

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

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## **SUPPLEMENTAL METHODS**

### ***Cell separation, immunophenotyping and cell sorting***

B lymphocytes were routinely separated from peripheral blood using Ficoll-Paque PLUS (GE Healthcare) gradient centrifugation coupled with a depletion of non-B cells by RosetteSep® Kits (Human B cell Enrichment Kit, CD3+ Depletion Kit; StemCell). Purity assesment and detailed immunophenotyping of separated cells were performed on CellLab Quanta™ SC or FC500 (Beckman Coulter Inc.). An aliquot of cells was stained with monoclonal antibodies against CD5, CD10, CD19, CD20, CD23, CD38, CD43, CD79b, FMC7, HLA-DR, slgK and slgL.

In specific cases (case 319, table 3), fluorescence activated cell sorting was performed using FACSaria III (BD Biosciences). B lymphocytes were stained against CD19 (anti-CD19-PE-Cy5, Beckman Coulter), slgK (polyclonal anti-slgK-FITC, ABserotech) and slgL (polyclonal anti-slgL-PE, ABserotech). Populations were sorted by the light chain isotype.

### ***Equipment and software used for fragment analysis and sequencing***

Either ABI PRISM 3130XL or 310 Genetic Analyzer (Applied Biosystems) were used for both methods. Results obtained from fragment analysis were processed by GeneMapper v4.1 or GeneScan v3.1.2. software. Sequences were inspected and processed in ABI PRISM® DNA Sequencing Analysis Software v5.3.1 and further handled in BioEdit v7.1.3.

### ***Cloning strategy***

Cloning of PCR amplicons was used if multiple IGHV-IGHD-IGHJ gene rearrangements were amplified from the same *IGHV* specific primer. PCR products were cloned into the pCR®2.1 vector which was used for transformation of competent *E. coli* (TA Cloning® Kit with One Shot® INVaF' Chemically Competent *E. coli*; Invitrogen), and multiple subcloned sequences were analyzed.

## ***Allele-specific oligonucleotide assay experiments and calculation of IGH rearrangement proportions in MP-IGH CLL cases***

Allele-specific oligonucleotide assays (ASO-qPCR) enabled assessment of clone proportion and long-term molecular monitoring of clonal dynamics. *IGHV* subgroup-specific TaqMan probes were used; the sense primer was targeted to the *IGHV* gene, while the antisense primer, which conferred specificity for individual rearrangements, was targeted to the CDR3 of each rearrangement. The set of assays for an individual patient was required to have efficiency difference  $<0.2$ . Albumin was used as a reference gene. ASO-qPCR assays were performed with FastStart TaqMan Probe Master (Roche) master mix using RotorGene 6000 (Corbett). Results and efficiency of the individual assays were evaluated by RotorGene 6000 Series Software 1.7.

The proportion  $R$  of individual *IGHV-IGHD-IGHJ* gene rearrangements was calculated as follows, based on the known value of an assay efficiency  $E$  and a  $C_t$  value when each of the rearrangements reached a certain number of copies  $c$  at certain level of detected signal intensity  $I$ . Hence, for each assay

$$I = c \cdot E^{C_t}$$

If assays for coexisting rearrangements reach equal level of intensity, then

$$c(1) \cdot E(1)^{C_t(1)} = c(2) \cdot E(2)^{C_t(2)}$$

For the proportion  $R$  of two IGH rearrangement

$$R = \frac{c(1)}{c(2)} = \frac{E(2)^{C_t(2)}}{E(1)^{C_t(1)}}$$

Considering that the relative number of copies gives  $c_r(1) + c_r(2) = 1$ , then

$$c_r(1) = \frac{R}{1+R} \quad \text{and} \quad c_r(2) = \frac{1}{1+R}$$

Similarly, in a case of three co-existing IGH rearrangements, it can be derived that

$$c_r(1) = \frac{1}{1+R_A^{-1}+R_C}, \quad c_r(2) = \frac{1}{1+R_A+R_B^{-1}}, \quad \text{and} \quad c_r(3) = \frac{1}{1+R_B+R_C^{-1}}$$

where

$$R_A = \frac{c(1)}{c(2)}, \quad R_B = \frac{c(2)}{c(3)}, \quad \text{and} \quad R_C = \frac{c(3)}{c(1)}$$

### ***Analysis of genomic defects***

Deletions 11q, 13q and 17p, and trisomy 12 were assessed using interphase FISH in 77% of cases. For the respective regions, following probes were used: LSI p53/LSI ATM and LSI D13S319/LSI 13q34/CEP12 Multi-Color Probe Sets (Abott Molecular-Vysis), or XL ATM/p53 and XL DLEU/LAMP/12cen (MetaSystems) according to the manufactures' recommendations. Identified chromosomal changes were classified according to the International System for Human Cytogenetic Nomenclature (ISCN) 2009.

Additionally, karyotype results from IL-2/CpG-stimulated metaphase cytogenetics were available in 52% of cases (Dicker F, Schnittger S, Haferlach T, et al. Immunostimulatory oligonucleotide-induced metaphase cytogenetics detect chromosomal aberrations in 80 % of CLL patients: a study of 132 CLL cases with correlation to FISH, IgVH status, and CD38 expression. Blood 2006;108(9):3152-60). Heparinized peripheral blood was cultured in RPMI-1640 (Sigma) supplemented with IL-2 (200 U/ml, Peprotech) and DSP30 (2 µmol/l, TIB MolBiol) for 72 hours at 37°C/5% CO<sub>2</sub>. Colchicine was added 5-6 hours prior to analysis. Further sample processing (hypotonic treatment and fixation) and chromosome preparation and staining was conducted according to the standard protocols (Haferlach C at al. 2011). The cultivation was considered successful when minimum of 5 metaphase cells were found. Evaluation of metaphase cells was performed using Lucia Karyo (Laboratory Imaging). Karyotypes were classified according to the ISCN 2009.

In the case 319, chromosomal aberrations were assessed in sorted populations using multiplex ligation-dependent probe amplification (MLPA) using SALSA MLPA P040 CLL probemix (MRC-Holland) according to the manufacturer's recommendations.

### ***Detection of TP53 gene mutations***

*TP53* mutations were identified by the yeast functional analysis (FASAY) described by Flaman and colleagues (Flaman JM, Frebourg T, Moreau V, et al. A simple p53 functional assay for screening cell lines, blood, and tumors. Proc Natl Acad Sci U S A. 1995;92:3963-7.). In this assay, the central part of the *TP53* gene (amplified from cDNA between exons 4

and 10, ie. codons 42 to 374) is introduced into an *ADE2*<sup>-</sup> yeast strain carrying a reporter with a p53-binding site upstream of the *ADE2* gene. On the plates containing a low level of adenine, the p53 wild-type (wt) samples form large white colonies, whereas the colonies with the *TP53* mutations (mut-p53) are small and red, which is attributable to limited growth and an accumulation of a reddish product of adenine metabolism. Samples containing a wt-p53 account for 90% or more of white colonies in the assay; the remaining 10% represent background, a consequence of PCR-induced mutations or a low-quality RNA. All *TP53* mutations were confirmed using sequencing from red colonies and a direct sequencing from corresponding patient genomic DNA (gDNA) (whole coding region, exons 2-11).

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Supplementary Table S1	Detailed characteristics of MP-IGH cases	mut	mutated <i>TP53</i> 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
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Supplementary Table S1	Detailed characteristics of MP-IGH cases	wt	wild type <i>TP53</i> gene
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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 <i>IGHV</i>
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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
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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
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Supplemental Figure S2	Overview of immunogenetic and immunophenotypic analyses in MP-IGH cases		



Supplemental Table S2 - part A

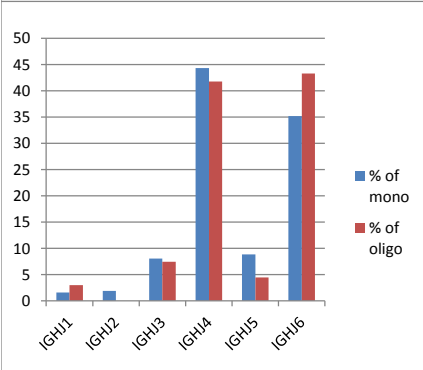
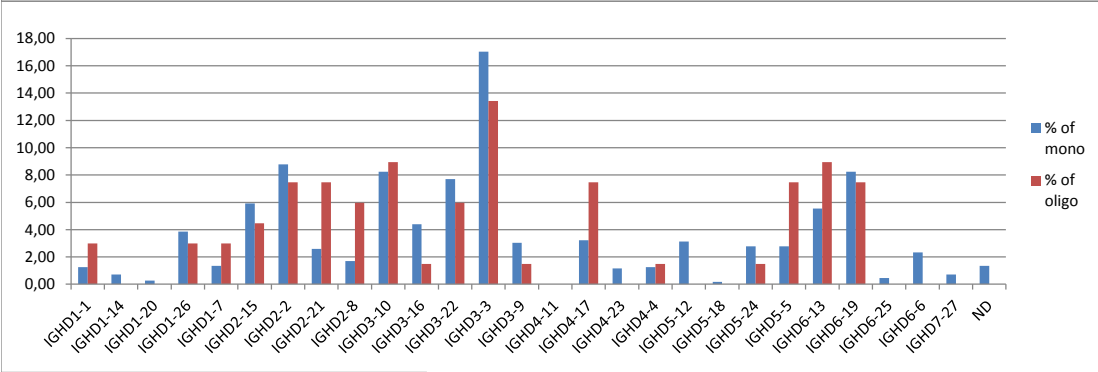
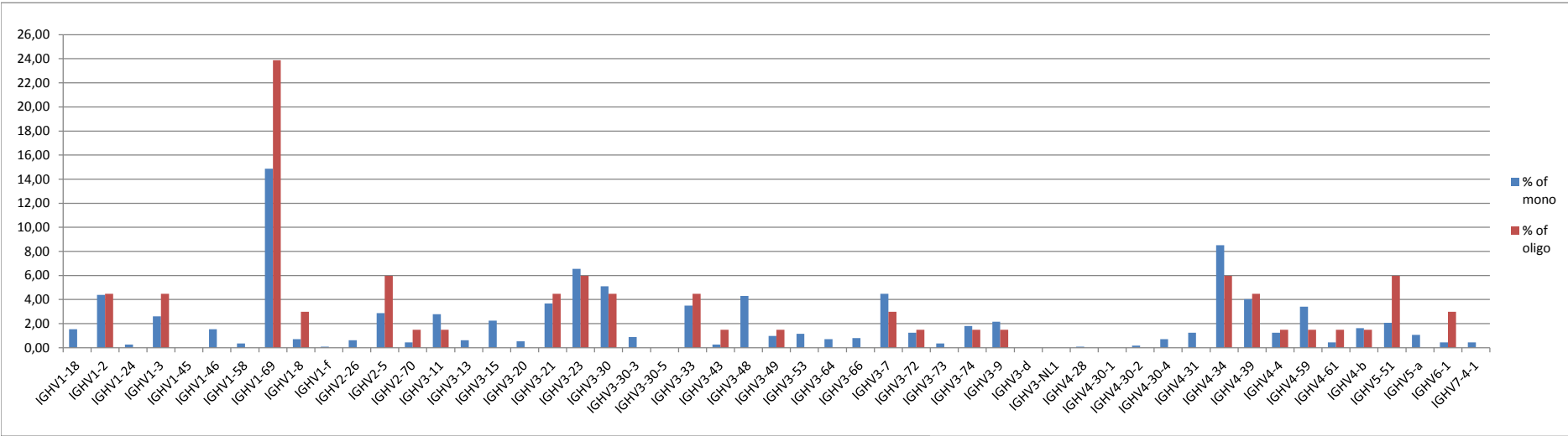
IGHV gene	# of mono cases	% of mono	# of oligo cases	% of oligo
IGHV1-18	17	1,52	0	0
IGHV1-2	49	4,39	3	4,48
IGHV1-24	3	0,27	0	0
IGHV1-3	29	2,60	3	4,48
IGHV1-45	0	0	0	0
IGHV1-46	17	1,52	0	0
IGHV1-58	4	0,36	0	0
IGHV1-69	166	14,87	16	23,88
IGHV1-8	8	0,72	2	2,99
IGHV1-f	1	0,09	0	0
IGHV2-26	7	0,63	0	0
IGHV2-5	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)	CDRS-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)	CDRS-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 HCDRL length	M-IGHV (1) = 1; U-IGHV (1) = 0	M-IGHV (2) = 1; U-IGHV (2) = 0	resulting mutational status	at least one stereotyped HCDR3	over time analysis	clonal drift observed	preference of stereotyped BcR	diminishing of stereotyped BcR
BRND0276	IGHV1-69*01	100	IGHD3-1*01	IGHH4*02	23	CATPTVYDFWSSVYVYVYVYVYVYVMDVW	7	P	IGHV1-69*01	100	IGHD3-1*01	IGHH4*02	23	CARILVDFWSSVYVYVYVYVYVYVMDVW	D	D	141	0	0.00	6	0	0	U	YES	YES	YES	YES	NO
BRND0279	IGHV1-69*01	100	IGHD3-2*02	IGHH4*02	23	CARHNDVDFWSSVYVYVYVYVYVYVMDVW	1	A	IGHV3-23*01	88.61	IGHD3-3*01	IGHH4*02	9	CARVDFWSSVYVYVYVYVYVYVMDVW	2	D	143	4	1.26	13	0	0	U	YES	YES	YES	YES	YES
BRND0307	IGHV1-3*01	98.61	IGHD3-10*01	IGHH4*02	11	CARVDFWSSVYVYVYVYVYVYVMDVW	1	N	IGHV3-23*01	85.00	IGHD3-1*01	IGHH4*02	13	CARVDFWSSVYVYVYVYVYVYVMDVW	N	N	143	4	1.63	0	0	1	DB	YES	NO	YES	YES	YES
BRND0319	IGHV3-30*03 or IGHV3-30*18	100	IGHD3-13*01	IGHH4*02	11	CARVDFWSSVYVYVYVYVYVYVMDVW	A	N	IGHV3-33*01 or IGHV3-33*06	100	IGHD3-17*01	IGHH4*02	14	CARVDFWSSVYVYVYVYVYVYVMDVW	P	P	343	2	0.00	1	0	0	U	NO	YES	YES	NO	NO
BRND0319	IGHV3-30*03 or IGHV3-30*18	100	IGHD3-13*01	IGHH4*02	11	CARVDFWSSVYVYVYVYVYVYVMDVW	A	N	IGHV3-33*01 or IGHV3-33*06	100	IGHD3-17*01	IGHH4*02	14	CARVDFWSSVYVYVYVYVYVYVMDVW	P	P	344	4	0.00	1	0	0	U	NO	YES	YES	NO	NO
BRND0319	IGHV3-33*01 or IGHV3-33*06	100	IGHD3-13*01	IGHH4*02	14	CARVDFWSSVYVYVYVYVYVYVMDVW	P	P	IGHV4-39*01	100	IGHD3-17*01	IGHH4*02	15	CARVDFWSSVYVYVYVYVYVYVMDVW	P	P	344	4	0.00	2	0	0	U	NO	YES	NO	NO	NO
BRND0447	IGHV3-2*02	92.16	IGHD3-11*01	IGHH4*02	13	CVRVGLSSVYVYVYVYVYVYVMDVW	P	P	IGHV4-34*01	100	IGHD3-5*01	IGHH4*02	16	CARVDFWSSVYVYVYVYVYVYVMDVW	D	D	344	4	1.68	2	1	1	M	NO	YES	YES	NO	NO
BRND0511	IGHV1-2*02	100	IGHD3-1*01	IGHH4*02	26	CARVDFWSSVYVYVYVYVYVYVMDVW	N	N	IGHV1-69*01	100	IGHD3-17*01	IGHH4*02	24	CARVDFWSSVYVYVYVYVYVYVMDVW	7	N	141	2	0.00	2	0	0	U	YES	NO	YES	NO	NO
BRND0511	IGHV1-2*02	100	IGHD3-1*01	IGHH4*02	26	CARVDFWSSVYVYVYVYVYVYVMDVW	N	N	IGHV1-69*01	100	IGHD3-17*01	IGHH4*02	27	CARVDFWSSVYVYVYVYVYVYVMDVW	7	N	141	2	0.00	1	0	0	U	NO	YES	NO	NO	NO
BRND0511	IGHV1-69*01	100	IGHD3-1*01	IGHH4*02	24	CARVDFWSSVYVYVYVYVYVYVMDVW	7	N	IGHV1-69*01	100	IGHD3-17*01	IGHH4*02	27	CARVDFWSSVYVYVYVYVYVYVMDVW	7	N	141	0	0.00	3	0	0	U	YES	NO	YES	NO	NO
BRND0511	IGHV1-51*01	94.44	IGHD3-15*01	IGHH4*02	27	CARVDFWSSVYVYVYVYVYVYVMDVW	D	D	IGHV4-39*01	93.27	IGHD3-17*01	IGHH4*02	10	CARVDFWSSVYVYVYVYVYVYVMDVW	P	P	506	4	1.57	17	1	1	M	NO	YES	YES	NO	NO
BRND0504	IGHV1-69*01	99.81	IGHD3-15*01	IGHH4*02	20	CARVDFWSSVYVYVYVYVYVYVMDVW	5	P	IGHV1-69*01	100	IGHD3-15*01	IGHH4*02	24	CARVDFWSSVYVYVYVYVYVYVMDVW	P	P	141	0	0.69	4	0	0	U	YES	YES	YES	NO	NO
BRND0504	IGHV1-69*01	99.81	IGHD3-15*01	IGHH4*02	20	CARVDFWSSVYVYVYVYVYVYVMDVW	5	P	IGHV2-5*10	96.51	IGHD3-21*01	IGHH4*02 or IGHH4*03	12	CARVDFWSSVYVYVYVYVYVYVMDVW	D	D	142	4	2.45	8	0	0	U	YES	YES	YES	YES	NO
BRND0504	IGHV1-69*01	99.81	IGHD3-15*01	IGHH4*02	24	CARVDFWSSVYVYVYVYVYVYVMDVW	5	P	IGHV2-5*10	96.51	IGHD3-21*01	IGHH4*02 or IGHH4*03	12	CARVDFWSSVYVYVYVYVYVYVMDVW	D	D	142	4	3.05	12	0	1	DB	NO	YES	YES	YES	NO
BRND0504	IGHV1-69*01	99.81	IGHD3-15*01	IGHH4*02	24	CARVDFWSSVYVYVYVYVYVYVMDVW	5	P	IGHV2-5*10	96.51	IGHD3-21*01	IGHH4*02 or IGHH4*03	12	CARVDFWSSVYVYVYVYVYVYVMDVW	D	D	142	4	3.05	12	0	1	DB	NO	YES	YES	YES	NO
BRND0504	IGHV1-69*01	99.81	IGHD3-15*01	IGHH4*02	15	CARVDFWSSVYVYVYVYVYVYVMDVW	5	P	IGHV4-34*01	99.81	IGHD3-22*01	IGHH4*02	17	CARVDFWSSVYVYVYVYVYVYVMDVW	P	P	144	4	6.24	2	1	0	DB	NO	YES	NO	NO	NO
BRND0504	IGHV1-69*01	100	IGHD3-15*01	IGHH4*02	16	CARVDFWSSVYVYVYVYVYVYVMDVW	5	P	IGHV5-51*01	100	IGHD3-13*01	IGHH4*02	15	CARVDFWSSVYVYVYVYVYVYVMDVW	1	N	145	0	0.00	0	0	0	U	YES	NO	YES	NO	NO
BRND0504	IGHV1-69*01	100	IGHD3-15*01	IGHH4*02	21	CARVDFWSSVYVYVYVYVYVYVMDVW	5	P	IGHV5-51*01	92.18	IGHD3-13*01	IGHH4*02	17	CARVDFWSSVYVYVYVYVYVYVMDVW	P	P	142	4	2.22	4	0	1	DB	NO	YES	YES	NO	NO
BRND0504	IGHV3-30*03 or IGHV3-30*18	100	IGHD3-2*02	IGHH4*02	26	CARVDFWSSVYVYVYVYVYVYVMDVW	3	P	IGHV5-51*01	100	IGHD3-5*01	IGHH4*02	24	CARVDFWSSVYVYVYVYVYVYVMDVW	D	D	344	4	0.00	2	0	0	U	NO	YES	YES	NO	NO
BRND0504	IGHV1-69*01	100	IGHD3-2*02	IGHH4*02	23	CARVDFWSSVYVYVYVYVYVYVMDVW	3	P	IGHV4-69*01	96.46	IGHD3-18*01	IGHH4*02	14	CARVDFWSSVYVYVYVYVYVYVMDVW	D	D	144	4	2.16	6	0	1	DB	YES	YES	YES	YES	NO
BRND0504	IGHV1-2*02	100	IGHD3-8*01	IGHH4*02	13	CARVDFWSSVYVYVYVYVYVYVMDVW	3	P	IGHV1-69*01	100	IGHD3-31*01	IGHH4*02	24	CARVDFWSSVYVYVYVYVYVYVMDVW	D	D	141	2	0.00	19	0	0	U	NO	YES	YES	NO	NO
BRND0504	IGHV1-2*02	100	IGHD3-8*01	IGHH4*02	13	CARVDFWSSVYVYVYVYVYVYVMDVW	3	P	IGHV1-69*01	100	IGHD3-31*01	IGHH4*02	24	CARVDFWSSVYVYVYVYVYVYVMDVW	D	D	141	2	0.00	19	0	0	U	NO	YES	YES	NO	NO
BRND0504	IGHV1-2*02	100	IGHD3-8*01	IGHH4*02	13	CARVDFWSSVYVYVYVYVYVYVMDVW	3	P	IGHV1-69*01	100	IGHD3-31*01	IGHH4*02	24	CARVDFWSSVYVYVYVYVYVYVMDVW	D	D	141	2	0.00	19	0	0	U	NO	YES	YES	NO	NO
BRND0504	IGHV1-2*02	100	IGHD3-8*01	IGHH4*02	13	CARVDFWSSVYVYVYVYVYVYVMDVW	3	P	IGHV1-69*01	100	IGHD3-31*01	IGHH4*02	24	CARVDFWSSVYVYVYVYVYVYVMDVW	D	D	141	2	0.00	19	0	0	U	NO	YES	YES	NO	NO
BRND0504	IGHV1-2*02	100	IGHD3-8*01	IGHH4*02	13	CARVDFWSSVYVYVYVYVYVYVMDVW	3	P	IGHV1-69*01	100	IGHD3-31*01	IGHH4*02	24	CARVDFWSSVYVYVYVYVYVYVMDVW	D	D	141	2	0.00	19	0	0	U	NO	YES	YES	NO	NO
BRND0504	IGHV1-2*02	100	IGHD3-8*01	IGHH4*02	13	CARVDFWSSVYVYVYVYVYVYVMDVW	3	P	IGHV1-69*01	100	IGHD3-31*01	IGHH4*02	24	CARVDFWSSVYVYVYVYVYVYVMDVW	D	D	141	2	0.00	19	0	0	U	NO	YES	YES	NO	NO
BRND0504	IGHV1-2*02	100	IGHD3-8*01	IGHH4*02	13	CARVDFWSSVYVYVYVYVYVYVMDVW	3	P	IGHV1-69*01	100	IGHD3-31*01	IGHH4*02	24	CARVDFWSSVYVYVYVYVYVYVMDVW	D	D	141	2	0.00	19	0	0	U	NO	YES	YES	NO	NO
BRND0504	IGHV1-2*02	100	IGHD3-8*01	IGHH4*02	13	CARVDFWSSVYVYVYVYVYVYVMDVW	3	P	IGHV1-69*01	100	IGHD3-31*01	IGHH4*02	24	CARVDFWSSVYVYVYVYVYVYVMDVW	D	D	141	2	0.00	19	0	0	U	NO	YES	YES	NO	NO
BRND0504	IGHV1-2*02	100	IGHD3-8*01	IGHH4*02	13	CARVDFWSSVYVYVYVYVYVYVMDVW	3	P	IGHV1-69*01	100	IGHD3-31*01	IGHH4*02	24	CARVDFWSSVYVYVYVYVYVYVMDVW	D	D	141	2	0.00	19	0	0	U	NO	YES	YES	NO	NO
BRND0504	IGHV1-2*02	100	IGHD3-8*01	IGHH4*02	13	CARVDFWSSVYVYVYVYVYVYVMDVW	3	P	IGHV1-69*01	100	IGHD3-31*01	IGHH4*02	24	CARVDFWSSVYVYVYVYVYVYVMDVW	D	D	141	2	0.00	19	0	0	U	NO	YES	YES	NO	NO
BRND0504	IGHV1-2*02	100	IGHD3-8*01	IGHH4*02	13	CARVDFWSSVYVYVYVYVYVYVMDVW	3	P	IGHV1-69*01	100	IGHD3-31*01	IGHH4*02	24	CARVDFWSSVYVYVYVYVYVYVMDVW	D	D	141	2	0.00	19	0	0	U	NO	YES	YES	NO	NO
BRND0504	IGHV1-2*02	100	IGHD3-8*01	IGHH4*02	13	CARVDFWSSVYVYVYVYVYVYVMDVW	3	P	IGHV1-69*01	100	IGHD3-31*01	IGHH4*02	24	CARVDFWSSVYVYVYVYVYVYVMDVW	D	D	141	2	0.00	19	0	0	U	NO	YES	YES	NO	NO
BRND0504	IGHV1-2*02	100	IGHD3-8*01	IGHH4*02	13	CARVDFWSSVYVYVYVYVYVYVMDVW	3	P	IGHV1-69*01	100	IGHD3-31*01	IGHH4*02	24	CARVDFWSSVYVYVYVYVYVYVMDVW	D	D	141	2	0.00	19	0	0	U	NO	YES	YES	NO	NO
BRND0504	IGHV1-2*02	100	IGHD3-8*01	IGHH4*02	13	CARVDFWSSVYVYVYVYVYVYVMDVW	3	P	IGHV1-69*01	100	IGHD3-31*01	IGHH4*02	24	CARVDFWSSVYVYVYVYVYVYVMDVW	D	D	141	2	0.00	19	0	0	U	NO	YES	YES	NO	NO
BRND0504	IGHV1-2*02	100	IGHD3-8*01	IGHH4*02	13	CARVDFWSSVYVYVYVYVYVYVMDVW	3	P	IGHV1-69*01	100	IGHD3-31*01	IGHH4*02	24	CARVDFWSSVYVYVYVYVYVYVMDVW	D	D	141	2	0.00	19	0	0	U	NO	YES	YES	NO	NO
BRND0504	IGHV1-2*02	100	IGHD3-8*01	IGHH4*02	13	CARVDFWSSVYVYVYVYVYVYVMDVW	3	P	IGHV1-69*01	100	IGHD3-31*01	IGHH4*02	24	CARVDFWSSVYVYVYVYVYVYVMDVW	D	D	141	2	0.00	19	0	0	U	NO	YES	YES	NO	NO
BRND0504	IGHV1-2*02	100	IGHD3-8*01	IGHH4*02	13	CARVDFWSSVYVYVYVYVYVYVMDVW	3	P	IGHV1-69*01	100	IGHD3-31*01	IGHH4*02	24	CARVDFWSSVYVYVYVYVYVYVMDVW	D	D	141	2	0.00	19	0	0	U	NO	YES	YES	NO	NO
BRND0504	IGHV1-2*02	100	IGHD3-8*01	IGHH4*02	13	CARVDFWSSVYVYVYVYVYVYVMDVW	3	P	IGHV1-69*01	100	IGHD3-31*01	IGHH4*02	24	CARVDFWSSVYVYVYVYVYVYVMDVW	D	D	141	2	0.00	19	0	0	U	NO	YES	YES	NO	NO
BRND0504	IGHV1-2*02	100	IGHD3-8*01	IGHH4*02	13	CARVDFWSSVYVYVYVYVYVYVMDVW	3	P	IGHV1-69*01	100	IGHD3-31*01	IGHH4*02	24															

Supplemental Table S4 - part A

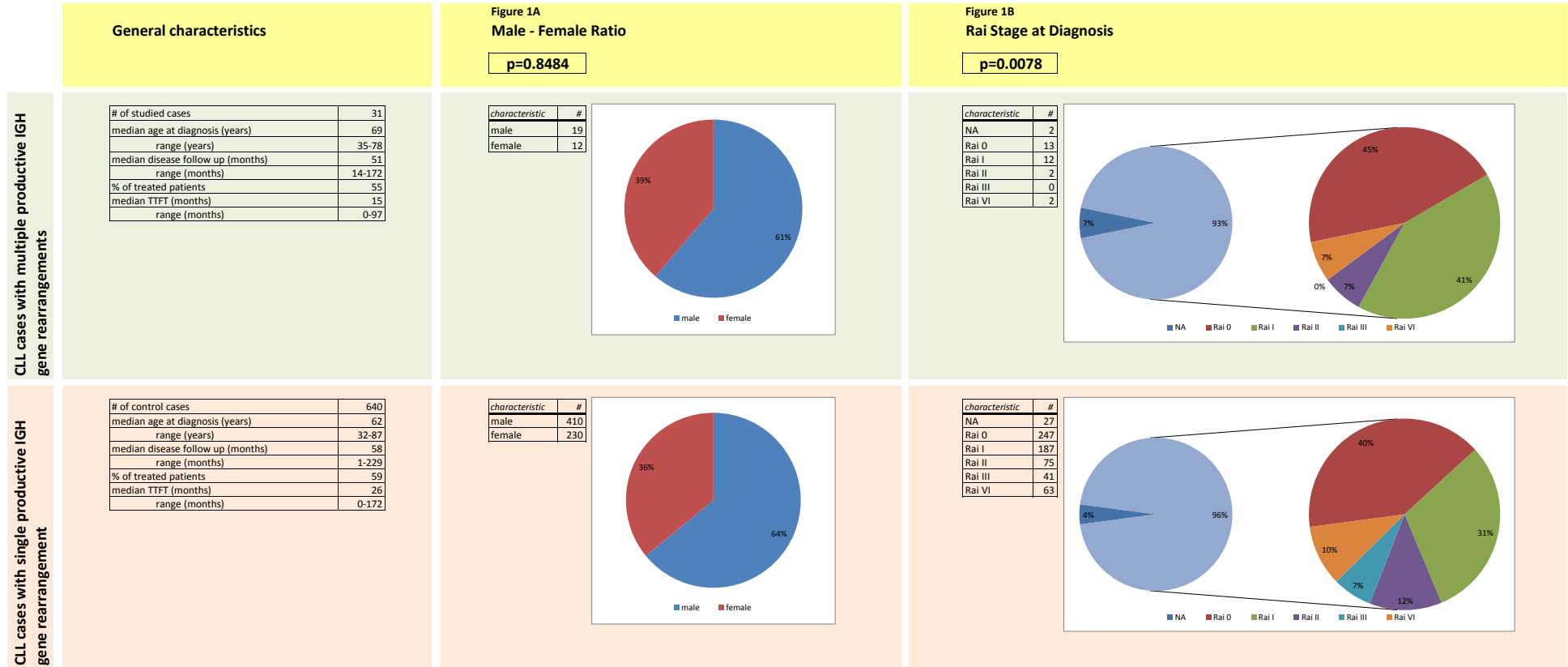
Patient ID	Group	Ig chain	Type of junction	V-GENE and allele	V-REGION identity %	D-GENE and allele	J-GENE and allele	CDR3-IMGT length	AA JUNCTION	Stereotyped subset	Functionality	Cause of unproductivity
BRN00261	I	heavy	IGHV-IGHD-IGHJ	IGHV1-69*01	100	IGHD3-3*01	IGHD*02	23	CATPYDFWSDGYPNYYNYGMDWV	7	productive	
BRN00261	I	heavy	IGHV-IGHD-IGHJ	IGHV1-69*01	100	IGHD3-3*01	IGHD*02	29	CAPRILDFWSDGYPNYYNYGMDWV	7	productive	
BRN00261	I	light	IGKJ-C-INTRON-KDE								allele inactivation	
BRN00261	I	light	IGKV-KDE	IGKV1-5*01, or IGVK1-5*02 or IGVK1-5*03	100						allele inactivation	
BRN00261	I	light	IGKV-KDE	IGKV1-5*03	99.62		IGKJ2*01, or IGKJ2*02 or IGKJ2*04	7	COQYNSYTF	7	productive	
BRN00261	I	heavy	IGHV-IGHD-IGHJ	IGHV1-69*01	100	IGHD3-3*01	IGHD*02	10	EDWVSDGSGVDF	7	productive	
BRN00279	I	heavy	IGHV-IGHD-IGHJ	IGHV1-69*01	100	IGHD3-2*02	IGHD*03	22	CARSPYVWVPSYNYNYGMDWV	3	productive	
BRN00279	I	heavy	IGHV-IGHD-IGHJ	IGHV1-21*01	98.61	IGHD5-24*01	IGHD*02	9	CARDTGDGMWV	2	productive	
BRN00279	I	light	IGKJ-C-INTRON-KDE								allele inactivation	
BRN00279	I	light	IGKV-KDE	IGHV1-39*01, or IGVK1D-39*01	98.52		IGHV1*01	9	COQYSYTRTF	9	productive	
BRN00279	I	light	IGKV-KDE	IGHV1-51*01, or IGVK1D-51*01	100		IGLJ1*01, or IGLJ3*01 or IGLJ3*02	11	COTWSSKSRVTF	11	productive	
BRN00307	II	heavy	IGHD-IGHJ			IGHD6-13*01	IGHD*02				incomplete	
BRN00307	II	heavy	IGHV-IGHD-IGHJ	IGHV1-3*01	98.61	IGHD3-10*01	IGHD*02	13	CARVQVWFYDFWV	13	productive	
BRN00307	II	heavy	IGHV-IGHD-IGHJ	IGHV1-39*01	89.93	IGHD1-1*01	IGHV1*01	13	CANADSWGDFYRHW	13	productive	
BRN00307	II	light	IGKV-KDE	IGHV1-39*01, or IGVK1D-39*01	100		IGHD*02				incomplete	
BRN00307	II	light	IGKV-KDE	IGHV1-18*01 P	100		IGHK4*01				unproductive	out-of-frame, IGVK pseudogene
BRN00319	I	heavy	IGHD-IGHJ			IGHD2-2*02	IGHD*02				incomplete	
BRN00319	I	heavy	IGHV-IGHD-IGHJ	IGHV1-69*01	100	IGHD3-9*01	IGHD*02				incomplete	
BRN00319	I	heavy	IGHV-IGHD-IGHJ	IGHV1-39*01, or IGVK1D-39*01	100	IGHD6-13*01	IGHD*02				incomplete	
BRN00319	I	heavy	IGHV-IGHD-IGHJ	IGHV1-39*03, or IGVK1-39*18	100	IGHD6-13*01	IGHD*02	13	CANAREQLPPEDW	13	productive	
BRN00319	I	heavy	IGHV-IGHD-IGHJ	IGHV1-33*01, or IGVK1-33*06	100	IGHD4-17*01	IGHD*02	14	CARGSGHGYVGMDFW	14	productive	
BRN00319	I	heavy	IGHV-IGHD-IGHJ	IGHV1-39*01	100	IGHD3-3*02	IGHD*02	16	CASDGLGSRMRSYDFW	16	productive	
BRN00319	I	light	IGKJ-C-INTRON-KDE								allele inactivation	
BRN00319	I	light	IGKV-KDE	IGHV1-15*01, or IGVK1-15*01	100		IGHK1*01	9	COQANSLRFG	9	productive	
BRN00319	I	light	IGKV-KDE	IGHV1-12*01, or IGVK1D-12*02	100		IGHK1*01, or IGVK1*01	9	COQSRNWRPFG	9	productive	
BRN00319	I	light	IGKV-KDE	IGHV1-11*01	100		IGHK1*01	9	COQYNSYTRTF	9	productive	
BRN00319	I	light	IGLV-IGLJ	IGLV3-23*01	100		IGLJ2*01, or IGLJ3*01	11	COVWSSSDVWVF	11	productive	
BRN00319	I	light	IGKV-KDE	IGHV1-39*01, or IGVK1D-39*01	100		IGHK2*01				unproductive	out-of-frame
BRN00319	I	light	IGLV-IGLJ	IGLV3-22*01	100		IGLJ1*01				unproductive	out-of-frame
BRN00319	I	light	IGLV-IGLJ	IGLV3-21*01(P)	100		IGLJ1*01, or IGLJ3*01	10	CCVTSATWVTF	10	productive	
BRN00447	III	heavy	IGHV-IGHD-IGHJ	IGHV1-39*01, or IGVK1D-39*01	92.36	IGHD6-13*01	IGHD*02	13	CARGSGSSVWDFW	13	productive	stop codons
BRN00447	III	heavy	IGHV-IGHD-IGHJ	IGHV1-34*01	94.04	IGHD5-5*01	IGHD*02	15	CASRFNGNYGYDFW	15	productive	
BRN00447	III	light	IGKV-KDE	IGHV1D-13*01	94.09		IGHK3*01, or IGHK4*01	9	COQYNSYTRTF	9	productive	
BRN00447	III	light	IGLV-IGLJ	IGLV3-23*01, or IGVK1D-23*02 or IGVK1D-23*03	98.76		IGHK3*01, or IGHK4*01	11	CCVTSATWVTF	11	productive	
BRN00511	II	heavy	IGHV-IGHD-IGHJ	IGHV1-2*02	100	IGHD3-3*01	IGHD*02	24	CARDTGSDGSSVWDFW	24	productive	
BRN00511	II	heavy	IGHV-IGHD-IGHJ	IGHV1-69*01	100	IGHD3-3*01	IGHD*02	11	CARDLYDFWSDYRPTGAYNYGMDWV	7	productive	
BRN00511	II	heavy	IGHV-IGHD-IGHJ	IGHV1-69*01	100	IGHD2-8*01	IGHD*02	27	CARAAAPYCTGVNGLYLAAYNYGMDWV	27	productive	
BRN00511	II	light	IGKJ-C-INTRON-KDE			IGHD4-17*01	IGHD*02				unproductive	out-of-frame, stop codons
BRN00511	II	light	IGKV-KDE	IGHV1-33*01, or IGVK1D-33*01	100						allele inactivation	
BRN00511	II	light	IGKV-KDE	IGHV1-12*01, or IGVK1D-12*02	100						unproductive	out-of-frame
BRN00511	II	light	IGKV-KDE	IGHV1-12*01, or IGVK1D-12*02 or IGVK1D-12*02	100		IGHK1*01				unproductive	out-of-frame
BRN00511	II	light	IGLV-IGLJ	IGLV3-01*01	100		IGLJ2*01, or IGLJ3*01				unproductive	out-of-frame
BRN00523	II	heavy	IGHV-IGHD-IGHJ	IGHV1-51*01	94.44	IGHD3-16*01	IGHD*02	27	CARLAAPPYMYVRSVDRHTNYGMDWV	27	productive	
BRN00523	II	heavy	IGHV-IGHD-IGHJ	IGHV1-1*01	93.27	IGHD1-1*01	IGHD*02	10	CARGSGDFW	10	productive	
BRN00523	II	light	IGKV-KDE	IGHV1D-20*01	100						allele inactivation	
BRN00523	II	light	IGKV-KDE	IGHV1-1*01	95.67		IGHD*02				incomplete	
BRN00604	II	heavy	IGHD-IGHJ			IGHD4-23*01	IGHD*02				incomplete	
BRN00604	II	heavy	IGHV-IGHD-IGHJ	IGHV1-69*01	100	IGHD3-10*01	IGHD*02	20	CARAMVGVGSYNYNYGMDWV	5	productive	
BRN00604	II	heavy	IGHV-IGHD-IGHJ	IGHV1-69*01	100	IGHD2-15*01	IGHD*02	24	CARLFFPSSGSSGVWAFSDFW	24	productive	
BRN00604	II	heavy	IGHV-IGHD-IGHJ	IGHV1-51*02	96.91	IGHD2-21*01	IGHD*02, or IGHK4*03	12	CANRRSGDFW	12	productive	
BRN00604	II	light	IGKJ-C-INTRON-KDE								allele inactivation	
BRN00604	II	light	IGKV-KDE	IGHV1-1*01	100						allele inactivation	
BRN00604	II	light	IGLV-IGLJ	IGLV1-44*01	99.62		IGLJ2*02	10	CAAWDNLNWF	10	productive	
BRN00604	II	light	IGLV-IGLJ	IGLV1-44*01	100		IGLJ1*01, or IGLJ3*01	9	CCVTSATWVTF	9	productive	
BRN00604	II	light	IGKV-KDE	IGHV1-1*01	100		IGHK1*01				unproductive	out-of-frame
BRN00604	II	light	IGKV-KDE	IGHV1-2*01	99.07		IGHK2*01				unproductive	out-of-frame
BRN00604	II	light	IGLV-IGLJ	IGLV1-44*01	100		IGLJ2*01, or IGLJ3*01 or IGLJ3*02				unproductive	out-of-frame
BRN00625	II	heavy	IGHD-IGHJ			IGHD5-2*01	IGHD*02				incomplete	
BRN00625	II	heavy	IGHV-IGHD-IGHJ	IGHV1-18*01	97.31	IGHD2-2*01	IGHD*02	25	CARDGQSSYSCYEGEYNYNYGMDWV	25	productive	
BRN00625	II	heavy	IGHV-IGHD-IGHJ	IGHV1-69*01, or IGVK1-69*12	93.06	IGHD5-5*01	IGHD*02	15	CARVLRYSYAELDFW	15	productive	
BRN00625	II	heavy	IGHV-IGHD-IGHJ	IGHV1-69*01	99.30	IGHD3-22*01	IGHD*02	17	CARAGGGKSSVWDFW	17	productive	
BRN00625	II	light	IGKV-KDE	IGHV1-16, or IGVK1D-16	100						allele inactivation	
BRN00625	II	light	IGKV-KDE	IGHV1-33*01, or IGVK1D-33*01	100		IGHK2*02	9	COQYNSYTRTF	9	productive	
BRN00625	II	light	IGLV-IGLJ	IGLV2-14*01	94.55		IGLJ3*02				unproductive	out-of-frame
BRN00625	II	light	IGKV-KDE	IGHV1-39*01, or IGVK1D-39*01	100		IGHD*02				unproductive	out-of-frame
BRN00814	II	heavy	IGHV-IGHD-IGHJ	IGHV1-69*01	100	IGHD2-15*01	IGHD*02	18	CARLGGVATVNYNYGMDWV	18	productive	
BRN00814	II	heavy	IGHV-IGHD-IGHJ	IGHV1-51*01	100	IGHD3-19*01	IGHD*02	13	CARDGSDGLDFW	13	productive	1
BRN00814	II	heavy	IGHV-IGHD-IGHJ	IGHV1-30*2*01	100	IGHD3-16*02	IGHD*02				unproductive	out-of-frame, stop codons
BRN00814	II	light	IGKV-KDE	IGHV1-33*01, or IGVK1D-33*01	100		IGHK1*01				productive	
BRN00814	II	light	IGKV-KDE	IGHV1-33*01, or IGVK1D-33*01	100		IGHK1*01				productive	
BRN00814	II	light	IGKV-KDE	IGHV1-39*01, or IGVK1D-39*01	100		IGHK1*01				productive	
BRN00814	II	light	IGKV-KDE	IGHV1-11*01	100		IGHK1*01				productive	
BRN00833	III	heavy	IGHV-IGHD-IGHJ	IGHV1-69*01	100	IGHD3-21*01	IGHD*02	21	CARVAGVALLGGLLNYNYGMDWV	21	productive	
BRN00833	III	heavy	IGHV-IGHD-IGHJ	IGHV1-69*01	92.78	IGHD3-22*01	IGHD*02	17	CARTYFSSDGHVDFW	17	productive	
BRN00833	III	light	IGKV-KDE	IGHV1-33*01, or IGVK1D-33*01	100		IGHK4*01	9	COQYNSYTRTF	9	productive	
BRN00833	III	light	IGKV-KDE	IGHV1-28*01, or IGVK1D-28*01	100		IGHK1*01	9	CANALGTRTF	9	productive	
BRN00845	III	heavy	IGHV-IGHD-IGHJ	IGHV1-39*03, or IGVK1-39*18	100	IGHD2-2*01	IGHD*02	26	CANLTPYCYSSYNYNYGMDWV	26	productive	
BRN00845	III	heavy	IGHV-IGHD-IGHJ	IGHV1-51*03	100	IGHD5-5*01	IGHD*02	24	CARHAEVQLWHPRRNYNYGMDWV	24	productive	
BRN00845	III	light	IGKV-KDE	IGHV1-8*01	100		IGHK2*01	9	COQYSYPTTF	9	productive	
BRN00846	II	heavy	IGHD-IGHJ			IGHD4-17*01	IGHD*02				incomplete	
BRN00846	II	heavy	IGHV-IGHD-IGHJ	IGHV1-69*01	100	IGHD2-2*01	IGHD*02	22	CARDPVPVWPAAMYYNYGMDWV	3	productive	
BRN00846	II	heavy	IGHV-IGHD-IGHJ	IGHV1-59*01	96.84	IGHD1-26*01	IGHD*02	14	CARTDYSYGYDFW	14	productive	
BRN00846	II	light	IGKV-KDE	IGHV1-13*01, or IGVK1D-17*01 or IGVK1D-17*02	100						allele inactivation	
BRN00846	II	light	IGKV-KDE	IGHV1-1*01	100		IGHD*02				incomplete	
BRN00862	II	heavy	IGHV-IGHD-IGHJ	IGHV1-2*02	100	IGHD2-8*01	IGHD*02	33	CARSLFYCTGVNCPVAAAGTGYNYGMDWV	33	productive	
BRN00862	II	heavy	IGHV-IGHD-IGHJ	IGHV1-69*01	100	IGHD2-21*02	IGHD*02	14	CASYGGDCYCLMDWV	14	productive	
BRN00862	II	light	IGKV-KDE	IGHV1-5*01, or IGVK1-5*02 or IGVK1-5*03	100						allele inactivation	
BRN00862	II	light	IGKV-KDE	IGHV1-5*01	100						allele inactivation	
BRN00862	II	light	IGKV-KDE	IGHV1-33*01, or IGVK1D-33*01	100		IGHK5*01	10	COQYNSYTRTF	10	productive	
BRN00862	II	light	IGKV-KDE	IGHV1-28*01, or IGVK1D-28*01	100		IGHK5*01	9	CMQALGTRTF	9	productive	
BRN00862	II	light	IGKV-KDE	IGHV1D-26*01	99.55		IGHK3*01	10	CMQADQPPPTF	10	productive	
BRN00877	III	heavy	IGHV-IGHD-IGHJ	IGHV1-2*02	93.47	IGHD3-18*01	IGHD*02	18	CARLSPSSDGHVDFW	18	productive	148C
BRN00877	III											

Supplemental Table S4 - part B

BRNO1037	I	heavy	IGHV-IGHD-IGHJ	IGHV3-H*01	99.53	IGHD2-21*01	IGHH*03		CARERRHHVVITLPTTWFFYMDVW		unproductive	out-of-frame, IGHV pseudogene
BRNO1037	I	light	IGK1-C-INTRON-KDE								allele inactivation	
BRNO1037	I	light	IGKV-KDE	IGKV3-3*01	100						allele inactivation	
BRNO1037	I	light	IGKV-IGKJ	IGKV1-3*02	96.14		IGKJ3*01		9	CQQVYTLPTF	productive	
BRNO1037	I	light	IGLV-IGLJ	IGLV3-1*01	98.20		IGLJ2*01, or IGLJ3*01		9	CQAWDSGVVF	productive	
BRNO1037	I	light	IGKV-IGKJ	IGKV3-2*01	100		IGKJ4*01			CQKYNALALVF	unproductive	out-of-frame
BRNO1037	I	light	IGKV-IGKJ	IGKV3-15*01	91.74		IGKJ3*01			CQQFNWVWTF	unproductive	out-of-frame, stop codons
BRNO1049	II	heavy	IGHD-IGHJ			IGHD1-26*01	IGHH*02				unproductive	
BRNO1049	II	heavy	IGHV-IGHD-IGHJ	IGHV1-3*01	100	IGHD1-26*01	IGHH*02		17	CARMYSGSYHYVYMDVW	productive	
BRNO1049	II	heavy	IGHV-IGHD-IGHJ	IGHV3-33*01, or IGHV3-33*06	95.83	IGHD4-17*01	IGHH*02		15	CVRVYGGNREDFYD	productive	
BRNO1049	II	heavy	IGHV-IGHD-IGHJ	IGHV4-39*01	100	IGHD6-13*01	IGHH*02		19	CARRGYSYSHYGRNWFDPW	productive	
BRNO1049	II	light	IGK1-C-INTRON-KDE								allele inactivation	
BRNO1049	II	light	IGKV-IGKJ	IGKV4-1*01	100		IGKJ1*01		9	CQQYVTPWTF	productive	
BRNO1049	II	light	IGKV-IGKJ	IGKV1-8*01	100		IGKJ4*02		8	CQQYVYPPFF	productive	
BRNO1054	I	heavy	IGHD-IGHJ			IGHD3-2*01, or IGHD2-2*02	IGHH*03, or IGHH*03				incomplete	
BRNO1054	I	heavy	IGHV-IGHD-IGHJ	IGHV3-23*04	93.75	IGHD6-13*01	IGHH*03		17	CAKUGHLYQYHYHVDVW	productive	
BRNO1054	I	heavy	IGHV-IGHD-IGHJ	IGHV5-51*01	95.14	IGHD5-5*01	IGHH*02		16	CARLYSYGLSTPADYD	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	CQYVNWPPGTF	productive	
BRNO1054	I	light	IGLV-IGLJ	IGLV3-1*01	95.85		IGLJ1*01, or IGLJ3*01		9	CQAWDSGVVF	productive	
BRNO1072	I	heavy	IGHD-IGHJ			IGHD6-25*01	IGHH*02				incomplete	
BRNO1072	I	heavy	IGHV-IGHD-IGHJ	IGHV1-2*02	100	IGHD6-19*01	IGHH*02		13	CAREQWLALSHFDYD	productive	1
BRNO1072	I	heavy	IGHV-IGHD-IGHJ	IGHV3-21*01	99.31	IGHD3-3*01	IGHH*02		20	CARDIRSVWSSGGQYGMVDW	productive	
BRNO1072	I	light	IGK1-C-INTRON-KDE								allele inactivation	
BRNO1072	I	light	IGKV-IGKJ	IGKV1-39*01, or IGVK1D-39*01	100		IGKJ1*01		10	CQQSYTHPWTF	productive	
BRNO1072	I	light	IGLV-IGLJ	IGLV1-51*02	98.56		IGLJ3*02		12	CXTWDSLSAHWVF	productive	
BRNO1072	I	light	IGKV-IGKJ	IGKV2-28*01, or IGVK2D-28*01	100		IGKJ5*01			CMGALDTL	unproductive	out-of-frame, stop codons
BRNO1087	II	heavy	IGHD-IGHJ			IGHD6-13*01	IGHH*02				incomplete	
BRNO1087	II	heavy	IGHV-IGHD-IGHJ	IGHV3-43*01	100	IGHD4-17*01	IGHH*02		14	CAKAGSGDYVCMVDW	productive	
BRNO1087	II	heavy	IGHV-IGHD-IGHJ	IGHV6-1*01	95.29	IGHD4-17*01	IGHH*02		12	CARDAGGGYVFDYD	productive	
BRNO1087	II	light	IGK1-C-INTRON-KDE								allele inactivation	
BRNO1087	II	light	IGKV-IGKJ	IGKV3-15*01	100		IGKJ2*01		10	CQQVNWPLVTF	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	IGHH*01, or IGHH*02		14	CAREGAGTDLDFDSD	productive	
BRNO1132	I	heavy	IGHV-IGHD-IGHJ	IGHV3-7*01	94.10	IGHD2-21*02	IGHH*02		17	CALSGLDLYYYYDMVDW	productive	
BRNO1132	I	light	IGK1-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 Hommap-IGKV1D-33*01	99.28		IGKJ1*01		9	CQQVNSYRGWTF	productive	
BRNO1132	I	light	IGLV-IGLJ	IGLV3-27*01	97.83		IGLJ2*01, or IGLJ3*01		9	CYSAADNKKF	productive	
BRNO1132	I	light	IGKV-IGKJ	IGKV3-11*01	100		IGKJ4*01			CQQNSRWKTF	unproductive	out-of-frame
BRNO1132	I	light	IGKV-IGKJ	IGKV4-1*01	100		IGKJ1*01			CQQYVTPWTF	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	IGHH*02		17	CVRREKNSDWGGCFNHW	productive	1488
BRNO1137	III	heavy	IGHV-IGHD-IGHJ	IGHV3-30-3*01	92.36	IGHD1-7*01	IGHH*01		16	CVRREKNSHCYKFNGLW	productive	
BRNO1137	III	light	IGLV-IGLJ	IGLV2-14*01	95.14		IGLJ3*01		11	CSYSGSLVVF	productive	
BRNO1137	III	light	IGKV-IGKJ	IGKV3-20*01, or IGVK3D-20*01	100		IGKJ4*01			CQQVSGPPALTF	unproductive	out-of-frame

Supplemental Table S5

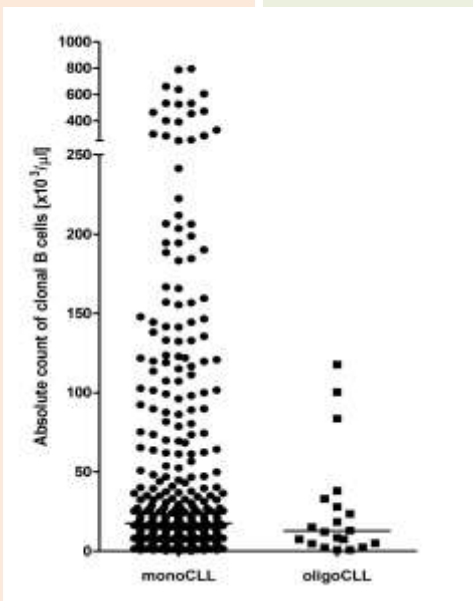
Patient ID	Time point	Absolute count of clonal B cells [x10 <sup>3</sup> /μl]	ASO-qPCR									Flow cytometry measurement				
			R1	Proportion of R1	Absolute count of cells with R1 [x10 <sup>3</sup> /μl]	R2	Proportion of R2	Absolute count of cells with R2 [x10 <sup>3</sup> /μl]	R3	Proportion of R3	Absolute count of cells with R3 [x10 <sup>3</sup> /μl]	No. of detected populations	Kappa expressing B cells	Absolute count of Kappa+ cells [x10 <sup>3</sup> /μl]	Lambda expressing B cells	Absolute count of Lambda+ cells [x10 <sup>3</sup> /μl]
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



**Figure 1C**  
**Absolute Count of Clonal B cells**

**p=0.1120**

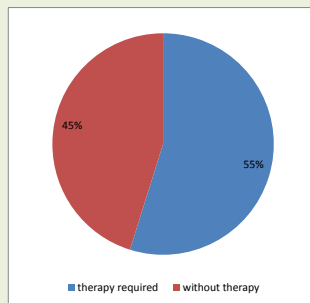
characteristic	monoCLL	oligoCLL
median (x10 <sup>3</sup> /μl)	18	13
range (x10 <sup>3</sup> /μl)	1-795	1-118



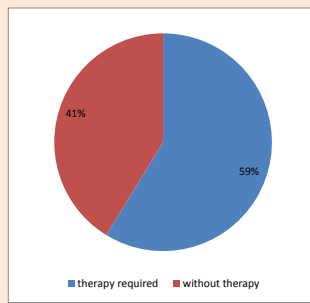
**Figure 1D**  
**Need for Treatment**

**p=0.7108**

characteristic	#
therapy required	17
without therapy	14



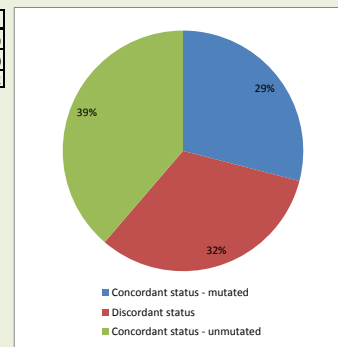
characteristic	#
therapy required	376
without therapy	264



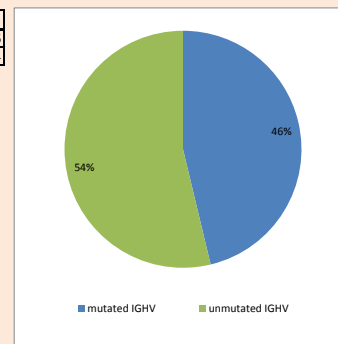
**Figure 1E**  
**Overall IGHV Mutational Status**

**p=0.6999**

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



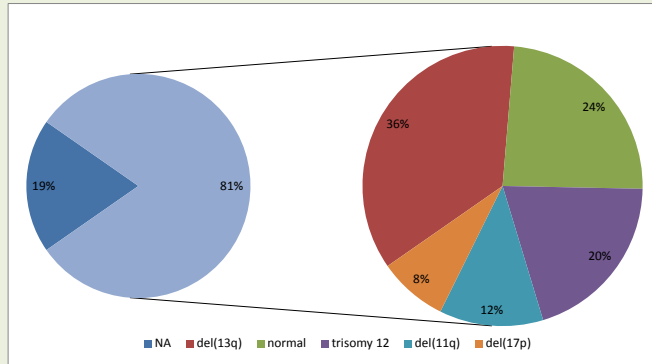
characteristic	#
mutated IGHV	296
unmutated IGHV	344



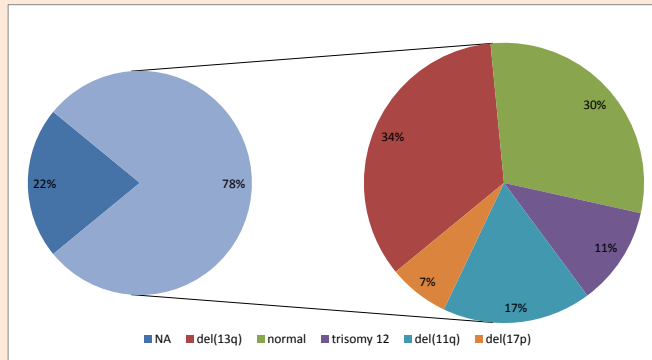
**Figure 1F**  
**FISH - Hierarchic Model**

**p=0.1014**

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



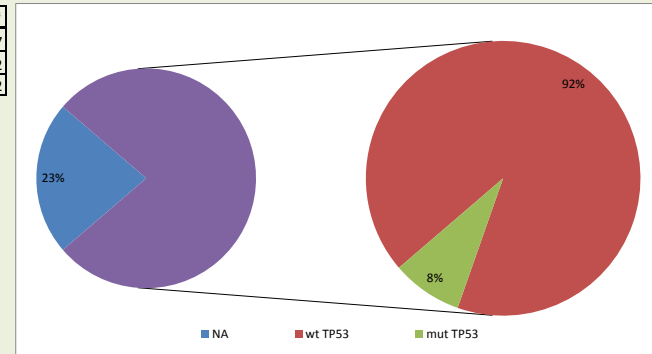
characteristic	#
NA	140
del(13q)	172
normal	150
trisomy 12	57
del(11q)	86
del(17p)	35



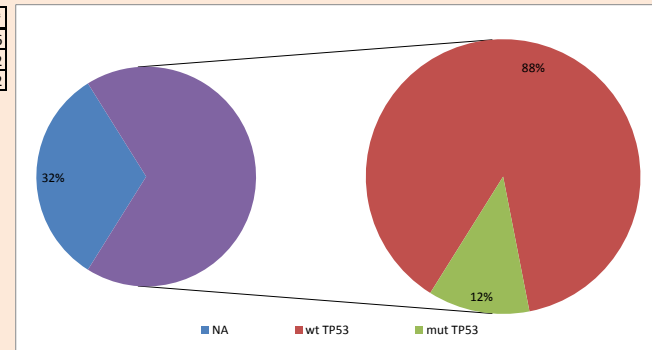
**Figure 1G**  
**TP53 Gene Mutation Status**

**p=1**

characteristic	#
NA	7
wt TP53	22
mut TP53	2



characteristic	#
NA	206
wt TP53	382
mut TP53	52





## Immunogenetic and Immunophenotypic Analysis

