# Dic(9;20)(p13;q11) in childhood acute lymphoblastic leukaemia is related to low cellular resistance to asparaginase, cytarabine and corticosteroids

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Dic(9;20)(p13;q11) was first described as a nonrandom chromosome abnormality in B-cell precursor acute lymphoblastic leukaemia (BCP ALL) in the mid 1990s,[1,2] and 71 dic(9;20)-positive cases have since then been reported.[3–5] Approximately 90% of these cases were children or adolescents, with dic(9;20) occurring in about 2% of childhood BCP ALL.[6] The recent review by Forestier *et al*.[5] describes that dic(9;20)-leukaemias are of B-cell precursor immunophenotype, never have a high hyperdiploid modal number, show a female predominance, and have a significant age incidence peak at 3 years. Most patients are allocated to non-standard risk treatment arms due to high WBC (median $24 \times 10^9$/l) and a relatively high frequency of CNS disease or other extra-medullary leukaemia (EML) at diagnosis. The prognostic implications of dic(9;20) are to a large extent unknown. A relatively large proportion of the relapses reported in the literature have been extra-medullary, and post-relapse treatment including block therapy has been successful in several patients, as illustrated by a p-EFS of 0.62 and a predicted overall survival of 0.82 at 5 years for the 24 Nordic cases.[5]

The optimal treatment strategy for children with dic(9;20)-positive BCP ALL is not known. Test of cellular drug resistance *in vitro* might help to elucidate this problem, and we have therefore analysed samples from patients with and without dic(9;20) to see if this aberration is associated with a specific drug sensitivity profile. To our knowledge, no such data have been published so far.

Leukaemic cells from bone marrow or peripheral blood of children (aged 1–17 years) with newly diagnosed BCP ALL were used in this study. Nordic centres for paediatric oncology participated and provided samples for test of *in vitro* drug resistance from 719 patients between January 1996 and December 2006. The patients were representative of all children in this age group diagnosed with BCP ALL in the Nordic countries during the study period, as evidenced by similarities in sex, age, WBC count at diagnosis, immunophenotype, cytogenetics and p-DFS (not shown). During this time period, two closely related protocols have been used, NOPHO-ALL-1992 and NOPHO-ALL-2000, the former described in detail by Gustafsson *et al*.[7] Dic(9;20) has not been a risk stratifying aberration in any of the two protocols. Instead, the choice of treatment intensity for patients with dic(9;20)-positive ALL was made according to age, WBC count, the presence of EML and morphologic response during induction therapy.

Chromosome banding analyses were performed using standard methods in 15 cytogenetic laboratories in the Nordic countries, and all abnormal karyotypes have annually been centrally reviewed (since 1996 in Sweden and since 2000 in all five Nordic countries). To retrieve as many dic(9;20)-positive ALL as possible, cases with either probable dic(9;20) or with abnormalities suggesting the presence of this aberration, such as monosomy 20 and deletion of 9p,[3] and with metaphase cells in fixative, surplus from the initial cytogenetic investigations, were screened by FISH using the LSI 9p21/CEP-9 dual-colour and the CEP-20 probes according to the manufacturer's instructions (Vysis, Stockholm, Sweden). In total, 37 such cases were analysed. The same FISH probes were also applied to confirmed dic(9;20)-positive ALL.[5]

Leukaemic cells were tested by the fluorometric microculture cytotoxicity assay (FMCA), a rapid and reproducible method for determination of *in vitro* drug sensitivity. It is based on the measurement of fluorescence generated from hydrolysis of fluorescein diacetate to fluorescein by cells with intact plasma membranes and has been described in detail.[8,9] Drugs were tested in triplicate. Six wells without drugs served as controls and six wells containing culture medium only served as blanks. The results are presented as survival index (SI), defined as fluorescence in test wells/fluorescence in control wells (blank values subtracted) $\times 100$. Thus, a low numerical value indicates high sensitivity to the cytotoxic effect of the drug.

Cytotoxic drugs were obtained from commercial sources and tested at empirically derived concentrations, chosen to produce a large scatter of SI values among the samples (see Table 2). These concentrations were adopted from previous studies of leukaemic cells.[8]

Non-parametric statistical methods were used throughout. The SPSS 14.0 software package was used for the calculations. All analyses were two-tailed and the level of statistical significance was set at $P<0.05$. Local ethics committees approved the study.

The FMCA was successful in 546 (76%) of the samples, and the reasons for failure were low proportion of lymphoblasts in control wells after incubation ($n=21$); low signal-to-noise ratio ($n=108$); high coefficient of variation in controls ($n=29$) and other technical problems ($n=15$).

Eleven out of the 546 samples successfully tested for *in vitro* drug resistance were diagnosed with dic(9;20). Table 1 shows the distribution of some important clinical and biological parameters within this group, as compared with the 24 Nordic cases described in a recent publication, and literature data from previous publications. A low median age of 3 years, female preponderance, high WBC count at diagnosis and a prevalence of 2% in our patient population, are characteristics that agree very well with published data.

Table 2 shows the *in vitro* drug resistance in samples from patients with or without dic(9;20). The dic(9;20)-positive samples were significantly more sensitive to asparaginase, cytarabine, dexamethasone and prednisolone, and there was a nonsignificant trend in the same direction also for the other drugs tested.

The next step in the data analysis was to exclude patients with other chromosomal aberrations known to be of prognostic significance and present exclusively in the dic(9;20)-negative group: high hyperploidy ($n=184$), t(12;21)(p13;q22) ($n=109$), t(9;22)(q34;q11) ($n=12$), t(1;19)(q23;p13) ($n=19$) and 11q23 translocations ($n=10$). As shown in Table 3, dic(9;20)-positive samples were much more sensitive to cytarabine and prednisolone than negative samples, with significant differences also for asparaginase, dexamethasone and 6-thioguanine.

Figures 1a–c show data for dic(9;20) compared with major cytogenetic subgroups in this material for three important ALL drugs. Dic(9;20) and t(1;19) samples were clearly among the most sensitive to cytarabine and prednisolone. A different pattern was seen with vincristine, where t(1;19) samples had a median SI of 19, whereas all other subgroups, including

**Table 1** Characteristics of children with dic(9;20) ALL tested for *in vitro* cellular drug resistance ($n=11$) compared with the whole Nordic material ($n=24$) and other published cases of dic(9;20)-positive BCP ALL ($n=47$)

| | In vitro *tested* N = *11* | *Whole Nordic material* N = *24* | *Literature data* N = *47* |
|---|---|---|---|
| *Age (years)* | | | |
| Median | 3.3 | 3 | 3 |
| Range | 1.3–14.1 | 0–15 | 0–17 |
| *Female/male (quotient)* | 2.7 | 1.7 | 2.1 |
| *Percentage of BCP ALL* | 2.0 | 1.3 | 2 |
| *WBC ($10^9$/l)* | | | |
| Median | 34 | 24 | 24 |
| Range | 2.6–121 | 0.9–303 | 2.3–536 |
| *Risk group (n)* | | | |
| SR | 3 | 5 | |
| IR | 3 | 9 | |
| HR | 5 | 9 | |
| Infant | 0 | 1 | |
| *Treatment protocol* | | | |
| NOPHO ALL-92 | 4 | 11 | |
| NOPHO ALL-2000 | 7 | 12 | |
| Interfant 99 | | 1 | |
| *Follow-up time (months)* | | | |
| Median | 50 | 61 | |
| Range | 24–135 | 24–135 | |
| *Relapse rate (%)* | 9 | 29 | 25 |

Abbreviations: ALL, acute lymphoblastic leukaemia; BCP ALL, B-cell precursor acute lymphoblastic leukaemia; HR, high risk; IR, intermediate risk; SR, standard risk.
The 11 patients tested for *in vitro* drug resistance are part of the Nordic material ($n=24$).
Data for the Nordic group and literature data were summarized by Forestier *et al*.[5]
Follow-up time for patients in continuous complete remission.

**Table 2** *In vitro* drug resistance in children with dic(9;21) ($n=11$) compared with other children with BCP ALL ($n=535$)

| *Drug* | *Concentration* | *Dic(9;20)* | | *All other BCP ALL* | | |
|---|---|---|---|---|---|---|
| | | *Median* | *(25th–75th)* | *Median* | *(25th–75th)* | P-*value* |
| Amsacrine | 1 | 21 | (16–35) | 27 | (16–43) | 0.42 |
| Asparaginase | 1 | 39 | (27–56) | 67 | (50–82) | 0.017 |
| Cytarabine | 0.5 | 35 | (28–41) | 56 | (39–72) | 0.005 |
| Dexamethasone | 1.4 | 26 | (16–50) | 50 | (35–66) | 0.025 |
| Doxorubicin | 0.5 | 19 | (12–52) | 30 | (17–44) | 0.33 |
| Etoposide | 5 | 25 | (19–80) | 37 | (22–57) | 0.64 |
| Melphalane | 2.5 | 16 | (9–21) | 28 | (18–38) | |
| Prednisolone | 50 | 20 | (16–42) | 47 | (31–61) | 0.010 |
| Vincristine | 0.5 | 36 | (31–66) | 50 | (34–71) | 0.56 |
| 6-Thioguanine | 10 | 23 | (17–40) | 38 | (22–55) | 0.12 |

Abbreviations: BCP ALL, B-cell precursor acute lymphoblastic leukaemia; SI, survival index.
Concentrations of the drugs are in microgram per millilitre, except for asparaginase (units per millilitre).
Median and 25th–75th percentile SI values denote percentage of surviving cells compared with controls.
Only four dic(9;20)-positive samples were tested with melphalane.
*P*-values were determined by the Mann–Whitney *U*-test.

dic(9;20), had median values between 36 and 53. When the dic(9;20) and t(1;19) groups were compared, similar SI values were found for most of the 10 drugs, except that the t(1;19) samples were significantly more sensitive to vincristine ($P=0.005$).

At the latest follow-up in January 2008, one patient with dic(9;20), initially treated as standard risk, had relapsed with EML in CNS 2.5 years after diagnosis. The patient is in second remission 12 months later. The median follow-up time for dic(9;20) patients in continuous complete remission is 50 months (range 24–135 months), so further relapses may still occur.

We have previously reported data on *in vitro* cellular drug resistance in 370 Nordic children with ALL and correlated the findings to risk group.[10] The specific drug sensitivity profile of two cytogenetic subgroups, t(12;21) and t(1;19), has also been

described.[11,12] These patients, together with new cases added after the publication of the reports, here form the cohort of 535 BCP ALL patients, which is compared with the eleven cases with dic(9;20).

**Table 3** *In vitro* drug resistance in children with dic(9;20) ($n=11$) compared with other BCP ALL patients ($n=201$) excluding high hyperploidy, t(12;21), t(9;22), t(1;19) and 11q23 rearrangement

| *Drug* | *Dic(9;20)* | | *Other BCP ALL* | | |
|---|---|---|---|---|---|
| | *Median* | *(25th–75th)* | *Median* | *(25th-75th)* | P-*value* |
| Amsacrine | 21 | (16–35) | 26 | (16–42) | 0.49 |
| Asparaginase | 39 | (27–56) | 69 | (55–89) | 0.013 |
| Cytarabine | 35 | (28–41) | 65 | (48–76) | <0.001 |
| Dexamethasone | 26 | (16–50) | 51 | (34–69) | 0.029 |
| Doxorubicin | 19 | (12–52) | 33 | (20–47) | 0.20 |
| Etoposide | 25 | (19–80) | 35 | (22–59) | 0.73 |
| Melphalane | 16 | (9–21) | 31 | (18–43) | |
| Prednisolone | 20 | (16–42) | 49 | (33–65) | 0.007 |
| Vincristine | 36 | (31–66) | 52 | (37–73) | 0.40 |
| 6-thioguanine | 23 | (17–40) | 43 | (30–61) | 0.016 |

Abbreviations: BCP ALL, B-cell precursor acute lymphoblastic leukaemia; SI, survival index.
The concentrations of the drugs are shown in Table 2.
Median and 25th–75th percentile SI values denote % surviving cells compared to controls.
*P*-values were determined by the Mann–Whitney *U*-test.

Our main finding was that tumour cells with the dic(9;20)-aberration were significantly more sensitive to asparaginase, cytarabine, dexamethasone and prednisolone, than other BCP ALL samples. Although the number of dic(9;20)-positive patients tested was limited, the findings were highly significant. The difference between dic(9,20)-positive and dic(9,20)-negative patients remained significant after elimination of unevenly distributed other cytogenetic subsets, such as high hyperploidy, t(1;19), t(9;22), t(12;21) and 11q23 translocations. One could speculate that dic(9;20)-positive cells are generally more susceptible to handling and various unspecific stimuli, thus explaining the results, but this is strongly contradicted by the finding that control cell survival was similar in the dic(9;20)-positive and dic(9;20)-negative groups.

Any conclusions regarding clinical treatment based on *in vitro* data must be drawn with great caution. Whether dic(9;20) patients benefit from intensified therapy including high-dose cytarabine, or from more extended treatment with asparaginase, as suggested by this data, can only be demonstrated by clinical trials. The fact that children with dic(9;20) have received treatment of varying intensity in different treatment protocols might help to answer the question of optimal therapy. To shed some light on this the 'Ponte di Legno' Working Group has, at its latest workshop in Atlanta in December 2007, initiated a retrospective meta-analysis of survival data for patients with this newly identified cytogenetic aberration.

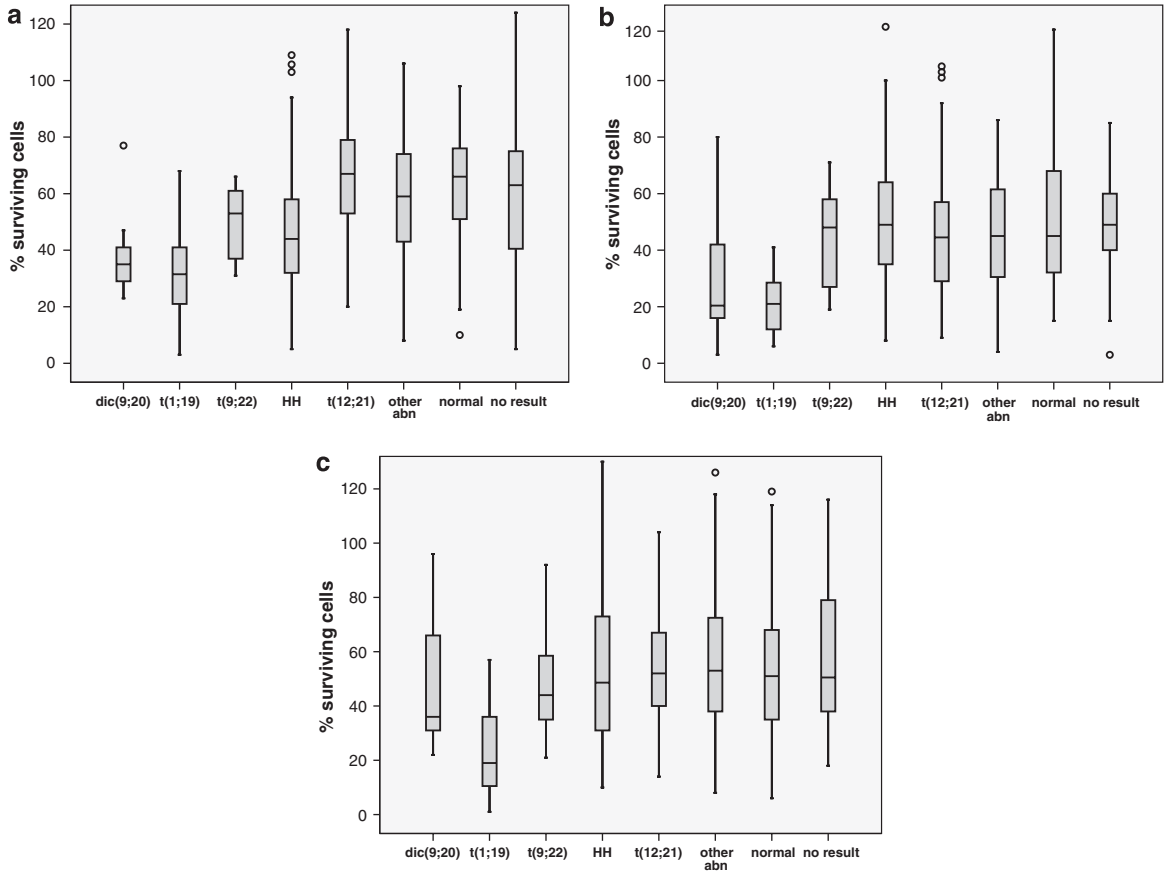


**Figure 1** *In vitro* drug resistance in 11 children with dic(9;20)-positive ALL compared with other major cytogenetic subgroups. (**a**) cytarabine 0.5 μg/ml, (**b**) prednisolone 50 μg/ml and (**c**) vincristine 0.5 μg/ml. Dic(9;20) $n=11$; t(1;19) $n=19$, t(9;22) $n=12$, HH (high hyperploidy with >51 chromosomes) $n=184$, t(12;21) $n=109$, other abn (other clonal abnormalities) $n=89$, normal $n=70$, no result $n=35$. The box-and-whisker plot shows median, first and third quartiles; whiskers extend to the highest and lowest value, excluding outliers, which are denoted by circles.

In summary, we found that the presence of dic(9;20) in childhood ALL was associated with low cellular drug resistance to asparaginase, cytarabine and corticosteroids. These data might help to understand the effect of different treatment regimens and determine the most efficient therapy for this group of patients.

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G Lönnerholm[1], A Nordgren[2], B-M Frost[1], OG Jonsson[3], J Kanerva[4], R Nygaard[5], K Schmiegelow[6], R Larsson[7] and E Forestier[8] on behalf of the Nordic Society for Paediatric Haematology and Oncology
[1]*Department of Women's and Children's Health, University Children's Hospital, Uppsala, Sweden;*
[2]*Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden;*
[3]*Department of Paediatrics, Landspitali University Hospital, Reykjavik, Iceland;*
[4]*Department of Hematology/Oncology and Stem Cell Transplantation, Hospital for Children and Adolescents, University of Helsinki, Helsinki, Finland;*
[5]*Department of Paediatrics, St Olav University Hospital, Trondheim, Norway;*
[6]*Paediatric Clinic II, Rigshospitalet, and the Medical Faculty, the University of Copenhagen, Copenhagen, Denmark;*
[7]*Department of Medical Sciences, Section of Pharmacology, University Hospital, Uppsala, Sweden and*
[8]*Department of Clinical Sciences, Pediatrics, University of Umeå, Umeå, Sweden*
*E-mail: gudmar.lonnerholm@kbh.uu.se*

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# A subset of Binet stage A CLL patients with TP53 abnormalities and mutated *IGHV* genes have stable disease

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*TP53* abnormalities consistently emerge as the most significant adverse prognostic factor in multivariate analyses of both prospective and retrospective studies in early and advanced chronic lymphocytic leukaemia (CLL).[1] In view of the higher response rates often achieved with alemtuzumab-containing regimens compared with alkylating agent and/or purine analogue therapy[2] and the possibility of long-term disease-free survival following allogeneic transplantation in some patients, there is an increasing consensus for screening for TP53 abnormalities in CLL patients who fulfil the International Workshop on Chronic Lymphocytic Leukemia (IWCLL) recommendations for initiating therapy. It has also been suggested that the detection of a TP53 abnormality in early CLL should be an indication for treatment with an alemtuzumab-containing regimen.[3] However, a small subset of patients with TP53 abnormalities pursue a benign clinical course.

Since 1994, we have examined fresh or dimethyl sulphoxide frozen cells from CLL patients presenting to the Royal Bournemouth Hospital, both for *TP53* loss by fluorescence *in situ* hybridization (FISH) and karyotypic abnormalities determined by G-banding. Analysis of G-banded metaphases was from peripheral blood lymphocyte cultures stimulated with Phorbol-