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Multiple productive immunoglobulin heavy chain gene rearrangements in chronic lymphocytic leukemia are mostly derived from independent clones

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Multiple productive immunoglobulin heavy chain gene rearrangements in chronic lymphocytic leukemia are mostly derived from independent clones

Running title: Multiple IGH Rearrangements in CLL

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ABSTRACT

In chronic lymphocytic leukemia, usually a monoclonal disease, multiple productive immunoglobulin heavy chain gene rearrangements are identified sporadically. Prognostication of such cases based on immunoglobulin heavy variable gene mutational status can be problematic, especially if the different rearrangements have discordant mutational status. To gain insight into the possible biological mechanisms underlying origin of the multiple rearrangements, we performed a comprehensive immunogenetic and immunophenotypic characterization of 31 cases with the multiple rearrangements identified in a cohort of 1147 chronic lymphocytic leukemia patients. For the majority of cases (25/31), we provide evidence for the co-existence of at least two B lymphocyte clones with chronic lymphocytic leukemia phenotype. We also identified clonal drifts in serial samples, likely driven by selection forces. More specifically, higher immunoglobulin variable gene identity to germline and longer complementarity determining region 3 were preferred in persistent or newly appearing clones, a phenomenon more pronounced in patients with stereotyped B cell receptors. Finally, we report that other factors, such as *TP53* gene defects and therapy administration, influence clonal selection. Our findings are relevant to clonal evolution in the context of antigen stimulation and transition of monoclonal B-cell lymphocytosis to chronic lymphocytic leukemia.

INTRODUCTION

Chronic lymphocytic leukemia (CLL) is characterized by the expression of restricted sets of B cell receptors (BcR), often with highly similar, stereotyped antigen-binding sites[1,2], strongly indicating the role of antigen in CLL development[3-5]. Further evidence in support of this notion has been amply provided by the fact that the mutational status of the clonotypic immunoglobulin heavy variable gene (*IGHV*) stratifies CLL patients into two groups with markedly different prognosis[6,7]. This implies that signals conveyed through BcRs with distinct molecular structure likely affect the biological behavior of the CLL clone[8,9], thus contributing to the eventual clinical outcome.

A subgroup of CLL cases carry multiple (mostly double) productive IGH rearrangements (MP-IGH), and pose an exception to the rule 'single clone – single rearrangement'. Such cases have been reported repeatedly and independently by several groups, and they are estimated to account for approximately 2% of the entire CLL cohort[10]. The true biological and clinical implications are currently unknown, especially when one of the rearrangements is mutated while the other unmutated.

Several mechanisms have been previously linked with the phenomenon of double productive IGH rearrangements. These mechanisms involve two main themes – either (i) lack of allelic exclusion at the IG loci[11] or (ii) the presence of two clonal populations[12-16].

Allelic exclusion regulates the expression of IG genes in any given B lymphocyte so that it may express a single heavy and a single light chain. This mechanism is critical for the process of clonal selection and the generation of high-affinity, antigen-specific antibodies. In a minority of B cells, the mechanism can be disrupted, leading to lack of allelic exclusion which is characterized by the production of two functional IGH or IGK/L molecules in a single B lymphocyte[17].

In CLL, lack of allelic exclusion on heavy chains leading to the presence of double productive IGH rearrangements was first reported in 1997[11]. At roughly the same time, *IGHV* gene replacement was suggested as another molecular mechanism that could lead to the

presence of double productive IGH rearrangements in CLL[18]. Whatever the precise molecular mechanism(s) underlying double IGH rearrangements in a single cell/clone, these have been interpreted as evidence for the possible operation of receptor editing in CLL. This is similar to what has been reported for normal B cells, where secondary rearrangements occurring after the expression of a potentially harmful BcR can offer the cell the opportunity to evade apoptotic death[19].

Double productive IGH rearrangements can also indicate the presence of two clonal populations, each expressing distinct BcR. Several cases with coexistence of two CLL clones have been reported[12-16], challenging the prevailing notion of CLL being a monoclonal disease. On the other hand, detection of double productive IGH gene rearrangements could also represent co-existence of CLL with another B lymphoproliferative disorder[12].

Clonal drift is a phenomenon in lymphoid malignancies with multiple productive antigen receptor gene rearrangements, in particular T-cell large granular lymphocyte leukemia, referring to a dynamic process of alterations in the proportion of the malignant clones[20]. Clonal drift has never been examined in CLL, though potentially relevant given evidence that the proliferation and overall biological behavior of CLL cells may differ between clones with mutated or unmutated *IGHV*[21,22].

Previous studies analyzing CLL cases with multiple productive IGH rearrangements lack a detailed genomic analysis of IG light chains and partial IGHD-IGHJ rearrangements that can be extremely informative about the molecular status of the IG loci, thus contributing to the clarification of the implicated mechanisms. In the present study, we performed a comprehensive analysis of IG heavy and light chain gene rearrangements in MP-IGH CLL patients with an aim of obtaining molecular insight into the biological causes of this phenomenon. Also, for the first time, we attempted a systematic study of clonal drift in MP-IGH CLL by tracing each rearrangement at different time-points in the natural history of the disease.

METHODS

Study group

MP-IGH cases were sought among 1147 CLL patients tested for *IGHV* gene mutational status at the University Hospital Brno, Czech Republic, from 2003 to 2011. All patients included in this study fulfilled the iwCLL/NCI diagnostic criteria for CLL[23]. Blood samples were taken after written informed consent in accordance with the Declaration of Helsinki under protocols approved by the Ethical Committee of the University Hospital Brno.

Sample processing, nucleic acid isolation and cDNA synthesis

B lymphocytes were routinely separated using RosetteSep kits (StemCell) from peripheral blood. The purity of enriched B cells and expression of surface markers were evaluated by flow-cytometry; all separated samples contained >98% CLL cells. Genomic DNA (gDNA) was isolated using the DNeasy Blood & Tissue Kit (Qiagen). For RNA isolation, either TriReagent (MRC, Inc.), or the RNA mini Kit (Qiagen) were used. Complementary DNA (cDNA) was synthesized using SuperScript II Reverse Transcriptase (Invitrogen) from 500 ng of total cellular RNA.

PCR amplification of IG gene rearrangements

IGHV-IGHD-IGHJ rearrangements were amplified by reverse transcription PCR using primers specific for the leader region of *IGHV* genes along with a consensus primer for the *IGHJ* genes. In cases with multiple amplicons, the PCR for IGHV-IGHD-IGHJ rearrangements was repeated on gDNA with a combination of FR1 primers and a consensus IGHJ primer[24].

PCR amplification of partial IGHD-IGHJ rearrangements was performed on gDNA utilizing seven subgroup-specific IGHD primers in combination with a consensus IGHJ primer[24]. Amplification of IGKV-IGKJ and IGLV-IGLJ rearrangements was performed with

primers specific for the framework region 1 (FR1) and the *IGKJ* or *IGLJ* genes[25,26], and/or following the BIOMED-2 protocol[24], both on cDNA and gDNA.

IG sequence analysis and interpretation

PCR amplicons were subjected to direct sequencing on both strands. If multiple IGHV-IGHD-IGHJ rearrangements were amplified from the same *IGHV* specific primer, subcloning was performed following recommended strategies[10]. Obtained sequences were analyzed using IMGT® and the IMGT/V-QUEST tool (http://www.imgt.org)[27]. For partial IGHD-IGHJ rearrangements, sequence analysis was performed by a multistep procedure using BLAST (http://blast.ncbi.nlm.nih.gov/), ExPASy (http://au.expasy.org/), and IMGT® tools.

Molecular monitoring of B cell clones over time

For long-term molecular monitoring of clonal dynamics, allele-specific oligonucleotide assays for quantitative real-time PCR (ASO-qPCR) were designed. gDNA was used for quantification of IG rearrangement proportions. If gDNA was not available in serial samples or ASO-qPCR assay design was not successful, clonal dynamics was assessed semi-quantitatively based on fragment analysis from cDNA using consensus FR1 and IGHJ primers. In such cases, clone size was estimated according to the size of the area under the curve.

For further details see Supplemental Methods that are available on the Haematologica Web page.

RESULTS

Multiple productive IGHV-IGHD-IGHJ gene rearrangements in CLL: Incidence and overview of IGH gene repertoires

Within a cohort of 1147 CLL patients analyzed for *IGHV* mutational status in this study, 548 (46.3%) sequences carried mutated and 635 (53.7%) unmutated *IGHV*, following the 98% identity cut-off value. The skewing to unmutated cases results from the fact that our Department is a tertiary center where cases with a less favorable clinical course are referred.

Multiple productive IGHV-IGHD-IGHJ rearrangements (MP-IGH) were identified in 31/1147 cases (2.7%). For patient characteristics see Supplemental Table S1 and Figure S1. Two or three transcribed productive IGHV-IGHD-IGHJ rearrangements were found in 26 (84%) and 5 (16%) cases, respectively, resulting in a total of 67 sequences. Of these, 29/67 (43%) carried mutated *IGHV*, while the remainder (38/67, 57%) carried unmutated *IGHV*; 27/38 unmutated rearrangements had *IGHV* with 100% identity to germline ('truly unmutated'[3]). Hence, the distribution of the sequences obtained in MP-IGH cases with regards to *IGHV* mutational status was similar to that of the entire cohort.

The *IGHV*, *IGHD*, and *IGHJ* gene repertoires in MP-IGH cases did not differ significantly from cases with single productive rearrangements (Table S2). Notably, 15/31 (48%) of MP-IGH cases harbored at least one rearrangement with stereotyped VH CDR3 region; three of 15 such cases carried two IGHV-IGHD-IGHJ rearrangements assigned to different subsets. From the perspective of individual sequences, 18/67 (26%) of rearrangements from MP-IGH cases were stereotyped.

We compared the IG gene repertoire, somatic hypermutation (SHM) status and CDR3 features between co-existing rearrangements within each case. Altogether, 41 pairs of rearrangements were analyzed; in the 5 cases with three rearrangements, all three possible pairs were included in the analysis. The main finding concerning *IGHV* usage was that the predominant pairings were IGHV3+IGHV4 and IGHV1+IGHV3 (8/41 pairs each) whereas the

frequency of the IGHV3+IGHV3 was low (2/41 pairs). With regards to SHM, concordant *IGHV* mutational status was seen in 21/31 cases (67.7%), of which 9 (29%) carried only mutated while the remaining 12 (38.7%) carried only unmutated *IGHV* genes. 10/31 cases (32.3%) were discordant for SHM since they carried rearrangements of different mutational status (according to the 98% cut-off value). Detailed results from this assessment are listed in Table S3.

Multiple IGHV-IGHD-IGHJ gene rearrangements in CLL: Immunophenotypic and molecular hints regarding their origin

We questioned whether MP-IGHs could co-exist in a single clone, alluding to a lack of allelic exclusion, or whether they derived from multiple co-existing clonal B cell populations. First, we performed detailed flow-cytometry analysis in 22 cases (Figure 1). 20/22 cases had a homogeneous phenotypic profile suggestive of CLL. Interestingly, 7/20 cases had clear evidence of two co-existing CLL clones with different light chain restriction. In the remaining 2/22 cases, we could document the presence of a CLL population co-existing with another clonal B cell population with a distinct immunophenotype (cases #1037 and 1054; Figure 1).

We then performed immunogenetic gDNA-based analysis in 26 MP-IGH cases. The reasoning behind this approach is that since a single cell and, by inference, a single clone carries only two IGH alleles, then the expected maximum number of IGH rearrangements per cell/clone is only two. Hence, the detection of partial IGHD-IGHJ (P-DJ) or non-transcribed/unproductive IGHV-IGHD-IGHJ rearrangements in MP-IGH CLL cases might constitute as convincing molecular evidence in favor of the existence of multiple clonal B cell populations, even when displaying a uniform immunophenotype. To exclude coincidental amplification or amplification of germline IGHD7-IGHJ1 region, all PCR products were sequenced and particular *IGHD* and *IGHJ* genes were assigned.

Overall, 20/26 cases were positive for P-DJ, indicating the existence of multiple clones. In 6/26 cases (four of them positive for P-DJ), this presumption was further supported

by detection of an additional IGHV-IGHD-IGHJ rearrangement that was either non-transcribed productive (1 case), or unproductive due to an out-of-frame junction (5 cases).

We subsequently extended the immunogenetic analysis to the IG light chains for all 31 MP-IGH cases. Overall, 74 IGKV-IGKJ and IGLV-IGLJ clonal rearrangements were amplified. Twenty-one cases (68%) carried multiple light chain rearrangements; at least two rearrangements were productive in 18/21 cases (86%); multiple productive and transcribed rearrangements were detected in 16/18 cases (52% from all 31 patients).

Additionally, in 26 cases we analyzed rearrangements of the IGK loci involving the kappa-deleting element (KDE). Among kappa-expressing cases (14/26), 10 had PCR evidence for KDE rearrangements. In the only one lambda-expressing case, both IGKV-KDE and IGKJ-C-INTRON-KDE rearrangements were detected. All cases expressing both kappa and lambda light chains (11/26) were positive for KDE rearrangements. In total, all 26 analyzed MP-IGH cases had at least one rearranged IGK allele.

When combining immunophenotypic and molecular results, we could categorize the 31 MP-IGH cases as follows (Table 1 and S4; Figure S2): *Group I* – Definite co-existence of two clonal B cell populations: 9 cases (29% from MP-IGH cases; 0.79% from the whole CLL cohort), of which 7 concerned co-existing CLL populations with different light chain restriction, while the remaining 2 concerned coexisting CLL+other B cell clone (cases #1037 and #1054); *Group II* – Highly likely co-existence of at least two clonal populations with CLL-like phenotype: 16 cases (52% from MP-IGH cases; 1.40% from the whole CLL cohort); *Group III* – Indeterminate as to one or more CLL-like populations: 6 cases (19% from MP-IGH cases; 0.52% from the whole CLL cohort), in which we failed to obtain conclusive evidence of more than one clone.

Tumor dynamics: Molecular monitoring over time suggesting clonal drift

In 22/31 MP-IGH patients (71%), reverse transcription PCR analysis of IGHV-IGHD-IGHJ rearrangements was performed repeatedly in several time points during the disease course (the median number of tests 2.5; range 2-6). The median interval from the first to the

last analysis was 24 months (range 8-74 months). Additionally, in 11 of the 22 cases with available consecutive gDNA samples, ASO-qPCR assays were designed for all IGHV-IGHD-IGHJ rearrangements to verify the results of PCR analysis and to quantify changes in the relative proportion of the clonal populations over time (Figure 2; Table S5). The observed changes were evaluated through categorizing the detected clonal IGHV-IGHD-IGHJ rearrangements as (i) diminishing (decreasing tendency in subsequent samples, state detectable→undetectable, >10% proportion change using ASO-qPCR), (ii) persistent (present constantly, stable or expanding compared to other persistent or diminishing rearrangement, respectively), or (iii) appearing (originally undetectable, expanding tendency).

Altogether, a clonal drift represented by changes in the proportions of the rearrangements was detected in 19/22 MP-IGH cases (86%). IGHV-IGHD-IGHJ rearrangement absent at initial testing appeared in 3/19 patients besides the original rearrangement(s), whereas in 18/19 patients one of initially multiple detected IGHV-IGHD-IGHJ rearrangements diminished during the disease course (Table S1). Importantly, among the latter cases, diminishing of the IGHV-mutated clone with concurrent persistence of the co-existing IGHV-unmutated clone resulted to re-categorization of 7/10 patients (70%) with originally discordant mutational status to the group with unmutated *IGHV* genes (Table 2).

Considering the clinical course of the 19 patients with clonal drift, we noted that the changes in the proportions of clonal populations were frequently related to progressive lymphocytosis or overall disease progression (8 out of 13 cases with progressive disease; 62%). Furthermore, eradication of a clone was observed in relation to therapy administration when one of the clones present before therapy was not detected at disease relapse while the other clone expanded (5 of 8 treated patients; in the remaining 3 patients one clone had diminished before therapy). Altogether, clonal drift was associated with disease progression/therapy in 12 of 13 progressive cases (92%; 63% of all cases with clonal drift).

Since the molecular features of IGHV-IGHD-IGHJ rearrangements attest to the role of selection by antigen in CLL development[4,5], they could also be relevant to the emergence, persistence, and drift of individual clones. Thus, in the 19 patients with clonal drift, we

compared IGHV-IGHD-IGHJ rearrangements in co-existing pairs (24 in total). Significantly, this analysis was suggestive of selection for clones with (i) higher *IGHV* gene identity to germline and/or (ii) longer VH CDR3 (cumulative preference in 71% of pairs, p=0.005, Chi-square test; Figure 3).

Moreover, we observed that the IG rearrangement bearing a VH CDR3 assigned to a stereotyped subset was preferred in 9 pairs (82% of the pairs with at least one stereotyped BcR; 38% of all pairs with clonal drift) and, strikingly, the aforementioned tendency towards selection for higher *IGHV* identity and longer VH CDR3 was particularly pronounced in this subgroup (p=0.01; Chi-square test) (Figure 3; Table S3).

Genomic background: influence of TP53 gene defects on clonal drift

Genomic abnormalities have been documented to impact on CLL patient survival and time to first therapy[28], with defects of the *TP53* gene shown to have the strongest impact on clinical outcome[29,30]. Having interest in whether such defects present in co-existing clones could influence selection of one over the other, we detected *TP53* defects consecutively during the disease course in 5 patients among the MP-IGH cohort (16%). In these patients, *TP53* mutation and/or 17p deletion and other genomic abnormalities were assigned to individual clones based on (i) changes in consecutive cytogenetic results that were correlated with changes in the relative proportions of IG rearrangements, and (ii) multiplex ligation-dependent probe amplification and *TP53* sequencing of individual FACS-sorted populations. Of significance, in all cases, the *TP53* defective clone expanded to the detriment of the *TP53* unaffected clone (Table 3). In 2 cases, the selection for the *TP53* defect was therapy-related. Notably, both selected clones harbored stereotyped rearrangements of the *IGHV1-69* gene (subset 3 and 7)[31] (Figure 2).

DISCUSSION

CLL is usually a monoclonal disease, hence, the entire CLL population constituting the progeny of a single B lymphocyte can be characterized by a unique IGHV-IGHD-IGHJ rearrangement. Significantly, patient stratification in groups with distinct prognosis is possible through determination of *IGHV* mutational status[32]. In MP-IGH CLL cases, assignment to the IGHV-mutated or unmutated category can be difficult, especially when the rearrangements have discordant mutational status, precluding conclusive interpretation[10]. In line with our observations, MP-IGH incidence reaches approximately 2% of CLL cases[10].

We attempted the first systematic assessment of MP-IGH CLL by performing a detailed immunophenotypic and molecular profiling with the aim to elucidate their biological cause. Based on the evidence for co-existence of multiple B cell clones, we assigned these cases into three groups. *Group I* (29% of MP-IGH cases; 0.79% from the whole CLL cohort) included cases for which we were able to confirm biclonal expansions differing in light chain restriction. The majority displayed typical CLL immunophenotype for both clonal populations. In two cases, the CLL clone co-existed with another B cell clone expressing an immunophenotype atypical for CLL. Sanchez and colleagues[12] previously reported a higher incidence of CLL cases with 2 or more phenotypically distinct B-cell clones (~4% when considering typical and atypical CLL together) than was observed in our study. This discrepancy is most likely attributed to differences in methodological design. In particular, Sanchez et al. investigated the incidence of more B-cell clones in a cohort of patients with leukemic chronic lymphoproliferative disorders characterized by detailed flow cytometry[12], whereas we undertook a comprehensive molecular immunogenetic profiling complemented by FISH and flow cytometry studies. Furthermore, we followed a stringent approach for assigning patients to *Group I,* requiring different light chain restriction, because alterations in the expression of other markers could be linked to intraclonal diversification or clonal evolution.

Group II (52% of MP-IGH cases; 1.40% from the whole CLL cohort) consisted of cases with immunogenetic evidence for more than one B cell clonal population based on the number of detected IG rearrangements exceeding the allele capacity of a single cell/clone, yet in which only one homogeneous population was assessed by flow-cytometry. This probably reflected either a very similar immunophenotype of the clones with the same light chain restriction, or low proportion of one clone in the sample, or both. We were able to document this presumption using ASO-qPCR in 5 cases. In addition, changes in the relative proportions of the IGH rearrangements were observed over time.

In *Group III* (19% of MP-IGH cases; 0.52% from the whole CLL cohort), we did not obtain definitive evidence mainly due to lack of available material. Potential explanations could still relate to molecular mechanisms such as BcR editing through *IGHV* gene replacement[18] or lack of allelic exclusion[11] leading to the expression of multiple IGHV-IGHD-IGHJ rearrangements in a single cell. The first possibility (i.e. *IGHV* gene replacement) was effectively ruled out since we did not identify common VH CDR3 motifs co-existing in any patient[33].

Although the evidence in Groups I and II suggests the presence of multiple B cell clones, alternative options must be considered, including: (i) presence of extra copies of the IGH locus due to trisomy 14 or amplification of 14q; nonetheless, this possibility is not supported in any case with available cytogenetic data (FISH and/or metaphase cytogenetics in 84% of MP-IGH cases); and, (ii) as already mentioned for *Group III*, lack of allelic exclusion leading to dual IGH-expressing B cells, as reported for autoreactive B cells[17] as well as indirectly for CLL[11,18]. It is worth mentioning that in *Group II*, we identified one case (#974) with dual expression of surface IgKappa and IgLambda light chains as a possible consequence of receptor editing or allelic inclusion in light chain loci[34]. So far, we have not been able to document whether both IG heavy transcripts detected in this patient were also translated and expressed. Admittedly, however, only analysis at the single cell level could reliably identify the underlying mechanism(s) in the above mentioned case and also confirm the presumption made for the whole *Group II*.

Monoclonal B cell lymphocytosis (MBL) is regarded as a pre-malignant state of CLL[35]. In contrast to the monoclonal nature of CLL, two or more “low-count” co-existing MBL clones have been documented at a high frequency[36]. Moreover, persistent as well as transient MBL clones have been observed[37]. Thus, CLL cases with multiple productive IGH gene rearrangements might represent the co-existence of CLL with a CLL-like MBL population, at least for a subset of cases. Following this line of reasoning, our cohort of MP-IGH CLL also features a number of low-count clones (see *Group II* above), which may signify borderline MBL/CLL clones co-existing for a certain time period with eventually prevailing CLL clones. This hypothesis is also supported by our experience with a case carrying two mutated IGHV-IGHD-IGHJ rearrangements that was originally classified as clinical MBL and, thus, excluded from the herein studied cohort. CLL eventually developed with only one of the original two rearrangements identified at the CLL stage.

Moreover, the idea of multiple B lymphocyte clones initiating CLL is in the line with the process of antigen stimulation generally considered to contribute to CLL development[3-5,38]. Initially, several B lymphocytes with different BcR specificity could target different epitopes of the same (auto)antigen. Later on, only some of these clones eventually gain additional abnormalities driving clonal expansion and profit from favorable interactions within their microenvironment[39]. Thus, although only one clone prevails in the majority of CLL cases, many B lymphocyte clones could be expanded at the beginning. Our present results support this notion since multiple rearrangements were often detected in early stages of the disease at diagnosis (see Figure S1 reporting a comparison between the MP-IGH cases and cases with only a single productive IGH rearrangement).

Clonal drift as a dynamic process of altering proportions of malignant lymphocyte clones is highly relevant to the understanding of the co-existence of multiple clones and the eventual prevalence of one over the other. It was first described in T-cell large granular lymphocyte leukemia harboring two T cell receptor beta chain gene rearrangements[20]. In CLL, clonal drift had not been referenced until now. A previously published study rather delineated relative stability of neoplastic clones[12]. Based on our data, it seems that the

emergence of an additional clone is possible but less likely. Similarly, the disappearance of a clone seems to occur more frequently if it co-exists with a more aggressive one. Our results show that higher *IGHV* gene identity and/or longer VH CDR3 regions were preferred over time, a phenomenon more pronounced in patients with stereotyped BcR. Relevant to these observations, telomere length measurements[21] have shown that proliferation of leukemic cells with unmutated *IGHV* is more intense compared to those with mutated *IGHV*. Furthermore, it is worth mentioning that longer VH CDR3s are a frequent feature of auto- and multi-reactive cells[40,41], which is also in line with the reactivity profile of IGHV-unmutated CLL clones. This indicates that antigen drive may underline clonal drift leading to selection for more aggressive clones with distinctive molecular features. Overall, based on the results of our study, clonal drift, predominantly leading to shrinkage or disappearance of clones detected at diagnosis, could be one of the factors contributing to the differing proportions of IGHV-mutated and IGHV-unmutated cases in MBL vs. CLL[35,42].

From a different perspective, it is reasonable to presume that genomic abnormalities might also impact on clonal drift. We document that the presence of a *TP53* defect in individual clones can also be implicated in clonal drift, further supporting the aggressiveness of p53 defective clones, which may underline selection leading to clonal predominance[29] and monoclonal CLL. In such cases, the p53 defect might represent a stronger phenotype prevailing over recognized unfavorable immunogenetic features. The impact of other genomic abnormalities, such as the 11q deletion, another strong marker of clinical outcome, remains to be elucidated due to low number of studied cases with the respective defects.

Finally, clonal drift might have important implications for decisions related to stratification and clinical management of MP-IGH CLL, especially in cases with discordant *IGHV* mutational status which are inconclusive regarding prognosis[10]. We observed a shift in favor of IGHV-unmutated status in majority of cases with discordant status. It might be an explanation for recently published data indicating that patients with discordant status present with adverse clinical course similar to IGHV-unmutated cases[43]. Moreover, based on our observations, gradual prevailing of one clone over other, also in cases with concordant

status, was often accompanied by disease progression. Therefore, we advocate repeated testing of *IGHV* mutational status in MP-IGH CLL since it can alert to changes in disease behavior.

In conclusion, our results suggest that most CLL cases with multiple IGHV-IGHD-IGHJ rearrangements can be accounted for by the presence of multiple B lymphocyte clones with CLL or CLL-like phenotype co-existing within the same patient. Importantly, we document for the first time that their proportions may change over time and that these changes are likely influenced by the molecular features of the BcR, including *IGHV* mutational status and VH CDR3 composition and length, and by the genomic make-up of the clones as well. This is potentially critical for understanding what drives B lymphocyte clonal evolution and could therefore provide insights and the means to influence or even halt this process.

AUTHORSHIP AND DISCLOSURES

Information on authorship, contributions and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

AUTHORS' CONTRIBUTION

KP designed and performed experiments, analyzed and interpreted data, generated figures and wrote the manuscript; HSF performed experiments, analyzed data; KB performed experiments; YB and MD provided samples and clinical data; SPav performed experiments, interpreted data, edited the manuscript; JMal performed experiments, edited the manuscript; JMay supervised clinical part of the study; BT designed the study, performed statistical analyses, edited the manuscript; SPos coordinated and supervised the study, edited the manuscript

DISCLOSURES

The authors declare no relevant conflict of interest.

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TABLES

Table 1. Immunogenetic and immunophenotypic data and resulting category referring to the possibility of co-existence of multiple clones in the respective patient.

Patient	Productive IGHV-IGHD-IGHJ	Partial IGHD-IGHJ	Unproductive IGHV-IGHD-IGHJ	Productive IGKV-IGKJ	Unproductive IGKV-IGKJ	IGKV-KDE	IGKJ-C-INTRON-KDE	Productive IGLV-IGLJ	Unproductive IGLV-IGLJ	Clones by flow-cytometry	Group
261	2			1		1	1	1		2	**I**
279	2			1			1	1		2	**I**
319	3	3		3	1	1	1	1	2	2	**I**
948	2	2		1	1	2		1		2	**I**
1030	2	1	1	1	1	1		1		2	**I**
1037	3	1	1	1	2	1	1	1		2	**I**
1054	2	1		1		1		1		2	**I**
1072	2	1		1	1		1	1		2	**I**
1132	2	1		2	2		1	1		2	**I**
307	2	1		1	1					1	**II**
511	3		1	1	1				1	NA	**II**
523	2	1		1		1				1	**II**
604	3	1			2		1	2	1	1	**II**
625	3[#]	1		1	1	1		1		1	**II**
814	2		1	3						NA	**II**
846	2	1		1		1				1	**II**
862	2			3		2				1	**II**
885	2	1		1		2	1			1	**II**
892	2	1		1			1			1	**II**
912	2	1		1						NA	**II**
923	2	1		1		2				1	**II**
974	2	1	1	3		2		1		1	**II**
1029	2	1		2		1				1	**II**
1049	3	1		2			1			1	**II**
1087	2	1		1			1			1	**II**
447	2			2						NA	**III**
833	2			2						NA	**III**
845	2			1						NA	**III**
877	2			1						NA	**III**
922	2			1						NA	**III**
1137	2				1			1		NA	**III**

(I) definite co-existence of two clonal B cell populations; (II) highly likely co-existence of at least two clonal populations with CLL-like phenotype; (III) indeterminate as to one or more CLL-like populations.

NA – not available; #one of IGH rearrangements non-transcribed

Table 2. Overview of CLL MP-IGH cases with originally discordant mutational status and the impact of clonal drift on overall *IGHV* mutational status (clones prevailing during the disease course are highlighted in grey).

Patient	IGHV gene	IGHV status (identity %)	CDR3 length	Stereotype subset	Over time tendency	Resulting IGHV status
604	IGHV1-69	U (100)	24		→	U
	IGHV1-69	U (99,3)	20	5	→	
	IGHV2-5	M (96,9)	12		↓	
625	IGHV4-34	U (99,3)	17		→	D
	IGHV1-69	M (93,1)	15		→	
833	IGHV1-69	U (100)	21		→	U
	IGHV2-5	M (92,8)	17		↓	
846	IGHV1-69	U (100)	22	3	→	U
	IGHV4-59	M (96,8)	14		↓	
912	IGHV4-34	U (100)	23	B1	→	U
	IGHV3-7	M (95,1)	23		↓	
1030	IGHV1-69	U (100)	22		→	U
	IGHV4-4	M (93,4)	11	219	↓	
1037	IGHV4-39	U (99,0)	11		→	D
	IGHV1-8	M (96,9)	12		↓	
	IGHV3-72	M (94,9)	16		→	
1049	IGHV4-39	U (100)	19	8	↑	U
	IGHV1-3	U (100)	17	28	→	
	IGHV3-33	M (95,8)	15		↓	
1087	IGHV3-43	U (100)	14		→	U
	IGHV6-1	M (95,3)	12		↓	
307*	IGHV1-3	U (98,6)	13		NA	D
	IGHV3-23	M (89,9)	13		NA	

Mutational IGHV status: M – mutated, U – unmutated, D - discordant; "↓" – diminishing; "→" – persisting; "↑" – appearing; NA – not available

* monitoring of clones over time was not performed in this case

Table 3. Comparison of individual B lymphocyte clones in patients with clonal drift and a *TP53* defect. In all cases, a *TP53* defect was related to a distinct B lymphocyte clone whereas another clone harbored intact *TP53* genes.

Patient	Clone with wild-type *TP53*				Clone with mutated *TP53*				
	IGHV and VH CDR3	**Karyotype**	**17p-**	**Over time tendency**	**IGHV and VH CDR3**	**Karyotype**	**17p-**	***TP53* mutation**	**Over time tendency**
261	IGHV1-69 (100 %) 29 aa	46,XY	No	↓	IGHV1-69 (100 %) 23 aa Subset 7	45,XY,-17; complex karyotype changes	Yes	p. M133K	→
279	IGHV3-21 (98.61 %) 9 aa Subset 2	46,XY,del(5)(q14.1q21.3)	No	↓	IGHV1-69 (100 %) 22 aa Subset 3	43,X,-Y,t(3;6)(q28;p12), der(4)t(4;21)(p15q11.2), der(5)t(Y;5)(q11.2;q21), t(12;14)(q13;q32.3),-17,-21, der(22)t(17;22)(q13;p11.2)	Yes	p. S215R	↑
319	IGHV3-30 (100 %) 13 aa IGHV4-39 (100 %) 16 aa	46,XX,del(11q),del(13q)	No	→	IGHV3-33 (100 %) 14 aa	46,XX	No	p. Y234C	↑
948	IGHV1-69 (100 %) 22 aa Subset 34	normal	No	↓	IGHV3-11 (100 %) 22 aa	del(13q), del(17p), -12	Yes	c. 782+1G>A	→
1072	IGHV3-21 (99.31 %) 20 aa	46,XX; 46,XX,del(13q)	No	↓	IGHV1-2 (100 %) 13 aa Subset 1	46,XX,del(13q),del(17p); 46,XX,del(17p)	Yes	p. F113V	→

FISH data available in all cases, karyotype data from CpG stimulated metaphase cytogenetics available in all but one (948) cases.

261 and 279: *TP53* gene defect selected in relapse after therapy. In patient 261, subclone with complex karyotype (-17, *TP53* gene mutation) was selected inside the *IGHV1-69*/23aa clone. Originally major 46,XY and *TP53*-wt fraction of the *IGHV1-69*/23aa clone diminished together with the other clone *IGHV1-69*/29aa. Only subclone *IGHV1-69*/23aa with inactivated *TP53* remained. For details see also Figure 2.

319: Populations sorted by FACS according to the light chain expression; genomic alterations were assigned to the clones subsequently using MLPA. Not possible to assess whether karyotype 46,XX,del(11q),del(13q) was associated with IGHV3-30 or IGHV4-39 clone or both, because of uniform Lambda light chain expression.

"↓" – diminishing; "→" – persisting; "↑" – appearing

FIGURE LEGENDS

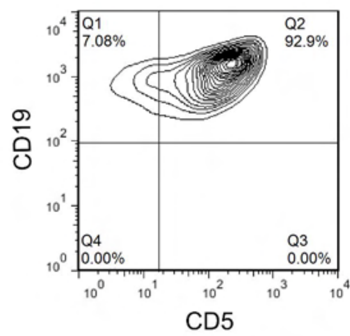
Figure 1. Immunophenotypig of MP-IGH CLL cases. In ***Patient 1132*** as an example of typical CLL immunophenotype, both malignant clones differed only in light chain isotype expression. ***Patient 862*** displayed typical immunophenotype of monoclonal CLL with IG kappa light chain expression. Nevertheless, changes in proportion of the two identified IGH rearrangements were observed (see Figure 2). In ***Patient 1037***, the malignant populations displayed distinct immunophenotypic features implying the co-existence of CLL/SLL with atypical CLL or other lymphoproliferative disorder. Mantle cell lymphoma was excluded in this patient because of Cyclin D1 negativity, and a diagnosis of SLL was established (bulky disease, absolute B lymphocyte count under 5,000 per µl). In ***Patient 1054***, two clonal populations were identified; the possibility of another indolent lymphoproliferative disease co-existing with CLL could not be excluded (CLL Matutes score for the atypical clone: 2 – 3).

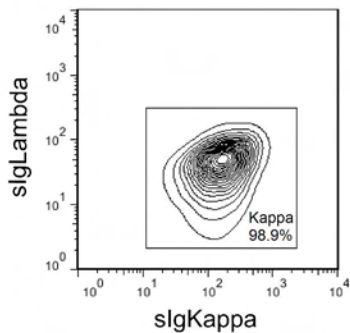
Figure 2. **Examples of evolution of biclonal/triclonal CLL.** Changes in the relative proportion of the malignant clones assessed using ASO-qPCR assays and flow-cytometry were usually accompanied by alterations in absolute count of clonal B lymphocytes (the additional axis "*B cell count*") and accelerated after therapy and/or by co-selection of unfavorable genomic aberrations, such as *TP53* defect. Time axes are scaled in *month/year*. ***Patient 604***: At diagnosis, three rearrangements – two with unmutated *IGHV1-69* (subset 5 with 20 amino acids in VH CDR3; non-stereotyped with VH CDR3 of 24 amino acids) and one with mutated *IGHV2-5* gene (in grey) – were assessed. The *IGHV2-5* gene rearrangement was minor and diminished during the disease course. Both *IGHV1-69* gene rearrangements have persisted over time. The patient did not receive any therapy during the follow-up. ***Patient 862***: At diagnosis, two IGHV-IGHD-IGHJ gene rearrangements utilizing unmutated *IGHV1-2* and *IGHV1-69* genes were detected. The *IGHV1-69* gene rearrangement was diminishing during the disease course. The patient did not receive any therapy during the follow-up. ***Patient 261***: Originally biclonal disease, both clones harbored

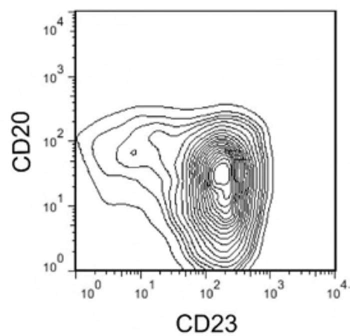
unmutated *IGHV1-69* gene rearrangements (subset 7 with VH CDR3 of 23 amino acids; non-stereotyped with 29 amino acids in VH CDR3). The proportion of the clones changed especially with therapy administration. After second-line therapy, one clone was diminishing and, at the same time, de novo *TP53* mutation/deletion was detected in the second persisting clone with stereotyped BCR. ***Patient 279***: Originally monoclonal CLL (unmutated *IGHV3-21*, subset 2); the second CLL clone harboring unmutated *IGHV1-69* (subset 3) and complex karyotype (including *TP53* defect) appeared in relapse of the disease after the first therapy. Selection of new malignant clone led to fulminant progression of the disease, and patient died of infection complications (†). mut – mutated *TP53* gene; wt – wild-type *TP53* gene, F – fludarabine, C – cyclophosphamide, R – rituximab, A – alemtuzumab.

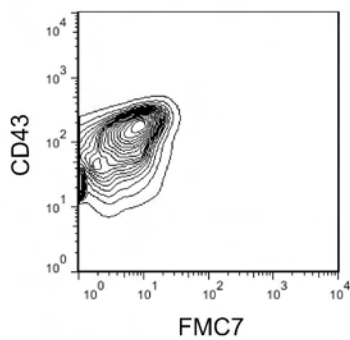
Figure 3. **Selection of higher *IGHV* gene identity and/or longer VH CDR3 in patients with clonal drift.** Each spot of the plot represents the difference in *IGHV* gene identity and VH CDR3 length in favor of the IG gene rearrangement that predominated in an individual patient during the disease course. Specifically, the position of a spot on the x-axis was calculated as the *IGHV* gene identity difference of persistent minus diminishing IG gene rearrangement in any individual patient, appearing minus persistent rearrangement, or appearing minus diminishing rearrangement, respectively. The position on the y-axis was calculated for VH CDR3 length difference in a similar way. White and black spots represent selection of stereotyped and non-stereotyped BcR IGs, respectively. The table lists the numbers of cases (counted per IG pairs) with similar behavior over time in relation to the preference/selection of the monitored immunogenetic features. Counts of cases with selection of a stereotyped BcR IG are shown in brackets.

Patient 862

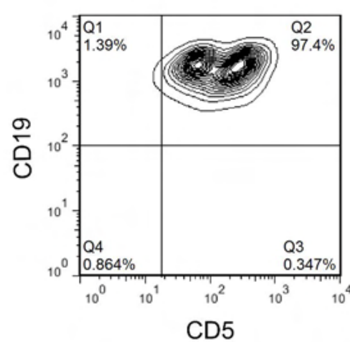


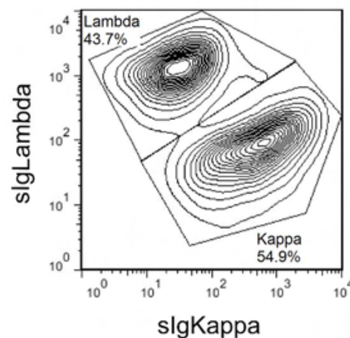


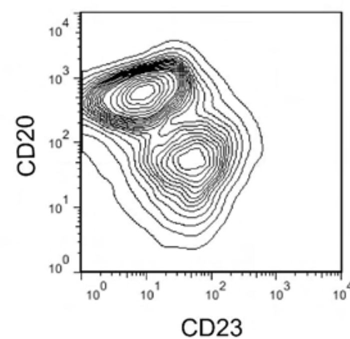


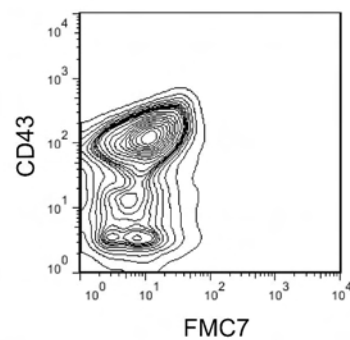


Patient 1037

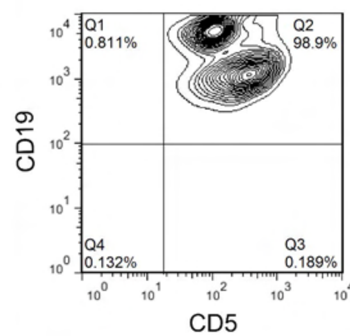


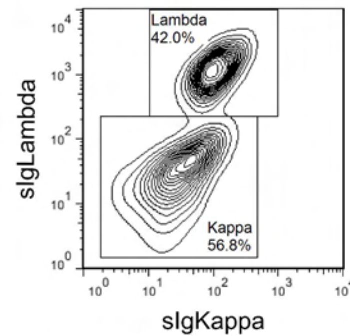


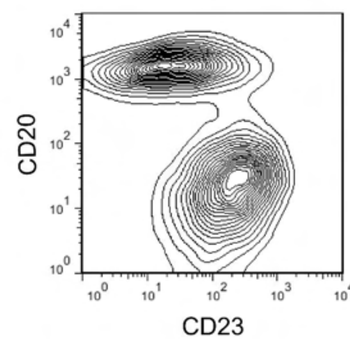


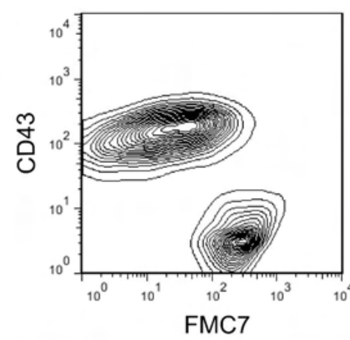


Patient 1054

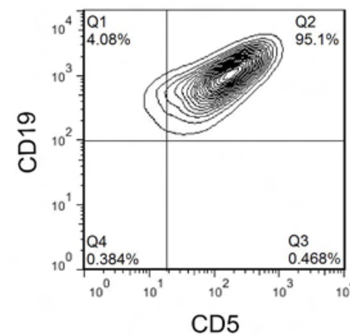


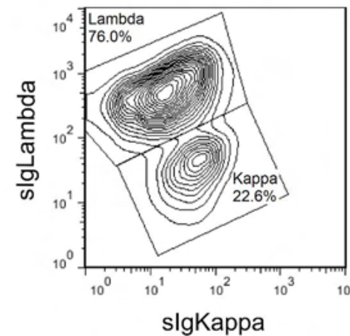


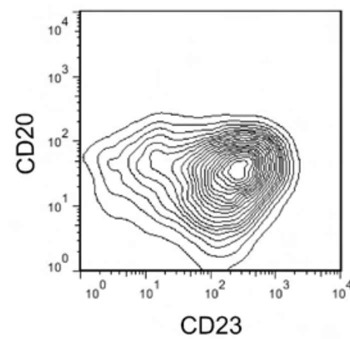


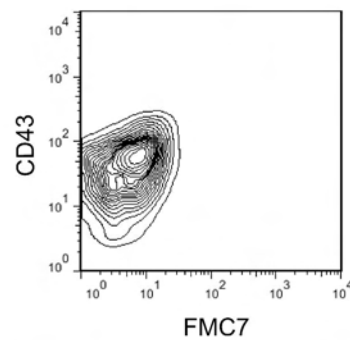


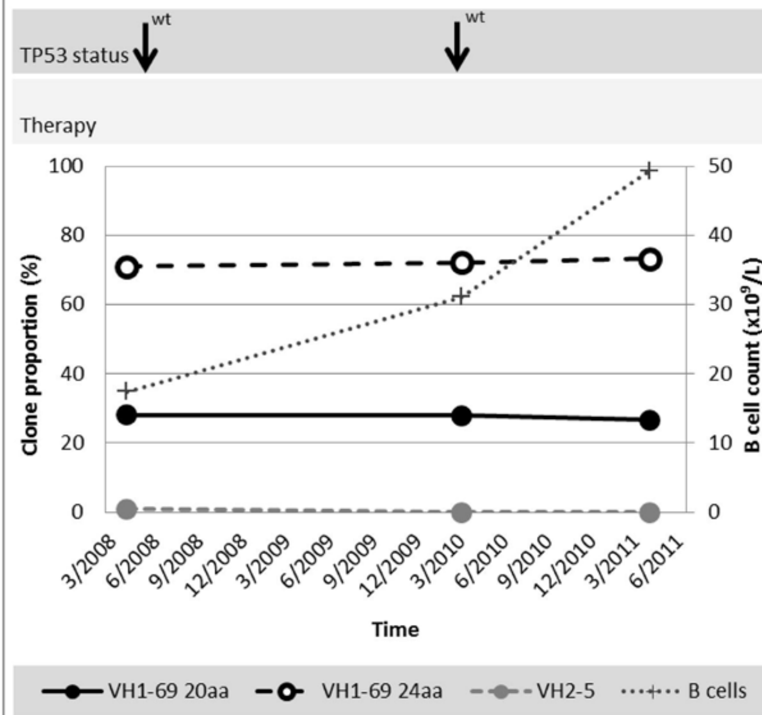
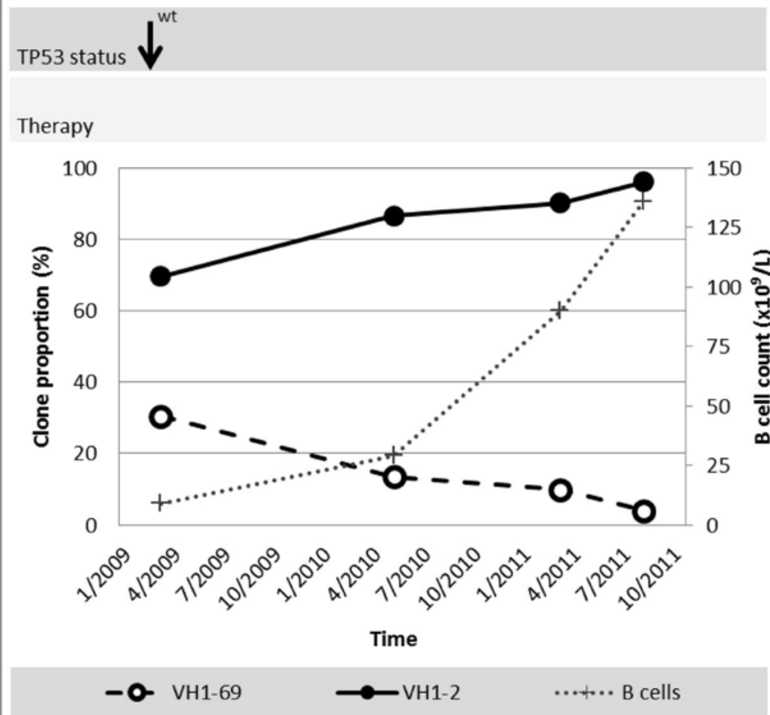
Patient 1132










Patient 604
TP53 status
wt
wt
Therapy
Clone proportion (%)
B cell count (x10⁹/L)
Time
VH1-69 20aa
VH1-69 24aa
VH2-5
B cells

Patient 862
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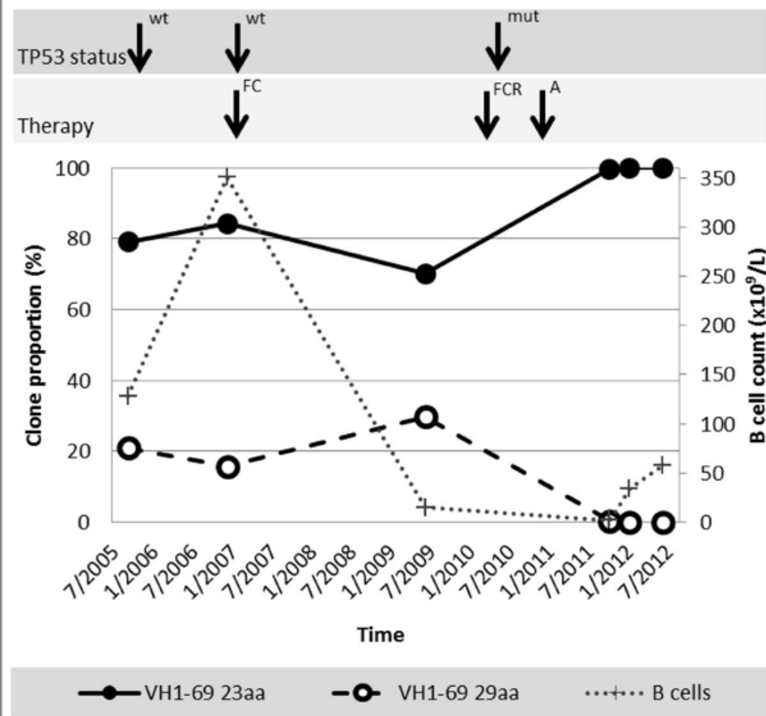
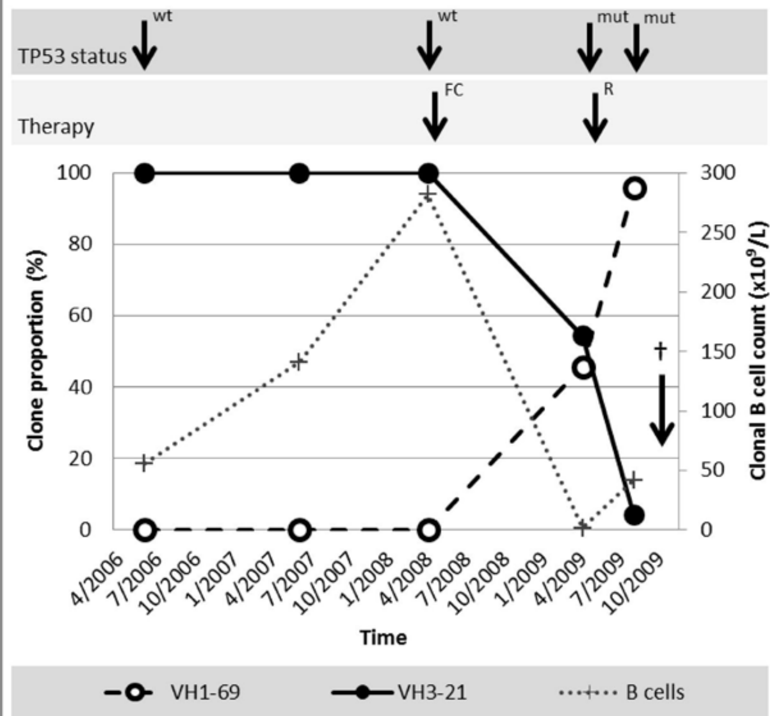
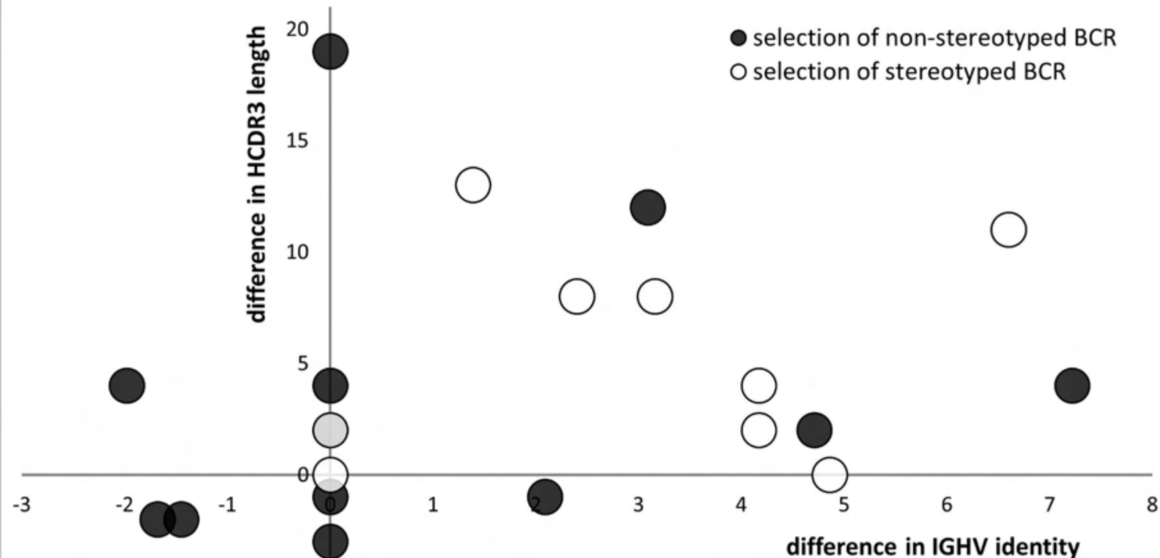
Therapy
Clone proportion (%)
B cell count (x10⁹/L)
Time
VH1-69
VH1-2
B cells

Patient 261
TP53 status
wt
wt
mut
Therapy
FC
FCR
A
Clone proportion (%)
B cell count (x10⁹/L)
Time
VH1-69 23aa
VH1-69 29aa
B cells

Patient 279
TP53 status
wt
wt
mut
mut
Therapy
FC
R
Clone proportion (%)
Clonal B cell count (x10⁹/L)
Time
VH1-69
VH3-21
B cells



preference	lower IGHV identity	equal IGHV identity	higher IGHV identity	Total
longer HCDR3	1 (0)	4 (1)	9 (5)	14 (58.3%)
equal HCDR3	0 (0)	1 (0)	1 (1)	2 (8.3%)
shorter HCDR3	3 (0)	3 (1)	2 (1)	8 (33.3%)
Total	4 (16.7%)	8 (33.3%)	12 (50%)	24

List of Tables and Figures

Table/Figure	Table/Figure Content	Abbreviation	Comment
Supplementary Table S1	Detailed characteristics of MP-IGH cases	A	appearing clone
Supplementary Table S1	Detailed characteristics of MP-IGH cases	D	diminishing clone
Supplementary Table S1	Detailed characteristics of MP-IGH cases	Dis	discordant IGHV mutational status
Supplementary Table S1	Detailed characteristics of MP-IGH cases	M	mutated *IGHV*
Supplementary Table S1	Detailed characteristics of MP-IGH cases	mut	mutated *TP53* gene
Supplementary Table S1	Detailed characteristics of MP-IGH cases	N	clonal drift not known
Supplementary Table S1	Detailed characteristics of MP-IGH cases	NA	not available
Supplementary Table S1	Detailed characteristics of MP-IGH cases	P	persistent clone
Supplementary Table S1	Detailed characteristics of MP-IGH cases	U	unmutated *IGHV*
Supplementary Table S1	Detailed characteristics of MP-IGH cases	wt	wild type *TP53* gene
Supplementary Table S2	Comparison of IG repertoire between groups of patients with single and multiple IG rearrangements	mono	CLL cases with single IGH rearrangement
Supplementary Table S2	Comparison of IG repertoire between groups of patients with single and multiple IG rearrangements	oligo	CLL cases with multiple IGH rearrangements
Supplementary Table S3	Molecular features of co-existing IGH rearrangement pairs	A	appearing clone
Supplementary Table S3	Molecular features of co-existing IGH rearrangement pairs	D	diminishing clone
Supplementary Table S3	Molecular features of co-existing IGH rearrangement pairs	Dis	discordant IGHV mutational status
Supplementary Table S3	Molecular features of co-existing IGH rearrangement pairs	M	mutated *IGHV*
Supplementary Table S3	Molecular features of co-existing IGH rearrangement pairs	N	clonal drift not known
Supplementary Table S3	Molecular features of co-existing IGH rearrangement pairs	P	persistent clone
Supplementary Table S3	Molecular features of co-existing IGH rearrangement pairs	U	unmutated *IGHV*
Supplementary Table S4	Complete list of IG rearrangements detected in individual patients		
Supplementary Table S5	Comparison of clone proportions detected using ASO-qPCR and flow cytometry	ASO-qPCR	allele-specific oligonucleotide assays for quantitative real-time PCR
Supplementary Table S5	Comparison of clone proportions detected using ASO-qPCR and flow cytometry	NA	not available
Supplementary Table S5	Comparison of clone proportions detected using ASO-qPCR and flow cytometry	NCP	no clonal population detected
Supplementary Table S5	Comparison of clone proportions detected using ASO-qPCR and flow cytometry	R1	IGH rearrangement 1
Supplementary Table S5	Comparison of clone proportions detected using ASO-qPCR and flow cytometry	R2	IGH rearrangement 2
Supplementary Table S5	Comparison of clone proportions detected using ASO-qPCR and flow cytometry	R3	IGH rearrangement 3
Supplementary Figure S1A	Male - Female ratio in the studied cohort compared to a control cohort of patients with single IG rearrangement		
Supplementary Figure S1B	Distribution of Rai stage at CLL diagnosis in the studied cohort compared to a control cohort of patients with single IG rearrangement	NA	not available
Supplementary Figure S1C	Absolute Count of Clonal B cells in the studied cohort compared to a control cohort of patients with single IG rearrangement	mono	CLL cases with single IGH rearrangement
Supplementary Figure S1C	Absolute Count of Clonal B cells in the studied cohort compared to a control cohort of patients with single IG rearrangement	oligo	CLL cases with multiple IGH rearrangements
Supplementary Figure S1D	Need for treatment in the studied cohort compared to a control cohort of patients with single IG rearrangement		
Supplementary Figure S1E	Overall IGHV mutational status in the studied cohort compared to a control cohort of patients with single IG rearrangement		
Supplementary Figure S1F	Distribution of chromosomal defects detected by interphase FISH in the studied cohort compared to a control cohort of patients with single IG rearrangement	NA	not available
Supplementary Figure S1G	Frequency of *TP53* mutations in the studied cohort compared to a control cohort of patients with single IG rearrangement	wt	wild type *TP53* gene
Supplementary Figure S1G	Frequency of *TP53* mutations in the studied cohort compared to a control cohort of patients with single IG rearrangement	mut	mutated *TP53* gene
Supplemental Figure S2	Overview of immunogenetic and immunophenotypic analyses in MP-IGH cases		

Supplemental Table S1

Patient ID	Case characteristics: gender	age at diagnosis	Rai stage at diagnosis	therapy administration	time to first therapy (months)	disease follow up (months)	current status	CLL related death	IGH analysis: overall IGHV status	max No. of IGHs per patient	combination of IGHV subgroups	follow up of IGHV testing (months)	TP53 gene and FISH analysis at diagnosis/prior therapy: TP53 status	13q deletion at diagnosis (% by FISH)	17q deletion at diagnosis (% by FISH)	11p deletion at diagnosis (% by FISH)	trisomy 12 at diagnosis (% by FISH)	Immunogenetic and immunophenotypic analysis: DNA analysis	flow cytometry analysis	over time analysis	clonal drift observed
BRNO0261	male	62	I	yes	31	105	dead	yes	U	2	1+1	74	wt	0	0	0	0	yes	yes	yes	yes
BRNO0279	male	72	0	yes	39	58	dead	yes	U	2	1+3	45	wt	0	0	0	0	yes	yes	yes	yes
BRNO0307	male	73	I	yes	19	31	dead	yes	Dis	2	1+3	0	NA	0	0	0	0	yes	yes	no	
BRNO0319	female	70	0	no		91	alive		U	3	3+3+4	67	wt	62	0	67	0	yes	yes	yes	yes
BRNO0447	male	74	I	no		52	alive		M	2	3+4	37	wt	NA	NA	NA	NA	no	no	yes	yes
BRNO0511	male	58	0	no		132	dead	no	U	3	1+1+1	2	wt	69	0	0	12	yes	no	no	
BRNO0523	male	35	I	yes	43	64	alive		M	2	5+6	43	wt	85	0	0	0	yes	yes	yes	yes
BRNO0604	male	61	0	no		65	alive		Dis	3	1+1+2	37	wt	20	0	35	0	yes	yes	yes	yes
BRNO0625	male	74	0	no		172	dead	no	Dis	2	1+4	35	NA	64	0	0	0	yes	yes	yes	no
BRNO0814	male	70	I	yes	12	23	dead	no	U	2	1+5	3	wt	0	0	0	0	yes	no	no	
BRNO0833	male	71	IV	yes	0	47	alive		Dis	2	1+2	15	wt	0	0	0	0	no	no	yes	yes
BRNO0845	male	69	II	yes	97	142	dead	no	U	2	3+5	30	wt	0	0	0	63	yes	no	yes	yes
BRNO0846	female	57	I	yes	15	52	alive		Dis	2	1+4	12	wt	40	0	78	0	yes	yes	yes	yes
BRNO0862	female	53	I	yes	37	51	alive		U	2	1+1	29	wt	0	0	0	35	yes	yes	yes	yes
BRNO0877	female	78	NA	yes	12	37	alive		M	2	2+3	0	NA	NA	NA	NA	NA	no	no	no	
BRNO0885	female	65	0	no		101	alive		U	2	1+2	24	wt	11	0	0	0	yes	yes	yes	yes
BRNO0892	female	59	0	no		50	alive		M	2	3+4	26	wt	NA	NA	NA	NA	yes	yes	yes	yes
BRNO0912	female	59	0	no		74	alive		Dis	2	3+4	16	wt	83	0	0	0	yes	no	yes	yes
BRNO0922	male	69	II	yes	4	47	alive		M	2	1+3	0	wt	0	0	0	36	no	no	no	
BRNO0923	female	74	I	yes	9	16	dead	yes	U	2	1+4	8	wt	75	0	0	0	yes	yes	yes	no
BRNO0948	male	70	0	no		27	dead	no	U	2	1+3	24	mut	18	53	0	45	yes	yes	yes	yes
BRNO0974	female	74	0	no		25	alive		M	2	1+3	20	NA	84	0	0	0	yes	yes	no	
BRNO1029	male	69	0	yes	4	42	alive		U	2	3+4	0	wt	0	0	0	9	yes	yes	no	
BRNO1030	male	72	I	yes	84	93	alive		Dis	2	1+4	19	wt	0	0	0	0	yes	yes	yes	yes
BRNO1037	male	59	I	no		91	alive		Dis	3	1+3+4	12	NA	NA	NA	NA	NA	yes	yes	yes	yes
BRNO1049	male	64	0	no		14	alive		Dis	3	1+3+4	11	NA	NA	NA	NA	NA	yes	yes	yes	yes
BRNO1054	male	66	NA	no		19	alive		M	2	3+5	13	wt	17	0	0	0	yes	yes	yes	no
BRNO1072	female	67	0	yes	15	20	dead	yes	U	2	1+3	14	mut	71	62	0	0	yes	yes	yes	yes
BRNO1087	female	71	I	yes	15	35	alive		Dis	2	3+6	12	wt	40	0	0	0	yes	yes	yes	yes
BRNO1132	female	58	I	no		85	alive		M	2	3+3	0	wt	NA	0	0	0	yes	yes	no	
BRNO1137	male	58	IV	yes	2	28	alive		M	2	2+3	3	NA	NA	NA	NA	NA	no	no	no	

Patient ID	Clone 1: V-GENE and allele (1)	V-REGION identity % (1)	D-GENE and allele (1)	J-GENE and allele (1)	CDR3-IMGT length (1)	AA JUNCTION (1)	stereotype subset (1)	over time tendency (1)
BRNO0261	IGHV1-69*01	100	IGHD3-3*01	IGHJ6*02	23	CATPPYDFWSGYYPNYYYYGMDVW	7	P
BRNO0279	IGHV1-69*01	100	IGHD3-3*02	IGHJ6*02	22	CARDRPDYVVPADYYYYGMDVW	3	A
BRNO0307	IGHV1-3*01	98,61	IGHD3-10*01	IGHJ4*02	13	CARVQWFGVYYFDYW	1	N
BRNO0319	IGHV3-30*03, or IGHV3-30*18	100	IGHD6-13*01	IGHJ4*02	13	CAKAREQQLPFFDYW		A
BRNO0447	IGHV4-34*01	94,04	IGHD5-5*01	IGHJ4*02	15	CASKFRGNTYGYYFDSW		D
BRNO0511	IGHV1-2*02	100	IGHD3-3*01	IGHJ6*02	26	CARDTRDDDFWSGYLPEYYYYYGMDVW		N
BRNO0523	IGHV5-51*01	94,44	IGHD3-16*01	IGHJ6*02	27	CARLAQPPMYYWRSDHTKKYSYYGMDVW		D
BRNO0604	IGHV1-69*01	100	IGHD2-15*01	IGHJ3*02	24	CARLFPPYCSGGSCYDWAPSGAFDIW		P
BRNO0625	IGHV4-34*01	99,30	IGHD3-22*01	IGHJ4*02	17	CARAGGRKDSGYYRSFDYW		P
BRNO0814	IGHV1-69*01	100	IGHD3-22*01	IGHJ6*02	18	CAREVGIVATLYYYYGMDVW		N
BRNO0833	IGHV1-69*01	100	IGHD1-21*01	IGHJ6*02	21	CARVADVEGLLLLGNYYYGMDVW		P
BRNO0845	IGHV3-30*03, or IGHV3-30*18	100	IGHD2-2*01	IGHJ6*02	26	CAKDLTPTYEYCSSTSCYYYYYYGMDVW		P
BRNO0846	IGHV1-69*01	100	IGHD2-2*01	IGHJ6*02	22	CARDPDIVVPAAMYYYYYGMDVW	3	P
BRNO0862	IGHV1-2*02	100	IGHD2-8*01	IGHJ6*02	33	CARSPLGYCTNGVCYDPPWVAAAGTGDYYYYGMDVW		P
BRNO0877	IGHV3-21*01	97,22	IGHD4-4*01	IGHJ6*02	19	CARDHGYSYYDDYYYYGMDVW		N
BRNO0885	IGHV2-70*01	100	IGHD3-22*01	IGHJ6*02	27	CARTLPGGYYYDSSGYYYPLNYYYGMDVW		P
BRNO0892	IGHV4-61*01	94,85	IGHD6-13*01	IGHJ4*02	15	CARDHRGYSSPWPFDNW		D
BRNO0912	IGHV4-34*01	100	IGHD3-3*01	IGHJ6*03	23	CARVGTYYDFWSGYSPYYYYMDVW	B1	P
BRNO0922	IGHV1-69*01	96,18	IGHD2-8*01	IGHJ4*02	17	CAREGTQYCTKDVCYMLYW		N
BRNO0923	IGHV1-3*01	100	IGHD6-19*01	IGHJ4*02	13	CAREQWLVTPNFDYW	1	P
BRNO0948	IGHV3-11*01	100	IGHD2-8*01	IGHJ6*02	22	CARDGWGYVPSBGYGYYYYGMDVW		P
BRNO0974	IGHV3-9*01	96,53	IGHD6-19*01	IGHJ4*02	13	CAKDGSIAVGPGDW		N
BRNO1029	IGHV3-23*01	100	IGHD3-10*01	IGHJ3*02	24	CAKDRGSPRIPLLWFGELGDGAFDIW		N
BRNO1030	IGHV1-69*01	100	IGHD2-2*01	IGHJ5*02	22	CAREAEDIVVVPAAWSVYNWFDPW		P
BRNO1037	IGHV4-39*01	98,97	IGHD3-9*01	IGHJ6*02	11	CASRDWGSGEDYW		P
BRNO1049	IGHV4-39*01	100	IGHD6-13*01	IGHJ5*02	19	CARRQGYSSWYGRSNWFDPW	8	A
BRNO1054	IGHV5-51*01	95,14	IGHD5-5*01	IGHJ4*02	16	CARLRYYGLSTSPADYW		N
BRNO1072	IGHV1-2*02	100	IGHD6-19*01	IGHJ4*02	13	CAREQWLALSHFDYW	1	P
BRNO1087	IGHV3-43*01	100	IGHD6-13*01	IGHJ6*02	14	CAKGASGDYYCMDVW		P
BRNO1132	IGHV3-23*01	94,44	IGHD6-13*01	IGHJ4*01, or IGHJ4*02	14	CAKEGAGTGLLDFDSW		N
BRNO1137	IGHV2-5*04, or IGHV2-5*07 or IGHV2-5*10	94,16	IGHD6-19*01	IGHJ5*01	17	CAYREKNSDWDGGCFNHW	148B	N

Patient ID	Clone 2: V-GENE and allele (2)	V-REGION identity % (2)	D-GENE and allele (2)	J-GENE and allele (2)	CDR3-IMGT length (2)	AA JUNCTION (2)	stereotype subset (2)	over time tendency (2)
BRNO0261	IGHV1-69*01	100	IGHD3-3*01	IGHJ6*02	29	CARPIILYDFWSGYYFGNGTDPYYYYGMDVW		D
BRNO0279	IGHV3-21*01	98,61	IGHD5-24*01	IGHJ6*02	9	CARDTDGMDVW	2	D
BRNO0307	IGHV3-23*01	89,93	IGHD1-1*01	IGHJ1*01	13	CAKADSNVEQYFRHW		N
BRNO0319	IGHV3-33*01, or IGHV3-33*06	100	IGHD4-17*01	IGHJ6*02	14	CARGQHGDYVYGMDVW		P
BRNO0447	IGHV3-74*01	92,36	IGHD6-13*01	IGHJ5*02	13	CVRGAIGSSVWFDPW		P
BRNO0511	IGHV1-69*01	100	IGHD3-3*01	IGHJ6*02	24	CARDLYDFWSGYRPTGAYYYYGMDVW	7H	N
BRNO0523	IGHV6-1*01	93,27	IGHD1-1*01	IGHJ4*02	10	CARGSIGIFDYW		P
BRNO0604	IGHV1-69*01	99,31	IGHD3-10*01	IGHJ6*03	20	CARAMVQGVISYYYYYYYMDVW	5	P
BRNO0625	IGHV1-69*01, or IGHV1-69*12	93,06	IGHD5-5*01	IGHJ4*02	15	CARVLRGYSYAEPLDYW		P
BRNO0814	IGHV5-51*01	100	IGHD6-19*01	IGHJ4*02	13	CARQQWLGNYYFDYW	1	N
BRNO0833	IGHV2-5*10	92,78	IGHD3-22*01	IGHJ4*02	17	CARTYFSDSSGYHKSFDCW		D
BRNO0845	IGHV5-51*03	100	IGHD5-5*01	IGHJ6*02	24	CARHAEWIQLWHPPRVYYYYYGMDVW		D
BRNO0846	IGHV4-59*01	96,84	IGHD1-26*01	IGHJ4*02	14	CARTDYSGSYYYFDSW		D
BRNO0862	IGHV1-69*01	100	IGHD2-21*02	IGHJ6*02	14	CASYGGDCYNLCMDVW		D
BRNO0877	IGHV2-5*10	93,47	IGHD3-10*01	IGHJ4*02	18	CAHRSPNSGSYWDGGYFDYW	148C	N
BRNO0885	IGHV1-69*01	100	IGHD2-2*01	IGHJ6*03	23	CARDNSDCSSTSCYEFYYYYYMDVW		D
BRNO0892	IGHV3-33*01, or IGHV3-33*03 or IGHV3-33*06	93,40	IGHD3-3*01	IGHJ3*02	13	CARDNPRVVRALEIW		P
BRNO0912	IGHV3-7*02, or IGHV3-7*03	95,14	IGHD2-15*01	IGHJ3*02	23	CARGDFVVVVGGTSYGDFIDAFDIW		D
BRNO0922	IGHV3-49*03, or IGHV3-49*05	94,56	IGHD1-7*01	IGHJ4*02	17	CARDPTSAYNWNLRHFDYW		N
BRNO0923	IGHV4-34*01	100	IGHD3-9*01	IGHJ6*02	17	CARLLAGAYYYYYYGMDVW	205	P
BRNO0948	IGHV1-69*01	100	IGHD3-10*02	IGHJ6*02	22	CARESAYYDILTGHNYYYYGMDVW	34	D
BRNO0974	IGHV1-8*01	95,14	IGHD3-22*01	IGHJ4*02	16	CAGGLGRYNDGNGLRDCW		N
BRNO1029	IGHV4-b*02	99,65	IGHD2-21*01	IGHJ4*02	18	CARDTIHSTLDRLGYRFDYW		N
BRNO1030	IGHV4-4*02	93,40	IGHD3-10*01	IGHJ3*01, or IGHJ3*02	11	CARDEGFQLLPLW	219	D
BRNO1037	IGHV1-8*01	96,88	IGHD4-17*01	IGHJ4*02	12	CARGWAYGDNADYW		D
BRNO1049	IGHV1-3*01	100	IGHD1-26*01	IGHJ6*02	17	CARMYSGSYYYYYYGMDVW	28A	P
BRNO1054	IGHV3-23*04	93,75	IGHD6-13*01	IGHJ6*03	17	CAKLGQHLVQIYYYHIDVW		P
BRNO1072	IGHV3-21*01	99,31	IGHD3-3*01	IGHJ6*02	20	CARDIRSVIIWSGQQNYGMDVW		D
BRNO1087	IGHV6-1*01	95,29	IGHD4-17*01	IGHJ4*02	12	CARDAGGGYLFDYW		D
BRNO1132	IGHV3-7*01	94,10	IGHD2-21*02	IGHJ6*02	17	CALSGLDLDYYYYYDMDVW		N
BRNO1137	IGHV3-30-3*01	92,36	IGHD1-7*01	IGHJ1*01	16	CVREDCNNCHCYKNFQLW		N

Patient ID	Clone 3: V-GENE and allele (3)	V-REGION identity % (3)	D-GENE and allele (3)	J-GENE and allele (3)	CDR3-IMGT length (3)	AA JUNCTION (3)	stereotype subset (3)	over time tendency (3)
BRNO0261								
BRNO0279								
BRNO0307								
BRNO0319	IGHV4-39*01	100	IGHD3-3*02	IGHJ4*02	16	CASDPGLGSRMRSYFDYW		P
BRNO0447								
BRNO0511	IGHV1-69*01	100	IGHD2-8*01	IGHJ6*02	27	CARAARPYCTNGVCYLEIAAYYYYGMDVW		N
BRNO0523								
BRNO0604	IGHV2-5*10	96,91	IGHD2-21*01	IGHJ4*02, or IGHJ4*03	12	CAHRRISIGGFDYW		D
BRNO0625								
BRNO0814								
BRNO0833								
BRNO0845								
BRNO0846								
BRNO0862								
BRNO0877								
BRNO0885								
BRNO0892								
BRNO0912								
BRNO0922								
BRNO0923								
BRNO0948								
BRNO0974								
BRNO1029								
BRNO1030								
BRNO1037	IGHV3-72*01	94,90	IGHD5-5*01	IGHJ4*02	16	CASFTAKFGSNIRKFDYW		P
BRNO1049	IGHV3-33*01, or IGHV3-33*06	95,83	IGHD4-17*01	IGHJ4*02	15	CVRSVYGDGNREDFDYW		D
BRNO1054								
BRNO1072								
BRNO1087								
BRNO1132								
BRNO1137								

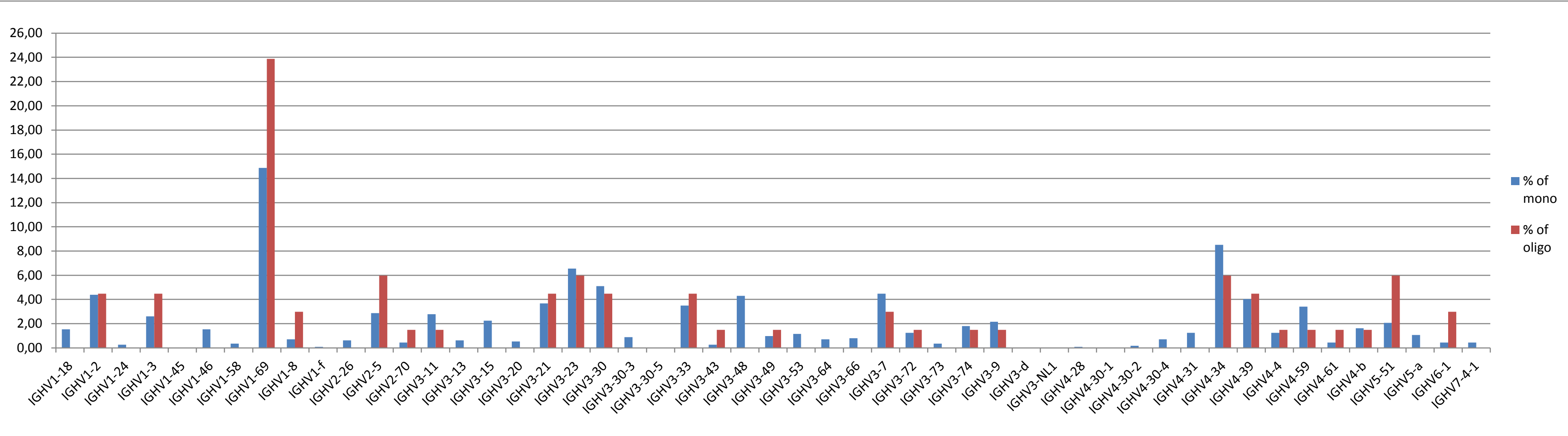
Supplemental Table S2 - part A

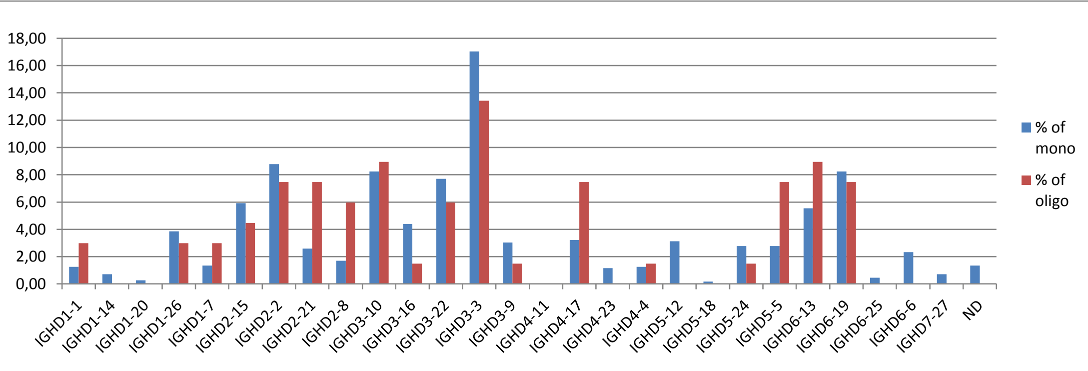
IGHV gene	# of mono cases	% of mono	# of oligo cases	% of oligo
IGHV1-18	17	1,52	0	0
IGHV1-2	49	4,39	3	4,48
IGHV1-24	3	0,27	0	0
IGHV1-3	29	2,60	3	4,48
IGHV1-45	0	0	0	0
IGHV1-46	17	1,52	0	0
IGHV1-58	4	0,36	0	0
IGHV1-69	166	14,87	16	23,88
IGHV1-8	8	0,72	2	2,99
IGHV1-f	1	0,09	0	0
IGHV2-26	7	0,63	0	0
IGHV2-5	32	2,87	4	5,97
IGHV2-70	5	0,45	1	1,49
IGHV3-11	31	2,78	1	1,49
IGHV3-13	7	0,63	0	0
IGHV3-15	25	2,24	0	0
IGHV3-20	6	0,54	0	0
IGHV3-21	41	3,67	3	4,48
IGHV3-23	73	6,54	4	5,97
IGHV3-30	57	5,11	3	4,48
IGHV3-30-3	10	0,90	0	0
IGHV3-30-5	0	0	0	0
IGHV3-33	39	3,49	3	4,48
IGHV3-43	3	0,27	1	1,49
IGHV3-48	48	4,30	0	0
IGHV3-49	11	0,99	1	1,49
IGHV3-53	13	1,16	0	0
IGHV3-64	8	0,72	0	0
IGHV3-66	9	0,81	0	0
IGHV3-7	50	4,48	2	2,99
IGHV3-72	14	1,25	1	1,49
IGHV3-73	4	0,36	0	0
IGHV3-74	20	1,79	1	1,49
IGHV3-9	24	2,15	1	1,49
IGHV3-d	0	0	0	0
IGHV3-NL1	0	0	0	0
IGHV4-28	1	0,09	0	0
IGHV4-30-1	0	0	0	0
IGHV4-30-2	2	0,18	0	0
IGHV4-30-4	8	0,72	0	0
IGHV4-31	14	1,25	0	0
IGHV4-34	95	8,51	4	5,97
IGHV4-39	45	4,03	3	4,48
IGHV4-4	14	1,25	1	1,49
IGHV4-59	38	3,41	1	1,49
IGHV4-61	5	0,45	1	1,49
IGHV4-b	18	1,61	1	1,49
IGHV5-51	23	2,06	4	5,97
IGHV5-a	12	1,08	0	0
IGHV6-1	5	0,45	2	2,99
IGHV7-4-1	5	0,45	0	0
	1116	**100**	**67**	**100**

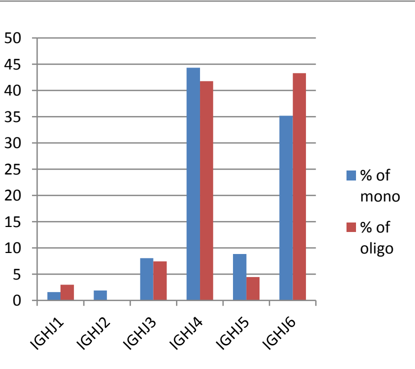
IGHD gene	# of mono cases	% of mono	# of oligo cases	% of oligo
IGHD1-1	14	1,25	2	2,99
IGHD1-14	8	0,72	0	0
IGHD1-20	3	0,27	0	0
IGHD1-26	43	3,85	2	2,99
IGHD1-7	15	1,34	2	2,99
IGHD2-15	66	5,91	3	4,48
IGHD2-2	98	8,78	5	7,46
IGHD2-21	29	2,60	5	7,46
IGHD2-8	19	1,70	4	5,97
IGHD3-10	92	8,24	6	8,96
IGHD3-16	49	4,39	1	1,49
IGHD3-22	86	7,71	4	5,97
IGHD3-3	190	17,03	9	13,43
IGHD3-9	34	3,05	1	1,49
IGHD4-11	0	0	0	0
IGHD4-17	36	3,23	5	7,46
IGHD4-23	13	1,16	0	0
IGHD4-4	14	1,25	1	1,49
IGHD5-12	35	3,14	0	0
IGHD5-18	2	0,18	0	0
IGHD5-24	31	2,78	1	1,49
IGHD5-5	31	2,78	5	7,46
IGHD6-13	62	5,56	6	8,96
IGHD6-19	92	8,24	5	7,46
IGHD6-25	5	0,45	0	0
IGHD6-6	26	2,33	0	0
IGHD7-27	8	0,72	0	0
ND	15	1,34	0	0
	1116	**100**	**67**	**100**

IGHJ gene	# of mono cases	% of mono	# of oligo cases	% of oligo
IGHJ1	18	1,61	2	2,99
IGHJ2	21	1,88	0	0
IGHJ3	90	8,06	5	7,46
IGHJ4	495	44,35	28	41,79
IGHJ5	99	8,87	3	4,48
IGHJ6	393	35,22	29	43,28
	1116	**100**	**67**	**100**

Supplemental Table S2 - part B







Supplemental Table S3

Patient ID	Rearrangement 1								Rearrangement 2								Results of detailed analysis											
	V-GENE and allele (1)	V-REGION identity % (1)	D-GENE and allele (1)	J-GENE and allele (1)	CDR3-IMGT length (1)	AA JUNCTION (1)	Stereotyped subset (1)	Over time tendency (1)	V-GENE and allele (2)	V-REGION identity % (2)	D-GENE and allele (2)	J-GENE and allele (2)	CDR3-IMGT length (2)	AA JUNCTION (2)	Stereotyped subset (2)	Over time tendency (2)	combination of IGHV subgroups	phylogenetic relation of used IGHV alleles	difference in mutational status	difference in HCDR3 length	M-IGHV (1) = 1; U-IGHV (1) = 0	M-IGHV (2) = 1; U-IGHV (2) = 0	resulting mutational status	at least one stereotyped HCRD3	over time analysis	clonal drift observed	preference of stereotyped BcR	diminishing of stereotyped BcR
BRNO0261	IGHV1-69*01	100	IGHD3-3*01	IGHJ6*02	23	CATPPYYDFWSGYYPNYYYYGMDVW	7	P	IGHV1-69*01	100	IGHD3-3*01	IGHJ6*02	29	CARPIILYDFWSGYYFGNGTDPYYYYGMDVW		D	1+1	0	0,00	6	0	0	U	yes	yes	yes	yes	no
BRNO0279	IGHV1-69*01	100	IGHD2-2*02	IGHJ6*02	22	CARDRPDIVVVPADIYYYYGMDVW	3	A	IGHV3-21*01	98,61	IGHD5-24*01	IGHJ6*02	9	CARDTDGMDVW	2	D	1+3	4	1,39	13	0	0	U	yes	yes	yes	yes	yes
BRNO0307	IGHV1-3*01	98,61	IGHD3-10*01	IGHJ4*02	13	CARVQWFGVYYFDYW	1	N	IGHV3-23*01	89,93	IGHD1-1*01	IGHJ1*01	13	CAKADSNVEQYFRHW		N	1+3	4	8,68	0	0	1	Dis	yes	no			
BRNO0319	IGHV3-30*03, or IGHV3-30*18	100	IGHD6-13*01	IGHJ4*02	13	CAKAREQQLPPEDYW		A	IGHV3-33*01, or IGHV3-33*06	100	IGHD4-17*01	IGHJ6*02	14	CARGQHGDYVYGMDVW		P	3+3	2	0,00	1	0	0	U	no	yes	yes	no	no
BRNO0319	IGHV3-30*03, or IGHV3-30*18	100	IGHD6-13*01	IGHJ4*02	13	CAKAREQQLPPEDYW		A	IGHV4-39*01	100	IGHD3-3*02	IGHJ4*02	16	CASDPGLGSRMRSYFDYW		P	3+4	4	0,00	3	0	0	U	no	yes	yes	no	no
BRNO0319	IGHV3-33*01, or IGHV3-33*06	100	IGHD4-17*01	IGHJ6*02	14	CARGQHGDYVYGMDVW		P	IGHV4-39*01	100	IGHD3-3*02	IGHJ4*02	16	CASDPGLGSRMRSYFDYW		P	3+4	4	0,00	2	0	0	U	no	yes	no		
BRNO0447	IGHV3-74*01	92,36	IGHD6-13*01	IGHJ5*02	13	CVRGAIGSSVWFDPW		P	IGHV4-34*01	94,04	IGHD5-5*01	IGHJ4*02	15	CASRFRGNTYGYYFDSW		D	3+4	4	1,68	2	1	1	M	no	yes	yes	no	no
BRNO0511	IGHV1-2*02	100	IGHD3-3*01	IGHJ6*02	26	CARDTRDDDFWSGYLPEYYYYYGMDVW		N	IGHV1-69*01	100	IGHD3-3*01	IGHJ6*02	24	CARDLYDFWSGYRPTGAYYYYGMDVW	7	N	1+1	2	0,00	2	0	0	U	yes	no			
BRNO0511	IGHV1-2*02	100	IGHD3-3*01	IGHJ6*02	26	CARDTRDDDFWSGYLPEYYYYYGMDVW		N	IGHV1-69*01	100	IGHD2-8*01	IGHJ6*02	27	CARAARPYCTNGVCYLEIAAYYYYGMDVW		N	1+1	2	0,00	1	0	0	U	no	no			
BRNO0511	IGHV1-69*01	100	IGHD3-3*01	IGHJ6*02	24	CARDLYDFWSGYRPTGAYYYYGMDVW	7	N	IGHV1-69*01	100	IGHD2-8*01	IGHJ6*02	27	CARAARPYCTNGVCYLEIAAYYYYGMDVW		N	1+1	0	0,00	3	0	0	U	yes	no			
BRNO0523	IGHV5-51*01	94,44	IGHD3-16*01	IGHJ6*02	27	CARLAQPPMKYVWRSDHTKKYSYFGMDVW		D	IGHV6-1*01	93,27	IGHD1-1*01	IGHJ4*02	10	CARGSIGIFDYW		P	5+6	4	1,17	17	1	1	M	no	yes	yes	no	no
BRNO0604	IGHV1-69*01	99,31	IGHD3-10*01	IGHJ6*03	20	CARAMVQGVISYYYYYYYMDVW	5	P	IGHV1-69*01	100	IGHD2-15*01	IGHJ3*02	24	CARLFPPYCSGGSCYGWAPSGAFDIW		P	1+1	0	0,69	4	0	0	U	yes	yes	no		
BRNO0604	IGHV1-69*01	99,31	IGHD3-10*01	IGHJ6*03	20	CARAMVQGVISYYYYYYYMDVW	5	P	IGHV2-5*10	96,91	IGHD2-21*01	IGHJ4*02, or IGHJ4*03	12	CAHRRISIGGFDYW		D	1+2	4	2,40	8	0	1	Dis	yes	yes	yes	yes	no
BRNO0604	IGHV1-69*01	100	IGHD2-15*01	IGHJ3*02	24	CARLFPPYCSGGSCYGWAPSGAFDIW		P	IGHV2-5*10	96,91	IGHD2-21*01	IGHJ4*02, or IGHJ4*03	12	CAHRRISIGGFDYW		D	1+2	4	3,09	12	0	1	Dis	no	yes	yes	no	no
BRNO0625	IGHV1-69*01, or IGHV1-69*12	93,06	IGHD5-5*01	IGHJ4*02	15	CARVLRGYSYAEPLDYW		P	IGHV4-34*01	99,30	IGHD3-22*01	IGHJ4*02	17	CARAGGRKGSGYYRSFDYW		P	1+4	4	6,24	2	1	0	Dis	no	yes	no		
BRNO0814	IGHV1-69*01	100	IGHD2-15*01	IGHJ6*02	18	CAREVGIVATLYYYYGMDVW		N	IGHV5-51*01	100	IGHD6-19*01	IGHJ4*02	13	CARQQWLGNYYFDYW	1	N	1+5	3	0,00	5	0	0	U	yes	no			
BRNO0833	IGHV1-69*01	100	IGHD2-21*01	IGHJ6*02	21	CARVAGVEGLLLLGNYYYGMDVW		P	IGHV2-5*10	92,78	IGHD3-22*01	IGHJ4*02	17	CARTYFSDSSGYHKSFDCW		D	1+2	4	7,22	4	0	1	Dis	no	yes	yes	no	no
BRNO0845	IGHV3-30*03, or IGHV3-30*18	100	IGHD2-2*01	IGHJ6*02	26	CAKDLTPTEYCSSTSCYYYYYYYGMDVW		P	IGHV5-51*03	100	IGHD5-5*01	IGHJ6*02	24	CARHAEWIQLWHPPRVYYYYYGMDVW		D	3+5	4	0,00	2	0	0	U	no	yes	yes	no	no
BRNO0846	IGHV1-69*01	100	IGHD2-2*01	IGHJ6*02	22	CARDPDIVVVPAAMYYYYYGMDVW	3	P	IGHV4-59*01	96,84	IGHD1-26*01	IGHJ4*02	14	CARTDYSGSYYYFDSW		D	1+4	4	3,16	8	0	1	Dis	yes	yes	yes	yes	no
BRNO0862	IGHV1-2*02	100	IGHD2-8*01	IGHJ6*02	33	CARSFLGYCTNGVCYDPPWVAAAGTGYYYYGMDVW		P	IGHV1-69*01	100	IGHD2-21*02	IGHJ6*02	14	CASYGGDCYNLCMDVW		D	1+1	2	0,00	19	0	0	U	no	yes	yes	no	no
BRNO0877	IGHV2-5*10	93,47	IGHD3-10*01	IGHJ4*02	18	CAHRSPNSGSYWDGGYFDYW	148C	N	IGHV3-21*01	97,22	IGHD4-4*01	IGHJ6*02	19	CARDHDYSYYDDYFYYGMDVW		N	2+3	4	3,75	1	1	1	M	yes	no			
BRNO0885	IGHV1-69*01	100	IGHD2-2*01	IGHJ6*03	23	CARDNSDCSSTSCYEFYYYYYMDVW		D	IGHV2-70*01	100	IGHD3-22*01	IGHJ6*02	27	CARTLPGQYYYDSSGYYYPLNYYYGMDVW		P	1+2	4	0,00	4	0	0	U	no	yes	yes	no	no
BRNO0892	IGHV3-33*01, or IGHV3-33*03 or IGHV3-33*06	93,40	IGHD3-3*01	IGHJ3*02	13	CARDNPRVVRALEIW		P	IGHV4-61*01	94,85	IGHD6-13*01	IGHJ4*02	15	CARDHRGYSSPWPFDNW		P	3+4	4	1,45	2	1	1	M	no	yes	no		
BRNO0912	IGHV3-7*02, or IGHV3-7*03	95,14	IGHD2-15*01	IGHJ3*02	23	CARGDFVVVVGGTSYGDFIDAFDIW		D	IGHV4-34*01	100	IGHD3-3*01	IGHJ6*03	23	CARVQTYYDFWSGYSPYYYYYMDVW	B1	P	3+4	4	4,86	0	1	0	Dis	yes	yes	yes	yes	no
BRNO0922	IGHV1-69*01	96,18	IGHD2-8*01	IGHJ4*02	17	CAREGTQYCTKGVCYMLYW		N	IGHV3-49*03, or IGHV3-49*05	94,56	IGHD1-7*01	IGHJ4*02	17	CARDPTSAYNWNLRHFDYW		N	1+3	4	1,62	0	1	1	M	no	no			
BRNO0923	IGHV1-3*01	100	IGHD6-19*01	IGHJ4*02	13	CAREQWLVTPNFDYW	1	P	IGHV4-34*01	100	IGHD3-9*01	IGHJ6*02	17	CARLLAGAYYYYYYGMDVW	205	P	1+4	4	0,00	4	0	0	U	yes	yes	no		
BRNO0948	IGHV1-69*01	100	IGHD3-10*02	IGHJ6*02	22	CARESAYYDILTGHNYYYYGMDVW	34	D	IGHV3-11*01	100	IGHD2-8*01	IGHJ6*02	22	CARDGWDIVPSKGYGYYYYGMDVW		P	1+3	4	0,00	0	0	0	U	yes	yes	yes	no	yes
BRNO0974	IGHV1-8*01	95,14	IGHD3-22*01	IGHJ4*02	16	CAGGLGRYNDGNGLRDCW		N	IGHV3-9*01	96,53	IGHD6-19*01	IGHJ4*02	13	CAKDIGSIAVGPGDW		N	1+3	4	1,39	3	1	1	M	no	no			
BRNO1029	IGHV3-23*01	100	IGHD3-10*01	IGHJ3*02	24	CAKDRGSPRIPLLWFGELGDDAFDIW		N	IGHV4-b*02	99,65	IGHD3-10*01	IGHJ4*02	18	CARDTIHSTLDRLGYRFDYW		N	3+4	4	0,35	6	0	0	U	no	no			
BRNO1030	IGHV1-69*01	100	IGHD2-2*01	IGHJ5*02	22	CAREAEDIVVVPAAWSVYNWFDPW		P	IGHV4-4*02	93,40	IGHD3-10*01	IGHJ3*01, or IGHJ3*02	11	CARDEGFQLLPLW	219	D	1+4	4	6,60	11	0	1	Dis	yes	yes	yes	no	yes
BRNO1037	IGHV1-8*01	96,88	IGHD4-17*01	IGHJ4*02	12	CARGWAYGDNADYW		P	IGHV3-72*01	94,90	IGHD5-5*01	IGHJ4*02	16	CASFTAKFGSNIRKFDYW		P	1+3	4	1,98	4	1	1	M	no	yes	no		
BRNO1037	IGHV1-8*01	96,88	IGHD4-17*01	IGHJ4*02	12	CARGWAYGDNADYW		P	IGHV4-39*01	98,97	IGHD3-3*01	IGHJ4*02	11	CASRDWGSGEDYW		P	1+4	4	2,09	1	1	0	Dis	no	yes	no		
BRNO1037	IGHV3-72*01	94,90	IGHD5-5*01	IGHJ4*02	16	CASFTAKFGSNIRKFDYW		P	IGHV4-39*01	98,97	IGHD3-3*01	IGHJ4*02	11	CASRDWGSGEDYW		P	3+4	4	4,07	5	1	0	Dis	no	yes	no		
BRNO1049	IGHV1-3*01	100	IGHD1-26*01	IGHJ6*02	17	CARMYSGSYYYYYGMDVW	28	P	IGHV3-33*01, or IGHV3-33*06	95,83	IGHD4-17*01	IGHJ4*02	15	CVRSVYGDGNREDFDYW		D	1+3	4	4,17	2	0	1	Dis	yes	yes	yes	yes	no
BRNO1049	IGHV1-3*01	100	IGHD1-26*01	IGHJ6*02	17	CARMYSGSYYYYYGMDVW	28	P	IGHV4-39*01	100	IGHD6-13*01	IGHJ5*02	19	CARRQGYSSSWYGRSNWFDPW	8	A	1+4	4	0,00	2	0	0	U	yes	yes	yes	yes	no
BRNO1049	IGHV3-33*01, or IGHV3-33*06	95,83	IGHD4-17*01	IGHJ4*02	15	CVRSVYGDGNREDFDYW		D	IGHV4-39*01	100	IGHD6-13*01	IGHJ5*02	19	CARRQGYSSSWYGRSNWFDPW	8	A	3+4	4	4,17	4	1	0	Dis	yes	yes	yes	yes	no
BRNO1054	IGHV3-23*04	93,75	IGHD6-13*01	IGHJ6*03	17	CAKLGQHLVQIYYYHIDVW		P	IGHV5-51*01	95,14	IGHD5-5*01	IGHJ4*02	16	CARLRYSYGLSTSPADYW		P	3+5	4	1,39	1	1	1	M	no	yes	no		
BRNO1072	IGHV1-2*02	100	IGHD6-19*01	IGHJ4*02	13	CAREQWLALSHFDYW	1	P	IGHV3-21*01	99,31	IGHD3-3*01	IGHJ6*02	20	CARDIRSVIIWSGQQNYGMDVW		D	1+3	4	0,69	7	0	0	U	yes	yes	yes	yes	no
BRNO1087	IGHV3-43*01	100	IGHD4-17*01	IGHJ6*02	14	CAKGASGDYVFCMDVW		P	IGHV6-1*01	95,29	IGHD4-17*01	IGHJ4*02	12	CARDAGGGYLFDYW		D	3+6	4	4,71	2	0	1	Dis	no	yes	yes	no	no
BRNO1132	IGHV3-7*01	94,10	IGHD2-21*02	IGHJ6*02	17	CALSGLDLDYYYYDMDVW		N	IGHV3-23*01	94,44	IGHD6-13*01	IGHJ4*01, or IGHJ4*02	14	CAREGAGTDLLDFDSW		N	3+3	2	0,34	3	1	1	M	no	no			
BRNO1137	IGHV2-5*04, or IGHV2-5*07 or IGHV2-5*10	94,16	IGHD6-19*01	IGHJ4*02	17	CAYRREKNSDWDGGCFNHW	148B	N	IGHV3-30-3*01	92,36	IGHD1-7*01	IGHJ1*01	16	CVREDCNNCHCYKNFQLW		N	2+3	4	1,80	1	1	1	M	yes	no			

Supplemental Table S4 - part A

Patient ID	Group	Ig chain	Type of junction	V-GENE and allele	V-REGION identity %	D-GENE and allele	J-GENE and allele	CDR3-IMGT length	AA JUNCTION	Stereotyped subset	Functionality	Cause of unproductivity
BRNO0261	I	heavy	IGHV-IGHD-IGHJ	IGHV1-69*01	100	IGHD3-3*01	IGHJ6*02	23	CATPPYYDFWSGYYPNYYYYGMDVW	7	productive	
BRNO0261	I	heavy	IGHV-IGHD-IGHJ	IGHV1-69*01	100	IGHD3-3*01	IGHJ6*02	29	CARPIILYDFWSGYYFGNGTDPYYYYGMDVW		productive	
BRNO0261	I	light	IGKJ-C-INTRON-KDE								allele inactivation	
BRNO0261	I	light	IGKV-KDE	IGKV1-5*01, or IGKV1-5*02 or IGKV1-5*03	100						allele inactivation	
BRNO0261	I	light	IGKV-IGKJ	IGKV1-5*03	99,62		IGKJ2*01, or IGKJ2*02 or IGKJ2*03 or IGKJ2*04	7	CQQYNSYTF		productive	
BRNO0261	I	light	IGLV-IGLJ	IGLV3-9*01	100		IGLJ1*01	10	CQVWDSSTGGVF		productive	
BRNO0279	I	heavy	IGHV-IGHD-IGHJ	IGHV1-69*01	100	IGHD2-2*02	IGHJ6*02	22	CARDRPDIVVVPADIYYYYGMDVW	3	productive	
BRNO0279	I	heavy	IGHV-IGHD-IGHJ	IGHV3-21*01	98,61	IGHD5-24*01	IGHJ6*02	9	CARDTDGMDVW	2	productive	
BRNO0279	I	light	IGKJ-C-INTRON-KDE								allele inactivation	
BRNO0279	I	light	IGKV-IGKJ	IGKV1-39*01, or IGKV1D-39*01	98,52		IGKJ1*01	9	CQQSYSTPRTF		productive	
BRNO0279	I	light	IGLV-IGLJ	IGLV1-51*01, or IGLV1-51*02	100		IGLJ2*01, or IGLJ3*01 or IGLJ3*02	11	CGTWDSSLSARVF		productive	
BRNO0307	II	heavy	IGHD-IGHJ			IGHD6-13*01	IGHJ6*02				incomplete	
BRNO0307	II	heavy	IGHV-IGHD-IGHJ	IGHV1-3*01	98,61	IGHD3-10*01	IGHJ4*02	13	CARVQWFGVYYFDYW	1	productive	
BRNO0307	II	heavy	IGHV-IGHD-IGHJ	IGHV3-23*01	89,93	IGHD1-1*01	IGHJ1*01	13	CAKADSNVEQYFRHW		productive	
BRNO0307	II	light	IGKV-IGKJ	IGKV1-39*01, or IGKV1D-39*01	100		IGKJ3*01	10	CQQSYSTPGFTF		productive	
BRNO0307	II	light	IGKV-IGKJ	IGKV2-18*01 P	100		IGKJ4*01		CMQATQFPTW#TF		unproductive	out-of-frame, IGKV pseudogene
BRNO0319	I	heavy	IGHD-IGHJ			IGHD2-2*02	IGHJ6*02				incomplete	
BRNO0319	I	heavy	IGHD-IGHJ			IGHD3-9*01	IGHJ5*02				incomplete	
BRNO0319	I	heavy	IGHD-IGHJ			IGHD6-13*01	IGHJ5*02				incomplete	
BRNO0319	I	heavy	IGHV-IGHD-IGHJ	IGHV3-30*03, or IGHV3-30*18	100	IGHD6-13*01	IGHJ4*02	13	CAKAREQQLPPEDYW		productive	
BRNO0319	I	heavy	IGHV-IGHD-IGHJ	IGHV3-33*01, or IGHV3-33*06	100	IGHD4-17*01	IGHJ6*02	14	CARGQHGDYVYGMDVW		productive	
BRNO0319	I	heavy	IGHV-IGHD-IGHJ	IGHV4-39*01	100	IGHD3-3*02	IGHJ4*02	16	CASDPGLGSRMRSYFDYW		productive	
BRNO0319	I	light	IGKJ-C-INTRON-KDE								allele inactivation	
BRNO0319	I	light	IGKV-KDE	IGKV3-15*01, or IGKV3-15*01	100						allele inactivation	
BRNO0319	I	light	IGKV-IGKJ	IGKV1-12*01, or IGKV1-12*02 or IGKV1D-12*02	100		IGKJ1*01	9	CQQANSFLRGF		productive	
BRNO0319	I	light	IGKV-IGKJ	IGKV3-11*01	100		IGKJ1*01, or IGKJ5*01	9	CQQRSNWPPGF		productive	
BRNO0319	I	light	IGKV-IGKJ	IGKV3-15*01	99,06		IGKJ1*01	10	CQQYNNWPLWTF		productive	
BRNO0319	I	light	IGLV-IGLJ	IGLV3-21*01	100		IGLJ2*01, or IGLJ3*01	11	CQVWDSSSDXVVF		productive	
BRNO0319	I	light	IGKV-IGKJ	IGKV1-39*01, or IGKV1D-39*01	100		IGKJ2*01		CQQSYST#YTF		unproductive	out-of-frame
BRNO0319	I	light	IGLV-IGLJ	IGLV3-22*01	100		IGLJ1*01		CLSGDEDN		unproductive	out-of-frame
BRNO0319	I	light	IGLV-IGLJ	IGLV2-5*01(P)	100		IGLJ2*01, or IGLJ3*01		CCSYTSSAT*R#VF		unproductive	out-of-frame, stop codons
BRNO0447	III	heavy	IGHV-IGHD-IGHJ	IGHV3-74*01	92,36	IGHD6-13*01	IGHJ5*02	13	CVRGAIGSSVWFDPW		productive	
BRNO0447	III	heavy	IGHV-IGHD-IGHJ	IGHV4-34*01	94,04	IGHD5-5*01	IGHJ4*02	15	CASRFRGNTYGYYFDSW		productive	
BRNO0447	III	light	IGKV-IGKJ	IGKV1D-13*01	94,09		IGKJ3*01, or IGKJ4*01	9	CQQYNNYPLTF		productive	
BRNO0447	III	light	IGLV-IGLJ	IGLV2-11*01, or IGLV2-11*02 or IGLV2-11*03	98,75		IGLJ2*01, or IGLJ3*01	11	CCSYAGTHLFVLF		productive	
BRNO0511	II	heavy	IGHV-IGHD-IGHJ	IGHV1-2*02	100	IGHD3-3*01	IGHJ6*02	26	CARDTRDDDFWSGYLPEYYYYYYGMDVW		productive	
BRNO0511	II	heavy	IGHV-IGHD-IGHJ	IGHV1-69*01	100	IGHD3-3*01	IGHJ6*02	24	CARDLYDFWSGYRPTGAYYYYGMDVW	7	productive	
BRNO0511	II	heavy	IGHV-IGHD-IGHJ	IGHV1-69*01	100	IGHD2-8*01	IGHJ6*02	27	CARAARPYCTNGVCYLEIAAYYYYGMDVW		productive	
BRNO0511	II	heavy	IGHV-IGHD-IGHJ	IGHV3-72*01	100	IGHD4-17*01	IGHJ4*02		CARVTTVTTREAPS##DYW		unproductive	out-of-frame, stop codons
BRNO0511	II	light	IGKJ-C-INTRON-KDE								allele inactivation	
BRNO0511	II	light	IGKV-IGKJ	IGKV1-33*01, or IGKV1D-33*01	100						productive	
BRNO0511	II	light	IGKV-IGKJ	IGKV1-12*01, or IGKV1-12*02 or IGKV1D-12*02	100		IGKJ1*01		CQQANSFF#WTF		unproductive	out-of-frame
BRNO0511	II	light	IGLV-IGLJ	IGLV3-01*01	100		IGLJ2*01, or IGLJ3*01		CQAWDSSTA#VF		unproductive	out-of-frame
BRNO0523	II	heavy	IGHD-IGHJ			IGHD6-19*01	IGHJ4*02				incomplete	
BRNO0523	II	heavy	IGHV-IGHD-IGHJ	IGHV5-51*01	94,44	IGHD3-16*01	IGHJ6*02	27	CARLAQPPMKYVWRSDHTKKYSYFGMDVW		productive	
BRNO0523	II	heavy	IGHV-IGHD-IGHJ	IGHV6-1*01	93,27	IGHD1-1*01	IGHJ4*02	10	CARGSIGIFDYW		productive	
BRNO0523	II	light	IGKV-KDE	IGKV3D-20*01	100						allele inactivation	
BRNO0523	II	light	IGKV-IGKJ	IGKV4-1*01	95,67		IGKJ2*01	9	CQQNYNLPYTF		productive	
BRNO0604	II	heavy	IGHD-IGHJ			IGHD4-23*01	IGHJ5*02				incomplete	
BRNO0604	II	heavy	IGHV-IGHD-IGHJ	IGHV1-69*01	99,31	IGHD3-10*01	IGHJ6*03	20	CARAMVQGVISYYYYYYYMDVW	5	productive	
BRNO0604	II	heavy	IGHV-IGHD-IGHJ	IGHV1-69*01	100	IGHD2-15*01	IGHJ3*02	24	CARLFPPYCSGGSCYGWAPSGAFDIW		productive	
BRNO0604	II	heavy	IGHV-IGHD-IGHJ	IGHV2-5*10	96,91	IGHD2-21*01	IGHJ4*02, or IGHJ4*03	12	CAHRRISIGGFDYW		productive	
BRNO0604	II	light	IGKJ-C-INTRON-KDE								allele inactivation	
BRNO0604	II	light	IGKV-KDE	IGKV4-1*01	100						allele inactivation	
BRNO0604	II	light	IGLV-IGLJ	IGLV1-44*01	99,62		IGLJ3*02	10	CAAWDDNLNGVF		productive	
BRNO0604	II	light	IGLV-IGLJ	IGLV2-11*01	100		IGLJ2*01, or IGLJ3*01	9	CCSYAGSFVVF		productive	
BRNO0604	II	light	IGKV-IGKJ	IGKV4-1*01	100		IGKJ1*01		CQQYYSTP##TF		unproductive	out-of-frame
BRNO0604	II	light	IGKV-IGKJ	IGKV5-2*01	99,07		IGKJ2*01		CLQHDNFPL##TF		unproductive	out-of-frame
BRNO0604	II	light	IGLV-IGLJ	IGLV1-44*01	100		IGLJ2*01, or IGLJ3*01 or IGLJ7*02		CAAWDDSLNGP##VVF		unproductive	out-of-frame
BRNO0625	II	heavy	IGHD-IGHJ			IGHD6-6*01	IGHJ4*02				incomplete	
BRNO0625	II	heavy	IGHV-IGHD-IGHJ	IGHV1-18*01	97,31	IGHD2-2*01	IGHJ6*02	25	CARDQGYCSSTSCYEGNYYYYYGMDVW		productive	
BRNO0625	II	heavy	IGHV-IGHD-IGHJ	IGHV1-69*01, or IGHV1-69*12	93,06	IGHD5-5*01	IGHJ4*02	15	CARVLRGYSYAEPLDYW		productive	
BRNO0625	II	heavy	IGHV-IGHD-IGHJ	IGHV4-34*01	99,30	IGHD3-22*01	IGHJ4*02	17	CARAGGRKGSGYYRSFDYW		productive	
BRNO0625	II	light	IGKV-KDE	IGKV1-16, or IGKV1D-16	100						allele inactivation	
BRNO0625	II	light	IGKV-IGKJ	IGKV1-33*01, or IGKV1D-33*01	100		IGKJ2*02	9	CQQYDNLPRTF		productive	
BRNO0625	II	light	IGLV-IGLJ	IGLV2-14*01	94,55		IGLJ3*02	10	CSSFSDSSTWVF		productive	
BRNO0625	II	light	IGKV-IGKJ	IGKV1-39*01, or IGKV1D-39*01	100		IGKJ5*01		CQQSYSTP#ITF		unproductive	out-of-frame
BRNO0814	II	heavy	IGHV-IGHD-IGHJ	IGHV1-69*01	100	IGHD2-15*01	IGHJ6*02	18	CAREVGIVATLYYYYGMDVW		productive	
BRNO0814	II	heavy	IGHV-IGHD-IGHJ	IGHV5-51*01	100	IGHD6-19*01	IGHJ4*02	13	CARQQWLGNYYFDYW	1	productive	
BRNO0814	II	heavy	IGHV-IGHD-IGHJ	IGHV4-30-2*01	100	IGHD3-16*02	IGHJ6*02		CASTPKTVL*LHLGELSF##YYYYYGMDVW		unproductive	out-of-frame, stop codons
BRNO0814	II	light	IGKV-IGKJ	IGKV1-33*01, or IGKV1D-33*01	100		IGKJ1*01	9	CQQYDNLPQTF		productive	
BRNO0814	II	light	IGKV-IGKJ	IGKV1-39*01, or IGKV1D-39*01	100		IGKJ1*01	10	CQQSYSTPPWTF		productive	
BRNO0814	II	light	IGKV-IGKJ	IGKV3-11*01	100		IGKJ1*01	9	CQQRSNWSWTF		productive	
BRNO0833	III	heavy	IGHV-IGHD-IGHJ	IGHV1-69*01	100	IGHD2-21*01	IGHJ6*02	21	CARVAGVEGLLLLGNYYYGMDVW		productive	
BRNO0833	III	heavy	IGHV-IGHD-IGHJ	IGHV2-5*10	92,78	IGHD3-22*01	IGHJ4*02	17	CARTYFSDSSGYHKSFDCW		productive	
BRNO0833	III	light	IGKV-IGKJ	IGKV1-33*01, or IGKV1D-33*01	100		IGKJ4*01	9	CQQYDNLPPTF		productive	
BRNO0833	III	light	IGKV-IGKJ	IGKV2-28*01, or IGKV2D-28*01	100		IGKJ2*01	9	CMQALQTPYTF		productive	
BRNO0845	III	heavy	IGHV-IGHD-IGHJ	IGHV3-30*03, or IGHV3-30*18	100	IGHD2-2*01	IGHJ6*02	26	CAKDLTPTEYCSSTSCYYYYYYGMDVW		productive	
BRNO0845	III	heavy	IGHV-IGHD-IGHJ	IGHV5-51*03	100	IGHD5-5*01	IGHJ6*02	24	CARHAEWIQLWHPPRVYYYYYGMDVW		productive	
BRNO0845	III	light	IGKV-IGKJ	IGKV1-8*01	100		IGKJ2*01	9	CQQYYSYPYTF		productive	
BRNO0846	II	heavy	IGHD-IGHJ			IGHD4-17*01	IGHJ1*01				incomplete	
BRNO0846	II	heavy	IGHV-IGHD-IGHJ	IGHV1-69*01	100	IGHD2-2*01	IGHJ6*02	22	CARDPDIVVVPAAMYYYYGMDVW	3	productive	
BRNO0846	II	heavy	IGHV-IGHD-IGHJ	IGHV4-59*01	96,84	IGHD1-26*01	IGHJ4*02	14	CARTDYSGSYYYFDSW		productive	
BRNO0846	II	light	IGKV-KDE	IGKV1-17*01, or IGKV1D-17*01 or IGKV1D-17*02	100						allele inactivation	
BRNO0846	II	light	IGKV-IGKJ	IGKV1-9*01	100		IGKJ2*01	10	CQQLNSYPMYTF		productive	
BRNO0862	II	heavy	IGHV-IGHD-IGHJ	IGHV1-2*02	100	IGHD2-8*01	IGHJ6*02	33	CARSFLGYCTNGVCYDPPWVAAAGTGYYYYGMDVW		productive	
BRNO0862	II	heavy	IGHV-IGHD-IGHJ	IGHV1-69*01	100	IGHD2-21*02	IGHJ6*02	14	CASYGGDCYNLCMDVW		productive	
BRNO0862	II	light	IGKV-KDE	IGKV1-5*01, or IGKV1-5*02 or IGKV1-5*03	100						allele inactivation	
BRNO0862	II	light	IGKV-KDE	IGKV2-30*01	100						allele inactivation	
BRNO0862	II	light	IGKV-IGKJ	IGKV1-33*01, or IGKV1D-33*01	100		IGKJ5*01	10	CQQYDNLPPITF		productive	
BRNO0862	II	light	IGKV-IGKJ	IGKV2-28*01, or IGKV2D-28*01	100		IGKJ5*01	9	CMQALQTPITF		productive	
BRNO0862	II	light	IGKV-IGKJ	IGKV2D-26*01	99,55		IGKJ3*01	10	CMQDAQDPPFTF		productive	
BRNO0877	III	heavy	IGHV-IGHD-IGHJ	IGHV2-5*10	93,47	IGHD3-10*01	IGHJ4*02	18	CAHRSPNSGSYWDGGYFDYW	148C	productive	
BRNO0877	III	heavy	IGHV-IGHD-IGHJ	IGHV3-21*01	97,22	IGHD4-4*01	IGHJ6*02	19	CARDHDYSYYDDYFYYGMDVW		productive	
BRNO0877	III	light	IGKV-IGKJ	IGKV3-11*01	96,86		IGKJ4*01	8	CQQRSNWVTF		productive	
BRNO0885	II	heavy	IGHD-IGHJ			IGHD3-9*01	IGHJ4*02				incomplete	
BRNO0885	II	heavy	IGHV-IGHD-IGHJ	IGHV1-69*01	100	IGHD2-2*01	IGHJ6*03	23	CARDNSDCSSTSCYEFYYYYYMDVW		productive	
BRNO0885	II	heavy	IGHV-IGHD-IGHJ	IGHV2-70*01	100	IGHD3-22*01	IGHJ6*02	27	CARTLPGQYYYDSSGYYYPLNYYYGMDVW		productive	
BRNO0885	II	light	IGKJ-C-INTRON-KDE								allele inactivation	
BRNO0885	II	light	IGKV-KDE	IGKV2-30*01	100						allele inactivation	
BRNO0885	II	light	IGKV-KDE	IGKV3-20*01, or IGKV3D-20*01	100						allele inactivation	
BRNO0885	II	light	IGKV-IGKJ	IGKV2-28*01, or IGKV2D-28*01	100		IGKJ1*01	10	CMQALQTLSWTF		productive	
BRNO0892	II	heavy	IGHD-IGHJ			IGHD5-12*01	NOT DETERMINED				incomplete	
BRNO0892	II	heavy	IGHV-IGHD-IGHJ	IGHV3-33*01, or IGHV3-33*03 or IGHV3-33*06	93,40	IGHD3-3*01	IGHJ3*02	13	CARDNPRVVRALEIW		productive	
BRNO0892	II	heavy	IGHV-IGHD-IGHJ	IGHV4-61*01	94,85	IGHD6-13*01	IGHJ4*02	15	CARDHRGYSSPWPFDNW		productive	
BRNO0892	II	light	IGKJ-C-INTRON-KDE								allele inactivation	
BRNO0892	II	light	IGKV-IGKJ	IGKV1-13*02, or IGKV1D-13*01	93,25		IGKJ2*01, or IGKJ2*03	10	CQQFHTYPPYNF		productive	
BRNO0912	II	heavy	IGHD-IGHJ			IGHD2-15*01	IGHJ5*02				incomplete	
BRNO0912	II	heavy	IGHV-IGHD-IGHJ	IGHV3-7*02, or IGHV3-7*03	95,14	IGHD2-15*01	IGHJ3*02	23	CARGDFVVVVGGTSYGDFIDAFDIW		productive	
BRNO0912	II	heavy	IGHV-IGHD-IGHJ	IGHV4-34*01	100	IGHD3-3*01	IGHJ6*03	23	CARVQTYYDFWSGYSPYYYYYMDVW	B1	productive	
BRNO0912	II	light	IGKV-IGKJ	IGKV1-33*01, or IGKV1D-33*01	100		IGKJ1*01	9	CQQYDNLPQTF		productive	
BRNO0922	III	heavy	IGHV-IGHD-IGHJ	IGHV1-69*01	96,18	IGHD2-8*01	IGHJ4*02	17	CAREGTQYCTKGVCYMLYW		productive	
BRNO0922	III	heavy	IGHV-IGHD-IGHJ	IGHV3-49*03, or IGHV3-49*05	94,56	IGHD1-7*01	IGHJ4*02	17	CARDPTSAYNWNLRHFDYW		productive	
BRNO0922	III	light	IGKV-IGKJ	IGKV1-39*01, or IGKV1D-39*01	92,48		IGKJ5*01	10	CQQSFTTPSITF		productive	
BRNO0923	II	heavy	IGHD-IGHJ			IGHD2-2*02	IGHJ6*02				incomplete	
BRNO0923	II	heavy	IGHV-IGHD-IGHJ	IGHV1-3*01	100	IGHD6-19*01	IGHJ4*02	13	CAREQWLVTPNFDYW	1	productive	
BRNO0923	II	heavy	IGHV-IGHD-IGHJ	IGHV4-34*01	100	IGHD3-9*01	IGHJ6*02	17	CARLLAGAYYYYYYGMDVW	205	productive	
BRNO0923	II	light	IGKV-KDE	IGKV2-28*01, or IGKV2D-28*01	100						allele inactivation	
BRNO0923	II	light	IGKV-KDE	IGKV4-1*01	100						allele inactivation	
BRNO0923	II	light	IGKV-IGKJ	IGKV1-39*01, or IGKV1D-39*01	100		IGKJ2*01	10	CQQSYSTPPYTF		productive	
BRNO0948	I	heavy	IGHD-IGHJ			IGHD2-15*01	IGHJ6*02				incomplete	
BRNO0948	I	heavy	IGHD-IGHJ			IGHD5-18*01	IGHJ6*02				incomplete	
BRNO0948	I	heavy	IGHV-IGHD-IGHJ	IGHV1-69*01	100	IGHD3-10*02	IGHJ6*02	22	CARESAYYDILTGHNYYYYGMDVW	34	productive	
BRNO0948	I	heavy	IGHV-IGHD-IGHJ	IGHV3-11*01	100	IGHD2-8*01	IGHJ6*02	22	CARDGWDIVPSKGYGYYYYGMDVW		productive	
BRNO0948	I	light	IGKV-KDE	IGKV1D-43*01	100						allele inactivation	
BRNO0948	I	light	IGKV-KDE	IGKV5-2*01	100						allele inactivation	
BRNO0948	I	light	IGKV-IGKJ	IGKV1-5*03	100		IGKJ2*01	9	CQQYNSYSYTF		productive	
BRNO0948	I	light	IGLV-IGLJ	IGLV3-21*01	99,61		IGLJ1*01	11	CQVWDSSSDHHVF		productive	
BRNO0948	I	light	IGKV-IGKJ	IGKV3-11*01	100		IGKJ3*01		CQQRSNC#FTF		unproductive	out-of-frame
BRNO0974	II	heavy	IGHD-IGHJ			IGHD2-15*01	IGHJ2*01				incomplete	
BRNO0974	II	heavy	IGHV-IGHD-IGHJ	IGHV1-8*01	95,14	IGHD3-22*01	IGHJ4*02	16	CAGGLGRYNDGNGLRDCW		productive	
BRNO0974	II	heavy	IGHV-IGHD-IGHJ	IGHV3-9*01	96,53	IGHD6-19*01	IGHJ4*02	13	CAKDIGSIAVGPGDW		productive	
BRNO0974	II	heavy	IGHV-IGHD-IGHJ	IGHV4-30-4*01	100	IGHD2-8*01	IGHJ6*02		CATIVLMV*PNGR#YYYGMDVW		unproductive	out-of-frame, stop codons
BRNO0974	II	light	IGKV-KDE	IGKV2-28, or IGKV2D-28	100						allele inactivation	
BRNO0974	II	light	IGKV-KDE	IGKV3-20, or IGKV3D-20	100						allele inactivation	
BRNO0974	II	light	IGKV-IGKJ	IGKV2-30*01	100		IGKJ4*01	9	CMQGTHWPLTF		productive	
BRNO0974	II	light	IGKV-IGKJ	IGKV3D-20*01	95,42		IGKJ2*01	9	CQQYGNSPNTF		productive	
BRNO0974	II	light	IGKV-IGKJ	IGKV4-1*01	100		IGKJ4*01	9	CQQYYSTPLTF		productive	
BRNO0974	II	light	IGLV-IGLJ	IGLV2-23*02	96,55		IGLJ2*01, or IGLJ3*01	11	CCSYAGSSNLMVF		productive	
BRNO1029	II	heavy	IGHD-IGHJ			IGHD4-23*01	IGHJ4*02				incomplete	
BRNO1029	II	heavy	IGHV-IGHD-IGHJ	IGHV3-23*01	100	IGHD3-10*01	IGHJ3*02	24	CAKDRGSPRIPLLWFGELGDDAFDIW		productive	
BRNO1029	II	heavy	IGHV-IGHD-IGHJ	IGHV4-b*02	99,65	IGHD3-10*01	IGHJ4*02	18	CARDTIHSTLDRLGYRFDYW		productive	
BRNO1029	II	light	IGKV-KDE	IGKV1-33*01, or IGKV1D-33*01	100						allele inactivation	
BRNO1029	II	light	IGKV-IGKJ	IGKV6-21*01(ORF), or IGKV6D-21*01(ORF)	99,19		IGKJ1*01	9	CHQSSSLPRTF		productive	
BRNO1029	II	light	IGKV-IGKJ	IGKV1-33*01, or IGKV1D-33*01	100		IGKJ5*01	9	CQQYDNLPITF		productive	
BRNO1030	I	heavy	IGHD-IGHJ			IGHD5-18*01	IGHJ6*02				incomplete	
BRNO1030	I	heavy	IGHV-IGHD-IGHJ	IGHV1-69*01	100	IGHD2-2*01	IGHJ5*02	22	CAREAEDIVVVPAAWSVYNWFDPW		productive	
BRNO1030	I	heavy	IGHV-IGHD-IGHJ	IGHV4-4*02	93,40	IGHD3-10*01	IGHJ3*01, or IGHJ3*02	11	CARDEGFQLLPLW	219	productive	
BRNO1030	I	heavy	IGHV-IGHD-IGHJ	IGHV3-22*01, or IGHV3-22*02	100	IGHD5-12*01	IGHJ4*02		CSRERTNVDIVATIP#DYW		unproductive	out-of-frame, stop codons
BRNO1030	I	light	IGKV-KDE	IGKV2-30*01	100						allele inactivation	
BRNO1030	I	light	IGKV-IGKJ	IGKV3-11*01	100		IGKJ4*01	10	CQQRSNWPALTF		productive	
BRNO1030	I	light	IGLV-IGLJ	IGLV2-8*01	93,80		IGLJ2*01, or IGLJ3*01 or IGLJ3*02	9	CSSYAGGSKVF		productive	
BRNO1030	I	light	IGKV-IGKJ	IGKV2-30*01	100		IGKJ1*01		CMQGTHWPP#TF		unproductive	out-of-frame
BRNO1037	I	heavy	IGHD-IGHJ			IGHD6-19*01	IGHJ3*02				incomplete	
BRNO1037	I	heavy	IGHV-IGHD-IGHJ	IGHV1-8*01	96,88	IGHD4-17*01	IGHJ4*02	12	CARGWAYGDNADYW		productive	
BRNO1037	I	heavy	IGHV-IGHD-IGHJ	IGHV3-72*01	94,90	IGHD5-5*01	IGHJ4*02	16	CASFTAKFGSNIRKFDYW		productive	
BRNO1037	I	heavy	IGHV-IGHD-IGHJ	IGHV4-39*01	98,97	IGHD3-3*01	IGHJ4*02	11	CASRDWGSGEDYW		productive	

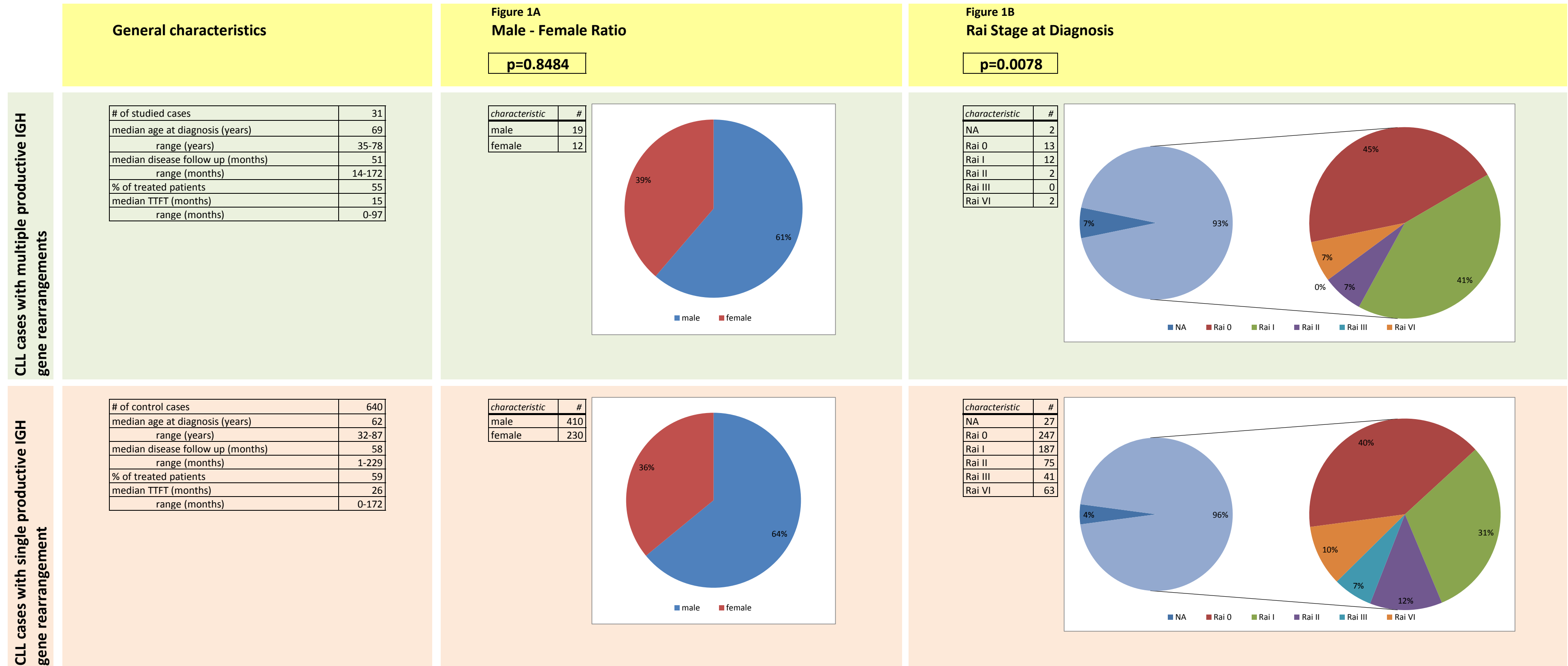
Supplemental Table S4 - part B

BRNO1037	I	heavy	IGHV-IGHD-IGHJ	IGHV3-h*01	99,53	IGHD2-21*01	IGHJ6*03		CARERERIHIVVVITLPTTW#FYYMDVW		unproductive	out-of-frame, IGHV pseudogene
BRNO1037	I	light	IGKJ-C-INTRON-KDE								allele inactivation	
BRNO1037	I	light	IGKV-KDE	IGKV7-3*01	100						allele inactivation	
BRNO1037	I	light	IGKV-IGKJ	IGKV1-16*02	96,14		IGKJ3*01	9	CQQYTTYPLTF		productive	
BRNO1037	I	light	IGLV-IGLJ	IGLV3-1*01	98,20		IGLJ2*01, or IGLJ3*01	9	CQAWDSSGVVF		productive	
BRNO1037	I	light	IGKV-IGKJ	IGKV1-27*01	100		IGKJ4*01		CQKYNSAL#LTF		unproductive	out-of-frame
BRNO1037	I	light	IGKV-IGKJ	IGKV3-15*01	91,74		IGKJ2*01		CQQFHNW##TF		unproductive	out-of-frame, stop codons
BRNO1049	II	heavy	IGHD-IGHJ			IGHD1-26*01	IGHJ4*02				incomplete	
BRNO1049	II	heavy	IGHV-IGHD-IGHJ	IGHV1-3*01	100	IGHD1-26*01	IGHJ6*02	17	CARMYSGSYYYYYYGMDVW	28	productive	
BRNO1049	II	heavy	IGHV-IGHD-IGHJ	IGHV3-33*01, or IGHV3-33*06	95,83	IGHD4-17*01	IGHJ4*02	15	CVRSVYGDGNREDFDYW		productive	
BRNO1049	II	heavy	IGHV-IGHD-IGHJ	IGHV4-39*01	100	IGHD6-13*01	IGHJ5*02	19	CARRQGYSSSWYGRSNWFDPW	8	productive	
BRNO1049	II	light	IGKJ-C-INTRON-KDE								allele inactivation	
BRNO1049	II	light	IGKV-IGKJ	IGKV4-1*01	100		IGKJ1*01	9	CQQYYSTPWTF		productive	
BRNO1049	II	light	IGKV-IGKJ	IGKV1-8*01	100		IGKJ4*02	8	CQQYYSYPPF		productive	
BRNO1054	I	heavy	IGHD-IGHJ			IGHD2-2*01, or IGHD2-2*02	IGHJ6*02, or IGHJ6*03				incomplete	
BRNO1054	I	heavy	IGHV-IGHD-IGHJ	IGHV3-23*04	93,75	IGHD6-13*01	IGHJ6*03	17	CAKLGQHLVQIYYYHIDVW		productive	
BRNO1054	I	heavy	IGHV-IGHD-IGHJ	IGHV5-51*01	95,14	IGHD5-5*01	IGHJ4*02	16	CARLRYSYGLSTSPADYW		productive	
BRNO1054	I	light	IGKV-KDE	IGKV3-11*01	100						allele inactivation	
BRNO1054	I	light	IGKV-IGKJ	IGKV3-15*01	97,34		IGKJ2*02	10	CQQYNKWPPGTF		productive	
BRNO1054	I	light	IGLV-IGLJ	IGLV3-1*01	95,85		IGLJ2*01, or IGLJ3*01	9	CQAWDSSTLIF		productive	
BRNO1072	I	heavy	IGHD-IGHJ			IGHD6-25*01	IGHJ4*02				incomplete	
BRNO1072	I	heavy	IGHV-IGHD-IGHJ	IGHV1-2*02	100	IGHD6-19*01	IGHJ4*02	13	CAREQWLALSHFDYW	1	productive	
BRNO1072	I	heavy	IGHV-IGHD-IGHJ	IGHV3-21*01	99,31	IGHD3-3*01	IGHJ6*02	20	CARDIRSVIIWSGQQNYGMDVW		productive	
BRNO1072	I	light	IGKJ-C-INTRON-KDE								allele inactivation	
BRNO1072	I	light	IGKV-IGKJ	IGKV1-39*01, or IGKV1D-39*01	100		IGKJ1*01	10	CQQSYSTHPWTF		productive	
BRNO1072	I	light	IGLV-IGLJ	IGLV1-51*02	98,56		IGLJ3*02	12	CXTWDSSLSAHWVF		productive	
BRNO1072	I	light	IGKV-IGKJ	IGKV2-28*01, or IGKV2D-28*01	100		IGKJ5*01		CMQALQTL		unproductive	out-of-frame, stop codons
BRNO1087	II	heavy	IGHD-IGHJ			IGHD6-13*01	IGHJ6*02				incomplete	
BRNO1087	II	heavy	IGHV-IGHD-IGHJ	IGHV3-43*01	100	IGHD4-17*01	IGHJ6*02	14	CAKGASGDYVFCMDVW		productive	
BRNO1087	II	heavy	IGHV-IGHD-IGHJ	IGHV6-1*01	95,29	IGHD4-17*01	IGHJ4*02	12	CARDAGGGYLFDYW		productive	
BRNO1087	II	light	IGKJ-C-INTRON-KDE								allele inactivation	
BRNO1087	II	light	IGKV-IGKJ	IGKV3-15*01	100		IGKJ2*01	10	CQQYNNWPLYTF		productive	
BRNO1132	I	heavy	IGHD-IGHJ			IGHD3-3*01	NOT DETERMINED				incomplete	
BRNO1132	I	heavy	IGHV-IGHD-IGHJ	IGHV3-23*01	94,44	IGHD6-13*01	IGHJ4*01, or IGHJ4*02	14	CAREGAGTDLLDFDSW		productive	
BRNO1132	I	heavy	IGHV-IGHD-IGHJ	IGHV3-7*01	94,10	IGHD2-21*02	IGHJ6*02	17	CALSGLDLDYYYYYDMDVW		productive	
BRNO1132	I	light	IGKJ-C-INTRON-KDE								allele inactivation	
BRNO1132	I	light	IGKV-IGKJ	IGKV1-9*01	97,74		IGKJ1*01	10	CQQVNSYRGWTF		productive	
BRNO1132	I	light	IGKV-IGKJ	IGKV1-33*01, or Homsap_IGKV1D-33*01	99,28		IGKJ1*01	9	CQQYDNLPQTF		productive	
BRNO1132	I	light	IGLV-IGLJ	IGLV3-27*01	97,83		IGLJ2*01, or IGLJ3*01	9	CYSAADNNKIF		productive	
BRNO1132	I	light	IGKV-IGKJ	IGKV3-11*01	100		IGKJ4*01		CQQRSNW#LTF		unproductive	out-of-frame
BRNO1132	I	light	IGKV-IGKJ	IGKV4-1*01	100		IGKJ3*01		CQQYYSTP#FTF		unproductive	out-of-frame
BRNO1137	III	heavy	IGHV-IGHD-IGHJ	IGHV2-5*04, or IGHV2-5*07 or IGHV2-5*10	94,16	IGHD6-19*01	IGHJ4*02	17	CAYRREKNSDWDGGCFNHW	148B	productive	
BRNO1137	III	heavy	IGHV-IGHD-IGHJ	IGHV3-30-3*01	92,36	IGHD1-7*01	IGHJ1*01	16	CVREDCNNCHCYKNFQLW		productive	
BRNO1137	III	light	IGLV-IGLJ	IGLV2-14*01	95,14		IGLJ1*01	11	CSSYSGSSTLFVF		productive	
BRNO1137	III	light	IGKV-IGKJ	IGKV3-20*01, or IGKV3D-20*01	100		IGKJ4*01		CQQYGSSPP#ALTF		unproductive	out-of-frame

Supplemental Table S5

Patient ID	Time point	Absolute count of clonal B cells [$x10^3/\mu l$]	ASO-qPCR									Flow cytometry measurement				
			R1	Proportion of R1	Absolute count of cells with R1 [$x10^3/\mu l$]	R2	Proportion of R2	Absolute count of cells with R2 [$x10^3/\mu l$]	R3	Proportion of R3	Absolute count of cells with R3 [$x10^3/\mu l$]	No. of detected populations	Kappa expressing B cells	Absolute count of Kappa+ cells [$x10^3/\mu l$]	Lambda expressing B cells	Absolute count of Lambda+ cells [$x10^3/\mu l$]
BRNO0261	9/2005	128,18	VH1-69/23aa	79,13%	101,43	VH1-69/29aa	20,87%	26,75				NA	NA		NA	
BRNO0261	12/2006	350,51	VH1-69/23aa	84,26%	295,34	VH1-69/29aa	15,74%	55,17				NA	NA		NA	
BRNO0261	6/2009	14,83	VH1-69/23aa	70,21%	10,41	VH1-69/29aa	29,79%	4,42				2	38,41%	5,69	60,21%	8,93
BRNO0261	10/2011	1,85	VH1-69/23aa	99,61%	1,85	VH1-69/29aa	0,39%	0,01				1	NCP	0	99,72%	1,85
BRNO0261	1/2012	33,86	VH1-69/23aa	NA		VH1-69/29aa	NA					1	NCP	0	99,35%	33,64
BRNO0261	6/2012	57,55	VH1-69/23aa	NA		VH1-69/29aa	NA					1	NCP	0	99,10%	57,03
BRNO0279	4/2008	281,63	VH1-69	0%	0	VH3-21	100%	281,63				1	NCP	0	99,15%	279,23
BRNO0279	4/2009	1,61	VH1-69	NA		VH3-21	NA					2	44,60%	0,72	52,90%	0,85
BRNO0279	8/2009	41,97	VH1-69	95,74%	40,18	VH3-21	4,26%	1,79				2	97,15%	40,77	1,78%	0,75
BRNO0319	3/2010	22,23	VH3-30	38,95%	8,66	VH3-33	54,49%	12,11	VH4-39	6,56%	1,46	2	29,84%	6,63	68,70%	15,27
BRNO0319	4/2011	37,99	VH3-30	39,21%	14,89	VH3-33	55,77%	21,19	VH4-39	5,02%	1,91	2	29,62%	11,25	69,36%	26,35
BRNO0319	10/2011	44,41	VH3-30	43,39%	19,27	VH3-33	53,02%	23,55	VH4-39	3,59%	1,59	2	31,25%	13,88	66,95%	29,73
BRNO0604	4/2008	17,44	VH1-69/20aa	28,07%	4,89	VH1-69/24aa	71,03%	12,39	VH2-5	0,90%	0,16	1	NCP	0	99,00%	17,26
BRNO0604	3/2010	31,05	VH1-69/20aa	27,91%	8,67	VH1-69/24aa	72,08%	22,38	VH2-5	0%	0	NA	NA		NA	
BRNO0604	4/2011	49,35	VH1-69/20aa	26,69%	13,17	VH1-69/24aa	73,31%	36,18	VH2-5	0%	0	1	NCP	0	98,92%	48,82
BRNO0846	5/2009	38,15	VH1-69	99,98%	38,14	VH4-59	0,02%	0,01				1	98,82%	37,70	NCP	0
BRNO0846	2/2010	210,22	VH1-69	100%	210,22	VH4-59	0%	0				1	99,56%	209,29	NCP	0
BRNO0862	3/2009	8,92	VH1-2	69,58%	6,20	VH1-69	30,42%	2,71				1	96,25%	8,58	NCP	0
BRNO0862	5/2010	29,15	VH1-2	86,54%	25,22	VH1-69	13,46%	3,92				1	99,23%	28,92	NCP	0
BRNO0862	3/2011	89,96	VH1-2	90,17%	81,12	VH1-69	9,83%	8,84				1	98,40%	88,52	NCP	0
BRNO0862	8/2011	135,90	VH1-2	96,08%	130,57	VH1-69	3,92%	5,33				1	99,59%	135,34	NCP	0
BRNO0885	5/2008	98,83	VH1-69	1,86%	1,84	VH2-70	98,14%	97,00				1	99,62%	98,46	NCP	0
BRNO0885	4/2009	161,34	VH1-69	1,13%	1,82	VH2-70	98,87%	159,52				1	99,16%	159,98	NCP	0
BRNO0885	4/2010	273,46	VH1-69	0,45%	1,23	VH2-70	99,55%	272,23				1	99,52%	272,14	NCP	0
BRNO0923	6/2009	91,30	VH1-3	98,40%	89,84	VH4-34	1,60%	1,46				1	99,26%	90,62	NCP	0
BRNO0923	3/2010	185,85	VH1-3	98,67%	183,37	VH4-34	1,33%	2,47				1	98,07%	182,26	NCP	0
BRNO0948	9/2009	27,93	VH1-69	18,46%	5,16	VH3-11	81,54%	22,77				2	30,43%	8,50	68,78%	19,21
BRNO0948	8/2011	72,53	VH1-69	2,70%	1,96	VH3-11	97,30%	70,57				2	4,17%	3,02	94,78%	68,74
BRNO1030	4/2010	25,58	VH1-69	99,84%	25,54	VH4-4	0,16%	0,04				NA	NA		NA	
BRNO1030	4/2011	51,16	VH1-69	99,93%	51,12	VH4-4	0,07%	0,04				2	96,81%	49,53	2,72%	1,39
BRNO1030	10/2011	67,32	VH1-69	99,97%	67,30	VH4-4	0,03%	0,02				2	98,04%	66,00	1,19%	0,80
BRNO1072	7/2010	104,01	VH1-2	68,01%	70,73	VH3-21	31,99%	33,27				2	70,67%	73,50	28,45%	29,59
BRNO1072	4/2011	386,93	VH1-2	89,64%	346,84	VH3-21	10,36%	40,09				2	91,21%	352,91	8,33%	32,23
BRNO1072	8/2011	2,81	VH1-2	100%	2,8	VH3-21	0%	0				1	98,75%	2,77	NCP	0

Supplemental Figure S1 - General Characteristics, S1A, S1B



Supplemental Figure S1 - S1C, S1D, S1E

Figure 1C

Absolute Count of Clonal B cells

p=0.1120

characteristic	*monoCLL*	*oligoCLL*
median ($x10^3/\mu l$)	18	13
range ($x10^3/\mu l$)	1-795	1-118

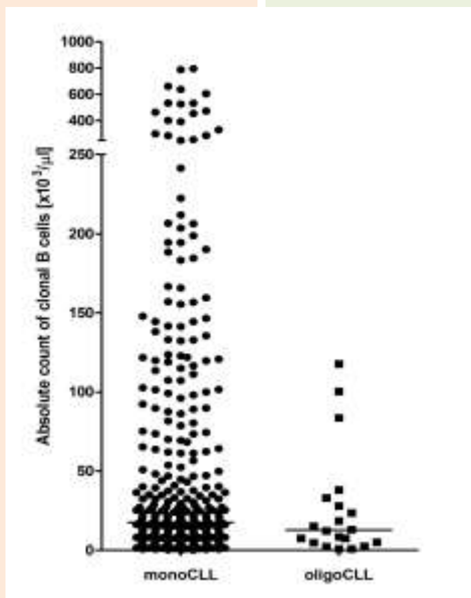
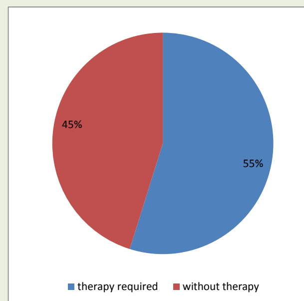


Figure 1D

Need for Treatment

p=0.7108

characteristic	*#*
therapy required	17
without therapy	14



characteristic	*#*
therapy required	376
without therapy	264

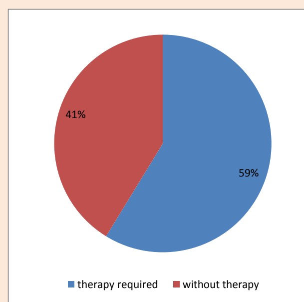
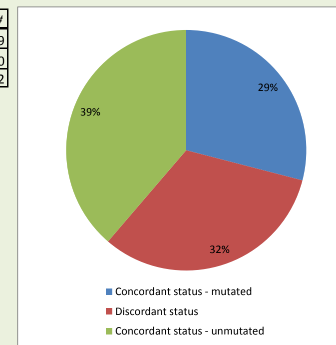


Figure 1E

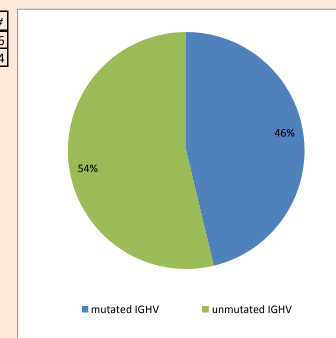
Overall IGHV Mutational Status

p=0.6999

characteristic	*#*
Concordant status - mutated	9
Discordant status	10
Concordant status - unmutated	12



characteristic	*#*
mutated IGHV	296
unmutated IGHV	344



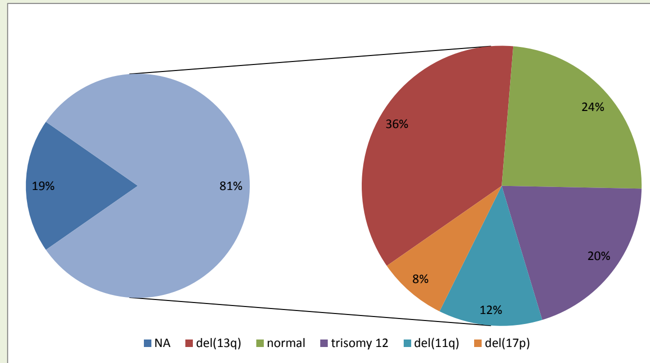
Supplemental Figure S1 - S1F, S1G

Figure 1F

FISH - Hierarchic Model

p=0.1014

characteristic	*#*
NA	6
del(13q)	9
normal	6
trisomy 12	5
del(11q)	3
del(17p)	2



characteristic	*#*
NA	140
del(13q)	172
normal	150
trisomy 12	57
del(11q)	86
del(17p)	35

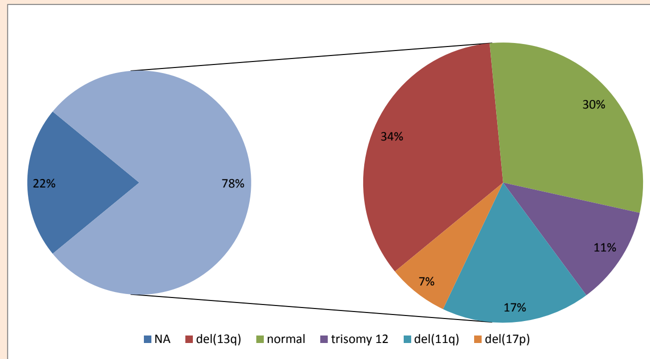
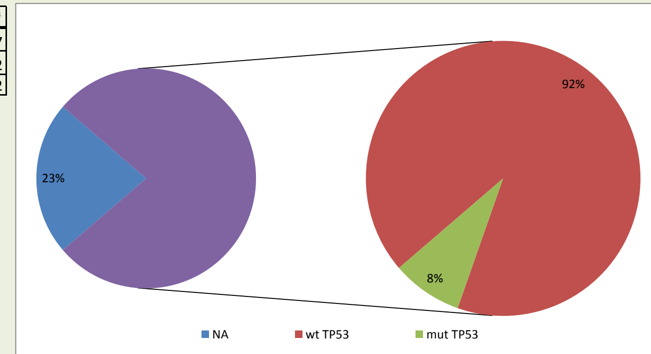


Figure 1G

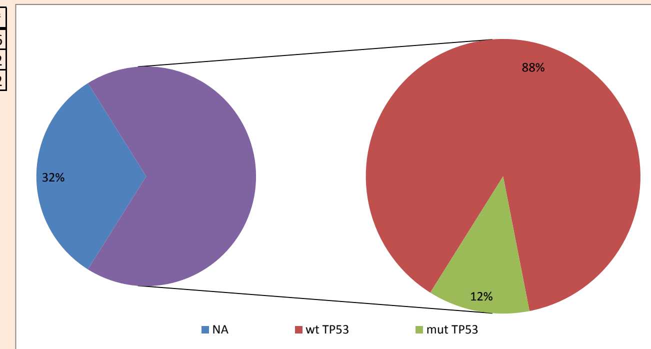
TP53 Gene Mutation Status

p=1

characteristic	*#*
NA	7
wt TP53	22
mut TP53	2



characteristic	*#*
NA	206
wt TP53	382
mut TP53	52



Supplemental Figure S2

Immunogenetic and Immunophenotypic Analysis