Should IGHV status and FISH testing be performed in all CLL patients at diagnosis? A systematic review and meta-analysis

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Since the first description of the natural history of chronic lymphocytic leukemia (CLL) by David Galton in 1966, the considerable heterogeneity in the disease course has been well recognized. The Rai and Binet staging systems described ~40 years ago have proven to be robust prognostic tools. Over the past 2 decades, several novel biological, genetic, and molecular markers have been shown to be useful adjuncts to the Rai and Binet staging systems. In this systematic review, we examined the role of immunoglobulin heavy-chain variable region gene (*IGHV*) mutation status and genetic abnormalities determined by interphase fluorescence in situ hybridization (FISH) in patients with newly diagnosed CLL. The cumulative evidence presented in this systematic review is sufficient to recommend that FISH and *IGHV* be performed as standard clinical tests for all patients with newly diagnosed CLL in those countries with the resources to do so. In addition to clinical stage, these parameters could represent the minimal standard initial prognostic evaluation for patients with CLL. This approach will allow the application of powerful, recently developed prognostic indices (all of which are dependent on *IGHV* and FISH results) to all patients with newly diagnosed CLL. (*Blood*. 2016;127(14):1752-1760)

Case presentation

Case 1. A 72-year-old man was incidentally discovered to have a white blood cell (WBC) count of 22 × 10^9/L during a routine annual visit to his primary care physician. The absolute lymphocyte count was 20 × 10^9/L. Peripheral blood flow cytometry revealed a clonal population of lymphocytes that coexpressed CD19, CD5, CD23, and CD20 (dim), consistent with a diagnosis of chronic lymphocytic leukemia (CLL). Serum β2-microglobulin level was normal. He was referred to a hematologist for further treatment. The patient was asymptomatic; physical examination demonstrated no organomegaly or lymphadenopathy. He was diagnosed with Rai 0 CLL.

Case 2. A 62-year-old woman was incidentally noted to have a WBC count of 15 × 10^9/L, with an absolute lymphocyte count of 14 × 10^9/L. Peripheral blood flow cytometry confirmed a diagnosis of CLL. The patient was asymptomatic; physical examination demonstrated no organomegaly or lymphadenopathy. Serum β2-microglobulin was 4.1 μg/mL (normal, 1.2-2.7 μg/mL). The patient was diagnosed with Rai 0 CLL.

What is the role of obtaining immunoglobulin heavy-chain variable region gene (IGHV) mutation status and an interphase fluorescence in situ hybridization (FISH) study at the time of CLL diagnosis in routine practice?

Background

Approximately 16 000 new cases of CLL are diagnosed each year in the United States.[1] There is considerable heterogeneity in the disease course of CLL—some patients have an indolent course and live for decades without therapy, whereas others experience relatively rapid progression and succumb to the disease within a few years despite maximal therapy.[2] Effective approaches to stratifying a patient's prognosis can enable the treating physician to provide more accurate patient counseling, tailor the frequency of follow-up, and, in some cases, inform therapy selection.

The Rai and Binet staging systems were developed ~40 years ago using readily available clinical and laboratory parameters to stratify patient risk.[3,4] Despite the enduring utility of clinical staging, there remains significant clinical heterogeneity among patients within each Rai and Binet stage category. Furthermore, approximately three quarters of newly diagnosed CLL patients are diagnosed at the Rai 0/Binet A stage, where the staging systems are unable to determine the likelihood or pace of disease progression.

Although a plethora of prognostic parameters have been proposed to address this limitation over the last 35 years, *IGHV* mutation status and cytogenetic abnormalities identified by FISH have been the most widely studied. In 1999, 2 independent groups reported that patients with higher levels of somatic mutation in the *IGHV* genes of their CLL clone experienced longer progression-free survival (PFS) and overall survival (OS).[5,6] Roughly 1 year after these 2 reports, Dohner and colleagues proposed a new prognostic model that categorized patients into 5 risk categories based on FISH. Using a hierarchical classification scheme, they demonstrated the shortest survival for patients with del17p13 (32 months), followed by patients with del11q23 (79 months), trisomy 12 (111 months), normal karyotype (114 months), and del13q14 as the sole abnormality (133 months).[7] After these seminal observations, several studies have demonstrated the consistent and robust ability of *IGHV* mutation status and interphase FISH to stratify patient outcome. Here, we performed a systematic review evaluating the prognostic utility of these 2 parameters in patients with newly diagnosed CLL.

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Methods

Eligibility criteria and literature search

We performed a literature search to identify studies on the prognostic value of *IGHV* mutation status and FISH in CLL with the aid of an experienced medical librarian. We applied no language restrictions. We searched 5 databases (Ovid MEDLINE, Ovid EMBASE, Ovid CENTRAL, Web of Science, and Scopus) to identify all citations from January 1999 to April 2015 describing the role of *IGHV* mutation testing and FISH in predicting prognosis for CLL. Ovid MEDLINE was used to design the strategy, using a combination of MeSH-controlled vocabulary and text words for each concept. The following terms were used to perform the search: immunoglobulin variable region, immunoglobulin heavy chain, genes, immunoglobulin, or *IGHV* (as text words); FISH, in situ hybridization, fluorescent; leukemia, lymphocytic, chronic, B-cell, or CLL. The results were imported into EndNote, and duplicate results were removed.

Full-length publications reporting on the prognostic value (eg, PFS and/or OS) of *IGHV* and/or FISH in patients with newly diagnosed CLL, and which included at least 200 patients, were included in the systematic review. Studies that included <200 patients, focused on treated patients, or did not report on PFS or OS were excluded. Preliminary results reported only in abstract form were excluded. Manuscripts that described the prognostic impact of *IGHV* mutation and FISH in the context of patients starting treatment on a clinical trial were not included, because ~30% to 50% of patients with newly diagnosed CLL never require therapy and the focus of such studies is evaluating the impact of therapy on OS as opposed to the use of prognostic parameters for risk stratification.

Study evaluation

Two reviewers working independently considered the potential eligibility of each of the abstracts generated by the search strategy. Each abstract was evaluated independently for final study inclusion. For discrepancies arising in the data abstracting process, a third reviewer returned to the source document to determine the accurate information.

Data extraction

Data were extracted using a standardized form to enter study participant characteristics, proportion of patients who had *IGHV* mutation status and FISH testing performed, and PFS and OS for all patients. Data extraction was performed in duplicate by 2 reviewers.

Meta-analysis

We performed generic inverse variance meta-analyses using random-effects models to calculate pooled hazard ratios (HR) with 95% confidence intervals (CI) for PFS and OS from multivariable study results for both IGHV mutation status and FISH results. Heterogeneity was assessed using the I^2 statistic. Statistical analyses were performed using Review Manager 5.3 (Cochrane Collaboration) software.

Results

The search criteria described in "Methods" identified 1450 citations. After independent evaluation of all 1450 studies by 2 reviewers, 31 studies met the criteria for inclusion in this study (Figure 1). Of these, 7 reported outcomes for *IGHV* mutation status only, 2 for FISH only, and 22 for both *IGHV* mutation status and FISH. Table 1 shows the baseline characteristics of patients, and the median PFS and median OS for both *IGHV* mutation status and interphase FISH for all studies.[7-35] Table 2 shows the HR for PFS and OS reported for multivariate models that included both *IGHV* mutation status and FISH.[7-35]

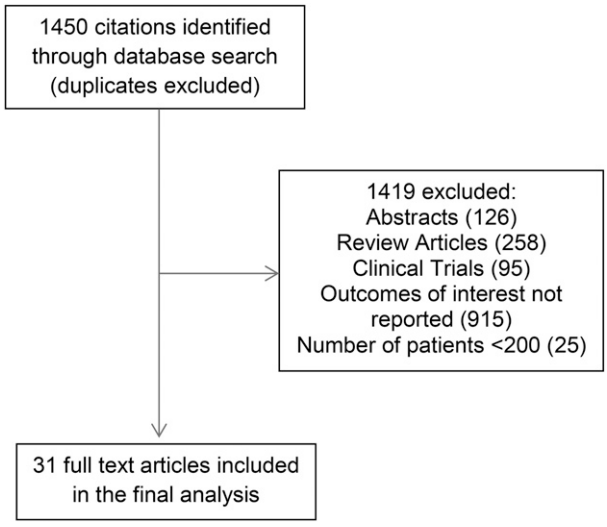


Figure 1. Literature search data.

IGHV mutation status

The median PFS for patients with unmutated *IGHV* genes (range, 1-5 years) was significantly shorter than for those with mutated *IGHV* genes (range, 9.2-18.9 years) across all studies. Similarly, the median OS for patients with unmutated *IGHV* (range, 3.2-10 years) was also significantly shorter than for those with mutated *IGHV* (range, 17.9-25.8 years) across all studies.

Interphase FISH

The median PFS of patients with high-risk FISH (including del17p13 and del11q23; range, 0.1-5.2 years) was significantly shorter than those with low/intermediate-risk FISH (including del13q, normal, and trisomy 12; range, 1.5-22 years). The median OS of patients with high-risk FISH (range, 3.3-9.7 years) was also significantly shorter than those with low/intermediate-risk FISH (range, 7.5-20.5 years).

Meta-analyses

In multivariable analyses, the HR for unmutated *IGHV* ranged from 2.0 to 10.7 for PFS and 1.6 to 6.9 for OS compared with mutated *IGHV*. *IGHV* remained an independent predictor of PFS in 15 of 18 studies reporting the results of multivariable analysis, including 12 of 15 studies adjusting for the prognostic impact of FISH. The pooled HR for PFS was 3.2 (95% CI, 2.8-3.7; $P < .0001$; $I^2 = 20\%$; Figure 2A). With respect to OS, *IGHV* remained an independent predictor of OS in 11 of 15 studies reporting the results of multivariable analysis, including 10 of 14 studies adjusting for the prognostic impact of FISH. The pooled HR for OS was 2.4 (95% CI, 2.0-3.0; $P < .0001$; $I^2 = 50\%$; Figure 2B). Heterogeneity was not explained by differing inclusion criteria (such as disease stage) across studies, and summary HR estimates were similar across study classes.

In multivariable analyses, the hazard ratio for high-risk FISH (defined as the presence of either del11q23 or del17p13) ranged from 1.3 to 4.7 for PFS and from 0.9 to 8.2 for OS. High-risk FISH remained an independent predictor of PFS in 8 of 17 studies reporting the results of multivariable analysis, including in 6 of 15 studies adjusting for the prognostic impact of *IGHV*. The pooled HR for PFS for studies reporting del11q23 FISH was 1.8 (95% CI, 1.5-2.2; $P < .0001$; $I^2 = 33\%$; Figure 3A). The pooled HR for PFS for studies reporting

Table 1. Median PFS and OS for *IGHV* and FISH of all studies included in this analysis

								Progression-free survival (y)							Overall survival (y)						
			Median		Rai/Binet stage (%)			*IGHV* median*		FISH median*					*IGHV* median*		FISH median*				
No.	Reference	n	age (y)	Males (%)	0 or A	I/II or B	III/IV or C	Mutated	Unmutated	13q⁻	NL	+12	11q⁻	17p⁻	Mutated	Uunmutated	13q⁻	NL	+12	11q⁻	17p-
***IGHV* only**																					
1	6	84	63	58	74	11	15	—	—						24.5	9.7					
2	5	47	63	67	37	58	5	—	—						—	9					
3	8	307	52	65	—	—	—	—	—						—	—					
4	19	303	63	54	34	62	2	10 y: 41%	10 y: 9%						10 y: 87%	10 y: 43%					
5	10	262	65	61	100	0	0	3 y: 93%	3 y: 66%						—	—					
6	11	337	64	60	71		29	NR	2						NR	7					
7	12	224	63	68	77		23	18.9	2.5						17.9	9.8					
FISH only																					
8	7	325	62	61	19	60	20			7.7	4.1	2.7	1.1	0.7			11	9.3	9.5	6.6	2.7
9	13	231	68	63	71	27	2			—	—	—	—	—			—	—	—	—	—
Both *IGHV* and FISH (OS only)																					
10	14	325	62	61	20	60	20								—	7.5	—	—	—	2.5	5.8
11	15	205	62	58	82	10	7								25.8	10	17.5	24.4	10.2	9.7	3.9
12	16	1948	60	68	21	56	23								5 y: 89%	5 y: 71%	5 y: 85%	5 y: 87%	5 y: 83%	5 y: 75%	5 y: 34%
Both *IGHV* and FISH (PFS only)																					
13	17	351	61	61	80		20	11.8	4	12	5	5	4	3							
14	18	930	59	61	87		13	—	—	5	4	4	3	1							
15	19	384	61	58	100	0	0	—	4	—	—	—	—	4							
16	20	3490	65	63	77		23	—	3.5	12	—	4	3	—							
Both *IGHV* and FISH (PFS and OS)																					
17	21	706	66	57	76	24		10.5	2		9		1		NR	8		20.5		6.8	
18	22	482	67	65	—	—	—	9.2	1.5	12	6.6	NR	1	1.8	5 y: 88%	5 y: 81%	NR	NR	NR	NR	2.5
19	23	2487	64	68	52	39	6	11	2.8	NR	8.7	5.4	2.4	5.2	NR	9.7	NR	NR	10.9	8.4	7.6
20	24	252	64	63	79	16	5	NR	1.1	NR	NR	1.5	0.5	0.1	NR	7	NR	NR	7	7.6	4
21	25	255	61	64	95		5	NR	5	—	—	—	4	2.6	—	3.2	—	—	—	3.3	5
22	26	1154	66	—	100	0	0	NR	5		22		3.6		23	13		23		13	
23	27	620	65	60	48	44	8	—	—	—	—	—	—	—	—	—	—	—	—	—	—
24	28	292	66% >60 y	55	72		28	3 y: 58%	3 y: 23%	3 y: 49%	3 y: 37%	3 y: 28%	3 y: 21%	3 y: 24%	10 y: 75%	10 y: 41%	10 y: 93%	10 y: NR	10 y: 72%	10 y: 67%	10 y: 0%
25	29	265	64	64	78		22	NR	1	NR	NR	2	1	<1	NR	7	NR	NR	10	9	4
26	30	311	66	61	78	15	6	NR	2		5.2		1.5		—	—	—	—	—	—	—
27	31	1274	66	55	80		20	—	—	NR	8	8	—	—	—	—	5 y: 84%	5 y: 78%	5 y: 63%	5 y: 54%	5 y: 48%
28	32	949	65	58	57	33	10	—	—	—	—	—	—	—	—	—	—	—	—	—	—
29	33	468	53% >60 y	59	100	0	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—
30	34	288	—	—	95		5	—	—	—	—	—	—	—	—	—	—	—	—	—	—
31	35	1160	65	65	—	—	—	—	—	9.1	8.4	4.2	2.3	3.7	—	—	7.5	7.5	7.5	5.7	4.2

FISH, fluorescence in situ hybridization; *IGHV*: immunoglobulin heavy-chain variable region gene mutation status; NR, not reached; OS, overall survival; PFS: progression-free survival.
*In studies where the median was not reported, outcomes at specific time points (e.g., 3 y PFS or 10 y OS, when available) were included

Table 2. Multivariable analysis results of studies included in this analysis

No.	Reference	n	Variables included in multivariable models	HR for PFS		HR for OS		Other variables significant on multivariable analysis	
				Unmutated *IGHV*	High-risk FISH	Unmutated *IGHV*	High-risk FISH	PFS	OS
***IGHV* only**									
1	6	84	—	—		—		—	—
2	5	47	—	—		—		—	—
3	8	307	ZAP-70, *IGHV*	2.5		—		Zap-70, *IGHV*	—
4	9	303	Age, Rai stage, CD49d, ZAP-70, *IGHV*	—		6.5		Rai stage, β2M, CD49d, *IGHV*	Age, CD49d, *IGHV*
5	10	262	*IGHV*, ZAP-70, CD38	2.5		—		*IGHV*, ZAP-70, CD38	—
6	11	337	—	—		—		—	—
7	12	224	—	—		—		—	—
FISH only									
8	7	325	LDH, WBC, Binet stage, FISH		—		2.9 (del11q) 8.1 (del17p)	—	LDH, WBC, Binet stage, FISH
9	13	231	β2M, morphology, FISH		4.7 (del11q)		—	β2M, morphology, FISH	—
Both *IGHV* and FISH (OS only)									
10	14	325	CD38, Age, *IGHV*, FISH, LDH, WBC count			2.9	1.8 (del11q) 8.2 (del17p)		*IGHV*, FISH, age, LDH, WBC
11	15	205	Stage, *IGHV*, FISH, Sex, CD38, morphology			6.9	2.0 (del11q) 3.3 (del17p)		Stage, *IGHV*, FISH
12	16	1948	Age, sex, stage, ECOG performance status, CBC, lymphocyte count, LDH, TK, β2M, ZAP-70, CD38, *IGHV*, FISH			1.9	1.4 (del11q) 6.0 (del17p)		Age, sex, ECOG performance status, β2M, TK, *IGHV*, FISH
Both *IGHV* and FISH (PFS only)									
13	17	351	CCL3, stage, CD38, *IGHV*, FISH	NS	2.4 (del11q) 2.4 (del17p)			CCL3, stage, CD38, FISH	
14	18	930	LDH, LN number and size, *IGHV*, FISH	10.7	1.9 (del11q) 2.1 (del17p)			LDH, LN number and size, *IGHV*, FISH	
15	19	384	CLL vs MBL, *IGHV*, *NOTCH1*, FISH	3.9	1.5 (del11q)			CLL, *IGHV*	
16	20	3490	*NOTCH1*, *SF3B1*, *TP53*, *IGHV*, FISH	3.7	1.4 (del11q)			*IGHV*, *SF3B1*, *TP53*	
Both *IGHV* and FISH (PFS and OS)									
17	21	706	Age, CD38, ZAP70, FISH, *IGHV*, β2M and GFR-adjusted β2M	NS	3.3 (del11q or del17p)	NS	3.7 (del17p or del11q)	FISH, GFR-adjusted β2M	Age, FISH, GFR-adjusted β2M
18	22	482	Age, WBC, *IGHV*, karyotype, FISH, t(14;18)	4.8	NS	NS	5.2 (del17p)	WBC, *IGHV*	Age, WBC, t(14;18)
19	23	2487	Rai stage, *IGHV*, ALC, CD38, ZAP-70, FISH	2.8	1.3 (del11q or del17p)	2.8	2.9 (del11q or del17p)	Stage, ALC, CD38, *IGHV*	CD38, FISH
20	24	252	LPL expression, age, gender, Binet stage, *IGHV*, FISH, CD38	2.4	1.4 (del11q) 2.0 (del17p)	1.9	0.9 (del11q) 4.6 (del17p)	CD38, *IGHV*	Age, stage, *IGHV*, FISH, LPL expression
21	25	255	SNP, *IGHV*, FISH	—	—	1.8	1.5 (del11q or del17p)	—	SNP
22	26	1154	CD38, age, LDT, *IGHV*, FISH, ZAP-70	3.3	NS	2.7	NS	Age, LDT, CD38, *IGHV*	

β2M, serum β2-microglobulin; CCL3, chemokine ligand 3; CGH, comparative genomic hybridization; ECOG, Eastern Cooperative Oncology Group; GFR, glomerular filtration rate; FISH, fluorescence in situ hybridization; HR, hazard ratio; *IGHV*, immunoglobulin heavy-chain variable region gene mutation status; LAIR1, leukocyte-associated immunoglobulin-like receptor 1; LDH, lactate dehydrogenase; LPL, lipoprotein lipase; MBL, monoclonal B-cell lymphocytosis; OS, overall survival; PFS, progression-free survival; SNP, single-nucleotide polymorphism; TK, serum thymidine kinase; WBC, white cell count.

*del17p identified by array CGH also.

†*TP53* mutation included with del17p.

Table 2. (continued)

No.	Reference	n	Variables included in multivariable models	HR for PFS		HR for OS		Other variables significant on multivariable analysis	
				Unmutated *IGHV*	High-risk FISH	Unmutated *IGHV*	High-risk FISH	PFS	OS
23	27	620	Age, sex, ALC, β2M, stage, CD38, lymph node regions, ZAP-70, year of diagnosis, therapy	3.1	2.0 (del11q)	2.0	2.1 (del17p)	β2M, *IGHV*, FISH	Age, sex, β2M, stage, *IGHV*, FISH
24	28	292	LDH, sex, β2M, IL-10 SNP, *IGHV*	2.0	—	2.1	—	Sex, LDH, β2M, IL-10 SNP, *IGHV*	Stage, IL-10 SNP, *IGHV*
25	29	265	Age, Binet Stage, Sex, CD38, FISH, telomere length, *TP53*/*NOTCH1*/*SF3B1* mutation	—	2.1 (del11q)	—	1.2 (del11q)	CD38, genetic profile, telomere length, FISH	Age, stage, CD38, genetic profile, telomere length
26	30	311	LAIR1 expression, *IGHV*, Binet stage, CD49d	2.7	2.0 (del11q or del17p)	—	—	LAIR1, stage, *IGHV*	—
27	31	1274	age, stage, integrated cytogenetic and mutational model, *IGHV*	—	—	1.6	—	—	Age, stage, *IGHV*, integrated cytogenetic and mutational model
28	32	949	Stage, β2M, FISH, ZAP-70, *IGHV*, *NOTCH1*, *SF3B1*, CIRS	NS	NS	—	NS	Stage, β2M, ZAP-70	Stage, β2M, ZAP-70, CIRS
29	33	468	Rai stage, ALC, β2M, CD38, gene profile, *IGHV*, FISH	2.6	1.7 (del11q or del17p)	—	—	Rai stage, ALC, β2M, progression-risk score	—
30	34	288	Array CGH, *IGHV*	5.6	-	6.6	3.5 (del17p)*	Array CGH, *IGHV*	
31	35	1160	*SF3B1*, *NOTCH1*, *IGHV*, *TP53*, FISH, sex, age, CD38, ZAP-70	3.0	1.5 (del11q)	2.2	2.2 (del17p)†	*SF3B1*, *IGHV*, FISH	*SF3B1*, *TP53*, age, CD38

β2M, serum β2-microglobulin; CCL3, chemokine ligand 3; CGH, comparative genomic hybridization; ECOG, Eastern Cooperative Oncology Group; GFR, glomerular filtration rate; FISH, fluorescence in situ hybridization; HR, hazard ratio; *IGHV*, immunoglobulin heavy-chain variable region gene mutation status; LAIR1, leukocyte-associated immunoglobulin-like receptor 1; LDH, lactate dehydrogenase; LPL, lipoprotein lipase; MBL, monoclonal B-cell lymphocytosis; OS, overall survival; PFS, progression-free survival; SNP, single-nucleotide polymorphism; TK, serum thymidine kinase; WBC, white cell count.

*del17p identified by array CGH also.

†*TP53* mutation included with del17p.

A

Study or subgroup	HR	SE	Weight (%)	Hazard ratio IV, random, 95% CI	Year
Rassenti 2004	0.92	0.23	7.7	2.51 [1.60, 3.94]	2004
Morabito 2009	0.92	0.36	3.6	2.51 [1.24, 5.08]	2009
Haferlach 2010	1.57	0.25	6.8	4.81 [2.94, 7.85]	2010
Shanafelt 2010	1.03	0.32	4.5	2.80 [1.50, 5.24]	2010
Kaderi 2011	0.89	0.30	5.0	2.44 [1.35, 4.38]	2011
Wierda 2011	2.37	0.72	1.0	10.70 [2.61, 43.87]	2011
Bulian 2012	1.13	0.29	5.3	3.10 [1.75, 5.47]	2012
Pepper 2012	1.19	0.17	11.9	3.29 [2.36, 4.59]	2012
Lech-Maranda 2013	0.69	0.28	5.6	1.99 [1.15, 3.45]	2013
Perbellini 2013	0.98	0.40	3.0	2.66 [1.22, 5.84]	2013
Jeromin 2014	1.11	0.16	12.9	3.03 [2.22, 4.15]	2014
Houldsworth 2014	1.73	0.28	5.6	5.64 [3.26, 9.76]	2014
Gentile 2014	0.96	0.26	6.3	2.61 [1.57, 4.35]	2014
Lionetti 2014	1.37	0.28	5.6	3.94 [2.27, 6.81]	2014
Baliakas 2015	1.31	0.14	15.1	3.71 [2.82, 4.88]	2015
Total (95% CI)			**100.0**	**3.22 [2.80, 3.72]**	

Heterogeneity: Tau^2=0.02; Chi^2=17.55, df=14 (*P*=.23); I^2=20%
Test for overall effect: Z=16.15 (*P*<.00001)

B

Study or subgroup	HR	SE	Weight (%)	Hazard ratio IV, random, 95% CI	Year
Krober 2002	1.07	0.27	8.4	2.92 [1.72, 4.95]	2002
Oscier 2002	1.93	0.50	3.7	6.89 [2.59, 18.36]	2002
Gattei 2008	1.88	0.53	3.3	6.55 [2.32, 18.52]	2008
Shanafelt 2010	1.03	0.57	3.0	2.80 [0.92, 8.56]	2010
Kaderi 2011	0.66	0.31	7.2	1.93 [1.05, 3.55]	2011
Ouillette 2011	0.59	0.51	3.5	1.80 [0.66, 4.90]	2011
Bulian 2012	0.71	0.20	11.0	2.03 [1.37, 3.01]	2012
Pepper 2012	0.99	0.21	10.6	2.69 [1.78, 4.06]	2012
Rossi 2013	0.49	0.17	12.4	1.63 [1.17. 2.28]	2013
Lech-Maranda 2013	0.74	0.30	7.5	2.10 [1.16, 3.77]	2013
Pflug 2014	0.64	0.13	14.2	1.90 [1.47, 2.45]	2014
Jeromin 2014	0.77	0.22	10.2	2.16 [1.40, 3.32	2014
Houldsworth 2014	1.88	0.40	5.1	6.55 [2.99, 14.35]	2014
Total (95% CI)			**100.0**	**2.43 [1.97, 2.99]**	

Heterogeneity: Tau^2=0.06; Chi^2=23.98, df=12 (*P*=.02); I^2=50%
Test for overall effect: Z=8.27 (*P*<.00001)

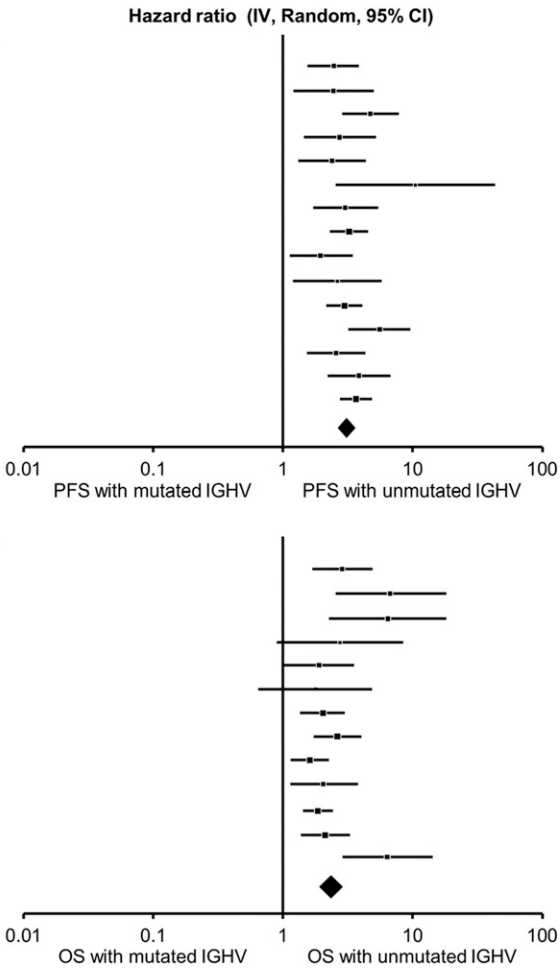


Figure 2. Meta-analysis of studies according to IGHV mutation status. Forest plot of studies reporting (A) progression-free survival (PFS) according to IGHV mutation status, and (B) overall survival (OS) according to IGHV mutation status. df, degree of freedom; IV, inverse variance; Random, random-effects model; SE, standard error; Z, *Z* value.

del17p13 FISH was 2.1 (95% CI, 1.6-2.7; $P < .0001$; $I^2 = 32\%$; Figure 4A). With respect to OS, FISH remained an independent predictor of OS in 10 of 14 studies reporting the results of multivariable analysis, including in 10 of 13 studies adjusting for the prognostic impact of *IGHV*. The pooled HR for OS for studies reporting del11q23 FISH was 1.7 (95% CI, 1.2-3.3; $P = .001$; $I^2 = 54\%$; Figure 3B). The pooled HR for OS for studies reporting del17p13 FISH was 3.0 (95% CI, 2.1-4.2; $P < .0001$; $I^2 = 56\%$; Figure 4B). Heterogeneity was not explained by differing inclusion criteria across studies or specific high-risk FISH definitions, and summary HR estimates were similar across study classes.

Discussion

The clinical course of individuals with early-stage CLL is highly variable and difficult to predict. Although the Rai/Binet staging classifications have been the international gold standards for CLL prognostication for the last 40 years, both staging systems lack the ability to predict outcomes for individual patients.[3,4] Several additional novel prognostic parameters, such as sequencing for recurrent genetic abnormalities, have been identified over the last decade.[36] When determining the role for these new markers, it is critical to assess their incremental value relative to existing prognostic tools. Thus, consensus on the standard prognostic evaluation is necessary to define the platform that these new tests aim to improve upon.

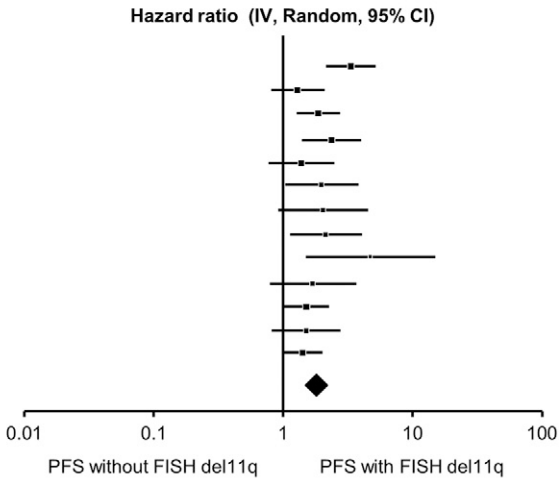
IGHV and FISH were first reported as prognostic parameters ~15 to 17 years ago. Nonetheless, at the time of the last consensus guidelines reported in 2008, they were not recommended as standard tests in the routine care of patients with CLL.[37] It is notable that ~90% of patients included in this analysis were reported after the 2008 International Workshop on Chronic Lymphocytic Leukemia (IWCLL) guidelines were released. Several recent efforts have attempted to develop comprehensive approaches incorporating clinical, serum, genetic, and molecular markers with independent prognostic value into a single risk score for patients with CLL (eg, CLL International Prognostic Index, German CLL Index, MD Anderson Prognosis Score).[16,18,38] It should be noted that *IGHV* and FISH were selected for inclusion in all of these models based on the incremental and independent prognostic information they provide.

There are several caveats to the routine use of these tests in standard clinical practice. Although the results of this analysis suggest that both *IGHV* mutation testing and FISH results are powerful prognostic tests for all patients with CLL, they should not be used to initiate

A

Study or subgroup	HR	SE	Weight (%)	Hazard ratio IV, random, 95% CI	Year
Delgado 2009	1.20	0.22	10.1	3.32 [2.16, 5.11]	2009
Shanafelt 2010	0.26	0.24	9.1	1.30 [0.81, 2.08]	2010
Wierda 2011	0.62	0.19	11.9	1.86 [1.28, 2.70]	2011
Sivina 2011	0.86	0.26	8.2	2.36 [1.42, 3.93]	2011
Kaderi 2011	0.32	0.29	7.0	1.38 [0.78, 2.43]	2011
Bulian 2012	0.68	0.33	5.8	1.97 [1.03, 3.77]	2012
Perbellini 2013	0.71	0.40	4.3	2.03 [0.93, 4.45]	2013
Mansouri 2013	0.76	0.32	6.1	2.14 [1.14, 4.00]	2013
Oliveira 2014	1.55	0.58	2.2	4.71 [1.51, 14.68]	2014
Gentile 2014	0.53	0.39	4.5	1.70 [0.79, 3.65]	2014
Jeromin 2014	0.41	0.20	11.3	1.51 [1.02, 2.23]	2014
Lionetti 2014	0.41	0.31	6.4	1.51 [0.82, 2.77]	2014
Baliakas 2015	0.35	0.17	13.3	1.42 [1.02, 1.98]	2015
Total (95% CI)			**100.0**	**1.83 [1.53, 2.19]**	

Heterogeneity: Tau^2=0.03; Chi^2=17.90, df=12(*P*=.12); I^2=33%
Test for overall effect: Z=6.65 (*P*<.00001)



B

Study or subgroup	HR	SE	Weight (%)	Hazard ratio IV, random, 95% CI	Year
Oscier 2002	0.69	0.48	8.3	1.99 [0.78, 5.11]	2002
Krober 2002	0.58	0.26	15.8	1.79 [1.07, 2.97]	2002
Delgado 2009	1.31	0.30	14.1	3.71 [2.06, 6.67]	2009
Shanafelt 2010	1.06	0.40	10.4	2.89 [1.32, 6.32]	2010
Ouillette 2011	0.41	0.71	4.6	1.51 [0.37, 6.06]	2011
Kaderi 2011	-0.09	0.30	14.1	0.91 [0.51, 1.65]	2011
Mansouri 2013	0.19	0.34	12.5	1.21 [0.62, 2.35]	2013
Pflug 2014	0.34	0.17	20.2	1.40 [1.01, 1.96]	2014
Total (95% CI)			**100.0**	**1.71 [1.24, 2.39]**	

Heterogeneity: Tau^2=0.11; Chi^2=15.10, df=7 (*P*=.03); I^2=54%
Test for overall effect: Z=3.23 (*P*<.001)

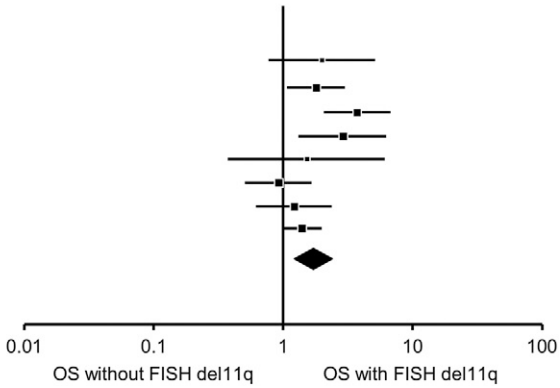


Figure 3. Meta-analysis of studies according to del11q23 status by FISH. Forest plot of studies reporting (A) progression-free survival (PFS) according to del11q23 status by FISH, and (B) overall survival (OS) according to del11q23 status by FISH. df, degree of freedom; IV, inverse variance; Random, random-effects model; SE, standard error; Z, *Z* value. Dohner and collegues[7] and Krober and collegues[17] report on the influence of FISH on OS among the same group of patients (n = 325). For the purposes of this meta-analysis, we included data reported by Krober and colleagues only.

CLL-specific therapy. Only patients with CLL who meet indication for therapy based on the 2008 IWCLL guidelines[37] should receive treatment, regardless of the information obtained by prognostic testing. The only exception to this would be in the context of a clinical trial where an early intervention strategy is being used for patients at "high-risk" for CLL who do not meet the traditional indications for therapy, as was done in the recently reported CLL12 trial.[39] In addition, the median age of patients included in this analysis was 64 years, which is younger than an average patient with CLL seen in practice (~72 years). It is unclear whether *IGHV* mutation and FISH are equally powerful prognostic markers in these older individuals with CLL. Finally, it should be noted that the treatment landscape for CLL has dramatically improved with the approval of novel signal inhibitors, such as ibrutinib[40] and idelalisib.[41] Although these treatments may improve the OS of patients with CLL, they will not influence the utility of *IGHV* and FISH testing for predicting time to first therapy in patients with newly diagnosed CLL.

Collectively, the results of this systematic review illustrate the robust and consistent prognostic value of both *IGHV* and FISH independent of clinical stage in patients with newly diagnosed and/or previously untreated CLL. The bulk of the evidence also indicates that *IGHV* and FISH provide complementary information with respect to both PFS and OS. A greater understanding of the risk of disease progression at the time of CLL diagnosis can help (1) counsel patients appropriately; (2) define the appropriate follow-up interval (shorter interval for high-risk patients); and (3) potentially treat high-risk patients on early intervention protocols.

Recommendations

Based on the experience summarized in this review, we believe the evidence is sufficient to recommend that FISH and *IGHV* be recommended as standard clinical tests for all patients with newly diagnosed CLL in those countries with the resources to do so. This change will help define the minimal standard initial prognostic evaluation for patients with CLL and help facilitate use of the powerful, recently developed, integrated prognostic indices,[16,18,38] all of which are dependent on these 2 variables.

Resolution of cases

Case 1

The patient underwent additional testing for *IGHV* gene mutation and interphase FISH. The CLL B cells showed mutated *IGHV*, and FISH demonstrated loss in the long arm of chromosome 13 (del13q). When

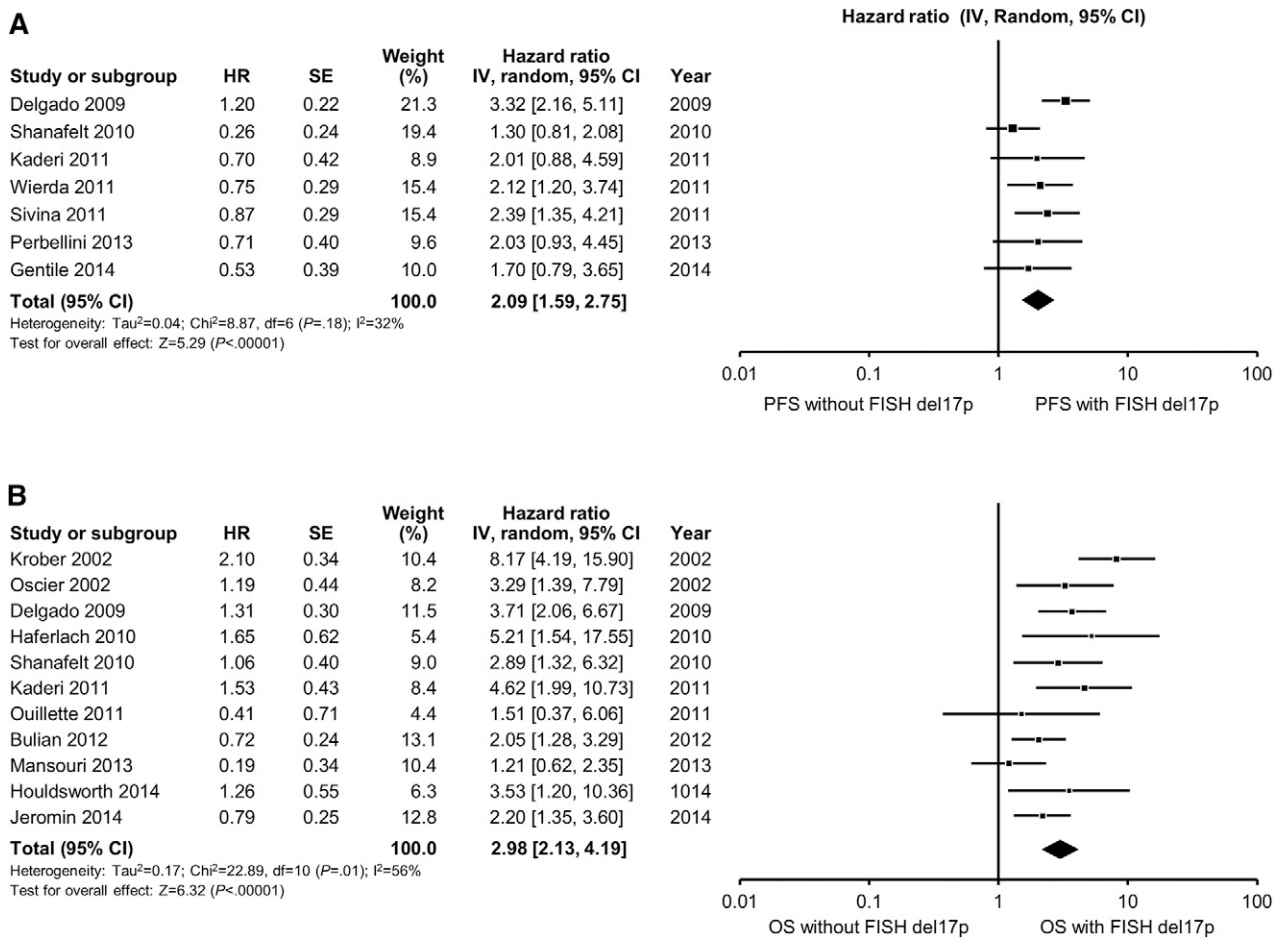


Figure 4. Meta-analysis of studies according to del17p13 status by FISH. Forest plot of studies reporting (A) progression-free survival (PFS) according to del17p13 status by FISH, and (B) overall survival (OS) according to del17p13 status by FISH. df, degree of freedom; IV, inverse variance; Random, random-effects model; SE, standard error; Z, *Z* value. Dohner and collegues[7] and Krober and collegues[17] report on the influence of FISH on OS among the same group of patients (n = 325). For the purposes of this meta-analysis, we included data reported by Krober and colleagues only.

we considered the patient's age, clinical stage, and β2-microglobulin level, he was deemed to have minimal risk disease as categorized by the recently devised CLL International Prognostic Index,[38] with ~95% 5-year life expectancy and only ~20% likelihood of requiring treatment within the following 5 years. The patient was advised to have an annual follow-up visit with his hematologist to assess for disease progression. Supportive care measures including age-appropriate cancer screening (including annual whole-body skin examination) and appropriate vaccinations for immunocompromised patients were also recommended.[42]

Case 2

The patient underwent additional testing for *IGHV* gene mutation and interphase FISH. The CLL B-cells showed an unmutated *IGHV* and FISH demonstrated a loss in the short arm of chromosome 17 (del17p13). When considered with the patient's age, clinical stage, and β2-microglobulin level, she was considered to have high risk of progressive disease as categorized by the recently devised CLL International Prognostic Index, with ~25% life expectancy and a very high likelihood of requiring treatment within the following 12 months. Although the patient was classified as having high-risk disease, she did not meet the 2008 IWCLL guidelines for starting therapy, and was therefore advised to follow-up with a hematologist every 3 months for the first year to assess for disease progression. In addition to the supportive care measures outlined for the patient in Case 1, the patient underwent human leukocyte antigen typing.

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Authorship

Contribution: S.A.P., P.S., M.T., C.P.W., and T.D.S. designed research and analyzed data; S.A.P., P.S., and M.T. performed research; and S.A.P. and T.D.S. wrote the paper.

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International and Pharmacyclics. He was not personally compensated for the research support. The remaining authors declare no competing financial interests.

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