, 20q13, 21q22 | 1.26 | 2               | 2              | 3              |
| 287/89 | FCL I                   | M   | 6q14–22.1  | 2         | 1.03      |                |                |                |
| Case No. | Diagnosis | Gender | Age | Chromosomes | FISH Changes | EV | PV | CR |
|----------|-----------|--------|-----|-------------|--------------|----|----|----|
| 521/89   | FCL II    | F      | None | 17p         | 4q11-ter, 7p13-ter | 1.00 | 2 2 2 |
| 372/90   | FCL II    | F      | None | 2q21-22, p13-ter | 1.07 |
| 377/90   | FCL II    | M      | None | 1q41-ter, 17q21-ter | 1.00 |
| 416/90   | FCL II    | M      | None | None | 1.00 |
| 577/90   | FCL II    | M      | 5, 9p, 17p | 3q, 12, X | 1.97 |
| 581/90   | FCL II    | F      | 17p | 1q35, 5q12, 17q, 18q12.3-21.1, X | 1.22 |
| 635/90   | FCL II*   | M      | None | None | 1.00 |
| 382/91   | FCL II    | M      | None | 17p | 1.00 |
| 436/91   | FCL II    | F      | None | None | 1.00 |
| 488/91   | FCL II    | M      | 1q41-ter, 17q21-ter | 1.03 |
| 488/91   | FCL II*   | M      | None | None | 1.00 |
| 497/91   | FCL II    | F      | None | None | 1.00 |
| 521/91   | FCL II    | F      | None | None | 1.00 |

**Mantle cell lymphoma**

| Case No. | Diagnosis | Gender | Age | Chromosomes | FISH Changes | EV | PV | CR |
|----------|-----------|--------|-----|-------------|--------------|----|----|----|
| 123/84   | MCL*      | M      | None | 9p21-ter, 11q23-ter, 13q21.3-ter, 14q21-ter, 22q12.2-ter | 3q22-ter, 10cen-p13, 10p12, Xq27-ter | 1.04 | 2 |
| 358/87   | MCL*      | F      | None | 2q8q21-ter, 9p13-ter, 11q12-ter, 13q14q21-ter | 3.6p, 7q21-ter, 15q21-ter | 1.94 | 3 |
| 154/88   | MCL       | M      | None | 1p21–3.1q3q13.1, 8p12q13q, 15q13-19q | 1p34-ter, 16p13.3, 19q21-ter | 0.95 | 2 1 2 |
| 265/88   | MCL*      | F      | None | 1p9q21-ter, 11q12-ter, 18q12-ter | 1q13qter, 18q12-ter | 1.00 | 2 2 2 |
| 285/88   | MCL       | M      | None | 1p21–3.1q3q21-23.1, 13cen-p21, 1.18p | 3q21-ter, 1q21-ter | 1.00 | 1 2 |
| 129/89   | MCL*      | M      | None | 3cen-p14q3, 1q14-23 | 3q21-ter, 1q21-ter, 6p | 1.02 | 3 2 2 |
| 309/89   | MCL*      | M      | None | 1p13q3-31q4p3q21-ter, 13q3q21-ter, 14q21-ter, 22q12.2-ter | 1.83 | 3+4 2+4 1+2 |
| 472/90   | MCL       | M      | None | None | 2p11-ter, 5q22-ter, 7p, 8q23-ter, 8q23-ter, 10p12-ter, 12q14-ter, 13p12-ter | 1.16 | 2 2 |
| 383/91   | MCL       | M      | None | 9p13qter-ter, 13q14q21-ter | 9q24-ter | 0.97 | 1 |
| 428/91   | MCL       | M      | 1p21–3.1q3q25 | None | 2.00 | 4 4 4 |
| 037/92   | MCL*      | F      | 7q31–32q, 17p | 17q21-ter, 21q21-ter | 1.00 | 2 2 1 |
| 067/92   | MCL*      | M      | 1p21, 8p9qter-q31, 11q22–23.1, 13q22q12-ter | 3q21–23q26-ter, 8q15q21.2-ter, 15q24-ter | 0.98 | 3 1 2 |
| 245/92   | MCL*      | M      | None | None | 3q, 3q21-ter | 1.03 | 2 2 |

**Diffuse large B-cell lymphoma**

| Case No. | Diagnosis | Gender | Age | Chromosomes | FISH Changes | EV | PV | CR |
|----------|-----------|--------|-----|-------------|--------------|----|----|----|
| 122/84   | DLBCL     | F      | None | 1p21–31.1q22-ter, 2q23-ter, 2q23-ter | 7q23-21.3q23.3, 16p1q31-14q32-ter | 1.23 | 2 2 1 |
| 255/85   | DLBCL     | F      | None | 6q, 10p13q12, 12p | 2q23-35.5q6, 8q2ter-q21, 15cen-q21, 17p | 3.6q, 7p7q, 18q12-ter | 1.04 | 2 2 |
| 377/87   | DLBCL     | M      | None | 13q13-14 | None | 0.97 | 1 2 |
| 390/87   | DLBCL     | M      | None | 1q11q4-ter, 11q22.2-ter | None | 1.00 | 2 2 2 |
| 399/87   | DLBCL     | F      | None | 1p31qter-q14q13ter, 15q23q13ter, 17q21.3ter, 19q | 1.06 | 2 2 2 |
| 258/88   | DLBCL     | M      | None | 5q17q | 5q, 8q23-ter, 12q21.3-ter, 13q21-ter, 21qXp | 1.07 | 3+4 2+4 1+2 |
| 287/88   | DLBCL     | F      | None | 4q17p, 14p | 8cen-p12, 12.1q21-ter, 18cen-q22, Xp | 1.00 |
| 454/88   | DLBCL     | F      | None | 6q11q15-21ter, 17p | 10q17p, 22q | 1.02 | 2 1 |
| 131/89   | DLBCL     | M      | None | 1p21–32q, 8p12q12 | 3q27p11, 8q23-ter, 9q22-ter, 16p18 | 2.23 | 8 4 4 |
| 438/89   | DLBCL     | F      | None | 2q24–34, 14q24.3-ter | None | 1.00 | 2 2 2 |
Table 1 Continued

| Case  | Diagnosis  | Gender | Chromosomes | Copy Numbers |
|-------|------------|--------|--------------|--------------|
| 525/89 | DLBCL     | F      | 1q42-ter,5cen-q14,9p21.3-22,Xp | 1cen-q31,3,6p,18q21-ter,Xq21-ter |
| 034/90 | DLBCL + SLL | M      | 1q42-ter,15q15-21 | 1cen-q25,3,7q,9q33-ter,22q13-ter |
| 612/90 | DLBCL     | F      | 9pter-q22      | 3q26.3-ter,8q24-ter,19p |
| 340/91 | DLBCL     | M      | None          | 7q          |
| 070/92 | DLBCL     | F      | None          | 1q21-33,3,6p21.3-ter,13q21.3-ter,3,7p,22q13-ter |
| 117/92 | DLBCL*    | F      | 5q,9pter-q23,9pter-q21,13q21.3-32,17p20 | 3,17q19 |
| 050/93 | DLBCL     | M      | None          | 1q21-33,3,6p21.3-ter,13q21.3-ter,3,7p,22q13-ter |

Burkitt’s and Burkitt-like lymphoma

| Case  | Diagnosis  | Gender | Chromosomes | Copy Numbers |
|-------|------------|--------|--------------|--------------|
| 458/88 | BL-like   | F      | 9p21-ter,10p11-qter,16,X | 5p15,8q13-ter,13q,19q |
| 277/89 | BL        | F      | None          | None          |
| 042/92 | BL-like   | M      | None          | None          |
| 008/92 | BL        | F      | 2q,5p14-ter,5q14-21,6q7q3,13ter,8pter-q12,12p13q,14,3-22,Xp22.1-qter | 4q28-ter,6p,7q11,23-31.1,18 |

Unclassified B-cell lymphoma

| Case  | Diagnosis  | Gender | Chromosomes | Copy Numbers |
|-------|------------|--------|--------------|--------------|
| 214/88 | M         | 6q     | None          | 8q          |
| 537/91 | BL-like   | None   | None          | None          |
| 008/92 | BL        | F      | 2q,5p14-ter,5q14-21,6q7q3,13ter,8pter-q12,12p13q,14,3-22,Xp22.1-qter | 4q28-ter,6p,7q11,23-31.1,18 |

*Regions where the green/red ratio exceeded 1.5 ('high-level' amplifications) are repeated in bold. bThe DNA index was measured by flow cytometry as described in 'Materials and methods'. Cases with a DNA index between 0.93 and 1.07 were considered DNA diploid, since only one G1 peak could be identified in the ungated DNA histograms. DNA tetraploid cases have a DNA index in the region 1.9–2.1. dData were taken from Galteland et al (1999) or assessed as described therein.

**Associations between CGH aberrations**

The frequency distribution of the number of aberrations was bimodal, with a natural cut-off between the groups at approximately four aberrations (Figure 3). Attempts to fit this distribution to a single Poisson distribution failed ($R^2 = 0.94$, $P = 0.0001$). A linear correlation of two aberrations per chromosome can be defined, one with a low number of aberrations (0–3), and one with a high number of aberrations (4–6).

**Frequency distribution of CGH aberrations**

The frequency distribution of the number of aberrations was bimodal, with a natural cut-off between the groups at approximately four aberrations (Figure 3). Attempts to fit this distribution to a single Poisson distribution failed ($R^2 = 0.94$, $P = 0.0001$). A linear correlation of two aberrations per chromosome can be defined, one with a low number of aberrations (0–3), and one with a high number of aberrations (4–6).
the other (Figure 1). For these chromosomes, the loss of one arm was associated with gain of the other (Table 2). Gain of 12cen-q15 was negatively correlated with the majority of the other aberrations, but none of these negative associations were statistically significant.

Gain of 12cen-q15 and 18q13.3–21 were the only aberrations which were found in more than 10% of the cases with a low number (0–3) of aberrations. The frequencies of these aberrations were less than two-fold higher among the cases with a high number of aberrations ($P = 0.25$ and 0.11, respectively). The 11q21–23.1 deletion was found 2.7 times more frequently in the lymphomas with many aberrations compared to the ones with few, but the difference was not significant ($P = 0.16$). However, while the loss of 11q21–23.1 occurred together with several other aberrations in 4 MCL cases, three of the 4 losses at 11q21–23.1 in SLL were found in tumours with a low number of aberrations. For the remaining CGH aberrations, the frequency in the tumours with many aberrations was >3.2 times higher than in the cases with few aberrations ($P < 0.05$ in all cases).

**Lymphoma subtype and CGH aberrations**

A high number of CGH aberrations was associated with MCL and DLBCL ($P < 0.001$). Figure 4 shows the distribution of the aberrations according to lymphoma sub-types. Loss of 1p21–31.1 ($P < 0.001$), 8p ($P = 0.02$), 9p21-ter ($P < 0.001$), 11q21–23.1 ($P = 0.03$), and 13q13–21.1 ($P < 0.001$) occurred more frequently in MCL than in the other subtypes. Gain of 6p ($P = 0.04$) was associated with DLBCL, and gain of 12cen-q15 was found more frequently in SLL than in the other lymphoma groups ($P = 0.04$).

**The impact of CGH aberrations on cellular phenotypes**

The tumour-specific S phase and apoptotic fractions of the tumours studied here have been published earlier (Stokke et al, 1998a). A high number of CGH aberrations ($P < 0.001$; Mann-Whitney), as well as loss of 8p ($P = 0.03$) and 17p ($P = 0.005$), and
gain of 3q21-ter \( (P < 0.001) \), 6p \( (P = 0.02) \), 7p \( (P = 0.007) \), and 8q23-ter \( (0.01) \) were associated with a high S phase fraction. None of the CGH aberrations were associated with apoptotic fraction \( (P \geq 0.13) \).

### CGH aberrations and patient survival

The prognostic values of the different gains and losses are given in Table 3. The relative risks associated with a high number of CGH aberrations, loss of 6cen-q24, 8p, 9p21-ter, 11q21–23.1, 13q13–21.1 and 17p, and gain of 3q21-ter and 6p, were significantly higher than one. Multivariate survival analysis revealed that the only independent prognostic factors were loss of 11q21–23.1 and 17p, and gain of 6p (Table 3).

The CGH parameters with independent prognostic value were analysed by Cox multivariate regression analysis together with lymphoma sub-type, as well as clinical and other biological parameters (Table 4). All parameters except for treatment had significant prognostic value in univariate analysis (Table 4), but only –11q21–23.1, –17p, +6p, and IPI had independent prognostic value. S phase fraction had marginal significance in multivariate analysis; further investigations have shown that the patients with the CGH aberrations with independent prognostic value survive a shorter time if the tumour S phase fraction is higher than 3% (data not shown). The survival was similar for the patients receiving different treatments (chemotherapy without doxorubicin, chemotherapy with doxorubicin, ‘other’ treatments; \( P > 0.75 \) for pairwise comparisons), and it will be demonstrated later (Figure 5) that the prognostic value of the CGH parameters and IPI is high in all these three treatment groups.

In an attempt to construct a combined parameter, we noticed that the –11q21–23.1, –17p, and +6p CGH parameters were negatively correlated (Table 2), which was not unexpected in view of their independent prognostic value (Tables 3, 4). The most logical way of combining these parameters would therefore be to include cases in the ‘poor survival’ group if they had any of these aberrations (and/or). This CGH combination parameter (Figure 5A) was substituted in an analysis similar to the one shown in Table 4 instead of the 3 individual CGH aberrations, and was the only parameter together with IPI which came out with significant prognostic value (data not shown). In a similar fashion as above, a single binary combined ‘CGH/IPI’ parameter was constructed such that cases were allocated to the ‘poor survival’ group if they had any of the three prognostic CGH aberrations and/or a high IPI score (i.e. IPI > 2). The prognostic value of the combined CGH/IPI parameter is shown in Figure 5C. When this parameter was entered into the multivariate analysis as shown in Table 4 instead of the CGH aberrations and IPI, it emerged as the only significant parameter, while S phase again had marginal significance (data not shown). This ‘backtesting’ of the combined parameter in multivariate analysis strongly suggests that we have chosen the best way (with this simple approach) of combining the individual parameters with independent prognostic value.

The survival according to the combined CGH/IPI parameter is shown for patients receiving different treatments (Figures 5D, E, F), with different lymphoma subtypes (Figures 6A, B, C, D), for the patients assessed at the first presentation (Figure 6E), as well as for the patients with relapsed NHL (Figure 6F). CGH/IPI had high prognostic value in all these sub-groups \( (P \leq 0.006) \).
DISCUSSION

Chromosomal aberrations have high prognostic value and are used for treatment stratification in acute lymphoblastic leukemia (Pui and Evans, 1998). Intensive combination chemotherapy can cure almost half of the patients with aggressive NHL (e.g., Fisher et al., 1993). It is therefore important to establish reliable prognostic factors for treatment stratification of NHL patients with large differences with respect to survival. Traditionally, histology and stage have been used as the criteria for selection of NHL patients for different types of therapy. Clinical parameters (IPI, The International Non-Hodgkin’s Lymphoma Prognostic Factors Project, 1993), and tumour cell proliferation (Stokke et al., 1998b and references therein) are known prognostic factors in B-cell NHL, which, however, have not yet been much used for treatment stratification. In this retrospective study, we have identified three chromosomal aberrations with high and independent prognostic value in B-cell NHL. Loss of 11q21–23.1 and 17p, and gain of 6p

Table 3 Prognostic values of CGH aberrations in B-cell NHL

| Aberration (No. of cases with aberration) | Univariate analysis | Multivariate analysis |
|------------------------------------------|---------------------|----------------------|
|                                          | Relative riska      | 95% Confidence interval | P-valuec | Relative riskb | 95% Confidence interval | P-valued |
| –1p21–31.1 (9)                           | 1.7                 | 0.78–3.9              | 0.18      | 0.80          | 3.1–15                   | < 0.001     |
| –6cen-q24 (11)                           | 2.1                 | 1.1–4.2               | 0.04      | 0.64          | 6.8                      | < 0.001     |
| –8p (10)                                 | 3.3                 | 1.6–6.8               | 0.002     | 0.27          | 4.4                      | < 0.001     |
| –9p21-ter (13)                           | 2.1                 | 1.1–4.0               | 0.02      | 0.67          | 2.3                      | 1.2–4.5     | 0.01     |
| –11q21–23.1 (10)                         | 3.8                 | 1.9–7.8               | < 0.001   | 6.8           | 3.1–15                   | < 0.001     |
| –13q13-21.1 (11)                         | 2.3                 | 1.2–4.5               | 0.01      | 0.57          | 4.4                      | 2.2–8.6     | < 0.001  |
| –17p (14)                                | 1.9                 | 1.0–3.5               | 0.04      | 0.52          | 1.9                      | 1.0–3.5     | 0.04     |
| +6p (10)                                 | 4.2                 | 2.0–8.8               | < 0.001   | 8.8           | 3.9–20                   | < 0.001     |
| +7p (11)                                 | 1.3                 | 0.60–3.0              | 0.47      | 0.92          | 1.9                      | 0.95–3.8    | 0.07     |
| +8q23-ter (12)                           | 0.99                | 0.42–2.3              | 0.98      |              | 1.0                      | 0.54–2.0    | 0.94     |
| +12cen-q15 (15)                          | 1.3                 | 0.58–2.9              | 0.54      |              | 1.3                      | 0.58–2.9    | 0.54     |
| +17q24-ter (12)                          | 1.0                 | 0.54–2.0              | 0.94      |              | 1.0                      | 0.54–2.0    | 0.94     |
| +18q13.3–21 (19)                         | 3.7                 | 2.1–6.6               | < 0.001   |              | 3.7                      | 2.1–6.6     | < 0.001  |
| #CGH aberrations > 3 (33)                |                     |                      |           |              |                         |            |

aSurvival data were available for 92 patients. bRelative to the cases without the respective aberration. Cox regression analysis was employed. Significant values are in bold print. cFrom Cox regression analysis. The log-rank test gave p-values with less than 15% deviation from the values in the table. dAberrations with P<0.20 in univariate analysis were included in Cox forward and backward multivariate regression analysis (identical results).
were all associated with relative risks larger than 5. The international Prognostic Index was the only other parameter with significant independent prognostic value, and a simple binary combination parameter of the three CGH aberrations and IPI had high prognostic value for groups of patients with various lymphoma sub-types, having received different treatments, and presenting with primary or relapse disease.

In agreement with earlier studies, we found a number of recurrent losses and gains common to several sub-types of B-cell NHL. CGH is a relatively new technique, and the sensitivity and specificity of the method in terms of absolute copy numbers of specific loci have only been documented in a few reports (Kallioniemi et al, 1994b; Bentz et al, 1995, 1996; Tirkkonen et al, 1998). For 64 of the cases reported in this study, we have also performed interphase FISH to directly copy the number of the c-MYC, RB1, and TP53 gene loci. The results obtained with c-MYC and TP53 document that, at least when employing the criteria described here, CGH is sensitive enough to detect gain and loss of one copy, respectively. However, this sensitivity depends on a predominance of tumor cells in the sample (Kallioniemi et al, 1994a). For 12 cases with RB1 deletion by FISH did not show a reduced ratio at 13q14 by CGH. This may imply a poor sensitivity of CGH at this locus, but it is more likely that the deleted regions around RB1 are so small in many cases that CGH is not able to detect them. The specificity of CGH appears to be high, since only one case without FISH aberration was found to have CGH aberration at the corresponding locus.

Twelve of the frequent (>10% of cases) CGH aberrations reported here have been observed in NHL before (Bentz et al, 1995, 1996, 2000; Monni et al, 1996, 1998; Dierlamm et al, 1997; Autio et al, 1998; Beà et al, 1999). Seven of these 12 aberrations, the losses at 6cen-q24, 11q21–23.1, and 13q13–21.1, and the gains at 3q21-ter, 7p, 12cen-q15, and 18q13.3–21, are frequent findings also in classical cytogenetic studies (Heim and Mitelman, 1995). Moreover, banding techniques have revealed frequent rearrangements involving the 5 other regions (1p, 6p, 9p, 8q, and 17p) in B-cell NHL (Heim and Mitelman, 1995). CGH studies in NHL indicate that the rearrangements at the latter five chromosomal regions are not balanced (this work; Bentz et al, 1995; 1996; 2000; Monni et al, 1996, 1998; Dierlamm et al, 1997; Beà et al, 1999). Some of the aberrations were local in the majority of cases where they occurred. Our data defined 1p21–31.1 as a consensus region for the losses at 1p, in excellent agreement with the results of others (Monni et al, 1996, 1998; Beà et al, 1999; Bentz et al, 2000). Interestingly, an almost identical consensus region for the losses at 1p was found in breast (Tirkkonen et al, 1998) and colorectal carcinomas (DeAngelis et al, 1999). The losses at 11q were also mostly local, with a consensus region at 11q21–23.1, again in perfect agreement with the minimal common region of deletion determined by others (Monni et al, 1998; Beà et al, 1999; Bentz et al, 2000).

Novel findings of this work include the frequent loss of 8p, and the gain of 17q24-ter. The loss of 8p was associated with the gain of 8q (Table 2). The 17q gains were associated with 17p losses, and may be the result of formation of an isochromosome 17q in some cases.

Suggested candidates for tumour suppressor genes and proto-oncogenes in the chromosomal regions which are frequently lost and gained, respectively, include the ATM gene at 11q23.1 (Bullrich et al, 1999; Schaffner et al, 2000), the TP53 gene at 17p13.1 (Stokke et al, 2000), and the BCL-2 gene at 18q21 (Monni et al, 1997). Other tumour suppressor genes and proto-oncogenes, which are located within the frequently altered regions, include p16INK4a (9p21), RB1 (13q14), BCL6 (3q27), CCND3 (6p), and c-MYC (8q24). We have investigated the expression of p16, pRB, bcl6, cyclin D3, and c-myc by immunoblotting and immunohistochemistry in the tumours presented in this paper (unpublished). Additionally, we have determined the methylation status of the p16INK4a gene (E Hovig and T Stokke, unpublished). We did not find any decreased expression of p16 and pRB in the tumors with 9p21-ter and 13q13–21.1 (RB1) deletions, respectively, compared to the others. Case 458/88 was the only case that did not express pRB; this case had high-level amplification of 13q by CGH, but no RB-1 alleles (Galteland et al, 1999). Also, none of 13 cases with 9p21-ter loss by CGH had detectable methylation of the p16INK4a gene. The cases with gain of 3q21-ter, 6p, and 8q23-ter did not show enhanced expression of bcl6, cyclin D3, and c-myc, respectively, compared to the others. Hence, the identities

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### Table 4

| Aberration (No. of cases with aberration or phenotype) | Univariate analysis | Multivariate analysis |
|-------------------------------------------------------|---------------------|----------------------|
|                                                       | Relative risk | 95% Confidence interval | P-value | Relative risk | 95% Confidence interval | P-value |
| −11q21–23.1 (10)                                      | 3.8          | 1.9–7.8               | <0.001  | 10            | 4.3–23                   | <0.001  |
| −17p (14)                                             | 4.4          | 2.2–8.6               | <0.001  | 5.0           | 2.4–11                   | <0.001  |
| +6p (10)                                              | 4.2          | 2.0–8.8               | <0.001  | 6.7           | 2.9–16                   | <0.001  |
| S phase fraction > 3% (30)                            | 3.1          | 1.8–5.6               | <0.001  | 3.7           | 1.8–7.5                  | <0.001  |
| IPI > 2 (25)                                          | 3.9          | 2.1–7.1               | <0.001  |               |                         |         |
| Lymphoma subtype FCL (36)*                            | 0.41         | 0.22–0.75             | 0.004   |               |                         |         |
| Relapse (vs. primary) biopsy (30)                     | 2.3          | 1.4–4.1               | 0.002   |               |                         |         |
|cyclin D1 expression (14)                             | 2.4          | 1.2–4.6               | 0.01    |               |                         |         |
| t(14;18) (37)                                         | 0.53         | 0.29–0.94             | 0.03    |               |                         |         |
| B symptoms (22)                                       | 2.7          | 1.5–4.9               | 0.001   |               |                         |         |
| Treatment†                                            | >0.75        |                       |         |               |                         |         |

*Survival data were available for 92 patients. †Relative to the cases without the respective aberration or phenotype. Cox regression analysis was employed.

**Aberrations with P < 0.20 in univariate analysis were included in Cox forward and backward multivariate regression analysis (identical results). FCL patients (median survival: 83 months) survived longer than the other patients (median survival: 33 months). There were no significant differences between the survival in the other lymphoma subtypes (P > 0.12). Patient treatments were essentially chemotherapy with (39 cases), or without (35 cases) doxorubicin, or other types of treatment (18 cases; interteron, radiation). The survival was not different in these 3 groups (P > 0.75 for all 3 pairwise comparisons; log-rank test).**
Figure 5  Survival in NHL according to CGH parameters and IPI status: combination of the parameters and prognostic value for patients receiving different treatments. Kaplan-Meyer plots were generated for all 92 patients for cases with the presence (solid lines), or absence (dotted lines) of −11q21–23.1 and/or −17p and/or +6p (A; relative risk (RR) = 13), IPI > 2 (B; RR = 3.9), and −11q21–23.1 and/or −17p and/or +6p and/or IPI > 2 (combined CGH/IPI parameter, C; RR = 26). Survival was also assessed for patients receiving chemotherapy without doxorubicin (D, 35 cases), with doxorubicin (E, 39 cases), or other therapy (F, 18 cases) according to the presence (solid lines), or absence (dotted lines) of −11q21–23.1 and/or −17p and/or +6p and/or IPI > 2 (combined CGH/IPI parameter). P-values were less than 0.001 for all comparisons.
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Figure 6 Survival in NHL according to the combined CGH/IPI parameter: prognostic value for different histologies and primary/recurrent disease. Survival was assessed for patients with small lymphocytic lymphoma (A, 17 cases), follicle centre lymphoma (B, 36 cases), mantle cell lymphoma (C, 13 cases), and diffuse large B-cell lymphoma (D, 17 cases), as well as for the primary biopsy (E, 62 cases) and rebiopsy (F, 30 cases) patients according to the presence (solid lines), or absence (dotted lines) of −11q21−23.1 and/or −17p and/or +6p and/or IPI>2 (combined CGH/IPI parameter). P-values were less than 0.001 for all comparisons, except for MCL (P = 0.006).
of the tumour suppressor genes and proto-oncogenes at these loci need yet to be determined.

Another novel finding in our study is that the number of aberrations per tumour showed a bimodal distribution. The tumours with a high number of aberrations (≥ 4) had a high S phase fraction. Also, the patients with tumours with a high number of aberrations had a much shorter survival time than the others, in agreement with the results of classical cytogenetic studies (reviewed by Knutsen, 1998). As expected, most of the aberrations were found more frequently in the tumours with many aberrations. However, several of these presumably secondary aberrations were associated (Table 2), indicating specific oncogene and/or tumor suppressor gene cooperation.

Candidates for primary CGH aberrations in B-cell lymphomas, i.e. aberrations found at a high frequency also in cases with few aberrations, include the gains at 12cen-q15 and 18q13.3–21, and possibly also the 11q21–23.1 deletions in small lymphocytic lymphomas. Among these aberrations, loss of 11q and gain of chromosome 12 have been defined as primary events from classical cytogenetic studies (Mitelman et al, 1994). Interestingly, the gains at 12cen–q15 and 18q13.3–21 did not have any prognostic value, suggesting that additional, ‘secondary’ events are required for evolution towards a more aggressive malignant lymphoma.

None of the aberrations detected by CGH were specific (i.e. diagnostic) for any lymphoma sub-type. However, several of the aberrations were found at a significantly higher frequency in some lymphoma sub-types, such as +12cen–q15 in SLL, −1p21–31.1, −8p, −9p21–ter, −11q21–23.1, −13q13–21.1, and +6p in DLBCL. Gain of chromosome 12 or parts of this chromosome has been observed by others in SLL (Bentz et al, 1995; Autio et al, 1998). However, +12cen–q15 gain was more frequent in SLL in this study (37%). The mantle cell lymphomas typically had a high number of aberrations, and the losses at 1p21–31.1, 8p, 9p21–ter, 11q21–23.1, and 13q13–21.1 were more frequent in this subset of NHL, in concordance with the results of others (Monni et al, 1998; Béa et al, 1999; Bentz et al, 2000). Interestingly, the losses at 1p21–31.1, 8p, 9p21–ter, and 13q13–21.1 typically occurred together (Table 2), and together with cyclin D1 over-expression caused by a t(11;14) translocation (unpublished results). Gain of 3q21-ter was found in almost 50% of the MCL cases, but this aberration is common in all types of NHL, including MZL (Monni et al, 1996; Dierlamm et al, 1997; Monni et al, 1998; Béa et al, 1999; Bentz et al, 2000; Figure 4). Concerning the diffuse large B-cell lymphomas, we found gain of 6p at a similar frequency (24%) as Monni et al (1996; 21%), but a higher incidence of 17p losses (29%) than Monni et al (1996; 3%).

Since patients with B-cell lymphomas with a high S phase fraction have a poor prognosis (Stokke et al, 1998b), it is of particular interest to identify the genetic aberrations responsible for this phenotype. The losses of 8p and 17p, and the gains of 3q21-ter, 6p, 7p and 8q23-ter were associated with a high S phase fraction (Table 4). Four of these aberrations are associated pairwise (−8p, +3q21-ter, +7p, +8q23-ter). Hence, not all four of these aberrations necessarily target genes that are important in the control of cell proliferation. The anti-proliferative activities of wild-type p53 are well documented in the literature (for reviews, see: Sherr, 1996; Levine, 1997), and our previous study showed that a high S phase fraction was characteristic for NHL cases with either mutations in TP53, loss of TP53, or clonal expression of p53 (Stokke et al, 2000).

Several aberrations with prognostic information were found in this study (Table 3). Multivariate analysis showed that loss of 11q21–23.1 (ATM) and 17p (TP53), and gain of 6p by CGH were independent prognostic factors. Other studies have shown that loss of 17p or any aberration in the TP53 pathway are strong prognostic factors in all subtypes of NHL (Sander et al, 1993; Tilly et al, 1994; Dohner et al, 1995; Schoch et al, 1995; Hernandez et al, 1996; Panayiotidis and Kotsi, 1999; Dohner et al, 2000; Stokke et al, 2000; reviewed by Knutsen, 1998), while 11q deletions are unfavorable for patients with SLL/CLL (Dohner et al, 1997; Panayiotidis and Kotsi, 1999; Dohner et al, 2000). Schouten et al (1990) described gain of chromosome 6 as an adverse prognostic sign in a group of patients with all types of NHL.

Interestingly, S phase fraction, presence of BCL1 and BCL2 translocations, lymphoma subtype, biopsy status (primary vs secondary), as well as B symptoms, became non-significant to predict prognosis when entered into Cox multivariate analysis together with the 3 CGH aberrations and IPI (Table 4). An ‘and/or’ combinatory scheme was found to incorporate the prognostic CGH aberrations and IPI into a single binary parameter which had high prognostic value for patients receiving different treatments, with various lymphoma subtypes, and primary as well as relapse cases (Figures 5, 6). The prognostic chromosomal imbalances identified here clearly warrant large prospective studies (e.g. by FISH) to evaluate whether prognostication and treatment stratification of NHL can be based on chromosomal aberrations.

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