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(54) Title: BIOMARKERS FOR DISCRIMINATING HEALTHY AND/OR NON-MALIGNANT NEOPLASTIC COLORECTAL CELLS FROM COLORECTAL CANCER CELLS

(57) Abstract: This disclosure relates to reagents, methods and kits for assessing sample tissue from an individual, wherein the sample tissue is suspected to be or to comprise colorectal cancer. More particularly, the disclosure provides the reagents, methods and kits to discriminate colorectal cancer and colorectal adenoma. Biomarkers disclosed include SLC7A5, IL8, CXCL1, MMP3, CA7, CHI3L1, IL1B, CXCL2, IL1RN, GREM1, and COL12A1. One or more polynucleotide(s) originating from a biomarker genetic locus were found to be useful to perform discriminating analysis. Particular embodiments relate to panels including these biomarkers. A specific embodiment is the assessment of one or more of the above biomarker(s), performed in comparison to a control of non-cancerous colorectal tissue, particularly healthy colorectal tissue. Particular embodiments relate to marker panels.

Biomarkers for discriminating healthy and/or non-malignant neoplastic colorectal cells from colorectal cancer cells

This disclosure relates to reagents, methods and kits for assessing sample tissue from an individual, wherein the sample tissue is suspected to be or to comprise colorectal cancer. More particularly, the disclosure provides the reagents, methods and kits to discriminate colorectal cancer and colorectal adenoma. Biomarkers disclosed include SLC7A5, IL8, CXCL1, MMP3, CA7, CHI3L1, IL1B, CXCL2, IL1RN, GREM1, and COL12A1.

One or more polynucleotide(s) originating from a biomarker genetic locus were found to be useful to perform discriminating analysis. Particular embodiments relate to panels including these biomarkers. A specific embodiment is the assessment of one or more of the above biomarker(s), performed in comparison to a control of non-cancerous colorectal tissue, particularly healthy colorectal tissue. Particular embodiments relate to marker panels. This disclosure further relates to reagents, methods and kits for assessing sample tissue from an individual, wherein the sample tissue is suspected to be or to comprise colorectal cancer. More particularly, the disclosure provides the reagents, methods and kits for determining, diagnosing, estimating disease progression or pathological staging of a colorectal cancer, in order to more accurately tailor therapy to an individual. Even more particularly, the disclosure provides the reagents, methods and kits to discriminate colorectal cancer and colorectal adenoma. Further, the disclosure relates to molecular biological reagents, methods and kits for monitoring and analyzing biological samples, particularly coloscopy biopsies, for quantifying expression of any one or informative combination of biomarkers selected from the group of human genes/gene products consisting of SLC7A5, IL8, CXCL1, MMP3, CA7, CHI3L1, IL1B, CXCL2, IL1RN, GREM1, and COL12A1. In a specific embodiment, assessing one or more of the above biomarker(s) is performed in comparison to a control of non-cancerous colorectal tissue, particularly healthy colorectal tissue.

Background of the Disclosure

The field of art of this disclosure concerns biomarkers for the assessment of colorectal cancer or a precursor thereof. These biomarkers are useful for risk

assessment, early detection, establishing prognosis, evaluation of intervention, recurrence of neoplastic tissue, and discovery of therapeutic intervention, and methods of use thereof.

In the field of medicine, clinical procedures providing for the risk assessment and early detection of colorectal cancer have been long sought. Currently, colorectal cancer is the second leading cause of cancer-related deaths in the Western world. One picture that has clearly emerged through decades of research into colorectal cancer is that early detection is critical to enhanced survival rates.

Colorectal cancer arises from non-malignant precursor stages, for which an important parameter is histology, particularly in terms of malignant potential. The two most common non-malignant histologic types are hyperplastic polyps (herein also referred to as colorectal polyps) and adenoma. Histologically, hyperplastic polyps contain an increased number of glandular cells with decreased cytoplasmic mucus, but lack nuclear hyperchromatism, stratification, or atypia. Adenomatous nuclei are usually hyperchromatic, enlarged, cigar-shaped, and crowded together in a palisade pattern. Adenomas are classified as tubular or villous. Histologically, tubular adenomas are composed of branched tubules, whereas villous adenomas contain digitiform villi arranged in a frond. Tubulovillous adenomas contain both elements.

Virtually all colorectal cancers arise from adenomas as demonstrated by multiple epidemiologic, clinical, and pathologic findings (Cappell, M.S., Med Clin N Am 89 (2005) 1-42; also see references cited therein). However, a relationship between hyperplastic polyps and colorectal cancer is controversial. Hyperplastic polyps may increase slightly the risk of colon cancer. Risk factors for malignancy in hyperplastic polyps include large polyp size (>1 cm diameter); location in the right colon; a focus of adenoma within the polyp (mixed hyperplasticadenomatous polyp); occurrence of more than 20 hyperplastic polyps in the colon; a family history of hyperplastic polyposis; and a family history of colon cancer (Jass, J.R., Dis Colon Rectum 44 (2001) 163–166).

As the ability to distinguish between non-malignant adenoma and colorectal cancer is deemed important in the assessment of colorectal neoplastic tissue, previous work tried to establish biomarkers for this purpose. Talieri, M., et al. (Cancer Letters 205 (2004) 97-106) observed that the intensity of histological staining specific for cathepsin B and cathepsin D antigens increased from adenoma to

adenocarcinoma. An immunohistochemical study of Fascin expression (Hashimoto, Y., et al., BMC Cancer 6 (2006) abstract, no pp presently known) revealed that Fascin is upregulated in a proportion of adenomas, where its expression is often focal. Strong and diffuse expression was seen in a subset of advanced colorectal adenocarcinomas that correlated with shorter survival in tumor stage III and IV patients. Fascin is an actin bundling protein with roles in the formation of cell protrusions and motility of mesenchymal and neuronal cells. Albrethsen, J., et al. (Molecular and Cellular Proteomics 9 (2010) 988-1005) present a comparative analysis of nuclear matrix proteins in colorectal adenoma and carcinoma tissue samples. Liu, C.-C., et al. (Zhongguo Yaowu Yu Linchuang (2011), 11(1), 23-26; English abstract) disclose an analysis based on immunohistochemistry showing that the expression of Yes-associated protein (YAP) in human colorectal adenocarcinoma is higher than in colorectal polyp and colorectal adenoma tissue. Lind, G. E., et al. (Molecular Cancer 10 (2011) 85) investigated a panel of epigenetic markers for the detection of colorectal cancer and adenomas. Promoter hypermethylation of the genes CNRIP1, FBN1, INA, MAL, SNCA, and SPG20 was studied. Zeng, Y., et al. (Chinese Medical Journal (English Edition) 124 (2011) 2144-2149) studied HIWI protein expression in formalin-fixed and paraffin-embedded colorectal cancer and non-cancerous specimens to establish potential prognostic use of the HIWI as a biomarker.

US 2009/0233295 A1 discloses a method for detecting a neoplasm comprising: a) obtaining a potentially neoplastic test sample and a non-neoplastic (non-cancerous) control sample; b) detecting a level of TRIM59 expression in the test sample and in the control sample; c) detecting a level of expression of at least one of UHRF1, TTK, SLC7A5, and/or KIF20A; and d) comparing in each of the two samples the level of TRIM59 expression and the level of expression of the other marker(s). The test sample is concluded to be neoplastic if in the test sample the levels of expression of TRIM59 and the other marker(s) are detectably greater than the respective levels in the control sample.

US 2011/0020370 A1 discloses methods for diagnosing cancer in a test cell sample by detecting an increase in the level of expression of SLC7A5 in the test cell sample as compared to the level of expression ofSLC7 A5 in a control cell sample isolated from a normal subject. A specific embodiment is the analysis of the level of SLC7A5 RNA in combination with the RNA level of any of the further markers UHRF1, TTK, SLC7A5, and/or KIF20A.

Birkenkamp-Demtroder K. et al. Gut 54 (2005) 374-384 disclose an analysis using oligonucleotide microarrays directed to differential gene expression in colon cancer. Notterman DA et al. Cancer Research 61 (2001) 3124-3130 disclose an analysis using oligonucleotide microarrays directed to differential gene expression of colorectal adenoma, adenocarcinoma and normal tissue.

In light of the state of the art the authors of the present disclosure pursued the goal to identify alternative and more advantageous biomarkers which could be helpful in differential diagnosis of colorectal cancer based on the analysis of biopsy specimens. Particularly, the biomarkers were intended to be nucleic acids. Further, the biomarkers were aimed at discriminating colorectal cancer from colorectal adenoma (neoplastic, but non-malignant and/or not-yet-malignant), and also at discriminating colorectal cancer from normal (healthy) tissue.

Summary of the Disclosure

In a first aspect, the disclosure provides reagents, particularly molecular biological reagents, methods and kits for the use of a polynucleotide originating from a genetic locus selected from any of SLC7A5, IL8, CXCL1, MMP3, CA7, IL1B, CHI3L1, CXCL2, IL1RN, GREM1 and COL12A1 as a biomarker for and/or in the discrimination of colorectal cancer from non-cancerous (=non-malignant) colorectal tissue.

In another aspect, the disclosure provides reagents, particularly molecular biological reagents, methods and kits for the use of a polynucleotide originating from a genetic locus selected from any of SLC7A5, IL8, CXCL1, MMP3, CA7, CHI3L1, IL1B, CXCL2, IL1RN and GREM1, and furthermore COL12A1, as a biomarker for and/or in the discrimination of colorectal cancer from colorectal adenoma.

In yet a further aspect, the disclosure provides reagents, particularly molecular biological reagents, methods and kits for the use of a panel of biomarkers for assessing any member of the group consisting of colorectal cancer, colorectal adenoma, and healthy colorectal tissue, particularly healthy colonic tissue, the panel comprising at least two polynucleotides, each polynucleotide corresponding to a different genetic locus, wherein at least one, and particulatly at least two of the polynucleotides originate(s) from a genetic locus selected from any of SLC7A5, IL8, CXCL1, MMP3, CA7, CHI3L1, IL1B, CXCL2, IL1RN, GREM1 and COL12A1.

In yet a further aspect, the disclosure provides reagents, particularly molecular biological reagents, methods and kits useful in a method for measuring the expression levels of a polynucleotide from a biomarker, the biomarker being capable of discriminating colorectal cancer from non-cancerous colorectal tissue in a human individual, the method comprising: selecting a biomarker comprising a contiguous polynucleotide originating from a genetic locus selected from any of SLC7A5, IL8, CXCL1, MMP3, CA7, IL1B, CHI3L1, CXCL2, IL1RN, GREM1 and COL12A1, a fragment thereof or its complement, and a variant thereof or its complement; obtaining (removing) from the human individual a biopsy sample suspected of being colorectal cancer; in vitro (ex vivo) isolating cellular RNA from the sample and reverse-transcribing isolated RNA to obtain cDNA; amplifying copies of cDNA for the biomarker originating from the sample; and quantifying the level of cDNA amplified from the sample, thereby measuring the expression level of the polynucleotide from a biomarker.

In alternative embodiments, the disclosure provides reagents, particularly molecular biological reagents, and methods for assessing non-malignant and malignant neoplastic colorectal tissue, performing differential diagnosis, conducting analysis of neoplastic disease, particularly detecting progression from colorectal adenoma to colorectal cancer, using sample tissue obtained from an individual. In this aspect, a tissue or cancer cell-containing sample from the patient is analyzed to detect expression of one or more biological markers. In particular embodiments, one or more biological marker(s) is/are selected from the group consisting of SLC7A5, IL8, CXCL1, MMP3, CA7, IL1B, CHI3L1, CXCL2, IL1RN, GREM1 and COL12A1. In these embodiments, colorectal neoplastic tissue is assessed wherein expression, particularly quantitative expression of one or more particularly a plurality of these biological markers differs from the expression, particularly quantitative expression of the one or more particularly a plurality of these biological markers from a non-cancer tissue or cell sample comprising normal and/or non-malignant but not cancer cells.

Particular use of the reagents, methods and kits of the disclosure is assessing the clinical stage of colorectal neoplastic tissue, wherein the biological markers include SLC7A5, IL8, CXCL1, MMP3, CA7, IL1B, CHI3L1, CXCL2, IL1RN, GREM1 and COL12A1. In a specific embodiment, a biomarker is a polynucleotide originating from a genetic locus selected from any of SLC7A5, IL8, CXCL1, MMP3, CA7, IL1B, CHI3L1, CXCL2, IL1RN, GREM1 and COL12A1. Also

falling within the scope of these aspects of the disclosure is quantitative detection of any one of these markers.

In specific aspects, the tissue sample is a small adenoma or adenomatous polyp. In particular, in these embodiments, the disclosure provides a measurement of an expression level, and in a specific embodiment, the expression level is compared to a control expression level; based on increased or decreased expression of a polynucleotide as disclosed herein, relative to a control expression level, non-malignant colorectal tissue in a cell sample can be discriminated from colorectal cancer.

In other embodiments, the colorectal neoplasy is an adenocarcinoma. In particular, in these embodiments, the disclosure provides a measurement of an expression level, and in a specific embodiment, the expression level is compared to a control expression level; based on increased or decreased expression of a polynucleotide as disclosed herein, relative to a control expression level, malignant colorectal tissue in a cell sample can be discriminated from healthy colorectal tissue, non-malignant colorectal tissue or a precursor stage of colorectal cancer.

In additional aspects, the disclosure provides a kit for the practice of the methods disclosed herein. In useful embodiments, said kits comprise at least two reagents, more specifically molecular biological reagents, for detecting expression, particularly for quantitatively measuring expression of biological markers (biomarkers) informative regarding the stage of progression of colorectal neoplastic tissue. In certain embodiments, the at least two reagents are useful for detecting biomarker expression, accordingly, and in a specific embodiment, a biomarker is a polynucleotide originating from a gene selected from the group consisting of SLC7A5, IL8, CXCL1, MMP3, CA7, IL1B, CHI3L1, CXCL2, IL1RN, GREM1 and COL12A1. Each embodiment of the kits of the present disclosure advantageously further comprise instructions for using the kits in the practice of the methods of the disclosure.

Specific embodiments will become evident from the following more detailed description of certain embodiments and the claims. Specific embodiments include the use of a polynucleotide originating from a genetic locus selected from the group consisting of SLC7A5, IL8, CXCL1, MMP3, CA7, IL1B, CHI3L1, CXCL2, IL1RN, GREM1 and COL12A1 as a biomarker for and/or in the discrimination of colorectal cancer from non-cancerous colorectal tissue. In a particular embodiment,

the non-cancerous colorectal tissue is healthy colonic tissue. In yet another particular embodiment, the non-cancerous colorectal tissue is dysplastic colorectal adenoma tissue. In yet another particular embodiment, the discrimination includes comparing the the level of a polynucleotide in the patient's tissue sample with the presence and/or the level and/or the quantity of the polynucleotide in a control. In yet another particular embodiment, the biomarker is selected from any of SLC7A5, IL8, CXCL1, MMP3, IL1B, CHI3L1, CXCL2, IL1RN, GREM1 and COL12A1, and compared to a control sample of non-cancerous colorectal tissue the level of the polynucleotide is increased in colorectal cancer. In yet another particular embodiment, the biomarker is CA7, and compared to a control sample of non-cancerous colorectal tissue the level of the polynucleotide is decreased in colorectal cancer. In yet another particular embodiment, colorectal cancer at a neoplastic stage selected from any of Tis N0 M0, T1-4 N0 M0, Dukes A, and Dukes B is discriminated from non-cancerous colorectal tissue. In yet another particular embodiment, the biomarker is selected from a group of polynucleotides, the group consisting of SEQ ID NOs: 1, 6, 11,16, 21, 26, 31, 36, 41, 46, and 51, a complement thereof, a fragment thereof or its complement, and a variant thereof or its complement. Another specific embodiment is a panel of biomarkers for assessing any member of the group consisting of colorectal cancer, colorectal adenoma and healthy colorectal tissue, the panel comprising at least two polynucleotides, each polynucleotide corresponding to a different genetic locus, wherein at least one, and particulatly at least two of the polynucleotides originate(s) from a genetic locus selected from any of SEQ ID NOs: 1, 6, 11, 16, 21, 26, 31, 36, 41, 46, and 51, a complement thereof, a fragment thereof or its complement, and a variant thereof or its complement. In a particular embodiment, the at least one, and particularly the at least two polynucleotide(s) is/are selected from any of SEQ ID NOs:4, 9, 14, 19, 24, 29, 34, 39, 44, 49, and 54, a complement thereof, a fragment thereof or its complement, and a variant thereof or its complement. Another specific embodiment is a method for measuring the expression levels of a polynucleotide from a biomarker, the biomarker being capable of discriminating colorectal cancer from non-cancerous colorectal tissue in a human individual, the method comprising: selecting a biomarker comprising a contiguous polynucleotide originating from a genetic locus selected from any of SLC7A5, IL8, CXCL1, MMP3, CA7, CHI3L1, IL1B, CXCL2, IL1RN, GREM1 and COL12A1; obtaining from the human individual a biopsy sample suspected of being colorectal cancer; in vitro (ex vivo) isolating cellular RNA from the sample and reverse-transcribing isolated RNA to obtain cDNA; amplifying copies of cDNA for the biomarker

originating from the sample; and quantifying the level of cDNA amplified from the sample, thereby measuring the expression level of the polynucleotide from a biomarker. In a particular embodiment, the biomarker is selected from the group consisting of SEQ ID NOs:1, 6, 11, 16, 21, 26, 31, 36, 41, 46, and 51, a fragment thereof or its complement, and a variant thereof or its complement. In yet another particular embodiment, the biomarker is selected from the group consisting of SEQ ID NOs:4, 9, 14, 19, 24, 29, 34, 39, 44, 49, and 54. In yet another particular embodiment, the step of amplifying copies of cDNA further comprises at least two pairs of primers chosen from the primer pairs of SEQ. ID NOs: 2 and 3, 7 and 8, 12 and 13, 17 and 18, 22 and 23, 27 and 28, 32 and 33, 37 and 38, 42and 43, 47 and 48, and 52 and 53. Another specific embodiment is a kit for the assessment of any member of the group consisting of colorectal cancer and colorectal adenoma, the kit comprising: at least one reagent that is used in analysis of polynucleotide expression level for a biomarker for a member selected from the group consisting of colorectal cancer, colorectal adenoma and colorectal polyp, wherein the biomarker comprises a polynucleotide originating from a genetic locus selected from any of SLC7A5, IL8, CXCL1, MMP3, CA7, CHI3L1, IL1B, CXCL2, IL1RN, GREM1 and COL12A1, a complement therof, a variant thereof, and a fragment thereof; and instructions for using the kit for analyzing the polynucleotide expression level.

Description of the Figures

The Figures 1 to 11 show box plots reflecting results with regards to biomarkers for assessing colorectal cancer (designated "CRC") compared with normal tissue (designated "N"), Figures 12 to 21 show the results for colorectal cancer (designated "CRC") compared with colorectal adenoma (designated "Ad"). In the box plots each box contains the middle 50% of the respective data. The middle line is the median, whiskers extend to the most extreme data point.

In each Figure, diagram "A" reflects quantitative readings of microarrays as described in Example 3, particularly the validation data. Diagram "B" in each Figure shows the data obtained by way of quantitative analysis according to the description in Examples 6 and 7.

Data points in the box plots are indicative of the relative amount of target cDNA polynucleotide amplified (y-axis). Data are shown for quantitative target sequence

determination normalized (“norm.”) against the level of a housekeeping gene (GAPDH).

Figure 1	SLC7A5
Figure 2	IL8
Figure 3	CXCL1
Figure 4	MMP3
Figure 5	CA7
Figure 6	CHI3L1
Figure 7	CXCL2
Figure 8	IL1RN
Figure 9	GREM1
Figure 10	COL12A1
Figure 11	IL1B
Figure 12	SLC7A5
Figure 13	IL8
Figure 14	CXCL1
Figure 15	MMP3
Figure 16	CA7
Figure 17	CHI3L1
Figure 18	IL1B
Figure 19	CXCL2
Figure 20	IL1RN
Figure 21	GREM1

Detailed Description of particular embodiments

5 This disclosure provides methods for assessing colorectal cancer progression in individuals, including cancer patients. In addition, this disclosure provides predictive biomarkers for assessing colorectal cancer progression. Furthermore, this disclosure provides methods for identifying a colorectal cancer tumor responsive to a chemotherapeutic agent. Moreover, this disclosure provides kits for assessing 10 colorectal cancer progression.

A cancer diagnosis, both an initial diagnosis of disease and subsequent monitoring of the disease course (before, during, or after treatment) is conventionally confirmed through histological examination of cell or tissue samples removed from a patient. Clinical pathologists need to be able to accurately determine whether 15 such samples are benign or malignant and to classify the aggressiveness of tumor samples deemed to be malignant, because these determinations often form the basis

for selecting a suitable course of patient treatment. Similarly, the pathologist needs to be able to detect the extent to which a cancer has grown or gone into remission, particularly as a result of or consequent to treatment, most particularly treatment with chemotherapeutic or biological agents.

5 Histological examination traditionally entails tissue-staining procedures that permit morphological features of a sample to be readily observed under a light microscope. A pathologist, after examining the stained sample, typically makes a qualitative determination of whether the tumor sample is malignant. It is difficult, however, to ascertain a tumor's aggressiveness merely through histological 10 examination of the sample, because a tumor's aggressiveness is often a result of the biochemistry of the cells within the tumor, such as protein expression or suppression and protein phosphorylation, which may or may not be reflected by the morphology of the sample. Therefore, it is important to be able to assess the biochemistry and/or molecular biology of the cells within a sample suspected to 15 comprise tumor cells. Particularly it is desired to discriminate malignant neoplastic tissue from non-malignant and/or healthy normal tissue.

The following additional definitions and explanations are provided for specific terms, which are used in the written description of the present disclosure.

As used in the specification and claims, the singular form “a”, “an” and “the” 20 include plural references unless the context clearly dictates otherwise. For example, the term “a molecule” also includes a plurality of molecules (i.e. one or more).

The expression “one or more” denotes a single item or a plurality thereof; in an embodiment, “one or more indicates 1 to 50; an embodiment thereof is 1 to 20, other embodiments thereof are 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, or 15.

25 The term “polynucleotide” denotes a single-stranded DNA or RNA molecule, or its respective complement, wherein in an embodiment the polynucleotide is a polymer of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 50, 75, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000 or more nucleoside monomers. A particular embodiment is a polymer of 15 to 35 nucleoside monomers. Another particular 30 embodiment is a polymer of 35 to 100 nucleoside monomers. Yet, another particular embodiment is a polymer of 50 to 150 nucleoside monomers. Yet, another particular embodiment is a polymer of 150 to 1500 nucleoside monomers. The term “polynucleotide” also encompasses homo- and heteroduplex double-stranded nucleic acid molecules of which each strand comprises 8 or more

nucleoside monomers. Further, heteroduplices are encompassed of which one strand comprises one or more non-natural nucleoside analog(s). In a particular embodiment, the strand with the nucleoside analog(s) comprises 8 or more monomers. An example for a non-natural nucleoside analog is a LNA (locked
5 nucleic acid) monomer. Other non-natural nucleoside analogs are known to the art and encompassed herein. Particular embodiments are non-natural nucleoside analogs capable of increasing the melting point of a heteroduplex with RNA or DNA. Being directed to polynucleotides capable of forming hybrids, or being hybridized or in the process of hybridization, the term "duplex" denotes the double
10 helix formed between a RNA or DNA molecule and its complement.

A "variant" of a polynucleotide differs from the polynucleotide by one or more mutations while preserving substantial sequence similarity as well as specificity in hybridization processes in the procedures disclosed herein. A mutation can be a point mutation wherein at a given position a first nucleobase is substituted by a
15 second nucleobase or an analog thereof. Further mutations are deletions or insertions. In particular embodiments, a polynucleotide and its variant have a sequence identity of a value selected from 95%, 96%, 97%, 98%, 99%, and a value higher than 95% and below 100%.

A "fragment", and particularly a "fragment of a polynucleotide" is understood to be
20 an incomplete or isolated portion of the polynucleotide, comprising at least a single strand with an undisrupted (contiguous) nucleotide sequence of 8 or more positions, according to the entire polynucleotide. In particular embodiments, according to the entire polynucleotide, the fragment thereof comprises an undisrupted nucleotide sequence of 17, 18, 19, 20, 25, 50, 75, 100, 200, 300, 500,
25 700, 1000 or more positions.

A polynucleotide or a nucleotide sequence "originating from" a genetic locus is understood to include a nucleic acid molecule (DNA or RNA) which results from transcription of the genetic locus, i.e. a transcript, or from reverse-transcription of a transcript. Taking a more operational perspective, the term polynucleotide
30 "originating from" a genetic locus encompasses a nucleic acid which is obtainable as a result of a sample preparation process in combination with one or more process(es) of treatment, that is treatment of the processed sample and/or treatment of the sample prior to applying the sample preparation process. Accordingly, the nucleic acid obtainable as a result of the processes is double-stranded or single-

stranded, and its sequence is comprised in or complementary to the sequence of any of the complements of the respective genetic locus.

The term “genetic locus” denotes a genomic region which is transcribed in a tumor cell and/or a non-tumor cell. A transcribed region of a gene can comprise any of non-coding leader, non-coding trailer, intron and exon sequences. However the term “genetic locus” further includes any transcribed region of a pseudogene, any transcribed region with established or putative regulatory function, and any other transcribed region. A transcribed region, generally, is genomic DNA which is a template for a DNA-dependent RNA polymerase at least once or more times in the lifetime of a tumor cell and/or a non-tumor cell.

As used with the teachings reagents, compositions, kits and methods disclosed herein, a nucleic acid probe is understood herein to be a collection of one or more nucleic acid fragments whose hybridization to a sample can be detected. The probe may be unlabeled or labeled so that its binding to the target or sample can be detected. The probe is produced from a source of nucleic acids from one or more particular (preselected) portions of the genome, e.g., one or more clones, an isolated genomic DNA fragment, or a collection of polymerase chain reaction (PCR) amplification products. The nucleic acid probe may also be isolated nucleic acids immobilized on a solid surface (e.g., nitrocellulose, glass, quartz, fused silica slides), as in an array. The probe may be a member of an array of nucleic acids as described, for instance, in WO 96/17958. Techniques capable of producing high density arrays can also be used for this purpose (see, e.g., Fodor, Science X (1991) 767-773; Johnston, Curr. Biol. 8 (1998) R171-R174; Schummer, Biotechniques 23 (1997) 1087-1092; Kern, Biotechniques 23 (1997) 120-124; US 5,143,854). One of skill will recognize that the precise sequence of the particular probes can be modified to a certain degree to produce probes that are substantially identical, but retain the ability to specifically bind to (i.e., hybridize specifically to) the same targets or samples as the probe from which they were derived.

A “biomarker” is a biological molecule which indicates a particular disease state or another physiological state of an organism. Thus, the term biomarker includes a substance whose detection indicates a particular disease state. A biomarker may also indicate a change in gene or protein expression that correlates with the risk or progression of a disease, or with the susceptibility of the disease to a given treatment. Biomarkers are characteristic biological properties that can be detected and measured in parts of the body like the blood or tissue. They may indicate either

normal or diseased processes in the body. Biomarkers can be specific cells, molecules, or genes, gene products, enzymes, or hormones. The term “biomarker” is encompassed in the broader term “marker” which denotes an object to indicate a position or a status or which serves as a standard of comparison or as an indication of what may be expected. A panel of biomarkers is a selection of two or more biomarkers.

A “marker of cancer” and in particular a “marker of colorectal cancer” in the sense of the present disclosure is any marker that, either alone or in combination with one or more further biomarker(s), adds relevant information in the assessment of colorectal cancer. Likewise, a “marker of colorectal adenoma” and a “marker of colorectal polyp” adds relevant information in the assessment of the respective neoplastic potential precursor of colorectal cancer. Any such information is particularly considered relevant or of additive value if at a given specificity the sensitivity, or if at a given sensitivity the specificity, respectively, for the assessment of colorectal cancer, colorectal adenoma or colorectal polyp can be improved by combining the marker with another biomarker or with a diagnostic result, or by including the marker into a panel of biomarkers. In an embodiment of any such assessment, the improvement in sensitivity or specificity, respectively, is statistically significant at a level of significance of $p = .05, .02, .01$ or lower.

The term “sample” as used herein refers to a biological sample obtained for the purpose of evaluation in vitro. In the embodied methods of the present disclosure, the sample or patient sample may be tissue. The sample may also be selected consiting from a group of peripheral blood plasma, serum, stool and tissue. It is understood that any such evaluation is made in vitro, which implies that the sample material is physically separated from the patient’s body in the process of such evaluation. The patient sample is discarded afterwards. The patient sample is solely used for the in vitro diagnostic method as disclosed, and the material of the patient sample is not transferred back into the patient’s body.

The expressions “assessment of” an object or “assess” or “assessing” an object, wherein the object is selected from colorectal cancer, colorectal adenoma, and colorectal polyp, are used to indicate that the disclosed subject-matter will (alone or together with other markers or variables, e.g., the classification criteria set forth by the AJCC or the Dukes stage classification, see below) e.g., aid the physician to establish or confirm the absence or presence of colorectal cancer, colorectal adenoma, and colorectal polyp, or aid the physician in the prognosis, the detection

of recurrence (follow-up of patients after surgery) and/or the monitoring of treatment, especially of chemotherapy. Thus, “assessing” and “assessment” encompass the use of technical items and execution of procedural steps which yield the results on which the physician establishes or confirms the presence or absence of an object selected from colorectal cancer, colorectal adenoma, and colorectal polyp.

A “colorectal polyp” is understood as being a neoplastic clump of cells on the inside (the lining) of the colon or the rectum. A colorectal polyp may give rise to colorectal cancer. Colorectal polyps are conventionally divided into two groups – non-neoplastic polyps and neoplastic polyps. Neoplastic polyps are also known as adenomatous polyps or adenomas. Non-neoplastic polyps include juvenile, hyperplastic, inflammatory, and lymphoid polyps. Non-neoplastic polyps have not been considered precursors of cancer while the neoplastic polyps bear the risk of being precursors of colorectal cancer. The gastroenterologist uses a colonoscopy to find and remove polyps and adenomas to prevent them from acquiring genetic changes that will lead to an invasive adenocarcinoma.

An “adenoma” is a benign tumor of glandular origin which can grow from many organs including the colon and/or the rectum (“colorectal adenoma”). Although these growths are benign, over time they may progress to become malignant, at which point they are referred to as “adenocarcinomas”. Most colorectal adenomas are polypoid. Large flat and depressed colorectal adenomas may be more likely to be severely dysplastic and give rise to malignancies. Colorectal adenomas are found commonly at colonoscopy. They are removed because of their tendency to become malignant and to lead to colorectal cancer. In medical practice it is desired to have alternative means to detect colon adenoma. A particular desire is to have a biomarker indicating the presence of colon adenoma.

Colorectal polyp tissue (= colorectal polyp) and colorectal adenoma are understood as each representing a non-malignant, precursor stage of colorectal cancer. In this regard, a precursor stage poses the risk of developing further into colorectal cancer, i.e. a malignant cancer.

In “dysplastic” colorectal tissue the crypts in the mucosa are usually distorted, and fail to mature in an orderly fashion. The nuclei are enlarged, tending to hyperchromatism and pseudostratification. Mucin is diminished. “Dysplasia” is a understood as being a precursor form of cancer. “Low grade dysplasia” is

characterized by moderate nuclear pleomorphism, hyperchromatism and loss of cell polarity. Where the superficial aspect of the crypt matures the surface epithelium often becomes villiform. High-grade dysplasia is the case when the loss of nuclear polarity is extreme, the nuclei being randomly located in the cell. The 5 nuclei stain deeply and mitoses may be seen throughout the crypt. Crypt branching is often complex, with foci of glands closely crowded 'back to back'.(Ian Talbot, et al., Biopsy Pathology in Colorectal Disease, Second edition (2007); 312-315). In terms of malignancy, high grade dysplastic colorectal adenoma in the sense as presented herein is considered to be different from adenocarcinoma.

10 "Adenocarcinoma" is defined as a malignant tumor that grows on the glandular epithelial cells of an internal organ. "Colorectal cancer" is a cancer of the colon and/or the rectum, a malignant tumor arising from the inner wall of the large intestine. The majority of colorectal cancer is of the adenocarcinoma type.

Colorectal cancer staging is performed for diagnostic and research purposes, and to 15 determine the best method of treatment. The systems for staging colorectal cancers depend on the extent of local invasion, the degree of lymph node involvement and whether there is distant metastasis.

A common staging system is the TNM (for tumors/nodes/metastases) system, from the American Joint Committee on Cancer (AJCC), AJCC Cancer Staging Manual 20 (Sixth ed.). Springer-Verlag New York, Inc. (2002).

AJCC stage	**TNM stage**	**TNM stage criteria for colorectal cancer**
Stage 0	Tis N0 M0	Tis: Tumor confined to mucosa; cancer-in-situ
Stage I	T1 N0 M0	T1: Tumor invades submucosa
Stage I	T2 N0 M0	T2: Tumor invades muscularis propria
Stage II-A	T3 N0 M0	T3: Tumor invades subserosa or beyond (without other organs involved)
Stage II-B	T4 N0 M0	T4: Tumor invades adjacent organs or perforates the visceral peritoneum
Stage III-A	T1-2 N1 M0	N1: Metastasis to 1 to 3 regional lymph nodes. T1 or T2.
Stage III-B	T3-4 N1 M0	N1: Metastasis to 1 to 3 regional lymph nodes. T3 or T4.
Stage III-C	any T, N2 M0	N2: Metastasis to 4 or more regional lymph nodes. Any T.
Stage IV	any T, any N, M1	M1: Distant metastases present. Any T, any N.

An alternative staging system for colorectal cancer is the Dukes classification. It identifies stages as

Dukes stage	stage criteria
A	Tumor confined to the intestinal wall
B	Tumor invading through the intestinal wall
C	With lymph node(s) involvement (this is further subdivided into C1 lymph node involvement where the apical node is not involved and C2 where the apical lymph node is involved)
D	With distant metastasis

It is a goal here to provide further and/or alternative and/or improved means to detect and assess neoplastic tissue of the colon and/or the rectum, including
5 adenoma and adenocarcinoma.

The starting point of the present disclosure was a search for biomarkers that have value in detection and/or treatment of colorectal cancer. Biomarkers for cancer in general and colorectal cancer in particular have several potential uses in the management of patient care. Ideally, they would be used for differential diagnosis,
10 early diagnosis, risk assessment, establishing prognosis, monitoring treatment, and detecting relapse. Additionally, such markers could play a valuable role in planning/designing and conducting therapeutic interventions.

The uses, methods, reagents and kits disclosed herein particularly involve the analysis of sampled tissue. An advantageous sample is tissue obtained as a biopsy.
15 Further, tissue obtained by way of resection is advantageous. Additionally, colonic tissue removed during coloscopy is specifically suitable to practice the teachings of the present disclosure. In this regard it is to be understood that these teachings collectively refer to an assessment ex-vivo, i.e. in vitro.

Clinically, the two criteria that are important for assessing the effectiveness of
20 biomarkers are specificity and sensitivity. “Specificity” of the biomarker is a percentage of correctly analysed healthy subjects of all healthy subjects or the probability for the negative diagnosis of healthy subjects. “Sensitivity” ist the percentage of correctly analysed diseased patients of all diseased patients or the probability for a positive diagnosis of diseased patients. Ideally, biomarkers would
25 have 100% clinical specificity and 100% clinical sensitivity. To date, no single biomarker has been identified that has an acceptably high degree of specificity and

sensitivity required to be effective in for the broad range of needs in patient care management.

It is highly desired to discriminate whether a cell sample contains colorectal cancer or non-malignant cells of a precursor stage such as colorectal adenoma and colorectal polyp. What is disclosed herein is based on discovery studies with mRNA isolated from biopsy samples from patients using an oligonucleotide array-based screening approach. Out of a large number of candidate sequences, a panel of biomarkers has been established to assess changes in human tissue from surgical and biopsy samples, and specifically against control tissue. Clinical samples from patients were used to identify significant genomic alterations as correlates of the emergence and progression of disease. To this end, the Affymetrix® platform of oligonucleotide arrays and analysis means served in the analysis of tumor RNA expression.

From these human subject studies, a plurality of biomarkers is disclosed herein. The biomarkers can be of medical use when used as single markers. However, their technical advantage in the assessment of colorectal cancer, precursor and early stages of colorectal cancer is increased when a panel of these biomarkers is used. Further, what is disclosed are methods for measuring gene expression levels based on individual markers and on a panel of markers.

A further embodiment disclosed herein therefore is a panel of biomarkers for assessing any member of the group consisting of colorectal cancer, colorectal adenoma and healthy colorectal tissue and particularly healthy colonic tissue, the panel comprising at least two polynucleotides, each polynucleotide corresponding to a different genetic locus, wherein at least one, and particulatly at least two of the polynucleotides originate(s) from a genetic locus selected from any of SLC7A5, IL8, CXCL1, MMP3, CA7, CHI3L1, IL1B, CXCL2, IL1RN, GREM1 and COL12A1.

In this regard, Example 9 discloses the surprisingly advantageous use of the marker CXCL2 (also see in Tables 1, 2 and 3 respective entry no. 3 for specific technical details and Figures 7 and 19). Discriminatory power of this marker was even increased by further including SLC7A5 (also see in Tables 1, 2 and 3 respective entry no. 1 for specific technical details and Figures 1 and 12), thereby showing unexpected positive results. Further improvement was achieved by additionally introducing IL8 (also see in Tables 1, 2 and 3 respective entry no. 2 for specific

technical details and Figures 2 and 13), which unpredictedly added to advantageous enhancement of discriminatory power. Thus, a particularly specific embodiment of a panel in line with the disclosures herein comprises CXCL2, specifically a combination of CXCL2 and SLC7A5, alternatively a combination of CXCL2 and IL8, and even more specifically a combination of CXCL2, SLC7A5 and IL8. Thus, the panel in this context comprises one or more polynucleotide(s) originating from a genetic locus selected from any of CXCL2, SLC7A5, and IL8 as biomarker(s) for and/or in the discrimination of colorectal cancer from non-cancerous colorectal tissue. Another embodiment is the panel in this context comprises one or more polynucleotide(s) originating from a genetic locus selected from any of CXCL2, SLC7A5, and IL8 as biomarker(s) for and/or in the discrimination of colorectal cancer from colorectal adenoma.

Additionally, another aspect of what is disclosed are kits which provide the reagents and instructions for measuring gene and protein expression levels based on the panel. The reagents, panels, methods and kits are useful in the management of patient care for colorectal cancer. Additionally, the reagents, panels, methods and kits are believed useful as the basis for discovery of therapeutic interventions for colorectal cancer.

Measuring gene expression means to quantify the level at which a particular gene is expressed within a cell, tissue or organism. While the amount of a final gene product may be indicative for the level of expression of the respective gene, measurement of gene expression in line with the present disclosure is typically made by detecting and quantifying mRNA, and infer gene expression level. Thus, the level of a given mRNA in a given sample is used as a biomarker.

An advantageous and frequently applied approach for measuring mRNA level is reverse transcription quantitative polymerase chain reaction (RT-qPCR), i.e. the combination of reverse transcription (RT) and quantitative PCR (qPCR), i.e. PCR which provides quantitative detection of the respective target nucleic acid. The RT step first generates a DNA template from the mRNA by reverse transcription, which is called cDNA. The cDNA serves as a template in the subsequent step of qPCR. In some qPCR embodiments fluorescence of a detection probe changes as the DNA amplification process progresses. Other qPCR embodiments make use of an intercalating dye which interacts with double-stranded DNA and which is capable of indicating newly formed DNA. In a calibrated system including controls qPCR can produce an absolute measurement such as number of copies of mRNA

present in the sample. The qPCR method is very sensitive as detection of a single mRNA molecule is possible.

Using DNA microarrays, highly parallel analysis can be performed. Arrays of different capture probes can be used to detect and/or quantify transcript levels for many genes at once (expression profiling). Recent advances in microarray technology allow for the quantitation, even on a single array, of transcript levels for every known gene in several organism's genomes, including the human genome. Alternatively, mRNA expression may be quantified by means of applying *in situ* hybridization methods.

For appropriate determination of gene expression levels, a normalization of measured data is required. In the context of the present invention, a normalization of the target gene expression was performed by determing expression values of housekeeping genes for between-sample comparisons.

The "in sample" quantification of these target genes can be performed after parallel measurement of the expression of a reference gene with a stabile amount/cell value from the same specimen. The determination of the expression level of these so-called "housekeeping" genes are the basis for the internal, in sample quantification. In the proposed setting internal housekeeping genes were 18S ribosomal RNA (18s), glycerinaldehyde-3-phosphate dehydrogenase (GAPDH), beta2-microglobulin (B2M), beta-actin (ACTB), hypoxanthine phosphoribosyl transferase 1 (HPRT1), ribosomal protein L13a (RPL13a), tyrosine 3-monooxygenase/tryptophan 5 monooxygenae activation protein, zeta polypeptide YWHAZ. However, any other described housekeeping gene can be used in the qPCR determinations.

Table 1 gives a first overview of the marker genes and genetic loci; nucleotide sequences originating from these loci are used as biomarkers, according to the present disclosure. A transcript of each marker gene or genetic locus alone advantageously serves as an indicator in the assessment of colorectal cancer or a precursor thereof. In another embodiment, a combination of two or more biomarkers as disclosed herein forms the basis for assessing colorectal cancer or a precursor thereof with enhanced specificity and sensitivity, and therefore provides enhanced management of patient care for colorectal cancer.

It is to be understood that fragments and variants of the biomarkers described in Table 1 and in the sequence listings are also useful biomarkers, either alone or in a

panel used for the analysis of colorectal cancer or a precursor thereof. What is meant by fragment is any incomplete or isolated portion of a polynucleotide in the sequence listing. It is recognized that almost daily, new discoveries are announced for gene variants, particularly for those genes under intense study, such as genes 5 implicated in diseases like cancer. Therefore, the sequence listings given are exemplary of what is now reported for a gene, but it is recognized that for the purpose of an analytical methodology, variants of the gene, and fragments of the genetic loci are also included. In a particular embodiment, the disclosure incorporates any nucleotide sequence which is in the range of 80% to 100% 10 identical to a nucleotide sequence disclosed herein or the complement thereof. In another embodiment, the identity is in the range of 90% to 100%, in yet another embodiment the identity is in the range of 95% and 100%.

Table 1

#	Marker gene designation	ENSEMBL Gene ID http://www.ensembl.org/Homo_sapiens/Info/Index	Genomic location (strand) of marker gene
1	SLC7A5	ENSG00000103257	16:87863622-87903125
2	IL8	ENSG00000169429	4:74606223-74609433
3	CXCL1	ENSG00000163739	4:74735110-74736959
4	MMP3	ENSG00000149968	11:102706532-102714534
5	CA7	ENSG00000168748	16:66878282-66888056
6	CHI3L1	ENSG00000133048	1:203148059-203155877
7	IL1B	ENSG00000125538	2:113587328-113594480
8	CXCL2	ENSG00000081041	4:74962793-74965010
9	IL1RN	ENSG00000136689	2:113864791-113891593
10	GREM1	ENSG00000166923	15:33010175-33026870
11	COL12A1	ENSG00000111799	6:75794042-75915767

Genomic locations are indicated by "Chromosome No.:start position-end position 15 as defined by Ensembl release 63 - June 2011 © Wellcome Trust Sanger Institute (WTSI) / EMBL-European Bioinformatics Institute (EBI); http://www.ensembl.org

The following presents some details concerning the marker genes found in the present study of biomarkers.

CA7 – [Synonyms: CAVII]

Carbonic anhydrases are a large family of zinc metalloenzymes that catalyze the reversible hydration of carbon dioxide. They participate in a variety of biological processes, including respiration, calcification, acid-base balance, bone resorption, and the formation of aqueous humor, cerebrospinal fluid, saliva, and gastric acid. They show extensive diversity in tissue distribution and in their subcellular localization. The cytosolic protein encoded by this gene is predominantly expressed in the salivary glands. Alternative splicing in the coding region results in multiple transcript variants encoding different isoforms.

IL1B – [Synonyms: IL-1, IL1-BETA, IL1F2]

The protein encoded by this gene is a member of the interleukin 1 cytokine family. This cytokine is produced by activated macrophages as a proprotein, which is proteolytically processed to its active form by caspase 1 (CASP1/ICE). This cytokine is an important mediator of the inflammatory response, and is involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis. The induction of cyclooxygenase-2 (PTGS2/COX2) by this cytokine in the central nervous system (CNS) is found to contribute to inflammatory pain hypersensitivity. This gene and eight other interleukin 1 family genes form a cytokine gene cluster on chromosome 2.

Elevated expression of IL1B increases risk of non-small cell lung cancer. Although it is known that IL1B polymorphisms are associated with tumor recurrence in stage II colon cancers, the function of this gene has not been clarified in CRC.

IL1RN – [Synonyms: DIRA, ICIL-1RA, IL-1RN, IL-1ra, IL-1ra3, IL1F3, IL1RA, IRAP, MVCD4]

The protein encoded by this gene is a member of the interleukin 1 cytokine family. This protein inhibits the activities of interleukin 1, alpha (IL1A) and interleukin 1, beta (IL1B), and modulates a variety of interleukin 1 related immune and inflammatory responses. This gene and five other closely related cytokine genes form a gene cluster spanning approximately 400 kb on chromosome 2. A polymorphism of this gene is reported to be associated with increased risk of osteoporotic fractures and gastric cancer. Four alternatively spliced transcript variants encoding distinct isoforms have been reported.

Interleukin 8 – [Synonyms: CXCL8, GCP-1, GCP1, LECT, LUCT, LYNAP, MDNCF, MONAP, NAF, NAP-1, NAP1

The protein encoded by this gene is a member of the CXC chemokine family. This chemokine is one of the major mediators of the inflammatory response. This
5 chemokine is secreted by several cell types. It functions as a chemoattractant, and is also a potent angiogenic factor. This gene is believed to play a role in the pathogenesis of bronchiolitis, a common respiratory tract disease caused by viral infection. This gene and other ten members of the CXC chemokine gene family form a chemokine gene cluster in a region mapped to chromosome 4q.

10 IL8 promotes cell proliferation and migration of human colon carcinoma through metalloproteinase-cleavage proHB-EGF. Overexpression of IL-8 is associated with tumor growth, metastasis, chemoresistance and angiogenesis.

GREM1 – [Synonyms: PIG2, CKTSF1B1, DAND2, DRM, GREMLIN, GREMLIN 1, IHG-2]

15 This gene encodes a member of the BMP (bone morphogenic protein) antagonist family. Like BMPs, BMP antagonists contain cystine knots and typically form homo- and heterodimers. The CAN (cerberus and dan) subfamily of BMP antagonists, to which this gene belongs, is characterized by a C-terminal cystine knot with an eight-membered ring. The antagonistic effect of the secreted
20 glycosylated protein encoded by this gene is likely due to its direct binding to BMP proteins. As an antagonist of BMP, this gene may play a role in regulating organogenesis, body patterning, and tissue differentiation. In mouse, this protein has been shown to relay the sonic hedgehog (SHH) signal from the polarizing region to the apical ectodermal ridge during limb bud outgrowth. Alternatively
25 spliced transcript variants encoding different isoforms have been found for this gene.

Gremlin 1 as an antagonist of bone morphogenic proteins has been shown to regulate early development, tumorgenesis. It was overexpressed in various human tumors and plays an oncogenic role especially in carcinomas including CRC.

CXCL1 – [Synonyms: FSP, GRO1, GROa, GRO-alpha, MGSA, MGSA-a, NAP-3, SCYB1]

Chemokines are a group of small (approximately 8 to 14 kD), mostly basic, structurally related molecules that regulate cell trafficking of various types of leukocytes through interactions with a subset of 7-transmembrane, G protein-coupled receptors. Chemokines also play fundamental roles in the development, homeostasis, and function of the immune system, and they have effects on cells of the central nervous system as well as on endothelial cells involved in angiogenesis or angiostasis. Chemokines are divided into 2 major subfamilies, CXC and CC, based on the arrangement of the first 2 of the 4 conserved cysteine residues; the 2 cysteines are separated by a single amino acid in CXC chemokines and are adjacent in CC chemokines. CXC chemokines are further subdivided into ELR and non-ELR types based on the presence or absence of a glu-leu-arg sequence adjacent and N terminal to the CXC motif. ELR types are chemotactic for neutrophils, while non-ELR types are chemotactic for lymphocytes.

Secretion of this interleukin-like GRO-alpha oncogene promote tumor initiation and growth. Elevated expression of GRO-alpha is frequent in colorectal carcinoma, whereas inhibition of GROalpha expression is associated with apoptosis. Colon carcinoma cells expressing higher levels of CXCL1, which can be associated with metastatic potential and modulate colon cancer cell proliferation resulting invasive phenotype and an inverse association can be found between CXCL1 overexpression and recurrence-free survival in stage III CRC patients.

CXCL2 – [Synonyms: CINC-2a, GRO2, GROb, MGSA-b, MIP-2a, MIP2, MIP2A, SCYB2]

Chemokine showed in previous studies a highly significant upregulation in CRC compared to normal colonic mucosa which could be already detected also in benign adenoma referring to involvement of CXCL2 in early stage of colorectal carcinogenesis.

COL13A1 – [Synonyms: RP1-238D15.1, BA209D8.1, COL12A1L, DJ234P15.1]

This gene encodes the alpha chain of type XII collagen, a member of the FACIT (fibril-associated collagens with interrupted triple helices) collagen family. Type XII collagen is a homotrimer found in association with type I collagen, an

association that is thought to modify the interactions between collagen I fibrils and the surrounding matrix. Alternatively spliced transcript variants encoding different isoforms have been identified.

CHI3L1 – [Synonyms: Chitinase 3 like-1, ASRT7, CGP-39, GP-39, GP39, HC-gp39, HCGP-3P, YKL-40, YKL40, YYL-40, hCGP-39]

Chitinases catalyze the hydrolysis of chitin, which is an abundant glycopolymer found in insect exoskeletons and fungal cell walls. The glycoside hydrolase 18 family of chitinases includes eight human family members. This gene encodes a glycoprotein member of the glycosyl hydrolase 18 family. The protein lacks chitinase activity and is secreted by activated macrophages, chondrocytes, neutrophils and synovial cells. The protein is thought to play a role in the process of inflammation and tissue remodeling. Chitinase 3 like-1 can protect cancer or/and stromal cells against apoptosis.

The mRNA expression level of CHI3L1 is upregulated in colorectal cancer tissue and elevated protein level can be found in the plasma of CRC patients. Chitinase 3 like-1 can have a role in macrophage recruitment and angiogenesis, furthermore it can protect cancer or/and stromal cells against apoptosis. CHI3L1may contribute to the proliferation, migration, and neoplastic progression of colon epithelial cells under inflammatory conditions in patients with IBD.

SLC7A5 – [Synonyms: 4F2LC, CD98, D16S469E, E16, LAT1, MPE16, hLAT1]

Expression of this cationic amino acid transporter was also found to be significantly associated with cell proliferation and angiogenesis, moreover it seems to play an important role in enhancing the tumor growth in vivo.

MMP3 – [Synonyms: Matrix-metalloproteinase 3, CHDS6, MMP-3, SL-1, STMY, STMY1, STR1]

Proteins of the matrix metalloproteinase (MMP) family are involved in the breakdown of extracellular matrix in normal physiological processes, such as embryonic development, reproduction, and tissue remodeling, as well as in disease processes, such as arthritis and metastasis. Most MMP's are secreted as inactive proproteins which are activated when cleaved by extracellular proteinases. This gene encodes an enzyme which degrades fibronectin, laminin, collagens III, IV, IX,

and X, and cartilage proteoglycans. The enzyme is thought to be involved in wound repair, progression of atherosclerosis, and tumor initiation. The gene is part of a cluster of MMP genes which localize to chromosome 11q22.3. Matrix-metalloproteinase 3 secretion promotes tumor initiation and growth.

5 Surprisingly, it has been found that sequences originating from a genetic locus according to the present disclosure and as given by the entries ## 1-11 of Table 1 are helpful to assess colorectal cancer or a precursor thereof. An embodiment of what is disclosed is therefore a biomarker for the assessment of any member of the group consisting of colorectal cancer and colorectal adenoma, the assessment 10 including detection of any of these members, wherein the biomarker origins from a gene or a genetic locus selected from the group consisting of SLC7A5, IL8, CXCL1, MMP3, CA7, CHI3L1, IL1B, CXCL2, IL1RN and GREM1. Thus, an embodiment is the use wherein colorectal cancer is discriminated from healthy tissue, the biomarker is selected from any of SLC7A5, IL8, CXCL1, MMP3, CA7, 15 CHI3L1, IL1B, CXCL2, IL1RN, GREM1, and COL12A1, and compared to the control sample of healthy colorectal tissue the level of the polynucleotide is increased or decreased in the sample obtained from the patient.

Quantitative analysis of the respective markers revealed that in the case of colorectal cancer (i.e. when colorectal canceris present in the patient’s sample) the 20 level of a marker is either higher or lower than in healthy tissue. Table 2 indicates the deviation for each maker locus.

Table 2

#	**Marker gene designation**	**Deviation of marker level in a sample of colorectal cancer, relative to healthy tissue**
1	SLC7A5	Up-regulated in CRC (increased)
2	IL8	Up-regulated in CRC (increased)
3	CXCL1	Up-regulated in CRC (increased)
4	MMP3	Up-regulated in CRC (increased)
5	CA7	Down-regulated in CRC (decreased)
6	CHI3L1	Up-regulated in CRC (increased)
7	IL1B	Up-regulated in CRC (increased)
8	CXCL2	Up-regulated in CRC (increased)
9	IL1RN	Up-regulated in CRC (increased)
10	GREM1	Up-regulated in CRC (increased)
11	COL12A1	Up-regulated in CRC (increased)

A particular embodiment is the use of any of the above markers in the assessment of an early stage of colorectal cancer in the sample, particularly a stage selected from Tis N0 M0, T1-4 N0 M0, Dukes A, and Dukes B. A further embodiment is a
5 method for measuring the expression levels of a polynucleotide from a biomarker, the biomarker being capable of discriminating colorectal cancer from non-cancerous colorectal tissue in a human individual, the method comprising: selecting a biomarker comprising a contiguous polynucleotide originating from a genetic locus selected from any of SLC7A5, IL8, CXCL1, MMP3, CA7, CHI3L1, IL1B,
10 CXCL2, IL1RN, and GREM1, and COL12A1, a fragment thereof or its complement, and a variant thereof or its complement; obtaining (removing) from the human individual a biopsy sample suspected of being colorectal cancer; in vitro (ex vivo) isolating cellular RNA from the sample and reverse-transcribing isolated RNA to obtain cDNA; amplifying copies of cDNA for the biomarker originating
15 from the sample; and quantifying the level of cDNA amplified from the sample, thereby measuring the expression level of the polynucleotide from a biomarker.

Another embodiment of what is disclosed is a panel of biomarkers with the specificity and sensitivity suitable for managing patient care for colorectal cancer, and particularly for discriminating colorectal cancer against non-malignant
20 neoplastic tissue such as colorectal adenoma and healthy (normal) colorectal tissue. In Tables 1 and 2, entries ## 1-11 refer to biomarkers which are believed to be of advantage. Encompassed are the transcripts and the polynucleotide sequences

therof, of the respective genes and genetic loci. The surprising advantage in the use of any of these biomarkers is obtained when a marker listed in Table 1 and Table 2 is used alone or in a panel of biomarkers.

A particular embodiment is a panel of biomarkers comprising two or more of the aforementioned polynucleotides, that is to say a panel of biomarkers for assessing any member of the group consisting of colorectal cancer and colorectal adenoma, the panel comprising at least two polynucleotides, each polynucleotide corresponding to a different genetic locus, wherein at least one, and particulatly at least two of the polynucleotides originate(s) from a genetic locus selected from any of SLC7A5, IL8, CXCL1, MMP3, CA7, CHI3L1, IL1B, CXCL2, IL1RN, GREM1, and COL12A1.

In a specific embodiment, for measuring expression levels as disclosed herein, two or more biomarkers are selected from the group consisting of SLC7A5, IL8, CXCL1, MMP3, CA7, CHI3L1, IL1B, CXCL2, IL1RN, and GREM1, and COL12A1. Accordingly, a number selected from 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11 biomarkers is used in the measurements, wherein each biomarker comprises a contiguous polynucleotide originating from therespective genetic locus, a fragment thereof or its complement, or a variant thereof or its complement.

In another embodiment of this disclosure, expression levels of polynucleotides for the biomarkers indicated in SEQ ID NOs: of SEQ ID NOs: 1, 6, 11, 16, 21, 26, 31, 36, 41, 46, and 51, and particularly SEQ ID NOs:4, 9, 14, 19, 24, 29, 34, 39, 44, 49, and 54, are used in the assessment of colorectal cancer or colorectal adenoma. An advantageous analysis of polynucleotide expression levels is frequently referred to in the art as gene expression profiling. In gene expression profiling, mRNA of a sample is first reverse-transcribed in a quantitative manner into a complementary DNA (cDNA). That is to say, the relative amount of cDNA derived from a given RNA species and resulting from the process of reverse transcription corresponds to the relative amount of the RNA species relative to all RNAs which are reverse-transcribed in the process. In a subsequent step, the cDNA serves as template for qPCR using specific oligonucleotides as primers and a quantitative detection method for the DNA which is newly generated after each amplification round. The deduced level of original target mRNA in the sample, also in comparison to other samples, is a leading indicator of a biological state; as disclosed herein, the level of target mRNA is used as an indicator of colorectal cancer or a precursor thereof in comparison to healthy individuals or individuals with none of the above neoplastic

growths. Pairs of primers advantageously used in qPCR, as a subsequent step after the reverse transcription process and for sequences originating from each gene locus are listed in entries 1-11 of Tables 1 and 2.

Since the combination of RT and qPCR amplifies copies of cDNA proportional to the original level of the corresponding mRNA in a sample, it has become a standard method that allows the analysis of even low levels of mRNA present in a biological sample. Compared to a reference sample target sequences (polynucleotides) originating from certain genetic loci may either be up-regulated or down-regulated in any particular biological state, and hence mRNA levels shift accordingly.

Methods and kits for determination of any of the polynucleotides shown, and expression profiling for a panel of molecular markers are part of the present disclosure. As used herein, the term "determination" encompasses qualitative demonstration, e.g. as "presence" or "absence", and quantitative determination, i.e. measuring the absolute or relative amount of a target analyte, e.g. a polynucleotide.

In one embodiment, a method for gene expression profiling comprises measuring the level of one or more a polynucleotide(s) originating from a genetic locus selected from any of SLC7A5, IL8, CXCL1, MMP3, CA7, CHI3L1, IL1B, CXCL2, IL1RN, GREM1 and COL12A1. Accordingly, methods are included which facilitate the determination of a level of mRNA directly, or of a level of cDNA, for one or more biomarker(s) disclosed herein. Such a method requires the use of primers, enzymes, and other reagents for the preparation, detection, and quantitation of mRNAs/cDNAs. The method of creating cDNA from mRNA in a sample is referred to as reverse transcription (RT). The primer pairs of SEQ. ID NOs: 2 and 3, 7 and 8, 12 and 13, 17 and 18, 22 and 23, 27 and 28, 32 and 33, 37 and 38, 42and 43, 47 and 48, and 52 and 53 are particularly suited for use in gene expression profiling using RT-qPCR based on the claimed panel. However, use of a primer pair for the assessment of a single biomarker is also an intended use. In addition to the primers, reagents such as one including a desoxynucleoside triphosphate mixture having all four desoxynucleoside triphosphates (e.g. dATP, dGTP, dCTP, and dTTP), one having the reverse transcriptase enzyme, and one having a thermostable DNA polymerase are required for RT-qPCR. Additionally buffers, inhibitors and activators are also required for the RT-qPCR process. Once the cDNA has been sufficiently amplified to a specified end point, the cDNA sample must be prepared for detection and quantitation. Though a number of

detection schemes are contemplated, as will be discussed in more detail below, one method contemplated for detection of polynucleotides is fluorescence spectroscopy, and therefore chromophores that are suited for fluorescence spectroscopy are desirable for labeling polynucleotides. One example of such a fluorescent label is the intercalating dye SYBR Green, though numerous related chromophores capable of forming complexes with newly formed nucleic acids by way of intercalating exist, and are known in the art.

In another embodiment, the hydrolysis probe principle is used. It is also known as the TaqMan® format. It relies on the 5′–3′ exonuclease activity of Taq polymerase to cleave a dual-labeled probe (hydrolysis probe) during hybridization to the complementary target sequence and fluorophore-based detection. As in other real time PCR methods, the resulting fluorescence signal permits quantitative measurements of the accumulation of the product during the exponential stages of the PCR; however, the hydrolysis (TaqMan®) probe significantly increases the specificity of the detection. A hydrolysis probes consists of a fluorophore covalently attached to the 5'-end of the oligonucleotide probe and a quencher at the 3'-end. Several different combinations of fluorophores and quenchers are known to the art and commercially available. In an intact hydrolysis probe the quencher molecule absorbs the fluorescence emitted by the fluorophore. As long as the fluorophore and the quencher remain in proximity, the quencher inhibit the fluorescence signal and therefore prevents its detection.

Hydrolysis probes are designed such that they anneal within a DNA region amplified by a specific set of primers. Particularly suited hydrolysis probes to practice the teachings found herein are selected from any of SEQ ID NOs: 5, 10, 15, 20, 25, 30, 35, 40, 4, 50, and 55. As the Taq polymerase extends the primer and synthesizes the nascent strand, the 5' to 3' exonuclease activity of the polymerase degrades the probe that has annealed to the template. Degradation of the probe releases the fluorophore from it and breaks the close proximity to the quencher, thus relieving the quenching effect and allowing fluorescence of the fluorophore to be detected. Hence, the amount of fluorescence detected by way of spectroscopy in a real-time PCR thermal cycler is dependent on the fluorophore released, hence the amount of DNA template present in the PCR.

Examples of detection modes contemplated for the disclosed methods include, but are not limited to spectroscopic techniques, such as fluorescence and UV-Vis spectroscopy, scintillation counting, and mass spectroscopy. Complementary to

these modes of detection, examples of labels for the purpose of detection and quantitation used in these methods include, but are not limited to chromophoric labels, scintillation labels, and mass labels.

The expression levels of polynucleotides and polypeptides measured using these methods may be normalized to a control established for the purpose of the targeted determination. These methods are believed useful in providing determinations as the basis of effective management of patient care for colorectal cancer. These methods may also be used in the discovery of therapeutic interventions for colorectal cancer. Additionally, not only biopsy samples from sigmoidoscopy, colonoscopy, or surgery may be analyzed by these methods, but biological samples from non-invasive or minimally evasive collection methods are indicated for these methods, as well.

It is further contemplated in what is disclosed to provide kits having the reagents and procedures that facilitate the ready implementation of the methods, and provide consistency and quality control thereby.

In another embodiment, a kit for gene expression analysis in the assessment of colorectal cancer or a precursor thereof, wherein the kit comprises the reagents and instructions necessary for the detection and quantitative analysis of a polynucleotide originating from any one of the genetic loci selected from the group consisting of SLC7A5, IL8, CXCL1, MMP3, CA7, CHI3L1, IL1B, CXCL2, IL1RN, GREM1 and COL12A1. Yet another embodiment is a kit for gene expression profiling of a panel comprising SLC7A5, IL8, CXCL1, MMP3, CA7, CHI3L1, IL1B, CXCL2, IL1RN, GREM1 and COL12A1. Thus, for example, the reagents may include primers, enzymes, and other reagents for the preparation, detection, and quantitation of cDNAs for the claimed panel of biomarkers, or individual biomarkers. As discussed above, the method of creating cDNA from mRNA in a sample is referred to as the combination of reverse transcription and quantitative polymerase chain reaction (RT-qPCR). The primer pairs of SEQ. ID NOs: 2 and 3, 7 and 8, 12 and 13, 17 and 18, 22 and 23, 27 and 28, 32 and 33, 37 and 38, 42and 43, 47 and 48, and 52 and 53 are particularly suited for use in gene expression profiling using RT-qPCR based on the claimed panel. These primer pairs were specifically designed, selected, and tested accordingly. In addition to the primers, reagents such as one including a desoxynucleoside triphosphate mixture having all four desoxynucleoside triphosphates (e.g. dATP, dGTP, dCTP, and dTTP), one having the reverse transcriptase enzyme, and one having a thermostable

DNA polymerase are required for RT-qPCR. Additionally buffers, inhibitors and activators used for the RT-qPCR process are suitable reagents for inclusion in the kit embodiment. In another embodiment, the reverse transcriptase and the thermostable DNA polymerase are provided together in a mixture, to enable one-
5 step RT-qPCR. In a variation of the embodiment, the thermostable DNA polymerase aditionally comprises as a further enzymatic activity that of a reverse transcriptase.

One method contemplated for detection of polynucleotides is fluorescence spectroscopy, and therefore fluorophores that are suited for fluorescence
10 spectroscopy are desirable for labeling polynucleotides and may also be included in reagents of the kit embodiment. Alternatively, labeled hydrolysis probes which can be used in the TaqMan® detection format may be included in the kits. Such hydrolysis probes may comprise LNA nucleosides to facilitate provision of shorter hydrolysis probes.

15 Further detection formats are known to the art and can be applied to practice the quantitation of biomarkers as disclosed herein.

Instructions included with the kit embodiment for gene expression profiling teach the user the following steps: to obtain a biological sample; to isolate cellular RNA from the sample; to synthesize cDNA from the isolated RNA; to amplify copies of
20 cDNA from the sample for the biomarker(s), and the panel for which the reagents are provided; and to quantify levels of cDNA amplified from the sample. Various samples including whole blood from a variety of procedures may be used. The instructions for obtaining a biological sample typically instruct the user to obtain a sample of blood (or another sample material as indicated elsewhere herein),
25 optionally to stabilize the sample material, and to subject the sample to a nucleic acid preparation procedure. The instructions may also include the step of comparing the cDNA levels quantified to a control.

Additionally, consumable labware required for sample collection, preparation, and analysis may be provided with the kits.

30 The Examples that follow are illustrative of specific embodiments of the disclosure, and various uses thereof. They set forth for explanatory purposes only, and are not to be taken as limiting the disclosure.

The following items further provide aspects of the disclosure, and specific embodiments to practice the teachings provided herein.

1. Use of a (one or more) polynucleotide(s) originating from a genetic locus selected from the group consisting of SLC7A5, IL8, CXCL1, MMP3, CA7, IL1B, CHI3L1, CXCL2, IL1RN, GREM1 and COL12A1 as a biomarker for and/or in the discrimination of colorectal cancer from non-cancerous colorectal tissue.

2. The use according to item 1, wherein two or more polynucleotides originating from two or more genetic loci are used as a biomarker panel for and/or in the discrimination of colorectal cancer from non-cancerous colorectal tissue, wherein the two or more genetic loci are selected from the group consisting of SLC7A5, IL8, CXCL1, MMP3, CA7, IL1B, CHI3L1, CXCL2, IL1RN, GREM1 and COL12A1.

3. The use according to item 1, wherein three or more polynucleotides originating from three or more genetic loci are used as a biomarker panel for and/or in the discrimination of colorectal cancer from non-cancerous colorectal tissue, wherein the three or more genetic loci are selected from the group consisting of SLC7A5, IL8, CXCL1, MMP3, CA7, IL1B, CHI3L1, CXCL2, IL1RN, GREM1 and COL12A1.

4. The use according to any of the items 2 and 3, wherein the polynucleotides are selected from different loci.

5. The use according to item 2, wherein a polynucleotide originating from SLC7A5 and a polynucleotide originating from CXCL2 are used as biomarker panel for and/or in the discrimination of colorectal cancer from non-cancerous colorectal tissue.

6. The use according to item 2, wherein a polynucleotide originating from SLC7A5, a polynucleotide originating from CXCL2, and a polynucleotide originating from IL8 are used as biomarker panel for and/or in the discrimination of colorectal cancer from non-cancerous colorectal tissue.

7. The use according to any of the items 5 or 6, wherein one or more further polynucleotide(s) is/are used in the biomarker panel for and/or in the discrimination of colorectal cancer from non-cancerous colorectal tissue.

8. The use according to any of the items 1 to 7, wherein the discrimination of colorectal cancer from non-cancerous colorectal tissue comprises assaying in vitro (ex-vivo) a tissue sample obtained from a patient (a human individual), particularly a biopsy sample, particularly a biopsy sample comprising colorectal tissue suspected of being colorectal cancer.

9. The use according to item 8, wherein the discrimination of colorectal cancer from non-cancerous colorectal tissue comprises assaying a polynucleotide selected from RNA, cDNA, and a complement thereof, the polynucleotide being isolated or prepared from the tissue sample or from the biopsy sample.

10. The use according to any of the items 8 and 9, wherein the discrimination of colorectal cancer from non-cancerous colorectal tissue includes quantification of a polynucleotide.

11. The use according to any of the items 8 to 10, wherein the discrimination of colorectal cancer from non-cancerous colorectal tissue includes comparing the presence and/or the level and/or the quantity of a polynucleotide in the patient's tissue sample with the presence and/or the level and/or the quantity of the polynucleotide in a control.

12. The use according to item 11, wherein the control is derived from non-cancerous colorectal tissue from the same patient, particularly the control is derived from healthy colorectal tissue.

13. The use according to any of the items 1 to 12, wherein the colorectal cancer is discriminated from a non-cancerous colorectal tissue selected from the group consisting of colorectal adenoma, colorectal polyp, and healthy (non-neoplastic) colorectal tissue.

14. The use according to any of the items 1 to 13, wherein colorectal cancer at a neoplastic stage selected from any of Tis N0 M0, T1-4 N0 M0, Dukes A, and Dukes B is discriminated from non-cancerous colorectal tissue.

15. The use according to any of the items 1 to 14, wherein colorectal cancer at a neoplastic stage more advanced than any of T4 N0 M0, and Dukes B is discriminated from non-cancerous colorectal tissue.

16. The use according to any of the items 1 to 15, wherein the discrimination comprises detection of, or establishment of, or confirmation of a neoplastic stage of colorectal cancer.

17. The use according to any of the items 11 to 16, wherein colorectal cancer is discriminated from non-cancerous colorectal tissue, the (one or more) biomarker is a polynucleotide originating from a genetic locus selected from the group consisting of SLC7A5, IL8, CXCL1, MMP3, IL1B, CHI3L1, CXCL2, IL1RN, GREM1 and COL12A1, and compared to the control sample of non-cancerous colorectal tissue the level of the respective polynucleotide originating from the genetic locus is increased in colorectal cancer.

18. The use according to any of the items 11 to 17, wherein colorectal cancer is discriminated from non-cancerous colorectal tissue, the biomarker is is a polynucleotide originating from the genetic locus CA7, and compared to the control sample of non-cancerous colorectal tissue the level of the polynucleotide originating from the genetic locus CA7 is decreased in the sample obtained from the patient, with the proviso that the control sample excludes colorectal adenoma.

19. The use according to any of the items 17 or 18, wherein the control sample is selected from the group consisting of colon tissue obtained from a healthy control individual, and healthy (non-neoplastic) colon tissue obtained from the same patient.

20. Use of a (one or more) polynucleotide(s) originating from a genetic locus selected from the group consisting of SLC7A5, IL8, CXCL1, MMP3, CA7, CHI3L1, IL1B, CXCL2, IL1RN and GREM1, and COL12A1, as a biomarker for and/or in the discrimination of colorectal cancer from colorectal adenoma, particularly high grade dysplastic colorectal adenoma.

21. The use according to item 20, wherein two or more polynucleotides originating from two or more genetic loci are used as a biomarker panel for and/or in the discrimination of colorectal cancer from colorectal adenoma, particularly dysplastic colorectal adenoma, particularly high grade dysplastic colorectal adenoma, wherein the two or more genetic loci are selected from the group consisting of SLC7A5, IL8, CXCL1, MMP3, CA7, IL1B, CHI3L1, CXCL2, IL1RN, GREM1 and COL12A1.

22. The use according to item 20, wherein three or more polynucleotides originating from three or more genetic loci are used as a biomarker panel for and/or in the discrimination of colorectal cancer from colorectal adenoma, particularly dysplastic colorectal adenoma, particularly high grade dysplastic colorectal adenoma, wherein the three or more genetic loci are selected from the group consisting of SLC7A5, IL8, CXCL1, MMP3, CA7, IL1B, CHI3L1, CXCL2, IL1RN, GREM1 and COL12A1.

23. The use according to any of the items 21 and 22, wherein the polynucleotides are selected from different loci.

24. The use according to any of the items 20 to 23, wherein the discrimination of colorectal cancer from colorectal adenoma comprises assaying in vitro (ex-vivo) a tissue sample obtained from a patient (a human individual), particularly a biopsy sample, particularly a biopsy sample comprising colorectal tissue suspected of being colorectal adenoma or colorectal cancer.

25. The use according to any of the items 20 and 24, wherein discrimination of colorectal cancer from colorectal adenoma comprises assaying a polynucleotide selected from RNA, cDNA, and a complement thereof, the polynucleotide being isolated or prepared from the tissue sample or from the biopsy sample.

26. The use according to any of the items 20 to 25, wherein the discrimination of colorectal cancer from colorectal adenoma includes quantification of a polynucleotide.

27. The use according to any of the items 24 to 26, wherein the discrimination of colorectal cancer from colorectal adenoma includes comparing the presence and/or the level and/or the quantity of a polynucleotide in the patient's tissue sample with the presence and/or the level and/or the quantity of the polynucleotide in a control.

28. The use according to item 27, wherein the control is derived from non-cancerous colorectal tissue from the same patient, particularly the control is derived from healthy colorectal tissue.

29. The use according to any of the items 20 to 28, wherein colorectal cancer at a neoplastic stage selected from any of Tis N0 M0, T1-4 N0 M0, Dukes A, and Dukes B is discriminated from colorectal adenoma.

30. The use according to any of the items 20 to 28, wherein colorectal cancer at a neoplastic stage more advanced than any of T4 N0 M0, and Dukes B is discriminated from colorectal adenoma.

31. The use according to any of the items 20 to 30, wherein the discrimination of colorectal cancer from colorectal adenoma comprises detection of, or establishment of, or confirmation of a neoplastic stage of colorectal cancer.

32. The use according to any of the items 20 to 31, wherein colorectal cancer is discriminated from colorectal adenoma, the (one or more) biomarker is a polynucleotide originating from a genetic locus selected from the group consisting of SLC7A5, IL8, CXCL1, MMP3, CA7, CHI3L1, IL1B, CXCL2, IL1RN and GREM1, and compared to the control sample of non-cancerous colorectal tissue the level of the respective polynucleotide originating from the genetic locus is increased in the sample obtained from the patient.

33. The use according to any of the the items 1 to 32, wherein the biomarker is combined with one or more further biomarker(s).

34. The use according to item 33, wherein the one or more further biomarker(s) is/are one or more polynucleotide(s) originating from a genetic locus selected from any of SLC7A5, IL8, CXCL1, MMP3, CA7, CHI3L1, IL1B, CXCL2, IL1RN and GREM1, and furthermore COL12A1.

35. The use according to any of the items 1 to 34, wherein the polynucleotide originating from the genetic locus SLC7A5 is selected from SEQ ID NO:1, the complement of SEQ ID NO:1, a fragment thereof or its complement, and a variant thereof or its complement, and particularly any of SEQ ID NOs:2, 3, 4, and 5.

36. The use according to any of the items 1 to 34, wherein the polynucleotide originating from the genetic locus IL8 is selected from SEQ ID NO:6, the complement of SEQ ID NO:6, a fragment thereof or its complement, and a variant thereof or its complement, and particularly any of SEQ ID NOs:7, 8, 9, and 10.

37. The use according to any of the items 1 to 34, wherein the polynucleotide originating from the genetic locus CXCL1 is selected from SEQ ID NO:11, the complement of SEQ ID NO:11, a fragment thereof or its complement, and a variant thereof or its complement, and particularly any of SEQ ID NOs:12, 13, 14, and 15.

38. The use according to any of the items 1 to 34, wherein the polynucleotide originating from the genetic locus MMP3 is selected from SEQ ID NO:16, the complement of SEQ ID NO:16, a fragment thereof or its complement, and a variant thereof or its complement, and particularly any of SEQ ID NOs:17, 18, 19, and 20.

39. The use according to any of the items 1 to 34, wherein the polynucleotide originating from the genetic locus CA7 is selected from SEQ ID NO:21, the complement of SEQ ID NO:21, a fragment thereof or its complement, and a variant thereof or its complement, and particularly any of SEQ ID NOs:22, 23, 24, and 25.

40. The use according to any of the items 1 to 34, wherein the polynucleotide originating from the genetic locus CHI3L1 is selected from SEQ ID NO:26, the complement of SEQ ID NO:26, a fragment thereof or its complement, and a variant thereof or its complement, and particularly any of SEQ ID NOs:27, 28, 29, and 30.

41. The use according to any of the items 1 to 34, wherein the polynucleotide originating from the genetic locus IL1B is selected from SEQ ID NO:31, the complement of SEQ ID NO:31, a fragment thereof or its complement, and a variant thereof or its complement, and particularly any of SEQ ID NOs:32, 33, 34, and 35.

42. The use according to any of the items 1 to 34, wherein the polynucleotide originating from the genetic locus CXCL2 is selected from SEQ ID NO:36, the complement of SEQ ID NO:36, a fragment thereof or its complement, and a variant thereof or its complement, and particularly any of SEQ ID NOs:37, 38, 39, and 40.

43. The use according to any of the items 1 to 34, wherein the polynucleotide originating from the genetic locus IL1RN is selected from SEQ ID NO:41, the complement of SEQ ID NO:41, a fragment thereof or its complement, and a variant thereof or its complement, and particularly any of SEQ ID NOs:42, 43, 44, and 45.

44. The use according to any of the items 1 to 34, wherein the polynucleotide originating from the genetic locus GREM1 is selected from SEQ ID NO:46, the complement of SEQ ID NO:46, a fragment thereof or its complement, and a variant thereof or its complement, and particularly any of SEQ ID NOs:47, 48, 49, and 50.

45. The use according to any of the items 1 to 34, wherein the polynucleotide originating from the genetic locus COL12A1 is selected from SEQ ID NO:51, the complement of SEQ ID NO:51, a fragment thereof or its complement, and a variant thereof or its complement, and particularly any of SEQ ID NOs:52, 53, 54, and 55.

46. A panel of biomarkers for assessing any member of the group consisting of colorectal cancer, colorectal adenoma and healthy colorectal tissue and particularly healthy colonic tissue, the panel comprising at least two polynucleotides, each polynucleotide corresponding to a different genetic locus, wherein at least one, and particulatly at least two, three, four, five, six, seven, eight, nine ten or eleven of the polynucleotide(s) originate(s) from a genetic locus selected from any of SLC7A5, IL8, CXCL1, MMP3, CA7, CHI3L1, IL1B, CXCL2, IL1RN, GREM1 and COL12A1.

47. The panel according to item 46, wherein the at least one, and particularly the at least two polynucleotide(s) is/are selected from any of SEQ ID NOs: 1, 6, 11, 16, 21, 26, 31, 36, 41, 46, and 51, a complement thereof, a fragment thereof or its complement, and a variant thereof or its complement.

48. The panel according to any of the items 46 and 47, wherein the at least one, and particularly the at least two polynucleotide(s) is/are selected from any of SEQ ID NOs:4, 9, 14, 19, 24, 29, 34, 39, 44, 49, and 54, a complement thereof, a fragment thereof or its complement, and a variant thereof or its complement.

49. The panel according to any of items 46 to 48, wherein the panel is selected for analysis of polynucleotide expression levels for any member of the group consisting of colorectal cancer and colorectal adenoma.

50. The panel of item 49, wherein the polynucleotide expression levels are or reflect the expression levels of mRNAs.

51. The panel of item 50, wherein the polynucleotide expression levels are the levels of cDNAs produced by the process of reverse transcription of mRNAs.

52. The panel of any of items 46 to 51, wherein at least one of the polynucleotides is a fragment of a polynucleotide originating from a genetic locus selected from any of SLC7A5, IL8, CXCL1, MMP3, CA7, CHI3L1, IL1B, CXCL2, IL1RN, GREM1 and COL12A1, or a variant thereof.

53. The panel of any of items 46 to 51, wherein at least one of the polynucleotides is a variant of a polynucleotide originating from a genetic locus selected from any of SLC7A5, IL8, CXCL1, MMP3, CA7, CHI3L1, IL1B, CXCL2, IL1RN, GREM1 and COL12A1, or a fragment thereof.

54. The panel of any of items 46 to 53, wherein the panel is used for the management of patient care in any of the group consisting of colorectal cancer and colorectal adenoma.

55. The panel of any of items 46 to 54, wherein the management of patient care includes one or more of risk assessment, diagnosis, establishing prognosis, monitoring patient treatment, and detecting relapse.

56. The panel of any of items 46 to 55, wherein the panel is used in discovery of therapeutic intervention of any of the group consisting of colorectal cancer, colorectal adenoma, and colorectal polyp .

57. A method for measuring the expression levels of a polynucleotide from a biomarker, the biomarker being capable of discriminating colorectal cancer from non-cancerous colorectal tissue in a human individual, the method comprising: selecting a biomarker comprising a contiguous polynucleotide included in a member of the group consisting of SEQ ID NOs:1, 6, 11, 16, 21, 26, 31, 36, 41, 46, and 51, a fragment thereof or its complement, and a variant thereof or its complement; obtaining (removing) from the human individual a biopsy sample suspected of being colorectal cancer; in vitro (ex

vivo) isolating cellular RNA from the sample and reverse-transcribing isolated RNA to obtain cDNA; amplifying copies of cDNA for the biomarker originating from the sample; and quantifying the level of cDNA amplified from the sample, thereby measuring the expression level of the polynucleotide from the biomarker.

58. The method according to item 57, wherein the expression levels of two or more polynucleotides from two or more different biomarkers are measured, particularly polynucleotides of two, three, four, five, six, seven, eight, nine ten or eleven biomarkers originating from a genetic locus selected from the group consisting of SLC7A5, IL8, CXCL1, MMP3, CA7, IL1B, CHI3L1, CXCL2, IL1RN, GREM1 and COL12A1.

59. The method according to item 58, wherein the method includes the step of selecting two or more biomarkers, each comprising a contiguous polynucleotide included in a member of the group consisting of SEQ ID NOs:1, 6, 11, 16, 21, 26, 31, 36, 41, 46, and 51, a fragment thereof, a complement thereof, and a variant thereof.

60. The method according to any of the items 57 to 59, wherein the method includes the step of selecting a biomarker comprising at least one polynucleotide from SEQ ID NOs:4, 9, 14, 19, 24, 29, 34, 39, 44, 49, and 54.

61. The method according to item 60, wherein the step of amplifying copies of cDNA further comprises at least two pairs of primers chosen from the primer pairs of SEQ. ID NOs: 2 and 3, 7 and 8, 12 and 13, 17 and 18, 22 and 23, 27 and 28, 32 and 33, 37 and 38, 42and 43, 47 and 48, and 52 and 53.

62. A method for measuring the expression levels of a polynucleotide from a biomarker, the biomarker being capable of discriminating colorectal cancer from colorectal adenoma in a human individual, the method comprising: selecting a biomarker comprising a contiguous polynucleotide included in a member of the group consisting of SEQ ID NOs:1, 6, 11, 16, 21, 26, 31, 36, 41, and 46, a fragment thereof or its complement, and a variant thereof or its complement; obtaining (removing) from the human individual a biopsy sample suspected of being colorectal cancer or colorectal adenoma; in vitro (ex vivo) isolating cellular RNA from the sample and reverse-transcribing isolated RNA to obtain cDNA; amplifying copies of cDNA for the biomarker originating from the sample; and quantifying the level of cDNA amplified

from the sample, thereby measuring the expression level of the polynucleotide from the biomarker.

63. The method according to item 62, wherein the expression levels of two or more polynucleotides from two or more biomarkers are measured, particularly polynucleotides of two, three, four, five, six, seven, eight, nine or ten biomarkers originating from a genetic locus selected from the group consisting of SLC7A5, IL8, CXCL1, MMP3, CA7, IL1B, CHI3L1, CXCL2, IL1RN, and GREM1.

64. The method according to item 63, wherein the method includes the step of selecting two or more biomarkers, each comprising a contiguous polynucleotide included in a member of the group consisting of SEQ ID NOs:1, 6, 11, 16, 21, 26, 31, 36, 41, and 46, a fragment thereof, a complement thereof, and a variant thereof.

65. The method according to any of the items 62 to 64, wherein the method includes the step of selecting a biomarker comprising at least one polynucleotide from SEQ ID NOs:4, 9, 14, 19, 24, 29, 34, 39, 44, and 49.

66. The method according to item 65, wherein the step of amplifying copies of cDNA further comprises at least two pairs of primers chosen from the primer pairs of SEQ. ID NOs: 2 and 3, 7 and 8, 12 and 13, 17 and 18, 22 and 23, 27 and 28, 32 and 33, 37 and 38, 42and 43, and 47 and 48.

67. The method according to any of the items 57 to 66, wherein the step of reverse-transcribing isolated RNA to obtain cDNA comprises using enzymes and reagents for the preparation of cDNAs.

68. The method according to any of the items 57 to 67, wherein the step of quantifying the level of cDNA further comprises labeling cDNA.

69. The method according to item 68, wherein labeling cDNA includes at least one fluorophore capable of interacting with double stranded nucleic acid by way of intercalation.

70. The method according to item 68, wherein labeling cDNA includes hybridization of a dual-labeled (fluorophore and quencher) hydrolysis probe to a the cDNA.

71. The method according to any of items 57 to 70, wherein the cDNA level(s) determined for the sample is/are compared to a control.

72. The method according to item 71, wherein the comparison is used in the management of patient care in any of colorectal cancer and colorectal adenoma.

73. The method according to item 72, wherein the management of patient care includes one or more of risk assessment, diagnosis, establishing prognosis, monitoring patient treatment, and detecting relapse.

74. The method according to item 71, wherein the comparison is used in discovery of therapeutic intervention of any of colorectal cancer and colorectal adenoma.

75. A kit for the assessment of any member of the group consisting of colorectal cancer and colorectal adenoma, the kit comprising: at least one reagent that is used in analysis of polynucleotide expression level for a biomarker for a member selected from the group consisting of colorectal cancer, colorectal adenoma and colorectal polyp, wherein the biomarker comprises a polynucleotide selected from SEQ ID NOs:1, 6, 11, 16, 21, 26, 31, 36, 41, 46, and 51, a complement therof, a variant thereof, and a fragment thereof; and instructions for using the kit for analyzing the polynucleotide expression level.

76. The kit according to item 75, comprising two or more reagents for use in analysis of polynucleotide expression levels for a plurality of biomarkers.

77. The kit according to item 76, wherein the plurality of biomarkers comprises two or more polynucleotides selected from SEQ ID NOs:4, 9, 14, 19, 24, 29, 34, 39, 44, 49, and 54.

78. The kit according to any of the items 75 to 77, wherein the polynucleotide expression levels is/are mRNA(s) level(s).

79. The kit according to any of the items 75 to 77, wherein the polynucleotide expression level(s) is/are cDNA(s).

80. The kit according to any of the items 75 to 78, wherein the reagent comprises one or more pair(s) of primers chosen from the primer pairs of SEQ. ID NOs:

2 and 3, 7 and 8, 12 and 13, 17 and 18, 22 and 23, 27 and 28, 32 and 33, 37 and 38, 42and 43, 47 and 48, and 52 and 53.

81. The kit according to item 80, further comprising reagents for the preparation of cDNA.

5 82. The kit according to any of items 75 to 81, comprising a reagent that is capable of detecting and quantifying polynucleotides.

83. The kit according to item 82, wherein the reagent includes at least one chromophore, particularly a fluorophore capable of interacting with double-stranded nucleic acid by way of intercalation.

10 84. The kit according to item 82, wherein the reagent includes at least one (dual-labeled) TaqMan® hydrolysis probe, particularly a hydrolysis probe selected from any of SEQ ID NOs: 5, 10, 15, 20, 25, 30, 35, 40, 4, 50, and 55.

85. The kit according to any of the items 75 to 84, further comprising consumable labware for at least one member of the group consisting of of
15 sample collection, sample preparation, and sample analysis.

The following examples, figures and sequence listing are provided to aid the understanding of the present disclosure, the true scope of which is set forth in the appended claims. It is understood that modifications can be made in the procedures set forth without departing from the spirit of the disclosure.

20 **Example 1**

Colon biopsy samples

All samples were taken on the basis of informed consent given by patients and healthy persons, likewise. Tissue was sampled during the course of coloscopy (endoscopic intervention) or resection of neoplastic tissue. Altogether 147 biopsy
25 specimen (53 in a first set, an additional 94 samples fresh frozen) were analyzed.

Colon biopsy samples were taken during endoscopic intervention and stored in RNALater reagent (Qiagen Inc. Germantown, USA) at -80°C. Fresh frozen tissue and FFPE tissue were obtained from surgical or endoscopic intervention.

Colon biopsy samples from untreated patients (approximately 5 mg each) were taken during endoscopic intervention and stored in RNALater Reagent (Qiagen Inc, Germantown, US) at –80°C.

From collected formalin fixed, paraffin-embedded (FFPE) tissue samples 10 µm
5 slices were cut.

Tissue samples obtained from surgically removed colon tissues were snap-frozen in liquid nitrogen and stored on -80°C until use. The paired control healthy specimen originated from the histologically normal area of the resected sample farest available from the tumor.

10 Clinicopathological diagnosis was made for each sample by a pathologist.

Example 2
RNA isolation

Total RNA was extracted from the biopsy samples by using the RNeasy Mini Kit (Qiagen), and from slides with FFPE tissue using the HIGH PURE RNA Paraffin
15 kit (Roche Diagnostics GmbH, Mannheim, Germany) and with the MAGNA PURE 96 system (Roche Diagnostics GmbH, Mannheim, Germany) using the RNA Cellular Large Volume kit according to the manufacturer's instructions.

Quantity of the isolated RNA was measured with a NanoDrop spectrophotometer (NanoDrop Technologies Inc., Montchanin, US). The quality of isolated RNA was
20 analyzed with capillary gel electrophoresis using 2100 Bioanalyzer and RNA 6000 Pico Kit (Agilent Inc, Santa Clara, US).

Example 3
Microarray analysis of biopsy samples

Total RNA was extracted from biopsy samples using RNeasy Mini Kit (Qiagen,
25 US) according to the manufacturer's instructions. Quantity and quality of the isolated RNA were tested by measuring of the absorbance and agarose gelelectrophoresis or capillary gelelectrophoresis using the 2100 Bioanalyzer and the RNA 6000 Pico Kit (Agilent Inc, Santa Clara, US). Biotinylated cRNA probes were synthesized from 5-8 µg total RNA and fragmented using One-Cycle Target
30 Labeling and Control Kit according to the Affymetrix GeneChip Expression Analysis Technical Manual, Section 2: "Eukaryotic Sample and Array Processing". 10 µg of each fragmented cRNA sample was hybridized into HGU133 Plus2.0

array (Affymetrix Inc.) at 45°C for 16 hours. The slides were washed and stained using Fluidics Station 450 and antibody amplification staining method according to the manufacturer's instructions. The fluorescent signals were detected by a GeneChip Scanner 3000.

5 Statistical evaluation of mRNA expression profiles: Pre-processing and quality control: Quality control analyses were performed according to the suggestions of The Tumor Analysis Best Practices Working Group (Nature Reviews Genetics 5 (2004) 229-237). Scanned images were inspected for artifacts, percentage of present calls (>25%) and control of the RNA degradation were evaluated. Based on 10 the evaluation criteria all biopsy measurements fulfilled the minimal quality requirements. The Affymetrix expression arrays were pre-processed by gcRMA with quantile normalization and median polish summerization.

Further analyses: To identify differentially expressed features "Significance Analysis of Microarrays" (SAM) was used. The Prediction Analysis for 15 Microarrays (PAM) which uses the nearest shrunken centroid method was applied for sample classification from gene expression data. The pre-processing and analysis was performed using R-environment with Bioconductor libraries.

Validation of the results was performed with a first set of histologically confirmed biopsies (total of 53, among these 22 colorectal cancer, 20 colorectal adenoma, 11 20 healthy colon tissue), and a second set of independently obtained and confirmed biopsies (total of 94, among these 27 colorectal cancer, 29 colorectal adenoma, 38 healthy colon tissue).

Example 4

Marker identification and validation

25 The marker set was identified using HGU133Plus2.0 microarrays (Affymetrix, Santa Clara, US) as described in Example 3, and initially on the basis of 53 biopsies. Of these 22 were CRC, 20 were colorectal adenoma, and 11 were normal colon tissue. The classificatory power of the discriminatory markers was further tested on 94 independent biopsies (27 CRC, 29 colorectal adenoma, 38 normal 30 colorectal tissue). The set of 11 markers (SLC7A5, IL8, CXCL1, MMP3, CA7, IL1B, CHI3L1, CXCL2, IL1RN, GREM1 and COL12A1) was determined which could correctly discriminate between high-grade dysplastic colorectal adenoma and CRC samples (sensitivity: 100%, specificity: 88,9%). This finding was to the surprise of the authors and proves a technical advantage and clinical utility.

In order to test the discriminatory power of the markers, 98 independent biopsies (39 CRC, 24 adenoma, 35 normal) and 67 independent FFPE sample (35 CRC, 32 normal) were analysed with individual markers and marker panels using TaqMan® RT-PCR .

5 **Example 5**

Reverse transcription

Using the Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics GmbH, Mannheim, Germany; Roche, Cat. No. 04 379 012 001) 2.5 µg total RNA from each of the analysed healthy, CRC (= colorectal cancer) and colorectal
10 adenoma biopsy/resectate specimens were reverse-transcribed, The quality of the cDNA samples was checked by realt-time PCR for SDHA (succinate dehydrogenase complex, subunit A, flavoprotein) housekeeping gene.

Example 6

Analysis of mRNA from biopsy samples using the TaqMan® RT-PCR format

15 TaqMan® real-time PCR was used to measure the expression of selected genes using an Applied Biosystems Micro Fluidic Card System. The measurements were performed using an ABI PRISM® 7900HT Sequence Detection System as described in the products User Guide. For data analysis the SDS 2.2 software was used. The extracted delta Ct values (which represent the expression normalized to
20 the ribosomal 18S expression) were grouped according to the histologic groups. Then the Student's t-test was performed to compare the expression values between groups.

Validation of was performed with a first set of histologically confirmed biopsy samples (total of 98, among these 39 colorectal cancer, 24 colorectal adenoma, 35
25 healthy colon tissue), and a second set of independently obtained and confirmed samples of formalin fixed paraffin embedded tissue material (total of 67 slides, among these 35 colorectal cancer, 32 healthy colon tissue).

Example 7

Re-Design of RT-qPCR assays with LNA detection probes as an embodiment
30 **of the TaqMan® real-time format**

In order to provide assays for screening with enhanced throughput, RT-PCR assays were designed making use of LNA-based hydrolysis probes of the "Universal

Probe Library" (= UPL; Roche Applied Science, Roche Diagnostics GmbH, Mannheim, Germany. A UPL probe is a short hydrolysis probes. Such a probe is labeled at the 5' end with fluorescein (FAM) and at the 3' end with a dark quencher dye. The length of a UPL probe is just 8-9 nucleotides and comprises a selected
5 sequence. In order to maintain the specificity and melting temperature (Tm) that hybridizing qPCR probes require, Locked Nucleic Acids (LNA) are incorporated into the sequence of each UPL probe. LNA's are DNA nucleotide analogues with increased binding strengths compared to standard DNA nucleotides.

The basis for the design of primers and probes as listed in Table N were the
10 sequences targeted by the capture probes of the Affymetrix hybridization microarrays of Example 5 and listed in Table 4.

Table 3

gene designation	Forward (left) primer; SEQ ID NO:	Reverse (right) primer; SEQ ID NO:	Amplicon sequence; SEQ ID NO:	UPL probe sequence; SEQ ID NO:
SLC7A5	2	3	4	5
IL8	7	8	9	10
CXCL1	12	13	14	15
MMP3	17	18	19	20
CA7	22	23	24	25
CHI3L1	27	28	29	30
IL1B	32	33	34	35
CXCL2	37	38	39	40
IL1RN	42	43	44	45
GREM1	47	48	49	50
COL12A1	52	53	54	55

Example 8

Biostatistics approach to assay validation

15 Accuracy of a diagnostic method is best described by its receiver-operating characteristics (ROC) (see especially Zweig, M. H., and Campbell, G., Clin. Chem. 39 (1993) 561-577). The ROC graph is a plot of all of the sensitivity/specificity pairs resulting from continuously varying the decision threshold over the entire range of data observed.

The clinical performance of a laboratory test depends on its diagnostic accuracy, or the ability to correctly classify subjects into clinically relevant subgroups. Diagnostic accuracy measures the test's ability to correctly distinguish two different conditions of the subjects investigated. Such conditions are for example health and disease or benign versus malignant disease.

In the Figures 1 to 21 herein, box plots are shown. Each box contains the middle 50% of the respective data. The middle line is the median, whiskers extend to the most extreme data point.

A ROC plot depicts the overlap between the two distributions by plotting the sensitivity versus 1 - specificity for the complete range of decision thresholds. On the y-axis is sensitivity, or the true-positive fraction [defined as (number of true-positive test results)/(number of true-positive + number of false-negative test results)]. This has also been referred to as positivity in the presence of a disease or condition. It is calculated solely from the affected subgroup. On the x-axis is the false-positive fraction, or 1 - specificity [defined as (number of false-positive results)/(number of true-negative + number of false-positive results)]. It is an index of specificity and is calculated entirely from the unaffected subgroup. Because the true- and false-positive fractions are calculated entirely separately, by using the test results from two different subgroups, the ROC plot is independent of the prevalence of disease in the sample. Each point on the ROC plot represents a sensitivity/1-specificity pair corresponding to a particular decision threshold. A test with perfect discrimination (no overlap in the two distributions of results) has an ROC plot that passes through the upper left corner, where the true-positive fraction is 1.0, or 100% (perfect sensitivity), and the false-positive fraction is 0 (perfect specificity). The theoretical plot for a test with no discrimination (identical distributions of results for the two groups) is a 45° diagonal line from the lower left corner to the upper right corner. Most plots fall in between these two extremes. (If the ROC plot falls completely below the 45° diagonal, this is easily remedied by reversing the criterion for "positivity" from "greater than" to "less than" or vice versa.) Qualitatively, the closer the plot is to the upper left corner, the higher the overall accuracy of the test.

One way to quantify the diagnostic accuracy of a laboratory test is to express its performance by a single number. Such an overall parameter e.g. is the so-called "total error" or alternatively the "area under the curve = AUC". The most common global measure is the area under the ROC plot. By convention, this area is always >

0.5 (if it is not, one can reverse the decision rule to make it so). Values range between 1.0 (perfect separation of the test values of the two groups) and 0.5 (no apparent distributional difference between the two groups of test values). The area does not depend only on a particular portion of the plot such as the point closest to the diagonal or the sensitivity at 90% specificity, but on the entire plot. This is a quantitative, descriptive expression of how close the ROC plot is to the perfect one (area = 1.0).

Example 9

Marker panel

For RT-PCR discriminant analysis was performed in order to find the best marker/s to differentiate Normal and CRC samples. In each step, one new marker was involved into the analysis. The three best markers to discriminate normal tissue and CRC were CXCL2, SLC7A5 and IL8. Their differentiation can be seen in the Tables 4, 5, and 6, where 0 refers to "Normal" (normal, i.e healthy tissue) and 1 refers to CRC. In the first row the amount of samples can be seen (which is 20 Normal and 24 CRC samples). In the second row the percentage can be seen. In case of CXCL2 alone 95% of Normal and 91.7% of CRC samples classified surprisingly well.

Table 4

CXCL2

Classification Results[b,c]

			Predicted Group Membership		
		V1	0	1	Total
Original	Count	0	19	1	20
		1	2	22	24
	%	0	95,0	5,0	100,0
		1	8,3	91,7	100,0
Cross-validated[a]	Count	0	19	1	20
		1	2	22	24
	%	0	95,0	5,0	100,0
		1	8,3	91,7	100,0

a. Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.

b. 93,2% of original grouped cases correctly classified.

c. 93,2% of cross-validated grouped cases correctly classified.

Table 5

CXCL2 and SLC7A5

Classification Results[b,c]

		V1	Predicted Group Membership 0	1	Total
Original	Count	0	19	1	20
		1	1	23	24
	%	0	95,0	5,0	100,0
		1	4,2	95,8	100,0
Cross-validated[a]	Count	0	18	2	20
		1	1	23	24
	%	0	90,0	10,0	100,0
		1	4,2	95,8	100,0

a. Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.

b. 95,5% of original grouped cases correctly classified.

c. 93,2% of cross-validated grouped cases correctly classified.

Table 6

5 CXCL2, SLC7A5 and IL8

Classification Results[b,c]

		V1	Predicted Group Membership 0	1	Total
Original	Count	0	19	1	20
		1	1	23	24
	%	0	95,0	5,0	100,0
		1	4,2	95,8	100,0
Cross-validated[a]	Count	0	18	2	20
		1	1	23	24
	%	0	90,0	10,0	100,0
		1	4,2	95,8	100,0

a. Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.

b. 95,5% of original grouped cases correctly classified.

c. 93,2% of cross-validated grouped cases correctly classified.

This set of CXCL2, SLC7A5 and IL8 allowed to differentiate 90% of Normal and 95.8% of CRC samples.

Table 7

Variables in the Analysis

Step		Tolerance	F to Remove	Wilks' Lambda
1	CXCL2	1,000	122,348	
2	CXCL2	,863	14,144	,255
	SLC7A5	,863	13,338	,251
3	CXCL2	,452	,303	,164
	SLC7A5	,833	14,843	,224
	IL8	,515	6,210	,188
4	SLC7A5	,984	23,722	,261
	IL8	,984	22,281	,255
5	SLC7A5	,780	29,667	,261
	IL8	,614	26,876	,250
	IL1RN	,504	4,113	,164
6	SLC7A5	,753	27,620	,187
	IL8	,610	13,109	,145
	IL1RN	,243	17,063	,157
	IL1B	,326	13,998	,148

Patent Claims

1. Use of three polynucleotides, each polynucleotide originating from a different genetic locus, each locus being selected from the group consisting of SLC7A5, IL8, CXCL1, MMP3, CA7, IL1B, CHI3L1, CXCL2, IL1RN, GREM1 and COL12A1, as a panel of biomarkers for and/or in the discrimination of colorectal cancer from non-cancerous colorectal tissue.

2. The use according to claim 1, wherein the non-cancerous colorectal tissue is healthy colonic tissue.

3. The use according to claim 1, wherein the non-cancerous colorectal tissue is dysplastic colorectal adenoma tissue.

4. The use according to any of the claims 2 to 3, wherein the discrimination of colorectal cancer from non-cancerous colorectal tissue includes comparing the the level of a polynucleotide in the patient's tissue sample with the presence and/or the level and/or the quantity of the polynucleotide in a control.

5. The use according to any of the claims 2 to 4, wherein a genetic locus is selected from any of SLC7A5, IL8, CXCL1, MMP3, IL1B, CHI3L1, CXCL2, IL1RN, GREM1 and COL12A1, and compared to a control sample of non-cancerous colorectal tissue the level of the polynucleotide originating from the genetic locus is increased in colorectal cancer.

6. The use according to any of the claims 2 to 4, wherein the genetic locus is CA7, and compared to a control sample of non-cancerous colorectal tissue the level of the polynucleotide originating from the genetic locus CA7 is decreased in colorectal cancer.

7. The use according to any of the claims 1 to 6, wherein colorectal cancer at a neoplastic stage selected from any of Tis N0 M0, T1-4 N0 M0, Dukes A, and Dukes B is discriminated from non-cancerous colorectal tissue.

8. The use according to any of the claims 1 to 7, wherein a biomarker is selected from a group of polynucleotides, the group consisting of SEQ ID NOs: 1, 6, 11,16, 21, 26, 31, 36, 41, 46, and 51, a complement thereof, a fragment thereof or its complement, and a variant thereof or its complement.

9. A panel of biomarkers for assessing any member of the group consisting of colorectal cancer, colorectal adenoma and healthy colorectal tissue, the panel comprising at least two polynucleotides, each polynucleotide corresponding to a different genetic locus, wherein at least three of the polynucleotides are selected from the group consisting of SEQ ID NOs: 1, 6, 11, 16, 21, 26, 31, 36, 41, 46, and 51, a complement of any member thereof, a fragment of any member thereof or its complement, and a variant of any member thereof or its complement.

10. The panel according to claim 9, wherein a polynucleotide is selected from the group consisting of SEQ ID NOs:4, 9, 14, 19, 24, 29, 34, 39, 44, 49, and 54, a complement of any member thereof, a fragment of any member thereof or its complement, and a variant of any member thereof or its complement.

11. A method for measuring the expression levels of a polynucleotide from a biomarker, the biomarker being capable of discriminating colorectal cancer from non-cancerous colorectal tissue in a human individual, the method comprising: selecting a biomarker comprising a contiguous polynucleotide originating from a genetic locus selected from any of SLC7A5, IL8, CXCL1, MMP3, CA7, CHI3L1, IL1B, CXCL2, IL1RN, GREM1 and COL12A1; obtaining from the human individual a biopsy sample suspected of being colorectal cancer; in vitro (ex vivo) isolating cellular RNA from the sample and reverse-transcribing isolated RNA to obtain cDNA; amplifying copies of cDNA for the biomarker originating from the sample; and quantifying the level of cDNA amplified from the sample, thereby measuring the expression level of the polynucleotide from a biomarker.

12. The method according to claim 11, wherein the biomarker is selected from the group consisting of SEQ ID NOs:1, 6, 11, 16, 21, 26, 31, 36, 41, 46, and 51, a complement of any member thereof, a fragment of any member thereof or its complement, and a variant of any member thereof or its complement.

13. The method according to claim 12, wherein the biomarker is selected from the group consisting of SEQ ID NOs:4, 9, 14, 19, 24, 29, 34, 39, 44, 49, and 54.

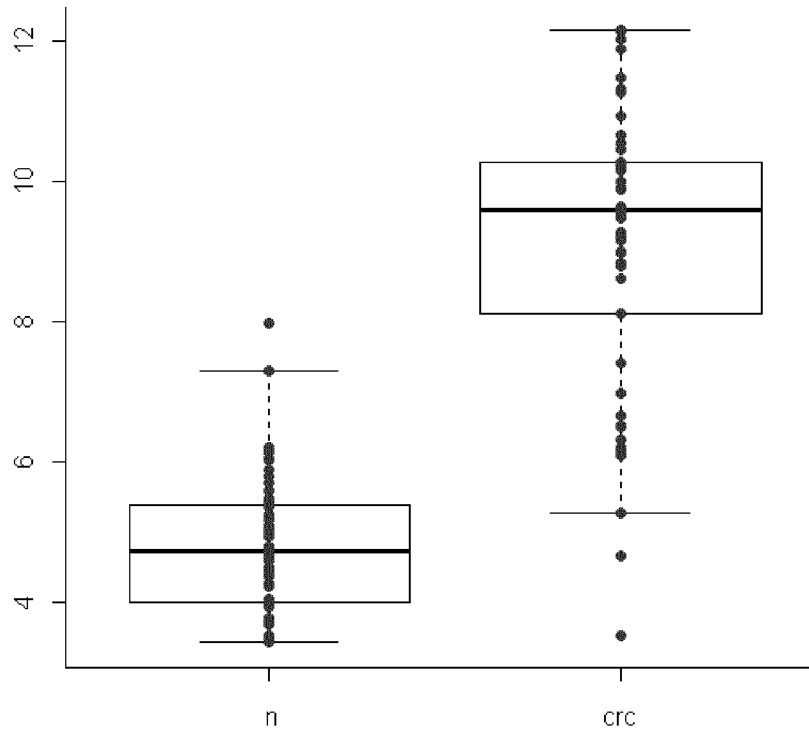
14. The method according to claim 13, wherein the step of amplifying copies of cDNA further comprises at least two pairs of primers chosen from the primer pairs of SEQ. ID NOs: 2 and 3, 7 and 8, 12 and 13, 17 and 18, 22 and 23, 27 and 28, 32 and 33, 37 and 38, 42and 43, 47 and 48, and 52 and 53.

5 15. A kit for the assessment of any member of the group consisting of colorectal cancer and colorectal adenoma, the kit comprising: at least reagents that are used in analysis of polynucleotide expression levels for a panel of three biomarkers for a member selected from the group consisting of colorectal cancer, colorectal adenoma and colorectal polyp, wherein the biomarkers 10 comprise polynucleotides selected from the group consisting of SEQ ID NOs: 1, 6, 11, 16, 21, 26, 31, 36, 41, 46, and 51 and COL12A1, a complement of any member thereof, a fragment of any member thereof or its complement, and a variant of any member thereof or its complement; and instructions for using the kit for analyzing polynucleotide expression level.

15

Fig. 1

A



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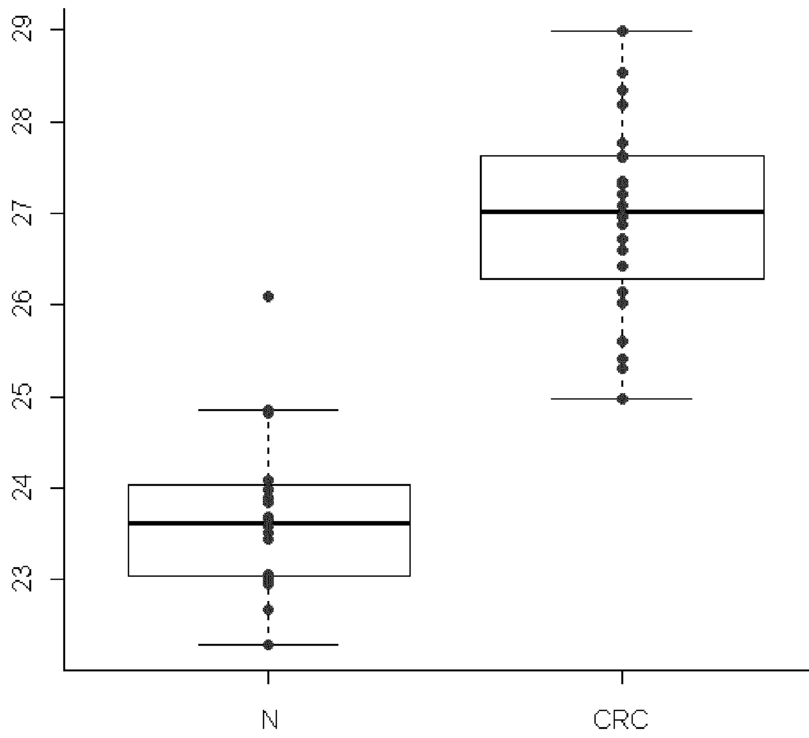

Fig. 2

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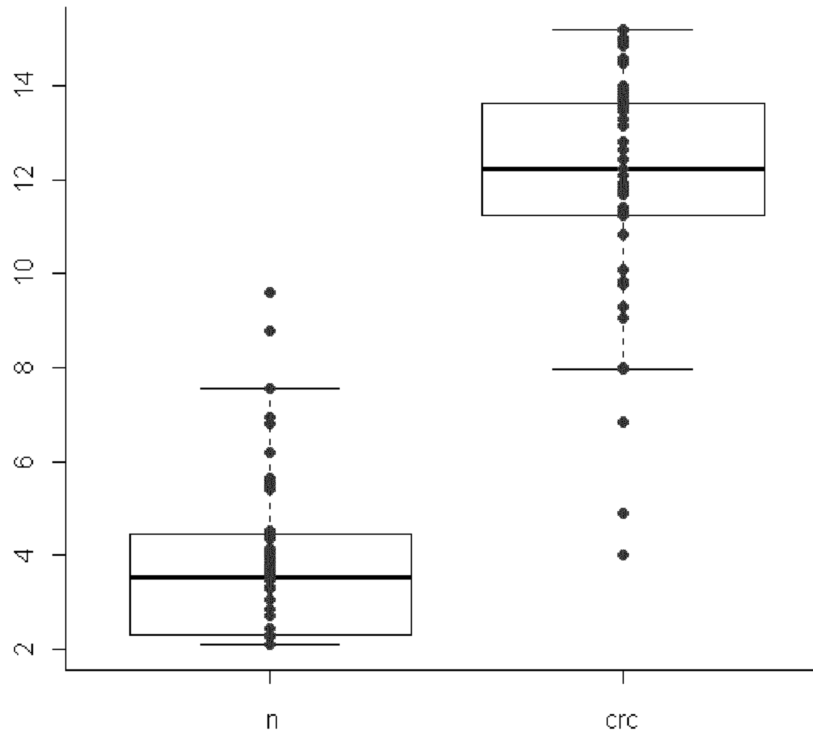

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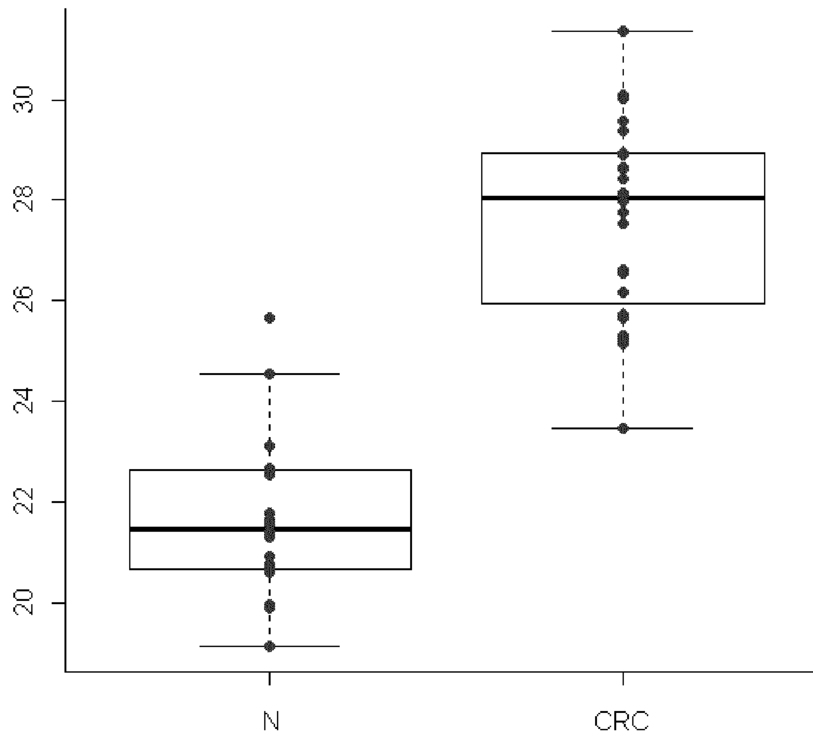

Fig. 3

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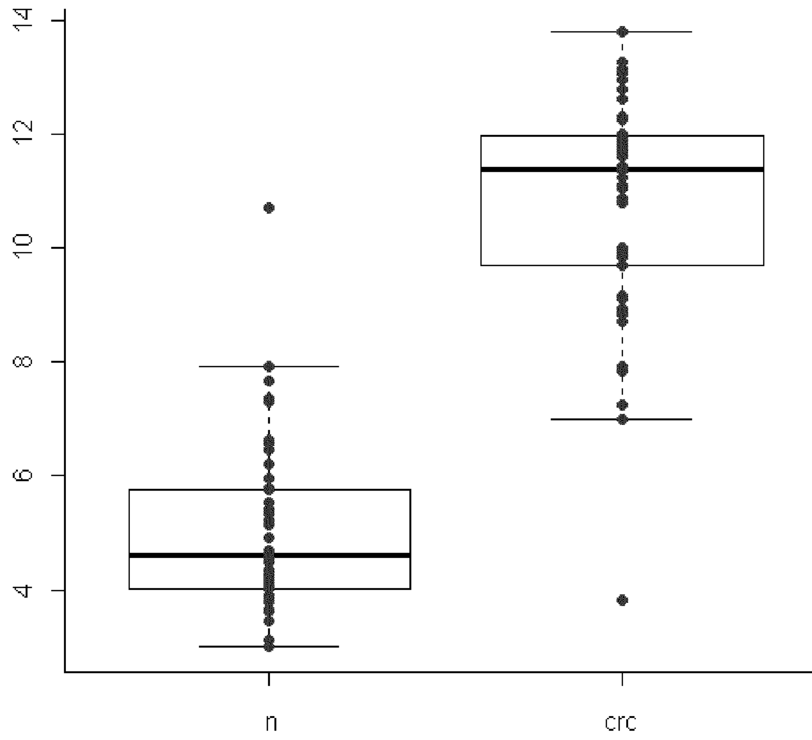

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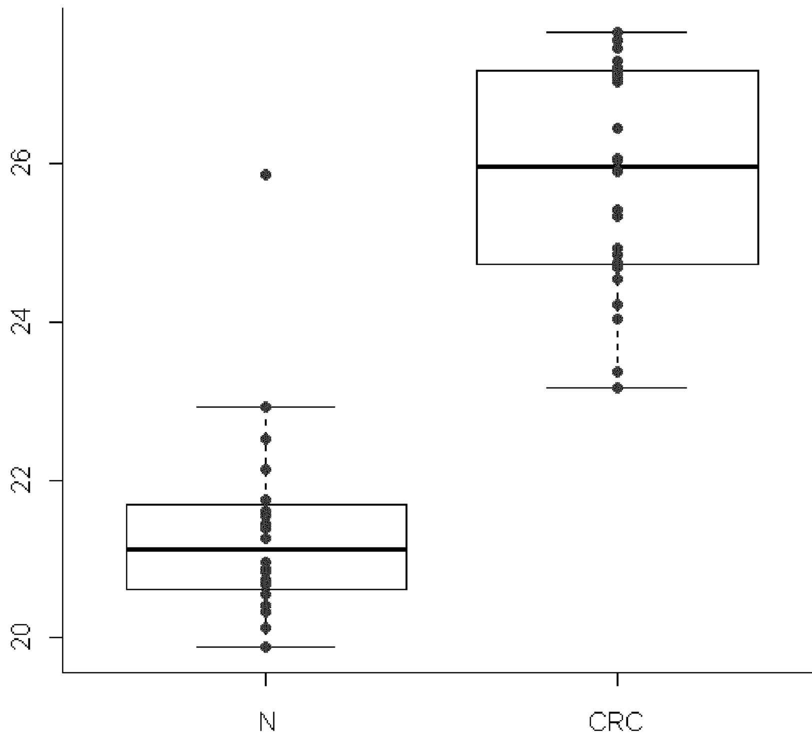

Fig. 4

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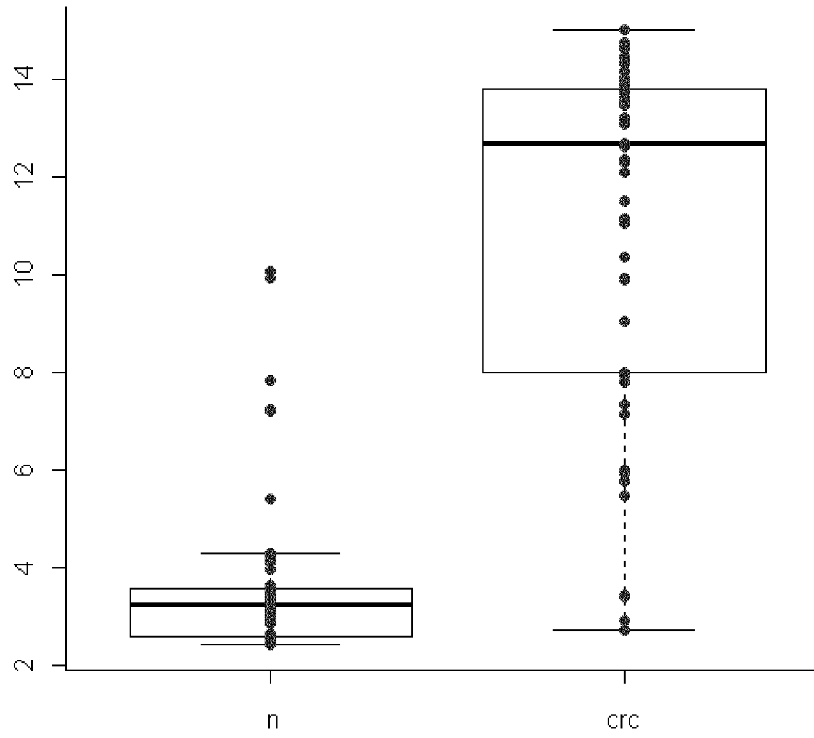

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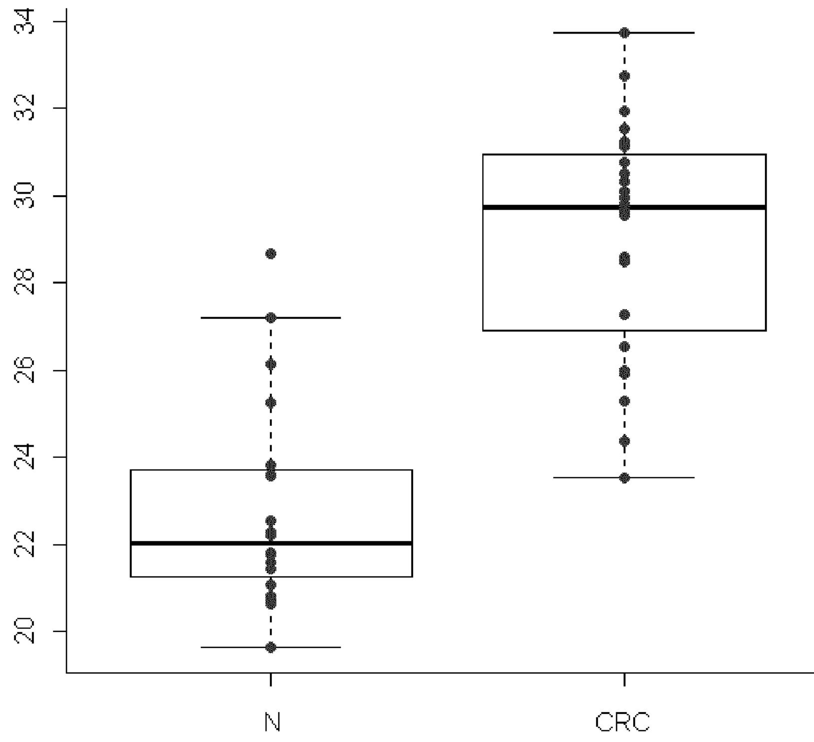

Fig. 5

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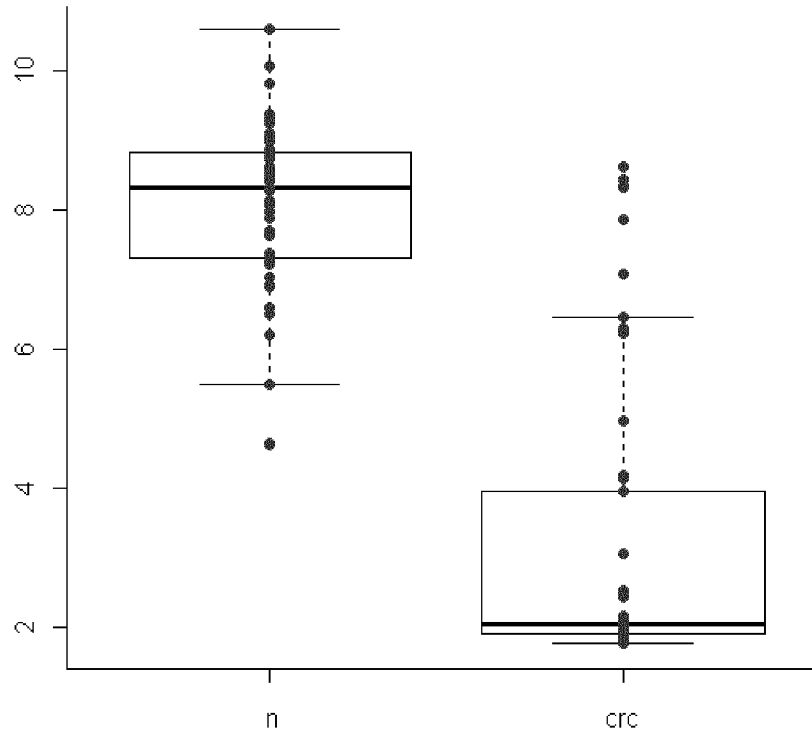

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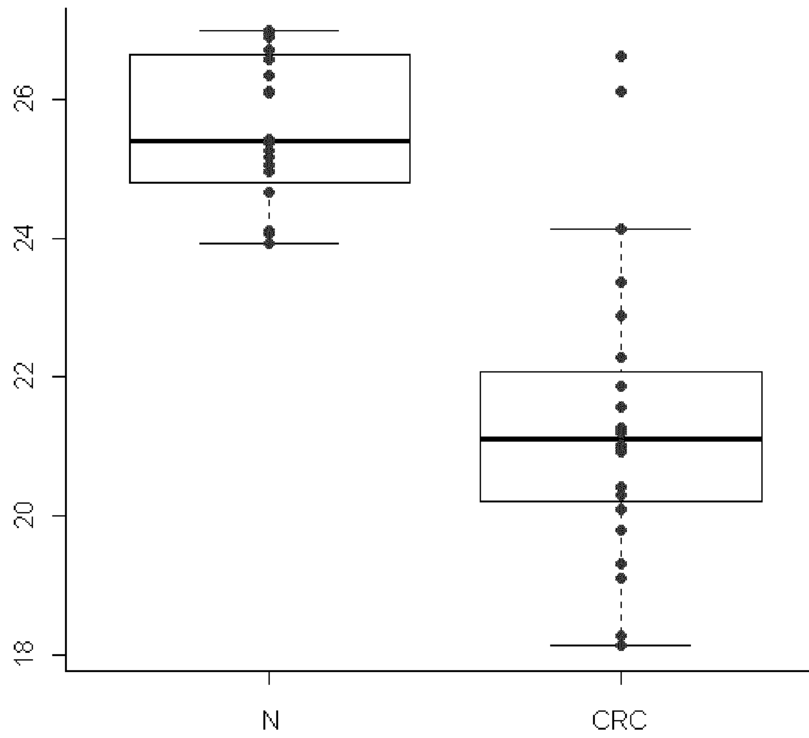

Fig. 6

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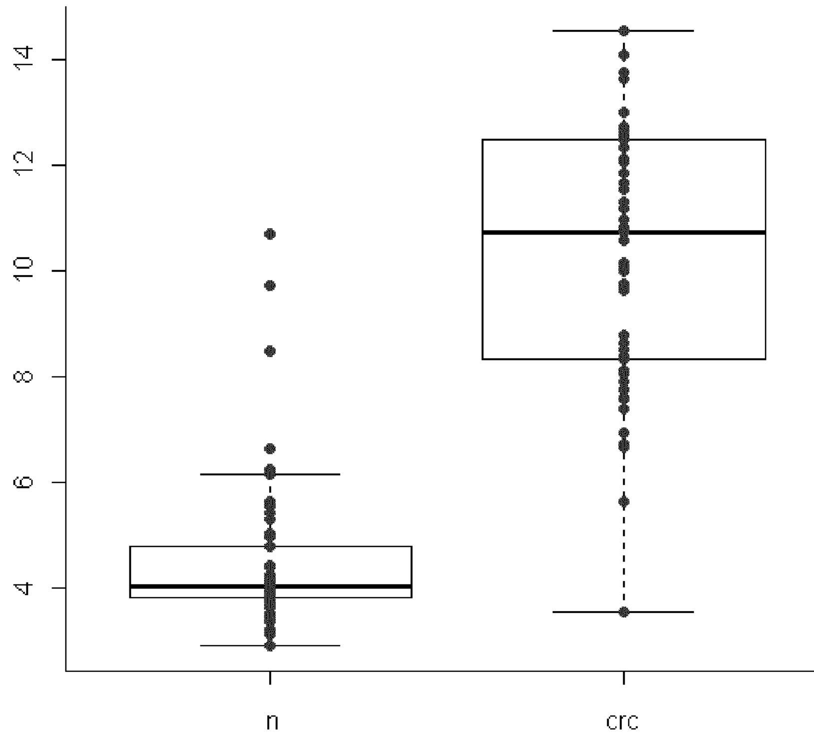

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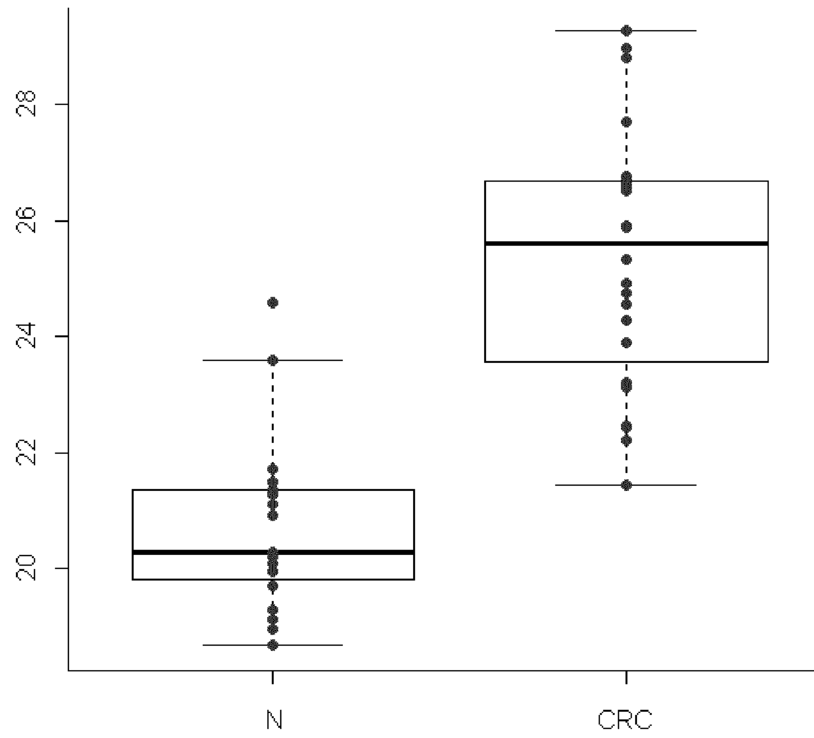

Fig. 7

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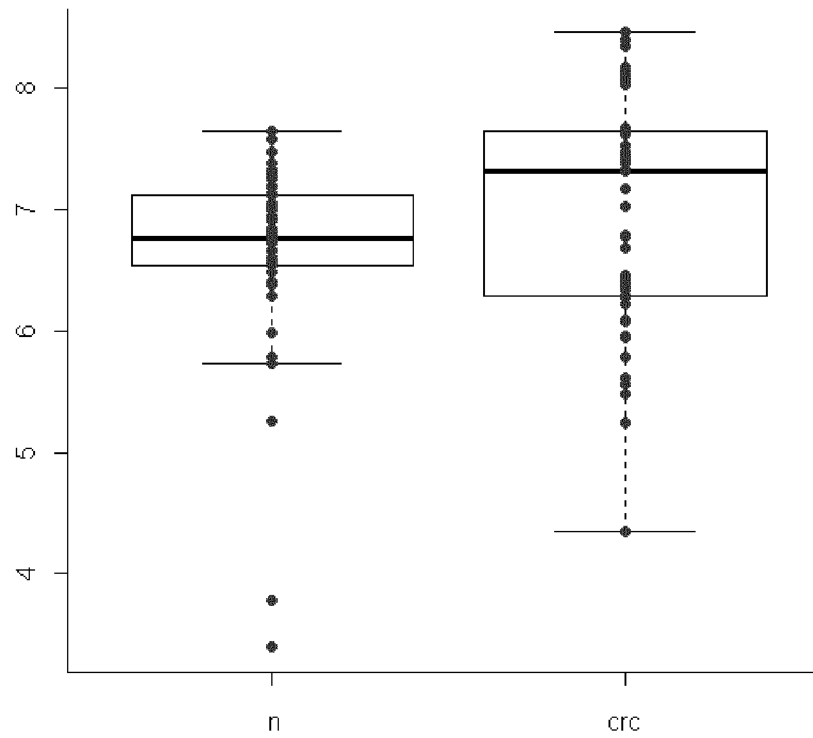

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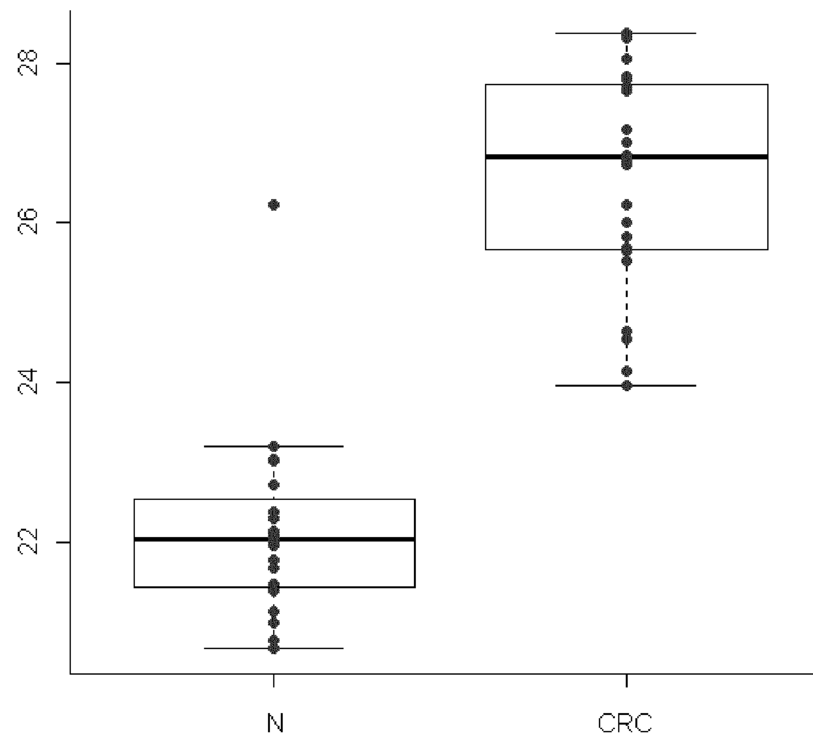

Fig. 8

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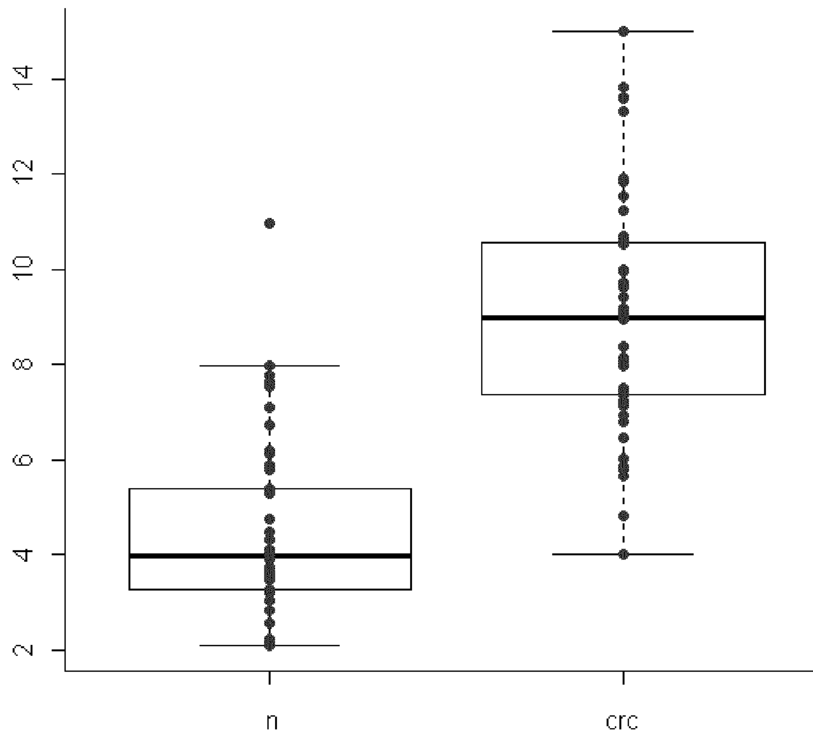

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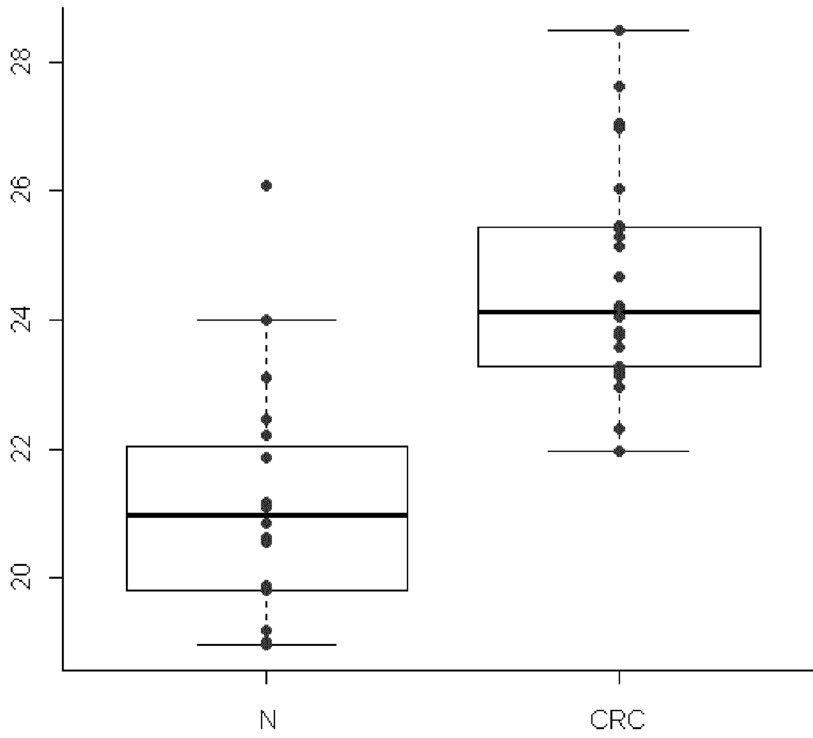

Fig. 9

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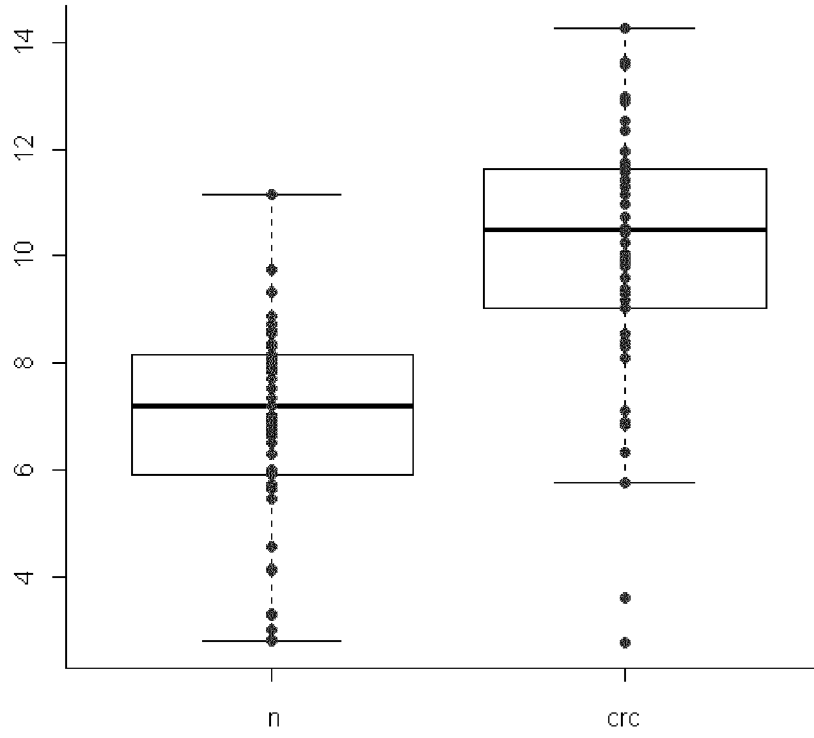

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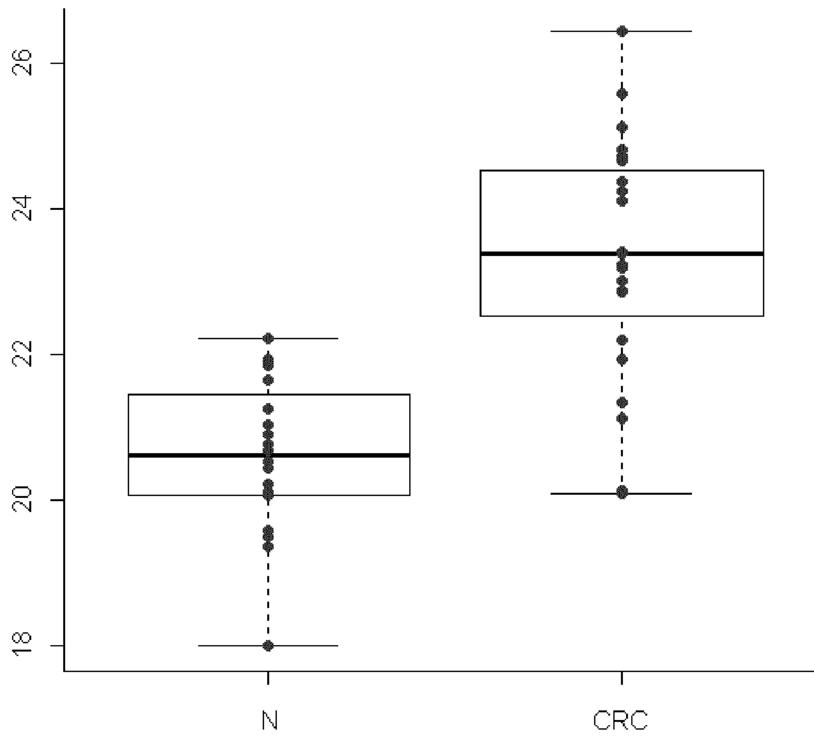

Fig. 10

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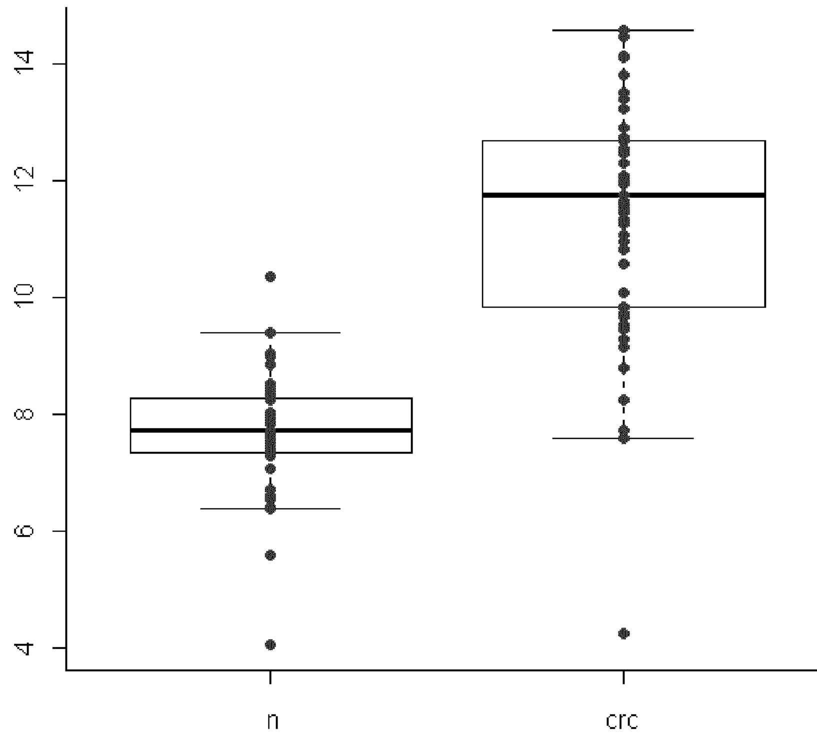

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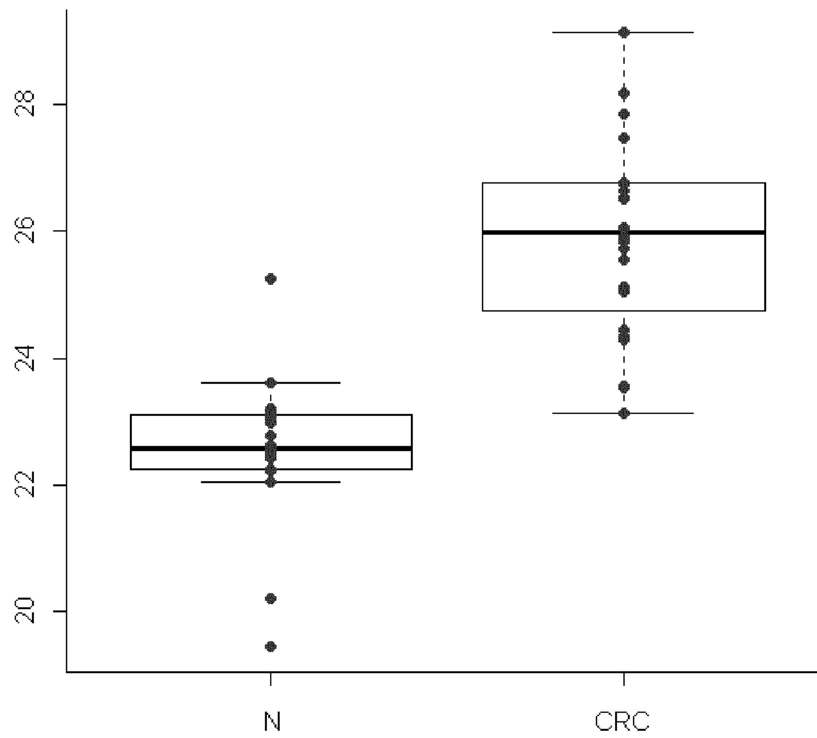

Fig. 11

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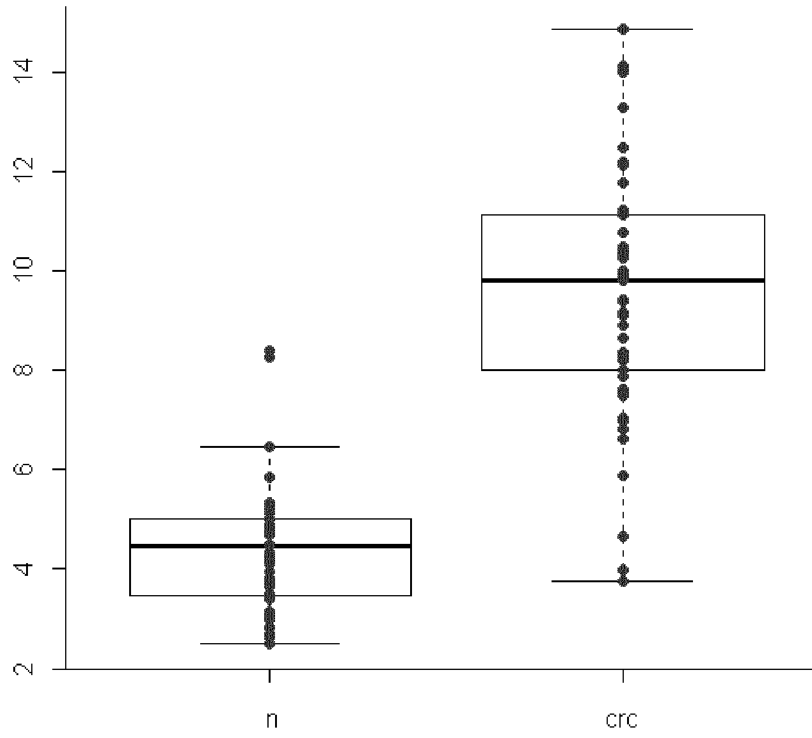

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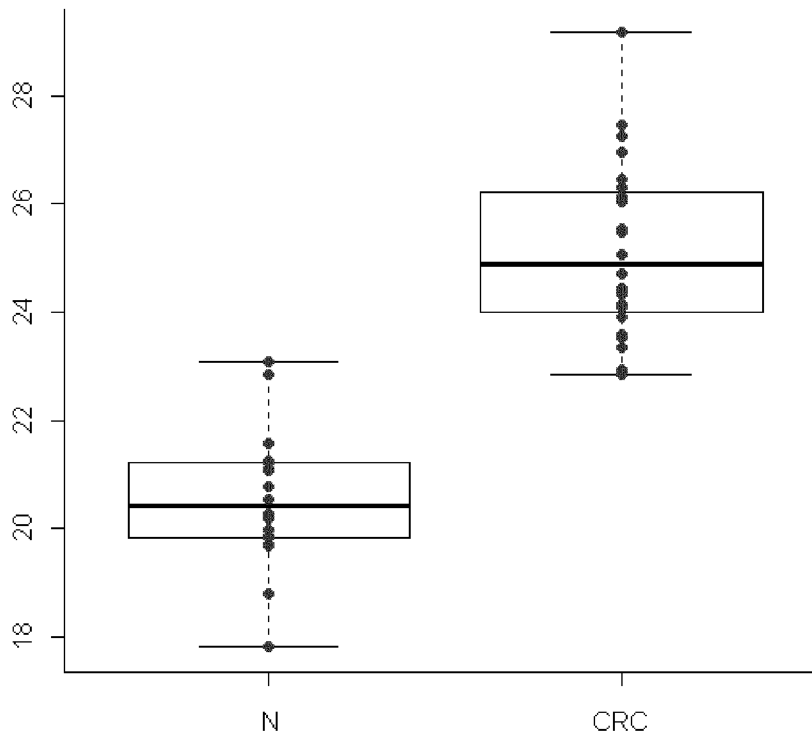

Fig. 12

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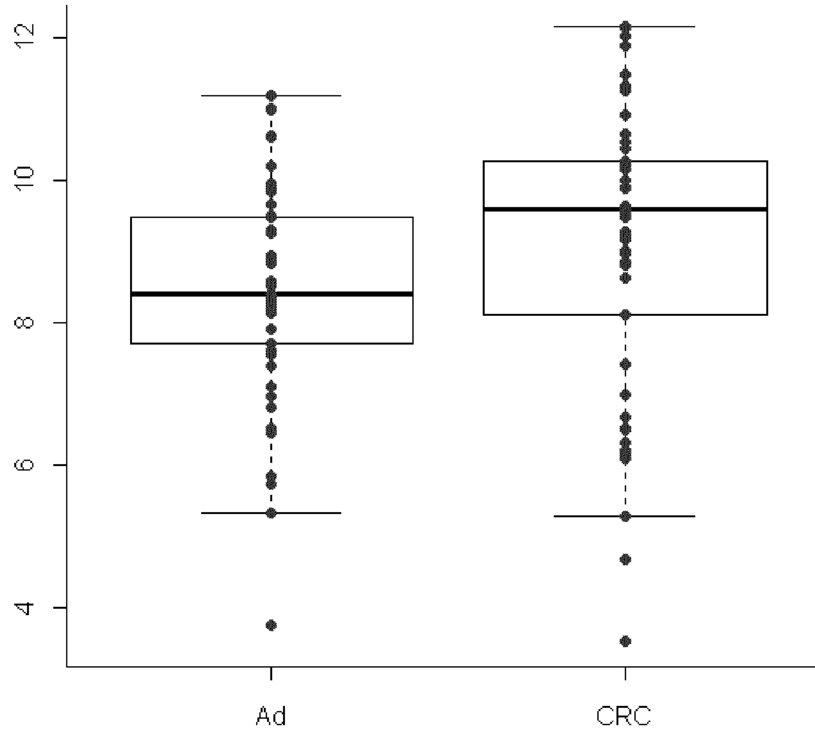

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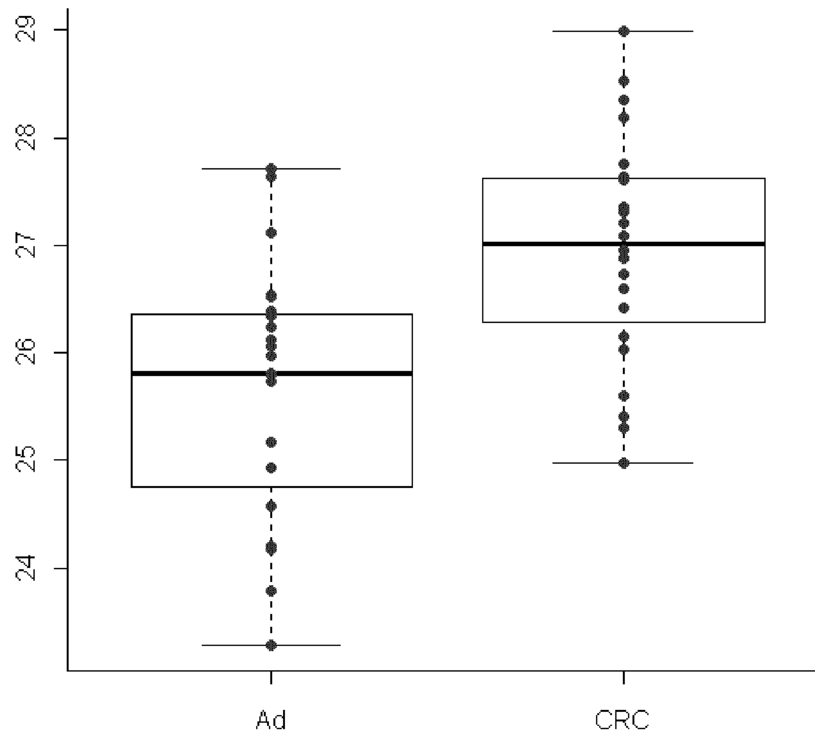

Fig. 13

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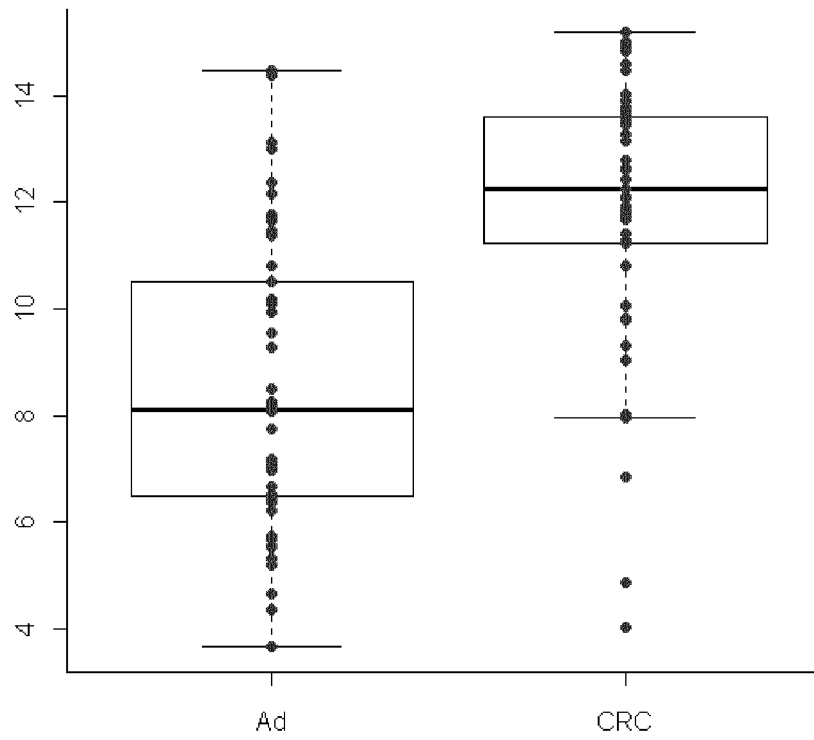

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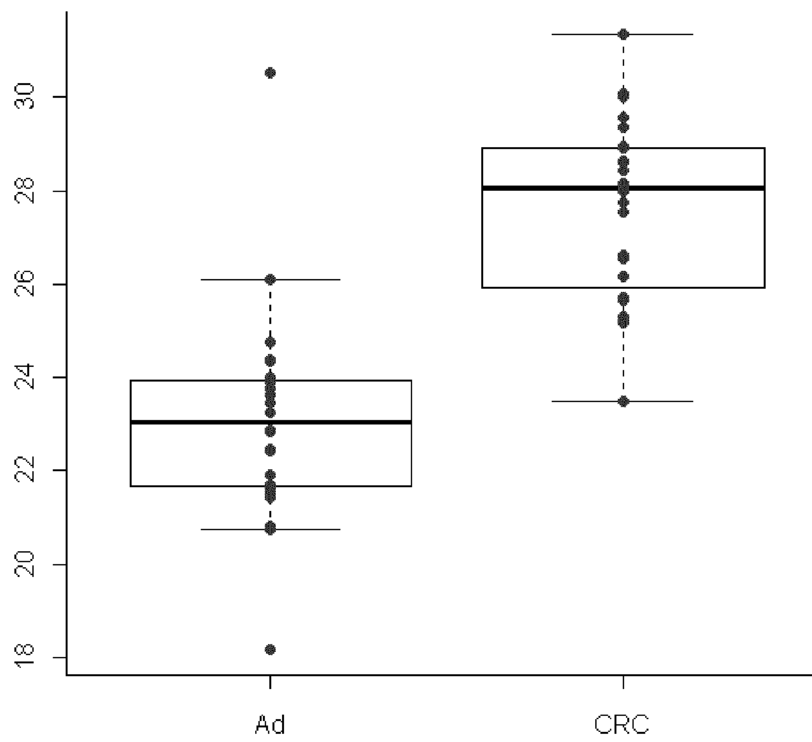

Fig. 14

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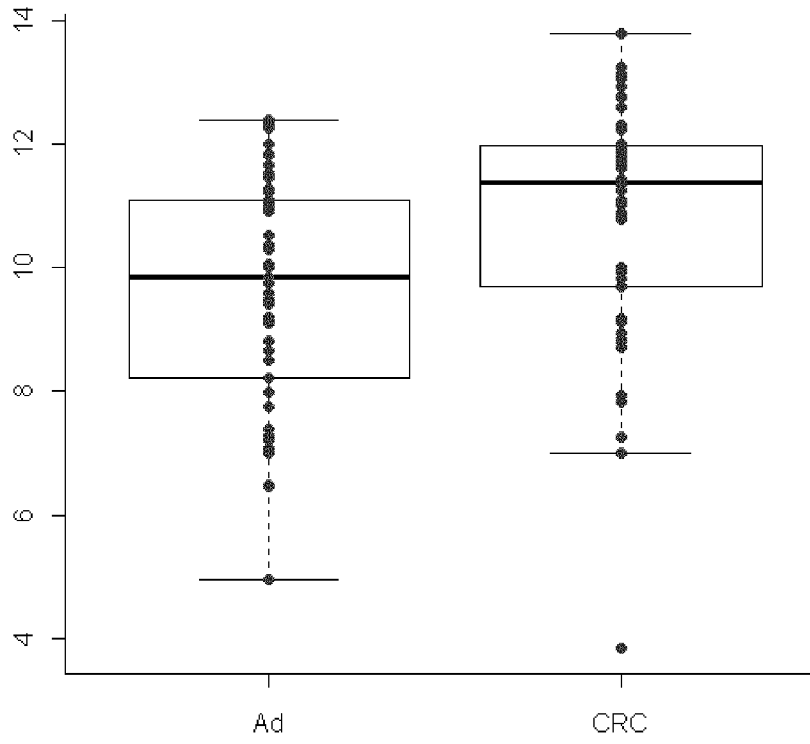

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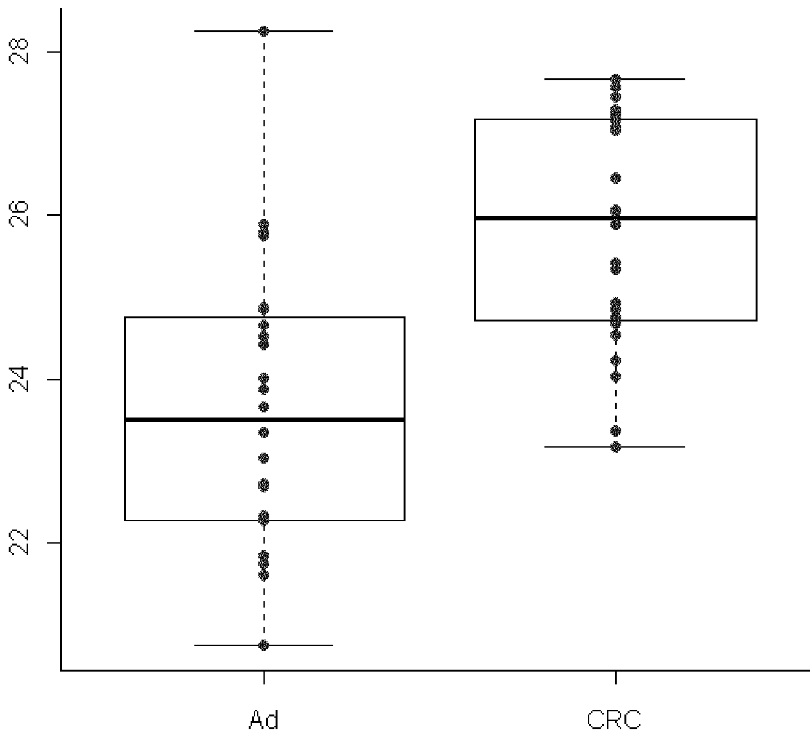
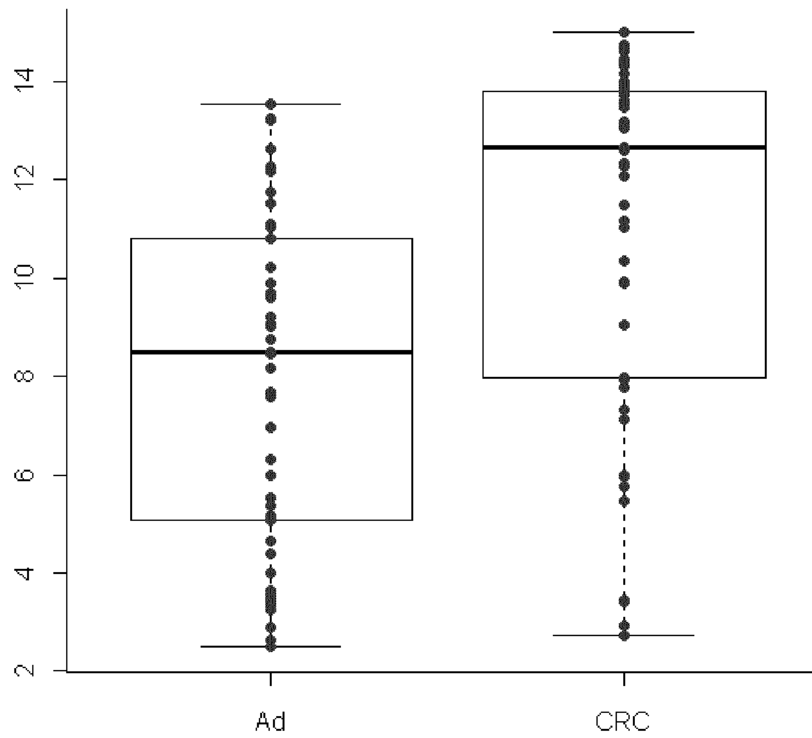


Fig. 15

A



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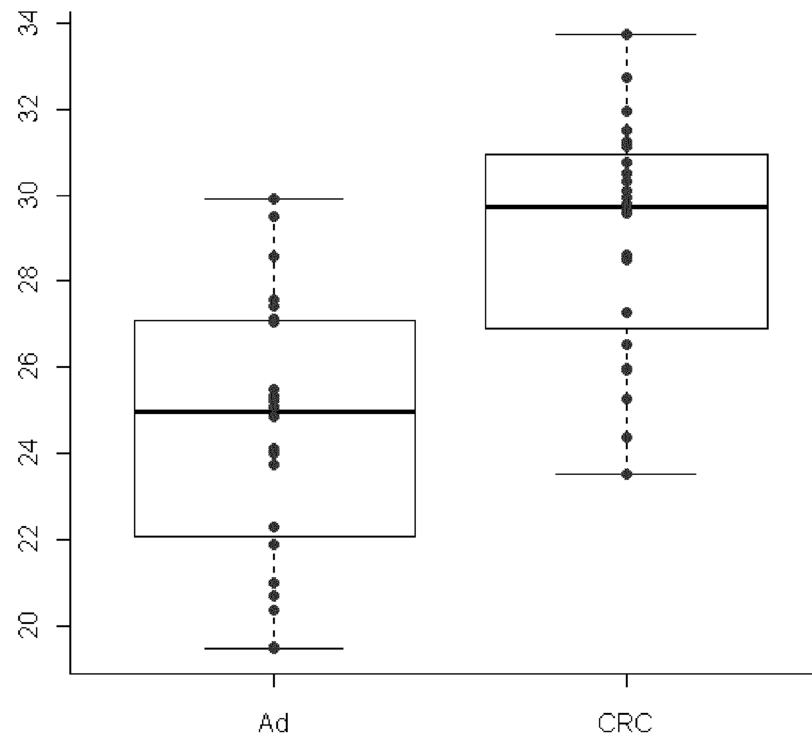
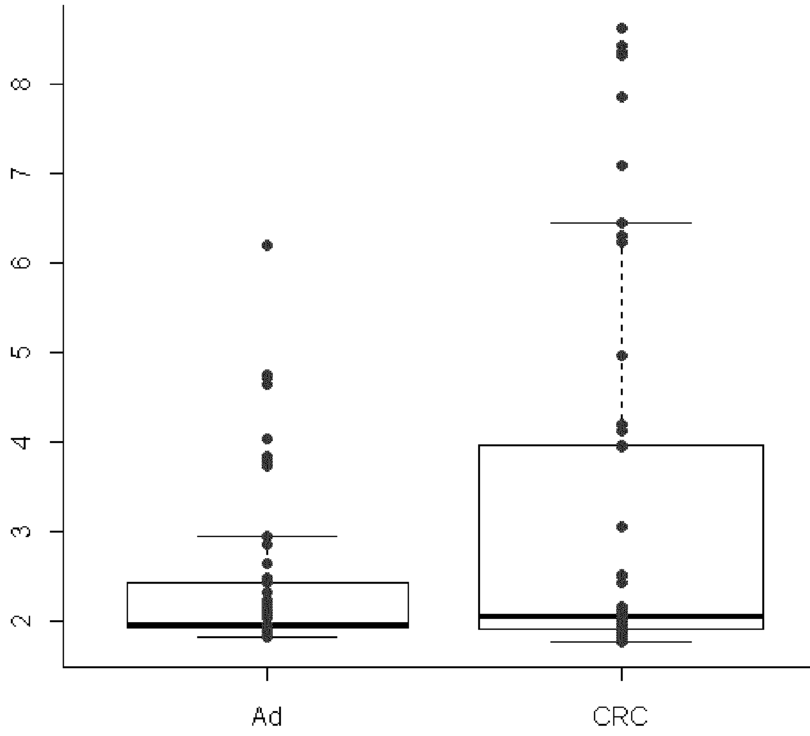


Fig. 16

A



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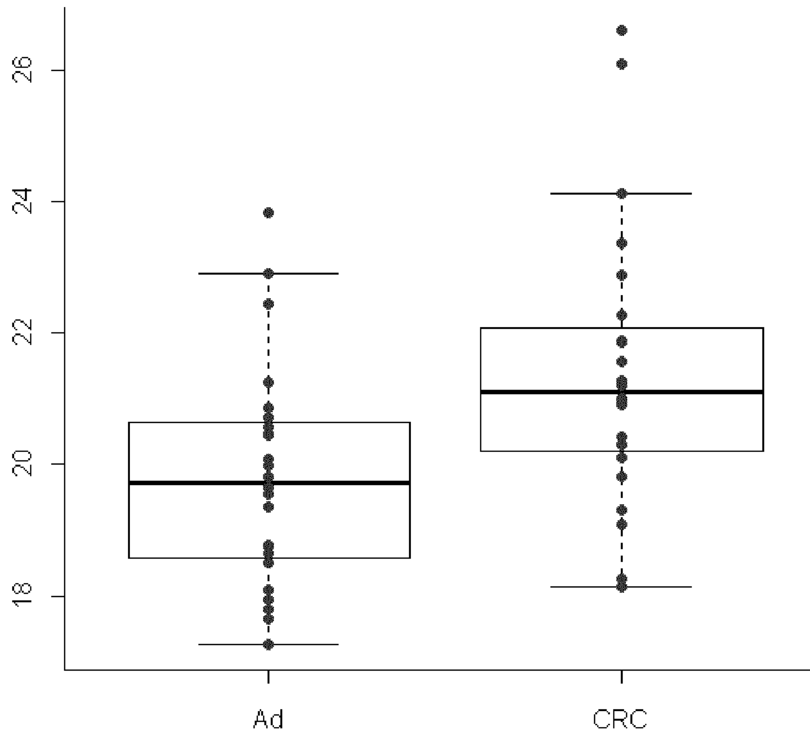
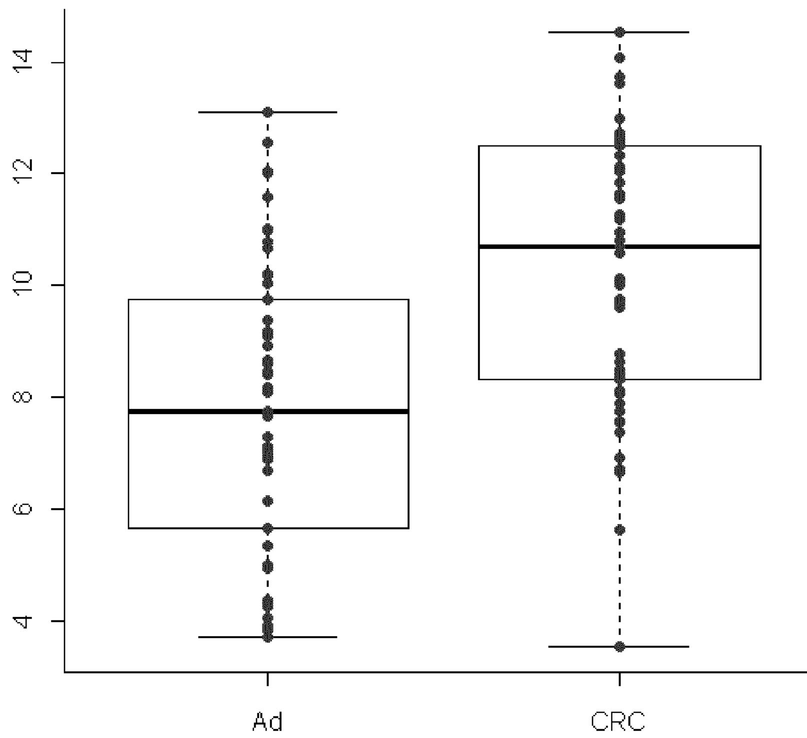


Fig. 17

A



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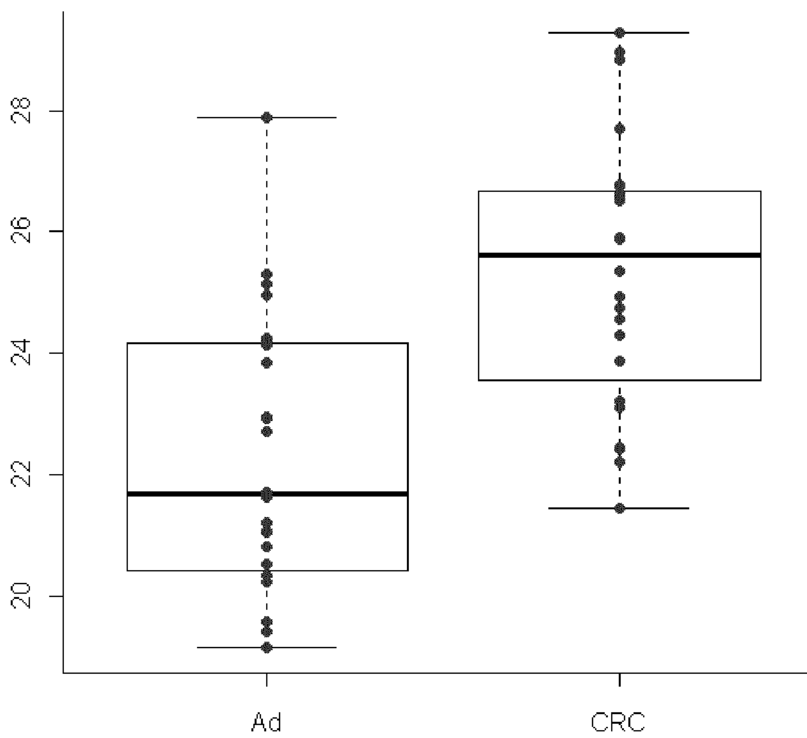
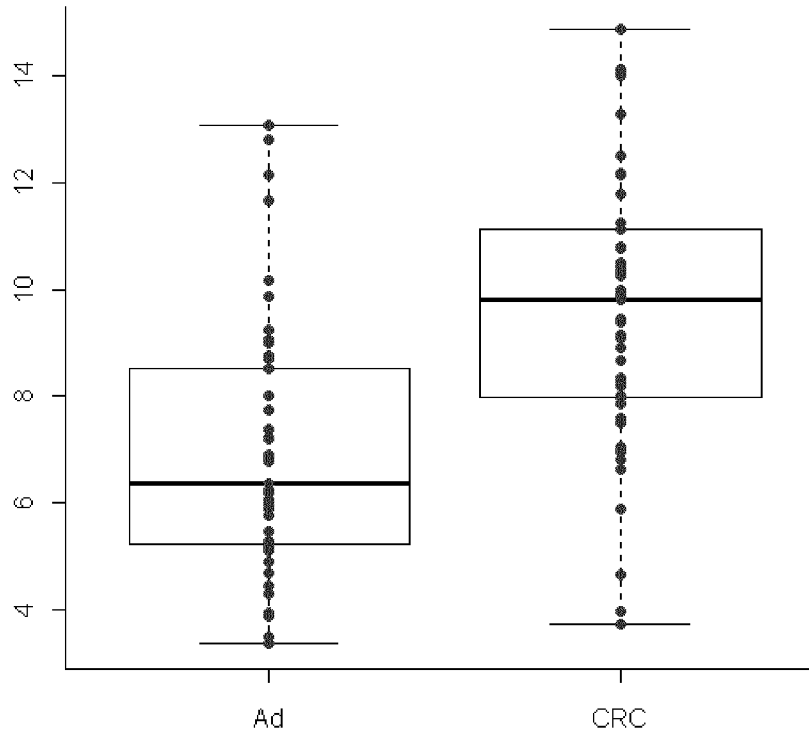


Fig. 18

A



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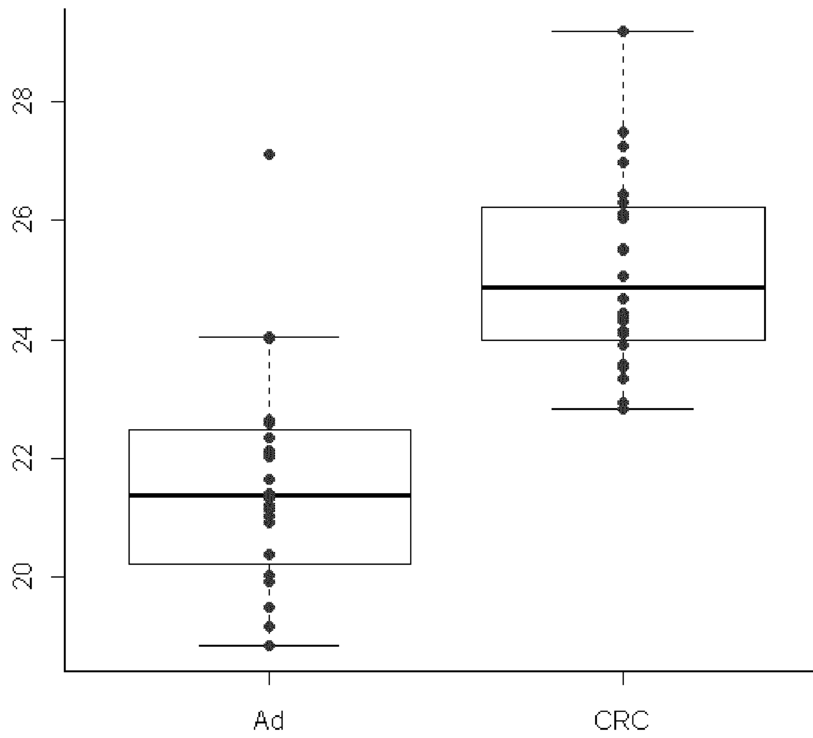
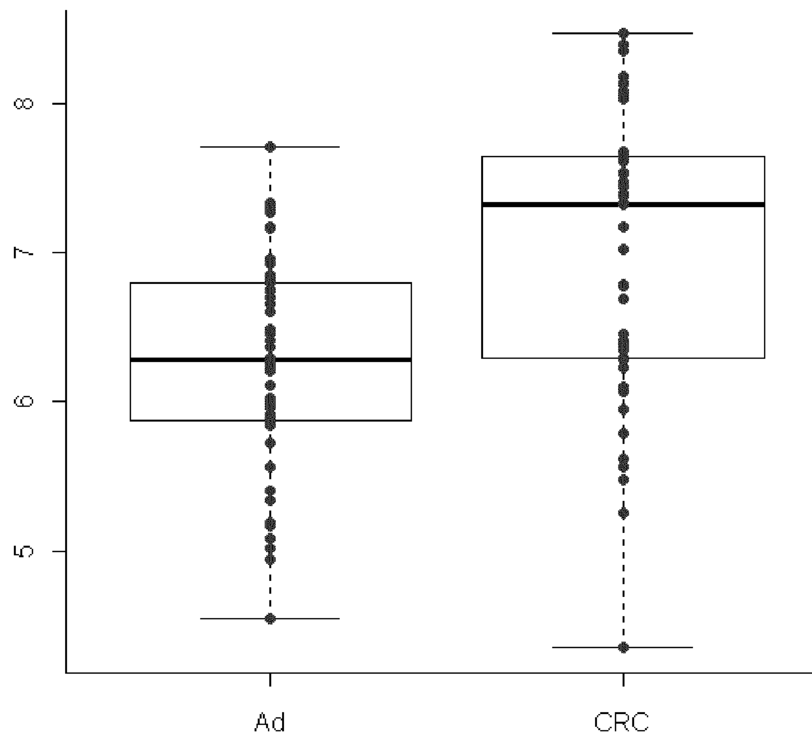


Fig. 19

A



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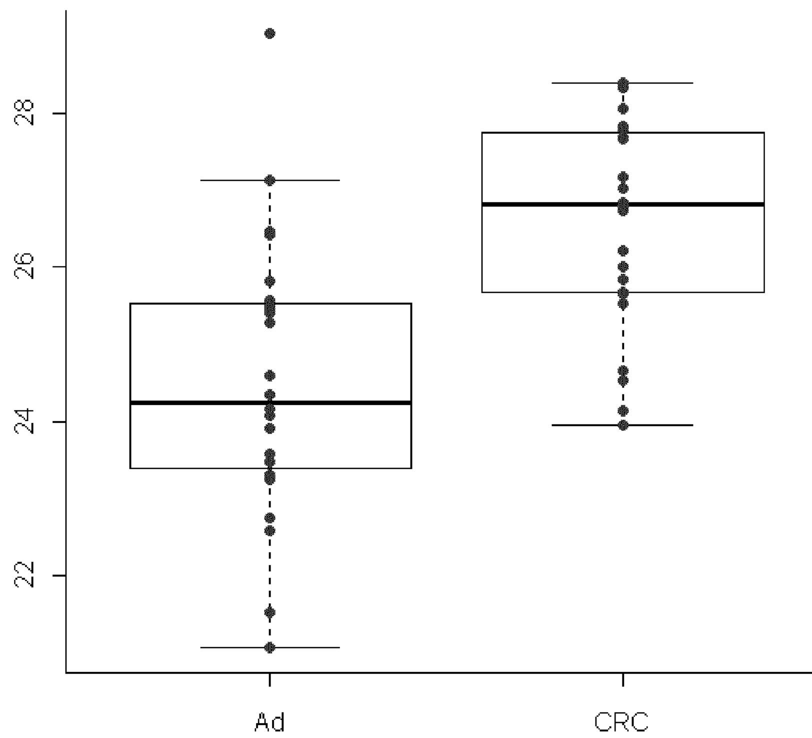
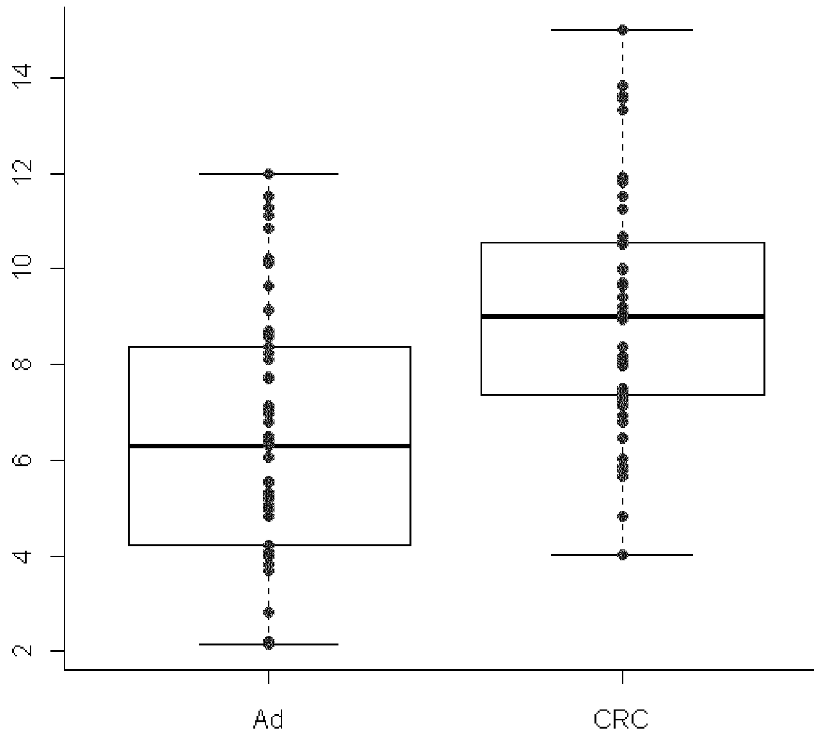


Fig. 20

A



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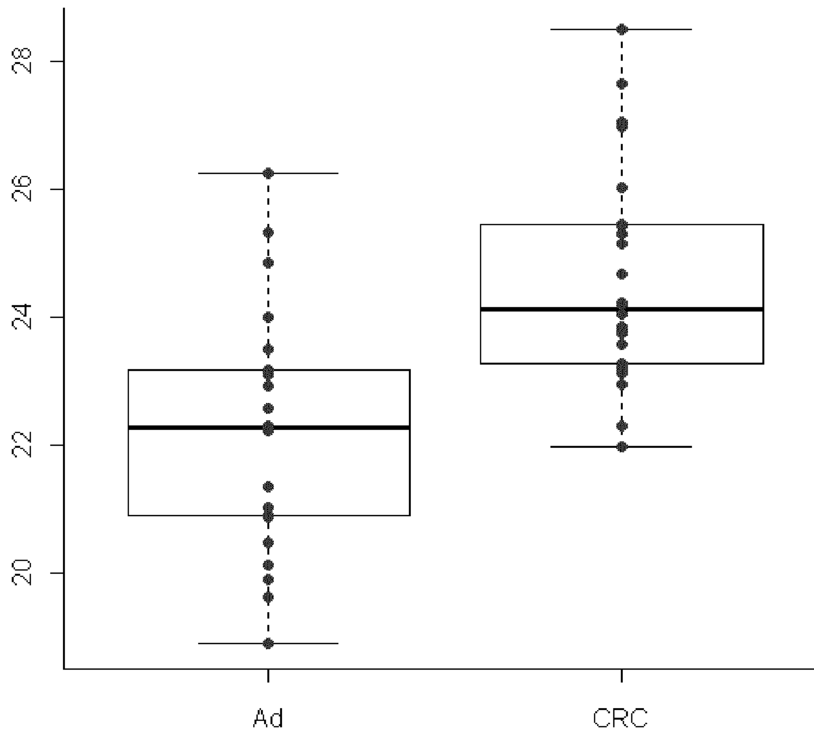
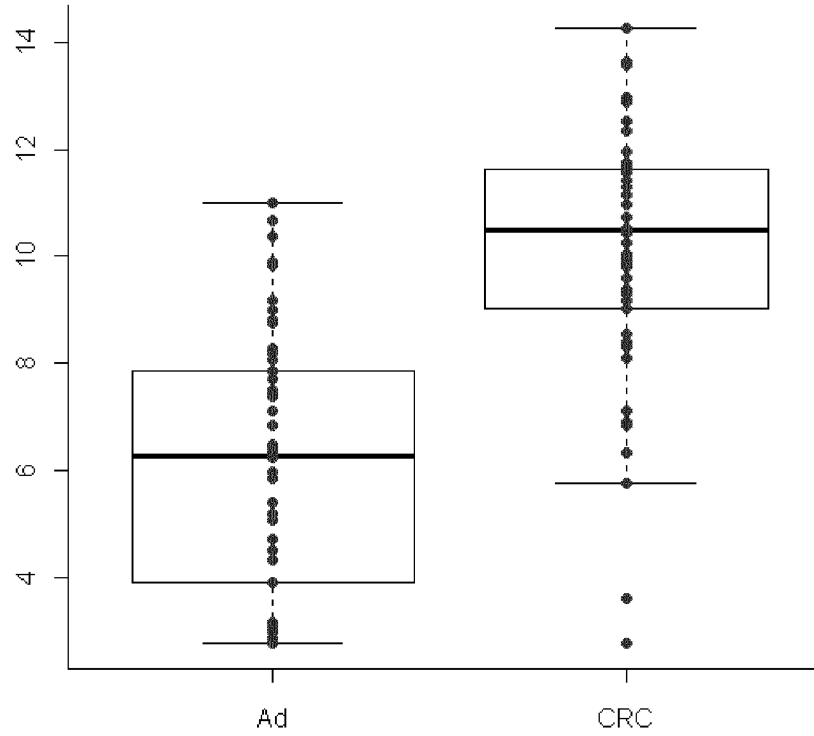


Fig. 21

A



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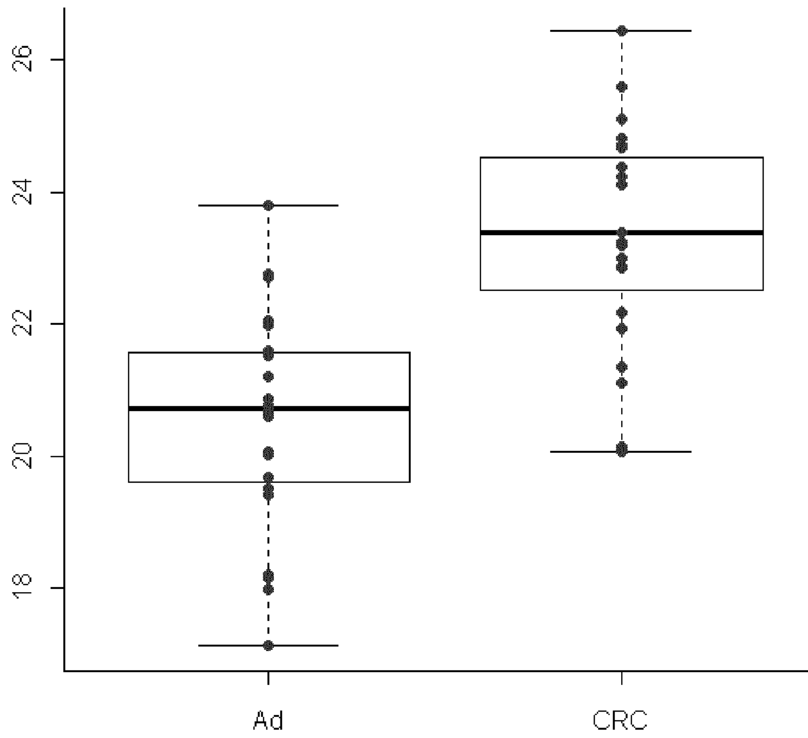

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2013/056515

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/68
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2009/233295 A1 (GEORGES ELIAS [CA] ET AL) 17 September 2009 (2009-09-17) abstract, [0016]-[0022] and whole document	6,8-10, 12-15
X	US 2011/020370 A1 (GEORGES ELIAS [CA] ET AL) 27 January 2011 (2011-01-27) abstract, claims 1-38 and th whole document	6,8-10, 12-15
X	WO 2008/079269 A2 (NIKOLSKY YURI [US]; BUGRIM ANDREJ [US]; NIKOLSKAYA TATIANA [US]) 3 July 2008 (2008-07-03) the whole document	1-15

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
25 June 2013	08/07/2013

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Lapopin, Laurence

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BIRKENKAMP-DEMTRODER K ET AL: "Differential gene expression in colon cancer of the caecum versus the sigmoid and rectosigmoid", GUT, vol. 54, no. 3, March 2005 (2005-03), pages 374-384, XP002683781, ISSN: 0017-5749 abstract, tab. 1 and 2, and the whole) -----	6,8-10, 12-15

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2013/056515

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2009233295 A1	17-09-2009	NONE	
US 2011020370 A1	27-01-2011	NONE	
WO 2008079269 A2	03-07-2008	EP 2094719 A2	02-09-2009
		RU 2009127709 A	27-01-2011
		US 2010216660 A1	26-08-2010
		WO 2008079269 A2	03-07-2008