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B-cell receptor stereotyped subsets and outcome for patients with chronic lymphocytic leukemia in the HOVON 68 trial

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Abstract

Aim: We here assessed the impact of B-cell receptor stereotypy on progression-free survival (PFS) and overall survival in patients from the HOVON 68 trial.



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Methods: Based on IGHV mutational analyses from participating centers in Sweden, Norway, Finland, Denmark, Poland, and the Netherlands, B-cell receptor stereotyped subsets were assigned using the ARResT/AssignSubsets software. Analysis for recurrent mutations was performed by next-generation sequencing by a 454-base platform. All other clinical data were extracted from the HOVON database by November 2016.

Results: In total, 178 out of 192 patients with sequences available were technically suitable for analysis. Thirty-eight patients (21%) were assigned to one of the 19 major subsets: Subset #2 ($n = 12$, 6.7%), Subset #8 ($n = 7$, 3.9%), Subset #6 ($n = 6$, 3.4%), and Subset #1 ($n = 5$, 2.8%). Other subsets found were: Subsets #3, #5, #31, and #64B. By November 2016, a PFS event had occurred for 150 patients (84%) and 79 patients (44%) had died. The median follow-up time for patients still alive was 78.9 months. Patients with UM-IGHV belonging to Subset #2 had significantly longer PFS than UM-IGHV 3-21-utilizing non-Subset #2 patients [UM-IGHV Subset #2 median PFS 61.3 months ($n = 8$) vs. UM-IGHV 3-21 non-Subset #2 median PFS 22.3 months ($n = 6$), $P = 0.01$]. Overall, no significant differences in PFS between groups were found for patients with M-IGHV.

Conclusion: In the HOVON 68 trial. Subset #2 patients had a good treatment outcome comparable to the outcome for non-high-risk patients with chronic lymphocytic leukemia following fludarabine-cyclophosphamide-rituximab-based treatment.

Keywords: Chronic lymphocytic leukemia, B-cell stereotypy subsets, treatment outcome

INTRODUCTION

The impact of B-cell receptor stereotypy on treatment outcome for patients with chronic lymphocytic leukemia (CLL) has recently been examined across multicenter clinical trials of both early stage and patients in need of treatment, treated with chemo-immunotherapies^[1]. The HOVON 68 trial compared chemotherapy with fludarabine and cyclophosphamide (FC) to chemo-immunotherapy with the addition of low-dose alemtuzumab as first-line treatment in high-risk patients with CLL^[2].

Approximately one third of patients with CLL carry a stereotyped B-cell receptor. These receptors can be divided into subsets based on homology of their complementarity determining region 3 (CDR3) sequence^[3]. At present, 19 major subsets have been described accounting for approximately 13% of patients with CLL^[4]. Subsets #1 and #2 are associated with particularly poor prognosis similar to TP53-aberrant cases, while Subset #8 is the subset with the highest risk of Richters transformation^[5]. Subset #2 is defined by usage of IGHV3-21/IGLV3-21 genes, a mixed somatic hypermutation status, and a particularly short CDR3^[3,6]. The HOVON 68 trial included high-risk patients with CLL defined as having 17p deletion [del(17p)], 11q deletion [del(11q)], trisomy 12, unmutated IGHV, and/or IGHV3-21^[2] (see trial registration at www.trialregister.nl as trial No. NTR529). We here assessed the impact of B-cell receptor stereotypy on progression-free survival (PFS) and overall survival (OS) in patients from the HOVON 68 trial. Our data show a favorable outcome for patients with CLL belonging to the stereotyped Subset #2 in this selected high-risk population.

METHODS

Based on IGHV mutational analyses from participating centers in Sweden, Norway, Finland, Denmark, Poland, and the Netherlands, B-cell receptor stereotyped subsets were assigned using the ARResT/AssignSubsets software^[7]. Analysis for recurrent mutations was performed by next-generation sequencing by a 454-base platform (Roche Diagnostics Corporation, Indianapolis, USA)^[8]. All other clinical data were extracted from the HOVON database at closure of the trial by November 2016. Chi square or Fisher's exact tests were used for contingency table analysis. OS and PFS from date of randomization were

examined by Kaplan-Meier curves, and log-rank tests as well as graphical illustrations were performed in Graph Pad Prism 9 (GraphPad software Inc., La Jolla, CA, USA) and SAS enterprise guide 7.1 (SAS institute, Cary, USA). A PFS event was defined as no response to treatment, progression, or death. *P*-values were two-sided and considered statistically significant if $P < 0.05$.

RESULTS

IGHV sequences from 192 out of 272 patients were available. Of these, 178 (93% of all patients) were technically suitable for analysis; all further analyses were restricted to this population. The original full sequences had to be available for analysis to be technically suitable for analysis. Thirty-eight patients (21%) could be assigned to one of the 19 major subsets: Subset #2 was the most frequent ($n = 12$, 6.7%), followed by Subset #8 ($n = 7$, 3.9%), Subset #6 ($n = 6$, 3.4%), and Subset #1 ($n = 5$, 2.8%). Other subsets found were: Subsets #3, #5, #31, and #64B. By November 2016, a PFS event had occurred for 150 patients (84%) and 79 patients (44%) had died. The median follow-up time for patients still alive was 78.9 months. Compared with the 94 patients excluded, the 178 patients included had similar baseline characteristics such as age group above 65 years, sex, treatment arm, Binet stage, WHO performance status, or CLL-IPI risk groups [Table 1]. Furthermore, there were no differences in PFS or OS between the excluded and included patients (PFS: $P = 0.96$; OS: $P = 0.70$).

Patients with unmutated immunoglobulin heavy chain variable genes (UM-IGHV) belonging to Subset #2 tended to have a longer median PFS than patients with UM-IGHV not utilizing IGHV3-21 [UM-IGHV Subset #2 median PFS 61.3 months ($n = 8$) vs. non-IGHV3-21 UM IGHV patients median PFS 32.1 months ($n = 139$), log rank $P = 0.12$, Figure 1A]. However, when PFS for patients with UM-IGHV belonging to Subset #2 were compared with PFS for UM-IGHV 3-21-utilizing non-Subset #2 patients, they had a significantly longer PFS [UM-IGHV 3-21 non-Subset #2 median PFS 22.3 months ($n = 6$), $P = 0.01$, Figure 1A]. Overall, no significant differences in PFS between groups were found for patients with M-IGHV (M-IGHV Subset #2 median PFS 49.6 months, M-IGHV non-Subset #2 median PFS 38.1 months, $P = 0.6$, Figure 1A) and for all patients with regard to OS (UM-IGHV Subset #2 median OS not reached, Figure 1B and Table 1). The survival rate of the 12 Subset #2 patients treated with either chemo- or chemoimmunotherapy in the HOVON 68 trial was comparable to that reported in other recent chemo- or chemoimmunotherapy trials^[9-11] [Table 1]. In addition, there were no differences in PFS or OS between treatment arms for patients belonging to Subset #2 (data not shown). Compared with patients with UM-IGHV not utilizing IGHV3-21, Subset #2 patients did not differ significantly with regard to most of their baseline characteristics. However, they were older, had more advanced disease, did not harbor del17p or trisomy 12, and more often had *SF3B1* mutations [Table 1].

DISCUSSION

The longer PFS after chemo- or chemoimmunotherapy for Subset #2 patients with UM-IGHV compared with patients with UM-IGHV or non-Subset #2 IGHV3-21 utilizing patients in this high-risk CLL cohort is somehow surprising when considering that Subset #2 patients are reported to have a median time to first treatment of only 22-24 months^[12,13]. However, a report based on a large cohort of patients with CLL showed a median survival after first line treatment of 7.3 years (88 months) in the pre-chemoimmunotherapy era and 10.7 years (128 months) in the chemoimmunotherapy era for patients belonging to Subset #2^[14]. In contrast to this, Jaramillo *et al.*^[1] found that advanced stage CLL patients belonging to Subset #2 with both M-IGHV and UM-IGHV had PFS similar to patients with UM-IGHV after chemotherapy- or rituximab chemoimmunotherapy-based regimens, but at the same time these advanced stage CLL patients belonging to Subset #2 tended to have a longer OS. Thus, Subset #2 patients might have longstanding benefit from first-line chemo- or chemoimmunotherapy, as administered in the HOVON 68 trial. The lack of *TP53*

Table 1. Characteristics at trial entry by subset #2, IGHV3-21 utilizing patients, IGHV mutational status and not utilizing IGHV3-21 as well as total and excluded patients

| | Subset #2 | IGHV3-21 non-subset #2 | M-IGHV non-IGHV3-21 | UM-IGHV non-IGHV3-21 | Total | Excluded patients |
|----------------------------|-------------|------------------------|---------------------|----------------------|--------------|-------------------|
| N | 12 | 7 | 20 | 139 | 178 | 94 |
| FCA | 7 (58%) | 5 (71%) | 15 (75%) | 65 (47%) | 92 (52%) | 41 (44%) |
| CR | 5/11 (45%) | 2/6 (33%) | 10/17 (59%) | 65/136 (48%) | 83/170 (47%) | 47 (50%) |
| Median PFS | 54.5 | 29.2 | 31.9 | 32.1 | 33.4 | 33.0 |
| Median OS | Not reached | 90.9 | Not reached | 88.8 | 90.9 | 82.1 |
| Age above 65 year | 6 (50%) | 0 (0%) | 8 (40%) | 35 (25%) | 49 (28%) | 17 (18%) |
| Male sex | 9 (75%) | 5 (71%) | 14 (70%) | 103 (74%) | 131 (74%) | 72 (77%) |
| Binet C | 10 (83%) | 4 (57%) | 11 (55%) | 39 (28%) | 64 (36%) | 29 (31%) |
| del(17p) | 0/10 (0%) | 0/6 (0%) | 4/20 (20%) | 15/128 (12%) | 19/164 (12%) | 9/84 (11%) |
| del(11q) | 3/10 (30%) | 2/6 (33%) | 2/20 (10%) | 32/128 (25%) | 39/164 (24%) | 27/84 (32%) |
| Tri12 | 0/10 (0%) | 0/6 (0%) | 14/20 (70%) | 30/128 (23%) | 44/164 (27%) | 14/84 (17%) |
| del(13q) | 4/10 (40%) | 3/6 (50%) | 0/20 (0%) | 27/128 (21%) | 29/164 (18%) | 20/84 (24%) |
| SF3B1mut | 4/7* (57%) | 1/3* (33%) | 0/13* (10%) | 9/78* (12%) | 14/101* (8%) | 3/16* (19%) |
| UM-IGHV | 8 (67%) | 6 (86%) | 0 (0%) | 139 (100%) | 153 (86%) | 77 (94%) |
| WHO 0 | 9 (75%) | 2 (29%) | 11/20 (55%) | 94/139 (68%) | 116 (65%) | 51 (54%) |
| Beta2m \geq 3.5 mg/L | 6/9 (67%) | 3 (43%) | 10/16 (63%) | 60/108 (56%) | 79/140 (56%) | 45 (56%) |
| CLL-IPI high and very high | 5/7 (71%) | 2/6 (33%) | 5/16 (31%) | 67/106 (63%) | 79/129 (61%) | 45/66 (68%) |

* Only a subgroup of the patients were analyzed for recurrent mutations limited by availability of samples. The total number of analyzed patients is given after /, when data was not available for all patients. FCA: Fludarabine, cyclophosphamide, and alemtuzumab; ND: not done; PFS: progression free survival; OS: overall survival; CR: complete remission; IGHV: immunoglobulin heavy chain variable genes; M-IGHV: mutated IGHV; UM-IGHV: unmutated IGHV; WHO: world health organization performance status; Beta2m: beta2microglobulin; CLL-IPI: CLL international prognostic index.

aberrations among Subset #2 patients may have contributed further to the improved efficacy of chemo- or chemoimmunotherapy in this trial, comparable to the efficacy of chemoimmunotherapy in the CLL10 trial that did not include patients with del(17p)^[9]. In line with this, Jeromin *et al.*^[10] found that TP53 aberration and del(11q) had an impact on OS in a large study of 267 IGHV3-21 cases and that del(11q) had a negative impact on OS for those belonging to Subset #2. In the HOVON 68 study, the presence of del(11q) had a marginal effect on PFS and no effect on OS, while the presence of del(17p) did impact both PFS and OS^[2]. In addition, in the large multicenter study examining B-cell receptor stereotyped subsets in relation to treatment outcome by Baliakas *et al.*^[14], neither del(17p) nor del(11q) had an impact on survival after treatment with chemo- or chemoimmunotherapy in Subset #2 patients. However, the study was based mainly on patients receiving rituximab-containing chemoimmunotherapy (FCR), and the impact from selection bias should always be considered when assessing biomarkers in different trial populations.

In summary, we found that 21% of the high-risk CLL patients treated in HOVON 68 belonged to a major B-cell receptor stereotyped subset, most frequently Subset #2. The Subset #2 patients had a good treatment outcome comparable to the outcome for non-high-risk patients with CLL following FCR-based treatment. The present findings emphasize the fact that chemoimmunotherapy may still have a place as first-line

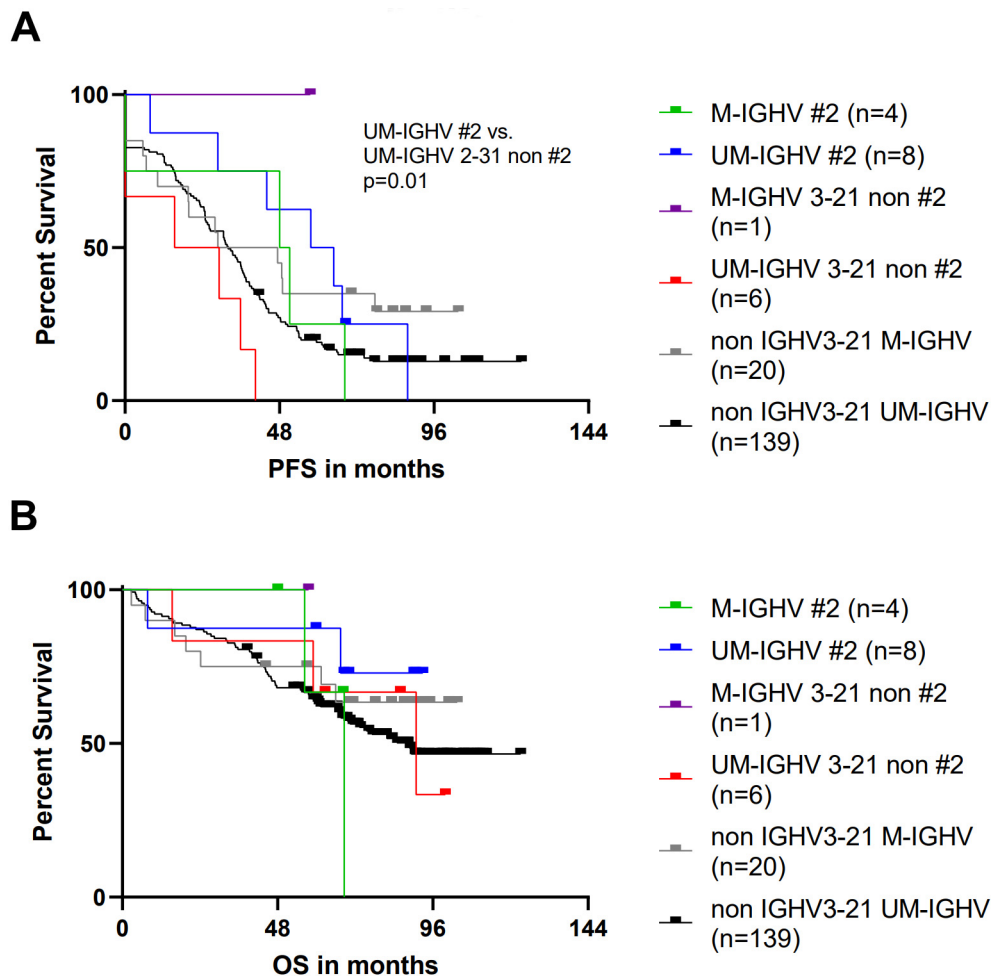


Figure 1. Outcome in the HOVON68 trial for patients belonging to subset #2 or utilizing IGHV3-21 by mutational status of IGHV. M-IGHV: Mutated immunoglobulin heavy chain genes; UM-IGHV: unmutated immunoglobulin heavy chain genes; #2: subset #2; 3-21: utilizing IGHV3-21; PFS: progression free survival; OS: overall survival.

treatment for particular groups of patients with high-risk CLL. However, these results should be interpreted with caution as low-dose alemtuzumab and FC alone are no longer used in clinical practice and due to the very small numbers of patients. Thus, the need for assessing the impact of targeted treatment for this group of patients is still warranted, and our observations from the HOVON 68 trial can serve as a contribution to the discussion of different treatment options for groups of patients belonging to specific B-cell receptor stereotyped subsets.

DECLARATIONS

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Authors' contributions

Performed the research: Pedersen LB, te Raa D, Juvonen V, Rosenquist R, Langerak AW, Evers LM, Zenz T
 Designed the research study: Vojdeman FJ, Pedersen LB, Niemann CU, Geisler CH
 Contributed essential reagents or tools: te Raa D, Juvonen V, Tjønnfjord GE, Walewski J, Itälä-Remes M, Kimby E, Rosenquist R, Langerak AW, Evers LM, Zenz T, Kater AP, van Oers MHJ, Geisler CH

Analysed the data: Vojdeman FJ, Pedersen LB, van Norden Y

Wrote the paper: Vojdeman FJ, Niemann CU

All authors have commented and approved the manuscript.

Availability of data and materials

All data is available upon specific inquiry to Vojdeman FJ.

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Niemann CU: Novo Nordisk Foundation NNF16OC0019302, Danish Cancer Society.

Conflicts of interest

Vojdeman FJ, Pedersen LB, te Raa D, Juvonen V, van Norden Y, Itälä-Remes M, Rosenquist R, Evers LM, Zenz T, van Oers MHJ, Geisler CH declared that there are no conflicts of interest.

Tjønnfjord GE: advisory board (Abbvie) and Lecture honoraria (Janssen Cilag).

Kimby E: advisory board: Roche, Celgene, Janssen, Abbvie, Pfizer, Mei Pharma. Educational lectures: Gilead, Roche, Celgene, Janssen, Abbvie. Research funding: Pfizer.

Langerak AW: research funding (Roche-Genentech and Gilead), advisory committee (AbbVie), lecture honoraria (Janssen).

Walewski J: advisory role: Roche, Celgene, Takeda, Janssen-Cilag, Servier, Amgen, BMS, Incyte; research funding: Roche, GSK/Novartis, Takeda, Janssen-Cilag, Abbvie; lecture honoraria: Roche, Celgene, Takeda, Janssen-Cilag, Servier; travel support: Roche.

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Ethical approval and consent to participate

The HOVON 68 study was approved by the national ethical committees as described in (Geisler *et al.*^[2] 2014).

Consent for publication

Not applicable.

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