Mutated or non-mutated? Which database to choose when determining the IgVH hypermutation status in chronic lymphocytic leukemia?

It has been accepted that the hypermutation status of immunoglobulin heavy chain genes (*IgVH*) is one of the most important independent prognostic factors in chronic lymphocytic leukemia (CLL). According to the degree of *IgVH* hypermutaion, CLL patients can be stratified into prognostic groups. Given the impact of *IgVH* mutation status on clinical setting, it has become highly desirable to standardize the laboratory methodologies used for *IgVH* mutation status determination. To check the reliability of our laboratory results, we performed a random interlaboratory testing. From 10 CLL samples tested, in 9 cases identical results were obtained in both laboratories. In one case, the result was discordant. The discrepancy was caused by the *IgVH* database used. This finding prompted us to double-check our cohort of 624 CLL patients, using IgBLAST and IMGT databases. The results showed 7.5% (47/624) discrepancies between both databases. In 21 out of 47 cases, the degree of hypermutation has changed in regard to the database used, resulting in major changes in the prognostic subgroup. Other irregularities between both databases were identified, with yet to be determined significance. In the light of presented data we would like to stress the necessity to identify/compile the most comprehensive *IgVH* database to be used for the determination of *IgVH* mutation status in CLL.

Haematologica 2006; 91:(1)e11-e12

It has been widely accepted that the mutation status of immunoglobulin heavy chain genes (*IgVH*) is one of the most important independent prognostic factors in chronic lymphocytic leukemia (CLL).[1,2] According to the *IgVH* hypermutation status (the threshold being 2% difference between the particular *IgVH* sequence and its closest germinal counterpart), CLL patients can be divided into favorable and unfavorable subgroups, respectively.[3-5] Great effort is being devoted to the harmonization of laboratory methodologies and implementation of a good laboratory practice, with the aim to gather comparable laboratory data for large multi-centric clinical trials. In 2003, van Dongen et al.6 summarized the recommendations for laboratory protocols used for the *IgVH* hypermutation detection in CLL, with the aim to harmonize obtained results to be comparable at interlaboratory level.

To test the factual degree of harmonization, we randomly selected ten cDNA samples from our cohort of CLL patients and sent them for an interlaboratory testing, in accordance with the regulations of the Institutional Board at the Homolka Hospital.

The samples were accompanied by a clear annotation on their respective *IgVH* mutation status and the *IgVH* subfamily used, as it was revealed in our laboratory previously.[7]

For 9 out of 10 samples, identical results were obtained in both laboratories, with one exception, which was identified as VH3-48, hypermutation 3.5%, in our laboratory, but VH3-48, germline, in the testing laboratory. Surprisingly, the explanation for this discrepancy was not a technical obstacle, but the *IgVH* database employed. We used IgBLAST,[8] whereas the other laboratory used IMGT.[9]

Intrigued by this finding, we decided to double-check the *IgVH* mutation status in our cohort of 624 CLL patients, to determine the degree of correspondence between both databases. Each of 624 *IgVH* sequences was screened against both IgBLAST and IMGT databases. In the majority of cases, both databases gave the same result. Nevertheless, in 47 out of 624 samples (7.5%), the results were discordant (Table 1):

In one case out of 47 (#C141), a different subfamily was identified (VH3-48, hypermutation 2.1% versus VH3-21, hypermutation 4.2%). Given the fact that VH3-21 is considered prognostically unfavorable,[10] this change is of a major prognostic impact.

In 21 out of 47 cases (#C541, B473, C141, F7, C831, C006, C796, F4, C705, C093, C743, C516, 3031, 2565, F1, F2, F5, C076, B400, 3066, C314 and F3), different *IgVH* gene/allele has been identified, with a significant change in the hypermutation status and prognostic subgroup. Importantly, the previously suggested *provisional IgVH* genes 4-b and 5-a (#C516, 3031, 2565, C306, 2725, C584, F1, F2, C755, B400, 3066, C314 and C952) are fully functional in CLL, as the sequencing analyses in this study have been carried out at the RNA (cDNA) level, and truly clonal V-D-J rearrangements have been corroborated. These *provisional* sequences are not included in the IgBLAST database, though IMGT database contains them.

In the remaining 25 out of 47 cases (#C786, C845, F6, 2361, C713, C934, A996, C637, 2370, 2449, B075, C306, 2725, C584, B578, 2218, C489, C686, C819, 2552, C623, C092, C755, C952 and C483), changes in the *IgVH* gene/allele, without impact on the prognostic category, have been revealed.

Recently, Lane et coworkers[11] reported in their paper in Leukemia that discrepancies between *IgVH* databases might exist. They used VBASE and IMGT to compare their data against. On our cohort of 624 CLL patients, we present similar findings here, with the exception that the databases employed in our study were IMGT and IgBLAST.

Our data clearly indicate that there are discrepancies among the concurrently available *IgVH* databases, in respect to the closest germinal sequences identified.

Since the *IgVH* mutation status has been considered the *golden standard* in CLL prognostication, we believe that it is highly desirable to identify/compile the most comprehensive data resource for the detection of *IgVH* hypermutation status in CLL. It seems that the IMGT database might be a candidate data-mining tool, given the fact that it contains more germline sequences, from which the *provisional* 4-b and 5-a genes have been found repeatedly in our study. Nevertheless, there are other irregularities between IgBLAST and IMGT, in respect to the *IgVH* subgroup identified. They might be clinically insignificant, but as it has been shown for VH3-21, the precise identification of the *IgVH* subgroup used by individual CLL is highly relevant.

Thus, we would like to stress the necessity to identify the most comprehensive *IgVH* data

resource to be used for the determination of *IgVH* mutation status in CLL, and wish to call for a discussion to clarify this issue.

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Table 1. Discrepancies found between IgBLAST and IMGT databases.

Sample Nr.	*Subfamily IgBLAST*	*Hypermutation IgBLAST (%)*	*Subfamily IMGT*	*Hypermutation IMGT (%)*
*C541	1-3	2	1-3*01	0
*B473	3-11*01	2.7	3-11*03	0
°C786	3-13	17.8	3-47	7.6
*C141	3-21	4.2	3-48	2.1
°C845	3-30	1.4	3-30*01	0
*F7	3-30	2.0	3-30	0
*C831	3-48	2	3-48*03	0
°F6	3-48	5.0	3-48	3.0
*C006	3-48*02	2	3-48*03	0
*C796	3-64	2.9	3-64*05	0.9
°2361	4-28	17.2	4-4	12.4
*F4	4-28	6.0	4-4	1.0
*C705	4-31	2.5	4-30-4*01	0
*C093	4-31	3.2	4-30	1
°C713	4-31	11.3	4-30-4*05	4.1
°C934	4-31	6.6	4-30-4*05	3.5
°A996	4-34	10.3	4-4	7.9
°C637	4-34	10.5	4-4	10
°2370	4-34	10.6	4-4	11.9
°2449	4-34	11.7	4-4	8.9
°B075	4-34	14.4	4-61	16.4
*C743	4-34	2.8	4-4	0
*C516	4-39	2	4-b	0
*3031	4-39	10.8	4-b	0
*2565	4-39	4.7	4-b	0
°C306	4-39	7.8	4-b	5.7
°2725	4-39	9.3	4-b	10.2
°C584	4-39	9.8	4-b	7.7
*F1	4-39	4.0	4-b	0
*F2	4-39	5.0	4-b	0
*F5	4-39	2.0	4-39	0
*C076	4-4	2.4	4-61	1.8
°B578	4-59	0	4-61	0
°2218	4-59	1.6	4-61	1.5
°C489	4-59	3.4	4-61	3.6
°C686	4-61	0	4-59	0
°C819	4-61	0	4-59	0
°2552	4-61	11.2	4-59	16.8
°C623	4-61	4.1	4-59	4.1
°C092	4-61	8.1	4-59	9.8
°C755	5-51	10.6	5-a	6
*B400	5-51	4.3	5-a	0
*3066	5-51	4.5	5-a	0
*C314	5-51	5.4	5-a	0
°C952	5-51	9.2	5-a	4.4
°C483	7-81	7.6	7-4-1	5.4
*F3	7-81	8.0	7-4-1	0

**Different IgVH gene or allele, clilnically relevant change in the prognostic subgroup;° different IgVH gene or allele, no clinically relevant change in the prognostic subgroup.*

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