recurrence over time. Moreover, many tumours are heterogeneous, resulting in potential false-positive or false-negative genetic characterization of biopsies of such tumours.

Recently, the use of circulating tumour cells for diagnosis and monitoring of tumour progression or recurrence showed the use of blood as a source of tumour-derived material, notably tissue fragments in the form of cells. However, the use of circulating tumour cells is inefficient for most cancers.

In general, a disease marker is defined as a compound of which the concentration is altered, preferably elevated, in a biological fluid from a diseased patient when compared to a normal healthy subject, and which may subsequently be used as a marker compound indicative of a disease. Yet, the identification of specific compounds, for instance proteins, in various body fluids as markers of disease, such as cancer, has been hampered by the lack of suitable techniques therefore.

Also in case of diseases other than cancer, markers may be available that are difficult to detect. This hampers early diagnosis of the disease.

The present invention aims to overcome the problem of the prior art that not all diseased tissues or disease types (e.g. tumours) result in circulating disease cells (e.g. circulating tumour cells). The present invention also aims to overcome the problem that protein markers for detecting diseases such as cancer are difficult to detect. Further, the present invention aims to provide methods that do not require biopsies, and allow extensive monitoring of patients.

SUMMARY ~~OF THE INVENTION~~

The present ~~invention~~disclosure ~~in a first aspect~~ provides a method of analysing a blood sample of a subject for the presence of a disease marker, said method comprising the steps of a) extracting nucleic acid from anucleated blood cells, preferably thrombocytes, in said blood sample to provide an

anucleated blood cell-extracted nucleic acid fraction, and b) analysing said anucleated blood cell-extracted nucleic acid fraction for the presence of a disease marker, wherein said disease marker is a disease-specific mutation in a gene of a nucleated cell of said subject, or wherein said disease marker is a disease-specific expression profile of genes of a nucleated cell of said subject.

In a first aspect, there is provided a method of analysing a blood sample of a subject for the presence of a cancer marker, said method comprising the steps of

a) extracting nucleic acid from anucleated blood cells, in said blood sample to provide an anucleated blood cell-extracted nucleic acid fraction, and

b) analysing said anucleated blood cell-extracted nucleic acid fraction for the presence of a cancer marker,

wherein said cancer marker is a cancer-specific mutation in a gene of a nucleated cell of said subject, or

wherein said cancer marker is a cancer-specific expression profile of genes of a nucleated cell of said subject, wherein said nucleic acid is cancer-derived nucleic acid.

The term "anucleated blood cell" as used herein refers to a cell that lacks a nucleus. The term includes reference to both erythrocyte and thrombocyte. Preferred embodiments of anucleated cells in aspects of this invention are thrombocytes. The term "anucleated blood cell" preferably does not include reference to cells that lack a nucleus as a result of faulty cell division.

The term "nucleated cell" as used herein refers to a cell having a nucleus. The term includes reference to somatic cells, germ cells and stem cells, and may include cells from colon, pancreas, brain, bladder, breast, prostate, lung, breast, ovary, uterus, liver, kidney, spleen, thymus, thyroid, nerve tissue, connective tissue, blood, epithelial tissue, lymph node, bone, muscle and skin tissues. The nucleated cell is preferably a cell from a diseased tissue.

Thus, the present ~~invention~~disclosure is generally aimed at analysing nucleic acids that have been transferred from cells that have a nucleus into cells that have no nucleus, wherein the cells that have no nucleus can be easily isolated from the blood stream and contain nucleic acid from the nucleated cells.

The term "nucleus" refers to the membrane-enclosed organelle found in eukaryotic cells that contains most of the cell's genetic material organized in the form of chromosomes. The genes within these chromosomes are the cell's nuclear genome. The interior of the nucleus contains a number of subnuclear bodies including the RNA-comprising nucleolus, which is mainly involved in the assembly of RNA-comprising ribosomes. After being produced in the nucleolus, ribosomes are exported to the cytoplasm where they translate mRNA.

An anucleated blood cell-extracted nucleic acid fraction preferably refers to a fraction comprising chromosomal DNA, ribosomal RNA, nucleolus RNA, and/or messenger RNA.

The term "gene" as used herein, and in particular in the phrasing "mutation in a gene of a nucleated cell" is meant to refer to any nucleic acid sequence, both chromosomal and extra-chromosomal, of a nucleated (somatic) cell, preferably a nuclear nucleic acid sequence, and may include transcribed and non-transcribed sequences as well as ribosomal RNA sequences, most preferably chromosomal sequences that are transcribed into RNA.

In a preferred embodiment of a method of the ~~invention~~disclosure said disease-specific mutation is in a chromosomal gene.

In another preferred embodiment, said gene is not a gene from an anucleated blood cell.

In a preferred embodiment of a method of the ~~invention~~disclosure said disease-specific expression profile is the expression profile of chromosomal genes. In particular of chromosomal genes from a nucleated cell the mRNA of which is present in a thrombocyte.

In another preferred embodiment of a method of the ~~invention~~disclosure said nucleic acid is ribonucleic acid (RNA), more preferably messenger ribonucleic acid (mRNA).

In a preferred embodiment of a method of the ~~invention~~disclosure said nucleic acid is not mtDNA. Hence, mitochondrial nucleic acid is preferably not an aspect of the present ~~invention~~disclosure.

In another preferred embodiment of a method of analysing a blood sample according to the ~~invention~~disclosure said step b) of analysing said anucleated blood cell-extracted nucleic acid fraction for the presence of a disease marker comprises the selective amplification of:

i) said mutation by reverse transcriptase polymerase chain reaction amplification using at least one nucleic acid mutation-specific amplification primer or probe, or

ii) a plurality of mRNAs by reverse transcriptase polymerase chain reaction amplification to determine the expression level of the chromosomal genes encoding said mRNAs to thereby provide an expression profile for said genes and comparing said expression profile to a reference profile.

The blood sample is preferably outside the body.

In a preferred embodiment of a method of the ~~invention~~disclosure the disease is selected from the group consisting of cancer, autoimmune disease, skin diseases, eye disease, endocrine diseases, neurological disorders, and cardiovascular diseases.

In another preferred embodiment of a method of the ~~invention~~disclosure said disease is selected from the group consisting of autoimmune disease, skin diseases, eye disease, endocrine diseases, neurological disorders, and cardiovascular diseases.

In another preferred embodiment of a method of the ~~invention~~disclosure said disease is cancer.

In yet another preferred embodiment of a method of the ~~invention~~disclosure said cancer is a solid tumour cancer, preferably selected from colon, pancreas, brain, bladder, breast, prostate, lung, breast, ovary, uterus, liver, kidney, spleen, thymus, thyroid, nerve tissue, epithelial tissue, lymph node, bone, muscle and skin.

In preferred embodiments of aspects of the ~~invention~~disclosure the auto-immune disease is selected from the group consisting of Achlorhydra Autoimmune Active Chronic Hepatitis; Acute Disseminated Encephalomyelitis; Acute hemorrhagic leukoencephalitis; Addison's Disease; Agammaglobulinemia; Alopecia areata; Amyotrophic Lateral Sclerosis; Ankylosing Spondylitis; Anti-GBM/TBM Nephritis; Antiphospholipid syndrome; Antisynthetase syndrome; polyarticular Arthritis; Atopic allergy; Atopic Dermatitis; Autoimmune Aplastic Anemia; Autoimmune cardiomyopathy; Autoimmune enteropathy; Autoimmune hemolytic anemia; Autoimmune hepatitis; Autoimmune inner ear disease; Autoimmune

disease; Lou Gehrig's Disease; Lupoid hepatitis; Lupus erythematosus; Majeed syndrome; Ménière's disease; Microscopic polyangiitis; Miller-Fisher syndrome; Mixed Connective Tissue Disease; Morphea; Mucha-Habermann disease; Muckle–Wells syndrome; Multiple Myeloma; Multiple Sclerosis; Myasthenia gravis; Myositis; Narcolepsy; Neuromyelitis optica; Neuromyotonia; Occular cicatricial pemphigoid; Opsoclonus myoclonus syndrome; Ord thyroiditis; Palindromic rheumatism; PANDAS; Paraneoplastic cerebellar degeneration; Paroxysmal nocturnal hemoglobinuria; Parry Romberg syndrome; Parsonnage-Turner syndrome; Pars planitis; Pemphigus; Pemphigus vulgaris; Pernicious anaemia; Perivenous encephalomyelitis; POEMS syndrome; Polyarteritis nodosa; Polymyalgia rheumatica; Polymyositis; Primary biliary cirrhosis; Primary sclerosing cholangitis; Progressive inflammatory neuropathy; Psoriasis; Psoriatic Arthritis; Pyoderma gangrenosum; Pure red cell aplasia; Rasmussen's encephalitis; Raynaud phenomenon; Relapsing polychondritis; Reiter's syndrome; Restless leg syndrome; Retroperitoneal fibrosis; Rheumatoid arthritis; Rheumatoid fever; Sarcoidosis; Schizophrenia; Schmidt syndrome; Schnitzler syndrome; Scleritis; Scleroderma; Sjögren's syndrome; Spondyloarthropathy; Sticky blood syndrome; Still's Disease; Stiff person syndrome; Subacute bacterial endocarditis (SBE); Susac's syndrome; Sweet syndrome; Sydenham Chorea; Sympathetic ophthalmia; Takayasu's arteritis; Temporal arteritis; Tolosa-Hunt syndrome; Transverse Myelitis; Ulcerative Colitis; Undifferentiated connective tissue disease; Undifferentiated spondyloarthropathy; Vasculitis; Vitiligo; Wegener's granulomatosis; Wilson's syndrome; and Wiskott-Aldrich syndrome.

In other preferred embodiments of aspects of the ~~invention~~disclosure the skin disease is selected from the group consisting of Acneiform eruptions; Autoinflammatory syndromes; Chronic blistering; Conditions of the mucous membranes; Conditions of the skin appendages; Conditions of the subcutaneous fat; Congenital anomalies; Connective tissue diseases (such as

Abnormalities of dermal fibrous and elastic tissue); Dermal and subcutaneous growths; Dermatitis (including Atopic Dermatitis, Contact Dermatitis, Eczema, Pustular Dermatitis, and Seborrheic Dermatitis); Disturbances of pigmentation; Drug eruptions; Endocrine-related skin disease; Eosinophilic; Epidermal nevi, neoplasms, cysts; Erythemas; Genodermatoses; Infection-related skin disease; Lichenoid eruptions; Lymphoid-related skin disease; Melanocytic nevi and neoplasms (including Melanoma); Monocyte- and macrophage-related skin disease; Mucinoses; Neurocutaneous; Noninfectious immunodeficiency-related skin disease; Nutrition-related skin disease; Papulosquamous hyperkeratotic (including Palmoplantar keratodermas); Pregnancy-related skin disease; Pruritic; Psoriasis; Reactive neutrophilic; Recalcitrant palmoplantar eruptions; Resulting from errors in metabolism; Resulting from physical factors (including Ionizing radiation-induced); Urticaria and angioedema; Vascular-related skin disease.

In other preferred embodiments of aspects of the ~~invention~~disclosure the endocrine disease is selected from the group consisting of Adrenal disorders; Glucose homeostasis disorders; Thyroid disorders; Calcium homeostasis disorders and Metabolic bone disease; Pituitary gland disorders ; and Sex hormone disorders.

In other preferred embodiments of aspects of the ~~invention~~disclosure the eye disease is selected from the group consisting of H00-H06 Disorders of eyelid, lacrimal system and orbit; H10-H13 Disorders of conjunctiva; H15-H22 Disorders of sclera, cornea, iris and ciliary body; H25-H28 Disorders of lens; H30-H36 Disorders of choroid and retina (including H30 Chorioretinal inflammation, H31 Other disorders of choroid, H32 Chorioretinal disorders in diseases classified elsewhere, H33 Retinal detachments and breaks, H34 Retinal vascular occlusions, H35 Other retinal disorders, and H36 Retinal disorders in diseases classified elsewhere); H40-H42 Glaucoma; H43-H45 Disorders of vitreous body and globe; H46-H48 Disorders of optic nerve and visual pathways; H49-H52 Disorders of ocular muscles, binocular movement,

accommodation and refraction; H53-H54.9 Visual disturbances and blindness; and H55-H59 Other disorders of eye and adnexa.

In other preferred embodiments of aspects of the ~~invention~~disclosure the neurological disorder is selected from the group consisting of Abarognosis; Acquired Epileptiform Aphasia; Acute disseminated encephalomyelitis; Adrenoleukodystrophy; Agenesis of the corpus callosum; Agnosia; Aicardi syndrome; Alexander disease; Alien hand syndrome; Allochiria; Alpers' disease; Alternating hemiplegia; Alzheimer's disease; Amyotrophic lateral sclerosis (see Motor Neurone Disease); Anencephaly; Angelman syndrome; Angiomatosis; Anoxia; Aphasia; Apraxia; Arachnoid cysts; Arachnoiditis; Arnold-Chiari malformation; Arteriovenous malformation; Ataxia Telangiectasia; Attention deficit hyperactivity disorder; Auditory processing disorder; Autonomic Dysfunction; Back Pain; Batten disease; Behcet's disease; Bell's palsy; Benign Essential Blepharospasm; Benign Intracranial Hypertension; Bilateral frontoparietal polymicrogyria; Binswanger's disease; Blepharospasm; Bloch-Sulzberger syndrome; Brachial plexus injury; Brain abscess; Brain damage; Brain injury; Brain tumor; Brown-Séquard syndrome; Canavan disease; Carpal tunnel syndrome; Causalgia; Central pain syndrome; Central pontine myelinolysis; Centronuclear myopathy; Cephalic disorder; Cerebral aneurysm; Cerebral arteriosclerosis; Cerebral atrophy; Cerebral gigantism; Cerebral palsy; Cerebral vasculitis; Cervical spinal stenosis; Charcot-Marie-Tooth disease; Chiari malformation; Chorea; Chronic fatigue syndrome; Chronic inflammatory demyelinating polyneuropathy (CIDP); Chronic pain; Coffin Lowry syndrome; Coma; Complex regional pain syndrome; Compression neuropathy; Congenital facial diplegia; Corticobasal degeneration; Cranial arteritis; Craniosynostosis; Creutzfeldt-Jakob disease; Cumulative trauma disorders; Cushing's syndrome; Cytomegalic inclusion body disease (CIBD); Cytomegalovirus Infection; Dandy-Walker syndrome; Dawson disease; De Morsier's syndrome; Dejerine-Klumpke palsy; Dejerine-Sottas disease; Delayed sleep phase syndrome; Dementia; Dermatomyositis;

syndrome; Sleep apnea; Sleeping sickness; Snatiation; Sotos syndrome; Spasticity; Spina bifida; Spinal cord injury; Spinal cord tumors; Spinal muscular atrophy; Spinocerebellar ataxia; Steele-Richardson-Olszewski syndrome; Stiff-person syndrome; Stroke; Sturge-Weber syndrome; Subacute sclerosing panencephalitis; Subcortical arteriosclerotic encephalopathy; Superficial siderosis; Sydenham's chorea; Syncope; Synesthesia; Syringomyelia; Tarsal tunnel syndrome; Tardive dyskinesia; Tarlov cyst; Tay-Sachs disease; Temporal arteritis; Tetanus; Tethered spinal cord syndrome; Thomsen disease; Thoracic outlet syndrome; Tic Douloureux; Todd's paralysis; Tourette syndrome; Toxic encephalopathy; Transient ischemic attack; Transmissible spongiform encephalopathies; Transverse myelitis; Traumatic brain injury; Tremor; Trigeminal neuralgia; Tropical spastic paraparesis; Trypanosomiasis; Tuberous sclerosis; Von Hippel-Lindau disease; Viliuisk Encephalomyelitis ; Wallenberg's syndrome; Werdnig-Hoffman disease; West syndrome; Whiplash; Williams syndrome; Wilson's disease; and Zellweger syndrome.

In other preferred embodiments of aspects of the ~~invention~~disclosure the cardiovascular disease is selected from the group consisting of Aneurysm; Angina; Atherosclerosis; Cerebrovascular Accident (Stroke); Cerebrovascular disease; Congestive Heart Failure; Coronary Artery Disease; Myocardial infarction (Heart Attack); and Peripheral vascular disease.

In another aspect, the present ~~invention~~disclosure provides a method of diagnosing disease in a subject using the method of analysing a blood sample according to the ~~invention~~disclosure. Hence, in another preferred embodiment of a method of the ~~invention~~disclosure, said method of analysing a blood sample according to the ~~invention~~disclosure is part of a method of diagnosing disease in a subject, and wherein the presence of said disease marker in said anucleated blood cell-extracted nucleic acid fraction is indicative of said subject suffering from said disease.

TIn another aspect, the present inventiondisclosure provides a method for determining the efficacy of a disease treatment in a subject, comprising the steps of:

- analysing a blood sample of a subject for the presence of a disease marker using the method of analysing a blood sample according to the inventiondisclosure at a first time point to thereby provide a first value for the level of said disease marker in said subject;
- analysing a blood sample of said subject for the presence of a disease marker using the method of analysing a blood sample according to the inventiondisclosure at a second time point that is earlier or later, preferably later, than said first time point, to thereby provide a second value for the level of said disease marker in said subject, wherein said subject has been subjected to a disease treatment between said first and second time point, and
- comparing said first and second value to determine the efficacy of said disease treatment in said subject.

In a second aspect, there is provided a method for determining the stage of cancer or the efficacy of a cancer treatment in a subject, comprising the steps of:

- analysing a blood sample of a subject for the presence of a cancer marker using the method as defined above at a first time point to thereby provide a first value for the level of said cancer marker in said subject,
- analysing a blood sample of said subject for the presence of a cancer marker using the method as defined above at a second time point to thereby provide a second value for the level of said cancer marker in said subject, wherein said subject has been subjected to a cancer treatment between said first and second time point, and
- comparing said first and second value to determine the efficacy of said cancer treatment in said subject.

The skilled artisan will understand that treatment prior to the first time point and subsequent measurements at a second, later, time point without any disease treatment having occurred between said time points, is included in ~~aspects of the invention~~the present disclosure for determining the efficacy of a disease treatment.

T~~In another aspect, t~~he present ~~invention~~disclosure provides a method for determining the stage of disease. In order to determine the stage of disease, it is beneficial to correlate disease marker values as determined by methods of this ~~invention~~disclosure to disease stages. A single measurement of the disease marker may than be compared to one or more reference values to obtain an indication of the stage of the disease.

T~~In another aspect, t~~he present ~~invention~~disclosure provides a method for determining the stage of disease in a subject, comprising the steps of:

- analysing a blood sample of a subject for the presence of a disease marker using the method of analysing a blood sample of a subject for the presence of a

disease marker according to the present ~~invention~~disclosure to thereby provide a test value for the level of said disease marker in said subject,

- providing a reference value for the level of said disease marker wherein said reference value is correlated to a particular stage of disease, and
- comparing said test and reference value to determine the stage of disease in said subject.

In a third aspect, there is provided a method for determining the stage of a cancer in a subject, comprising the steps of:

- analysing a blood sample of a subject for the presence of a cancer marker using the method as defined above to thereby provide a test value for the level of said cancer marker in said subject.
- providing a reference value for the level of said cancer marker wherein said reference value is correlated to a particular stage of cancer, and
- comparing said test and reference value to determine the stage of cancer in said subject.

T~~In yet another aspect, t~~he present ~~invention~~disclosure provides a kit of parts adapted for performing a method of the ~~invention~~disclosure as described herein above, the kit comprising a packaging material which comprises at least one of:

- a container for holding anucleated blood cells, preferably thrombocytes, separated from a blood sample;
- an agent for extracting nucleic acids from said anucleated blood cells;
- an agent for selectively amplifying from said nucleic acids extracted from said anucleated blood cells a disease-specific marker as described herein above, such as a disease-specific mutation in a gene of a nucleated cell of a subject or a disease-specific expression profile of nucleic acid from a nucleated cell of said subject, for instance by reverse transcriptase polymerase chain reaction amplification, and
- a printed or electronic instruction for performing a method of the ~~invention~~disclosure as described herein above,

the kit further comprising:

- a reference for said disease marker, wherein said reference is indicative for the presence or absence of said disease marker in said anucleated blood cells-extracted nucleic acid fraction.

In a fourth aspect, there is provided a kit of parts adapted for performing the method recited above, the kit comprising a packaging material which comprises at least one of:

- a container for holding anucleated blood cells separated from a blood sample of a subject;
- an agent for extracting nucleic acids from said anucleated blood cells;
- an agent for selectively amplifying from said nucleic acids extracted from said anucleated blood cells a cancer-specific mutation in a gene of a nucleated cell of said subject by reverse transcriptase polymerase chain reaction amplification, and
- a printed or electronic instruction for performing the method recited above,

the kit further comprising:

- a reference for said cancer marker, wherein said reference is indicative for the presence or absence of said cancer marker in said anucleated blood cells-extracted nucleic acid fraction.

In a preferred embodiment of a kit according to the present ~~invention~~disclosure said reference is a reference value for the level of nucleic acids comprising said disease-specific mutation in anucleated blood cells in a healthy control subject or in a control subject suffering from disease, or wherein said reference is a reference expression profile, for instance for a plurality of

mRNAs in anucleated blood cells from a healthy control subject or from a control subject suffering from disease.

In another preferred embodiment of a kit according to the present ~~invention~~disclosure said agent or instruction is selected from a particle or fluorescent marker-labeled anti-anucleated blood cell antibody (preferably a fluorescent marker-labeled anti-thrombocyte antibody), an instruction for bead-based anucleated blood cells isolation (preferably thrombocyte isolation), an instruction for FACS sorting of anucleated blood cells (preferably of thrombocytes), an instruction for anucleated blood cell (preferably thrombocyte) recovery by centrifugation, or negative selection of non-anucleated blood cell components (preferably non-thrombocyte components).

~~In yet another aspect, t~~The present ~~invention~~disclosure provides a device for diagnosing disease, the device comprising a support and at least one agent for specifically determining a level and/or activity of at least one nucleic acid mutant in a anucleated blood cells sample of the subject, said agent being attached to said support, and a computer-readable medium having computer-executable instructions for performing a method of the ~~invention~~disclosure as described herein above.

In a fifth aspect, there is provided a device for diagnosing cancer, the device comprising a support and at least one agent for specifically determining a level and/or activity of at least one nucleic acid mutant in an anucleated blood cell sample of the subject attached to said support, and a computer-readable medium having computer-executable instructions for performing the method recited above.

In a preferred embodiment of a device according to the present ~~invention~~disclosure, said at least one agent is an oligonucleotide probe or sequencing primer.

In a preferred embodiment of a device according to the present ~~invention~~disclosure, the device comprises a lateral flow device, a dipstick or a cartridge for performing a nucleic acid hybridization reaction between an anucleated blood cells-extracted nucleic acid and at least one nucleic acid mutation-specific amplification primer or oligonucleotide probe, or between an anucleated blood cells-extracted nucleic acid and a plurality of gene-specific amplification primers or oligonucleotide probes for providing an disease-specific gene expression profile.

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Our letter of July 2, 2012

Amended claims

1. A method of analysing a blood sample of a subject for the presence of a cancer marker, said method comprising the steps of
a) extracting nucleic acid from anucleated blood cells, in said blood sample to provide an anucleated blood cell-extracted nucleic acid fraction, and
b) analysing said anucleated blood cell-extracted nucleic acid fraction for the presence of a cancer marker,
wherein said cancer marker is a cancer-specific mutation in a gene of a nucleated cell of said subject, or
wherein said cancer marker is a cancer-specific expression profile of genes of a nucleated cell of said subject, wherein said nucleic acid is cancer-derived nucleic acid.

2. The method of claim 1, wherein said anucleated blood cells are thrombocytes or erythrocytes.

3. The method of claim 1 wherein said cancer is a solid tumour cancer,.

4. The method of any one of the preceding claims, wherein said cancer-specific mutation is in a chromosomal gene, or wherein said cancer-specific expression profile is of chromosomal genes.

5. The method of any one of the preceding claims, wherein said nucleic acid is ribonucleic acid (RNA).

6. The method of any one of the preceding claims, wherein said step b) of analysing said anucleated blood cell-extracted nucleic acid fraction for the presence of a cancer marker comprises the selective amplification of
i) said mutation by reverse transcriptase polymerase chain reaction amplification using at least one nucleic acid mutation-specific amplification primer or probe, or
ii) a plurality of mRNAs by reverse transcriptase polymerase chain reaction amplification to determine the expression level of the chromosomal genes encoding said mRNAs to thereby provide an expression profile for said genes and comparing said expression profile to a reference profile.

7. The method of any one of the preceding claims, wherein said method is part of a method of diagnosing said cancer in a subject, and wherein the presence of said cancer marker in said anucleated blood cell-extracted nucleic acid fraction is indicative of said subject suffering from cancer.

8. A method for determining the stage of cancer or the efficacy of a cancer treatment in a subject, comprising the steps of:
- analysing a blood sample of a subject for the presence of a cancer marker using the method according to any one of claims 1-8 at a first time point to thereby provide a first value for the level of said cancer marker in said subject,
- analysing a blood sample of said subject for the presence of a cancer marker using the method according to any one of claims 1-7 at a second time point to thereby provide a second value for the level of said cancer marker in said subject, wherein said subject has been subjected to a cancer treatment between said first and second time point, and

- comparing said first and second value to determine the efficacy of said cancer treatment in said subject.

9. A method for determining the stage of a cancer in a subject, comprising the steps of:
- analysing a blood sample of a subject for the presence of a cancer marker using the method according to any one of claims 1-7 to thereby provide a test value for the level of said cancer marker in said subject,
- providing a reference value for the level of said cancer marker wherein said reference value is correlated to a particular stage of cancer, and
- comparing said test and reference value to determine the stage of cancer in said subject.

10. A kit of parts adapted for performing the method recited in any one of claims 1-9, the kit comprising a packaging material which comprises at least one of:
- a container for holding anucleated blood cells separated from a blood sample of a subject;
- an agent for extracting nucleic acids from said anucleated blood cells;
- an agent for selectively amplifying from said nucleic acids extracted from said anucleated blood cells a cancer-specific mutation in a gene of a nucleated cell of said subject by reverse transcriptase polymerase chain reaction amplification, and
- a printed or electronic instruction for performing the method recited in any one of claims 1-9,
the kit further comprising:
- a reference for said cancer marker, wherein said reference is indicative for the presence or absence of said cancer marker in said anucleated blood cells-extracted nucleic acid fraction.

11. The kit of claim 10, wherein said reference is a reference value for the level of nucleic acids comprising said cancer-specific mutation in thrombocytes in a healthy control subject or in a control subject suffering from said cancer, or
wherein said reference is a reference expression profile for said plurality of mRNAs in anucleated blood cells from a healthy control subject or from a control subject suffering from said cancer.

12. A kit of claim 10 or 11, wherein said agent is selected from a particle or fluorescent marker-labeled anti-anucleated blood cell antibody, or wherein said instruction is selected from an instruction for bead-based anucleated blood cells isolation, an instruction for FACS sorting of anucleated blood cells, an instruction for anucleated blood cell recovery by centrifugation, or negative selection of non-anucleated blood cell components.

13. A device for diagnosing cancer, the device comprising a support and at least one agent for specifically determining a level and/or activity of at least one nucleic acid mutant in an anucleated blood cell sample of the subject attached to said support, and
a computer-readable medium having computer-executable instructions for performing the method recited in any one of claims 1-9.

14. The device of claim 13, wherein said at least one agent is an oligonucleotide probe or sequencing primer.

15. The device of claim 13 or 14, comprising a lateral flow device, a dipstick or a cartridge for performing a nucleic acid hybridization reaction between:
- an anucleated blood cells-extracted nucleic acid and at least one nucleic acid mutation-specific amplification primer or oligonucleotide probe, wherein said

nucleic acid mutation-specific amplification primer or oligonucleotide probe is specific for a cancer-specific mutation, or

- an anucleated blood cells-extracted nucleic acid and a plurality of gene-specific amplification primers or oligonucleotide probes for providing an cancer-specific gene expression profile.