be widely used as an *MDS cell line*, and I recently received several manuscripts for review in which generalizations about MDS pathobiology were made on the basis of *in vitro* experiments performed in P39/Tsugane cells. In order to determine whether an aliquot of unique P39/Tsugane cells exists and to confirm the findings of the JCBR, DSMZ, and Sanger Institute, I obtained P39/Tsugane cells from two laboratory groups with publications that made use of these cells. (These laboratories graciously supplied the cells despite knowing that I intended to evaluate them for cross-contamination with HL-60 cells.)

In a laboratory area that had never worked with HL-60 cells, I grew the putative P39/Tsugane cells to confluence in RPMI 1640 media supplemented with 10% fetal calf serum, and compared them to HL-60 cells from the NCI (HL-60 cells courtesy of Scott. H. Kaufmann, MD PhD, Mayo Clinic, Rochester, MA, USA). Methods of comparison included G-banded karyotyping supplemented by fluorescent *in situ* hybridization (FISH, courtesy of Rhett Ketterling, MD, Cytogenetics Laboratory, Mayo Clinic) and a 12-marker DNA Variable Nucleotide Tandem Repeat (VNTR) panel used for forensic work (courtesy of W. Edward Highsmith, PhD, Division of Laboratory Genetics, Mayo Clinic).

Both of the P39/Tsugane aliquots tested exhibited karyotyping/FISH and VNTR results identical to the HL-60 cells. The published karyotype of P39/Tsugane cells is "45,XY,+del(6)(q15),9q+, t(14;16)-(q24;q21),-16,-17".

In contrast, among the aliquots I examined, there were no metaphases with this karyotype, FISH results for chromosome 17p deletion were normal, and the karyotype was instead female as for HL-60 cells (other investigators who are working with P39/Tsugane cells and wish to easily determine whether their aliquot is actually HL-60 cells might begin by assessing for presence of a Y chromosome by FISH, since P39/Tsugane cells were obtained from a man and HL-60 cells from a woman).

While it is possible that an aliquot of a unique P39/Tsugane cell line exists, and the published results might still retain some relevance even though the experiments were actually performed in HL-60 cells, MDS investigators should be aware that the P39/Tsugane cells available from major cell banks and in widespread use in research laboratories are contaminated by HL-60 cells. Drexler recently reviewed all putative MDS cell lines and found only 3 of them (MDS92, M-TAT, and TER-3) that had both been established in the MDS phase (i.e. rather than after AML transformation, as for P39/Tsugane) and might be valid, although more analysis at the genome and proteome levels is required.[4]

Obtaining a suitable cell line that mimics characteristics of MDS and is suitable for mechanistic biological analysis remains a research priority.

P39/Tsugane cells cannot provide any special insight into MDS and should not be used for this purpose.

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Autoimmune hemolytic anemia in patients with chronic lymphocytic leukemia is associated with IgVH status

Recent investigations in chronic lymphocytic leukemia (CLL) have correlated the mutational status of immunoglobulin heavy-chain variable-region (*IgVH*) gene to the development of secondary immune thrombocytopenia, the risk being higher for unmutated cases.[1,2] A similar association between autoimmune hemolytic anemia (AHA) and IgVH gene status has not yet been defined. Therefore, whereas it is well known that the prevalence of autoimmune hemolytic anemia is highest in the more advanced stages of the disease,[3] and may depend on the type of treatment administered,[4,5] the impact of this autoimmune complication on the outcome of patients with chronic lymphocytic leukemia remains controversial.[4-7]

To address this, we searched our database of 473 chronic lymphocytic leukemia patients consecutively referred to our Department from 1st January 2000 to 1st January 2009. We identified 35 chronic lymphocytic leukemia patients (7%) who developed overt autoimmune hemolytic anemia. All patients met the chronic lymphocytic leukemia diagnostic criteria of the National Cancer Institute. Autoimmune hemolytic anemia was defined with standard criteria: a fall in hemoglobin level of at least 2 g/dL, associated with a positive direct antiglobulin test and/or increased reticulocyte count, and a rise in indirect bilirubin with no other causes of anemia identified.

As part of the diagnostic work-up, IgVH DNA sequence analysis was performed on peripheral blood or bone marrow specimens of all the 238 consecutive chronic lymphocytic leukemia patients diagnosed at our Institution since June 2004. Mutational status was obtained retrospectively, using the samples collected at diagnosis, in 12 of the 35 cases of chronic lymphocytic leukemia with autoimmune hemolytic anemia occurring before June 2004. Sequences were aligned to IMGT and V-BASE directories and analyzed using DNAPLOT and IMGT/VQUEST software. Those differing more than 2% from the corresponding germ-line gene were considered mutated.

Median follow-up from diagnosis of chronic lymphocytic leukemia was 66 months (range 6-118). All patients with autoimmune hemolytic anemia except 2 presented with RAI stage 0 or 1, and developed autoimmune hemolytic anemia in a median time of 48 months (range 0-102), all requiring specific therapy for the anemia. In

order to avoid possible confounding effects due to patient selection in determining the association between occurrence of autoimmune hemolytic anemia and IgVH status, we performed a case-control study comparing the 35 patients with chronic lymphocytic leukemia and autoimmune hemolytic anemia (cases) with 35 chronic lymphocytic leukemia patients without autoimmune hemolytic anemia (controls). Cases and controls were matched for gender, age (± 2 years) and RAI stage at diagnosis, year of diagnosis, and length of follow-up to adjust for the time at risk of developing autoimmune hemolytic anemia. A higher prevalence of un-mutated IgVH was found in patients with autoimmune hemolytic anemia (23 of 35, 66%). In comparison only 10 of 35 (28%) controls showed un-mutated IgVH, as expected in an unselected cohort.[8] The odds ratio of developing autoimmune hemolytic anemia in un-mutated chronic lymphocytic leukemia could be estimated as 4.79 (95% CI 1.74–13.18, P=0.001 by Fisher's exact test). No significant difference was observed in VH family distribution between the two groups, with VH3 family being the most represented among patients with autoimmune hemolytic anemia (66%; Table 1).

Considering all chronic lymphocytic leukemia patients in our series, overall survival (OS) was similar in patients with or without autoimmune hemolytic anemia (5-year OS 70% *vs.* 80%, respectively, P=0.46) despite the association of autoimmune hemolytic anemia with un-mutated IgVH and the overall adverse impact of un-mutated IgVH on overall survival in our series. Of note, autoimmune hemolytic anemia developed significantly earlier in the course of chronic lymphocytic leukemia in patients with un-mutated IgVH compared to mutated patients (39 *vs.* 59 months, respectively, P=0.04). Patients developing

Table 1. IgVH mutational status in cases (CLL with AHA) and controls (CLL without AHA)

	Cases			Controls		
	VH gene	VH homology %	VH status	VH gene	VH homology %	VH status
1	V1-69	100	Unmutated	V1-2	93.4	Mutated
2	V1-69	100	Unmutated	V1-2	97.9	Mutated
3	V1-69	100	Unmutated	V1-3	99.7	Unmutated
4	V1-69	100	Unmutated	V1-58	99.7	Unmutated
5	V1-69	100	Unmutated	V1-69	100	Unmutated
6	V2-5	93.8	Mutated	V1-69	100	Unmutated
7	V3-11	100	Unmutated	V1-69	90.4	Mutated
8	V3-15	93.2	Mutated	V1-8	100	Unmutated
9	V3-20	100	Unmutated	V2-5	94.5	Mutated
10	V3-21	96.5	Unmutated	V3-15	93.8	Mutated
11	V3-21	87	Mutated	V3-21	97.1	Mutated
12	V3-23	95.2	Mutated	V3-21	96.6	Mutated
13	V3-23	99.6	Unmutated	V3-21	97.6	Mutated
14	V3-23	100	Unmutated	V3-23	97.3	Mutated
15	V3-30	100	Unmutated	V3-23	91.5	Mutated
16	V3-30	99.6	Unmutated	V3-30	93.8	Mutated
17	V3-30	100	Unmutated	V3-30	93.4	Mutated
18	V3-33	96.5	Mutated	V3-30	93	Mutated
19	V3-33	100	Unmutated	V3-30	99.5	Unmutated
20	V3-48	100	Unmutated	V3-33	94.8	Mutated
21	V3-48	96.3	Mutated	V3-53	95.1	Mutated
22	V3-48	93.2	Mutated	V3-64	99.3	Unmutated
23	V3-48	100	Unmutated	V3-7	93.4	Mutated
24	V3-7	100	Unmutated	V3-7	100	Unmutated
25	V3-7	93.4	Mutated	V3-7	93	Mutated
26	V3-7	96.2	Mutated	V3-72	93	Mutated
27	V3-72	99.4	Unmutated	V3-72	94	Mutated
28	V3-72	92	Mutated	V4-30	94.8	Mutated
29	V3-73	100	Unmutated	V4-34	90	Mutated
30	V4-30	100	Unmutated	V4-34	95.1	Mutated
31	V4-34	96.6	Mutated	V4-4	91	Mutated
32	V4-39	100	Unmutated	V4-59	100	Unmutated
33	V4-4	93.6	Mutated	V4-61	95.3	Mutated
34	V4-59	100	Unmutated	V4-B	100	Unmutated
35	V4-61	100	Unmutated	V4-B	96.6	Mutated

autoimmune hemolytic anemia within 48 months (early AHA) after chronic lymphocytic leukemia diagnosis (16 patients, 81% un-mutated) had a significantly inferior overall survival compared to patients developing autoimmune hemolytic anemia later in the course of the disease (47% *vs*. 94%, *P*=0.0001) or without autoimmune hemolytic anemia (47% *vs*. 81%, *P*=0.0008).

In summary, our data show that autoimmune hemolytic anemia is associated with un-mutated status in chronic lymphocytic leukemia patients and that patients with early onset of autoimmune hemolytic anemia have a shorter survival. Similar observations have been reported in patients developing secondary immune thrombocytopenia in the course of chronic lymphocytic leukemia, where time to immune thrombocytopenia development was also associated with overall survival.[9] The poorer outcome of patients with autoimmune complications arising early in the course of chronic lymphocytic leukemia might depend on the association with un-mutated status but also on the increased morbidity and mortality due to immunosuppressive treatments required for treating autoimmunity. Larger prospective cohorts are needed in order to verify this observation, and to tell us whether the association is directly causal or whether it reflects an increased tumor bulk at the time of autoimmune hemolytic anemia development.

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