Supplemental Methods

Selection of patients, histology and immunohistochemistry

Criteria for case selection were the presence of multiple samples at relapse (LN and BM), and tumor cell content of >50% in the LN specimens, as well as >30% in the BM specimens. Formalin fixed and paraffin-embedded (FFPE) specimens of the three patients, and fresh frozen (FF) LN specimens of two patients (patient 1 and 2) were available. The cases were selected from the files of the Institutes of Pathology of the Technical University of Muenchen, and the Julius Maximilians University of Wuerzburg, Germany.

FFPE LN and ethylen-diamine-tetra-acetate-decalcified BM specimens were cut into 4µm sections and stained with Haematoxylin & Eosin, Giemsa, and Gomori. Diagnostic immunostainings were performed on an automated immunostainer (Ventana Medical Systems, Tucson, AZ, USA) according to the company's protocols, with minor modifications. The applied panel of antibodies was directed against CD20 (L26, Dako, Copenhagen, Denmark), CD3 (polyclonal, Dako), CD10 (Novocastra Newcastle, UK), CD5 (Novocastra), BCL-2 (Dako), BCL-6 (Novocastra) and Ki67 (MIB1, Dako). The presence of the translocation t(14;18)(q32;q21) was confirmed by fluorescence in-situ hybridization (FISH) (Vysis BCL2 Break Apart Probe, Abbott, Germany). Selected cases were reevaluated by two hematopathologists (M.K. and F.F.).

DNA/RNA extraction

Genomic DNA was extracted from 20µm sections of FFPE LN tissues by overnight digestion with 25µl proteinase K (2mg/ml) in 500µl extraction buffer at 56°C, as described elsewhere. [1] Briefly, crude DNA extracts in a 1:10 dilution were used as templates to amplify

a 268 bp β-globin fragment for control of DNA quality. If no or only weak products of correct size were detected, the PCR was repeated with undiluted DNA extract. [2]

RNA was extracted using the RNeasy Mini Kit (Qiagen, Hilden, Germany) for the two fresh frozen LN specimens (patient 1 and 2), complemented by reverse transcription of extracted RNA with the Superscript III reverse transcriptase (Invitrogen, Karlsruhe, Germany). BM cells from FFPE BM tissues were likewise processed in the manner described above. Laser capture microdissection was performed repeatedly on neoplastic lymphoid infiltrates of the BM to increase the number of cells investigated. [1]

IgV_H-gene family-specific PCR

Amplification of the IgV_H-gene segments was performed as described elsewhere. [1, 3] Briefly, 2µl of purified cDNA of fresh frozen LN tissue extracts were used in 25µl PCR reaction volumes using framework I (FRI) family-specific V_H primers (VHL1-VHL6) and a J_H (JHa) consensus primer. DNA of FFPE LN and BM tissues was amplified in seminested PCRs, using a V_H framework II (FRIIa) consensus primer and a J_H (JHb - internal, JHc - external) consensus primer. In cases of seminested PCRs, DNA was diluted 1:10 for the external, and 1:100 for the internal rounds. 35 cycles of amplification were used, with annealing at 56°C and a final extension at 74°C for 10 minutes. For the second, intern reaction, the JHb-specific primer and the FR2a primer were applied. 20 cycles of amplification performed under the same conditions as for the extern reaction. To minimize Taq polymerase amplification errors, AmpliTaq Gold (PE Applied Biosystems, Foster City, CT, USA) was applied to all PCRs. Reactions were conducted with the Primus 96 Plus Thermal Cycler (MWG Biotech AG, Ebersberg, Germany) and the MJ Research PTC200 Peltier Thermal Cycler (Bio-Rad Laboratories GmbH, Germany). (Table 1)

Cloning and sequencing of IgV_H-gene segments

FRII amplicons were purified using a QIAquick PCR Purification Kit (Quiagen), whereas a QIAquick Gel Extraction Kit (Quiagen) was used for the FRI amplicons. Cloning was performed using the TOPO TA Cloning Kit (Invitrogen). Amplicons were introduced in plasmid vectors (pCR®2.1). Chemically competent E. coli bacteria (OneShot®TOP10F') were transformed with the recombinant plasmids. After plasmid extraction, EcoR1-restriction enzyme digestion and purification, sequencing of the IgV_H-gene segments was performed with an ABI Prism 7700 automated sequencer (PE Applied Biosystems), using the Big Dye-Terminator cycle kit (PE Applied Biosystems).

IgV_H-genes sequence analysis and definition of mutational patterns

The V_H-,D-, and J_H-gene segment families of the amplified IgV_H-gene segments were determined by comparing the obtained sequences with the published human immunoglobulin germline sequences using NCBI IgBLAST database (http://www.ncbi.nlm.mih.gov/igblast). [4] For each patient, only sequences with the dominant V_HDJ_H-gene rearrangement were further analyzed. [5] Mutational patterns of patients' sequences were established by comparison with the relevant germline sequnence. Those V_H-, D-, and J_H-gene segment sequences which were most similar to the determined germline sequence were postulated to be the naïve "wild type" sequence of the correspondent patient. Nucleotide residues without mutations were illustrated with "-", mutations were highlighted with the letter ("A", "T", "C" or "G") of the new nucleotide.

Calculation of the probability measurement

The population-specific relative frequency P_k of each mutation k, occurring among the sequenced clones was determined. The product of these relative mutational frequencies $\mathbf{\Pi}P_k$ was calculated for each HPC, representing a probability measure. As the number of mutations differed among the hypothetical clones, the calculated probabilities could not be simply compared to each other to reach a reasonable hierarchy of the HPCs. Because of this multiplicative operation, a mathematical bias was generated rendering hypothetical clones with higher mutational numbers a smaller probability than clones with fewer mutations, not reflecting evolutionary credibility. To stratify the probabilities to a comparable level, the root of the individual probabilities was applied. The exponent of the root was composed of three key figures: One representing the number of mutations of the considered HPC, one representing the number of repeats z of the HPC among the particular population (LN, BM or LN+BM), and one corresponding to the number of sequenced clones r covered by the relevant HPC. The final values represented the standardized probability measurements. These calculations were separately applied to the LN population, the BM population, and the LN and BM populations together, resulting in three tables of standardized pm-values illustrating the HPC-hierarchies. (Tables 2A-D; Tables 3A-D; Tables 4A-H)

Supplemental Results

Detailed compartment and inter-compartment evolution of lymphoma clones of patient 1

For the LN population of the first patient, where 10 different FL clones (a2, a6, a7, b1, b2, a8, b3, b7, b6 and b4) where detectable, a total of 48 HPCs was calculated. The HPC with the highest pm-value (HPC-LN1) was derived from a group of four sequenced LN-clones (clone a2, a6, a7, b1). Thus, these four clones were considered to be the core group of the LN-population that is believed to have evolved from HPC-LN1. (Table 2A)

The HPC with the second highest pm-value (HPC-LN2) comprised a total of five sequenced LN-clones, the already referred clones a2, a6, a7, b1, and in addition clone b2. Thus, HPC-LN2 constituted the predecessor clone of a2, a6, a7, b1, HPC-LN1 and b2. The pm-guided hierarchy of HPCs of the LN-population was analyzed for further HPCs assuming that already constituted groups of sequenced clones could not be separated by a HPC with a subordinate pm-value, unless such a HPC was a successor clone of an already established HPC. All sequenced clones and the chosen nine HPCs finally converged on clone b4.

The same procedure was applied to the eight sequenced BM clones that yielded a total of 24 BM-HPCs. Out of these, nine BM-HPCs (HPC-BM1 – HPC-BM9) were chosen, to reconstruct the pm-guided clonal evolution of the BM. Interestingly, unlike the LN clones, all BM clones shared one common mutation illustrated by HPC-BM1. (Table 2B)

To evaluate inter-compartment migration between the LN and the BM population, a HPC hierarchy comprising all (LN and BM) sequenced clones was generated. This set of HPCs was named “inter-compartment population of HPCs” and comprised 108 HPCs. (Table 2C) Two HPCs with the highest pm-value (HPC-LN+BM1 and HPC-LN+BM2) were identical to HPC-LN1 and HPC-LN2 from the LN HPCs. The third inter-compartment HPC (HPC-

LN+BM3) comprised sequenced LN (a2, a6, a7, b1) and BM (k5) clones. The fourth inter-compartment HPC (HPC-LN+BM4) comprised sequenced LN (a8) and BM (k1, k12, k2, k3) clones. Most sequenced clones, except for LN clones b6 and a8 and BM clones k4 and k5, evolved from inter-compartment HPCs with the same mutation pattern already established among the compartment-specific HPC-hierarchies (for example HPC-LN+BM1 equaled HPC-LN1, HPC-LN+BM11 equaled HPC-BM2, etc.). In contrast, seven inter-compartment HPCs, (HPC-LN+BM3, HPC-LN+BM4, HPC-LN+BM9, HPC-LN+BM10, HPC-LN+BM13, HPC-LN+BM14 and HPC-LN+BM15), comprised sequenced LN- and BM-clones ([k5, a2, a6, a7,b1]; [k1, k12, k2, k3, a8]; [k5, a2, a6, a7, b1, b2]; [k7, b6]; [k5, a2, a6, a7, b1, b3, b7]; [k1,k12, k2, k3, k4, a2, a6, a7, a8, b1, b2, b3, b7]; [k1, k12, k2, k3, k4, k5, k7, k9, b6]), and thus were not included in the LN- or BM-specific HPC-hierarchies. (Figure 4; Tables 2C-D)

Detailed compartment and inter-compartment evolution of lymphoma clones of patient 3

The pre-therapeutic LN population of 2004 was composed of eleven sequenced clones (m2, m5, m6, m8, m9, m1, m4, m3, m11, m7, m10). Among these, clone m1 and m4 shared the identical mutation pattern.

The mutation pattern of clone m2 was verifiable in all other clones, identifying this clone as a "founder clone". Four HPCs of a total of ten generated LN-specific HPCs were introduced to derive the nodal evolution, whereby HPC LN1 (2004) was identical to the sequenced clone m2.

The pre-therapeutic BM population of 2004 was composed of nine sequenced clones (n1, n8, n10, n7, n9, n4, n6, n3, n5). Three out of six HPCs were introduced to retrace this evolution of BM clones, which shared 13 identical mutations (depicted by HPC BM1 04).

The BM population of the first relapse (2006) showed an early "founder clone" (x3) that displayed the identical mutation pattern as the LN "founder clone" m2 of the pre-therapeuic LN population of 2004. Four out of six HPCs were introduced in this population, of which three were identical to sequenced clones (HPC BM1 06 = x3; HPC BM3 06 = x8; HPC BM4 06 = x2). The remaining eight sequenced clones, (x8, x2, x6, x7, x4, x1, x5, x9) shared eleven identical mutations, depicted by HPC BM2 06.

The post-therapeutic LN population of 2007 comprised of eight FL clones (o8, o9, o2, o5 ,o4 ,o1, o6 ,o10) with eleven identical mutations. Four HPCs out of six determined HPCs were introduced to analyze the clonal evolution. Of note, HPC-LN3 07 was identical to sequenced clone o2.

The BM population of the second relapse (2007) comprised ten clones (s10, s1, s5, s6, s2, s3, s7, s9, s4, s8). The clones s1 and s5 showed the identical mutation pattern with 14 mutations, the clones s3 and s7 with 15 identical mutations. Five HPCs out of seven generated HPCs were introduced to illustrate this clonal evolution. HPC BM3 07 was identical to clone s6, and HPC BM5 07 was identical to clone s9. (Tables 4A-F)

Analysis of somatic hypermutation patterns

Patient 1:

Sequences of both compartments (LN and BM) showed BCR-preserving SH mutation patterns by exhibiting a statistically significant selection for silent mutations (S) in the framework region of their IgV_H-gene sequences with 10/10 (100%) of LN clones and 3/8 (37.5%) of BM clones. The sequences of the remaining five BM clones (k3, k4, k5, k9, k12) contained one or more in-frame stop-codons and thus were not analyzed further. (Table 5A)

Patient 2:

The six LN sequences of the year 2002 (6/6, 100%) presented a non-random excess of BCR-preserving S-mutations and contained no in-frame stop-codons. For the 14 BM sequences of 2005, ten contained in-frame stop-codons (k1, k2, k3, k4, k5, k6, k7, k8, k10, k11); the remaining four BM sequences (3/14, 21%), clones k9, k12, k13, and k14, exhibited p-values consistent with structural BCR preservation. (Table 5B)

Patient 3:

No in-frame stop-codons were detected in the entire sequence population of patient 3. For the LN population of the year 2004, four sequences exhibited p-values consistent with structural BCR preservation (4/11, 36%), for the BM population of 2004 it was 3/9 (33%), 3/9 (33%) for the BM population of 2006, 1/8 (13%) for LN population of 2007, and 3/10 (30%) for the BM population of 2007. Of note, 9/28 (32%) of all BM sequences compared to 5/19 (26%) of all LN sequences showed BCR-preserving SH. Grouping of treatment-naïve LN and BM sequences of 2004 and opposing them to treatment-experienced LN and BM sequences of 2007, showed a reduction of the proportion of BCR-preserving clones of about one third (7/20, [35%] in 2004 versus. 4/18, [22%] in 2007). (Table 5C)

Figures (titles and legends):

Figure 1.

Mutational hierarchy of intraclonal sequence heterogeneity and delineation of hypothetical predecessor clones.

Legend: On the basis of an example lymph node (LN) population composed of six clones/sequences (LN1 - LN6), aspects of the non-multiple sequence alignment (non-MSA) based algorithm "mutation evolution estimator" (MEE)/"follicular lymphoma evolution reconstructor" (FLER) developed for this study are presented. First the mutational hierarchy of intraclonal heterogeneity is created by arranging the clones in an ascending order of mutations (mutation number depicted in brackets). Then each nucleotide residue (numbered from 1 – 21 in the example) is scanned among the clonal population (only a LN population is depicted here) and each nucleotide residue the is at least mutated (equally) in two clones, defines a group of clones and an hypothetical predecessor clone (HPC) that contains all shared mutations of the very clone group (in the example clone LN5 and clone LN6 share a common mutation at nucleotide residue number 10, hereby defining HPC5 with three mutations). In this study, not only the pool of LN HPCs was derived, but also the pool of HPCs for bone marrow (BM) clones of the follicular lymphoma cells and for the inter-compartment population (LN and BM together). So three pools of HPCs were created, a LN pool, a BM pool and the inter-issue pool. The line Pk signifies the relative mutation frequency of the respective mutation at the nucleotide residue k among the considered population, here of six LN clones. "t" signifies the number of clones contained in the population.

Figure 2.

Delineation of the parameters defining the probability measure pm of an hypothetical predecessor clone.

Legend: By use of the crucial formula to calculate the probability measure pm as pivotal element for stratification of hypothetical predecessor clones (HPC) in the non-multiple sequence alignment (non-MSA) based algorithm "mutation evolution estimator" (MEE)/"follicular lymphoma evolution reconstructor" (FLER) created for this study to retrace follicular lymphoma evolution/dissemination, the generation of the input parameters can be reasoned in the figure above: Each HPC is defined by: r, the group strength meaning the number of sequenced clones contained in the HPC-defining group of clones (clone group); z, the number of repeats of a HPC-sequence among the considered population (i.e. LN-, BM- or LN+BM[inter-tissue]-population). In this example HPC3 could be delineated twice from the group of six LN clones, the other HPC were traced only once. i, the number of mutations of the considered HPC. These three parameters, r, z, i and t, the total number of (sequenced) clones contained in the considered population, defined a product that was operated to extract the root out of the product of relative frequencies Pk of mutations present in the considered HPC. (Pk signifies the relative mutation frequency of the respective mutation at the nucleotide residue k among the considered population, here of six LN clones. "t" signifies the number of clones contained in the population.

Figure 3.

Pedigree of calculated compartment-specific follicular lymphoma cell evolution of patient 1.

Legend: LN – lymph node; BM – bone marrow; In the left panel the lymph node specific evolution of 10 nodal sequenced clones is depicted. By introduction of ten hypothetical predecessor clones (HPCs) the evolution could be traced back to the unmutated sequence of clone b4. (HPC_)LN3 signified the identical sequences of clone a7 and b1.

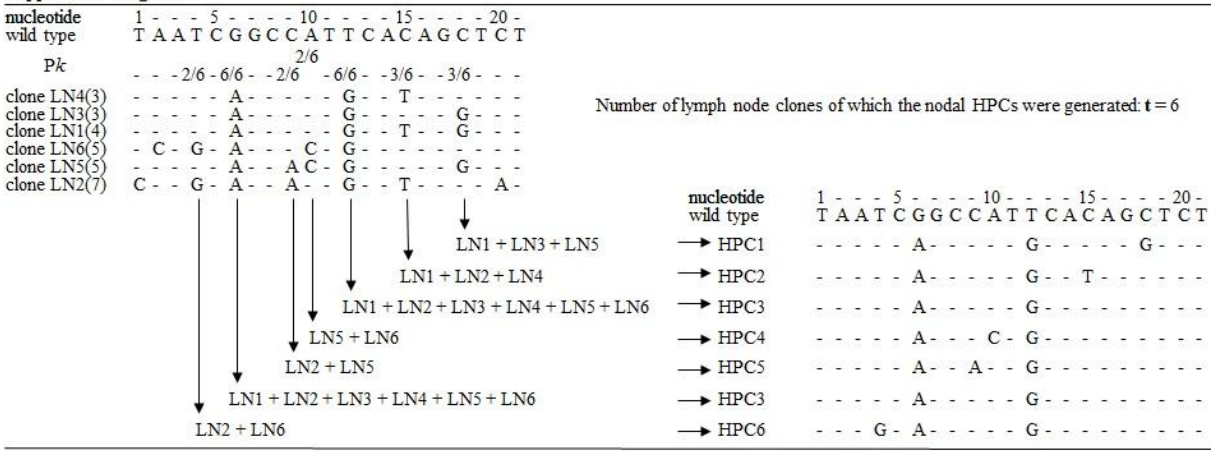
Figure 4.

Pedigree of calculated inter-compartment follicular lymphoma cell evolution of patient 1.

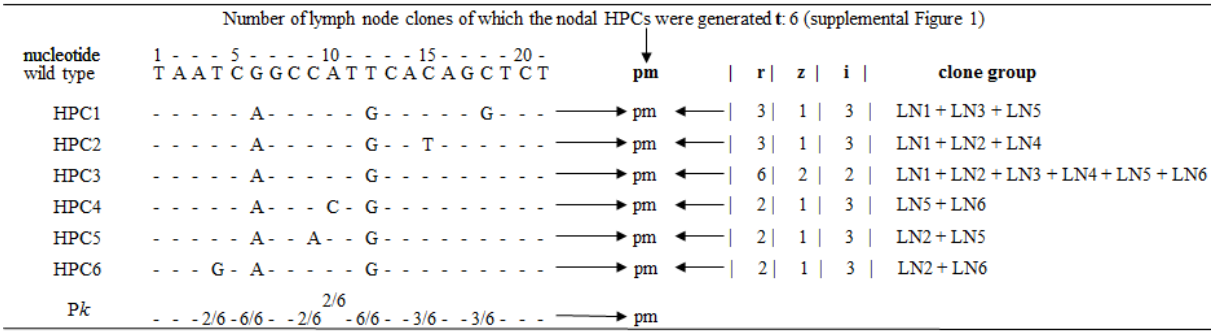
Legend:

LN – lymph node; BM – bone marrow; Model of inter-tissue evolution of all sequenced clones is depicted. By introduction of 16 hypothetical predecessor clones (HPCs) the evolution could be traced back to the unmutated sequence of clone b4. With BM clones k4 and k5 evolving from a HPCs of LN residency and LN clones b6 and a8 evolving from a HPCs of BM residency, a possible evolution by inter-tissue migration was indicated.

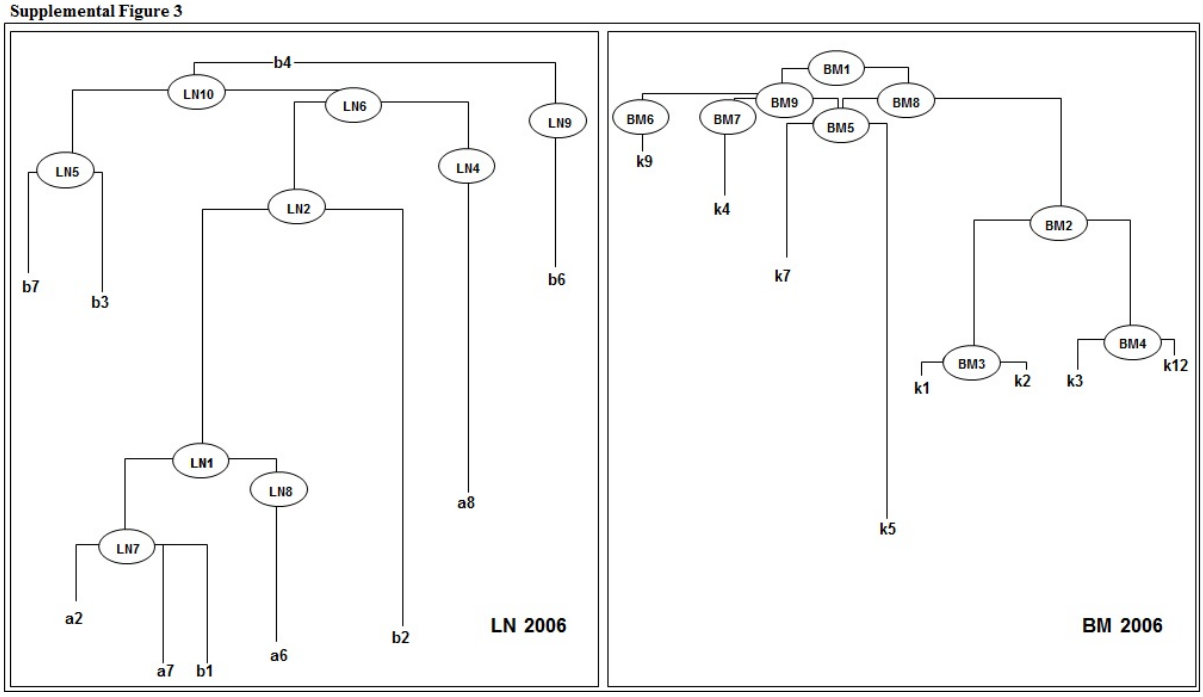
Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4

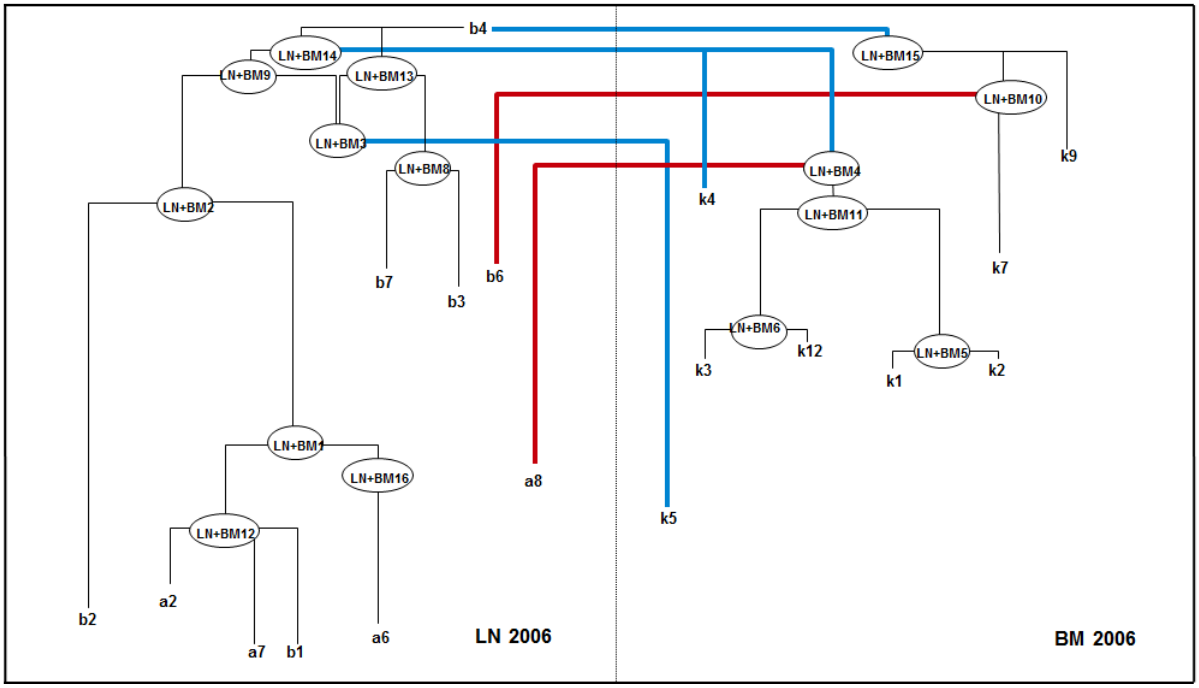


Table 1.
Sequences of applied primers for amplification of IgVH-gene segments

primer	location	sequence
IgV_H framework II consensus primer:	FRIIa	5'-TGG(A/G)TCCG(C/A)CAG(G/C)C(T/C)(T/C)CNGG-3'
JH consensus primer (extern):	JHc	5'-TGAGGAGACGGTGACC-3'
JH consensus primer (intern):	JHb	5'-GTGACCAGGGTNCCTTGGCCCCAG-3'
IgV_H framework I family-specific primers	FRI:	
	VHL1	5'-CCATGGACTGGACCTGGAGG-3'
	VHL2	5'-ATGGACATACTTTGTTCCAGC-3'
	VHL3	5'-CCATGGAGTTTGGGCTGAGC-3
	VHL4	5'-ATGAAACACCTGTGGTTCTT-3'
	VHL5	5'-ATGGGGTCAACCGCCATCCT-3'
	VHL6	5'-ATGTCTGTCTCCTTCCTCAT-3'
JH consensus primer:	JHa	5'-ACCTGAGGAGACGGTGACC-3'

Legend: For framework I (FRI) reactions, specific upstream primers corresponding to one of the six human immunoglobulin heavy chain variable region (IgVH)-gene family leader sequences (VHL1; VHL2; VHL3; VHL4; VHL5; VHL6) and a downstream primer (JHa) corresponding to a consensus sequence at the 3' end of the heavy chain joining (JH) region; for the semi-nested framework II (FRII) reactions a IgVH consensus upstream primer (FRIIa) and two downstream primers corresponding to a consensus sequence at the 3' end of the JH region (JHc for the extern round and JHb for the intern round) respectively, were applied.

Table 2A.
Pm-stratified hierarchy of hypothetical predecessor clones of lymph node clones of patient 1

Number of sequenced lymph node clones t: 10					
pm	r	z	i	clone group	clone name
0.996105691	**4**	**28**	**56**	**a2 a6 a7 b1**	**HPC_LN1**
0.993189382	**5**	**15**	**22**	**a2 a6 a7 b1 b2**	**HPC_LN2**
0.984105090	**2**	**6**	**83**	**a7=b1**	**HPC_LN3**
0.981687540	**2**	**6**	**16**	**a8 b2**	**HPC_LN4**
0.972807130	**2**	**4**	**17**	**b3 b7**	**HPC_LN5**
0.972313869	5	3	9	a2 a6 a7 a8 b1	
0.972228968	**6**	**3**	**5**	**a2 a6 a7 a8 b1 b2**	**HPC_LN6**
0.971310310	5	3	8	a2 a7 a8 b1 b2	
0.968139171	**3**	**3**	**67**	**a2 a7 b1**	**HPC_LN7**
0.967649031	**3**	**3**	**60**	**a6 a7 b1**	**HPC_LN8**
0.962002675	**2**	**4**	**7**	**a8 b6**	**HPC_LN9**
0.960847660	2	3	10	b2 b3	
0.957210100	3	2	5	a6 b3 b7	
0.956661709	2	2	7	a8 b7	
0.956483496	2	2	59	a2 a6	
0.952764866	2	2	6	a8 b3	
0.951930999	2	2	11	b2 b7	
0.951711575	3	2	6	b2 b3 b7	
0.936646498	4	2	2	a7 b1 b6 b7	
0.933262707	2	2	3	a2 b6	
0.930906252	3	1	7	a2 b3 b7	
0.930086848	7	1	2	a2 a6 a7 a8 b1 b3 b7	
0.930086848	7	1	2	a2 a6 a7 a8 b1 b2 b7	
0.929870818	6	1	3	a2 a6 a7 b1 b2 b7	
0.929870818	6	1	3	a2 a6 a7 b1 b3 b7	
0.929870818	6	1	3	a2 a7 a8 b1 b3 b7	
0.929744946	4	1	9	a2 a7 b1 b7	
0.929231928	6	1	2	a2 a7 b1 b2 b3 b7	
0.928370261	5	1	4	a2 a6 a7 b1 b3	
0.928335720	2	1	11	a6 a8	
0.928317767	**8**	**1**	**1**	**a2 a6 a7 a8 b1 b2 b3 b7**	**HPC_LN10**
0.926762506	2	1	29	a2 b2	
0.926399064	4	1	6	a7 b1 b2 b7	
0.925030139	5	1	6	a2 a7 b1 b3 b7	
0.924970437	2	1	25	a6 b2	
0.923777311	5	1	3	a7 b1 b2 b3 b7	
0.923040458	3	1	23	a2 a6 b2	
0.921835799	4	1	2	a8 b2 b3 b7	
0.920615315	3	1	30	a7 b1 b2	
0.914667404	3	1	2	a6 b2 b3	
0.913797189	4	1	27	a2 a7 b1 b2	
0.903086550	4	1	2	a6 a7 b1 b6	
0.902880451	6	1	1	a2 a6 a7 b1 b2 b6	
0.884157677	2	1	3	b2 b6	
0.877306662	4	1	1	a7 a8 b1 b6	
0.860280654	3	1	1	a8 b2 b6	
0.860280654	3	1	1	a6 a8 b6	
0.860280654	3	1	1	a6 b6 b7	

Legend: List of 48 lymph node-(LN) specific hypothetical predecessor clones (HPC) of patient 1 derived from the ten sequenced lymph node clones. Ten of these HPCs (HPC_LN1 – HPC_LN10) were necessary to recapitulate the pm-guided LN-specific follicular lymphoma cell evolution of patient 1. r, z and i - input parameters for the formula to calculate the probability measure pm. The HPCs chosen for the recapitulation of lymphoma cell evolution are depicted in bold letters. This followed the premise that already created clonal groups could not be disrupted by introduction of HPCs with a lower pm value.

Table 2B.
Pm-stratified hierarchy of hypothetical predecessor clones of bone marrow clones of patient 1

Number of sequenced bone marrow clones t: 8					
pm	r	z	i	clone group	clone name
1.000000000	**8**	**1**	**1**	**k1 k12 k2 k3 k4 k5 k7 k9**	**HPC_BM1**
0.993175882	**4**	**18**	**23**	**k1 k12 k2 k3**	**HPC_BM2**
0.991067728	**2**	**14**	**42**	**k1 k2**	**HPC_BM3**
0.989030389	**2**	**11**	**39**	**k12 k3**	**HPC_BM4**
0.965505424	**2**	**3**	**8**	**k5 k7**	**HPC_BM5**
0.962361334	5	2	4	k1 k12 k2 k3 k5	
0.957188732	2	2	7	k1 k5	
0.955689315	**3**	**2**	**5**	**k12 k3 k9**	**HPC_BM6**
0.954841604	**2**	**3**	**5**	**k4 k7**	**HPC_BM7**
0.953184293	**6**	**1**	**2**	**k1 k12 k2 k3 k5 k7**	**HPC_BM8**
0.953184293	6	1	2	k12 k2 k3 k4 k5 k9	
0.953184293	6	1	2	k1 k2 k3 k5 k7 k9	
0.942942048	5	1	2	k12 k3 k5 k7 k9	
0.942942048	5	1	2	k1 k12 k2 k3 k4	
0.938811585	5	1	3	k2 k3 k4 k5 k9	
0.936703374	4	1	3	k1 k2 k3 k9	
0.936703374	4	1	3	k1 k12 k2 k7	
0.933032992	4	1	2	k1 k2 k4 k9	
0.933032992	**4**	**1**	**2**	**k4 k5 k7 k9**	**HPC_BM9**
0.930123298	3	1	4	k12 k3 k7	
0.927763879	3	1	5	k1 k2 k7	
0.917078013	2	1	6	k1 k7	
0.915579611	2	1	9	k3 k9	
0.913105615	2	1	6	k12 k7	

Legend: List of 24 bone marrow-(BM) specific hypothetical predecessor clones (HPC) of patient 2 derived from the eight sequenced bone marrow clones. Nine of these HPCs (HPC_BM1 – HPC_BM9) were necessary to recapitulate the pm-guided BM-specific follicular lymphoma cell evolution of patient 1. r, z and i - input parameters for the formula to calculate the probability measure pm. The HPCs chosen for the recapitulation of lymphoma cell evolution are depicted in bold letters. This followed the premise that already created clonal groups could not be disrupted by introduction of HPCs with a lower pm value.

Table 2C.
Pm-stratified hierarchy of hypothetical predecessor clones of lymph node and bone marrow clones of patient 1

Number of sequenced lymph node and bone marrow clones t: 18					
pm	r	z	i	clone group	clone name
0.995272941	**4**	**17**	**56**	**a2 a6 a7 b1**	**HPC_LN+BM1**
0.991628452	**5**	**9**	**22**	**a2 a6 a7 b1 b2**	**HPC_LN+BM2**
0.991025389	**5**	**9**	**15**	**k5 a2 a6 a7 b1**	**HPC_LN+BM3**
0.989239843	**5**	**6**	**17**	**k1 k12 k2 k3 a8**	**HPC_LN+BM4**
0.986316210	3	5	23	k12 k3 a8	
0.985853674	**2**	**5**	**42**	**k1 k2**	**HPC_LN+BM5**
0.985009879	**2**	**5**	**39**	**k12 k3**	**HPC_LN+BM6**
0.984884900	**2**	**5**	**83**	**a7 b1**	**HPC_LN+BM7**
0.980565997	7	3	6	k1 k2 a2 a6 a7 b1 b2	
0.975237441	**2**	**3**	**17**	**b3 b7**	**HPC_LN+BM8**
0.974356182	9	2	6	k1 k12 k2 k3 a2 a6 a7 a8 b1	
0.973184689	**6**	**3**	**4**	**k5 a2 a6 a7 b1 b2**	**HPC_LN+BM9**
0.972329665	6	2	7	k1 k12 k2 k3 a8 b2	
0.972020049	2	2	7	a8 b7	
0.968692199	**2**	**3**	**6**	**k7 b6**	**HPC_LN+BM10**
0.967481700	**4**	**2**	**23**	**k1 k12 k2 k3**	**HPC_LN+BM11**
0.965860813	2	3	7	a8 b6	
0.965136330	2	2	11	b2 b7	
0.965037408	5	2	4	k7 a2 a6 a7 b1	
0.963132649	**3**	**2**	**67**	**a2 a7 b1**	**HPC_LN+BM12**
0.959659257	2	2	14	k5 b2	
0.959090016	2	2	10	k7 b3	
0.956044422	4	1	5	k1 k2 b3 b7	
0.955283010	5	2	2	k5 a7 b1 b6 b7	
0.954173030	6	1	3	k5 k7 a2 a7 b1 b7	
0.952794986	7	1	7	k1 k2 a2 a6 a7 a8 b1	
0.952046584	6	1	3	a2 a6 a7 b1 b2 b7	
0.951547296	4	1	3	k1 k2 k7 b3	
0.950165898	11	1	2	k1 k12 k2 k3 a2 a6 a7 a8 b1 b2 b7	
0.949658919	8	1	2	k1 k12 k2 k3 a8 b2 b3 b7	
0.949269604	10	1	3	k1 k12 k2 k3 a2 a6 a7 a8 b1 b2	
0.949230507	3	1	7	a2 b3 b7	
0.948882011	9	1	4	k1 k12 k2 k3 a2 a7 a8 b1 b2	
0.948801998	3	1	3	k7 a8 b3	
0.948463631	7	1	2	k1 k12 k2 k3 k5 a8 b3	
0.948463631	**7**	**1**	**2**	**k5 a2 a6 a7 b1 b3 b7**	**HPC_LN+BM13**
0.948325162	6	1	5	a2 a6 a7 a8 b1 b2	
0.947570874	2	2	3	a2 b6	
0.947530208	3	1	8	k12 a8 b2	
0.947452769	2	1	11	a6 a8	
0.947207528	13	1	1	k1 k12 k2 k3 k5 k7 a2 a6 a7 a8 b1 b3 b7	
0.947207528	**13**	**1**	**1**	**k1 k12 k2 k3 k4 a2 a6 a7 a8 b1 b2 b3 b7**	**HPC_LN+BM14**
0.947020496	3	1	14	k1 k2 a6	
0.946702330	6	1	2	k4 a2 a7 b1 b3 b7	
0.946702330	6	1	2	k7 a2 a6 a7 b1 b3	
0.945546243	3	2	2	k1 k5 b2	
0.945230398	5	1	7	k1 k2 a2 a6 b2	

0.944499440	3	1	7	k12 k3 b3	
0.944304319	6	1	3	k1 k12 k2 k3 b2 b3	
0.944244761	5	1	2	k1 k12 k2 k7 a8	
0.944244761	5	1	2	k1 k2 a6 b2 b3	
0.943803257	4	1	3	k1 k2 k3 k9	
0.943722057	12	1	1	k1 k2 k3 k5 k7 k9 a2 a7 a8 b1 b3 b7	
0.943717943	5	1	4	k1 k12 k2 k3 k5	
0.943637953	2	1	8	k5 k7	
0.942573820	3	1	21	k1 k2 a8	
0.942346363	2	1	9	k5 a8	
0.941098602	4	1	6	a7 b1 b2 b7	
0.940838927	4	1	2	k4 a6 a7 b1	
0.940838927	4	1	2	k4 a6 b3 b7	
0.940692201	3	1	5	a6 b3 b7	
0.940351656	2	1	8	k5 b3	
0.939125108	2	1	6	k1 k7	
0.936900984	2	1	10	k5 b7	
0.935979286	3	1	2	k4 a2 a6	
0.934761456	2	1	22	k5 a2	
0.934398034	3	1	4	k12 k7 a8	
0.934049609	2	1	16	a8 b2	
0.933787975	3	1	30	a7 b1 b2	
0.933653291	2	1	9	k3 k9	
0.933407112	6	1	2	k12 k2 k3 k4 k5 k9	
0.933032992	**9**	**1**	**1**	**k1 k12 k2 k3 k4 k5 k7 k9 b6**	**HPC_LN+BM15**
0.932075317	4	1	27	a2 a7 b1 b2	
0.931925043	5	1	2	k1 k12 k2 k3 b6	
0.931020120	2	1	10	b2 b3	
0.930943797	3	1	26	k5 a7 b1	
0.929866524	2	1	59	a2 a6	
0.929690438	2	1	18	k5 a6	
0.929484925	4	1	21	k5 a2 a7 b1	
0.929452696	5	1	3	k2 k3 k4 k5 k9	
0.927754113	2	1	9	k7 a8	
0.927371791	3	1	5	k5 a6 b2	
0.926257065	6	1	3	k5 a2 a7 a8 b1 b2	
0.926236185	**3**	**1**	**60**	**a6 a7 b1**	**HPC_LN+BM16**
0.924428320	6	1	2	k5 a7 b1 b2 b3 b7	
0.924312433	7	1	1	k5 a2 a7 b1 b2 b3 b7	
0.924312433	7	1	1	k5 k7 a2 a7 a8 b1 b2	
0.924312433	7	1	1	k5 a2 a6 a7 a8 b1 b2	
0.924312433	7	1	1	k1 k2 k4 k9 a6 b6 b7	
0.924312433	7	1	1	k12 k3 k5 k7 k9 a8 b6	
0.924201106	4	1	3	k5 b2 b3 b7	
0.924081156	2	1	3	k4 b2	
0.922899504	2	1	7	k4 a6	
0.918963718	6	1	1	a2 a6 a7 b1 b2 b6	
0.918963718	6	1	1	k12 k3 k9 a6 a7 b1	
0.918963718	6	1	1	k12 k3 k7 b2 b3 b7	
0.918046801	3	1	2	k7 a8 b2	
0.916900496	4	1	2	a6 a7 b1 b6	
0.915899410	2	1	5	k4 k7	
0.913635012	3	1	2	k7 b2 b3	
0.912565628	5	1	1	k4 k5 k7 k9 a6	

0.912565628	5	1	1	k12 k3 k9 a2 b2	
0.912565628	5	1	1	k5 a7 a8 b1 b6	
0.904591493	4	1	1	k4 k7 b2 b6	
0.894057698	3	1	1	k4 k7 b3	
0.894057698	3	1	1	a6 a8 b6	
0.894057698	3	1	1	a8 b2 b6	
0.894057698	3	1	1	k9 a8 b2	

Legend: List of 108 inter-compartment hypothetical predecessor clones (HPC) of patient 1 derived from the ten sequenced lymph node and the eight sequenced bone marrow clones altogether. Hereby 16 of these HPCs (HPC_LN+BM1 – HPC_LN+BM16) were necessary to recapitulate the pm-guided inter-compartment follicular lymphoma cell evolution of patient 1. r, z and i - input parameters for the formula to calculate the probability measure pm. The HPCs chosen for the recapitulation of lymphoma cell evolution are depicted in old letters. This followed the premise that already created clonal groups could not be disrupted by introduction of HPCs with a lower pm value.

Table 2D.
Pm-directed comparison of inter-compartment and compartment-specific hypothetical predecessor clones of patient 1

clone	pm(HPC LN+BM)	pm(HPC LN)	pm(HPC BM)
k4	0.947207528 (HPC_LN+BM14)	-----	**0.954841604 (HPC_BM7)**
b6	**0.968692199 (HPC_LN+BM10)**	0.962002675 (HPC_LN9)	-----
a8	**0.989239843 (HPC_LN+BM4)**	0.981687540 (HPC_LN4)	-----
k5	**0.991025389 (HPC_LN+BM3)**	-----	0.965505424 (HPC_BM5)

Legend: In case inter-compartment follicular lymphoma cell evolution and compartment-specific follicular lymphoma cell evolution of patient 1 differed for a sequenced clone (by suggestion of a migatory event in the inter-tissue evolution), the pm-values of the corresponding immediate HPCs (compartment-specific vs. inter-compartment) were compared. By this the most likely mode of evolution according to the highest pm value was determined. Chosen HPCs are presented in bold letters.

Table 3A.
Pm-stratified hierarchy of hypothetical predecessor clones of lymph node clones of patient 2

Number of sequenced lymph node clones t: 6					
pm	r	z	i	clone group	clone name
1.000000000	**6**	**27**	**27**	**a1 a2 a3 a4 a5 a6**	**HPC_LN1**
0.996861460	**5**	**2**	**29**	**a1 a2 a3 a5 a6**	**HPC_LN2**
0.990292390	**2**	**1**	**30**	**a3 a5**	**HPC_LN3**

Legend: List of three lymph node-(LN) specific hypothetical predecessor clones (HPC) of patient 2 derived from the six sequenced LN clones. All three HPCs (HPC_LN1 – HPC_LN3) were introduced in the corresponding pedigree to recapitulate the pm-guided LN-specific follicular lymphoma cell evolution of patient 2. r, z and i - input parameters for the formula to calculate the probability measure pm.

Table 3B.
Pm-stratified hierarchy of hypothetical predecessor clones of bone marrow clones of patient 2

Number of sequenced bone marrow clones t: 14					
pm	r	z	i	clone group	clone name
1.000000000	**14**	**22**	**22**	**k1 k10 k11 k12 k13 k14 k2 k3 k4 k5 k6 k7 k8 k9**	**HPC_BM1**
0.998390254	13	1	23	k1 k10 k11 k12 k13 k14 k2 k3 k4 k5 k6 k7 k9	
0.998390254	13	1	23	k1 k10 k11 k12 k13 k14 k3 k4 k5 k6 k7 k8 k9	
0.998390254	13	1	23	k1 k10 k11 k12 k13 k2 k3 k4 k5 k6 k7 k8 k9	
0.998390254	**13**	**1**	**23**	**k1 k10 k11 k13 k14 k2 k3 k4 k5 k6 k7 k8 k9**	**HPC_BM2**
0.996834761	**12**	**1**	**24**	**k1 k10 k11 k13 k14 k2 k3 k4 k5 k6 k7 k8**	**HPC_BM3**
0.996113791	11	1	25	k1 k10 k11 k2 k3 k4 k5 k6 k7 k8 k9	
0.996074069	9	1	25	k10 k11 k12 k13 k2 k3 k5 k6 k9	
0.996074069	9	1	25	k1 k10 k13 k14 k3 k4 k5 k8 k9	
0.996011853	7	1	24	k12 k13 k2 k5 k6 k8 k9	
0.994976559	**6**	**1**	**27**	**k1 k10 k11 k14 k3 k7**	**HPC_BM4**
0.994366030	3	1	26	k12 k7 k9	
0.993923350	2	4	32	k13 k14	
0.993218771	3	1	27	k11 k12 k9	
0.992973431	3	1	27	k13 k14 k8	
0.992785142	3	1	29	k11 k5 k9	
0.991806910	**3**	**1**	**28**	**k11 k14 k7**	**HPC_BM5**
0.989489587	2	1	27	k12 k7	

Legend: List of 18 bone marrow-(BM) specific hypothetical predecessor clones (HPC) of patient 2 derived from the 14 sequenced bone marrow clones. Five of these HPCs (HPC_BM1 – HPC_BM5) were necessary to recapitulate the pm-guided BM-specific follicular lymphoma cell evolution of patient 2. r, z and i - input parameters for the formula to calculate the probability measure pm. The HPCs chosen for the recapitulation of lymphoma cell evolution are depicted in bold letters. This followed the premise that already created clonal groups could not be disrupted by introduction of HPCs with a lower pm value.

Table 3C.
Pm-stratified hierarchy of hypothetical predecessor clones of lymph node and bone marrow clones of patient 2

Number of sequenced lymph node and bone marrow clones t: 20					
pm	r	z	i	clone group	clone name
1.000000000	**20**	**20**	**20**	**k8 k1 k10 k11 k12 k13 k14 k2 k3 k4 k5 k6 k7 k9 a1 a2 a3 a4 a5 a6**	**HPC_LN+BM1**
0.998779477	19	1	21	k8 k1 k10 k11 k12 k13 k2 k3 k4 k5 k6 k7 k9 a1 a2 a3 a4 a5 a6	
0.998779477	19	1	21	k8 k1 k10 k11 k12 k13 k14 k3 k4 k5 k6 k7 k9 a1 a2 a3 a4 a5 a6	
0.998779477	**19**	**1**	**21**	**k8 k1 k10 k11 k12 k13 k14 k2 k3 k4 k5 k6 k7 k9 a1 a2 a3 a5 a6**	**HPC_LN+BM2**
0.997629272	**18**	**1**	**22**	**k1 k10 k11 k12 k13 k14 k2 k3 k4 k5 k6 k7 k9 a1 a2 a3 a5 a6**	**HPC_LN+BM3**
0.997354361	14	1	22	k8 k1 k10 k11 k12 k13 k14 k2 k3 k4 k5 k6 k7 k9	
0.997264678	13	1	22	k8 k12 k13 k2 k5 k6 k9 a1 a2 a3 a4 a5 a6	
0.996854665	8	2	23	k13 k14 a1 a2 a3 a4 a5 a6	
0.996245617	7	2	25	k12 a1 a2 a3 a4 a5 a6	
0.995466541	**9**	**1**	**25**	**k10 k11 k12 k13 k2 k3 k5 k6 k9**	**HPC_LN+BM4**
0.995451944	13	1	23	k8 k1 k10 k11 k13 k14 k2 k3 k4 k5 k6 k7 k9	
0.994644658	3	1	26	k12 k7 k9	
0.994621201	2	1	25	k11 a3	
0.994387303	9	1	25	k8 k1 k10 k13 k14 k3 k4 k5 k9	
0.994066149	11	1	25	k8 k1 k10 k11 k2 k3 k4 k5 k6 k7 k9	
0.993771438	12	1	24	k8 k1 k10 k11 k13 k14 k2 k3 k4 k5 k6 k7	
0.993330480	6	1	27	k1 k10 k11 k14 k3 k4 k5 k6 k9 a1 a2 a3 a4 a5 a6	
0.993209287	**3**	**1**	**27**	**k11 k12 k9**	**HPC_LN+BM5**
0.991719981	3	1	29	k11 k5 k9	
0.991606417	3	1	27	k8 k13 k14	
0.991288488	2	2	32	k13 k14	
0.991269043	3	1	28	k13 a3 a5	
0.990900142	3	1	28	k11 k14 k7	
0.990656748	2	1	27	k12 k7	

Legend: List of 24 inter-compartment hypothetical predecessor clones (HPC) of patient 2 derived from the six sequenced lymph node (LN) and the 14 sequenced bone marrow (BM) clones altogether. Five of these HPCs (HPC_LN+BM1 – HPC_LN+BM5) were necessary to recapitulate the pm-guided inter-compartment follicular lymphoma cell evolution of patient 2. r, z and i - input parameters for the formula to calculate the probability measure pm. The HPCs chosen for the recapitulation of lymphoma cell evolution are depicted in bold letters. This followed the premise that already created clonal groups could not be disrupted by introduction of HPCs with a lower pm value.

Table 3D.
Pm-directed comparison of inter-compartment and compartment-specific hypothetical predecessor clones of patient 2

clone	pm(HPC LN+BM)	pm(HPC LN)
a1	0.997629272 (HPC_LN+BM3)	**1.000000000 (HPC_LN1)**
a6	0.997629272 (HPC_LN+BM3)	**1.000000000 (HPC_LN1)**
a2	**0.997629272 (HPC_LN+BM3)**	0.996861460 (HPC_LN2)
a3	**0.997629272 (HPC_LN+BM3)**	0.990292390 (HPC_LN3)
a5	**0.997629272 (HPC_LN+BM3)**	0.990292390 (HPC_LN3)

Legend: In case inter-compartment follicular lymphoma cell evolution and compartment-specific follicular lymphoma cell evolution of patient 2 differed for a sequenced clone (by suggestion of a migatory event in the inter-tissue evolution), the pm-values of the corresponding immediate hypothetical predecessor (HPC) clones (compartment-specific vs. inter-compartment) were compared. By this the most likely mode of evolution according to the highest pm-value was determined. Chosen HPCs are presented in bold letters.

Table 4A.
Pm-stratified hierarchy of hypothetical predecessor clones of lymph node clones of patient 3 year 2004

Number of sequenced lymph node clones t: 11					
pm	r	z	i	clone group	clone name
1.000000000	**11**	**5**	**5**	**(=m2) m1 m10 m11 m2 m3 m4 m5 m6 m7 m8 m9**	**HPC_LN1_2004**
0.993388763	**9**	**5**	**12**	**m1 m10 m11 m3 m4 m6 m7 m8 m9**	**HPC_LN2_2004**
0.993215251	**10**	**2**	**7**	**m1 m10 m11 m3 m4 m5 m6 m7 m8 m9**	**HPC_LN3_2004**
0.984217308	8	1	8	m1 m10 m11 m3 m4 m5 m6 m8	
0.979015847	4	1	13	m10 m11 m7 m9	
0.978450620	5	1	13	m1 m10 m3 m4 m7	
0.977948899	2	1	13	m7 m8	
0.977921971	3	1	9	m10 m11 m5	
0.974290904	3	1	14	m1 m4 m7	
0.972455583	**2**	**1**	**14**	**m7 m9**	**HPC_LN4_2004**

Legend: List of ten lymph node hypothetical predecessor clones (HPC) of patient 3 derived from the eleven sequenced lymph node clones of the year 2004. Four of these HPCs (HPC_LN1_2004 – HPC_LN4_2004) were necessary to recapitulate the pm-guided lymph node-(LN) specific follicular lymphoma cell evolution of the year 2004 of patient 3. Hereby HPC_LN1_2007 was identical to the sequenced clone m2. r, z and i - input parameters for the formula to calculate the probability measure pm. The HPCs chosen for the recapitulation of lymphoma cell evolution are depicted in bold letters. This followed the premise that already created clonal groups could not be disrupted by introduction of HPCs with a lower pm value.

Table 4B.
Pm-stratified hierarchy of hypothetical predecessor clones of bone marrow clones of patient 3 year 2004

Number of sequenced bone marrow clones t: 9					
pm	r	z	i	clone group	clone name
1.000000000	**9**	**13**	**13**	**n1 n10 n3 n4 n5 n6 n7 n8 n9**	**HPC_BM1_2004**
0.996081596	**8**	**2**	**15**	**n10 n3 n4 n5 n6 n7 n8 n9**	**HPC_BM2_2004**
0.992785701	6	1	14	n1 n10 n3 n4 n5 n9	
0.989760871	**5**	**1**	**16**	**n10 n3 n7 n8 n9**	**HPC_BM3_2004**
0.989760871	5	1	16	n3 n4 n6 n8 n9	
0.989158198	4	1	16	n3 n4 n5 n7	

Legend: List of six bone marrow hypothetical predecessor clones (HPC) of patient 3 derived from the nine sequenced bone marrow clones of the year 2004. Three of these HPCs (HPC_BM1_2004 – HPC_BM3_2004) were necessary to recapitulate the pm-guided bone marrow specific follicular lymphoma cell evolution of the year 2004 of patient 3. r, z and i - input parameters for the formula to calculate the probability measure pm. The HPCs chosen for the recapitulation of lymphoma cell evolution are depicted in bold letters. This followed the premise that already created clonal groups could not be disrupted by introduction of HPCs with a lower pm value.

Table 4C.
Pm-stratified hierarchy of hypothetical predecessor clones of bone marrow clones of patient 3 year 2006

Number of sequenced bone marrow clones t: 9					
pm	r	z	i	clone group	clone name
1.000000000	**9**	**5**	**5**	**(=x3) x1 x2 x3 x4 x5 x6 x7 x8 x9**	**HPC_BM1_2006**
0.994660532	**8**	**6**	**11**	**x1 x2 x4 x5 x6 x7 x8 x9**	**HPC_BM2_2006**
0.978737511	3	1	12	x1 x5 x9	
0.978737511	3	1	12	x1 x6 x9	
0.978656322	**5**	**1**	**12**	**(=x8) x5 x6 x7 x8 x9**	**HPC_BM3_2006**
0.978656322	**5**	**1**	**12**	**(=x2) x2 x4 x5 x7 x9**	**HPC_BM4_2006**

Legend: List of six bone marrow hypothetical predecessor clones (HPC) of patient 3 derived from the nine sequenced bone marrow clones of the year 2006. Four of these HPCs (HPC_BM1_2006 – HPC_BM4_2006) were necessary to recapitulate the pm-guided bone marrow specific follicular lymphoma cell evolution of the year 2006 of patient 3. Hereby HPC_BM1_2006 was identical to the sequenced clone x3, HPC_BM3_2006 was identical to the sequenced clone x8 and HPC_BM4_2006 was identical to the sequenced clone x2. r, z and i - input parameters for the formula to calculate the probability measure pm. The HPCs chosen for the recapitulation of lymphoma cell evolution are depicted in bold letters. This followed the premise that already created clonal groups could not be disrupted by introduction of HPCs with a lower pm value.

Table 4D.
Pm-stratified hierarchy of hypothetical predecessor clones of lymph node clones of patient 3 year 2007

Number of sequenced lymph node clones t: 8					
pm	r	z	i	clone group	clone name
1.000000000	**8**	**11**	**11**	**o6 o1 o10 o2 o4 o5 o8 o9**	**HPC_LN1_2007**
0.994451641	**7**	**1**	**12**	**o6 o1 o2 o4 o5 o8 o9**	**HPC_LN2_2007**
0.994451641	7	1	12	o6 o1 o10 o2 o4 o5 o8	
0.994451641	7	1	12	o6 o1 o10 o2 o4 o5 o9	
0.985522602	**4**	**1**	**15**	**(=o2) o6 o1 o2 o4**	**HPC_LN3_2007**
0.985282237	**2**	**2**	**14**	**o8 o9**	**HPC_LN4_2007**

Legend: List of six lymph node hypothetical predecessor clones (HPC) of patient 3 derived from the eight sequenced lymph node clones of the year 2007. Four of these HPCs (HPC_LN1_2007 – HPC_LN4_2007) were necessary to recapitulate the pm-guided lymph node specific follicular lymphoma cell evolution of the year 2007 of patient 3. Hereby HPC_LN3_2007 was identical to the sequenced clone o2. r, z and i - input parameters for the formula to calculate the probability measure pm. The HPCs chosen for the recapitulation of lymphoma cell evolution are depicted in bold letters. This followed the premise that already created clonal groups could not be disrupted by introduction of HPCs with a lower pm value.

Table 4E.
Pm-stratified hierarchy of hypothetical predecessor clones of bone marrow clones of patient 3 year 2007

Number of sequenced bone marrow clones t: 10					
pm	r	z	i	clone group	clone name
1.000000000	**10**	**11**	**11**	**s1 s10 s2 s3 s4 s5 s6 s7 s8 s9**	**HPC_BM1_2007**
0.995955872	**9**	**2**	**13**	**s1 s2 s3 s4 s5 s6 s7 s8 s9**	**HPC_BM2_2007**
0.991522379	6	1	12	s1 s10 s2 s3 s5 s7	
0.989745134	**6**	**1**	**14**	**(=s6) s3 s4 s6 s7 s8 s9**	**HPC_BM3_2007**
0.985658133	2	1	14	s2 s8	
0.982881660	**2**	**1**	**15**	**s4 s8**	**HPC_BM4_2007**
0.982881660	**2**	**1**	**15**	**(=s9) (s4 s9**	**HPC_BM5_2007**

Legend: List of seven bone marrow hypothetical predecessor clones (HPC) of patient 3 derived from the ten sequenced bone marrow clones of the year 2007. Five of these HPCs (HPC_BM1_2007 – HPC_BM5_2007) were necessary to recapitulate the pm-guided bone marrow specific follicular lymphoma cell evolution of the year 2007 of patient 3. Hereby HPC_BM3_2007 was identical to the sequenced clone s6 and, HPC_BM5_2007 was identical to the sequenced clone s9. r, z and i - input parameters for the formula to calculate the probability measure pm. The HPCs chosen for the recapitulation of lymphoma cell evolution are depicted in bold letters. This followed the premise that already created clonal groups could not be disrupted by introduction of HPCs with a lower pm value.

Table 4F.
Pm-stratified hierarchy of hypothetical predecessor clones of all lymph node and all bone marrow clones of patient 3

Number of sequenced lymph node and bone marrow clones t: 47					
pm	r	z	i	clone group	clone name
1.000000000	**47**	**5**	**5**	**(=m2) all 47**	**HPC_LN04BM04BM06BM07LN07_1**
0.997940936	**44**	**3**	**8**	**all 47 but x3 m2 m5**	**HPC_LN04BM04BM06BM07LN07_2**
0.997255524	44	1	6	all 47 but n1 x3 m2	
0.997255524	44	1	6	all 47 but s10 x3 m2	
0.996615443	**9**	**4**	**13**	**n1 n10 n3 n4 n5 n6 n7 n8 n9**	**HPC_LN04BM04BM06BM07LN07_3**
0.996180039	27	1	11	all 47 but n1 n10 n5 s1 s10 s2 s5 x1 x3 x6 x8 m1 m2 m3 m4 m5 m6 m8 o8	
0.996099851	18	1	11	n3 n4 n5 n7 s2 s8 x5 x6 x7 x8 x9 m1 m10 m3 m4 m7 o8 o9	
0.995918697	22	1	9	n1 n10 n3 n4 n5 n9 s1 s10 s2 s3 s5 s7 x1 x5 x9 m1 m4 m7 o1 o2 o4 o6	
0.995791318	37	1	8	all 47 but n1 n4 n5 n6 s10 x3 m2 m7 m9 o9	
0.995589252	36	1	10	all 47 but n1 x1 x2 x3 x4 x5 x6 x7 x8 m2 m5	
0.995396505	11	1	12	n5 m1 m10 m11 m3 m4 m5 m6 m7 m8 m9 o1	
0.995058751	7	1	11	n3 x1 x6 x9 m7 m8 o10	
0.995040990	**10**	**1**	**11**	**s1 s10 s2 s3 s4 s5 s6 s7 s8 s9**	**HPC_LN04BM04BM06BM07LN07_4**
0.994930261	**7**	**1**	**12**	**o1 o2 o4 o5 o6 o8 o9**	**HPC_LN04BM04BM06BM07LN07_5**
0.993811444	7	1	8	s4 s8 m10 m11 m5 o8 o9	
0.993447760	3	1	14	n3 s4 s9	
0.993351661	3	1	13	n6 m7 m9	
0.993345570	2	1	13	m3 o4	
0.991538640	2	1	14	n5 x9	

Legend: List of 19 inter-compartment hypothetical predecessor clones (HPC) of patient 3 derived from the 19 sequenced lymph node (LN) and the 28 sequenced bone marrow (BM) clones altogether. Five of these HPCs were necessary to recapitulate the pm-guided inter-compartment follicular lymphoma cell evolution of patient 3 over period of time from 2004 to 2007. Hereby the mutation pattern of the sequenced LN clone m2 of the initial lymph node population (year 2004) was included in all other sequenced clones and in all HPCs. Thus this clone was considered the “founder clone“, that could be retraced in all compartments of patient 3, either as an actual sequenced clone (lymph node population of 2004 [clone m2] and bone marrow population of 2006 [clone x3]), or as the most likely inter-tissue HPC (HPC_LN1_04 / HPC_LN04BM04BM06BM07LN07_1). r, z and i - input parameters for the formula to calculate the probability measure pm. The HPCs chosen for the recapitulation of lymphoma cell evolution are depicted in bold letters. This followed the premise that already created clonal groups could not be disrupted by introduction of HPCs with a lower pm value.

Table 4G.
Pm-stratified hierarchy of hypothetical predecessor clones of lymph node clones of 2004 and 2007 of patient 3 together

Number of sequenced lymph node clones t: 19					
pm	r	z	i	clone group	clone name
1.000000000	**19**	**5**	**5**	**all LN clones**	**HPC_LN04+LN07_1**
0.996145503	**18**	**2**	**7**	**m1 m10 m11 m3 m4 m5 m6 m7 m8 m9 o1 o2 o4 o5 o6 o8 o9 o10**	**HPC_LN04+LN07_2**
0.995819090	**17**	**4**	**11**	**m1 m10 m11 m3 m4 m6 m7 m8 m9 o1 o2 o4 o5 o6 o8 o9 o10**	**HPC_LN04+LN07_3**
0.991423906	15	1	8	m1 m10 m11 m3 m4 m5 m6 m8 o1 o2 o4 o5 o6 o8 o10	
0.990103363	**7**	**1**	**12**	**m1 m4 m7 m8 m9 o1 o2 o4 o5 o6 o8 o9 o10**	**HPC_LN04+LN07_4**
0.990103363	7	1	12	m1 m10 m11 m3 m4 m5 m6 m7 o1 o2 o4 o6	
0.990103363	**7**	**1**	**12**	**o1 o2 o4 o5 o6 o8 o9**	**HPC_LN04+LN07_5**
0.990091987	10	1	12	m1 m10 m11 m3 m4 m6 m7 m8 m9 o1	
0.989870351	11	1	12	m10 m11 m7 m9 o1 o2 o4 o5 o6 o9 o10	
0.988309733	3	1	12	m7 m8 o10	
0.988045896	5	1	8	m10 m11 m5 o8 o9	
0.987089545	2	1	13	m3 o4	
0.984280706	2	1	14	m7 m9	

Legend: List of 13 inter-compartment hypothetical predecessor clones (HPC) of patient 3 derived from the 19 sequenced lymph node (LN) clones (2004 and 2007 altogether). Five of these HPCs were necessary to recapitulate the pm-guided a possible follicular lymphoma cell evolution of patient 3 over period of time from 2004 to 2007 for a lymph node resident mode.. r, z and i - input parameters for the formula to calculate the probability measure pm. The HPCs chosen for the recapitulation of lymphoma cell evolution are depicted in bold letters. This followed the premise that already created clonal groups could not be disrupted by introduction of HPCs with a lower pm value.

Table 4H.
Pm-directed comparison of nodal relapse in 2007 from the bone marrow vs. nodal persistence for clones of patient 3

population	pm(HPC LN04+LN07)	pm(HPC LN04+BM04+BM06+BM07+LN07)
LN clones 2007	0.996145503 (HPC_LN04+LN07)	**0.997940936 (HPC_LN04+BM04+BM06+BM07+LN07_2)**

Legend: To estimate whether nodal persistence of follicular lymphoma clones from the primary nodal population in 2004 or "reinfiltration" of follicular lymphoma clones from the bone marrow to the lymph node was more likely to provoke the relapse in 2007, all lymph node clones (of 2004 and 2007) except of m2 were put together forming an inter-tissue population and all bone morrow clones (of 2004, 2006 and 2007) except of x3 were put together forming another inter-tissue population. Of both populations the second most likely hypothetical predecessor clone (after m2 or x3 respectively) were opposed concerning the pm-value. Reinfiltration from the bone marrow compartment to the lymph node was more likely (HPC_LN04+BM04+BM06+BM07+LN07_2) to be causing the relapse of 2007 than nodal persistence throughout the interval from 2004 to 2007 (HPC_LN04+LN07).

Table 5A.
Analysis of replacement and silent mutations of IgVH of patient 1

	FRII			FRI			FRII			FRI		
	FRW		p-value (FRW)	FRW		p-value (FRW)	CDR		p-value (CDR)	CDR		p-value (CDR)
	R	S		R	S		R	S		R	S	
lymph node clones of year 2006												
a2	14	25	**<0.005**	27	38	**<0.005**	18	20	0.95086	21	24	0.95239
a6	14	27	**<0.005**	27	44	**<0.005**	17	21	0.98773	20	27	0.98695
a7	16	26	**<0.005**	28	39	**<0.005**	19	22	0.96993	23	25	0.93649
a8	6	21	**<0.005**	13	27	**<0.005**	17	17	0.77305	20	22	0.64254
b1	16	26	**<0.005**	28	41	**<0.005**	19	22	0.96993	22	27	0.97144
b2	13	23	**<0.005**	26	38	**<0.005**	18	26	0.95880	19	27	0.98035
b3	4	7	**<0.005**	7	8	**<0.005**	9	14	0.79579	11	20	0.67921
b4	0	0	---	0	0	---	0	0	---	0	0	---
b6	4	4	**<0.005**	5	6	**<0.005**	11	12	0.36245	13	13	0.12210
b7	2	6	**<0.005**	3	8	**<0.005**	9	15	0.68546	10	17	0.70370
bone marrow clones of year 2006												
k1	7	12	**<0.005**	---	---	---	13	13	0.62905	---	---	---
k2	8	10	**<0.005**	---	---	---	13	13	0.59154	---	---	---
k7	2	5	**<0.005**	---	---	---	9	14	0.65408	---	---	---
k3			sc103(TGA)	---	---	---	---	---	---	---	---	---
k4			sc99(TAG)	---	---	---	---	---	---	---	---	---
k5			sc87(TGA)	---	---	---	---	---	---	---	---	---
k9			sc103(TAG)	---	---	---	---	---	---	---	---	---
k12			sc103(TGA)	---	---	---	---	---	---	---	---	---

Legend: Analysis of replacement (R) and silent (S) mutations according to multinomially modified Chang and Casali formula. For LN clones of patient 1, framework I (FRI) sequences were determined, too. So corresponding values for FRI and FRII are shown. Framework regions (FRW) of immunoglobulin-genes are considered to be essential for b-cell receptor integrity. Since the p-values for framework regions (FRW) of FRI and FRII sequences were <0,005 for all LN and for three BM clones of patient 1, b-cell receptor integrity and thus BCR restriction was considered for these clones. Since p-values for complementarity determinig regions (CDR) of FRI and FRII sequences were not <0.005, antigen selection of IgVH muations could not be proven. Calculation was done using "www.stat.stanford.edu/immunoglobin database/". Five of the eight bone marrow clones presented an in-frame stopcodon (sc; triplet number). Thus these clones were not considered to show b-cell receptor integrity and to be liberated from BCR restriction for survival. Codon numbering did not parallel the official NCBI IgBLAST numbering, but started at the first codon of FRI.

Table 5B.
Analysis of replacement and silent mutations of IgVH of patient 2

	FRII			FRI			FRII			FRI		
	FRW		p-value (FRW)	FRW		p-value (FRW)	CDR		p-value (CDR)	CDR		p-value (CDR)
	R	S		R	S		R	S		R	S	
lymph node clones of year 2002												
a1	4	8	**<0.005**	5	11	**<0.005**	9	8	0.54664	10	11	0.49653
a2	4	8	**<0.005**	5	11	**<0.005**	10	8	0.44953	11	11	0.39981
a3	4	10	**<0.005**	5	13	**<0.005**	9	9	0.68278	10	12	0.60967
a4	4	8	**<0.005**	5	11	**<0.005**	9	8	0.55311	10	11	0.50194
a5	4	5	**<0.005**	5	12	**<0.005**	9	9	0.64022	10	12	0.57324
a6	4	8	**<0.005**	5	12	**<0.005**	9	8	0.54664	10	11	0.53543
bone marrow clones of year 2005												
k9	9	7	**0.03437**	---	---	---	11	6	0.46866	---	---	---
k12	7	10	**<0.005**	---	---	---	11	7	0.53730	---	---	---
k13	6	12	**<0.005**	---	---	---	11	8	0.65800	---	---	---
k14	7	8	**<0.005**	---	---	---	11	8	0.50515	---	---	---
k1			sc49(TAA)	---	---	---	---	---	---	---	---	---
k2			sc49(TAA)	---	---	---	---	---	---	---	---	---
k3			sc49(TAA)	---	---	---	---	---	---	---	---	---
k4			sc49(TAA)	---	---	---	---	---	---	---	---	---
k5			sc49(TAA)	---	---	---	---	---	---	---	---	---
k6			sc49(TAA)	---	---	---	---	---	---	---	---	---
k7			sc49(TAA)	---	---	---	---	---	---	---	---	---
k8			sc49(TAA) sc82(TAA)	---	---	---	---	---	---	---	---	---
k10			sc49(TAA)	---	---	---	---	---	---	---	---	---
k11			sc49(TAA)	---	---	---	---	---	---	---	---	---

Legend: Analysis of replacement (R) and silent (S) mutations according to multinomially modified Chang and Casali formula. For LN clones of patient 2, framework I (FRI) sequences were determined, too. So corresponding values for FRI and FRII are shown. Framework regions (FRW) of immunoglobulin-genes are considered to be essential for b-cell receptor integrity. Since the p-values for FRW of FRI and FRII sequences were <0,05 for all LN clones and four BM clones of patient 2, b-cell receptor integrity and thus BCR restriction was considered for these clones. Since p-values for complementarity determinig regions (CDR) of FRI and FRII sequences were not <0.005, antigen selection of IgVH muations could not be proven. Calculation was done using “www.stat.stanford.edu/immunoglobin database/”. Ten of the 14 bone marrow clones presented an in-frame stopcodon (sc; triplet number). Thus these clones were not considered to show b-cell receptor integrity and to be liberated from BCR restriction for survival. Codon numbering did not parallel the official NCBI IgBLAST numbering, but started at the first codon of FRI.

Table 5C.
Analysis of replacement and silent mutations of IgVH of patient 3

	FRII			FRII		
	FRW		**p-value (FRW)**	**CDR**		**p-value (CDR)**
	R	**S**		**R**	**S**	
lymph node clones of year 2004						
m1	3	6	**0.02438**	3	3	0.78311
m2	0	2	**0.02380**	0	3	0.91282
m3	4	6	0.07436	2	3	0.91642
m4	3	6	**0.02438**	3	3	0.78311
m5	0	3	**0.00208**	3	3	0.40486
m6	3	5	0.05451	2	3	0.86353
m7	5	6	0.09638	3	3	0.85626
m8	4	5	0.10587	2	3	0.89289
m9	4	6	0.07724	2	3	0.91642
m10	4	6	0.05281	3	3	0.82832
m11	4	5	0.07436	3	3	0.78918
lymph node clones of year 2007						
o1	4	6	0.05125	3	3	0.82281
o2	4	5	0.07436	3	3	0.78311
o4	5	5	0.12794	3	3	0.82281
o5	4	5	0.07436	3	3	0.78219
o6	4	5	0.05125	3	4	0.82281
o8	3	6	**0.02438**	3	3	0.78918
o9	4	5	0.07677	3	3	0.78918
o10	5	6	0.13045	2	3	0.93512
bone marrow clones of year 2004						
n1	3	5	**0.02191**	3	4	0.77505
n3	6	7	0.05758	4	4	0.85250
n4	5	5	0.06469	4	4	0.74416
n5	7	9	0.05623	4	4	0.91951
n6	5	6	0.06204	3	4	0.88348
n7	4	6	**0.03304**	3	4	0.85551
n8	5	5	0.08826	3	4	0.85551
n9	5	5	0.06239	4	4	0.74416
n10	4	5	**0.03196**	4	4	0.69702
bone marrow clones of year 2006						
x1	3	4	0.05479	3	3	0.68269
x2	3	4	0.08198	2	3	0.82724
x3	0	2	**0.04908**	0	3	0.95184
x4	3	4	0.05506	3	3	0.68269
x5	3	5	**0.03758**	3	3	0.73655
x6	3	5	0.05625	2	3	0.86353
x7	3	5	0.05654	2	3	0.86353
x8	2	5	**0.02351**	2	3	0.82724
x9	6	5	0.11733	4	4	0.78766
bone marrow clones of year 2007						
s1	3	4	**0.03607**	4	3	0.52819
s2	3	5	**0.02438**	4	3	0.59169
s3	4	4	0.07436	4	3	0.59169
s4	4	5	0.05125	4	3	0.65815
s5	3	4	**0.03607**	4	3	0.52819
s6	4	4	0.10634	3	3	0.74032

s7	4	4	0.07436	4	3	0.59169
s8	4	5	0.05281	4	3	0.65815
s9	4	5	0.07436	3	3	0.78660
s10	3	3	0.08126	3	3	0.62549

Legend: Analysis of replacement (R) and silent (S) mutations according to multinomially modified Chang and Casali formula. For lymph node (LN) and bone marrow (BM) clones of patient 3 only framework II (FRII) sequences were determined. Framework regions (FRW) of immunoglobulin-genes are considered to be essential for b-cell receptor integrity. The p-values of framework regions (FRW) of FRII sequences of 14 clones were <0,05. For these clones (in bold letters) b-cell receptor integrity and thus BCR restriction was considered. Since p-values for complementarity determinig regions (CDR) of sequences were never <0.05, antigen selection of IgVH muations could not be proven. Calculation was done using "www.stat.stanford.edu/immunoglobin database/". None of the clones of patient 3 contained an in-frame stopcodon.

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