References


Response:

Interpretation of sequential minimal residual disease assessment in chronic lymphocytic leukemia

Esteve et al have presented interesting and informative observations regarding the assessment of minimal residual disease (MRD) following intensive therapy for chronic lymphocytic leukemia (CLL). Their report is consistent with our own experience in that the eradication of detectable disease in CLL is associated with prolonged progression-free survival. We also agree that CLL patients who have detectable MRD at any time following monoclonal antibody therapy or autologous stem cell transplantation will experience progressively increasing levels of CLL in their peripheral blood and will eventually relapse clinically. To determine whether it is possible to predict the time at which further therapy will be required, we have assessed sequential samples in 45 patients for up to 5 years following treatment with Alemtuzumab (MabCampath) for refractory CLL (manuscript in preparation). In summary, following Alemtuzumab therapy the kinetics of disease progression is biphasic in most (24 of 34) patients: there is a rapid increase in peripheral blood CLL cells within 2 months of stopping therapy, followed by a second phase characterized by more gradual increase in CLL cells. It appears that the first phase, with rapidly increasing peripheral CLL cells, is due to redistribution of CLL cells from bone marrow or lymphoid tissue to peripheral blood, while the second phase represents expansion of absolute numbers of CLL cells. During this second phase it is possible to calculate a doubling time for the CLL cells (in this series it was a median of 30 days [range, 5-289 days]), which can be used to predict when an individual with CLL is likely to progress clinically.

It is of considerable importance that Esteve et al have demonstrated persistent but stable levels of CLL in patients after allogeneic transplantation. This indicates that there must be continuing suppression of the CLL clone following an allograft, and therefore that graft-versus-leukemia is a clinically significant phenomenon in this setting. We therefore agree that to understand the clinical implication of detectable MRD after allograft in an individual patient requires sequential studies and should not in itself be used to define therapy. But we believe that both our own data and the data of Esteve et al indicate that after Alemtuzumab and/or after autograft, the presence of detectable MRD is always indicative of progressive disease and that the tumor burden at the end of therapy is predictive of outcome.

Andy C. Rawstron and Peter Hillmen

Correspondence: Andy C. Rawstron, HMDS, Academic Unit of Haematology and Oncology, Algernon Firth Building, University of Leeds, Leeds LS1 3EX, United Kingdom; e-mail: andy.rawstron@hmds.org.uk

To the editor:

Comprehensive cytogenetic and molecular genetic characterization of the TI-1 acute myeloid leukemia cell line reveals cross-contamination with K-562 cell line

The TI-1 cell line was reportedly established from peripheral blood blasts of a male patient with acute myeloid leukemia (AML) French-American-British subtype M2.1 It was described as having a complex karyotype with 65 to 71 chromosomes and the XXY sex chromosomes. The chromosome aberrations included 2q+, 6p+, 9p+, 9p- , 13p+, trisomy of chromosomes 1, 4, 5, 7, 8, 11, 12, 15, 16, and 19; and 11 unidentified marker chromosomes.1

One of the authors (M.A.C.) received the TI-1 cell line from the original investigators, and because it had not been hitherto fully characterized we performed cytogenetic and molecular genetic analyses. TI-1 cells were cultured in RPMI-1640 medium with 20% fetal bovine serum (GibcoBRL, Rockville, MD). Cells were harvested and chromosomes G-banded using standard methods. Spectral karyotyping (SKY) was performed according to the manufacturer’s protocol (Applied Spectral Imaging [ASI], Migdal Haemek, Israel). Fluorescence in situ hybridization (FISH) was performed using standard techniques with the LSI BCR/ABL Dual Color, Dual Fusion Translocation Probe (Vysis, Downers Grove, IL). Comparative genomic hybridization (CGH) was performed according to standard protocol.2 For analysis of copy-number changes, a combination of 3.0 standard deviations from the mean, and upper and lower thresholds of above 1.17 (gains) and below 0.83 (losses) were used.3 DNA isolation and restriction landmark genomic scanning (RLGS) were carried out according to standard protocols.15

Cytogenetic analysis of TI-1 revealed a complex karyotype with a modal chromosome number of 68 (range, 60-70 chromosomes) (Figure 1A-B). As in the original report,2 we observed trisomy of chromosomes 1, 4, 5, 7, 8, 11, 15, 16, and 19. One copy of
chromosome 12, recognized as normal by G-banding, was shown by SKY to be a cryptic der(12)t(12;21). Likewise, the chromosome recognized as a normal Y chromosome in Figure 4 of Taoka et al1 was demonstrated by SKY and FISH to be a der(22)t(9;13;22). Each of the 16 structurally altered chromosomes in Figure 4 of Taoka et al1 was present in our preparations (Figure 1A). Thus, the cells we studied had the same karyotype as TI-1 cells analyzed by Taoka et al,1 ruling out the possibility that the abnormalities arose during in vitro propagation in our laboratory.

The final karyotype description, based on G-banding, SKY, and FISH, is as follows: 63-70,3n,XX,-X,der(2)(2pter...hst(9q34;22q11.2)),der(2)(2pter...2q37::13q?::hsr(9q34;22q11.2)) [cp13]/60-69,idem,der(6)t(3;6)(q2?5;p2?5) [cp10] .

The CGH profile, showing 14 gains and 9 losses (Figure 1C), corresponds well with cytogenetics. The copy-number karyotype is as follows: +1p31.3-pter, +1q, +2q24.2-32.1, -2q33-35, +3p21-pter, -3p14.3-q24, +5p, +6p, +6q25.1-qter, +7q21.1-qter, +pter-q22.3, +9q34.1-qter, +10q11.2-22.3, -10q23-qter, -12p, -13q11-21.3, +13q31-32, -14, -17p, +18pter-q12, -18q12.3-qter, +21q, +22q11.2-13.2.

The RLGS profile of TI-1 had 5 loci with DNA amplification, 2 of which had strong homology to chromosome 22q11. Amplification of RLGS locus 3C71 on 22q11 is shown in Figure 1D; amplification of 22q11 was confirmed by CGH (Figure 1C). FISH localized the amplified BCR/ABL fusion gene on 2 acrocentric marker chromosomes and the der(2)t(2;9;22) (Figure 1E).

Surprisingly, our data are essentially identical to those in earlier reports on standard cytogenetics, 6-8 FISH with BCR/ABL probe, 7-9 multicolor FISH, 8,9 and CGH 8-10 of the K-562 chronic myelocytic leukemia (CML) cell line, established in 1970 from a female patient with CML in blast crisis. 11 Seventeen of 19 structurally aberrant chromosomes we detected were also present in K-562 cells analyzed by multicolor FISH. 8,9 Although Naumann et al8 and Gribble et al9 did not detect the der(2)pter...hst(9q34;22q11.2)) [cp13]/60-69,idem,der(6)t(3;6)(q2?5;p2?5) [cp10] .

Three key points allowed us to conclude that TI-1 is a derivative of K-562: (1) the presence of many identical, unique and frequently

Figure 1. Cytogenetic and molecular genetic characterization of TI-1 cell line. G-banding (A) and SKY in classification colors (B) demonstrate several numerical and structural aberrations. Arrows indicate clonal structural abnormalities. See text for complete description. The asterisk in A designates der(6)t(3;6)(q2?5;p2?5), an anomaly present in a minority of TI-1 cells. (C) CGH profile shows regions of gain and loss in TI-1. Upper and lower threshold limits are 3.0 standard deviations from the mean signal intensity. Note the high-level amplification of 22q11. (D) Portion of RLGS profile of normal blood (control) on top and TI-1 on bottom. RLGS locus 3C71 (arrow) on 22q11 is amplified in TI-1, as demonstrated by the increased signal intensity compared to the control. (E) Dual color FISH with probe for BCR on 22q11 (green signals on 2 normal chromosomes 22) and ABL on 9q34 (red signals; 1 signal located on del(9)(p13)p24) and 2 signals on both ends of der(9)(p13)p24) shows multiple, overlapping BCR/ABL fusion signals (yellow) localized on 2 der(22)(9;13;22) (arrows) and 1 der(2)(2;9;22) (arrowhead).
very complex structural aberrations; (2) the same pattern of chromo-
some gains and losses, including lack of the Y chromosome, whose
supposed presence was quoted by Taoka et al\textsuperscript{1} as an important feature
distinguishing TI-1 cells from the K-562 cells; and (3) identical CGH
profiles and chromosomal locations of amplified BCR/ABL in TI-1 and
K-562 cells. Because our G-banded karyotype is the same as the
karyotype published by Taoka et al,\textsuperscript{1} the cross-contamination most
likely occurred at the original source.

Importantly, Drexler and colleagues have estimated that 18% of
human tumor cell lines have intraspecies cross-contamination that
occurred at the source of cell line establishment, including other
cases in which K-562 was the contaminating culprit.\textsuperscript{1,12,13} It has
recently been recommended that short tandem repeat profiling be
used as an international reference standard for human cell lines
used in research settings.\textsuperscript{14} We wish to notify the scientific
community that the TI-1 cell line is a cross-contaminant of K-562
and should no longer be used for research on AML.

Laura J. Rush, Kristolina Heinonen, Krzysztof Mrózek, Bryan J. Wolf,
Mohamed Abdel-Rahman, Jadwiga Szymanska, Paivi Peltomäki,
Fehmida Kapadia, Clara D. Bloomfield, Michael A. Caligiuri, and
Christoph Plass

Correspondence: Christoph Plass, The Ohio State University, Division
of Human Cancer Genetics, Medical Research Facility 464A, 420 West 12th
Avenue, Columbus, OH 43210; e-mail: plass-1@medctr.osu.edu

Supported by grants CA82351 (B.J.W.), 5P30CA06058, CA09338 (M.A.C.),
and CA88111 (C.P.) from the National Cancer Institute, Bethesda, MD

References

1. Taoka T, Tasaka T, Tanaka T, Iriko S, Norman AW. Characterization, growth,
and differentiation of a human myeloid leukemia cell line, TI-1 cell. Blood. 1992;
80:46-52.

In: Taylor G, ed. Laboratory Methods for the Detection of Mutations and Poly-


In: Hayashizaki Y, Watanabe S, eds. Restriction Landmark Genomic Scanning

5. Costello JF, Fruhwald MC, Sinagilica DJ, et al. Aberrant Cpg-island methyl-
ation has non-random and tumor-type-specific patterns. Nat Genet. 2000;24:
132-138.

6. Chen TR. Modal karyotype of human leukemia cell line. K562 (ATCC CCL

7. Wu S-Q, Voelkering KV, Sabatini L, Chen X-R, Huang J, Meisner LF. Exten-
sive amplification of bcr/abl fusion genes clustered on three marker chromo-

8. Naumann S, Reutzel D, Speicher M, Decker H-J. Complete karyotype charac-
terization of the K-562 cell line by combined application of G-banding, multiplex-
fluorescence in situ hybridization, fluorescence in situ hybridization, and com-

9. Gröbler SM, Roberts I, Grace C, Andrews KM, Green AR, Nacheva EP. Cytoge-
netics of the chronic myeloid leukemia-derived cell line K562: karyotype clarifi-
cation by multicolor fluorescence in situ hybridization, comparative genomic
hybridization, and locus-specific fluorescence in situ hybridization. Cancer

10. Rodley P, McDonald M, Price B, Fright R, Morris C. Comparative genomic hy-
bridization reveals previously undescribed amplifications and deletions in the
chronic myeloid leukemia-derived K-562 cell line. Genes Chromosomes Can-
cer. 1997;19:36-42.

11. Lizzio CB, Lizzio BB. Human chronic myelogenous leukemia cell-line with

12. Drexler HG, Dirks WG, MacLeod RAF. False human hematopoietic cell lines:

13. MacLeod RAF, Dirks WG, Matsuo Y, Kaufmann M, Milch H, Drexler HG. Wide-
spread intraspecies cross-contamination of human tumor cell lines arising at

provides an international reference standard for human cell lines. Proc Natl

To the editor:

Why antiviral CD8 T lymphocytes fail to prevent progressive immunodeficiency
in HIV-1 infection

We would like to comment on the recent review by Lieberman et
al\textsuperscript{1} by offering an additional interpretation on the mechanisms that
contribute to lack of protection by antiviral CD8 T cells in HIV
infection. In the last few years we have been able to demonstrate that,
since the early phases of HIV infection, the pulmonary microenviron-
ment can be infected by the etiologic agent of AIDS and that the intra-alveolar presence of HIV evokes a discrete
immune response mediated by antiviral CD8\textsuperscript{+} cytotoxic T lympho-
cytes (CTLs).\textsuperscript{2} In asymptomatic patients the appearance of pulmo-
nary CD8 T cells is associated with the clearance of the virus from
the lung microenvironment. But with the progression of HIV
disease, the cytotoxic activity of pulmonary CTLs declines. Our
data published in 1995 emphasize the role of HIV infection in the
progressive functional impairment of CD8 T cells.\textsuperscript{3} Although
lymphocytes expressing the CD4 receptor are the principal cell
target for HIV, lung CD8\textsuperscript{+} T cells of most patients with AIDS show
an unexpected in vivo HIV infectivity.\textsuperscript{1} When proviral load on
pulmonary T-cell subsets is assessed using the DNA–polymerase
chain reaction (PCR) technique, most of the bronchoalveolar
lavage (BAL) proviral DNA can be found in the underrepresented
CD4 T-cell subset, but PCR analysis directly performed on highly
enriched CD8\textsuperscript{+} T cells shows that this population also carries
detectable amounts of HIV DNA. Circumstantial evidence ob-
tained evaluating peripheral blood CD8 also supports the hypo-
thesis that retroviral infection of CD8 cells may contribute to the
functional decline of this subset upon disease progression in
HIV-infected individuals.\textsuperscript{4} Interestingly, the proviral load of pulmo-
nary CD8\textsuperscript{+} T cells usually shows an upward trend with respect to the
corresponding samples isolated from the peripheral blood of the
same patient.\textsuperscript{5} Because we demonstrated that CD8\textsuperscript{+} T cells
accumulating in the lungs of HIV-infected patients are preactivated
Tc1 cells prone to spontaneous and activation-induced apoptosis,\textsuperscript{6}
it is tempting to relate the productive infection to the increased
apoptosis rate of CD8\textsuperscript{+} T cells.

Concerning the mechanisms that account for the infection of CD8\textsuperscript{+} T cells, at least 2 hypotheses can be proposed. The repeated
contacts occurring in the lung microenvironment between activated
HIV-specific CTLs and relevant targets might lead to the infection of
CD8 cells. This hypothesis is supported by in vitro studies showing that HIV may be transmitted through cell-to-cell contact
between persistently infected CD4 cells and CD8 CTLs.\textsuperscript{7} An
additional, though not necessarily alternative, hypothesis is that
lung CD8\textsuperscript{+} CTLs derive from T-cell precursors that transiently
coexpress both CD4 and CD8 determinants in secondary
Comprehensive cytogenetic and molecular genetic characterization of the TI-1 acute myeloid leukemia cell line reveals cross-contamination with K-562 cell line

Laura J. Rush, Kristiina Heinonen, Krzysztof Mrózek, Bryan J. Wolf, Mohamed Abdel-Rahman, Jadwiga Szymanska, Paivi Peltomäki, Fehmida Kapadia, Clara D. Bloomfield, Michael A. Caligiuri and Christoph Plass