**ORIGINAL ARTICLE**

High expression of Mcl-1 in ALK positive and negative anaplastic large cell lymphoma

R Rust, G Harms, T Blokzijl, M Boot, A Diepstra, J Kluiver, L Visser, S-C Peh, M Lim, W A Kamps, S Poppema, A van den Berg

**Aim:** To gain more insight into the genes involved in the aetiology and pathogenesis of anaplastic large cell lymphoma (ALCL).

**Methods:** Serial analysis of gene expression (SAGE) was undertaken on the CD4+ALK+ (anaplastic lymphoma kinase positive) ALCL derived cell line Karpas299 and as comparison on CD4+ T cells. Quantitative reverse transcription polymerase chain reaction (RT-PCR) and immunohistochemistry were performed on five ALCL derived cell lines and 32 tissue samples to confirm the SAGE data.

**Results:** High expression of Mcl-1 was seen in the Karpas299 cell line, whereas the two other antiapoptotic Bcl-2 family members, Bcl-2 and Bcl-XL, were not detected in the SAGE library. Quantitative RT-PCR confirmed the high expression of Mcl-1 mRNA and low expression of Bcl-2 and Bcl-XL in Karpas299 and in four other ALCL cell lines. To expand on these initial observations, primary tissue samples were analysed for Mcl-1, Bcl-XL, and Bcl-2 by immunohistochemistry. All 23 ALK+ and nine ALK− ALCL cases were positive for Mcl-1. Bcl-2 and Bcl-XL were expressed infrequently in ALK+ ALCL cases, but were present in a higher proportion of ALK− ALCL cases.

**Conclusion:** The consistent high expression of Mcl-1 in ALK+ and ALK− ALCL suggests that Mcl-1 is the main antiapoptotic protein in this disease. The high frequency of Mcl-1, Bcl-2, and Bcl-XL positive ALCL cases in the ALK− group compared with the ALK+ group indicates that ALK induced STAT3 activation is not the main regulatory pathway in ALCL.

Anaplastic large cell lymphoma (ALCL) is characterised by the presence of a cohesive proliferation of large CD30 positive cells with abundant cytoplasm and pleomorphic, often horseshoe shaped nuclei. Most ALCL cases are of the T cell type, but null cell type ALCL also occurs. Two forms of ALCL can be distinguished based on the presence or absence of translocations involving the anaplastic lymphoma kinase (ALK) gene, such as the (2;3)(p23;q35) translocation (NPM/ALK). More recently, variant translocation partners have been described that also lead to overexpression of the ALK protein. ALK positive cases occur more frequently in children and young adults and have a relatively good prognosis with appropriate chemotherapy. ALK negative ALCL occurs in older individuals and has a poor prognosis.

“Mcl-1 is a member of the Bcl-2 family of apoptosis regulating proteins (including Bcl-2, Bcl-XL, Bax, Bak, and Bad) and can inhibit apoptotic cell death.”

Little is known about the biology of this malignant disease and only few studies have been published identifying genes specifically expressed in ALCL. To gain more insight into the genes that are involved in the aetiology and pathogenesis of ALCL, we applied serial analysis of gene expression (SAGE). This technique allows the construction of a comprehensive expression profile and results in a quantitative overview of the expression of the genes corresponding to the SAGE tags. Comparison of the CD4+ALK+ ALCL derived cell line Karpas299 with flow cytometry sorted peripheral blood CD4+ T cells revealed high expression of Mcl-1 in Karpas299. Mcl-1 is a member of the Bcl-2 family of apoptosis regulating proteins (including Bcl-2, Bcl-XL, Bax, Bak, and Bad) and can inhibit apoptotic cell death. Mcl-1 is also involved in the programming of differentiation, promotes cell viability. In contrast to the apoptosis inhibiting potential of the full length Mcl-1 transcript, it has been suggested that differential splicing of the Mcl-1 gene yields a shorter protein with a Bcl-2 homology domain 3 that may promote cell death.

To study Mcl-1 and other antiapoptotic Bcl-2 family members Bcl-2 and Bcl-XL, we performed real time reverse transcription polymerase chain reaction (RT-PCR) and immunohistochemistry on ALK+ and ALK− ALCL cases.

**MATERIALS AND METHODS**

**Cell lines and tissues**

CD4+ T cells were isolated from the buffy coats of healthy donors using a fluorescence activated cell sorter (MoFlo Cytomation, Fort Collins, Colorado, USA) and were used directly for RNA isolation and SAGE analysis. The CD4+ALK+ ALCL derived cell line Karpas299 was obtained from ATCC (Rockville, Maryland, USA) and the NPM/ALK positive ALCL derived cell lines SU-DHL-1, SR786, SUP-M2, and DEL were obtained from DSMZ (Braunschweig, Germany). Based on the presence of the t(2;5) translocation, expression of CD4 and ALK, and lack of expression of B cell markers, we selected the Karpas299 cell line for the SAGE analysis. Frozen and paraffin wax embedded ALCL tissue specimens from 23 ALK+ and nine ALK− ALCL cases were obtained from the departments of pathology of the Groningen University Medical Centre, the Netherlands and the University of Malaysia. All protocols for obtaining and studying human...
Mcl-1 expression in ALCL

The SAGE procedure was performed as described previously.14 The SAGE libraries were compared and linked to the Unigene library to identify the corresponding genes.

Gene specific real time RT-PCR
Total RNA was isolated with Trizol (Life Technologies Inc, Gaithersburg, Maryland, USA) and first strand cDNA synthesis was performed using the protocol provided by the manufacturer (Life Technologies Inc). Assays-on-DemandTM gene expression products (Applied Biosystems, Foster City, California, USA) were used for Bcl-2 (Hs00236808_s1), Bcl-XL (Hs00236329_m1), and Mcl-1 (Hs00172031_m1). Real time PCR was performed in 1 × Taqman® Universal PCR master mix (Applied Biosystems). β2 Microglobulin (β2m) was used as a positive control and for normalisation; β2m forward (5’-gaaatggagacctcagctg-3’), β2m reverse (5’-gatgctgcttacatgctcgat-3’), and probe (5’-gcctcagctcagctgagctg-3’). The amounts of the target gene were measured by determining ΔCt (Ctgene − Ctc2m) and the SD of ΔCt (SD(ΔCt)) was calculated (SD(ΔCt) = ((SD(gene))² + (SD(2m))²)⁰⁵). The relative amount of target gene was measured by determining ΔΔCt (ΔΔCt = ΔCtcalibrator − ΔCttestsample) and the factor difference was calculated (2^−ΔΔCt). The range is given as 2^−ΔΔCt±SD(ΔΔCt) and 2^−ΔΔCt±SD(ΔΔCt).

Immunostaining
ALK, Bcl-2, Bcl-XL, and Mcl-1 positivity was assessed by immunohistochemistry using standard protocols and appropriate dilutions of monoclonal mouse antihuman ALK antibody, monoclonal mouse antihuman Bcl-2 antibody, polyclonal rabbit antihuman Mcl-1 antibody (Dako, Copenhagen, Denmark), and monoclonal rabbit antihuman Bcl-XL antibody (Zymed, San Francisco, California, USA). Peroxidase activity was visualised with diaminobenzidine and H2O2. For all analyses, negative controls (first incubation step without primary antibody) and positive control tissue sections were included. Differences in the number of positive cases between the ALK+ and ALK− group were tested using Fisher’s exact test.

RESULTS
We constructed gene expression profiles of Karpas299 and CD4+ T cells using the SAGE technique. For Karpas299 we sequenced 10 678 tags representing 5090 different genes and for the CD4+ T cells we obtained 8425 tags representing 4467 different genes. These expression profiles were compared and...
linked to the Unigene library to identify the corresponding genes. The SAGE tag belonging to the Mcl-1 gene was detected at a frequency of 0.04% in Karpas299, and 0.01% in the CD4+ T cells. The tag for the shorter splice variant of Mcl-1 was not detected in the SAGE libraries. No SAGE tags were seen for the other antiapoptotic members of the Bcl-2 family (Bcl-2 and Bcl-XL) in Karpas299 or in CD4+ T cells. To confirm this differential expression, ALK+ ALC cell lines Karpas299, SUP-M2, SR786, SU-DHL-1, and DEL were analysed using β2m, expressed at similar levels based on the SAGE libraries (0.29% in Karpas299 and 0.24% in CD4+ T cells), as a housekeeping gene. All five cell lines highly expressed Mcl-1 compared with tonsil and CD4+ T cells. Bcl-XL expression was moderate in the SUP-M2, SR786, SU-DHL-1, and DEL cell lines but was low in Karpas299 compared with CD4+ T cells and tonsil. Bcl-2 was slightly upregulated in the SUP-M2 and SR786 cell lines, but was reduced in the other ALC cell lines compared with the CD4+ T cells (fig 1).

Immunocytochemical staining for Mcl-1 revealed strong positivity in the Karpas299 cell line (fig 2A) and positive cytoplasmic staining in most of the tumour cells in all ALK+ and ALK− ALC cases. Staining for Bcl-2 was negative in all ALK+ cases, whereas four of the nine ALK− cases were positive. Bcl-XL was positive in eight of nine ALK− cases, but only in three of 18 ALK+ cases (table 1; fig 2). The ALK− group included significantly more Bcl-2 (p = 0.004) and Bcl-XL (p = 0.000) positive cases than the ALK+ group.

**DISCUSSION**

Using SAGE analysis, the antiapoptotic member of the Bcl-2 family Mcl-1 was shown to be highly expressed in the ALC cell line Karpas299 compared with CD4+ T cells. In contrast, tags corresponding to the other two members of the antiapoptotic Bcl-2 family, Bcl-XL and Bcl-2, were not identified in the SAGE libraries. Screening of the SAGE library for tags corresponding to proapoptotic genes revealed the presence only of a low frequency of tags corresponding to the Bak gene. The SAGE results were confirmed with quantitative RT-PCR, which indicated increased expression of the Mcl-1 gene in Karpas299 and in the four other ALC derived cell lines. Moreover, quantitative RT-PCR for Bcl-XL and Bcl-2 clearly showed that expression was much lower than that of Mcl-1, consistent with the SAGE results.

Immunohistochemical analysis of 32 ALC cases (23 ALK+ and nine ALK−) demonstrated the presence of the Mcl-1 protein in most tumour cells in all cases. No difference in Mcl-1 staining intensity or percentage of positive tumour cells was seen between ALK+ and ALK− cases. Mcl-1 protein expression was reported previously in 10 of 10 and 10 of 11 ALCs. In a more recent study, Rasidakis et al detected Mcl-1 positivity in 16 of 26 ALK− ALCs using a 10% cutoff. These data confirm the consistent high expression of Mcl-1 and strongly suggest that Mcl-1, rather than Bcl-2 or Bcl-XL, is the main antiapoptotic protein of the Bcl-2 family expressed in ALC, and in particular in ALK+ ALC. Analysis of the apoptotic rate in ALC revealed low levels of apoptotic cells, ranging from 1.2% to 3.2%, in ALK+ and ALK− cases.

A possible role for Mcl-1 in lymphomagenesis is supported by the finding of a variety of lymphomas in Mcl-1 transgenic mice. Moreover, high Mcl-1 expression has been reported in various other lymphoma subtypes, including angioimmunoblastic T cell lymphoma, myeloma cell lines, cutaneous T cell lymphoma, diffuse large B cell lymphoma, and mantle cell lymphoma. Treatment with the cyclin dependent kinase inhibitor flavopiridol, which results in a strong downregulation of Mcl-1 expression, has been shown to be effective in multiple myeloma, leukaemia cell lines, and in chronic lymphocytic leukaemia samples, indicating the importance of Mcl-1 as an antiapoptotic protein.

“Our findings argue against a role for ALK induced activation of STAT3 and suggest involvement of other pathways leading to the induction of Bcl-XL and Bcl-2 expression”

The inhibition of JAK3 in two ALK+ ALC derived cell lines resulted in downregulation of activated STAT3, decreased levels of Bcl-2 and Bcl-XL, and no changes in Mcl-1 values. This suggests that other mechanisms are involved in the induction of Mcl-1 in ALC cases. However, in a more recent study Amin et al demonstrated the downregulation of Bcl-2, Bcl-XL, and Mcl-1 upon transfection of a dominant negative STAT3 in two ALC cell lines, supporting the STAT3 mediated induction of Mcl-1 in these cells. Similar results were obtained in macrophages and large granular lymphocyte leukemia, which showed reduced Mcl-1 expression upon treatment with JAK inhibitors. Our data indicate the presence of the Mcl-1 protein in all ALC cases, independent of expression of the ALK protein, which suggests that besides the ALK induced activation of STAT3, other pathways might also contribute to the induction of Mcl-1 expression in ALC cases that lack ALK expression. This is supported by two studies that show the involvement of the phosphatidylinositol 3-kinase pathway in the upregulation of Mcl-1 expression.

In addition to positive regulation via survival signals such as activated STAT3 and the phosphatidylinositol 3-kinase pathway, Mcl-1 can also be downregulated via E2F1. Treatment with flavopiridol results in stabilisation of E2F1,

Table 1 Overview of the immunohistochemistry results of ALK+ and ALK− ALC cases

<table>
<thead>
<tr>
<th>ALC case</th>
<th>ALK</th>
<th>Mcl-1</th>
<th>Bcl-2</th>
<th>Bcl-XL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>NR</td>
</tr>
<tr>
<td>3</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>NR</td>
</tr>
<tr>
<td>4</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>NR</td>
</tr>
<tr>
<td>5</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>NR</td>
</tr>
<tr>
<td>6</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>NR</td>
</tr>
<tr>
<td>7</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>13</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>14</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>15</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>16</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>17</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>18</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>19</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>20</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>21</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>22</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>23</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>24</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>25</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>26</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>27</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>28</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>29</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>30</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>31</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>32</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

- No staining in the tumour cells; +, moderate staining in most of the tumour cells; ++, strong staining in most tumour cells; NR, no result; few cells, staining in <10% of the tumour cells; part of cells, staining in 10–30% of the tumour cells.

ALCL, anaplastic large cell lymphoma; ALC, anaplastic lymphoma kinase.
which acts as a transcriptional repressor of Mcl-1, and induces an effective downregulation of Mcl-1. Based on the balance between survival pathways and the effectiveness of Mcl-1 downregulation upon flavopiridol treatment, beneficial effects might be achieved by treatment of ALCL with flavopiridol.

As mentioned above, several studies suggest a role for ALK induction of STAT3 in the induction of Bcl-2 and Bcl-X expression in ALCL cases. Indeed, the presence of activated STAT3 was demonstrated in most ALK+ cases, but also in approximately half of the ALK- cases. These data indicate that STAT3 activation correlates with, but is not strictly dependent on, ALK expression in ALCL. The analysis of Bcl-2 and Bcl-X expression in ALCL demonstrated the complete absence of Bcl-2 in ALK+ cases and the expression of Bcl-2 in four of nine ALK- cases. These data indicate that Bcl-2 and Bcl-X are only infrequently expressed in ALK+ ALCL, but are present in a higher proportion of ALK- ALCLs. An inverse relation between ALK and Bcl-2/Bcl-X expression was previously noted in ALCL. These findings argue against a role for ALK induction of STAT3 and suggest involvement of other pathways leading to the induction of Bcl-Xi and Bcl-2 expression.

In summary, similar amounts of Mcl-1 protein are expressed in both ALK- and ALK+ ALCLs, whereas the expression of Bcl-2 and Bcl-Xi is limited to ALK- cases. Because activated STAT3 is not detectable in a small proportion of ALK+ cases and in half of the ALK- cases, it is very likely that other regulatory pathways are involved in the expression of these antiapoptotic Bcl-2 family members. The consistent expression of Mcl-1 as demonstrated in our study indicates that treatment with agents such as flavopiridol may provide a novel tool for the treatment of both ALK+ and ALK- patients with ALCL.

ACKNOWLEDGEMENTS

This study was supported by the Foundation for Pediatric Oncology Research Groningen (SKOG, grant number 99-04).

Authors’ affiliations

Rust, G Harms, T Blokzijl, M Boot, A Diepstra, J Kluiver, L Visser, S Poppema, P van den Berg, Department of Pathology and Laboratory Medicine, Universal Medical Centre Groningen and University of Groningen, 9700 RB Groningen, The Netherlands

Peh, Department of Pathology, University of Malaya, 50603 Kuala Lumpur, Malaysia

Lim, Department of Pathology, University of Utah Health Sciences Center, Salt Lake City, 84132 USA

W A Kamps, Department of Paediatric Oncology, Universal Medical Centre Groningen and University of Groningen

REFERENCES


15. Craig RW. MCL1 provides a window on the role of the BCL2 family in cell proliferation, differentiation and tumour suppression. Leukemia 2002;16:444–54.


Clinical Evidence — Call for contributors

Clinical Evidence is a regularly updated evidence-based journal available worldwide both as a paper version and on the internet. Clinical Evidence needs to recruit a number of new contributors. Contributors are healthcare professionals or epidemiologists with experience in evidence-based medicine and the ability to write in a concise and structured way.

Areas for which we are currently seeking authors:

- Child health: nocturnal enuresis
- Eye disorders: bacterial conjunctivitis
- Male health: prostate cancer (metastatic)
- Women’s health: pre-menstrual syndrome; pyelonephritis in non-pregnant women

However, we are always looking for others, so do not let this list discourage you.

Being a contributor involves:

- Selecting from a validated, screened search (performed by in-house Information Specialists) epidemiologically sound studies for inclusion.
- Documenting your decisions about which studies to include on an inclusion and exclusion form, which we keep on file.
- Writing the text to a highly structured template (about 1500–3000 words), using evidence from the final studies chosen, within 8–10 weeks of receiving the literature search.
- Working with Clinical Evidence editors to ensure that the final text meets epidemiological and style standards.
- Updating the text every six months using any new, sound evidence that becomes available. The Clinical Evidence in-house team will conduct the searches for contributors; your task is simply to filter out high quality studies and incorporate them in the existing text.
- To expand the topic to include a new question about once every 12–18 months.

If you would like to become a contributor for Clinical Evidence or require more information about what this involves please send your contact details and a copy of your CV, clearly stating the clinical area you are interested in, to Klara Brunnhuber (kbrunnhuber@bmjgroup.com).

Call for peer reviewers

Clinical Evidence also needs to recruit a number of new peer reviewers specifically with an interest in the clinical areas stated above, and also others related to general practice. Peer reviewers are healthcare professionals or epidemiologists with experience in evidence-based medicine. As a peer reviewer you would be asked for your views on the clinical relevance, validity, and accessibility of specific topics within the journal, and their usefulness to the intended audience (international generalists and healthcare professionals, possibly with limited statistical knowledge). Topics are usually 1500–3000 words in length and we would ask you to review between 2–5 topics per year. The peer review process takes place throughout the year, and our turnaround time for each review is ideally 10–14 days.

If you are interested in becoming a peer reviewer for Clinical Evidence, please complete the peer review questionnaire at www.clinicalevidence.com or contact Klara Brunnhuber (kbrunnhuber@bmjgroup.com).
High expression of Mcl-1 in ALK positive and negative anaplastic large cell lymphoma

R Rust, G Harms, T Blokzijl, M Boot, A Diepstra, J Kluiver, L Visser, S-C Peh, M Lim, W A Kamps, S Poppema and A van den Berg

*J Clin Pathol* 2005 58: 520-524
doi: 10.1136/jcp.2004.022335

Updated information and services can be found at:
http://jcp.bmj.com/content/58/5/520

These include:

**References**

This article cites 42 articles, 13 of which you can access for free at:
http://jcp.bmj.com/content/58/5/520#BIBL

**Email alerting service**

Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

**Topic Collections**

Articles on similar topics can be found in the following collections

- Immunochemistry (including allergy) (1664)
- Molecular genetics (355)

**Notes**

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/