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Bryja et al.

(54) **METHOD OF DETERMINATION OF DIAGNOSIS AND PROGNOSIS IN PATIENTS WITH B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA AND OLIGONUCLEOTIDES FOR USE IN THIS METHOD**

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(57) **ABSTRACT**

The invention provides a method of determination of diagnosis and prognosis of B-cell chronic lymphocytic leukemia from a biological sample collected from the body of a patient, wherein the status of Wnt/PCP signaling pathway is determined. Within the framework of the present invention the relation of CLL and molecular signaling pathway Wnt/PCP the components of which are markedly up-regulated in B-lymphocytes of the patients suffering from CLL was identified. The status of the Wnt/PCP signaling pathway can be determined, e.g., by the determination of the expression of components of said signaling pathway or by the determination of migration of CLL cells in the gradient of a chemokine in the presence of the ligand of said signaling pathway. The invention also relates to suitable oligonucleotides for use in the method of determination of expression of the signaling pathway components.

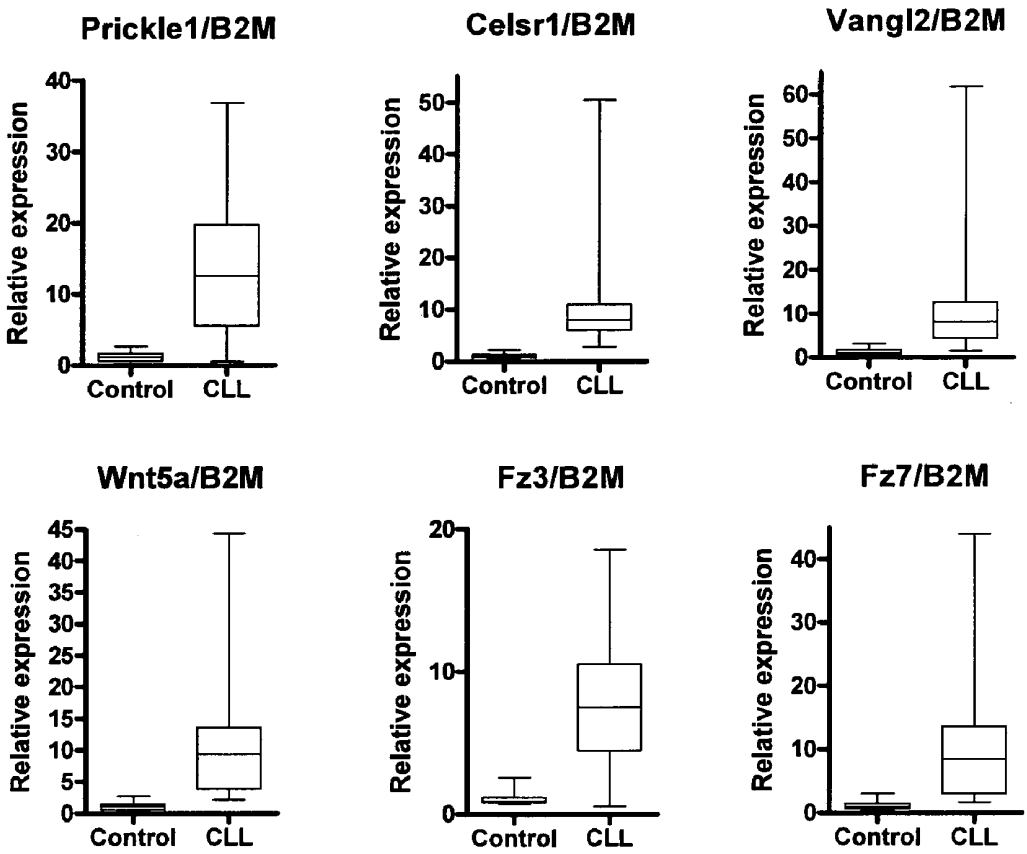

Prickle1/B2M
Relative expression
40
30
20
10
0
Control
CLL
Celsr1/B2M
Relative expression
50
40
30
20
10
0
Control
CLL
Vangl2/B2M
Relative expression
60
50
40
30
20
10
0
Control
CLL
Wnt5a/B2M
Relative expression
45
40
35
30
25
20
15
10
5
0
Control
CLL
Fz3/B2M
Relative expression
20
10
0
Control
CLL
Fz7/B2M
Relative expression
40
30
20
10
0
Control
CLL

Figure 1A

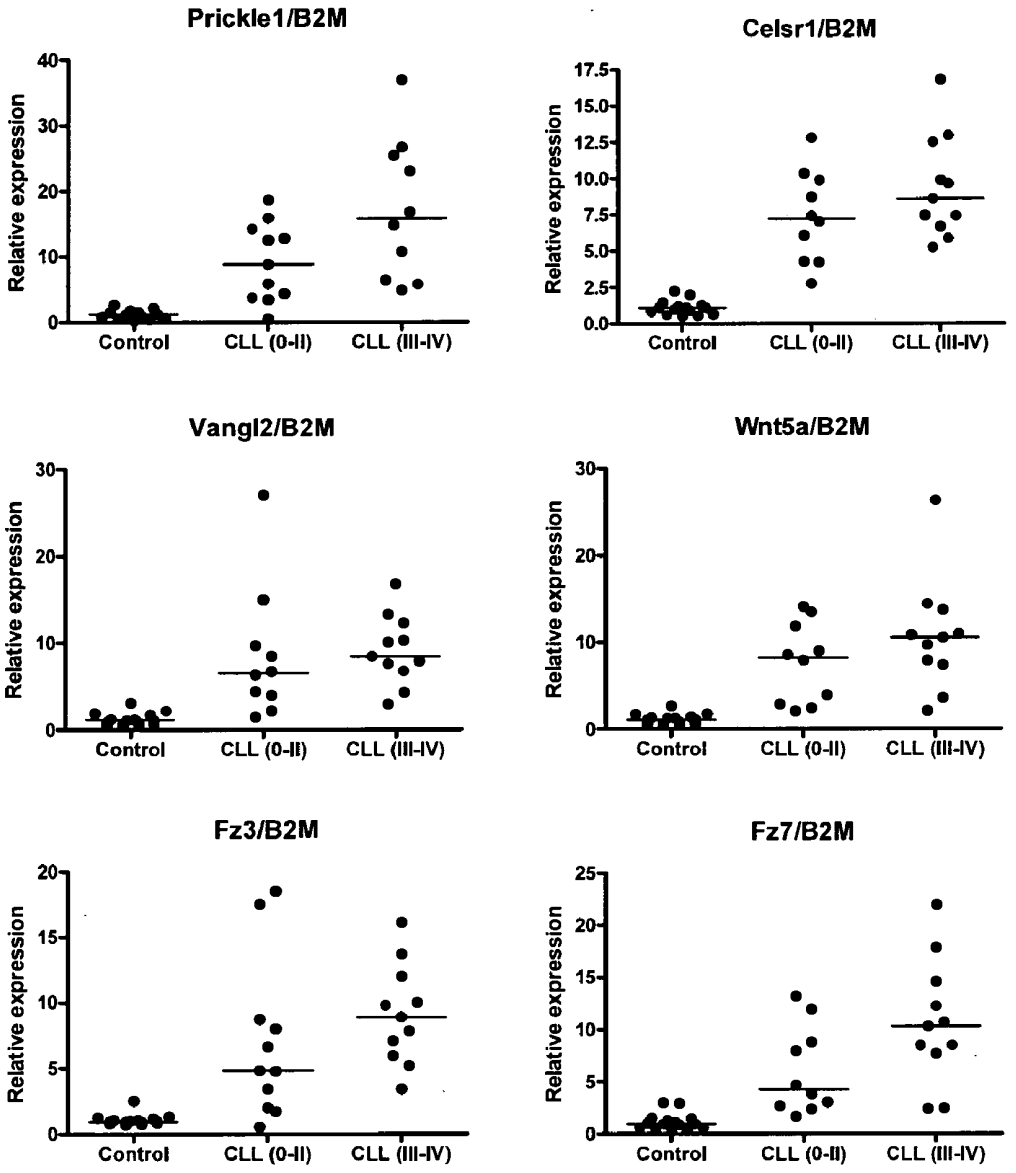
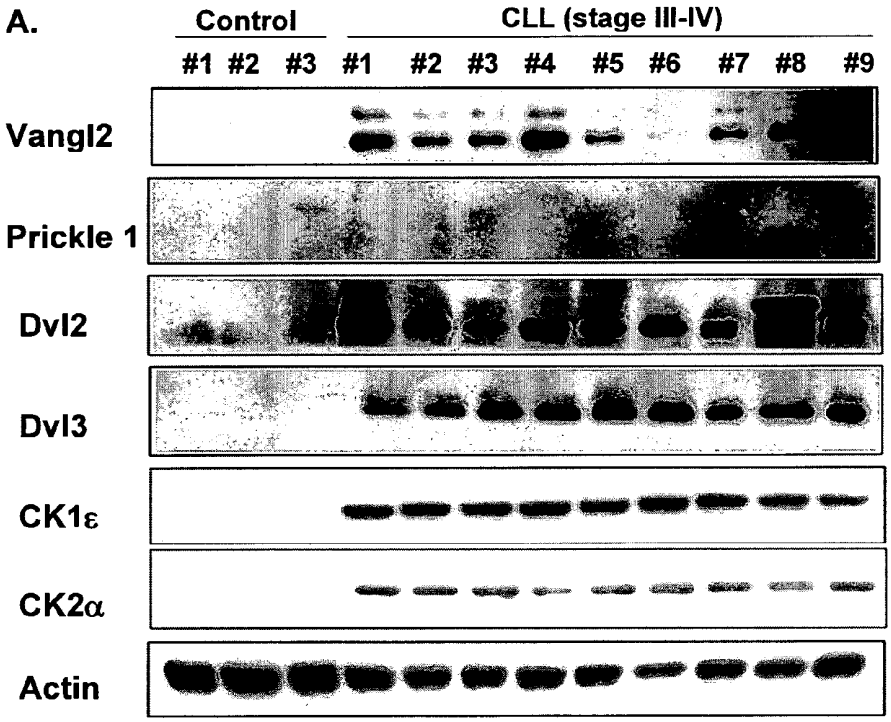

Prickle1/B2M
Relative expression
40
30
20
10
0
Control
CLL (0-II)
CLL (III-IV)
Celsr1/B2M
Relative expression
17.5
15.0
12.5
10.0
7.5
5.0
2.5
0.0
Control
CLL (0-II)
CLL (III-IV)
Vangl2/B2M
Relative expression
30
20
10
0
Control
CLL (0-II)
CLL (III-IV)
Wnt5a/B2M
Relative expression
30
20
10
0
Control
CLL (0-II)
CLL (III-IV)
Fz3/B2M
Relative expression
20
15
10
5
0
Control
CLL (0-II)
CLL (III-IV)
Fz7/B2M
Relative expression
25
20
15
10
5
0
Control
CLL (0-II)
CLL (III-IV)

Figure 1B

A.



B. **Summary of protein expression analysis in peripheral blood mononuclear cells**

	healthy controls	CLL (0-II)	CLL (III-IV)
Ror1	24/ 0 / 0	0 / 7 / 13	0/ 0 / 15
Vang	13 / 1 / 0	6 / 5 / 8	0 / 1 / 14
Ck1e	24 / 0 / 9	5/ 10 / 5	0 / 1 / 14
Ck2a	11/ 13 / 0	4/ 8 / 8	1 / 1 / 13
Dvl2	20 / 2 / 0	4 / 9 / 7	0 / 1 / 11
Dvl3	22/ 2 / 0	6 / 5 / 8	1 / 0 / 14

Figure 2

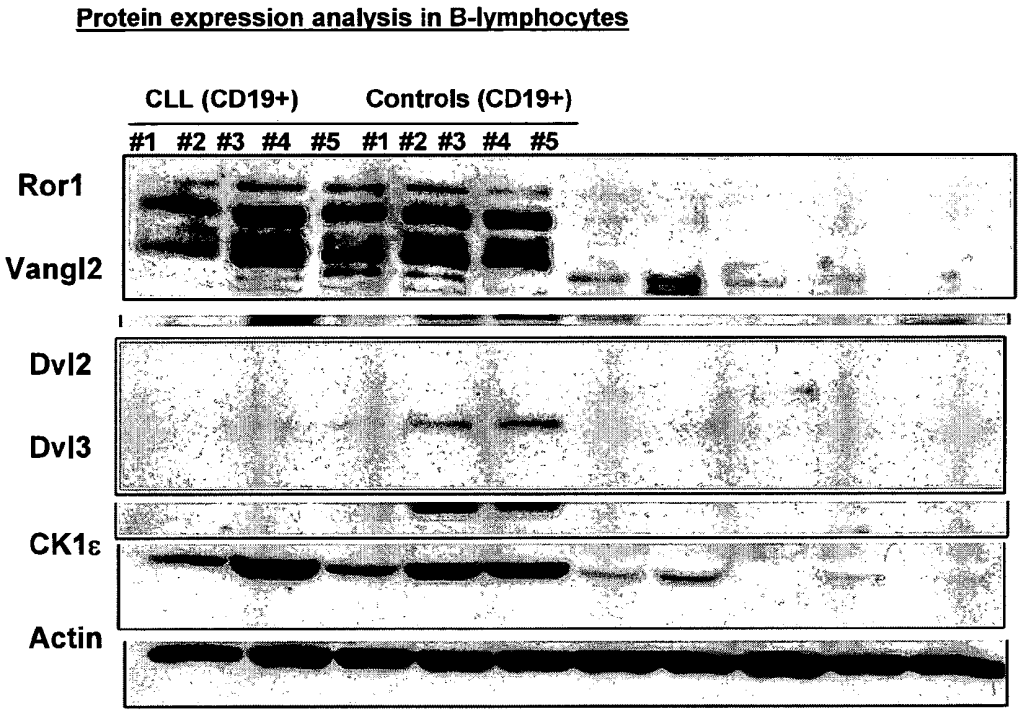

Protein expression analysis in B-lymphocytes
CLL (CD19+)
Controls (CD19+)
#1 #2 #3 #4 #5 #1 #2 #3 #4 #5
Ror1
Vangl2
Dvl2
Dvl3
CK1ε
Actin

Figure 3

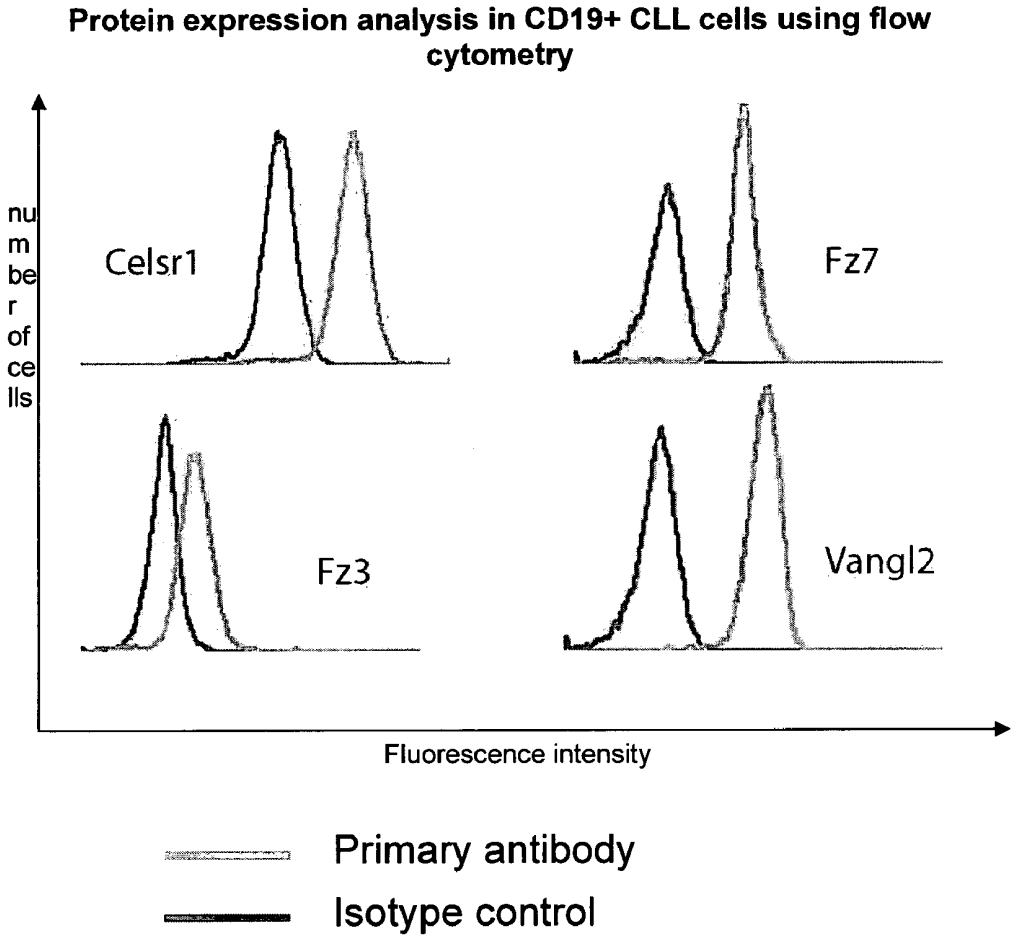
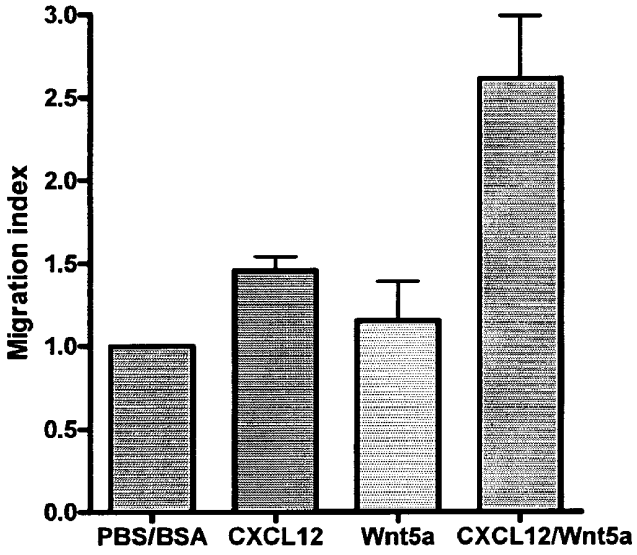

Protein expression analysis in CD19+ CLL cells using flow cytometry
number of cells
Celsr1
Fz7
Fz3
Vangl2
Fluorescence intensity
Primary antibody
Isotype control

Figure 4

A. **Wnt5a promotes migration of CLL cells in CXCL12 gradient**



B. **Key role of CK1 inhibition on migration of CLL cells**

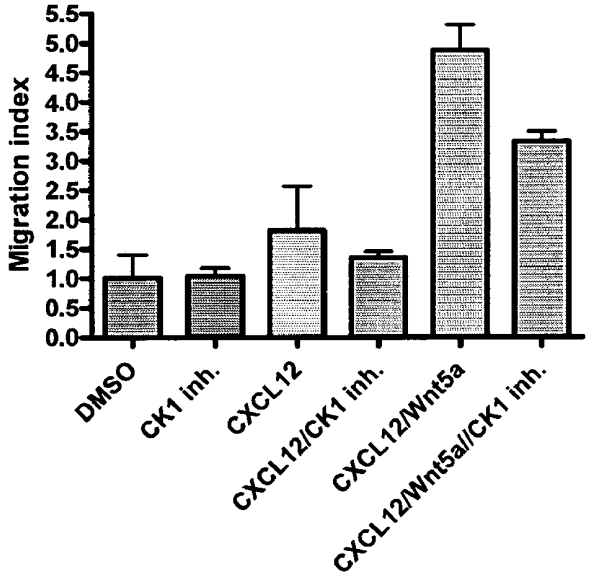
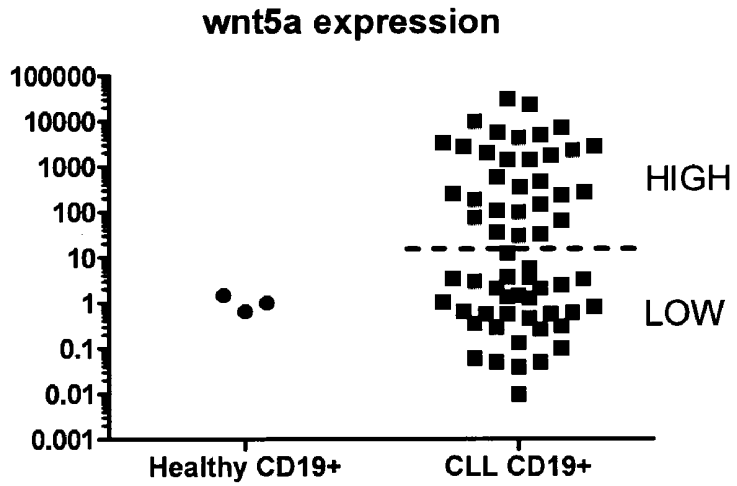
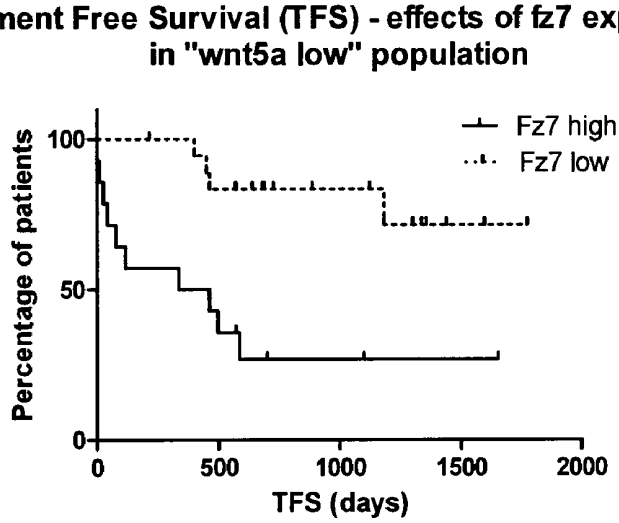


Figure 5


wnt5a expression
100000
10000
1000
100
10
1
0.1
0.01
0.001
HIGH
LOW
Healthy CD19+
CLL CD19+

A


Treatment Free Survival (TFS) - effects of fz7 expression in "wnt5a low" population
Fz7 high
Fz7 low
Percentage of patients
100
50
0
0
500
1000
1500
2000
TFS (days)

B

Figure 6

METHOD OF DETERMINATION OF DIAGNOSIS AND PROGNOSIS IN PATIENTS WITH B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA AND OLIGONUCLEOTIDES FOR USE IN THIS METHOD

FIELD OF ART

[0001] The invention relates to a method of determination of diagnosis and/or prognosis in patients suffering from B-cell chronic lymphocytic leukemia (CLL) and to oligonucleotides for use in this method.

BACKGROUND ART

[0002] B-cell chronic lymphocytic leukemia (CLL) is clinically a very heterogeneous disease with so far unclear pathogenesis. It ensues from the existing knowledge that it is a monoclonal expansion of B-lymphocytes that subsequently gather both in peripheral blood and in lymphatic organs, which further cause life-threatening complications in the form of enlargement of organs, a decreased function of immunity system, anaemia and others. It is supposed that the disease originates in the consequence of disturbance of apoptosis and changes in B lymphocytes migration. In 1975, a first prognostic system according to Rsi (Rai K R, Sawitsky A, Cronkite E P et al.: Clinical staging of chronic lymphocytic leukemia. Blood 1975 46: 219-234) was published and introduced, and subsequently in 1977 a similar classification of the disease according to Binet (Binet J L, Lepoprier M, Dighiero G et al.: A clinical staging system for chronic lymphocytic leukemia: prognostic significance. Cancer. 40, 855-64, 1977; Binet J L, Auquier A, Dighiero G et al.: A new prognostic classification of chronic lymphocytic leukemia derived from a multivariate survival analysis. Cancer 48:198-206, 1981) was introduced. Both systems are based on basic and clinically easy to observe features or available laboratory parameters (number and shape of lymphocytes, thrombocytes, values of haemoglobin, anaemia, infiltration of organs, etc.). In connection with modern molecular markers these systems are still used for the determination of prognosis.

[0003] With the introduction of cytogenetic methods, a correlation was observed between certain cytogenetic changes in the cells of patients and the development of their disease. For instance, deletions 17p and 11q are connected with a worse prognosis and, on the contrary, deletion 13q or trisomia of chromosome 12 are considered a positive prognostic feature. On the p arm of chromosome 17, there is located the tumour-suppressor gene p53 and the gene ATM (ataxia teleangiectasia mutated) is located on the q arm of chromosome 11. Both these genes act in the protection of the cell against damaging influences, therefore, their loss or mutation is connected with higher agressiveness of the disease. With regard to the fact that cytogenetic abnormalities occur in 75 to 80% of the patients, the examination by means of FISH probes panel has been included among standard examination procedures.

[0004] Another method available at present is the determination of the mutation status of the variable part of immunoglobulin chain by means of sequencing, and this method contributes significantly to the determination of the prognosis of the patients. It has been shown that the mutation status does not change during the disease. The patients with unmutated IgVH have a worse prognosis than those with the mutated one (Hamblin T J, Davis Z, Gardiner A et al.: Unmutated IgVH genes are associated with a more aggressive form of chronic lymphocytic leukemia. Blood 94, 1999, 1848-1854), wherein IgVH with less than 98% homology is considered to be mutated. The mutation status is a significant prognostic indicator regardless of the clinical stage at which the patients are. A significant improvement in the determination of the mutation status and subsequently of the prognosis of the patient was achieved by the recent identification of a molecular prognostic marker, cytoplasmatic protein ZAP-70 (Rosenwald A, Alizadeh A A, Widhopf G et al.: Relation of gene expression phenotype to immunoglobulin mutation genotype in B cell chronic lymphocytic leukemia. J Exp Med 194, 2001, 1639-1647), the high concentration of which correlates significantly with the unmutated IgVH status. Further developments leading to more accurate diagnosis include, e.g., the determination of expression of the surface molecule CD38 that is stable in time and the high expression of which is connected with a worse progression of the disease (Damle R N, Wasil T, Fais F et al.: IgV gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. Blood 94, 1999, 1840-1847). Several prognostic markers are known, however, they have not been tested in a larger group of patients yet. A complex use of the above-mentioned analyses contributes significantly to the determination of the prognosis of the patients, however, so far no specific marker is known that would enable to identify with certainty the patients whose disease will prograde in near future and distinguish them from those patients who will remain at the clinical stage for many years without any need for therapy.

[0005] The aim of the present invention is to provide a method of determination of the diagnosis and/or prognosis which could replace the current diagnostic and prognostic methods by introducing new and specific markers that will enable the determination of the diagnosis and the prognosis on the basis of the diagnosis. Based on the determined diagnosis and prognosis, the most suitable treatment procedure for each individual patient is selected. The determination of the diagnosis and prognosis can easily be repeated in the course of the disease. Furthermore, the present invention can form a basis for the preparation of targeted therapy at several levels of cell signalization taking account of the current condition of a particular patient.

DISCLOSURE OF THE INVENTION

[0006] Object of the present invention is a method for determination of the diagnosis and/or prognosis of B-cell chronic lymphocytic leukemia (CLL) from a biological sample collected from the body of a patient, wherein the status of the signaling pathway Wnt/PCP is determined.

[0007] The term "determination of the diagnosis and/or prognosis" as it is used in this description shall be understood as including the determination of the diagnosis of CLL, the determination of the acuity of CLL in the patient, the determination of the prognosis on the basis of the diagnosis, as well as the selection of a suitable treatment.

[0008] The term "determination of the status of the signaling pathway" as it is used in this description shall be understood to include the determination of the extent of expression of the signaling pathway components, the up-regulation of the signaling pathway components and/or the determination of the functionality or the activity of the signaling pathway as a whole.

[0009] The biological sample collected from the body of the patient is preferably a sample of peripheral blood.

[0010] Within the framework of the present invention, the relation between the CLL and the molecular signaling pathway Wnt/PCP, the components of which are significantly up-regulated in B-lymphocytes of the patients with CLL, was identified.

[0011] Wnt/planar cell polarity (PCP) is a signaling pathway that was originally described in the *drosophila Drosophila melanogaster* (Seifert J R, Mlodzik M: Frizzled/PCP signalling: a conserved mechanism regulating cell polarity and directed motility. Nat Rev Genet. 2007 February; 8(2): 126-38). The components of the Wnt/PCP pathway (the so-called PCP proteins) regulate particularly the cell polarity and orientation. This function was investigated in detail in the regulation of cell orientation in the wing and composite eye of *drosophila*. Only recently, the function of Wnt/PCP pathway was also characterized in vertebrates. In amphibians and mammals it regulates particularly the key cell movements (the so-called morphogenetic movements) in the early embryonic development (especially at the stages of gastrulation and neurulation) and in the adult organisms it regulates the polarity of capillaceous cells in the inner ear (Seifert J R, Mlodzik M: Frizzled/PCP signalling: a conserved mechanism regulating cell polarity and directed motility. Nat Rev Genet. 2007 February; 8(2):126-38; Torban E, Kor C, Gros P: Van Gogh-like2 (Strabismus) and its role in planar cell polarity and convergent extension in vertebrates. Trends Genet. 2004 November; 20(11):570-7). There is not much known about the role of the Wnt/PCP pathway in the pathogenesis of human diseases, with the exception of several findings of an increased or a decreased level of the individual components of this pathway in solid tumours (summarized in Essen J R: Non-canonical Wnt signaling in tumor progression and metastasis, Zebrafish 2009, 6: 1-8). In leukemic diseases, only one case is known so far in which it has been shown that the expression of the secreted ligand Wnt5a of this pathway is decreased in the patients with acute myeloid and acute lymphoblastic leukemia (Liang et al., Wnt5a inhibits B cell proliferation and functions as a tumor suppressor in haematopoietic tissue. Cancer Cell 2003, 4: 349-360). According to the information available to us, no data were available so far about the role of the PCP proteins in CLL.

[0012] The key components of the Wnt/PCP pathway include ligands from the family Wnt (especially, Wnt5a and Wnt11) that bind to their membrane receptors from the Frizzled family, further, membrane proteins Van Gogh like 2 (Vangl2; in *Drosophila* called Strabismus) and Celsr1 (in *Drosophila* called Flamengo), cytoplasmatic components Dishevelled (Dvl), Prickle 1, and kinases casein kinase 1 epsilon (CK1s) and casein kinase 2 alpha (CK2a) (Seifert J R, Mlodzik M: Frizzled/PCP signalling: a conserved mechanism regulating cell polarity and directed motility. Nat Rev Genet. 2007 February; 8(2):126-38; Torban E, Kor C, Gros P: Van Gogh-like2 (Strabismus) and its role in planar cell polarity and convergent extension in vertebrates. Trends Genet. 2004 November; 20(11):570-7).

[0013] Our results show that the following parts of the Wnt/PCP pathway are up-regulated in CLL: (i) membrane receptors—Vangl2, Celsr1, Frizzled 3 (Fz3) and Frizzled 7 (Fz7), (ii) cytoplasmatic proteins Dvl 2, Dvl 3 and Prickle1, (iii) kinases casein kinase 1 epsilon (CK1ϵ) and casein kinase 2 alpha (CK2α) and (iv) secreted ligand of that pathway Wnt5a.

[0014] The status of the signaling pathway Wnt/PCP can be determined, e.g., by determining the expression of at least one protein belonging to the signaling pathway Wnt/PCP and/or at least one gene encoding protein belonging to the signaling pathway Wnt/PCP.

[0015] Preferably, the protein belonging to the signaling pathway Wnt/PCP is selected from the group comprising membrane receptors Vangl2, Celsr1, Frizzled 3 and Frizzled 7, cytoplasmatic proteins Dvl 2, Dvl 3 and Prickle1, kinases casein kinase 1 epsilon and casein kinase 2 alpha and the secreted ligand of this pathway Wnt5a. The expression of the proteins can be determined, e.g., by the methods Western blotting or ELISA, the expression of the membrane receptors can be determined also by other methods such as flow cytometry.

[0016] Preferably, the gene encoding the protein belonging to the signaling pathway Wnt/PCP is selected from the group comprising wnt5a, frizzled 7, frizzled 3, vangl2, prickle1 and celsr1. The expression of the gene encoding the protein can be determined, e.g., by the method of quantitative PCR (real-time PCR).

[0017] For the detection of gene expression by the method of quantitative PCR the following oligonucleotides can be used as primers:

Gene	Forward primer (5' to 3')	Reverse primer (5' to 3')
celsr1	TACAACCTTTGGGCTCTGGCTG	CCTTCATCAGGGTCGTTAGCAC
frizzled3	TTGAGGATGTGCCAAGATTTGC	AGCCTACGACAGGGAAGTGTGAC
frizzled7	TACCACGGAGAGAAGGGCATC	GCATAAGAAAAAGCGGAGTTCGG
prickle1	TTCAGTGCTCAGCGGAAGAAAG	AGACAAAACAGGATGGGTGCC
vangl2	ACAGTAGTAACGGGCACCTCAGAGC	TTGAAGGCGACAGAGATGAAGAG
wnt5a	AACAGCCGCTTCAACTCGC	CGTAGCAGCACCAGTGGAACTTG

[0018] Oligonucleotides not fully identical with the primers specified herein above can be used, however, they should have at least 75%, preferably at least 85%, homology with the primers specified herein. The decrease of homology to 75% (85%) in comparison with the original sequence of nucleotides can be achieved, e.g., by extension or shortening of the primers by several nucleotides at the 5' and/or 3' end.

[0019] The status of the signaling pathway Wnt/PCP can be determined, in another embodiment of the invention, by the determination of migration of CLL cells in a chemokine gradient in the presence of Wnt5a.

[0020] It has been shown recently that Wnt5a, the key component of the Wnt/PCP pathway, is essential for cell polarization in the gradient of chemokine (CXCL12) (Witze E S, Litman E S, Argast G M, Moon R T, Ahn N G: Wnt5a control of cell polarity and directional movement by polarized redistribution of adhesion receptors. Science. 2008 Apr. 18; 320 (5874):365-9). This ability of Wnt5a is most probably mediated by the PCP proteins. It is important to mention that chemokines such as CXCL12, CCL19 and CCL21 regulate positively the invasiveness and transendothelial migration of CLL cells and create the gradient between peripheral blood and another different environment that is represented by lymphatic nodes and bone marrow. Our results show the direct effect of the PCP proteins on the migration of, namely, both normal CLL cells and CLL cells in the gradient of chemokine (see below Example 7). We have developed a procedure that enables the testing of the ability of the CLL cells to migrate in the presence and the absence of recombinant Wnt5a (200 ng/ml) using Transwell migration wells (Redondo-Muñoz J, Escobar-Díaz E, Samaniego R, Terol M J, García-Marco J A, García-Pardo A: MMP-9 in B-cell chronic lymphocytic leukemia is up-regulated by alpha4beta1 integrin or CXCR4 engagement via distinct signaling pathways, localizes to podosomes, and is involved in cell invasion and migration. Blood. 2006 Nov. 1; 108(9):3143-51; Richardson S J, Matthews C, Catherwood M A, Alexander H D, Carey B S, Farrugia J, Gardiner A, Mould S, Oscier D, Copplestone J A, Prentice A G: ZAP-70 expression is associated with enhanced ability to respond to migratory and survival signals in B-cell chronic lymphocytic leukemia (CLL). Blood. 2006 May 1; 107(9):3584-92). The extent of the migration of the CLL cells in the chemokine gradient after the stimulation by Wnt5a may be determined, e.g., by the procedure specified in Example 7 and used for the determination of the migration capacity of the CLL cells in vitro. The ability to migrate then closely correlates with the infiltration of lymphatic organs and with the aggressiveness of the disease (Till K J, Lin K, Zuzel M., Cawley J C. The chemokine receptor CCR7 and a4 integrin are important for the migration of chronic lymphocytic leukemia cells into lymph nodes. Blood. 2002 99: 2977-2984).

[0021] A further object of the present invention are oligonucleotides having at least 75%, preferably at least 85%, homology of the nucleotide sequence with sequences selected from the group comprising:

Forward primer (5' to 3')	Reverse primer (5' to 3')
TACAACCTTTGGGCTCTGGCTG	CCTTCATCAGGGTCGTTAGCAC
TTGAGGATGTGCCAAGATTTGC	AGCCTACGACAGGGAAGTGTGAC
TACCACGGAGAGAAGGGCATC	GCATAAGAAAAAGCGGAGTTCGG
TTCAGTGCTCAGCGGAAGAAAG	AGACAAAACAGGATGGGTGCC
ACAGTAGTAACGGGCACCTCAGAGC	TTGAAGGCGACAGAGATGAAGAG
AACAGCCGCTTCAACTCGC	CGTAGCAGCACCAGTGGAACTTG

[0022] Object of the present invention are further these oligonucleotides for use in the method of determination of the diagnosis and/or prognosis of B-cell chronic lymphocytic leukemia according to the present invention.

[0023] The contributions of the present invention over the prior art include the identification of a molecular pathway that has not yet been connected in any way with the pathogenesis of CLL. The components of the pathway concerned active in the CLL include both surface molecules and cytoplasmatic proteins, including several kinases.

[0024] The determination of the expression of the individual components of the pathway concerned (by means of RT-PCR and flow cytometry in the case of surface markers, or by the method RT-PCR, Western blotting, or ELISA in the case of cytoplasmatic components) will enable the use of the invention as a diagnostic marker.

[0025] We show herein that the expression of the components of the Wnt/PCP pathway (hereinafter referred to as PCP proteins) correlates with the progression of CLL, and that the Wnt/PCP pathway regulates the migration of CLL cells. It has been shown that the ability of CLL cells to migrate and infiltrate the secondary lymphatic organs contributes, in principle, to the progression of CLL. It will be possible to apply the invention by way of introduction of new diagnostic markers and of the determination of the migration capacity of CLL cells in a chemokine gradient.

BRIEF DESCRIPTION OF DRAWINGS

[0026] FIG. **1** summarizes the results of diagnostics of CLL from mononuclears of peripheral blood by the method RT-PCR according to Example 1. FIG. **1**A summarizes the relative expression of a set of tested genes in relation to an endogenic control B2M (Beta-2 microglobulin) in mononuclears of 14 controls and 25 patients with CLL.

[0027] FIG. **1**B shows the correlation of the expression of the markers with the stages of CLL.

[0028] FIG. **2** summarizes the results of diagnostics of CLL from mononuclears of peripheral blood by the method Western blotting according to Example 4. FIG. **2**A shows the results of the analysis of the samples from CLL patients and healthy controls by the method Western blotting. FIG. **2**B shows the comparison of the extent of the expression of the proteins in healthy controls, patients with CLL at stages 0-II and patients with CLL at stages III-IV on the scale: non-detectable expression, weak expression, strong expression.

[0029] FIG. **3** summarizes the results of diagnostics of CLL from B-lymphocytes of peripheral blood by the method Western blotting according to Example 5—results of the analyses of samples from CLL patients and healthy controls.

[0030] FIG. **4** shows the method of diagnostics of CLL from B-lymphocytes of peripheral blood by the method of flow cytometry according to Example 6.

[0031] FIG. **5** shows the results of the migration essay according to Example 7. In part A it shows that Wnt5a is able to positively affect the migration of CLL cells in the presence of the chemokine CXCL12, in part B it shows that essential for these effects is the component of the Wnt/PCP pathway CK1 (addition of CK1 inhibitor—D4476, 100 uM—blocks migration).

[0032] FIG. **6** presents results from qRT-PCR analysis performed on a group of CLL patients. FIG. **6**A shows that two groups of patients can be distinguished based on the expression level of the wnt5a gene. FIG. **6**B shows Kaplan-Meier survival curve (Treatment Free Survival—TFS) based on the expression of the fz7 gene in a group of CLL patients with a low expression of the wnt5a gene.

SEQUENCE LISTING

[0033] SEQ. ID. NO. 1: amino acid sequence of protein Dvl2
SEQ. ID. NO. 2: amino acid sequence of protein Dvl3
SEQ. ID. NO. 3: amino acid sequence of protein CK1ϵ
SEQ. ID. NO. 4: amino acid sequence of protein CK2α
SEQ. ID. NO. 5: amino acid sequence of protein Vangl2
SEQ. ID. NO. 6: cDNA sequence Wnt5a
SEQ. ID. NO. 7: cDNA sequence Frizzled3 (Fz3)
SEQ. ID. NO. 8: cDNA sequence Frizzled7 (Fz7)
SEQ. ID. NO. 9: cDNA sequence Prickle 1
SEQ. ID. NO. 10: cDNA sequence Vangl2
SEQ. ID. NO. 11: cDNA sequence Celsr1

EXAMPLES OF CARRYING OUT THE INVENTION

Example 1

Diagnostics of C LL from Mononuclears of Peripheral Blood by the Method RT-PCR

[0034] Peripheral blood of a CLL patient was transferred to a test tube and the volume of blood was measured. 6 ml of diluted blood were layered to 3 ml of Ficoll-Paque Plus® tempered to laboratory temperature. Centrifugation was performed for 30 minutes/400 g. The layer of lymphocytes was transferred to a clean test tube (15 cm) and completed with 2% FBS (fetal bovine serum in physiological solution with phosphate buffer) to 10 ml, the number of test tubes was selected so that the separated lymphocytes might be diluted at least 2×, the content of the test tubes was thoroughly mixed. After that, centrifugation was performed for 10-15 minutes/200 g, the supernatant was removed and the sediment was suspended in 1 ml of cooled lysing buffer (lysion of erythrocytes—154.9 mM NH4Cl, 9.9 mM NH4HCO3, 1 mM EDTA), the volume was completed to 10 ml and mixed by turning over. Sediments from more test tubes were combined in this step. Centrifugation 10-15 minutes/200 g was performed, supernatant removed and the sediment suspended in 2% FBS and transferred to a test micro-tube. After a further centrifugation 10 minutes/200 g the supernatant was removed and the sediment was lysed in 350 μl RLT buffer (lysing buffer, part of RNeasy Mini Kit of the company Qiagen intended for the isolation of total RNA).

[0035] Total RNA was isolated from the separated CLL cells by means of High Pure RNA Isolation Kit according to the instructions of the manufacturer (Roche). The quality and concentrations of the obtained total RNA were determined by spectrophotometry (NanoDrop) and electrophoresis (Bio-Analyzer 2100). Only the samples showing good parameters (RIN>8, Concentration>50 ng/μl) were used for further analysis. The system FastStart Taq DNA Polymerase (Roche) was used for the synthesis of the cDNA according to the instructions of the manufacturer. 500 ng of total RNA were transcribed. The obtained 20 μl cDNA were diluted by RNAse-free water to the final volume of 50 μl. Each sample was analyzed in triplicate by means of the system LightCycler® 480 SYBR Green I Master (Roche), according to the instructions of the manufacturer. The DNA amplification was subsequently detected by the device LightCycler® 480 Real-Time PCR System (Roche) and the obtained data were analyzed by means of the software LightCycler® 480 Software (Roche). The result was the number of the cycle during which the fluorescence achieves a pre-set threshold (Ct—fluorescence threshold cycle). The relative expression of the gene was normalized to the expression of the operating gene GAPDH (ΔCt). The obtained data were analyzed by means of non-parametric Wilcox test.

[0036] In the subsequent step we obtained samples from mononuclears of peripheral blood both of healthy controls and of patients at clinical stages 0-IV according to Rai, and we analyzed the expression of human homologues of the genes that are associated with non-canonical Wnt signalization (particularly the Wnt/PCP pathway). The expression of the genes prickle1, vangl2, celsr1, wnt5a, frizzled3 and frizzled7, and endogenous controls (B2M and actin) was determined by means of RT-PCR. We analyzed the expression in a panel of patients at various clinical stages according to Rai. FIG. **1**A summarizes the expression of these genes in mononuclears of 14 controls and 25 patients. The results of quantitative real time-PCR (qRT-PCR) analysis provide the evidence that some PCP genes are highly expressed at the level of transcription in CLL cells.

[0037] During qRT-PCR and the analysis of the protein expression we detected considerable variability in the expression of the individual markers. This is why we tested the possibility that the expression of PCP genes is associated with the progression of CLL. Therefore, we classified the patients according to the clinical stage (according to Rai), namely, into categories (i) stage 0-II and (ii) stage III-IV. As shown in FIG. **1**B, the expression of the majority of the markers determined by RT-PCR correlates with the stages of CLL. These trends are obvious in all genes, but the differences in the expression of the markers of the CLL patients at the stages 0-II and III-IV in our samples are statistically significant, especially, with prickle1 and frizzled7 (One way ANOVA, Tukey post test, $p<0.05$).

Example 2

Diagnostics of CLL from B-Lymphocytes of Peripheral Blood by Means of Quantitative RT-PCR

[0038] Peripheral blood of the patient suffering from CLL was collected to collection test tubes with anticoagulation substance (heparin). Mononuclear cells were separated by means of gradient centrifugation (Ficoll-Paque PLUS, GE Healthcare) which was preceded by incubation with a cocktail of bispecific antibodies (at one end anti CD2, CD3, CD16, CD36, CD56, CD66b and at the other end anti-glycophorin A) (RosetteSep® B Cell Enrichment Cocktail, StemCell). Undesirable cells form, by means of bispecific antibodies, together with erythrocytes, formations called rosettes.

[0039] The density of the formed rosettes is higher than the density of the individual undesirable cells and during gradient centrifugation their sedimentation occurs. Total RNA was isolated from the separated CLL cells by means of High Pure RNA Isolation Kit according to the instructions of the manufacturer (Roche). The quality and the concentration of the obtained total RNA were determined by spectrophotometry (NanoDrop) and electrophoretically (BioAnalyzer 2100). Only the samples with good parameters (RIN>8, concentration>50 ng/μl) were used for further analyses. The system FastStart Taq DNA Polymerase (Roche) was used for the synthesis of cDNA according to the instructions of the manufacturer. 500 ng of total RNA were transcribed. The obtained 20 μl of cDNA were diluted by RNAse-free water to the final volume of 50 μl. Each sample was analyzed in triplicate by means of the system LightCycler® 480 SYBR Green I Master

(Roche), according to the instructions of the manufacturer. DNA amplification was subsequently detected by the device LightCycler® 480 Real-Time PCR System (Roche) and the obtained data were analyzed by means of software LightCycler® 480 Software (Roche). The result was the number of the cycle in which the fluorescence achieves a pre-set threshold (CT—fluorescence threshold cycle). The relative expression of the gene was normalized to the expression of the operating gene GAPDH that was selected on the basis of the stability of its expression across the samples.

Example 3

Diagnostics of CLL from B-Lymphocytes of Peripheral Blood by Means of Quantitative RT-PCR

[0040] Peripheral blood of a CLL patient was transferred to a test tube and the volume of the blood was measured. Further, RosetteSep® Human B Cell Enrichment Cocktail (50 μl/ml of blood) was added, the mixture was mixed by circling and incubated for 20 minutes at laboratory temperature. After that at least the same volume of 2% FBS (fetal bovine serum in physiological solution with phosphate buffer) was added, mixed and 6 ml of the diluted blood were layered on 3 ml Ficoll-Paque Plus® tempered to laboratory temperature. Centrifugation was performed 30 minutes/400 g. The layer of lymphocytes was transferred to a clean test tube (15 cm) and completed with 2% FBS to 10 ml, the number of test tubes was selected so that the separated lymphocytes might be diluted at least 2×, the content of the test tubes was thoroughly mixed. After that, centrifugation was performed 10-15 minutes/200 g, the supernatant was removed and the sediment suspended in 1 ml of cooled lysing buffer (lysion of erythrocytes—154.9 mM NH4Cl, 9.9 mM NH4HCO3, 1 mM EDTA), the volume was completed to 10 ml and mixed by turning over. Sediments from several test tubes were combined in this step. Centrifugation was performed 10-15 minutes/200 g, the supernant was removed and the sediment was suspended in 2% FBS and transferred to a test micro-tube. After further centrifugation 10 minutes/200 g the supernant was removed and the sediment lysed in 350 μl RLT buffer (lysing buffer, a part of RNeasy Mini Kit of the company Qiagen intended for the isolation of total RNA).

[0041] Total RNA was isolated from the separated CLL cells by means of High Pure RNA Isolation Kit according to the instructions of the manufacturer (Roche). The quality and concentration of the obtained total RNA was determined spectrophotometrically (NanoDrop) and electrophoretically (BioAnalyzer 2100). Only the samples having good parameters were used for further analyses (RIN>8, concentration>50 ng/μl). FastStart Taq DNA Polymerase system (Roche) was used for the synthesis of cDNA according to the instructions of the manufacturer. 500 ng of total RNA were transcribed. The obtained 20 μl of cDNA were dissolved by RNAse-free water to the final volume of 50 μl. Each sample was analyzed in triplicate by means of the system LightCycler® 480 SYBR Green I Master (Roche) according to the instructions of the manufacturer. The DNA amplification was detected subsequently by the device LightCycler® 480 Real-Time PCR System (Roche) and the obtained data were analyzed by means of software LightCycler® 480 Software (Roche). The result was the number of the cycle during which the fluorescence achieves a pre-set threshold (Ct—fluorescence threshold cycle). The relative expression of the gene was normalized to the expression of the operating gene GAPDH (ΔCt). The obtained data were analyzed by means of non-parametric Wilcoxon test.

[0042] The following primers were used for the detection of gene expression in examples 1-3.

Gene	Forward primer (5' to 3')	Reverse primer (5' to 3')	Prod (bp)
celsr1	TACAACCTTTGGGCTCTGGCTG	CCTTCATCAGGGTCGTTAGCAC	197
frizzled3	TTGAGGATGTGCCAAGATTTGC	AGCCTACGACAGGGAAGTGTGAC	209
FZ7	TACCACGGAGAGAAGGGCATC	GCATAAGAAAAAGCGGAGTTCGG	207
prickle1	TTCAGTGCTCAGCGGAAGAAAG	AGACAAAACAGGATGGGTGCC	175
vangl2	ACAGTAGTAACGGGCACCTCAGAGC	TTGAAGGCGACAGAGATGAAGAG	248
wnt5a	AACAGCCGCTTCAACTCGC	CGTAGCAGCACCAGTGGAACTTG	232

Example 4

Diagnostics of CLL from Mononuclears of Peripheral Blood by Means of the Method Western Blotting

[0043] Peripheral blood of a CLL patient was transferred to a test tube and the volume of the blood was measured. 6 ml of the dissolved blood were layered on 3 ml Ficoll-Paque Plus® tempered to laboratory temperature. Centrifugation was performed 30 minutes/400 g. The layer of lymphocytes was transferred to a clean test tube (15 cm) and completed with 2% FBS to 10 ml, the number of the test tubes was selected so that the separated lymphocytes might be diluted at least 2×, the content of the test tubes was thoroughly mixed. After that, centrifugation was performed 10-15 minutes/200 g, the supernant was extracted and the sediment suspended in 1 ml of cooled lysing buffer (lysion of erythrocytes—154.9 mM NH4Cl, 9.9 mM NH4HCO3, 1 mM EDTA), the volume was completed to 10 ml and mixed by turning over. Sediments from several test tubes were combined in this step. Centrifugation was performed 10-15 minutes/200 g, the supernatant extracted and the sediment suspended in 2% FBS and transferred to a test micro-tube. After further centrifugation 10 minutes/200 g the supernatant was extracted and the sediment was lysed in lysing buffer (0.5% NP-40, 150 mM NaCl, 50 mM Tris pH 7.4, 1 mM EDTA). After sonication (Branson

Sonifier 150), the quantity of proteins was determined by means of Bio-Rad DC Protein Assay kit. The samples were adjusted to the unified concentration of proteins 1 μg/μl, and 20 μl of each sample were applied on 8% polyacrylamide gel and analyzed by the method SDS-PAGE/Western blotting according to standard procedure using polyvinylidenedifluoride (PVDF) membranes (Towbin H, Staehelin T, Gordon J. (1979): Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci USA.* 76 (9): 4350-4354; Pluskal M G, Przekop M B, Kavonian M R, Vecoli C, Hicks D A, Immobilon PVDF transfer membrane: A new membrane substrate for Western blotting of proteins. *BioTechniques* 4, 1986, pp. 272-282). After blotting, the membranes were blocked for 1 hr. in 5% non-fat milk (Promil, Laktino) in TBS-Tween (100 mM NaCl, 20 mM Tris (pH 7,6), 0.05% Tween 20, dissolved in distilled water pH 7.1-7.2)

[0044] The following combinations of antibodies diluted in 5% milk/TBS-Tween were used for the detection:

antigen	Primary antibody (manufacturer)	Dilution of primary antibody	Secondary antibody (manufacturer)	Dilution of secondary antibody
Dvl2	#3224 anti-rabbit Dvl2 (Cell Signaling Technology)	1:1000	A6667 anti-rabbit IgG (Sigma-Aldrich ®)	1:5000
Dvl3	SC-8027 anti-mouse Dvl3 (Santa Cruz Biotechnology, inc)	1:500	A6782 anti-mouse IgG (Sigma-Aldrich ®)	1:5000
Casein kinase 1 ϵ (CKlϵ)	SC-6471 anti-goat CK1 (Santa Cruz Biotechnology, inc.)	1:500	A4174 anti-goat IgG (Sigma-Aldrich ®)	1:5000
Casein kinase 2 α/α'	BD 611611 anti-mouse CK2 (BD Transduction Laboratories ™)	1:500	A6782 anti-mouse IgG (Sigma-Aldrich ®)	1:5000
Vangl2	AF 4815 anti-goat Vangl2 (R&D Systems ®)	1:1000	A4174 anti-goat IgG (Sigma-Aldrich ®)	1:5000
Prickle1	ab15577 anti-rabbit Prickle1 (Abcam ®)	1:500	A6667 anti-rabbit IgG (Sigma-Aldrich ®)	1:5000
Actin	SC-1615 anti-rabbit Actin (Santa Cruz Biotechnology, inc.)	1:5000	A6667 anti-rabbit IgG (Sigma-Aldrich ®)	1:5000

[0045] We present the analysis of the samples of 15 CLL patients and 15 healthy controls by the method Western blotting and the obtained data in FIG. **2**A. Increased levels of PCP proteins, such as Vangl2 and Prickle1, were detected, identified by means of qRT-PCR. It provides evidence that the increased levels of transcripts are transcribed to proteins the increased quantity of which can be detected by this method. The protein analysis of the other components of non-canonical Wnt signalling, such as Dvl2, Dvl3, CK1ϵ and CK2α, showed a significantly higher levels of those mediators in CLL patients. It is interesting that the transcripts encoding Dvl2 and Dvl3, and CK1ϵ and CK2α were not demonstrably higher in the primary analysis of mononuclears in CLL patients which means that the level of those proteins is regulated post-transcriptionally.

[0046] Because the WB analysis does not provide numerical data, we classified the data to 3 categories only: non-detectable expression, weak expression, strong expression. The summary of this analysis is in FIG. **2**B. CK1E has never been detected in healthy controls, Vangl2, Dvl2 and Dvl3 were detected in the healthy individuals only rarely (less than 10% of examples showed weak expression only) and CK2α was detected weakly in 50% of examples. In the samples of peripheral blood of the patients at stages 0-II protein levels in Dvl2, Dvl3, Vangl2, Ck1ϵ and CK2α differed from a non-detected signal to a strong signal (roughly by one third). The patients at stages III-IV showed a strong expression of all above-mentioned PCP proteins with the exception of very few patients only. Based on these results, we can conclude that the accumulation of PCP proteins and their transcripts occurs with the progression of CLL.

Example 5

Diagnostics of CLL of B-Lymphocytes of Peripheral Blood by Means of the Method Western Blotting

[0047] Peripheral blood of a CLL patient was transferred to test tubes and the volume of the blood was measured. RosetteSep® Human B Cell Enrichment Cocktail (500 ml of blood) was further added, the mixture was mixed by turning over and incubated for 20 minutes at laboratory temperature. After that minimally the same volume of 2% FBS (fetal bovine serum in physiological solution with phosphate buffer) was added, the solutions were stirred and 6 ml of diluted blood was layered on 3 ml Ficoll-Paque Plus® tempered to laboratory tempera-

ture. Centrifugation 30 minutes/400 g was performed. The layer of lymphocytes was transferred to a clean test tube (15 cm) and completed with 2% FBS to 10 ml, the number of test tubes was selected so that the separated lymphocytes might be diluted at least 2×, the content of the test tubes was thoroughly mixed. After that, centrifugation 10-15 minutes/200 g was performed, the supernant was extracted and the sediment suspended in 1 ml of cooled lysing buffer (lysion of erythrocytes—154.9 mM NH4Cl, 9.9 mM NH4HCO3, 1 mM EDTA), the volume was completed to 10 ml and mixed by turning over. The sediments from several test tubes were combined in this step. Centrifugation 10-15 minutes/200 g was performed, the supernatant extracted and the sediment suspended in 2% FBS and transferred to a test micro-tube. After further centrifugation 10 minutes/200 g the supernatant was extracted and the sediment lysed and processed in the same manner as in Example 5.

[0048] The procedure in Example 5 enables us to differentiate whether there is an increased quantity of the PCP proteins directly in the CLL cells or whether it is caused by a contamination by other cell types. The up-regulation of the PCP proteins may be in principle specific for the CLL cells or may reflect the enrichment of peripheral mononuclears of the patients with B-cells. To disprove the second possibility we analyzed the protein levels of Vangl2, Dvl2, Dvl3, Ck1ϵ and CK2α in formulas after the separation by means of RosetteSep Human B Cell Enrichment Cocktail in 5 controls and 5 patients and we obtained similar results as in the non-separated analyzed samples. The percentage of CD19+ cells in all analyzed separated samples was determined at 95% by FACS method. The results are shown in FIG. **3**.

Example 6

Determination of the Presence of PCP Proteins on the Surface of CLL Cells

[0049] Some PCP proteins are membrane proteins and therefore they are suitable for detection by means of flow cytometry. Therefore, we tested their expression in CD19+ B-cells.

[0050] Procedure: Peripheral blood of a CLL patient was transferred to a test tube and the volume of the blood was measured. RosetteSep® Human B Cell Enrichment Cocktail (50 μl/ml of blood) was added, the mixture was mixed by turning over and incubated for 20 minutes at laboratory temperature. At least the same volume of 2% FBS (fetal bovine serum in physiological solution with phosphate buffer) was then added, the mixture was stirred and 6 ml of the diluted blood layered on 3 ml Ficoll-Paque Plus® tempered to laboratory temperature. Centrifugation 30 minutes/400 g was performed. The layer of lymphocytes was transferred to a clean test tube (15 cm) and completed with 2% FBS to 10 ml, the number of test tubes was selected so that the separated lymphocytes might be diluted at least 2×, the content of the test tubes was thoroughly mixed. After that, centrifugation 10-15 minutes/200 g was performed, the supernatant was extracted and the sediment suspended in 1 ml of cooled lysing buffer (lysion of erythrocytes—154.9 mM NH4Cl, 9.9 mM NH4HCO3, 1 mM EDTA), the volume was completed to 10 ml and mixed by turning over. The sediments from several test tubes were combined in this step. Centrifugation 10-15 minutes/200 g was performed, the supernatant extracted and the sediment suspended in 2% FBS and transferred to a test micro-tube. After a further centrifugation 10 minutes/200 g the supernant was extracted, the sediment was washed with 1 ml PBS and after a further centrifugation suspended in a smaller quantity of PBS and fixed in 1 ml of cooled methanol (−20° C.). After fixation in ice-cold methanol the samples were washed in azide buffer (PBS, 1% BSA, 0.1% sodium azide) and centrifuged (200 g/5 min/RT). Each sample was diluted so that it might contain minimum 0.5×10^6 cells. After adding 50 μl of azide buffer with primary antibody in the ratio 1:50, the samples were incubated at least 1 hr at the temperature of 37° C. The samples were washed in azide buffer, centrifuged and left in 50 μl of azide buffer to which secondary antibody (anti-goat-Cy5 from Jackson ImmunoResearch 1:200, or anti-rabbit-alexa488 from Axxora 1:1000 or anti-mouse-alexa488 from Axxora 1:1000) was added. The samples were incubated for 1 hr at the temperature of 37° C. The measurement was performed with the device FACSCalibur (Becton Dickinson) and CellQuest 3.0 software was used for the assessment (Becton Dickinson).

[0051] We present the results in FIG. **4**. These results prove, using several methods, that the PCP proteins are up-regulated in CLL leukemic clones in comparison with B-cells of healthy controls and that they are present on the surface of CLL lymphocytes.

Example 7

Determination of Migration of CLL Cells by Means of Transwell Assay

[0052] To prove the direct effect of the PCP proteins on the migration of both normal CLL cells, and CLL cells in the gradient of chemokine, we tested the ability of CLL cells to migrate in the presence and the absence of recombinant Wnt5a (200 ng/ml) using Transwell migration wells (Redondo-Muñoz J, Escobar-Díaz E, Samaniego R, Terol M J, García-Marco J A, García-Pardo A: MMP-9 in B-cell chronic lymphocytic leukemia is up-regulated by alpha4beta1 integrin or CXCR4 engagement via distinct signaling pathways, localizes to podosomes, and is involved in cell invasion and migration. Blood. 2006 Nov. 1; 108(9):3143-51; Richardson S J, Matthews C, Catherwood M A, Alexander H D, Carey B S, Farrugia J, Gardiner A, Mould S, Oscier D, Copplestone J A, Prentice A G: ZAP-70 expression is associated with enhanced ability to respond to migratory and survival signals in B-cell chronic lymphocytic leukemia (CLL). Blood. 2006 May 1; 107(9):3584-92). As we show in FIG. **5**A, CXCL12 itself influences positively the migration of CLL cells, while Wnt-5a itself was not able to induce migration. The combination of CXCL12 and Wnt5a significantly increased the migration of CLL in Transwell assay in comparison with other experimental conditions. These data show that Wnt5a induces a better migration of CLL cells to the source of the chemokine CXCL12. To prove that the migration of CLL cells in the chemokine gradient is mediated by the PCP proteins specifically increased in CLL, we inhibited pharmacologically CK1ϵ. CK1ϵ is the key component of the Wnt/PCP pathway and its quantity in the patients suffering from CLL is increased. As we show in FIG. **5**B, the inhibition of CK1ϵ blocks the effects of Wnt5a on cell migration in the chemokine gradient. These results demonstrate that the migration of CLL cells is under the control of the PCP proteins and that the pharmacological intervention with the Wnt/PCP pathway blocks their migration which is a property inseparably connected with the progression of CLL. The extent of the migration of CLL cells in chemokine gradient after Wnt5a stimu-

lation may be determined by the following procedure and used for the determination of the migration capacity of CLL cells that correlates closely with the infiltration of lymphatic organs and the agressiveness of the disease (Till K J, Lin K, Zuzel M., Cawley J C. The chemokine receptor CCR7 and a4 integrin are important for migration of chronic lymphocytic leukemia cells into lymph nodes. Blood. 2002 99: 2977-2984).

Procedure:

[0053] CLL cells were isolated by the identical procedure as in Example 6. After isolation the cells were transferred to tissue culture and subjected to Transwell assay. 96 well plates were used for the experiments (Corning, HTS Transwell®-96 Permeable Support with 5.0 μm Pore Polycarbonate Membrane). These plates consist of a bottom part including the wells and a top part that includes the inserts, the bottom of which is formed by the membrane of a defined size of pores. Each sample was measured in triplicate. The wells in the bottom part of the plate always included 0.235 ml of the medium (RPMI 1640, 0.1% FBS, 1% Penicillin Streptomycin, 1% L-glutamine) and further the required concentration of the chemokine or solvent (0.1% BSA in PBS). The inserts that contained always 0.08 ml of the medium together with 0.5×10^6 cells (it is necessary to the cell suspension to be homogeneous) were inserted subsequently to the plates. Antibodies, solvents and Wnt5a at required concentrations were added to these inserts in parallel. The cultivation was performed in an incubator at 37° C. and 5% CO_2 for the period of 20 hours. After the cultivation the inserts were removed from the transwell plate, trypsin was added to each well (100 μl) and after 5 minutes of its activity the number of the cells that migrated through the membrane were counted in each well. The Coulter Counter device, model FN, was used for counting the cells but also Bürker chamber or FACS can be used.

Example 8

Patient Prognosis Based on PCP Gene Expression from the Peripheral Blood Sample of CLL Patient

[0054] Procedure: Peripheral blood of a CLL patient was transferred into a test tube and the volume of the blood was measured. RosetteSep® Human B Cell Enrichment Cocktail (50 μl/ml blood) was added to the blood sample and the mixture was mixed gently, and incubated for 20 minutes at the room temperature. At least the same volume of 2% FBS (fetal bovine serum in physiological solution with phosphate buffer) was added. After mixing, 6 ml of the diluted blood was transferred on the 3 ml layer of Ficoll-Paque Plus® tempered at room temperature, and spinned down for 30 min at 400 g.

[0055] The layer of B-lymphocytes was transferred into a new tube (15 cm) and filled with 2% FBS to 10 ml. The volume of the separated B-lymphocytes was diluted at least twice and was mixed properly. After centrifugation for 10-15 minutes at 200 g, the supernatant was removed and the sediment was first resuspended in 1 ml and then filled up to 10 ml by cooled lysis buffer (for lysis of erytrocytes—154.9 mM NH4Cl, 9.9 mM NH4HCO3, 1 mM EDTA). After this step, sediments of all tubes were put together and centrifuged for 10 min at 200 g. The supernatant was removed and the sediment was resuspended in 2% FBS and transferred to the microtube. After additional centrifugation for 10 min at 200 g, the supernatant was removed and the sediment was lysed in 350 ul of RLT buffer (lysis buffer from Rneasy Mini Kit, Qiagen, for isolation of total RNA). Total RNA from separated CLL B-lymphocytes was isolated using High Pure Isolation Kit according to the manufacturer instructions (Roche). The quality and concentration of isolated total RNA was determined spectrophotometrically (Nanodrop) and eletrophoretically (BioAnalyzer 2100). For next analysis only samples with good parametres were used (RIN>8, concentration>50 ng/μl).

[0056] For synthesis of cDNA, the FastStart Taq DNA Polymerase system (Roche) was used according to manufacturer instructions. 500 ng of total RNA was transcribed. Acquired 20u1 of cDNA was diluted with RNAse-free water to the volume of 50 μl. Each sample was analyzed in triplicate using the LightCycler® 480 SYBR Green I Master (Roche) system according to the manufacturer instructions. Subsequently, the amplification of DNA was detected using LightCycler® 480 Real-Time PCR System (Roche) and obtained data were analyzed using LightCycler® 480 Software (Roche). The result was the number of cycle, in which the fluorescence reached adjusted threshold (fluorescence threshold cycle). Relative gene expression was normalized to the expression of the house-keeping gene GAPDH (ΔCt).

[0057] Primers from the Example 3 were used for the detection of gene expression. The expression data have been obtained from 61 patients in clinical stages 0-IV. According to the expression level of wnt5a gene, patients were divided into two groups. First group "low wnt5a" was formed by patients with the Wnt5a expression less than 50-fold of average healthy CD19+ B-cells. Second group "high wnt5a" was formed by patients with the wnt5a expression more than 50-fold greater than in average healthy CD19+ B-cells.

[0058] In FIG. **6**A, Y axis represents group of 3 healthy donnors and a group of 61 patients with different disease progression. X axis represents logarithmic scale of wnt5a gene expression. Based on this result, patients were divided into two groups according to the wnt5a expression level. First group "low wnt5a" was formed by patients with the wnt5a expression value less than or equal to 50 on the logarithmic scale. Second group "high wnt5a" was formed by patients with the wnt5a expression value greater than 50 on the logarithmic scale.

[0059] Based on the division of patients according to the wnt5a expression level, additional marker from the list of PCP genes can be used for the prognosis assesment in the "wnt5a-low" group. FIG. **6**B presents Kaplan-Meier survival curves, which show the statistically significant shorter treatment free survival time in the patients with higher fz7 gene expression in a subset of patients with low wnt5a levels. X axis represents percentage of patients and Y axis represents treatment free survival in days. PCP gene expression level, when combined with the division of patients according to their wnt5a expression level, enabled to assess the prognosis of CLL.

INDUSTRIAL APPLICABILITY

[0060] The present invention can be used in diagnostics and treatment of B-cell chronic lymphocytic leukemia (CLL). With regard to the fact that the components of the Wnt/PCP signaling pathway described here are up-regulated in CLL and their expression in adult human tissues is relatively low, the determination of the expression of PCP proteins can be used as diagnostic/prognostic markers.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 11

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<211> LENGTH: 736
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 1

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            20                  25                  30

Val Pro Ala Glu Arg Ile Thr Leu Gly Asp Phe Lys Ser Val Leu Gln
        35                  40                  45

Arg Pro Ala Gly Ala Lys Tyr Phe Phe Lys Ser Met Asp Gln Asp Phe
    50                  55                  60

Gly Val Val Lys Glu Glu Ile Ser Asp Asp Asn Ala Arg Leu Pro Cys
65                  70                  75                  80

Phe Asn Gly Arg Val Val Ser Trp Leu Val Ser Ser Asp Asn Pro Gln
                85                  90                  95

Pro Glu Met Ala Pro Pro Val His Glu Pro Arg Ala Glu Leu Ala Pro
            100                 105                 110

Pro Ala Pro Pro Leu Pro Pro Leu Pro Pro Glu Arg Thr Ser Gly Ile
        115                 120                 125

Gly Asp Ser Arg Pro Pro Ser Phe His Pro Asn Val Ser Ser Ser His
    130                 135                 140

Glu Asn Leu Glu Pro Glu Thr Glu Thr Glu Ser Val Val Ser Leu Arg
145                 150                 155                 160

Arg Glu Arg Pro Arg Arg Arg Asp Ser Ser Glu His Gly Ala Gly Gly
                165                 170                 175

His Arg Thr Gly Gly Pro Ser Arg Leu Glu Arg His Leu Ala Gly Tyr
            180                 185                 190

Glu Ser Ser Ser Thr Leu Met Thr Ser Glu Leu Glu Ser Thr Ser Leu
        195                 200                 205

Gly Asp Ser Asp Glu Glu Asp Thr Met Ser Arg Phe Ser Ser Ser Thr
    210                 215                 220

Glu Gln Ser Ser Ala Ser Arg Leu Leu Lys Arg His Arg Arg Arg Arg
225                 230                 235                 240

Lys Gln Arg Pro Pro Arg Leu Glu Arg Thr Ser Ser Phe Ser Ser Val
                245                 250                 255

Thr Asp Ser Thr Met Ser Leu Asn Ile Ile Thr Val Thr Leu Asn Met
            260                 265                 270

Glu Lys Tyr Asn Phe Leu Gly Ile Ser Ile Val Gly Gln Ser Asn Glu
        275                 280                 285

Arg Gly Asp Gly Gly Ile Tyr Ile Gly Ser Ile Met Lys Gly Gly Ala
    290                 295                 300

Val Ala Ala Asp Gly Arg Ile Glu Pro Gly Asp Met Leu Leu Gln Val
305                 310                 315                 320

Asn Asp Met Asn Phe Glu Asn Met Ser Asn Asp Asp Ala Val Arg Val
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Leu Arg Asp Ile Val His Lys Pro Gly Pro Ile Val Leu Thr Val Ala
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-continued

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            340                 345                 350

Lys Cys Trp Asp Pro Ser Pro Gln Ala Tyr Phe Thr Leu Pro Arg Asn
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Glu Pro Ile Gln Pro Ile Asp Pro Ala Ala Trp Val Ser His Ser Ala
    370                 375                 380

Ala Leu Thr Gly Thr Phe Pro Ala Tyr Pro Gly Ser Ser Ser Met Ser
385                 390                 395                 400

Thr Ile Thr Ser Gly Ser Ser Leu Pro Asp Gly Cys Glu Gly Arg Gly
                405                 410                 415

Leu Ser Val His Thr Asp Met Ala Ser Val Thr Lys Ala Met Ala Ala
            420                 425                 430

Pro Glu Ser Gly Leu Glu Val Arg Asp Arg Met Trp Leu Lys Ile Thr
        435                 440                 445

Ile Pro Asn Ala Phe Leu Gly Ser Asp Val Val Asp Trp Leu Tyr His
    450                 455                 460

His Val Glu Gly Phe Pro Glu Arg Arg Glu Ala Arg Lys Tyr Ala Ser
465                 470                 475                 480

Gly Leu Leu Lys Ala Gly Leu Ile Arg His Thr Val Asn Lys Ile Thr
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Phe Ser Glu Gln Cys Tyr Tyr Val Phe Gly Asp Leu Ser Gly Gly Cys
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Glu Ser Tyr Leu Val Asn Leu Ser Leu Asn Asp Asn Asp Gly Ser Ser
        515                 520                 525

Gly Ala Ser Asp Gln Asp Thr Leu Ala Pro Leu Pro Gly Ala Thr Pro
    530                 535                 540

Trp Pro Leu Leu Pro Thr Phe Ser Tyr Gln Tyr Pro Ala Pro His Pro
545                 550                 555                 560

Tyr Ser Pro Gln Pro Pro Pro Tyr His Glu Leu Ser Ser Tyr Thr Tyr
                565                 570                 575

Gly Gly Gly Ser Ala Ser Ser Gln His Ser Glu Gly Ser Arg Ser Ser
            580                 585                 590

Gly Ser Thr Arg Ser Asp Gly Gly Ala Gly Arg Thr Gly Arg Pro Glu
        595                 600                 605

Glu Arg Ala Pro Glu Ser Lys Ser Gly Ser Gly Ser Glu Ser Glu Pro
    610                 615                 620

Ser Ser Arg Gly Gly Ser Leu Arg Arg Gly Gly Glu Ala Ser Gly Thr
625                 630                 635                 640

Ser Asp Gly Gly Pro Pro Pro Ser Arg Gly Ser Thr Gly Gly Ala Pro
                645                 650                 655

Asn Leu Arg Ala His Pro Gly Leu His Pro Tyr Gly Pro Pro Pro Gly
            660                 665                 670

Met Ala Leu Pro Tyr Asn Pro Met Met Val Val Met Met Pro Pro Pro
        675                 680                 685

Pro Pro Pro Val Pro Pro Ala Val Gln Pro Pro Gly Ala Pro Pro Val
    690                 695                 700

Arg Asp Leu Gly Ser Val Pro Pro Glu Leu Thr Ala Ser Arg Gln Ser
705                 710                 715                 720

Phe His Met Ala Met Gly Asn Pro Ser Glu Phe Phe Val Asp Val Met
                725                 730                 735
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<210> SEQ ID NO 2

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<211> LENGTH: 716
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 2

```
Met Gly Glu Thr Lys Ile Ile Tyr His Leu Asp Gly Gln Glu Thr Pro
1               5                   10                  15

Tyr Leu Val Lys Leu Pro Leu Pro Ala Glu Arg Val Thr Leu Ala Asp
            20                  25                  30

Phe Lys Gly Val Leu Gln Arg Pro Ser Tyr Lys Phe Phe Phe Lys Ser
        35                  40                  45

Met Asp Asp Asp Phe Gly Val Val Lys Glu Glu Ile Ser Asp Asp Asn
    50                  55                  60

Ala Lys Leu Pro Cys Phe Asn Gly Arg Val Val Ser Trp Leu Val Ser
65                  70                  75                  80

Ala Glu Gly Ser His Pro Asp Pro Ala Pro Phe Cys Ala Asp Asn Pro
                85                  90                  95

Ser Glu Leu Pro Pro Pro Met Glu Arg Thr Gly Gly Ile Gly Asp Ser
            100                 105                 110

Arg Pro Pro Ser Phe His Pro His Ala Gly Gly Gly Ser Gln Glu Asn
        115                 120                 125

Leu Asp Asn Asp Thr Glu Thr Asp Ser Leu Val Ser Ala Gln Arg Glu
    130                 135                 140

Arg Pro Arg Arg Arg Asp Gly Pro Glu His Ala Thr Arg Leu Asn Gly
145                 150                 155                 160

Thr Ala Lys Gly Glu Arg Arg Arg Glu Pro Gly Gly Tyr Asp Ser Ser
                165                 170                 175

Ser Thr Leu Met Ser Ser Glu Leu Glu Thr Thr Ser Phe Phe Asp Ser
            180                 185                 190

Asp Glu Asp Asp Ser Thr Ser Arg Phe Ser Ser Ser Thr Glu Gln Ser
        195                 200                 205

Ser Ala Ser Arg Leu Met Arg Arg His Lys Arg Arg Arg Arg Lys Gln
    210                 215                 220

Lys Val Ser Arg Ile Glu Arg Ser Ser Ser Phe Ser Ser Ile Thr Asp
225                 230                 235                 240

Ser Thr Met Ser Leu Asn Ile Ile Thr Val Thr Leu Asn Met Glu Lys
                245                 250                 255

Tyr Asn Phe Leu Gly Ile Ser Ile Val Gly Gln Ser Asn Glu Arg Gly
            260                 265                 270

Asp Gly Gly Ile Tyr Ile Gly Ser Ile Met Lys Gly Gly Ala Val Ala
        275                 280                 285

Ala Asp Gly Arg Ile Glu Pro Gly Asp Met Leu Leu Gln Val Asn Glu
    290                 295                 300

Ile Asn Phe Glu Asn Met Ser Asn Asp Asp Ala Val Arg Val Leu Arg
305                 310                 315                 320

Glu Ile Val His Lys Pro Gly Pro Ile Thr Leu Thr Val Ala Lys Cys
                325                 330                 335

Trp Asp Pro Ser Pro Arg Gly Cys Phe Thr Leu Pro Arg Ser Glu Pro
            340                 345                 350

Ile Arg Pro Ile Asp Pro Ala Ala Trp Val Ser His Thr Ala Ala Met
        355                 360                 365

Thr Gly Thr Phe Pro Ala Tyr Gly Met Ser Pro Ser Leu Ser Thr Ile
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    370                 375                 380

Thr Ser Thr Ser Ser Ser Ile Thr Ser Ser Ile Pro Asp Thr Glu Arg
385                 390                 395                 400

Leu Asp Asp Phe His Leu Ser Ile His Ser Asp Met Ala Ala Ile Val
                405                 410                 415

Lys Ala Met Ala Ser Pro Glu Ser Gly Leu Glu Val Arg Asp Arg Met
            420                 425                 430

Trp Leu Lys Ile Thr Ile Pro Asn Ala Phe Ile Gly Ser Asp Val Val
        435                 440                 445

Asp Trp Leu Tyr His Asn Val Glu Gly Phe Thr Asp Arg Arg Glu Ala
    450                 455                 460

Arg Lys Tyr Ala Ser Asn Leu Leu Lys Ala Gly Phe Ile Arg His Thr
465                 470                 475                 480

Val Asn Lys Ile Thr Phe Ser Glu Gln Cys Tyr Tyr Ile Phe Gly Asp
                485                 490                 495

Leu Cys Gly Asn Met Ala Asn Leu Ser Leu His Asp His Asp Gly Ser
            500                 505                 510

Ser Gly Ala Ser Asp Gln Asp Thr Leu Ala Pro Leu Pro His Pro Gly
        515                 520                 525

Ala Ala Pro Trp Pro Met Ala Phe Pro Tyr Gln Tyr Pro Pro Pro Pro
    530                 535                 540

His Pro Tyr Asn Pro His Pro Gly Phe Pro Glu Leu Gly Tyr Ser Tyr
545                 550                 555                 560

Gly Gly Gly Ser Ala Ser Ser Gln His Ser Glu Gly Ser Arg Ser Ser
                565                 570                 575

Gly Ser Asn Arg Ser Gly Ser Asp Arg Arg Lys Glu Lys Asp Pro Lys
            580                 585                 590

Ala Gly Asp Ser Lys Ser Gly Gly Ser Gly Ser Glu Ser Asp His Thr
        595                 600                 605

Thr Arg Ser Ser Leu Arg Gly Pro Arg Glu Arg Ala Pro Ser Glu Arg
    610                 615                 620

Ser Gly Pro Ala Ala Ser Glu His Ser His Arg Ser His His Ser Leu
625                 630                 635                 640

Ala Ser Ser Leu Arg Ser His His Thr His Pro Ser Tyr Gly Pro Pro
                645                 650                 655

Gly Val Pro Pro Leu Tyr Gly Pro Pro Met Leu Met Met Pro Pro Pro
            660                 665                 670

Pro Ala Ala Met Gly Pro Pro Gly Ala Pro Pro Gly Arg Asp Leu Ala
        675                 680                 685

Ser Val Pro Pro Glu Leu Thr Ala Ser Arg Gln Ser Phe Arg Met Ala
    690                 695                 700

Met Gly Asn Pro Ser Glu Phe Phe Val Asp Val Met
705                 710                 715


<210> SEQ ID NO 3
<211> LENGTH: 416
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 3

Met Glu Leu Arg Val Gly Asn Lys Tyr Arg Leu Gly Arg Lys Ile Gly
1               5                   10                  15
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Ser Gly Ser Phe Gly Asp Ile Tyr Leu Gly Ala Asn Ile Ala Ser Gly
            20                  25                  30

Glu Glu Val Ala Ile Lys Leu Glu Cys Val Lys Thr Lys His Pro Gln
        35                  40                  45

Leu His Ile Glu Ser Lys Phe Tyr Lys Met Met Gln Gly Gly Val Gly
    50                  55                  60

Ile Pro Ser Ile Lys Trp Cys Gly Ala Glu Gly Asp Tyr Asn Val Met
65                  70                  75                  80

Val Met Glu Leu Leu Gly Pro Ser Leu Glu Asp Leu Phe Asn Phe Cys
                85                  90                  95

Ser Arg Lys Phe Ser Leu Lys Thr Val Leu Leu Leu Ala Asp Gln Met
            100                 105                 110

Ile Ser Arg Ile Glu Tyr Ile His Ser Lys Asn Phe Ile His Arg Asp
        115                 120                 125

Val Lys Pro Asp Asn Phe Leu Met Gly Leu Gly Lys Lys Gly Asn Leu
    130                 135                 140

Val Tyr Ile Ile Asp Phe Gly Leu Ala Lys Lys Tyr Arg Asp Ala Arg
145                 150                 155                 160

Thr His Gln His Ile Pro Tyr Arg Glu Asn Lys Asn Leu Thr Gly Thr
                165                 170                 175

Ala Arg Tyr Ala Ser Ile Asn Thr His Leu Gly Ile Glu Gln Ser Arg
            180                 185                 190

Arg Asp Asp Leu Glu Ser Leu Gly Tyr Val Leu Met Tyr Phe Asn Leu
        195                 200                 205

Gly Ser Leu Pro Trp Gln Gly Leu Lys Ala Ala Thr Lys Arg Gln Lys
    210                 215                 220

Tyr Glu Arg Ile Ser Glu Lys Lys Met Ser Thr Pro Ile Glu Val Leu
225                 230                 235                 240

Cys Lys Gly Tyr Pro Ser Glu Phe Ser Thr Tyr Leu Asn Phe Cys Arg
                245                 250                 255

Ser Leu Arg Phe Asp Asp Lys Pro Asp Tyr Ser Tyr Leu Arg Gln Leu
            260                 265                 270

Phe Arg Asn Leu Phe His Arg Gln Gly Phe Ser Tyr Asp Tyr Val Phe
        275                 280                 285

Asp Trp Asn Met Leu Lys Phe Gly Ala Ala Arg Asn Pro Glu Asp Val
    290                 295                 300

Asp Arg Glu Arg Arg Glu His Glu Arg Glu Glu Arg Met Gly Gln Leu
305                 310                 315                 320

Arg Gly Ser Ala Thr Arg Ala Leu Pro Pro Gly Pro Pro Thr Gly Ala
                325                 330                 335

Thr Ala Asn Arg Leu Arg Ser Ala Ala Glu Pro Val Ala Ser Thr Pro
            340                 345                 350

Ala Ser Arg Ile Gln Pro Ala Gly Asn Thr Ser Pro Arg Ala Ile Ser
        355                 360                 365

Arg Val Asp Arg Glu Arg Lys Val Ser Met Arg Leu His Arg Gly Ala
    370                 375                 380

Pro Ala Asn Val Ser Ser Ser Asp Leu Thr Gly Arg Gln Glu Val Ser
385                 390                 395                 400

Arg Ile Pro Ala Ser Gln Thr Ser Val Pro Phe Asp His Leu Gly Lys
                405                 410                 415
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<210> SEQ ID NO 4
<211> LENGTH: 391
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 4

Met Ser Gly Pro Val Pro Ser Arg Ala Arg Val Tyr Thr Asp Val Asn
1 5 10 15

Thr His Arg Pro Arg Glu Tyr Trp Asp Tyr Glu Ser His Val Val Glu
20 25 30

Trp Gly Asn Gln Asp Asp Tyr Gln Leu Val Arg Lys Leu Gly Arg Gly
35 40 45

Lys Tyr Ser Glu Val Phe Glu Ala Ile Asn Ile Thr Asn Asn Glu Lys
50 55 60

Val Val Val Lys Ile Leu Lys Pro Val Lys Lys Lys Lys Ile Lys Arg
65 70 75 80

Glu Ile Lys Ile Leu Glu Asn Leu Arg Gly Gly Pro Asn Ile Ile Thr
85 90 95

Leu Ala Asp Ile Val Lys Asp Pro Val Ser Arg Thr Pro Ala Leu Val
100 105 110

Phe Glu His Val Asn Asn Thr Asp Phe Lys Gln Leu Tyr Gln Thr Phe
115 120 125

Thr Asp Tyr Asp Ile Arg Phe Tyr Met Tyr Glu Ile Leu Lys Ala Leu
130 135 140

Asp Tyr Cys His Ser Met Gly Ile Met His Arg Asp Val Lys Pro His
145 150 155 160

Asn Val Met Ile Asp His Glu His Arg Lys Leu Arg Leu Ile Asp Trp
165 170 175

Gly Leu Ala Glu Phe Tyr His Pro Gly Gln Glu Tyr Asn Val Arg Val
180 185 190

Ala Ser Arg Tyr Phe Lys Gly Pro Glu Leu Leu Val Asp Tyr Gln Met
195 200 205

Tyr Asp Tyr Ser Leu Asp Met Trp Ser Leu Gly Cys Met Leu Ala Ser
210 215 220

Met Ile Phe Arg Lys Glu Pro Phe Phe His Gly Arg Asp Asn Tyr Asp
225 230 235 240

Gln Leu Val Arg Ile Ala Lys Val Leu Gly Thr Glu Asp Leu Tyr Gly
245 250 255

Tyr Ile Asp Lys Tyr Asn Ile Glu Leu Asp Pro Arg Phe Asn Asp Ile
260 265 270

Leu Gly Arg His Ser Arg Lys Arg Trp Glu Arg Phe Val His Arg Glu
275 280 285

Asn Gln His Leu Val Ser Pro Glu Ala Leu Asp Phe Leu Asp Lys Leu
290 295 300

Leu Arg Tyr Asp His Gln Ser Arg Leu Thr Ala Arg Glu Ala Met Glu
305 310 315 320

His Pro Tyr Phe Tyr Thr Val Val Lys Asp Gln Ala Arg Met Gly Ser
325 330 335

Ser Ser Met Pro Gly Gly Ser Thr Pro Val Ser Ser Ala Asn Val Met
340 345 350

Ser Gly Ile Ser Ser Val Pro Thr Pro Ser Pro Leu Gly Pro Leu Ala
355 360 365

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Gly Ser Pro Val Ile Ala Ala Ala Asn Pro Leu Gly Met Pro Val Pro
    370                 375                 380

Ala Ala Ala Gly Ala Gln Gln
385                 390


<210> SEQ ID NO 5
<211> LENGTH: 521
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 5

Met Asp Thr Glu Ser Gln Tyr Ser Gly Tyr Ser Tyr Lys Ser Gly His
1               5                   10                  15

Ser Arg Ser Ser Arg Lys His Arg Asp Arg Arg Asp Arg His Arg Ser
            20                  25                  30

Lys Ser Arg Asp Gly Gly Arg Gly Asp Lys Ser Val Thr Ile Gln Ala
        35                  40                  45

Pro Gly Glu Pro Leu Leu Asp Asn Glu Ser Thr Arg Gly Asp Glu Arg
    50                  55                  60

Asp Asp Asn Trp Gly Glu Thr Thr Thr Val Val Thr Gly Thr Ser Glu
65                  70                  75                  80

His Ser Ile Ser His Asp Asp Leu Thr Arg Ile Ala Lys Asp Met Glu
                85                  90                  95

Asp Ser Val Pro Leu Asp Cys Ser Arg His Leu Gly Val Ala Ala Gly
            100                 105                 110

Ala Thr Leu Ala Leu Leu Ser Phe Leu Thr Pro Leu Ala Phe Leu Leu
        115                 120                 125

Leu Pro Pro Leu Leu Trp Arg Glu Glu Leu Glu Pro Cys Gly Thr Ala
    130                 135                 140

Cys Glu Gly Leu Phe Ile Ser Val Ala Phe Lys Leu Leu Ile Leu Leu
145                 150                 155                 160

Leu Gly Ser Trp Ala Leu Phe Phe Arg Arg Pro Lys Ala Ser Leu Pro
                165                 170                 175

Arg Val Phe Val Leu Arg Ala Leu Leu Met Val Leu Val Phe Leu Leu
            180                 185                 190

Val Val Ser Tyr Trp Leu Phe Tyr Gly Val Arg Ile Leu Asp Ala Arg
        195                 200                 205

Glu Arg Ser Tyr Gln Gly Val Val Gln Phe Ala Val Ser Leu Val Asp
    210                 215                 220

Ala Leu Leu Phe Val His Tyr Leu Ala Val Val Leu Leu Glu Leu Arg
225                 230                 235                 240

Gln Leu Gln Pro Gln Phe Thr Leu Lys Val Val Arg Ser Thr Asp Gly
                245                 250                 255

Ala Ser Arg Phe Tyr Asn Val Gly His Leu Ser Ile Gln Arg Val Ala
            260                 265                 270

Val Trp Ile Leu Glu Lys Tyr Tyr His Asp Phe Pro Val Tyr Asn Pro
        275                 280                 285

Ala Leu Leu Asn Leu Pro Lys Ser Val Leu Ala Lys Lys Val Ser Gly
    290                 295                 300

Phe Lys Val Tyr Ser Leu Gly Glu Glu Asn Ser Thr Asn Asn Ser Thr
305                 310                 315                 320

Gly Gln Ser Arg Ala Val Ile Ala Ala Ala Ala Arg Arg Arg Asp Asn
                325                 330                 335
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Ser His Asn Glu Tyr Tyr Tyr Glu Glu Ala Glu His Glu Arg Arg Val
            340                 345                 350

Arg Lys Arg Arg Ala Arg Leu Val Val Ala Val Glu Glu Ala Phe Thr
        355                 360                 365

His Ile Lys Arg Leu Gln Glu Glu Glu Gln Lys Asn Pro Arg Glu Val
    370                 375                 380

Met Asp Pro Arg Glu Ala Ala Gln Ala Ile Phe Ala Ser Met Ala Arg
385                 390                 395                 400

Ala Met Gln Lys Tyr Leu Arg Thr Thr Lys Gln Gln Pro Tyr His Thr
                405                 410                 415

Met Glu Ser Ile Leu Gln His Leu Glu Phe Cys Ile Thr His Asp Met
            420                 425                 430

Thr Pro Lys Ala Phe Leu Glu Arg Tyr Leu Ala Ala Gly Pro Thr Ile
        435                 440                 445

Gln Tyr His Lys Glu Arg Trp Leu Ala Lys Gln Trp Thr Leu Val Ser
    450                 455                 460

Glu Glu Pro Val Thr Asn Gly Leu Lys Asp Gly Ile Val Phe Leu Leu
465                 470                 475                 480

Lys Arg Gln Asp Phe Ser Leu Val Val Ser Thr Lys Lys Val Pro Phe
                485                 490                 495

Phe Lys Leu Ser Glu Glu Phe Val Asp Pro Lys Ser His Lys Phe Val
            500                 505                 510

Met Arg Leu Gln Ser Glu Thr Ser Val
        515                 520


<210> SEQ ID NO 6
<211> LENGTH: 1143
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 6

atgaagaagt ccattggaat attaagccca ggagttgctt tggggatggc tggaagtgca     60

atgtcttcca agttcttcct agtggctttg gccatatttt tctccttcgc ccaggttgta    120

attgaagcca attcttggtg gtcgctaggt atgaataacc ctgttcagat gtcagaagta    180

tatattatag gagcacagcc tctctgcagc caactggcag gactttctca aggacagaag    240

aaactgtgcc acttgtatca ggaccacatg cagtacatcg gagaaggcgc gaagacaggc    300

atcaaagaat gccagtatca attccgacat cgaaggtgga actgcagcac tgtggataac    360

acctctgttt ttggcagggt gatgcagata ggcagccgcg agacggcctt cacatacgcg    420

gtgagcgcag caggggtggt gaacgccatg agccgggcgt gccgcgaggg cgagctgtcc    480

acctgcggct gcagccgcgc cgcgcgcccc aaggacctgc cgcgggactg gctctggggc    540

ggctgcggcg acaacatcga ctatggctac cgctttgcca aggagttcgt ggacgcccgc    600

gagcgggagc gcatccacgc caagggctcc tacgagagtg ctcgcatcct catgaacctg    660

cacaacaacg aggccggccg caggacggtg tacaacctgg ctgatgtggc ctgcaagtgc    720

catggggtgt ccggctcatg tagcctgaag acatgctggc tgcagctggc agacttccgc    780

aaggtgggtg atgccctgaa ggagaagtac gacagcgcgg cggccatgcg gctcaacagc    840

cggggcaagt tggtacaggt caacagccgc ttcaactcgc ccaccacaca agacctggtc    900

tacatcgacc ccagccctga ctactgcgtg cgcaatgaga gcaccggctc gctgggcacg    960
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cagggccgcc tgtgcaacaa gacgtcggag ggcatggatg gctgcgagct catgtgctgc 1020

ggccgtggct acgaccagtt caagaccgtg cagacggagc gctgccactg caagttccac 1080

tggtgctgct acgtcaagtg caagaagtgc acggagatcg tggaccagtt tgtgtgcaag 1140

tag 1143

<210> SEQ ID NO 7
<211> LENGTH: 2001
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 7

atggctatga cttggattgt cttctctctt tggcccttga ctgtgttcat ggggcatata 60

ggtgggcaca gtttgttttc ttgtgaacct attaccttga ggatgtgcca agatttgcct 120

tataatacta ccttcatgcc taatcttctg aatcattatg accaacagac agcagctttg 180

gcaatggagc cattccaccc tatggtgaat ctggattgtt ctcgggattt ccggcctttt 240

ctttgtgcac tctacgctcc tatttgtatg gaatatggac gtgtcacact tccctgtcgt 300

aggctgtgtc agcgggctta cagtgagtgt tcgaagctca tggagatgtt tggtgttcct 360

tggcctgaag atatggaatg cagtaggttc ccagattgtg atgagccata tcctcgactt 420

gtggatctga atttagctgg agaaccaact gaaggagccc cagtggcagt gcagagagac 480

tatggttttt ggtgtccccg agagttaaaa attgatcctg atctgggtta ttcttttctg 540

catgtgcgtg attgttcacc tccttgtcca aatatgtact tcagaagaga agaactgtca 600

tttgctcgct atttcatagg attgatttca atcatttgcc tctcggccac attgtttact 660

tttttaactt ttttgattga tgtcacaaga ttccgttatc ctgaaaggcc tattatattt 720

tatgcagtct gctacatgat ggtatcctta attttcttca ttggattttt gcttgaagat 780

cgagtagcct gcaatgcatc catccctgca caatataagg cttccacagt gacacaagga 840

tctcataata aagcctgtac catgcttttt atgatactct atttttttac tatggctggc 900

agtgtatggt gggtaattct taccatcaca tggtttttag cagctgtgcc aaagtggggt 960

agtgaagcta ttgagaagaa agcattgctg tttcacgcca gtgcatgggg catccccgga 1020

actctaacca tcatcctttt agcgatgaat aaaattgaag gtgacaatat tagtggcgtg 1080

tgttttgttg gcctctacga tgttgatgca ttgagatatt ttgttcttgc tcccctctgc 1140

ctgtatgtgg tagttggggt ttctctcctc ttagctggca ttatatccct aaacagagtt 1200

cgaattgaga ttccattaga aaaggagaac caagataaat tagtgaagtt tatgatccgg 1260

atcggtgttt tcagcattct ttatctcgta ccactcttgg ttgtaattgg atgctacttt 1320

tatgagcaag cttaccgggg catctgggaa acaacgtgga tacaagaacg ctgcagagaa 1380

tatcacattc catgtccata tcaggttact caaatgagtc gtccagactt gattctcttt 1440

ctgatgaaat acctgatggc tctcatagtt ggcattccct ctgtattttg ggttggaagc 1500

aaaaagacat gctttgaatg ggccagtttt tttcatggtc gtaggaaaaa agagatagtg 1560

aatgagagcc gacaggtact ccaggaacct gattttgctc agtctctcct gagggatcca 1620

aatactccta tcataagaaa gtcaagggga acttccactc aaggaacatc cacccatgct 1680

tcttcaactc agctggctat ggtggatgat caaagaagca aagcaggaag catccacagc 1740

aaagtgagca gctaccacgg cagcctccac agatcacgtg atggcaggta cacgccctgc 1800

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agttacagag gaatggagga gagactacct catggcagca tgtcacgact aacagatcac 1860

tccaggcata gtagttctca tcggctcaat gaacagtcac gacatagcag catcagagat 1920

ctcagtaata atcccatgac tcatatcaca catggcacca gcatgaatcg ggttattgaa 1980

gaagatggaa ccagtgctta a 2001

<210> SEQ ID NO 8
<211> LENGTH: 1725
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 8

atgcgggacc ccggcgcggc cgctccgctt tcgtccctgg gcctctgtgc cctggtgctg 60

gcgctgctgg gcgcactgtc cgcgggcgcc ggggcgcagc cgtaccacgg agagaagggc 120

atctccgtgc cggaccacgg cttctgccag cccatctcca tcccgctgtg cacggacatc 180

gcctacaacc agaccatcct gcccaacctg ctgggccaca cgaaccaaga ggacgcgggc 240

ctcgaggtgc accagttcta cccgctggtg aaggtgcagt gttctcccga actccgcttt 300

ttcttatgct ccatgtatgc gcccgtgtgc accgtgctcg atcaggccat cccgccgtgt 360

cgttctctgt gcgagcgcgc ccgccagggc tgcgaggcgc tcatgaacaa gttcggcttc 420

cagtggcccg agcggctgcg ctgcgagaac ttcccggtgc acggtgcggg cgagatctgc 480

gtgggccaga acacgtcgga cggctccggg ggcccaggcg gcggccccac tgcctaccct 540

accgcgccct acctgccgga cctgcccttc accgcgctgc ccccgggggc ctcagatggc 600

agggggcgtc ccgccttccc cttctcatgc ccccgtcagc tcaaggtgcc cccgtacctg 660

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ggcctgatgt actttaagga ggaggagagg cgcttcgccc gcctctgggt gggcgtgtgg 780

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cgcttcagct acccagagcg gcccatcatc ttcctgtcgg gctgctactt catggtggcc 900

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gacgatggct accgcacggt ggcgcagggc accaagaagg agggctgcac catcctcttc 1020

atggtgctct acttcttcgg catggccagc tccatctggt gggtcattct gtctctcact 1080

tggttcctgg cggccggcat gaagtggggc cacgaggcca tcgaggccaa ctcgcagtac 1140

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caggtagacg gggacctgct gagcggggtg tgctacgttg gcctctccag tgtggacgcg 1260

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accgagaagc tggagaagct catggtgcgc atcggcgtct tcagcgtgct ctacacagtg 1440

cccgccacca tcgtcctggc ctgctacttc tacgagcagg ccttccgcga gcactgggag 1500

cgcacctggc tcctgcagac gtgcaagagc tatgccgtgc cctgcccgcc cggccacttc 1560

ccgcccatga gccccgactt caccgtcttc atgatcaagt acctgatgac catgatcgtc 1620

ggcatcacca ctggcttctg gatctggtcg ggcaagaccc tgcagtcgtg gcgccgcttc 1680

taccacagac ttagccacag cagcaagggg gagactgcgg tatga 1725

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<210> SEQ ID NO 9
<211> LENGTH: 2496
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 9

atgcctttgg agatggagcc caagatgagc aaactggcct ttggctgtca gagaagttcc 60

acatcagatg atgactctgg ctgtgcattg gaggagtacg cctgggtccc cccgggcctg 120

agaccagagc agatccagct ctattttgct tgcttaccag aggaaaaagt tccttacgtt 180

aacagccccg gagagaagca tcggattaaa cagcttttgt accagttacc accacatgat 240

aatgaggtac ggtattgcca gtctttgagt gaagaggaga aaaaagagtt gcaggtgttc 300

agtgctcagc ggaagaaaga agcactggga agaggaacaa ttaagcttct gtccagagca 360

gtcatgcatg ctgtgtgtga gcagtgtggt ttgaagataa atggaggtga agttgcagtg 420

ttcgcctccc gtgcgggccc tggtgtgtgc tggcacccat cctgttttgt ctgtttcacg 480

tgtaatgagc tgctggtcga cctcatctat ttttatcagg atggaaaaat tcactgtggc 540

aggcaccatg cagaactgct caaaccacgg tgctcagcat gtgacgagat aatttttgct 600

gatgagtgca cagaagctga gggtcgccat tggcacatga aacacttctg ctgccttgag 660

tgtgaaacgg tcctgggagg acagaggtat atcatgaagg acggccgccc cttctgctgt 720

ggctgttttg agtctctcta tgcggagtac tgtgaaacct gtggggaaca tattggtgtg 780

gaccatgcac agatgaccta tgacgggcag cactggcacg ccacggaagc ctgcttttct 840

tgtgcccagt gtaaagcctc tttgttggga tgtcccttcc ttcccaaaca gggtcagatt 900

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1-11. (canceled)

12. A method of determination of the diagnosis and prognosis of B-cell chronic lymphocytic leukemia from a biological sample collected from the body of a patient, characterized in that the expression of at least one protein selected from the group including membrane receptors Vangl2, Celsr1 and Frizzled 7, cytoplasmatic proteins Dvl 2, Dvl 3 and Prickle1, kinase casein kinase 1 epsilon and casein kinase 2 alpha and secreted ligand Wnt5a and/or of at least one gene encoding a protein selected from the group including membrane receptors Vangl2, Celsr1 and Frizzled 7, cytoplasmatic proteins Dvl 2, Dvl 3 and Prickle1, kinase casein kinase 1 epsilon and casein kinase 2 alpha and secreted ligand Wnt5a is determined, wherein the proteins and/or genes are up-regulated in B-lymphocytes of the patients with CLL, and their expression correlates with the progression of CLL.

13. The method according to claim **12**, characterized in that the biological sample collected from the body of the patient is a sample of peripheral blood.

14. The method according to claim **12**, characterized in that the expression of the protein is determined by a method selected from the group comprising Western blotting and ELISA.

15. The method according to claim **12**, characterized in that the protein is a membrane receptor selected from the group comprising Vangl2, Celsr1 and Frizzled 7, and in that the expression of the protein is determined by flow cytometry.

16. The method according to claim **12**, characterized in that the gene encoding a protein is selected from the group comprising wnt5a, frizzled 7, vangl2, prickle 1 and celsr1, and in that the expression of the gene encoding protein is determined by a method of quantitative PCR.

17. The method according to claim **16**, characterized in that oligonucleotides having at least 75%, preferably at least 85%, homology of nucleotide sequence with a sequence selected from the group comprising:

Forward primer (5' to 3')	Reverse primer (5' to 3')
TACAACCTTTGGGCTCTGGCTG	CCTTCATCAGGGTCGTTAGCAC
TACCACGGAGAGAAGGGCATC	GCATAAGAAAAAGCGGAGTTCGG
TTCAGTGCTCAGCGGAAGAAAG	AGACAAAACAGGATGGGTGCC
ACAGTAGTAACGGGCACCTCAGAGC	TTGAAGGCGACAGAGATGAAGAG
AACAGCCGCTTCAACTCGC	CGTAGCAGCACCAGTGGAACTTG

are used as primers for the quantitative PCR.

18. A method of determination of the diagnosis and prognosis of B-cell chronic lymphocytic leukemia from a biological sample collected from the body of a patient, characterized in that the migration of CLL cells in the gradient of a chemokine after adding Wnt5a is determined, wherein their ability to migrate correlates with the progression of CLL.

19. A method of determination of diagnosis and prognosis of B-cell chronic lymphocytic leukemia, comprising using oligonucleotides having at least 75%, preferably at least 85%, homology of nucleotide sequence with a sequence selected from the group comprising:

Forward primer (5' to 3')	Reverse primer (5' to 3')
TACAACCTTTGGGCTCTGGCTG	CCTTCATCAGGGTCGTTAGCAC
TACCACGGAGAGAAGGGCATC	GCATAAGAAAAAGCGGAGTTCGG
TTCAGTGCTCAGCGGAAGAAAG	AGACAAAACAGGATGGGTGCC
ACAGTAGTAACGGGCACCTCAGAGC	TTGAAGGCGACAGAGATGAAGAG
AACAGCCGCTTCAACTCGC	CGTAGCAGCACCAGTGGAACTTG

on a biological sample collected from a patient.

* * * * *

专利名称(译)	用于该方法的B细胞慢性淋巴细胞白血病和寡核苷酸患者的诊断和预后的测定方法		
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摘要(译)

本发明提供了一种从患者体内采集的生物样品中确定B细胞慢性淋巴细胞白血病的诊断和预后的方法，其中确定了Wnt / PCP信号传导途径的状态。在本发明的范围内，鉴定了CLL和分子信号传导途径Wnt / PCP的关系，其组分在患有CLL的患者的B淋巴细胞中显着上调。 Wnt / PCP信号传导途径的状态可以通过例如确定所述信号传导途径的组分的表达或通过在所述配体存在下测定趋化因子梯度中CLL细胞的迁移来确定。信号通路。本发明还涉及用于测定信号传导途径组分表达的方法的合适的寡核苷酸。

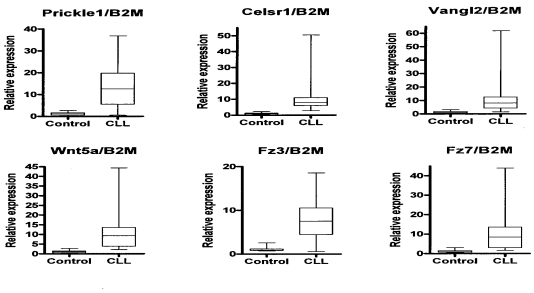

Figure 1A