(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
06 October 2022 (06.10.2022)

WIPO | PCT

(10) International Publication Number
WO 2022/207566 A1

(51) International Patent Classification:
C12Q 1/6886 (2018.01)

(21) International Application Number:
PCT/EP2022/058151

(22) International Filing Date:
28 March 2022 (28.03.2022)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
21305391.1 29 March 2021 (29.03.2021) EP

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*
— *in black and white; the international application as filed contained color or greyscale and is available for download from PATENTSCOPE*

(54) **Title:** NEW METHOD TO EVALUATE PANCREATIC CANCER PROGNOSIS

(57) **Abstract:** The present invention relates to the prediction of the survival time of a patient suffering of a pancreatic cancer. In this study, the inventors aimed to determine whether PDAC could be stratified on the basis of their GT gene expression profiles involved in the biosynthesis of glycoconjugates. By using bioinformatic analysis of RNA-seq data focused on 169 GT genes from patient-derived xenografts (PDX), they have identified a combination of 19 GT, which was able to discriminate 3 clusters of PDAC associated with specific molecular profiles and clinical features of patients. These GT genes were validated on public databases as a prognostic glyco-signature, which could allow best patient care in the future and also highlight new potential targets for diagnosis and prognosis of PDAC. Thus, the invention relates to a method for predicting the survival time of a patient suffering from a pancreatic cancer comprising i) determining in a sample obtained from the patient the expression level of at least one gene selected in the group consisting in GALNT9, A4GNT, B3GALT5, B3GNT6, C1GALT1, FUT2, FUT3, FUT4, FUT6, GALNT4, GALNT6, GALNT12, GCNT1, GYG2, LFNG, MGAT5, ST6GALNAC1, ST8SIA3 and XYLT.

WO 2022/207566 A1

NEW METHOD TO EVALUATE PANCREATIC CANCER PROGNOSIS

FIELD OF THE INVENTION:

The invention relates to a method for predicting the survival time of a patient suffering from a pancreatic cancer comprising i) determining in a sample obtained from the patient the expression level of at least one gene selected in the group consisting in GALNT9, A4GNT, B3GALT5, B3GNT6, C1GALT1, FUT2, FUT3, FUT4, FUT6, GALNT4, GALNT6, GALNT12, GCNT1, GYG2, LFNG, MGAT5, ST6GALNAC1, ST8SIA3 and XYLT1.

BACKGROUND OF THE INVENTION:

PDAC ranks seventh among the causes of cancer deaths (1 2) and is in path to become the second cause of global cancer mortality by 2030 (3). The 5-years survival rate is around 6% and varies depending on the cancer staging at the time of diagnosis. Despite advances in pancreatic cancer research field, PDAC is still characterised by a resistance to chemotherapies and presents a disparity in the response to treatments and survival depending mainly on the surgical management of the patient (4-6). The main cause of disparities in clinical evolution and resistance to conventional chemotherapies between patients is related to a significant molecular heterogeneity between their tumours. Therefore, stratification of PDAC appears as a favored strategy to improve patient care and allow the best clinical decision for precision medicine. Several studies have analysed gene expression of tumour and/or stroma from primary and metastatic PDAC by using mRNA expression microarray and RNA sequencing (RNA-seq); many molecular subtypes of PDAC have been proposed with potential prognostic relevance (7-12). Importantly, two clusters of PDAC corresponding to classical and basal-like subtypes are often highlighted with differences in overall survival (OS) and morphological patterns, constituting a well-established consensus.

Glycosylation in eukaryotic cells is the major post-translational modification of macromolecules and participate in the maturation and acquisition of their functions in the cell (13). This process involves mainly sequential actions of different families of glycosylation enzymes such as glycosyltransferases and glycosidases, whose expression and function are tightly regulated in each cell. These glyco-enzymes are able to produce numerous and various glycan chains from monosaccharides to form glycoproteins bearing mainly N- and O-linked glycans, glycolipids and proteoglycans. Glycosylation deregulation is one of the important mechanisms contributing to tumoral heterogeneity and is now widely accepted as one of the

hallmarks of cancer (14). A large number of studies have described significant changes in glycan chains of glycoconjugates and glycosylation processes in cancers (15 16). The appearance of aberrant glycosylated structures on tumour cells, but also on cells from the microenvironment, seems to be essential for malignant transformation, tumour growth, cell signaling, as well as cell adhesion and metastatic dissemination (17 18). Upstream, deregulation of this process occurs partly at the gene expression level of glycosyltransferases (GT): a deep modification of GT gene expression generates aberrant glycosylated antigens and deregulates the whole glycome of cancer cells contributing to their aggressive phenotype.

In PDAC, the most common changes in glycan structures affect glycosylated Lewis blood group antigens (19-21). The sialyl-Lewis x and sialyl-Lewis a antigens, whose biosynthesis is dependent from α1,3/4-fucosyltransferases (FUT) and α2,3-sialyltransferases (ST3GAL), are preferentially expressed on the surface of tumour cells, which, in the blood or lymphatic circulation, can be recognized by selectins, expressed at the membrane of endothelial cells and could thus promote the formation of metastases (22 23). The expression of truncated O-glycans such as Tn and sialyl-Tn antigens, described in many cancers, was also observed in PDAC (24 25). The expression of sialyl-Tn antigen is dependent on many GT such as polypeptide N-acetylgalactosaminyltransferase (GALNT) and α2,6-sialyltransferase (ST6GALNAC) families, but also on deregulated expression of chaperone and GT involved in the elongation of O-glycans such as COSMC, core 1 synthase glycoprotein-N-acetylgalactosamine 3-β-galactosyltransferase 1 (C1GALT1) or β1,6-N-acetylglucosaminyltransferases (GCNT1 or GCNT3). These truncated O-glycans could promote tumour growth and metastatic behavior (26).

SUMMARY OF THE INVENTION:

In a previous study, glycoprotein metabolism has been highlighted as one of the top biological processes significantly deregulated in PDAC (27) suggesting that relevant subtypes could be identified according to their aberrant glycosylation processes. In this study, the inventors aimed to determine whether PDAC could be stratified on the basis of their GT gene expression profiles involved in the biosynthesis of glycoconjugates. By using bioinformatic analysis of RNA-seq data focused on 169 GT genes from patient-derived xenografts (PDX), they have identified a combination of 19 GT, which was able to discriminate 3 clusters of PDAC associated with specific molecular profiles and clinical features of patients. These GT genes were validated on public databases as a prognostic glyco-signature, which could allow best

patient care in the future and also highlight new potential targets for diagnosis and prognosis of PDAC.

Thus, the invention relates to a method for predicting the survival time of a patient suffering from a pancreatic cancer comprising i) determining in a sample obtained from the patient the expression level of at least one gene selected in the group consisting in GALNT9, A4GNT, B3GALT5, B3GNT6, C1GALT1, FUT2, FUT3, FUT4, FUT6, GALNT4, GALNT6, GALNT12, GCNT1, GYG2, LFNG, MGAT5, ST6GALNAC1, ST8SIA3 and XYLT1. Particularly, the invention is defined by its claims.

DETAILED DESCRIPTION OF THE INVENTION:

Prognostic application

A first aspect of the invention relates to a method for predicting the survival time of a patient suffering from a pancreatic cancer comprising i) determining in a sample obtained from the patient the expression level of at least one gene selected in the group consisting in GALNT9, A4GNT, B3GALT5, B3GNT6, C1GALT1, FUT2, FUT3, FUT4, FUT6, GALNT4, GALNT6, GALNT12, GCNT1, GYG2, LFNG, MGAT5, ST6GALNAC1, ST8SIA3 and XYLT1 ii) comparing said expression level determined at step i) with their predetermined reference value and iii) providing a good prognosis when the expression level of A4GNT, B3GALT5, B3GNT6, C1GALT1, FUT2, FUT3, FUT4, FUT6, GALNT4, GALNT6, GALNT12, GCNT1, GYG2, LFNG, MGAT5, ST6GALNAC1, ST8SIA3 or XYLT1 determined at step i) is higher than their predetermined reference values, or providing a bad prognosis when the expression level of A4GNT, B3GALT5, B3GNT6, C1GALT1, FUT2, FUT3, FUT4, FUT6, GALNT4, GALNT6, GALNT12, GCNT1, GYG2, LFNG, MGAT5, ST6GALNAC1, ST8SIA3 or XYLT1 determined at step i) is lower than their predetermined reference values or providing a good prognosis when the expression level of GALNT9 determined at step i) is lower than its predetermined reference value, or providing a bad prognosis when the expression level of GALNT9 determined at step i) is higher than its predetermined reference value.

In a particular embodiment, the invention relates to a method for predicting the survival time of a patient suffering from a pancreatic cancer comprising i) determining in a sample obtained from the patient the expression level of the genes selected in the group consisting in A4GNT, B3GALT5, B3GNT6, C1GALT1, FUT2, FUT3, FUT4, FUT6, GALNT4, GALNT6, GALNT12, GCNT1, GYG2, LFNG, MGAT5, ST6GALNAC1, ST8SIA3 and XYLT1 ii)

comparing said expression level determined at step i) with their predetermined reference value and iii) providing a good prognosis when the expression level of A4GNT, B3GALT5, B3GNT6, C1GALT1, FUT2, FUT3, FUT4, FUT6, GALNT4, GALNT6, GALNT12, GCNT1, GYG2, LFNG, MGAT5, ST6GALNAC1, ST8SIA3 and XYLT1 determined at step i) is higher than its predetermined reference value, or providing a bad prognosis when the expression level of A4GNT, B3GALT5, B3GNT6, C1GALT1, FUT2, FUT3, FUT4, FUT6, GALNT4, GALNT6, GALNT12, GCNT1, GYG2, LFNG, MGAT5, ST6GALNAC1, ST8SIA3 and XYLT1 determined at step i) is lower than their predetermined reference value.

In a particular embodiment, the invention relates to a method for predicting the survival time of a patient suffering from a pancreatic cancer comprising i) determining in a sample obtained from the patient the expression of the gene GALNT9 ii) comparing said expression level determined at step i) with their predetermined reference value and iii) providing a good prognosis when the expression level of GALNT9 determined at step i) is lower than its predetermined reference value, or providing a bad prognosis when the expression level of GALNT9 determined at step i) is higher than its predetermined reference value.

In a particular embodiment, the invention relates to a method for predicting the survival time of a patient suffering from a pancreatic cancer comprising i) determining in a sample obtained from the patient the expression level of the genes selected in the group consisting in GALNT9, A4GNT, B3GALT5, B3GNT6, C1GALT1, FUT2, FUT3, FUT4, FUT6, GALNT4, GALNT6, GALNT12, GCNT1, GYG2, LFNG, MGAT5, ST6GALNAC1, ST8SIA3 and XYLT1 ii) comparing said expression level determined at step i) with their predetermined reference value and iii) providing a good prognosis when the expression level of A4GNT, B3GALT5, B3GNT6, C1GALT1, FUT2, FUT3, FUT4, FUT6, GALNT4, GALNT6, GALNT12, GCNT1, GYG2, LFNG, MGAT5, ST6GALNAC1, ST8SIA3 or XYLT1 determined at step i) is higher than its predetermined reference value and when the expression level of GALNT9 determined at step i) is lower than its predetermined reference value, or providing a bad prognosis when the expression level of A4GNT, B3GALT5, B3GNT6, C1GALT1, FUT2, FUT3, FUT4, FUT6, GALNT4, GALNT6, GALNT12, GCNT1, GYG2, LFNG, MGAT5, ST6GALNAC1, ST8SIA3 or XYLT1 determined at step i) is lower than their predetermined reference values and when the expression level of GALNT9 determined at step i) is higher than its predetermined reference value.

According to these particular embodiment, the sample can be blood, peripheral-blood, serum, plasma, tumoral circulating cells, tumor sample that is to say a sample obtained from the tumor or a biopsy obtained from the tumor. According to the invention, the tumoral sample can be a pancreatic tumor sample that is to say a sample obtained from the pancreatic tumor or a biopsy obtained from a pancreatic tumor. The sample can be obtained from a resected and unresectable pancreatic tumour.

According to the invention, the method of the invention can also be useful to stratify patients in function of the gravity of their pancreatic tumors. As used herein, the term "stratify" consists in, depending on the level of expression of the genes according to the invention, classifying the patients with a good or bad prognosis between them and in adapting the treatment to the patient according to their biological characteristics.

Thus the invention also relates to a method for stratifying a patient suffering from a pancreatic cancer comprising i) determining in a sample obtained from the patient the expression level of at least one gene selected in the group consisting in GALNT9, A4GNT, B3GALT5, B3GNT6, C1GALT1, FUT2, FUT3, FUT4, FUT6, GALNT4, GALNT6, GALNT12, GCNT1, GYG2, LFNG, MGAT5, ST6GALNAC1, ST8SIA3 and XYLT1 ii) comparing said expression level determined at step i) with their predetermined reference value and iii) providing a good prognosis when the expression level of A4GNT, B3GALT5, B3GNT6, C1GALT1, FUT2, FUT3, FUT4, FUT6, GALNT4, GALNT6, GALNT12, GCNT1, GYG2, LFNG, MGAT5, ST6GALNAC1, ST8SIA3 or XYLT1 determined at step i) is higher than their predetermined reference values, or providing a bad prognosis when the expression level of A4GNT, B3GALT5, B3GNT6, C1GALT1, FUT2, FUT3, FUT4, FUT6, GALNT4, GALNT6, GALNT12, GCNT1, GYG2, LFNG, MGAT5, ST6GALNAC1, ST8SIA3 or XYLT1 determined at step i) is lower than their predetermined reference values or providing a good prognosis when the expression level of GALNT9 determined at step i) is lower than its predetermined reference value, or providing a bad prognosis when the expression level of GALNT9 determined at step i) is higher than its predetermined reference value.

In one embodiment, the pancreatic cancer is a stage T1 pancreatic cancer, a stage T2 pancreatic cancer, a stage T3 pancreatic cancer or a stage T4 pancreatic cancer according to the UICC-TNM classification.

In another embodiment, the pancreatic cancer is a pancreatic ductal adenocarcinoma (PDAC), a pancreatic adenocarcinoma, a pancreatic serous cystadenomas (SCNs), a pancreatic

intraepithelial neoplasia (PanIN), pancreatic mucinous cystic neoplasms (MCNs), a non-resectable pancreatic ductal adenocarcinoma (PDAC) or a non-resectable pancreatic adenocarcinoma.

As used herein, the term "patient" denotes a human with a pancreatic cancer according to the invention.

As used herein, the term "survival time" denotes the percentage of people in a study or treatment group who are still alive for a certain period of time after they were diagnosed with or started treatment for a disease, such as pancreatic cancer (according to the invention). The survival time rate is often stated as a five-year survival rate, which is the percentage of people in a study or treatment group who are alive five years after their diagnosis or the start of treatment.

As used herein and according to the invention, the term "survival time" can regroup the term OS.

As used herein, the term "Overall survival (OS)" denotes the time from diagnosis of a disease such as pancreatic cancer (according to the invention) until death from any cause. The overall survival rate is often stated as a two-year survival rate, which is the percentage of people in a study or treatment group who are alive two years after their diagnosis or the start of treatment.

Gene symbol	**Gene name**	**Glycan synthesis / Function**	**Entrez (NCBI) Gene reference number**
GALNT9	Polypeptide N-acetylgalactosaminyltransferase 9	Transfers a GalNAc residue to serine or threonine residue of an acceptor core protein. Initiation of mucin-type O-linked protein glycosylation	50614
A4GNT	Alpha-1,4-N-acetylglucosaminyltransferase	Transfers an alpha-1,4-GlcNAc residue onto core 2	51146

		branched O-glycans. Biosynthesis of mucin-type O-glycan	
B3GALT5	Beta-1,3-galactosyltransferase 5	Transfers a beta-1,3-Gal residue to GlcNAc-based acceptors such as the core 3 O-glycan. Biosynthesis of type 1 Lewis antigens	10317
B3GNT6	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 6	Transfers a beta-1,3-GlcNAc residue to GalNAc-serine or -threonine. Biosynthesis of mucin-type core 3 O-glycan	192134
C1GALT1	Core 1 synthase, glycoprotein-N-acetylgalactosamine 3-beta-galactosyltransferase 1	Transfers a beta-1,3-Gal residue to O-linked GalNAc residue onto protein. Biosynthesis of mucin-type core 1 O-glycan	56913
FUT2	Fucosyltransferase 2	Transfers an alpha-1,2-Fuc residue to terminal Gal-based acceptors. Lewis and ABO blood group antigen biosynthesis	2524
FUT3	Fucosyltransferase 3 (Lewis blood group)	Transfers an alpha-1,3- or alpha-1,4-Fuc residue to GlcNAc-based acceptors. Last step of Lewis blood group antigen biosynthesis	2525
FUT4	Fucosyltransferase 4	Transfers an alpha-1,3-Fuc residue to GlcNAc-based	2526

		acceptors. Lewis x (CD15) antigen biosynthesis	
FUT6	Fucosyltransferase 6	Transfers an alpha-1,3-Fuc residue to GlcNAc of alpha-2,3 sialylated substrates. Sialyl-Lewis x antigen biosynthesis	2528
GALNT4	Polypeptide N-acetylgalactosaminyltransferase 4	Transfers a GalNAc residue to serine or threonine residue of an acceptor core protein. Initiation of mucin-type O-linked protein glycosylation	8693
GALNT6	Polypeptide N-acetylgalactosaminyltransferase 6	Transfers a GalNAc residue to serine or threonine residue of an acceptor core protein. Initiation of mucin-type O-linked protein glycosylation	11226
GALNT12	Polypeptide N-acetylgalactosaminyltransferase 12	Transfers a GalNAc residue to serine or threonine residue of an acceptor core protein. Initiation of mucin-type O-linked protein glycosylation	79695
GCNT1	Glucosaminyl (N-acetyl) transferase 1	Transfers a beta-1,6-GlcNAc residue onto mucin-type core 1 O-glycan. Biosynthesis of mucin-type core 2 branched O-glycan	2650

GYG2	Glycogenin 2	Self-glucosylation (Glc). Initiation reaction of glycogen biosynthesis	8908
LFNG	LFNG O-fucosylpeptide	Transfers a beta-1,3-GlcNAc residue to O-linked fucose residue onto Notch molecules. Regulation of Notch molecules activity (Notch signaling pathway)	3955
MGAT5	Alpha-1,6-mannosylglycoprotein 6-beta-N-acetylglucosaminyltransferase	Transfers a beta-1,6-GlcNAc residue to mannose of biantennary N-linked glycan present onto glycoproteins. Biosynthesis of tri- and tetra-antennary complexe N-glycans	4249
ST6GALNAC1	ST6 N-acetylgalactosaminide alpha-2,6-sialyltransferase 1	Transfers an alpha-2,6-NeuAc residue to O-linked GalNAc residues onto protein. Biosynthesis of cancer-associated sialyl-Tn antigen	55808
ST8SIA3	ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 3	Transfers an alpha-2,8-NeuAc residue to terminal NeuAc of glycolipids and N-linked glycan of glycoproteins. Biosynthesis of polysialic acid chains	51046
XYLT1	Xylosyltransferase 1	Transfers a Xyl residue to a serine residue of an acceptor core protein.	64131

		Biosynthesis of glycosaminoglycan chains	

Table1: list of the 19 GT genes of the invention use as prognostic markers (here "the genes of the invention"). Abreviations used: Gal : Galactose ; Fuc : Fucose ; Glc : Glucose ; Xyl : Xylose ; GalNAc : N-acetylgalactosamine ; GlcNAc : N-acetylglucosamine ; NeuAc : N-acetylneuraminic acid or sialic acid.

Measuring the expression level of the genes of the invention (see the table 1) can be done by measuring the gene expression level of them or by measuring the level of the protein of these genes and can be performed by a variety of techniques well known in the art.

Typically, the expression level of a gene may be determined by determining the quantity of mRNA. Methods for determining the quantity of mRNA are well known in the art. For example the nucleic acid contained in the samples (e.g., cell or tissue prepared from the patient) is first extracted according to standard methods, for example using lytic enzymes or chemical solutions or extracted by nucleic-acid-binding resins following the manufacturer's instructions. The extracted mRNA is then detected by hybridization (e. g., Northern blot analysis, in situ hybridization) and/or amplification (e.g., RT-qPCR).

Other methods of Amplification include ligase chain reaction (LCR), transcription-mediated amplification (TMA), strand displacement amplification (SDA) and nucleic acid sequence based amplification (NASBA).

Nucleic acids having at least 10 nucleotides and exhibiting sequence complementarity or homology to the mRNA of interest herein find utility as hybridization probes or amplification primers. It is understood that such nucleic acids need not be identical, but are typically at least about 80% identical to the homologous region of comparable size, more preferably 85% identical and even more preferably 90-95% identical. In certain embodiments, it will be advantageous to use nucleic acids in combination with appropriate means, such as a detectable label, for detecting hybridization.

Typically, the nucleic acid probes include one or more labels, for example to permit detection of a target nucleic acid molecule using the disclosed probes. In various applications, such as in situ hybridization procedures, a nucleic acid probe includes a label (e.g., a detectable label). A "detectable label" is a molecule or material that can be used to produce a detectable signal that indicates the presence or concentration of the probe (particularly the bound or hybridized probe) in a sample. Thus, a labeled nucleic acid molecule provides an indicator of the presence or concentration of a target nucleic acid sequence (e.g., genomic target nucleic

acid sequence) (to which the labeled uniquely specific nucleic acid molecule is bound or hybridized) in a sample. A label associated with one or more nucleic acid molecules (such as a probe generated by the disclosed methods) can be detected either directly or indirectly. A label can be detected by any known or yet to be discovered mechanism including absorption, emission and/ or scattering of a photon (including radio frequency, microwave frequency, infrared frequency, visible frequency and ultra-violet frequency photons). Detectable labels include colored, fluorescent, phosphorescent and luminescent molecules and materials, catalysts (such as enzymes) that convert one substance into another substance to provide a detectable difference (such as by converting a colorless substance into a colored substance or vice versa, or by producing a precipitate or increasing sample turbidity), haptens that can be detected by antibody binding interactions, and paramagnetic and magnetic molecules or materials.

Particular examples of detectable labels include fluorescent molecules (or fluorochromes). Numerous fluorochromes are known to those of skill in the art, and can be selected, for example from Life Technologies (formerly Invitrogen), e.g., see, The Handbook—A Guide to Fluorescent Probes and Labeling Technologies). Examples of particular fluorophores that can be attached (for example, chemically conjugated) to a nucleic acid molecule (such as a uniquely specific binding region) are provided in U.S. Pat. No. 5,866, 366 to Nazarenko et al., such as 4-acetamido-4'-isothiocyanatostilbene-2,2' disulfonic acid, acridine and derivatives such as acridine and acridine isothiocyanate, 5-(2'-aminoethyl) aminonaphthalene-1-sulfonic acid (EDANS), 4-amino -N- [3 vinylsulfonyl)phenyl]naphthalimide-3,5 disulfonate (Lucifer Yellow VS), N-(4-anilino-1-naphthyl)maleimide, antl1ranilamide, Brilliant Yellow, coumarin and derivatives such as coumarin, 7-amino-4-methylcoumarin (AMC, Coumarin 120), 7-amino-4-trifluoromethylcouluarin (Coumarin 151); cyanosine; 4',6-diarninidino-2-phenylindole (DAPI); 5',5"dibromopyrogallol-sulfonephthalein (Bromopyrogallol Red); 7 -diethylamino -3 - (4'-isothiocyanatophenyl)-4-methylcoumarin; diethylenetriamine pentaacetate; 4,4'-diisothiocyanatodihydro-stilbene-2,2'-disulfonic acid; 4,4'-diisothiocyanatostilbene-2,2'-disulfor1ic acid; 5-[dimethylamino] naphthalene-1-sulfonyl chloride (DNS, dansyl chloride); 4-(4'-dimethylaminophenylazo)benzoic acid (DABCYL); 4-dimethylaminophenylazophenyl-4'-isothiocyanate (DABITC); eosin and derivatives such as eosin and eosin isothiocyanate; erythrosin and derivatives such as erythrosin B and erythrosin isothiocyanate; ethidium; fluorescein and derivatives such as 5-carboxyfluorescein (FAM), 5-(4,6dicl1lorotriazin-2-yDarninofluorescein (DTAF), 2'7'dimethoxy-4'5'-dichloro-6-carboxyfluorescein (JOE),

fluorescein, fluorescein isothiocyanate (FITC), and QFITC Q(RITC); 2',7'-difluorofluorescein (OREGON GREEN®); fluorescamine; IR144; IR1446; Malachite Green isothiocyanate; 4-methylumbelliferone; ortho cresolphthalein; nitrotyrosine; pararosaniline; Phenol Red; B-phycoerythrin; o-phthaldialdehyde; pyrene and derivatives such as pyrene, pyrene butyrate and succinimidyl 1-pyrene butyrate; Reactive Red 4 (Cibacron Brilliant Red 3B-A); rhodamine and derivatives such as 6-carboxy-X-rhodamine (ROX), 6-carboxyrhodamine (R6G), lissamine rhodamine B sulfonyl chloride, rhodamine (Rhod), rhodamine B, rhodamine 123, rhodamine X isothiocyanate, rhodamine green, sulforhodamine B, sulforhodamine 101 and sulfonyl chloride derivative of sulforhodamine 101 (Texas Red); N,N,N',N'-tetramethyl-6-carboxyrhodamine (TAMRA); tetramethyl rhodamine; tetramethyl rhodamine isothiocyanate (TRITC); riboflavin; rosolic acid and terbium chelate derivatives. Other suitable fluorophores include thiol-reactive europium chelates which emit at approximately 617 nm (Heyduk and Heyduk, Analyt. Biochem. 248:216-27, 1997; J. Biol. Chem. 274:3315-22, 1999), as well as GFP, LissamineTM, diethylaminocoumarin, fluorescein chlorotriazinyl, naphthofluorescein, 4,7-dichlororhodamine and xanthene (as described in U.S. Pat. No. 5,800,996 to Lee et al.) and derivatives thereof. Other fluorophores known to those skilled in the art can also be used, for example those available from Life Technologies (Invitrogen; Molecular Probes (Eugene, Oreg.)) and including the ALEXA FLUOR® series of dyes (for example, as described in U.S. Pat. Nos. 5,696,157, 6, 130, 101 and 6,716,979), the BODIPY series of dyes (dipyrromethene boron difluoride dyes, for example as described in U.S. Pat. Nos. 4,774,339, 5,187,288, 5,248,782, 5,274,113, 5,338,854, 5,451,663 and 5,433,896), Cascade Blue (an amine reactive derivative of the sulfonated pyrene described in U.S. Pat. No. 5,132,432) and Marina Blue (U.S. Pat. No. 5,830,912).

In addition to the fluorochromes described above, a fluorescent label can be a fluorescent nanoparticle, such as a semiconductor nanocrystal, e.g., a QUANTUM DOTTM (obtained, for example, from Life Technologies (QuantumDot Corp, Invitrogen Nanocrystal Technologies, Eugene, Oreg.); see also, U.S. Pat. Nos. 6,815,064; 6,682,596; and 6,649, 138). Semiconductor nanocrystals are microscopic particles having size-dependent optical and/or electrical properties. When semiconductor nanocrystals are illuminated with a primary energy source, a secondary emission of energy occurs of a frequency that corresponds to the handgap of the semiconductor material used in the semiconductor nanocrystal. This emission can he detected as colored light of a specific wavelength or fluorescence. Semiconductor nanocrystals with different spectral characteristics are described in e.g., U.S. Pat. No. 6,602,671. Semiconductor nanocrystals that can he coupled to a variety of biological molecules (including

dNTPs and/or nucleic acids) or substrates by techniques described in, for example, Bruchez et al., Science 281 :20132016, 1998; Chan et al., Science 281:2016-2018, 1998; and U.S. Pat. No. 6,274,323. Formation of semiconductor nanocrystals of various compositions are disclosed in, e.g., U.S. Pat. Nos. 6,927, 069; 6,914,256; 6,855,202; 6,709,929; 6,689,338; 6,500,622; 6,306,736; 6,225,198; 6,207,392; 6,114,038; 6,048,616; 5,990,479; 5,690,807; 5,571,018; 5,505,928; 5,262,357 and in U.S. Patent Publication No. 2003/0165951 as well as PCT Publication No. 99/26299 (published May 27, 1999). Separate populations of semiconductor nanocrystals can he produced that are identifiable based on their different spectral characteristics. For example, semiconductor nanocrystals can he produced that emit light of different colors based on their composition, size or size and composition. For example, quantum dots that emit light at different wavelengths based on size (565 nm, 655 nm, 705 nm, or 800 nm emission wavelengths), which are suitable as fluorescent labels in the probes disclosed herein are available from Life Technologies (Carlshad, Calif.).

Additional labels include, for example, radioisotopes (such as 3 H), metal chelates such as DOTA and DPTA chelates of radioactive or paramagnetic metal ions like Gd3+, and liposomes.

Detectable labels that can he used with nucleic acid molecules also include enzymes, for example horseradish peroxidase, alkaline phosphatase, acid phosphatase, glucose oxidase, beta-galactosidase, beta-glucuronidase, or beta-lactamase.

Alternatively, an enzyme can he used in a metallographic detection scheme. For example, silver in situ hyhridization (SISH) procedures involve metallographic detection schemes for identification and localization of a hybridized genomic target nucleic acid sequence. Metallographic detection methods include using an enzyme, such as alkaline phosphatase, in combination with a water-soluble metal ion and a redox-inactive substrate of the enzyme. The substrate is converted to a redox-active agent by the enzyme, and the redoxactive agent reduces the metal ion, causing it to form a detectable precipitate. (See, for example, U.S. Patent Application Publication No. 2005/0100976, PCT Publication No. 2005/ 003777 and U.S. Patent Application Publication No. 2004/ 0265922). Metallographic detection methods also include using an oxido-reductase enzyme (such as horseradish peroxidase) along with a water soluble metal ion, an oxidizing agent and a reducing agent, again to form a detectable precipitate. (See, for example, U.S. Pat. No. 6,670,113).

Probes made using the disclosed methods can be used for nucleic acid detection, such as ISH procedures (for example, fluorescence in situ hybridization (FISH), chromogenic in situ

hybridization (CISH) and silver in situ hybridization (SISH)) or comparative genomic hybridization (CGH).

In situ hybridization (ISH) involves contacting a sample containing target nucleic acid sequence (e.g., genomic target nucleic acid sequence) in the context of a metaphase or interphase chromosome preparation (such as a cell or tissue sample mounted on a slide) with a labeled probe specifically hybridizable or specific for the target nucleic acid sequence (e.g., genomic target nucleic acid sequence). The slides are optionally pretreated, e.g., to remove paraffin or other materials that can interfere with uniform hybridization. The sample and the probe are both treated, for example by heating to denature the double stranded nucleic acids. The probe (formulated in a suitable hybridization buffer) and the sample are combined, under conditions and for sufficient time to permit hybridization to occur (typically to reach equilibrium). The chromosome preparation is washed to remove excess probe, and detection of specific labeling of the chromosome target is performed using standard techniques.

For example, a biotinylated probe can be detected using fluorescein-labeled avidin or avidin-alkaline phosphatase. For fluorochrome detection, the fluorochrome can be detected directly, or the samples can be incubated, for example, with fluorescein isothiocyanate (FITC)-conjugated avidin. Amplification of the FITC signal can be effected, if necessary, by incubation with biotin-conjugated goat antiavidin antibodies, washing and a second incubation with FITC-conjugated avidin. For detection by enzyme activity, samples can be incubated, for example, with streptavidin, washed, incubated with biotin-conjugated alkaline phosphatase, washed again and pre-equilibrated (e.g., in alkaline phosphatase (AP) buffer). For a general description of in situ hybridization procedures, see, e.g., U.S. Pat. No. 4,888,278.

Numerous procedures for FISH, CISH, and SISH are known in the art. For example, procedures for performing FISH are described in U.S. Pat. Nos. 5,447,841; 5,472,842; and 5,427,932; and for example, in Pir1kel et al., Proc. Natl. Acad. Sci. 83:2934-2938, 1986; Pinkel et al., Proc. Natl. Acad. Sci. 85:9138-9142, 1988; and Lichter et al., Proc. Natl. Acad. Sci. 85:9664-9668, 1988. CISH is described in, e.g., Tanner et al., Am.1. Pathol. 157:1467-1472, 2000 and U.S. Pat. No. 6,942,970. Additional detection methods are provided in U.S. Pat. No. 6,280,929.

Numerous reagents and detection schemes can be employed in conjunction with FISH, CISH, and SISH procedures to improve sensitivity, resolution, or other desirable properties. As discussed above probes labeled with fluorophores (including fluorescent dyes and QUANTUM DOTS®) can be directly optically detected when performing FISH. Alternatively, the probe can be labeled with a nonfluorescent molecule, such as a hapten (such as the following non-

limiting examples: biotin, digoxigenin, DNP, and various oxazoles, pyrrazoles, thiazoles, nitroaryls, benzofurazans, triterpenes, ureas, thioureas, rotenones, coumarin, courmarin-based compounds, Podophyllotoxin, Podophyllotoxin-based compounds, and combinations thereof), ligand or other indirectly detectable moiety. Probes labeled with such non-fluorescent molecules (and the target nucleic acid sequences to which they bind) can then be detected by contacting the sample (e.g., the cell or tissue sample to which the probe is bound) with a labeled detection reagent, such as an antibody (or receptor, or other specific binding partner) specific for the chosen hapten or ligand. The detection reagent can be labeled with a fluorophore (e.g., QUANTUM DOT®) or with another indirectly detectable moiety, or can be contacted with one or more additional specific binding agents (e.g., secondary or specific antibodies), which can be labeled with a fluorophore.

In other examples, the probe, or specific binding agent (such as an antibody, e.g., a primary antibody, receptor or other binding agent) is labeled with an enzyme that is capable of converting a fluorogenic or chromogenic composition into a detectable fluorescent, colored or otherwise detectable signal (e.g., as in deposition of detectable metal particles in SISH). As indicated above, the enzyme can be attached directly or indirectly via a linker to the relevant probe or detection reagent. Examples of suitable reagents (e.g., binding reagents) and chemistries (e.g., linker and attachment chemistries) are described in U.S. Patent Application Publication Nos. 2006/0246524; 2006/0246523, and 2007/ 01 17153.

It will be appreciated by those of skill in the art that by appropriately selecting labelled probe-specific binding agent pairs, multiplex detection schemes can he produced to facilitate detection of multiple target nucleic acid sequences (e.g., genomic target nucleic acid sequences) in a single assay (e.g., on a single cell or tissue sample or on more than one cell or tissue sample). For example, a first probe that corresponds to a first target sequence can he labelled with a first hapten, such as biotin, while a second probe that corresponds to a second target sequence can be labelled with a second hapten, such as DNP. Following exposure of the sample to the probes, the bound probes can he detected by contacting the sample with a first specific binding agent (in this case avidin labelled with a first fluorophore, for example, a first spectrally distinct QUANTUM DOT®, e.g., that emits at 585 nm) and a second specific binding agent (in this case an anti-DNP antibody, or antibody fragment, labelled with a second fluorophore (for example, a second spectrally distinct QUANTUM DOT®, e.g., that emits at 705 nm). Additional probes/binding agent pairs can he added to the multiplex detection scheme using other spectrally distinct fluorophores. Numerous variations of direct, and indirect (one step, two

step or more) can he envisioned, all of which are suitable in the context of the disclosed probes and assays.

Probes typically comprise single-stranded nucleic acids of between 10 to 1000 nucleotides in length, for instance of between 10 and 800, more preferably of between 15 and 700, typically of between 20 and 500. Primers typically are shorter single-stranded nucleic acids, of between 10 to 25 nucleotides in length, designed to perfectly or almost perfectly match a nucleic acid of interest, to be amplified. The probes and primers are "specific" to the nucleic acids they hybridize to, i.e. they preferably hybridize under high stringency hybridization conditions (corresponding to the highest melting temperature Tm, e.g., 50 % formamide, 5x or 6x SCC. SCC is a 0.15 M NaCl, 0.015 M Na-citrate).

The nucleic acid primers or probes used in the above amplification and detection method may be assembled as a kit. Such a kit includes consensus primers and molecular probes. A preferred kit also includes the components necessary to determine if amplification has occurred. The kit may also include, for example, PCR buffers and enzymes; positive control sequences, reaction control primers; and instructions for amplifying and detecting the specific sequences.

In a particular embodiment, the methods of the invention comprise the steps of providing total RNAs extracted from cumulus cells and subjecting the RNAs to amplification and hybridization to specific probes, more particularly by means of a quantitative or semi-quantitative RT-PCR (or q RT-PCR).

In another preferred embodiment, the expression level is determined by DNA chip analysis. Such DNA chip or nucleic acid microarray consists of different nucleic acid probes that are chemically attached to a substrate, which can be a microchip, a glass slide or a microsphere-sized bead. A microchip may be constituted of polymers, plastics, resins, polysaccharides, silica or silica-based materials, carbon, metals, inorganic glasses, or nitrocellulose. Probes comprise nucleic acids such as cDNAs or oligonucleotides that may be about 10 to about 60 base pairs. To determine the expression level, a sample from a test subject, optionally first subjected to a reverse transcription, is labelled and contacted with the microarray in hybridization conditions, leading to the formation of complexes between target nucleic acids that are complementary to probe sequences attached to the microarray surface. The labelled hybridized complexes are then detected and can be quantified or semi-quantified. Labelling may be achieved by various methods, e.g. by using radioactive or fluorescent labelling. Many variants of the microarray hybridization technology are available to the man skilled in the art (see e.g. the review by Hoheisel, Nature Reviews, Genetics, 2006, 7:200-210).

Expression level of a gene may be expressed as absolute expression level or normalized expression level. Typically, expression levels are normalized by correcting the absolute expression level of a gene by comparing its expression to the expression of a gene that is not a relevant for determining the cancer stage of the patient, e.g., a housekeeping gene that is constitutively expressed. Suitable genes for normalization include housekeeping genes such as the actin gene ACTB, ribosomal 18S gene, GUSB, PGK1, TFRC, GAPDH, TBP and ABL1. This normalization allows the comparison of the expression level in one sample, e.g., a patient sample, to another sample, or between samples from different sources.

According to the invention, the level of the proteins of the genes of the invention (here "the proteins of the invention") may also be measured and can be performed by a variety of techniques well known in the art. For measuring the expression level of the proteins of the invention, techniques like ELISA (see below) allowing to measure the level of the soluble proteins are particularly suitable.

In the present application, the "level of protein" or the "protein level expression" or the "protein concentration" means the quantity or concentration of said protein. In another embodiment, the "level of protein" means the level of the proteins fragments. In still another embodiment, the "level of protein" means the quantitative measurement of the proteins of the invention expression relative to a negative control.

According to the invention, the proteins of the invention may be measured at the surface of the tumor cells or in an extracellular context (for example in blood or plasma).

Typically protein concentration may be measured for example by capillary electrophoresis-mass spectroscopy technique (CE-MS) or ELISA performed on the sample.

Such methods comprise contacting a sample with a binding partner capable of selectively interacting with proteins present in the sample. The binding partner is generally an antibody that may be polyclonal or monoclonal, preferably monoclonal.

The presence of the protein can be detected using standard electrophoretic and immunodiagnostic techniques, including immunoassays such as competition, direct reaction, or sandwich type assays. Such assays include, but are not limited to, Western blots; agglutination tests; enzyme-labeled and mediated immunoassays, such as ELISAs; biotin/avidin type assays; radioimmunoassays; immunoelectrophoresis; immunoprecipitation, capillary electrophoresis-mass spectroscopy technique (CE-MS).etc. The reactions generally include revealing labels such as fluorescent, chemioluminescent, radioactive, enzymatic labels or dye molecules, or

other methods for detecting the formation of a complex between the antigen and the antibody or antibodies reacted therewith.

The aforementioned assays generally involve separation of unbound protein in a liquid phase from a solid phase support to which antigen-antibody complexes are bound. Solid supports which can be used in the practice of the invention include substrates such as nitrocellulose (e. g., in membrane or microtiter well form); polyvinylchloride (e. g., sheets or microtiter wells); polystyrene latex (e.g., beads or microtiter plates); polyvinylidine fluoride; diazotized paper; nylon membranes; activated beads, magnetically responsive beads, and the like.

More particularly, an ELISA method can be used, wherein the wells of a microtiter plate are coated with a set of antibodies against the proteins to be tested. A sample containing or suspected of containing the marker protein is then added to the coated wells. After a period of incubation sufficient to allow the formation of antibody-antigen complexes, the plate(s) can be washed to remove unbound moieties and a detectably labeled secondary binding molecule is added. The secondary binding molecule is allowed to react with any captured sample marker protein, the plate is washed and the presence of the secondary binding molecule is detected using methods well known in the art.

Methods of the invention may comprise a step consisting of comparing the proteins and fragments concentration in circulating cells with a control value. As used herein, "concentration of protein" refers to an amount or a concentration of a transcription product, for the proteins of the invention. Typically, a level of a protein can be expressed as nanograms per microgram of tissue or nanograms per milliliter of a culture medium, for example. Alternatively, relative units can be employed to describe a concentration. In a particular embodiment, "concentration of proteins" may refer to fragments of the proteins of the invention. Thus, in a particular embodiment, fragment of the proteins of the invention may also be measured.

In a particular embodiment, the detection of the level of the proteins of the invention can be performed by flow cytometry.

In another embodiment, the extracellular part of the proteins of the invention are detected.

Predetermined reference values used for comparison of the expression levels may comprise “cut-off” or “threshold” values that may be determined as described herein. Each reference (“cut-off”) value for the genes of the invention level may be predetermined by carrying out a method comprising the steps of:

a) providing a collection of samples from patients suffering of a pancreatic cancer;

b) determining the level of the genes of the invention for each sample contained in the collection provided at step a);

c) ranking the tumor tissue samples according to said level

d) classifying said samples in pairs of subsets of increasing, respectively decreasing, number of members ranked according to their expression level,

e) providing, for each sample provided at step a), information relating to the actual clinical outcome for the corresponding cancer patient;

f) for each pair of subsets of samples, obtaining a Kaplan Meier percentage of survival curve;

g) for each pair of subsets of samples calculating the statistical significance (p value) between both subsets

h) selecting as reference value for the level, the value of level for which the p value is the smallest.

For example the expression level of the genes of the invention has been assessed for 100 pancreatic cancer samples of 100 patients. The 100 samples are ranked according to their expression level. Sample 1 has the best expression level and sample 100 has the worst expression level. A first grouping provides two subsets: on one side sample Nr 1 and on the other side the 99 other samples. The next grouping provides on one side samples 1 and 2 and on the other side the 98 remaining samples etc., until the last grouping: on one side samples 1 to 99 and on the other side sample Nr 100. According to the information relating to the actual clinical outcome for the corresponding pancreatic cancer patient, Kaplan Meier curves are prepared for each of the 99 groups of two subsets. Also for each of the 99 groups, the p value between both subsets was calculated.

The reference value is selected such as the discrimination based on the criterion of the minimum p value is the strongest. In other terms, the expression level corresponding to the boundary between both subsets for which the p value is minimum is considered as the reference value. It should be noted that the reference value is not necessarily the median value of expression levels.

In routine work, the reference value (cut-off value) may be used in the present method to discriminate pancreatic cancer samples and therefore the corresponding patients.

Kaplan–Meier curves of percentage of survival as a function of time are commonly used to measure the fraction of patients living for a certain amount of time after treatment and are well known by the man skilled in the art.

The man skilled in the art also understands that the same technique of assessment of the expression level of a protein should of course be used for obtaining the reference value and thereafter for assessment of the expression level of a protein of a patient subjected to the method of the invention.

Such predetermined reference values of expression level may be determined for any genes of the invention defined above.

A further object of the invention relates to kits for performing the methods of the invention, wherein said kits comprise means for measuring the expression level of the genes of the invention in the sample obtained from the patient.

The kits may include probes, primers macroarrays or microarrays as above described. For example, the kit may comprise a set of probes as above defined, usually made of DNA, and that may be pre-labelled. Alternatively, probes may be unlabelled and the ingredients for labelling may be included in the kit in separate containers. The kit may further comprise hybridization reagents or other suitably packaged reagents and materials needed for the particular hybridization protocol, including solid-phase matrices, if applicable, and standards. Alternatively the kit of the invention may comprise amplification primers that may be pre-labelled or may contain an affinity purification or attachment moiety. The kit may further comprise amplification reagents and also other suitably packaged reagents and materials needed for the particular amplification protocol.

The present invention also relates to the genes of the invention as biomarkers for outcome of pancreatic cancer patients.

Therapeutics applications

In a further aspect, the invention also relates to a method for treating a pancreatic cancer in a patient with a bad prognosis as described above comprising the administration to said patient of an anti-cancer agent.

In another aspect, the invention also relates to a method for treating a pancreatic cancer in a patient with a good prognosis as described above comprising the administration to said patient of an anti-cancer agent.

Anti-cancer agent can be selected in the group consisting in cytarabine, anthracyclines, fludarabine, gemcitabine, capecitabine, methotrexate, taxol, taxotere, mercaptopurine, thioguanine, hydroxyurea, cyclophosphamide, ifosfamide, nitrosoureas, platinum complexes such as cisplatin, carboplatin and oxaliplatin, mitomycin, dacarbazine, procarbizine, etoposide,

teniposide, campathecins, bleomycin, doxorubicin, idarubicin, daunorubicin, dactinomycin, plicamycin, mitoxantrone, L-asparaginase, doxorubicin, epimbicm, 5-fluorouracil, taxanes such as docetaxel and paclitaxel, leucovorin, levamisole, irinotecan, estramustine, etoposide, nitrogen mustards, BCNU, nitrosoureas such as carmustme and lomustine, vinca alkaloids such as vinblastine, vincristine and vinorelbine, imatimb mesylate, hexamethyhnelamine, topotecan, kinase inhibitors, phosphatase inhibitors, ATPase inhibitors, tyrphostins, protease inhibitors, inhibitors herbimycm A, genistein, erbstatin, and lavendustin A. In one embodiment, additional anticancer agents may be selected from, but are not limited to, one or a combination of the following class of agents: alkylating agents, plant alkaloids, DNA topoisomerase inhibitors, anti-folates, pyrimidine analogs, purine analogs, DNA antimetabolites, taxanes, podophyllotoxin, hormonal therapies, retinoids, photosensitizers or photodynamic therapies, angiogenesis inhibitors, antimitotic agents, isoprenylation inhibitors, cell cycle inhibitors, actinomycins, bleomycins, MDR inhibitors and Ca2+ ATPase inhibitors.

Additional anti-cancer agent may be selected from, but are not limited to, cytokines, chemokines, growth factors, growth inhibitory factors, hormones, soluble receptors, decoy receptors, monoclonal or polyclonal antibodies, mono-specific, bi-specific or multi-specific antibodies, monobodies, polybodies.

Additional anti-cancer agent may be selected from, but are not limited to, growth or hematopoietic factors such as erythropoietin and thrombopoietin, and growth factor mimetics thereof.

In the present methods for treating cancer, additional therapeutic active agent can be added like an antiemetic agent. Suitable antiemetic agents include, but are not limited to, metoclopromide, domperidone, prochlorperazine, promethazine, chlorpromazine, trimethobenzamide, ondansetron, granisetron, hydroxyzine, acethylleucine monoemanolamine, alizapride, azasetron, benzquinamide, bietanautine, bromopride, buclizine, clebopride, cyclizine, dunenhydrinate, diphenidol, dolasetron, meclizme, methallatal, metopimazine, nabilone, oxypemdyl, pipamazine, scopolamine, sulpiride, tetrahydrocannabinols, thiefhylperazine, thioproperazine and tropisetron. In a preferred embodiment, the antiemetic agent is granisetron or ondansetron.

In another embodiment, the further therapeutic active agent can be an hematopoietic colony stimulating factor. Suitable hematopoietic colony stimulating factors include, but are not limited to, filgrastim, sargramostim, molgramostim and epoietin alpha.

In still another embodiment, the other therapeutic active agent can be an opioid or non-opioid analgesic agent. Suitable opioid analgesic agents include, but are not limited to,

morphine, heroin, hydromorphone, hydrocodone, oxymorphone, oxycodone, metopon, apomorphine, nomioiphine, etoipbine, buprenorphine, mepeddine, lopermide, anileddine, ethoheptazine, piminidine, betaprodine, diphenoxylate, fentanil, sufentanil, alfentanil, remifentanil, levorphanol, dextromethorphan, phenazodne, pemazocine, cyclazocine, methadone, isomethadone and propoxyphene. Suitable non-opioid analgesic agents include, but are not limited to, aspirin, celecoxib, rofecoxib, diclofinac, diflusinal, etodolac, fenoprofen, flurbiprofen, ibuprofen, ketoprofen, indomethacin, ketorolac, meclofenamate, mefanamic acid, nabumetone, naproxen, piroxicam and sulindac.

In yet another embodiment, the further therapeutic active agent can be an anxiolytic agent. Suitable anxiolytic agents include, but are not limited to, buspirone, and benzodiazepines such as diazepam, lorazepam, oxazapam, chlorazepate, clonazepam, chlordiazepoxide and alprazolam.

In yet another embodiment, the further therapeutic active agent can be a checkpoint blockade cancer immunotherapy agent.

Typically, the checkpoint blockade cancer immunotherapy agent is an agent which blocks an immunosuppressive receptor expressed by activated T lymphocytes, such as cytotoxic T lymphocyte-associated protein 4 (CTLA4) and programmed cell death 1 (PDCD1, best known as PD-1), or by NK cells, like various members of the killer cell immunoglobulin-like receptor (KIR) family, or an agent which blocks the principal ligands of these receptors, such as PD-1 ligand CD274 (best known as PD-L1 or B7-H1).

Typically, the checkpoint blockade cancer immunotherapy agent is an antibody.

In some embodiments, the checkpoint blockade cancer immunotherapy agent is an antibody selected from the group consisting of anti-CTLA4 antibodies, anti-PD1 antibodies, anti-PDL1 antibodies, anti-PDL2 antibodies, anti-TIM-3 antibodies, anti-LAG3 antibodies, anti-IDO1 antibodies, anti-TIGIT antibodies, anti-B7H3 antibodies, anti-B7H4 antibodies, anti-BTLA antibodies, and anti-B7H6 antibodies.

The invention also relates to a pharmaceutical composition comprising an anti-cancer treatment for use in the treatment of a pancreatic cancer in a subject with a bad prognosis as described above.

Any therapeutic agent of the invention may be combined with pharmaceutically acceptable excipients, and optionally sustained-release matrices, such as biodegradable polymers, to form therapeutic compositions.

"Pharmaceutically" or "pharmaceutically acceptable" refers to molecular entities and compositions that do not produce an adverse, allergic or other untoward reaction when administered to a mammal, especially a human, as appropriate. A pharmaceutically acceptable carrier or excipient refers to a non-toxic solid, semi-solid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type.

The form of the pharmaceutical compositions, the route of administration, the dosage and the regimen naturally depend upon the condition to be treated, the severity of the illness, the age, weight, and sex of the patient, etc.

The pharmaceutical compositions of the invention can be formulated for a topical, oral, intranasal, parenteral, intraocular, intravenous, intramuscular, intrathecal or subcutaneous administration and the like.

Particularly, the pharmaceutical compositions contain vehicles which are pharmaceutically acceptable for a formulation capable of being injected. These may be in particular isotonic, sterile, saline solutions (monosodium or disodium phosphate, sodium, potassium, calcium or magnesium chloride and the like or mixtures of such salts), or dry, especially freeze-dried compositions which upon addition, depending on the case, of sterilized water or physiological saline, permit the constitution of injectable solutions.

The doses used for the administration can be adapted as a function of various parameters, and in particular as a function of the mode of administration used, of the relevant pathology, or alternatively of the desired duration of treatment.

In addition, other pharmaceutically acceptable forms include, e.g. tablets or other solids for oral administration; time release capsules; and any other form currently can be used.

Typically the anti-cancer agent according to the invention are administered to the subject in a therapeutically effective amount.

By a "therapeutically effective amount" the anti-cancer agent is meant a sufficient amount of the anti-cancer agent for treating cancer at a reasonable benefit/risk ratio applicable to any medical treatment. It will be understood, however, that the total daily usage of the anti-cancer agent will be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective dose level for any particular subject will depend upon a variety of factors including the disorder being treated and the severity of the disorder; activity of (the specific) the anti-cancer agent employed; the specific composition employed, the age, body weight, general health, sex and diet of the subject; the time of administration, route of administration, and rate of excretion of the specific the anti-cancer

agent employed; the duration of the treatment; drugs used in combination or coincidental with the specific anti-cancer agent employed; and like factors well known in the medical arts. For example, it is well within the skill of the art to start doses of the anti-cancer agent at levels lower than those required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved. However, the daily dosage of the products may be varied over a wide range from 0.01 to 1,000 mg per adult per day. Typically, the compositions contain 0.01, 0.05, 0.1, 0.5, 1.0, 2.5, 5.0, 10.0, 15.0, 25.0, 50.0, 100, 250 and 500 mg of the the anti-cancer agent for the symptomatic adjustment of the dosage to the subject to be treated. A medicament typically contains from about 0.01 mg to about 500 mg of the anti-cancer agent, preferably from 1 mg to about 100 mg of the anti-cancer agent. An effective amount of the drug is ordinarily supplied at a dosage level from 0.0002 mg/kg to about 20 mg/kg of body weight per day, especially from about 0.001 mg/kg to 7 mg/kg of body weight per day.

In a particular embodiment, the anti-cancer agent may be used in a concentration between 0.01 μM and 20 μM, particularly, the anti-cancer agent may be used in a concentration of 0.01, 0.05, 0.1, 0.5, 1.0, 2.5, 5.0, 10.0, 15.0, 20.0 μM.

According to the invention, the anti-cancer agent is administered to the subject in the form of a pharmaceutical composition. Thus, the invention also relates to a therapeutic composition comprising the anti-cancer agent for use in the treatment of a cancer in a subject in need thereof.

Typically, the anti-cancer agent may be combined with pharmaceutically acceptable excipients, and optionally sustained-release matrices, such as biodegradable polymers, to form therapeutic compositions. "Pharmaceutically" or "pharmaceutically acceptable" refer to molecular entities and compositions that do not produce an adverse, allergic or other untoward reaction when administered to a mammal, especially a human, as appropriate. A pharmaceutically acceptable carrier or excipient refers to a non-toxic solid, semi-solid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type.

In the pharmaceutical compositions of the present invention for oral, sublingual, subcutaneous, intramuscular, intravenous, transdermal, local or rectal administration, the active principle, alone or in combination with another active principle, can be administered in a unit administration form, as a mixture with conventional pharmaceutical supports, to animals and human beings. Suitable unit administration forms comprise oral-route forms such as tablets, gel capsules, powders, granules and oral suspensions or solutions, sublingual and buccal administration forms, aerosols, implants, subcutaneous, transdermal, topical, intraperitoneal,

intramuscular, intravenous, subdermal, transdermal, intrathecal and intranasal administration forms and rectal administration forms.

Typically, the pharmaceutical compositions contain vehicles which are pharmaceutically acceptable for a formulation capable of being injected. These may be in particular isotonic, sterile, saline solutions (monosodium or disodium phosphate, sodium, potassium, calcium or magnesium chloride and the like or mixtures of such salts), or dry, especially freeze-dried compositions which upon addition, depending on the case, of sterilized water or physiological saline, permit the constitution of injectable solutions. The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions; formulations including sesame oil, peanut oil or aqueous propylene glycol; and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. Solutions comprising the NDPK-D protein or fragment thereof and/or an agent for NDPK-D protein expression of the invention or the anti-cancer agent of the invention as free base or pharmacologically acceptable salts can be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms. The NDPK-D protein or fragment thereof and/or an agent for NDPK-D protein expression of the present invention or the anti-cancer agent of the invention can be formulated into a composition in a neutral or salt form. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like. The carrier can also be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetables oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for

example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminium monostearate and gelatin. Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with several of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized agent of the present inventions into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the typical methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the anti-cancer agent of the invention plus any additional desired ingredient from a previously sterile-filtered solution thereof. The preparation of more, or highly concentrated solutions for direct injection is also contemplated, where the use of DMSO as solvent is envisioned to result in extremely rapid penetration, delivering high concentrations of the active agents to a small tumor area. Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms, such as the type of injectable solutions described above, but drug release capsules and the like can also be employed. For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. In this connection, sterile aqueous media which can be employed will be known to those of skill in the art in light of the present disclosure. Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject.

The invention will be further illustrated by the following figures and examples. However, these examples and figures should not be interpreted in any way as limiting the scope of the present invention.

FIGURES:

Figure 1: Clinical features of patients and their PDAC molecular profiles. (A) Boxplot showing Log2 normalized expression of 19 GT genes stratified by clusters 1 and 2. The lower and upper hinges correspond to the first and third quartiles (the 25th and 75th percentiles). Kruskal-Wallis p-value was used for the statistical significance between clusters. (B) Foresplot of the multivariate survival analysis including PDAC clustering, surgical resection and the disease stage.

Figure 2. Validation of the glyco-signature on ICGC patient's cohort (RNA-seq). Survival curves estimated by using the Kaplan-Meier method and comparing OS probabilities between clusters with the log-rank test (p=0.0042). Cluster 1 and 2 have shorter median OS of 304 and 359 days respectively compared to cluster 3 with median OS of 719 days.

Figure 3. Validation of the glyco-signature on ICGC patient's cohort (microarray). Survival curves estimated by using the Kaplan-Meier method and comparing OS probabilities between clusters with the log-rank test (p=0.0019). Cluster 1 and 2 have shorter median OS of 515 and 517 days respectively compared to cluster 3 with median OS of 1048 days.

Figure 4. Validation of the glyco-signature on Puleo patient's cohort. Survival curves estimated by using the Kaplan-Meier method and comparing OS probabilities between clusters with the log-rank test (p=0.0045). Cluster 1 and 2 have shorter median OS of 23.8 and 22.9 months respectively compared to cluster 3 with median OS of 46.4 months.

Figure 5. Comparison of patient survival with classical subtype PDAC. Patient survival curves estimated by using the Kaplan-Meier method and comparing OS probabilities between clusters using the log-rank test in (A) ICGC RNA-seq (p=0.048) with median OS of classical subtype in cluster 2 (429 days) shorter than median OS of classical subtype in cluster 3 (768 days), (B) ICGC microarray (p=0.032) with median OS of classical subtype in cluster 1 (518 days) and median OS of classical subtype in cluster 2 (517 days) shorter than median OS of classical subtype in cluster 3 (1048 days) and (C) Puleo patient's cohort (p=0.018) with median OS of classical subtype in cluster 1 (24.9 months) and median OS of classical subtype in cluster 2 (24.3 months) shorter than median OS of classical subtype in cluster 3 (46.4 months).

Figure 6. Comparison of GT expression levels of the glyco-signature between clusters. Boxplots showing Log2 normalized expression of 19 GT genes stratified by clusters 1, 2 and 3 in (A) ICGC RNA-seq, (B) ICGC affymetrix transcriptomic and (C) affymetrix transcriptomic from Puleo patient's cohort datasets. The lower and upper hinges correspond to the first and third quartiles (the 25th and 75th percentiles). The boxplots are annotated by statistical significance of p-values (pairwise comparisons) from Wilcoxon's test and Student's

t-test for ICGC RNA-seq and both affymetrix transcriptomic from Puleo's and ICGC's cohort datasets respectively. ns, non significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

EXAMPLE:

Material & Methods

PaCaOmics patient's cohort and PDX

The patient cohort was described in a previous study (28) and is part of the PaCaOmics clinical trial NCT01692873. It includes PDAC samples obtained from resected and unresectable tumours. The tumour samples of these patients were used to generate PDX. Outliers were excluded after a histological selection and 74 patients were retained for our study.

RNA-seq analysis and gene selection

RNA libraries were generated from 74 PDX samples, then RNA-seq data were acquired and processed as previously described (28). Analyses were performed on genes encoding main GT families involved in glycosylation machinery from human species-specific RNA reads. Therefore a gene set of 211 GT genes was extracted from GlycoGene DataBase (GGDB; https://acgg.asia/ggdb2/) and based on glycosyltransferase gene group section of Hugo Gene Nomenclature Committee (HGNC: https://www.genenames.org/data/genegroup/#!/group/424). Among available genes in our RNA-seq dataset, those belonging to UDP glucuronosyltransferase family were excluded as their expression values were close to 0. Analysis was performed on 169 genes (data not shown). Unsupervised hierarchical clustering was performed using Euclidean distance algorithm and Ward.D2's method for linkage. Heatmap representations of 74 PDX focusing on 169 GT genes were generated using ComplexHeatmap package on RStudio version 1.2.5033. Relative expression between samples for each gene is depicted in a color gradation and intensities varying from red to blue for upregulated and downregulated genes respectively.

Hierarchical Clustering on Principal Component (HCPC) analysis and glyco-signature definition

HCPC analyses were performed using 'FactoMineR' and 'Factoextra' packages of R programming language. To extract essential information and get rid of noise, only components that explain at least 5% of variances were retained for further analysis. Hierarchical clustering was then performed on PCA, using Euclidean distance algorithm and Ward's method for linkage. The link between the cluster variable and the quantitative variable corresponding to the GT genes was described with the square correlation coefficient of the F-test in a one-way

analysis of variance. A total of 19 GT genes showing the most significant p-values were selected as the most relevant GT markers in cluster definition constituting the glyco-signature (data not shown).

Validation on public datasets

The Australian cohort of International Cancer Genome Consortium (ICGC) was used as external validation cohort for the glyco-signature.9 Both RNA-seq and gene microarray expression datasets, including 91 and 269 resected pancreatic tumours respectively, were downloaded from ICGC Data Portal (data repositories, release 20 on http://dcc.icgc.org/). Gene expression raw values were normalized and gene-wise centered. Note that among the 19 GT markers, GALNT4 gene was not part of ICGC gene microarray expression dataset. The glyco-signature was validated on a second independent affymetrix transcriptomic dataset, obtained from the Puleo et al. (11) multi-centric cohort including 309 resected primary PDAC. Data were processed as previously described. In both affymetrix transcriptomic datasets, multiple probes are used to target the same gene. The probe showing the highest variance between samples was selected, since it was considered as the one providing the most amount of information.

Comparison with previously established classification

Basal-like/classical subtyping for each dataset was performed using Purity Independent Subtyping of Tumors (PurIST), an algorithm of a Single Sample Classifier (SSC) developed by Rashid et al (29). Proportions of different subtypes in clusters were determined for ICGC RNA-seq dataset and the association between both classifications was assessed using Fisher's exact test. Concerning the ICGC microarray dataset and Puleo patient's cohort, Pearson's chi-squared test was used to determine the significance of subtype distribution in clusters obtained through analysis of GT gene expression. Similarly, proportions of subtypes based on microenvironment features proposed by the authors in Puleo patient's cohort were assessed in clusters, using Pearson's chi-squared test. Standardized residuals for Pearson's chi-squared tests were represented on mosaic plots.

Survival analyses

Multivariate Cox proportional hazard regression model from the survival package was used to determine relative risk of death associated to different factors, including the 95% confidence interval. The follow-up starting point of survival time was defined at the diagnostic time. Concerning the Puleo's cohort and the ICGC affymetrix dataset, analyses were performed on the available survival data of 288 and 267 patients respectively.

Differential expression and functional analysis

Differential expression analysis comparing pairwise clusters were performed using DESeq2 and limma R packages for RNA-seq and microarray datasets respectively. Adjusted p-value < 0.05 and |log2(fold change)| > 1 were defined as cut-off criteria. Differentially expressed genes were represented on volcano-plots and Venn diagrams for comparisons between 2 clusters and between 3 clusters respectively.

Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed for upregulated and downregulated genes using ClusterProfiler R package.

Results:

Expression profiles of GT genes predict overall survival of PDAC patients

RNA-seq data of 169 GT genes revealed significant differences in gene expression between tumour cells from 74 PDX (data not shown) highlighting the tumoral heterogeneity in GT gene expression. Subsets of PDAC were distinguishable with a high expression of specific genes and low expression of others. To deeply explore these PDAC glyco-profiles, we performed a principal component analysis (PCA), followed by a hierarchical clustering (HCPC). Two different clusters of PDAC were identified (data not shown) based on a specific GT gene expression profile (Figure 1A). Cluster 1 and cluster 2 included respectively 21.6% (n=16/74) and 78.4% (n=58/74) of PDAC patients.

In order to assess whether specific GT gene expression profiles defined for each cluster could be related to prognosis, we performed a multivariate analysis including surgical resection and disease stage since those factors are the most relevant ones impacting survival, predicting at best patient prognosis. PDAC stratification through GT gene expression profile was significantly associated to patient OS as an independent prognostic factor (p=0.007) (Figure 1B). Indeed, PDAC patients in cluster 2 have a best survival compared to patients in cluster 1. This prognostic value, independent of other factors, suggests that GT gene expression profiles have a significant impact on the survival of patients.

Identification of GT genes as prognostic markers

To define GT genes with a major impact on the survival, the top GT genes whose expressions contributed significantly to cluster definition, were extracted from HCPC analysis. This results in a selection of 19 GT genes as the most relevant markers to stratify PDAC (table 1). Among these genes, several functional paralogues of fucosyltransferases including FUT2, FUT3, FUT4, FUT6, and of N-acetygalactosaminyltransferases GALNT4, GALNT6 and GALNT12 were significantly downregulated in cluster 1 compared to cluster 2 while GALNT9

was upregulated in cluster 1 (Figure 1A). Our results also showed a significant downregulation of A4GNT, B3GALT5, B3GNT6, C1GALT1, GCNT1, GYG2, LFNG, MGAT5, ST6GALNAC1, ST8SIA3 and XYLT in cluster 1 compared to cluster 2 (Figure 1A).

Clinical features of patients and their PDAC molecular profiles

The HCPC analysis allowed the identification of two prognostic clusters of PDAC with distinct clinical features of patients. Unresectable PDAC represented 75% (n=12/16) in cluster 1 whereas cluster 2 included 60% (n=35/58) of patients with resected tumours (data not shown). Among the unresectable PDAC, 25% and 17% were locally advanced tumours in clusters 1 and 2 respectively. Moreover, cluster 1 contains 63% (n=10/16) of metastatic tumours compared to cluster 2 with only 34% (n=20/58) of metastatic tumours (data not shown). The classification of PDAC into different transcriptomic subtypes using PurIST classifier showed that 90% of basal-like subtype were classified in cluster 1 and inversely cluster 2 regrouped 89% of classical subtype (p=9.347e-07, Fisher's exact test). However, the composition of cluster 1 is heterogeneous with 56% basal-like vs 44% classical subtypes and 81% basal-like vs 19% classical subtypes according to PurIST classifier and Nicolle et al. (30) proposed stratification respectively. On the other hand, cluster 2 contains around 2% of PDAC basal-like subtype whatever the classifier used. Then, the cluster 1 regroups basal-like PDAC subtypes and classical PDAC subtypes of patients with short survival for the majority of them (data not shown). This idea could be refined by the Pancreatic Adenocarcinoma Molecular Gradient (PAMG), (28) a more precise stratification of tumours represented by a gradient from pure basal-like to pure classical. Indeed, the PAMG confirms cluster 1 mixed composition including tumours with intermediate gradient, although it does contain the top scored basal-like tumours. These combined features allow a precise tumour characterisation, which is essential to dissect molecular diversity. Therefore, the glyco-signature of 19 GT genes was found relevant to stratify PDAC according to their aggressiveness.

Validation of the glyco-signature prognostic value on independent cohorts

To validate the prognostic accuracy of the proposed glyco-signature, we applied it on three independent external datasets of resected PDAC tumours from ICGC (RNA-seq and microarray) and Puleo patient's cohorts. Note that the glyco-signature was determined on PDX, considering exclusively the epithelial compartment of tumours, unlike the validation performed on resected whole tumour tissues, including stromal component of tumours. In this context, HCPC analyses identified systematically three clusters, through the 19 GT genes, with

significant differences in OS. Clusters 1 and 2 were associated with poor prognosis compared to the cluster 3 which showed about a double median OS (Figures 2, 3 and 4). Furthermore, statistical analyses showed that the three clusters were significantly associated with basal-like/classical subtyping. While the overwhelming majority of tumours in clusters 3 had a classical subtype, both clusters 1 and 2 included most of the basal-like subtype PDAC but contained also a large proportion of classical subtype PDAC having a poor prognosis (Figure 5A to C). Besides, expression differences of the 19 GT genes in the 3 clusters showed globally a trend of gradual expression from cluster 1 to cluster 3, except for GALNT9, which is downregulated in cluster 3 (Figure 6A to C). This is consistent with what was previously shown in PaCaOmics patient's cohort. More importantly, the expression of B3GALT5, FUT3, C1GALT1, GALNT4, GALNT12 and ST6GALNAC1 were strongly downregulated in cluster 1 compared to cluster 2.

These validation analyses on expression datasets, obtained from diverse technological platforms (RNA-seq or microarray) allowed to assess the glyco-signature accuracy on stratifying PDAC samples independently of the technological provenance of generated expression data and increased its robustness.

Tumour subtyping based on the epithelial compartment was not sufficient to explain the two poor prognostic clusters including simultaneously basal-like and classical subtype PDAC. Therefore, we assessed whether identified clusters through GT gene expression could be associated with specific microenvironment features as suggested by the Puleo et al. study (11). Interestingly, the stromal-driven subtype composition was different between the two poor prognostic clusters. Cluster 1 includes predominantly desmoplastic and immune classical microenvironment (46.7% and 18.9% respectively), whereas cluster 2 is significantly uncorrelated with immune classical subtype and is mainly enriched in stroma activated microenvironment (36.3%) (p-value<2.2e-16) (data not shown). Cluster 3, constituted of PDAC with a better prognosis of patients, was characterized by a predominant pure classical microenvironment (81%). These particular distributions highlight the importance of microenvironment features in prognosis related to specific GT gene expression profiles. Beyond the established basal-like/classical classification, the glyco-signature was then able to discriminate two clusters with similar poor prognosis but differing by their microenvironment features.

Identification of functional pathways

In order to better characterize the tumour biology and decipher global transcription patterns related to each cluster, a pairwise differential expression analysis was performed for each dataset allowing us to highlight lists of differentially expressed genes used further for the KEGG pathway analyses. Results showed the specificity and relevance of glycosylation process in the determination of the prognostic clusters. 'Mucin type O-glycan biosynthesis' was an enriched pathway for upregulated genes in clusters with better prognosis (data not shown). A large majority of the 19 GT gene markers is part of this pathway. In particular the glycosylation pathways involving GALNT4, GALNT6, GALNT12, GCNT1 and ST6GALNAC1 or B3GALT5, FUT2 and FUT3 were upregulated in cluster 3 and/or 2 vs cluster 2 and/or 1 in ICGC and Puleo patient's cohorts (data not shown). Similarly, a core of intrinsically connected pathways including 'Retinol metabolism', 'Chemical carcinogenesis' as well as 'Drug metabolism – cytochrome P450' and 'Metabolism of xenobiotics by cytochrome P450' were systematically identified as a KEGG pathway network of upregulated genes in clusters with better prognosis (data not shown). A focused analysis on redundant genes composing these pathways and identified across different datasets allowed us to bring out the most relevant ones. In this way, ADH1C, CYP2C18, CYP2S1, CYP3A5, GSTA1, GSTA2 and UGT2B17 were found as the major contact points within the network linking these pathways (data not shown). In particular CYP3A5 involved in these pathways is upregulated in cluster 2 vs cluster 1 in PaCaOmics patient's cohort and in cluster 3 and 2 vs cluster 1 in ICGC and Puleo patient's cohorts (data not shown).

Moreover, most of the enriched pathways of downregulated genes in clusters having the best prognosis were related to inflammatory process or immune system such as 'Cytokine-cytokine receptor interaction' and 'IL-17 signaling pathway' (data not shown). At the gene level, IL6 is common to several downregulated pathways in clusters with the higher OS across multiple comparisons of different datasets (data not shown).

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Throughout this application, various references describe the state of the art to which this invention pertains. The disclosures of these references are hereby incorporated by reference into the present disclosure.

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CLAIMS:

1. A method for predicting the survival time of a patient suffering from a pancreatic cancer comprising i) determining in a sample obtained from the patient the expression level of at least one gene selected in the group consisting in GALNT9, A4GNT, B3GALT5, B3GNT6, C1GALT1, FUT2, FUT3, FUT4, FUT6, GALNT4, GALNT6, GALNT12, GCNT1, GYG2, LFNG, MGAT5, ST6GALNAC1, ST8SIA3 and XYLT1 ii) comparing said expression level determined at step i) with their predetermined reference value and iii) providing a good prognosis when the expression level of A4GNT, B3GALT5, B3GNT6, C1GALT1, FUT2, FUT3, FUT4, FUT6, GALNT4, GALNT6, GALNT12, GCNT1, GYG2, LFNG, MGAT5, ST6GALNAC1, ST8SIA3 or XYLT1 determined at step i) is higher than their predetermined reference values, or providing a bad prognosis when the expression level of A4GNT, B3GALT5, B3GNT6, C1GALT1, FUT2, FUT3, FUT4, FUT6, GALNT4, GALNT6, GALNT12, GCNT1, GYG2, LFNG, MGAT5, ST6GALNAC1, ST8SIA3 or XYLT1 determined at step i) is lower than their predetermined reference values or providing a good prognosis when the expression level of GALNT9 determined at step i) is lower than its predetermined reference value, or providing a bad prognosis when the expression level of GALNT9 determined at step i) is higher than its predetermined reference value.

2. The method for predicting the survival time of a patient suffering from a pancreatic cancer according to the claim 1 comprising i) determining in a sample obtained from the patient the expression level of the genes selected in the group consisting in A4GNT, B3GALT5, B3GNT6, C1GALT1, FUT2, FUT3, FUT4, FUT6, GALNT4, GALNT6, GALNT12, GCNT1, GYG2, LFNG, MGAT5, ST6GALNAC1, ST8SIA3 and XYLT1 ii) comparing said expression level determined at step i) with their predetermined reference value and iii) providing a good prognosis when the expression level of A4GNT, B3GALT5, B3GNT6, C1GALT1, FUT2, FUT3, FUT4, FUT6, GALNT4, GALNT6, GALNT12, GCNT1, GYG2, LFNG, MGAT5, ST6GALNAC1, ST8SIA3 and XYLT1 determined at step i) is higher than its predetermined reference value, or providing a bad prognosis when the expression level of A4GNT, B3GALT5, B3GNT6, C1GALT1, FUT2, FUT3, FUT4, FUT6, GALNT4, GALNT6, GALNT12, GCNT1,

GYG2, LFNG, MGAT5, ST6GALNAC1, ST8SIA3 and XYLT1 determined at step i) is lower than their predetermined reference value.

3. The method for predicting the survival time of a patient suffering from a pancreatic cancer according to claim 1 comprising i) determining in a sample obtained from the patient the expression of the gene GALNT9 ii) comparing said expression level determined at step i) with their predetermined reference value and iii) providing a good prognosis when the expression level of GALNT9 determined at step i) is lower than its predetermined reference value, or providing a bad prognosis when the expression level of GALNT9 determined at step i) is higher than its predetermined reference value.

4. A method for predicting the survival time of a patient suffering from a pancreatic cancer comprising i) determining in a sample obtained from the patient the expression level of the genes selected in the group consisting in GALNT9, A4GNT, B3GALT5, B3GNT6, C1GALT1, FUT2, FUT3, FUT4, FUT6, GALNT4, GALNT6, GALNT12, GCNT1, GYG2, LFNG, MGAT5, ST6GALNAC1, ST8SIA3 and XYLT1 ii) comparing said expression level determined at step i) with their predetermined reference value and iii) providing a good prognosis when the expression level of A4GNT, B3GALT5, B3GNT6, C1GALT1, FUT2, FUT3, FUT4, FUT6, GALNT4, GALNT6, GALNT12, GCNT1, GYG2, LFNG, MGAT5, ST6GALNAC1, ST8SIA3 or XYLT1 determined at step i) is higher than its predetermined reference value and when the expression level of GALNT9 determined at step i) is lower than its predetermined reference value, or providing a bad prognosis when the expression level of A4GNT, B3GALT5, B3GNT6, C1GALT1, FUT2, FUT3, FUT4, FUT6, GALNT4, GALNT6, GALNT12, GCNT1, GYG2, LFNG, MGAT5, ST6GALNAC1, ST8SIA3 or XYLT1 determined at step i) is lower than their predetermined reference values and when the expression level of GALNT9 determined at step i) is higher than its predetermined reference value.

5. The method according to claims 1 to 4 wherein the pancreatic cancer is a pancreatic ductal adenocarcinoma (PDAC), a pancreatic adenocarcinoma, a pancreatic serous cystadenomas (SCNs), a pancreatic intraepithelial neoplasia (PanIN), pancreatic mucinous cystic neoplasms (MCNs), a non-resectable pancreatic ductal adenocarcinoma (PDAC) or a non-resectable pancreatic adenocarcinoma.

6. The method according to claims 1 to 5 wherein the sample can be blood, peripheral-blood, serum, plasma, tumoral circulating cells, tumor sample or a biopsy obtained from the tumor.

7. A method for treating a pancreatic cancer in a patient with a bad prognosis according to claim 1 to 6 comprising the administration to said patient of an anti-cancer agent.

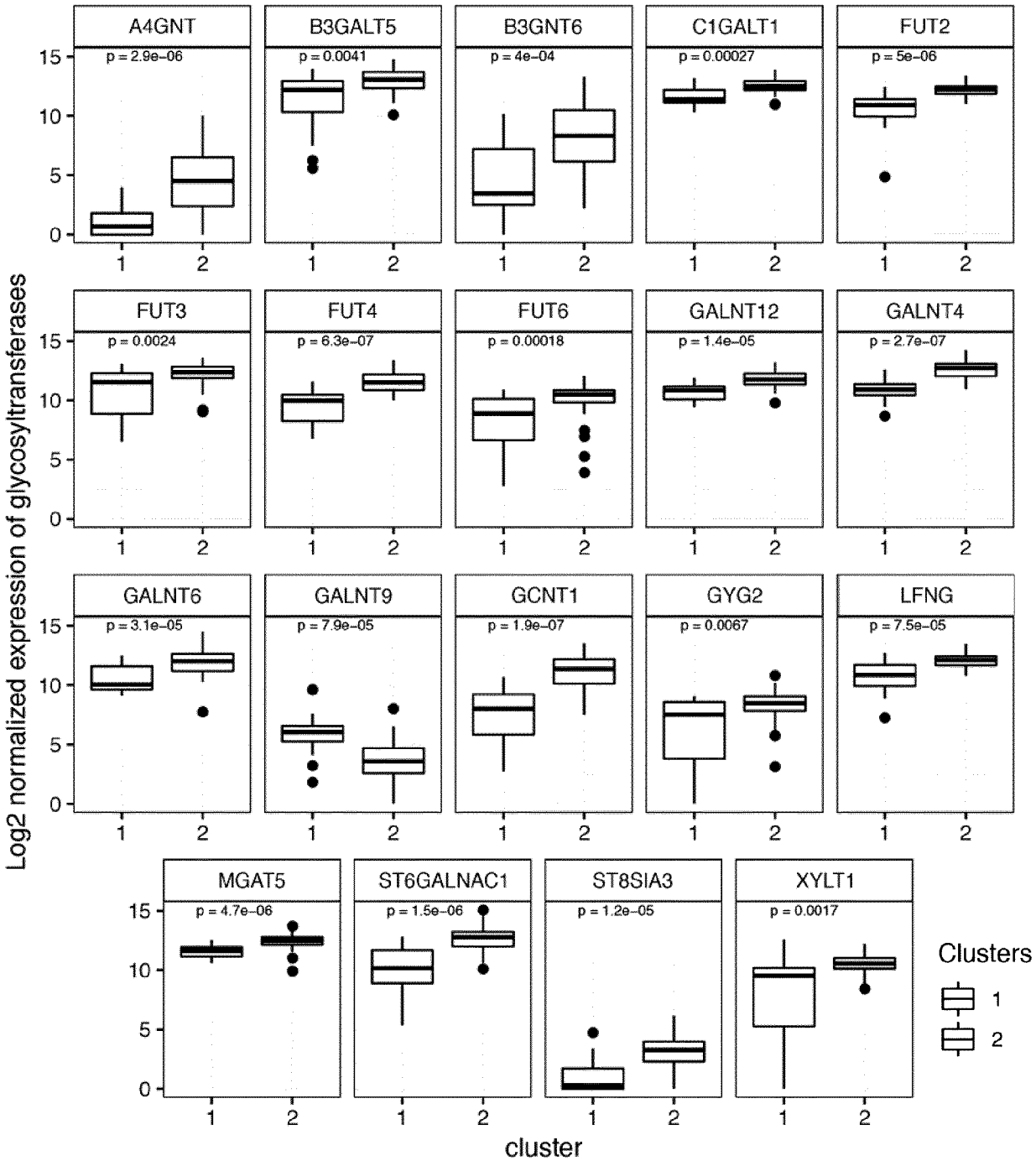
A4GNT
p = 2.9e−06
B3GALT5
p = 0.0041
B3GNT6
p = 4e−04
C1GALT1
p = 0.00027
FUT2
p = 5e−06
FUT3
p = 0.0024
FUT4
p = 6.3e−07
FUT6
p = 0.00018
GALNT12
p = 1.4e−05
GALNT4
p = 2.7e−07
GALNT6
p = 3.1e−05
GALNT9
p = 7.9e−05
GCNT1
p = 1.9e−07
GYG2
p = 0.0067
LFNG
p = 7.5e−05
MGAT5
p = 4.7e−06
ST6GALNAC1
p = 1.5e−06
ST8SIA3
p = 1.2e−05
XYLT1
p = 0.0017
Log2 normalized expression of glycosyltransferases
cluster
Clusters
1
2

Figure 1A

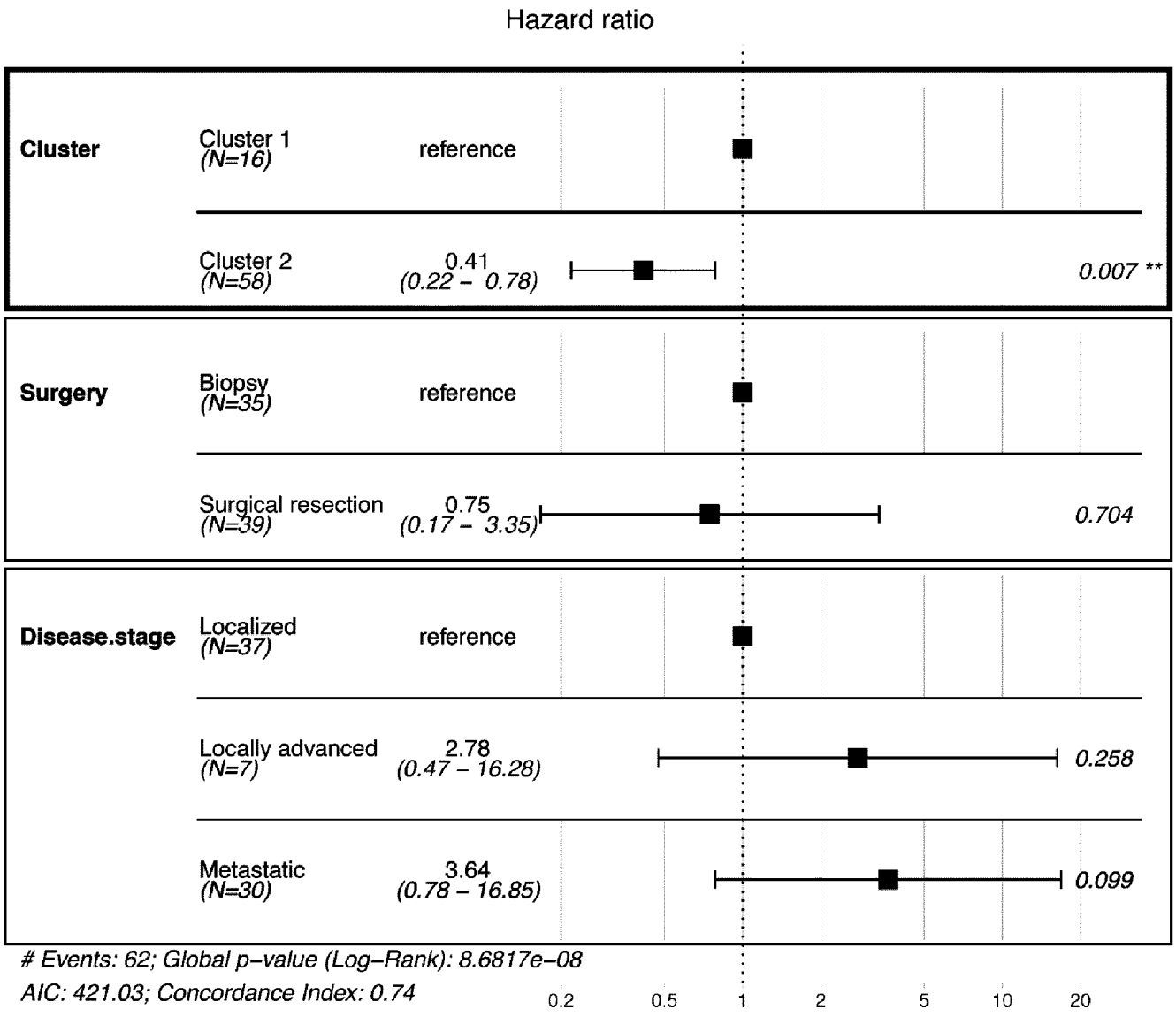
Hazard ratio
Cluster
Cluster 1 (N=16)
reference
Cluster 2 (N=58)
0.41 (0.22 – 0.78)
0.007 **
Surgery
Biopsy (N=35)
reference
Surgical resection (N=39)
0.75 (0.17 – 3.35)
0.704
Disease.stage
Localized (N=37)
reference
Locally advanced (N=7)
2.78 (0.47 – 16.28)
0.258
Metastatic (N=30)
3.64 (0.78 – 16.85)
0.099
Events: 62; Global p–value (Log–Rank): 8.6817e–08
AIC: 421.03; Concordance Index: 0.74
0.2
0.5
1
2
5
10
20

Figure 1B

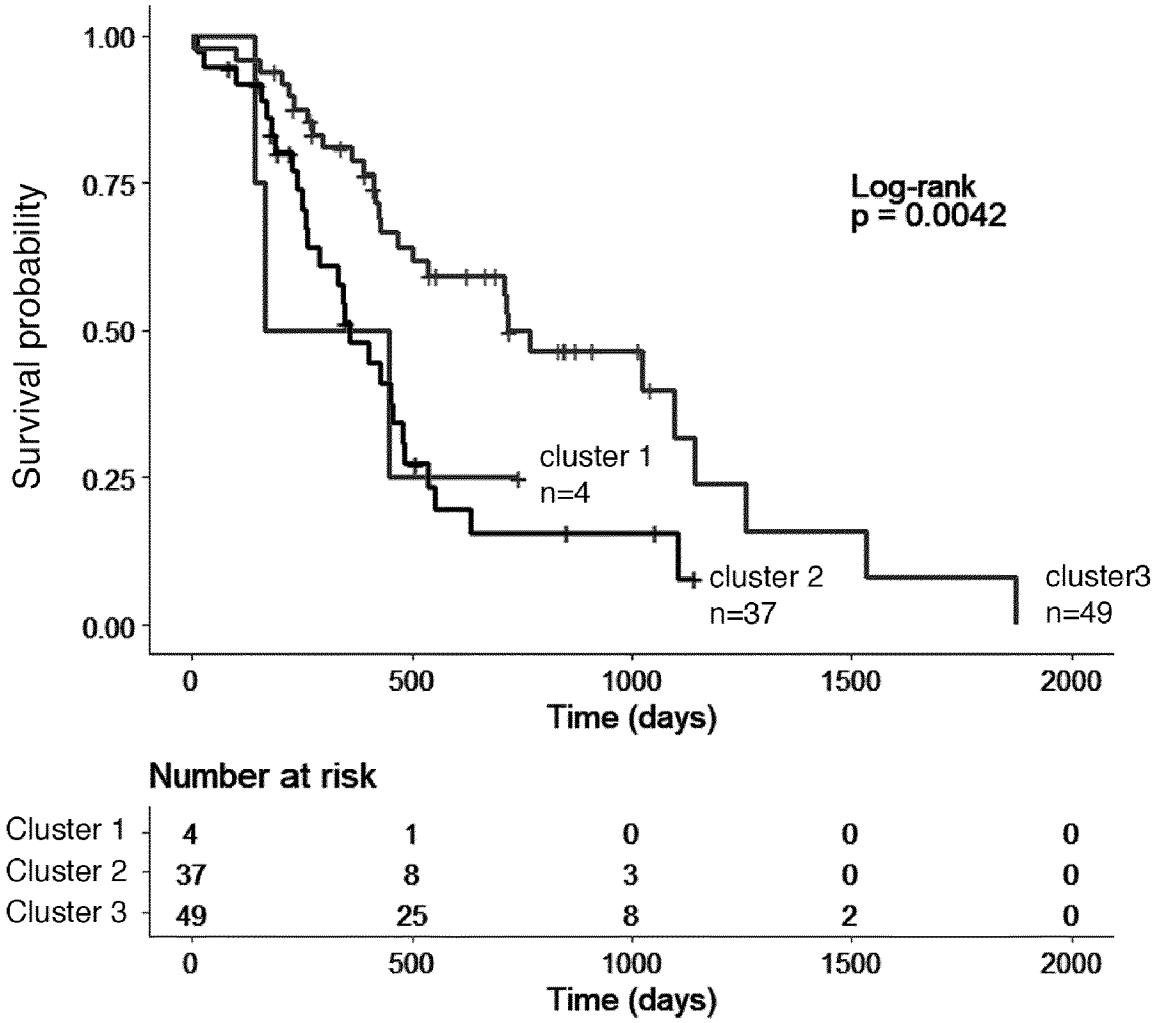
1.00
0.75
0.50
0.25
0.00
Survival probability
Log-rank
p = 0.0042
cluster 1
n=4
cluster 2
n=37
cluster3
n=49
0
500
1000
1500
2000
Time (days)
Number at risk
Cluster 1
Cluster 2
Cluster 3
4 1 0 0 0
37 8 3 0 0
49 25 8 2 0
0
500
1000
1500
2000
Time (days)

Figure 2

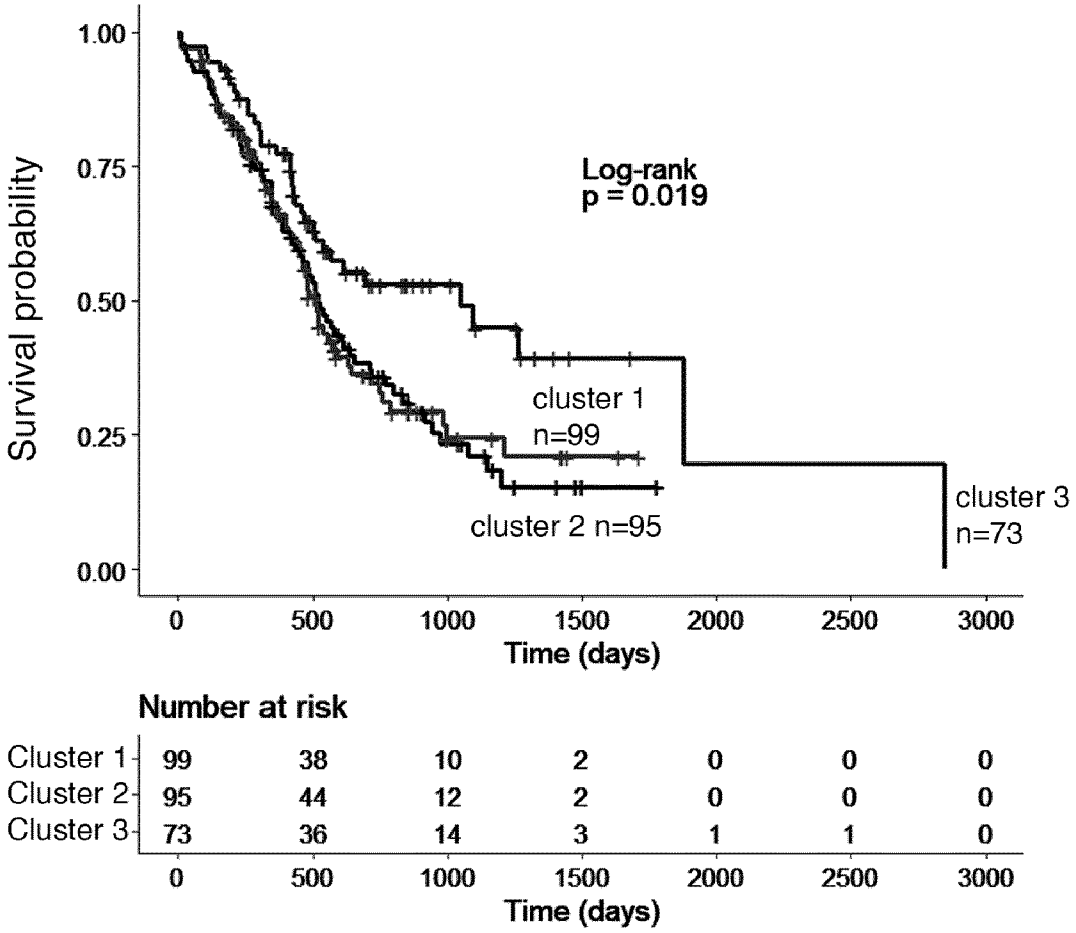
1.00
0.75
0.50
0.25
0.00
Survival probability
Log-rank
p = 0.019
cluster 1
n=99
cluster 2 n=95
cluster 3
n=73
0
500
1000
1500
2000
2500
3000
Time (days)
Number at risk
Cluster 1 99 38 10 2 0 0 0
Cluster 2 95 44 12 2 0 0 0
Cluster 3 73 36 14 3 1 1 0
0
500
1000
1500
2000
2500
3000
Time (days)

Figure 3

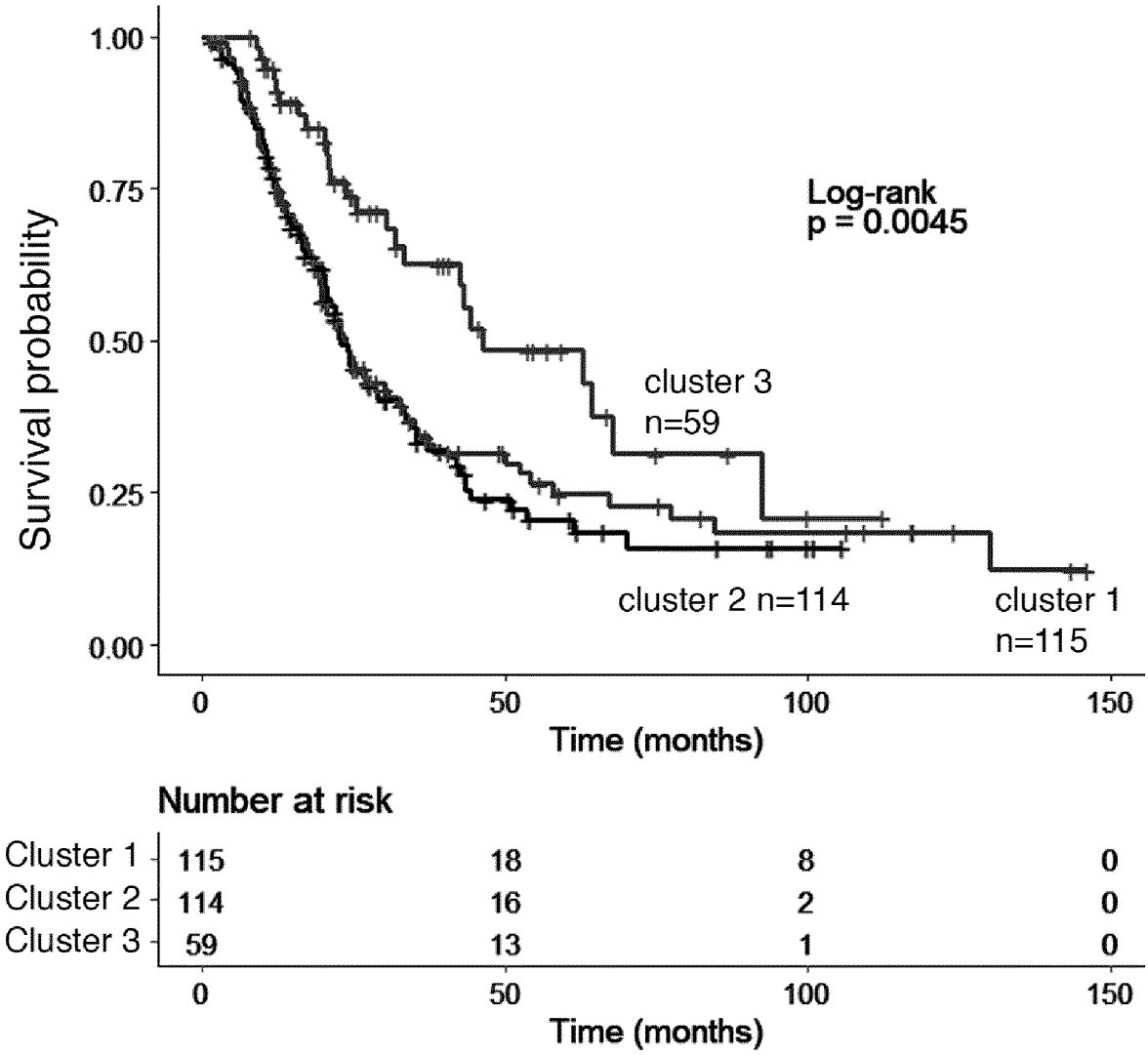
1.00
0.75
0.50
0.25
0.00
Survival probability
Log-rank
p = 0.0045
cluster 3
n=59
cluster 2 n=114
cluster 1
n=115
0
50
100
150
Time (months)
Number at risk
Cluster 1
Cluster 2
Cluster 3
115
18
8
0
114
16
2
0
59
13
1
0
0
50
100
150
Time (months)

Figure 4

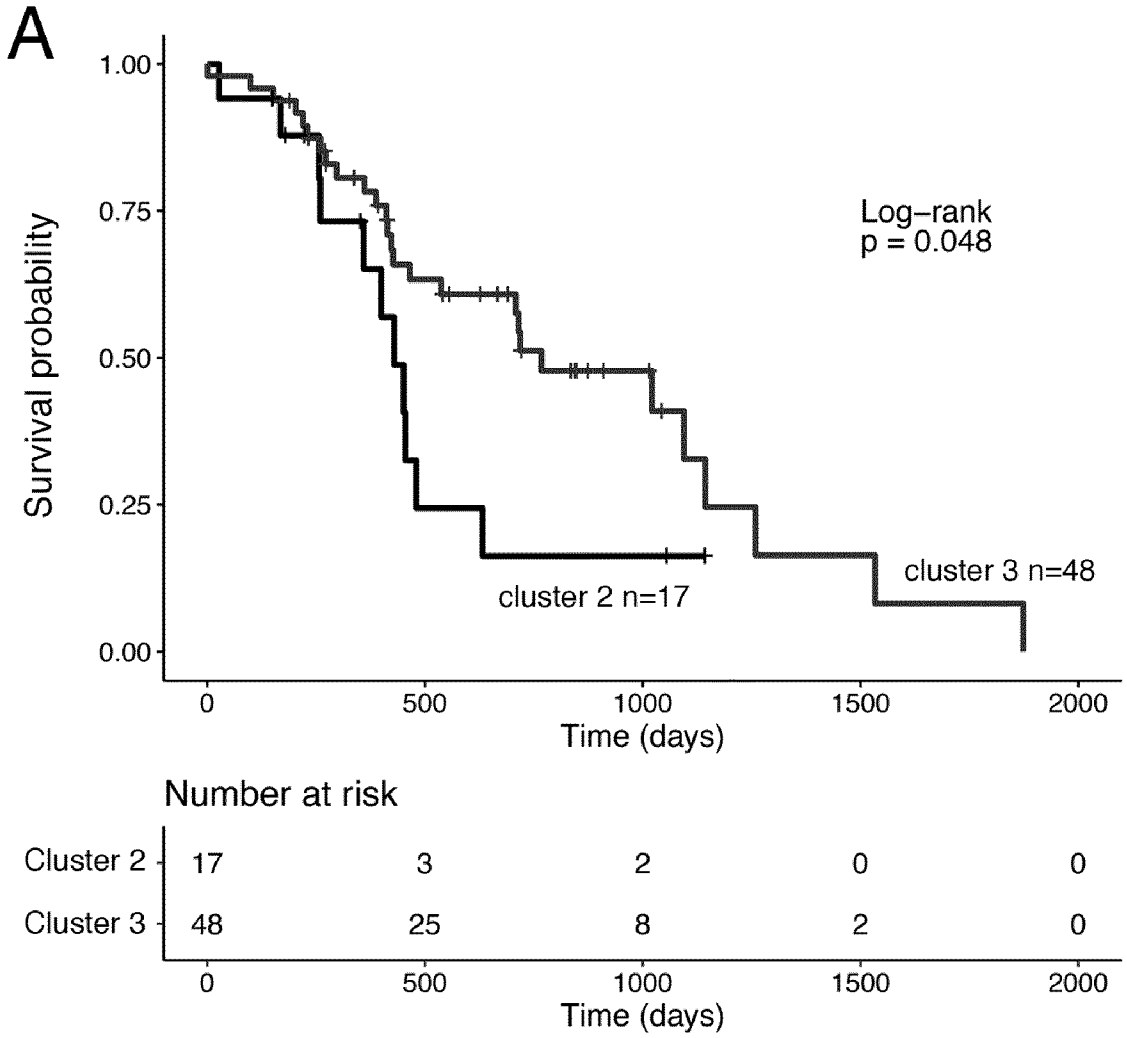
A
1.00
0.75
0.50
0.25
0.00
Survival probability
Log−rank
p = 0.048
cluster 3 n=48
cluster 2 n=17
0
500
1000
1500
2000
Time (days)
Number at risk
Cluster 2
17
3
2
0
0
Cluster 3
48
25
8
2
0
0
500
1000
1500
2000
Time (days)

Figure 5A

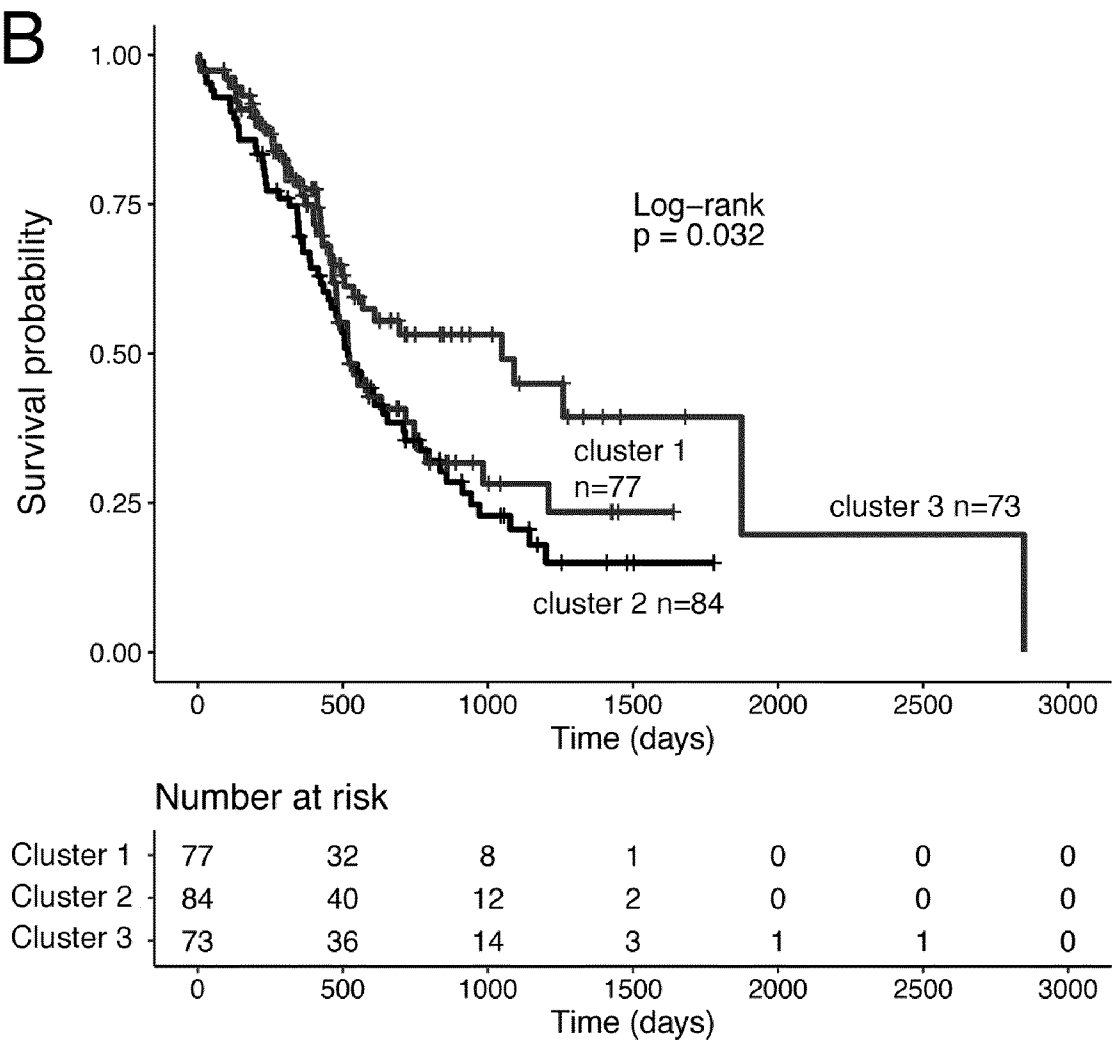
B
1.00
0.75
0.50
0.25
0.00
Survival probability
Log−rank
p = 0.032
cluster 1
n=77
cluster 3 n=73
cluster 2 n=84
0
500
1000
1500
2000
2500
3000
Time (days)
Number at risk
Cluster 1 77 32 8 1 0 0 0
Cluster 2 84 40 12 2 0 0 0
Cluster 3 73 36 14 3 1 1 0
0
500
1000
1500
2000
2500
3000
Time (days)

Figure 5B

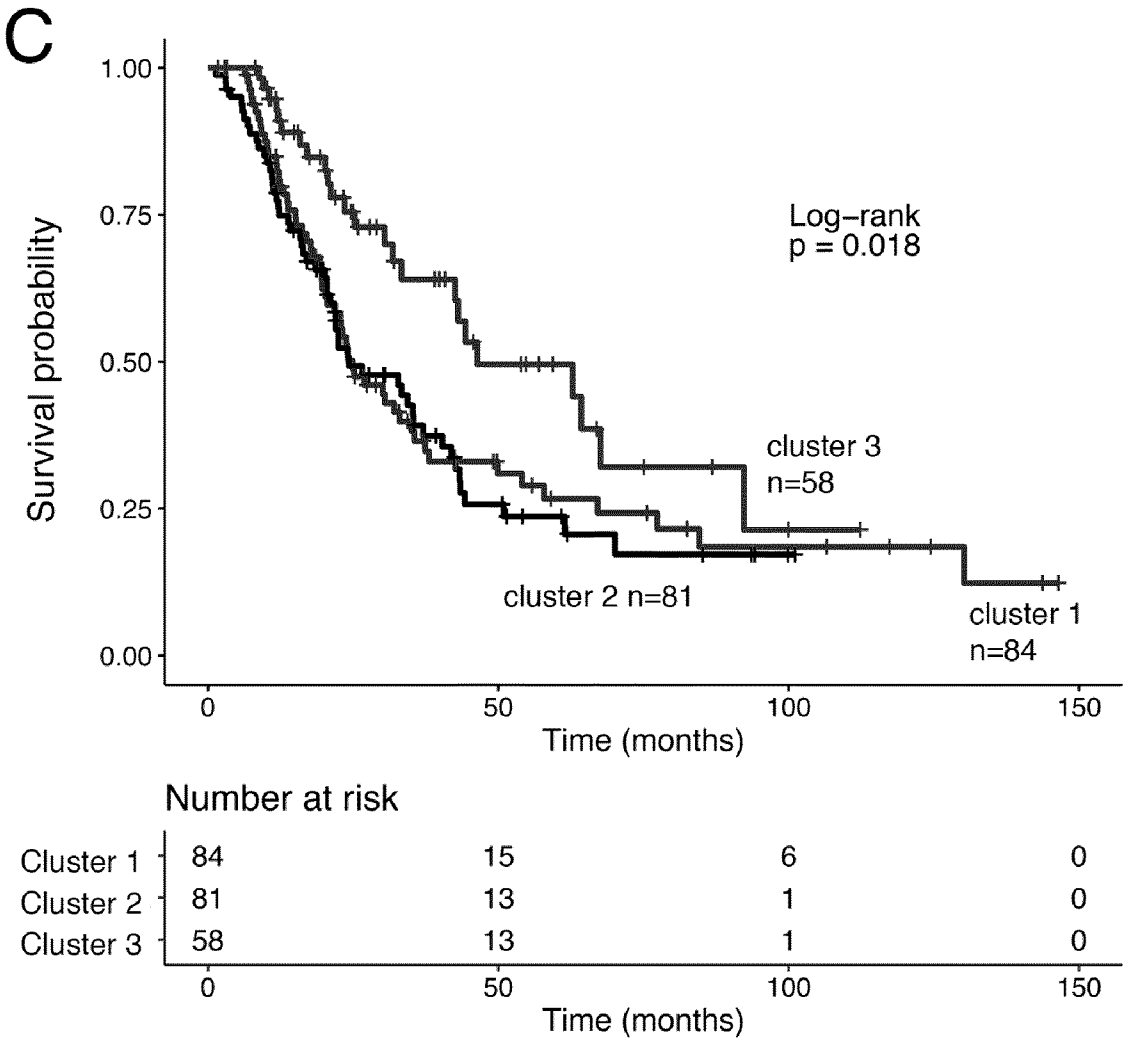
C
1.00
0.75
0.50
0.25
0.00
Survival probability
Log−rank
p = 0.018
cluster 3
n=58
cluster 2 n=81
cluster 1
n=84
0
50
100
150
Time (months)
Number at risk
Cluster 1
Cluster 2
Cluster 3
84
15
6
0
81
13
1
0
58
13
1
0
0
50
100
150
Time (months)

Figure 5C

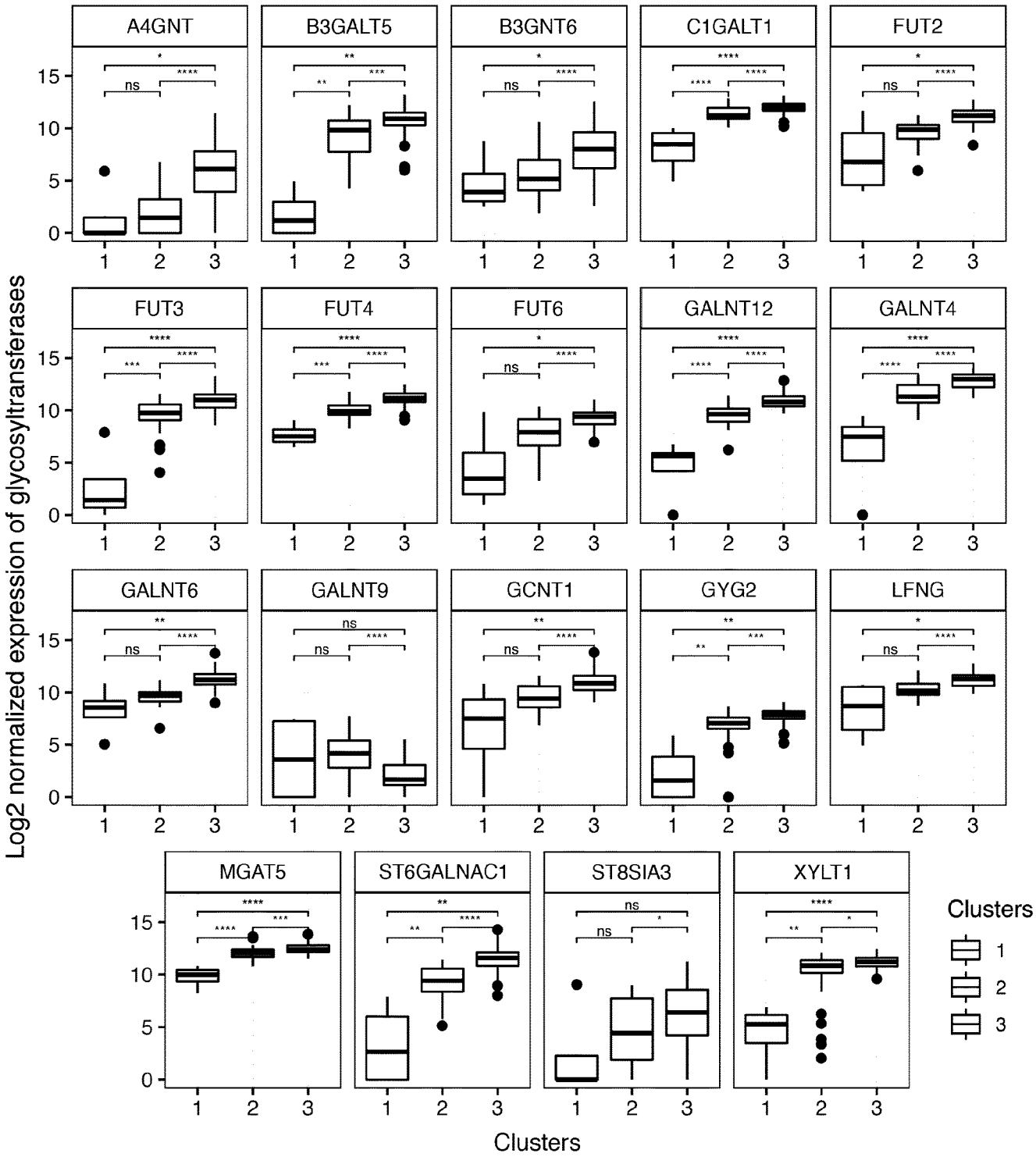
A4GNT
B3GALT5
B3GNT6
C1GALT1
FUT2
FUT3
FUT4
FUT6
GALNT12
GALNT4
GALNT6
GALNT9
GCNT1
GYG2
LFNG
MGAT5
ST6GALNAC1
ST8SIA3
XYLT1
Log2 normalized expression of glycosyltransferases
Clusters
Clusters
1
2
3

Figure 6A

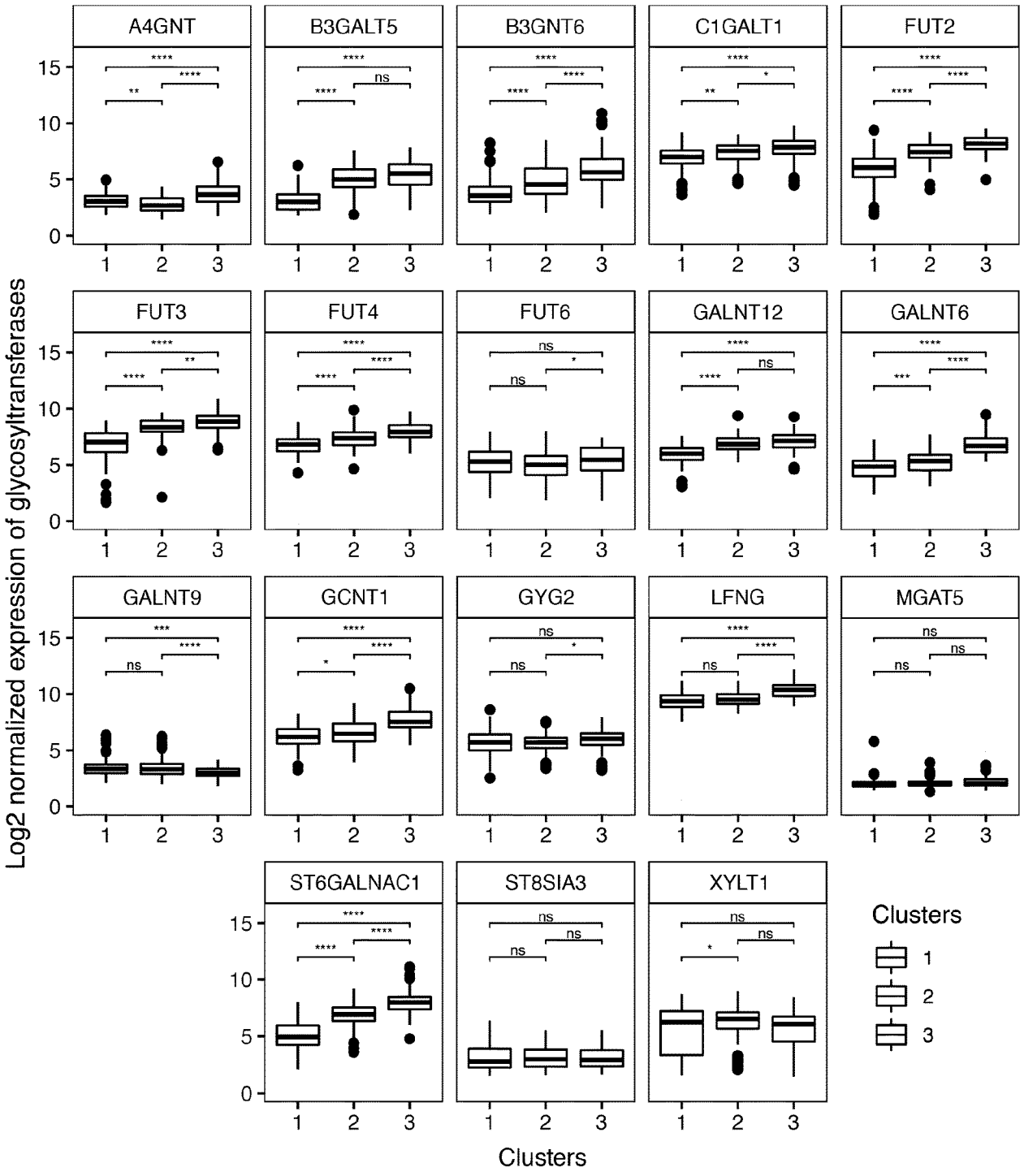
A4GNT
B3GALT5
B3GNT6
C1GALT1
FUT2
FUT3
FUT4
FUT6
GALNT12
GALNT6
GALNT9
GCNT1
GYG2
LFNG
MGAT5
ST6GALNAC1
ST8SIA3
XYLT1
Log2 normalized expression of glycosyltransferases
Clusters
Clusters
1
2
3

Figure 6B

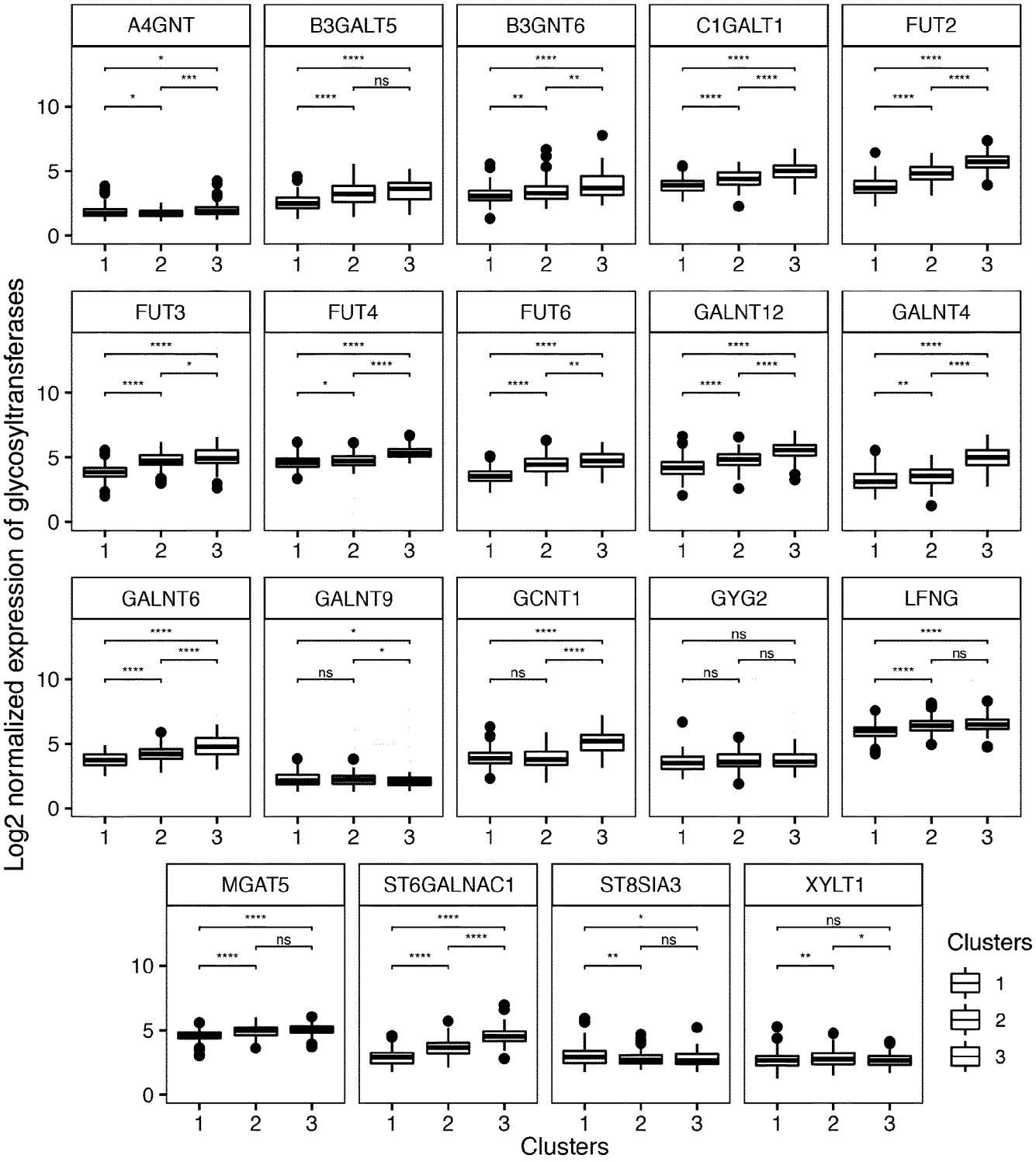
A4GNT
B3GALT5
B3GNT6
C1GALT1
FUT2
FUT3
FUT4
FUT6
GALNT12
GALNT4
GALNT6
GALNT9
GCNT1
GYG2
LFNG
MGAT5
ST6GALNAC1
ST8SIA3
XYLT1
Log2 normalized expression of glycosyltransferases
Clusters
1
2
3

Figure 6C

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2022/058151

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/6886
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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2015/049377 A1 (AB SCIENCE [FR]; ACOBIOM [FR]) 9 April 2015 (2015-04-09)	1-7
Y	WO 2016/091888 A2 (INST NAT SANTE RECH MED [FR] ET AL.) 16 June 2016 (2016-06-16) table 3	1,3,5-7

-/--

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

[x] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 June 2022	06/07/2022

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Cornelis, Karen

1

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2022/058151

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	GUPTA ROHITESH ET AL: "Global analysis of human glycosyltransferases reveals novel targets for pancreatic cancer pathogenesis", BRITISH JOURNAL OF CANCER, NATURE PUBLISHING GROUP, GB, vol. 122, no. 11, 19 March 2020 (2020-03-19), pages 1661-1672, XP037146798, ISSN: 0007-0920, DOI: 10.1038/S41416-020-0772-3 [retrieved on 2020-03-19] the whole document	1,3,5-7
A	SCHJOLDAGER KATRINE T ET AL: "Global view of human protein glycosylation pathways and functions", NAT REV MOL CELL BIOL, NATURE PUBLISHING GROUP UK, LONDON, vol. 21, no. 12, 21 October 2020 (2020-10-21), pages 729-749, XP037299641, ISSN: 1471-0072, DOI: 10.1038/S41580-020-00294-X [retrieved on 2020-10-21]	1-7

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2022/058151

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2015049377 A1	09-04-2015	AR 097909 A1	20-04-2016
		EP 3052103 A1	10-08-2016
		TW 201514311 A	16-04-2015
		US 2016244845 A1	25-08-2016
		WO 2015049377 A1	09-04-2015
WO 2016091888 A2	16-06-2016	NONE	