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(54) Title: METHOD OF PROGNOSTICATING, OR FOR DETERMINING THE EFFICIENCY OF A COMPOUND FOR TREATING CANCER

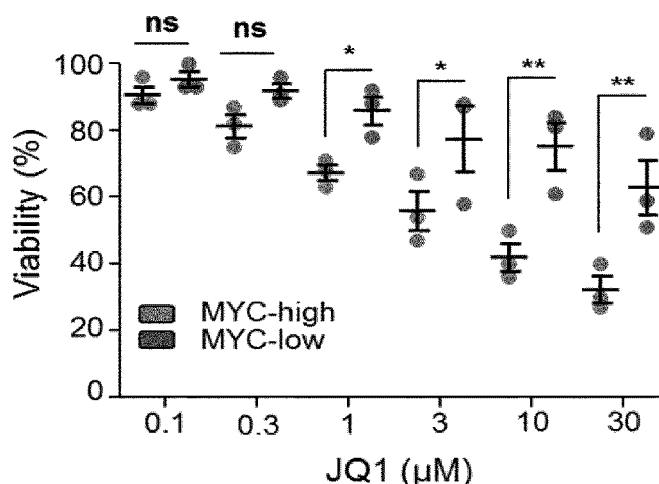


Fig. 5C

(57) Abstract: An *in vitro* or *ex vivo* method for determining the efficiency of a compound modulating the c-Myc oncogene activity for treating cancer, preferentially pancreatic adenocarcinoma, of a human in need thereof, or for prognosticating said cancer, comprising the step of measuring, in a biological sample from said human, the expression level of at least one specific marker gene selected from a group consisting of CDC20, KPNA2, PLK1, SRM, RFC4, MCM2, RUVBL2, MAD2L1, CCT4, CAD, VSIG2, BCL2L15, RAB25, TXNIP, CTSE and ERN2.

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METHOD OF PROGNOSTICATING, OR FOR DETERMINING THE
EFFICIENCY OF A COMPOUND FOR TREATING CANCER

TECHNICAL FIELD OF THE INVENTION

The invention relates to an *in vitro* or *ex vivo* method for determining the efficiency of a compound modulating
5 the c-Myc oncogene activity for treating cancer, preferentially pancreatic adenocarcinoma of a human in need thereof, and to an *in vitro* or *ex vivo* method for prognosticating such a cancer. It also relates to compositions, kits and solid supports, that are suitable
10 for use in said methods.

BACKGROUND OF THE INVENTION

Pancreatic ductal adenocarcinoma (PDAC) is one of the
15 most lethal cancers, and a major public issue since there are approximately 230,000 new PDAC cases per year worldwide with approximately the same number of death. Like others malignant diseases, PDAC results from a complex combination of genetic, epigenetic and
20 environmental factors which gives rise to a particularly heterogeneous disease, with patients having different set of symptoms, predisposition to early metastasis, and therapeutic responses. Current treatments for patients with a PDAC are not highly effective primarily due to the
25 recently discovered fact that these tumors are both molecularly and clinically heterogeneous. For example, the response of these tumors to gemcitabine and Folfirinox, the two gold standard therapies against PDAC, is of only 10 (Burris et al, 1997) and 31% (Conroy et al,

2011) respectively. The variability in this response seems to be due, on one hand, to the difficulty for drugs to reach the transformed cells because of the compact PDAC stroma (resulting in hypovascularization) and, on the other hand, to the marked differences in cellular susceptibility to drugs into the tumors. Therefore, it has become important to develop methods to stratify patients in a manner that allows to predicting their susceptibility to the treatments so as to increase their therapeutic responses which will results in increased survival expectancies. The patent document WO2016/091888 is an example of such strategy.

A frequently deregulated, although insufficiently therapeutically exploited pathway in PDAC involves the *c-Myc* oncogene. This transcription factor influences the expression of a significant number of genes involved in cell growth, proliferation and apoptosis. In fact, this oncogene has been implicated in the pathogenesis of one-third of all human malignancies. As it relates to pancreatic cancer, *c-Myc* is found to be amplified in more than 30% as well as overexpressed in more than 40% of tumors, with additional cases displaying rearrangement or changes in methylation of this locus. Early studies confirmed the oncogenic role of *c-Myc* in PDAC using genetically engineered mouse models, which upon overexpression of this gene display increased pancreatic tumorigenesis. In addition, using a variety of experimental models, it has been later shown that the *MYC* pathway activation induces tumor growth, DNA replication, protein synthesis and increases tumor cell metabolism, angiogenesis and suppression of the host immune response. Moreover, *MYC* activates stemness, blocks cellular

senescence, and its overexpression is frequently associated with poor clinical outcome and aggressiveness. This indicates that c-MYC behaves as a cancer driver gene for PDAC.

5

Consequently, many efforts have been dedicated to identify potent MYC inhibitors as new therapeutic options. Key to these efforts, the discovery that the Bromodomain and extra-terminal family of proteins (BET),
10 which are efficiently inhibited by a compound known as JQ1, are necessary for MYC activity. Notably, JQ1 suppresses PDAC development in mice by inhibiting both the MYC activity and inflammatory signals. Conversely, inhibition of MYC activity is thought to be also an
15 essential mechanism by which BET inhibitors suppress tumor progression in hematological malignancies. Thus, identifying a subgroup of pancreatic patients based on their MYC-high status and testing their response to JQ1 is timely and of paramount medical importance.

20

Based on this observation, there remains a need for discovering potential markers for patients stratification and, more precisely, potential markers involved by the c-Myc oncogene pathway. In other words, there remains a
25 need for identifying pathways that are deregulated in tumors. The blockage of these pathways with specific inhibitors should lead to cell growth arrest, death and tumor regression. Finally, there remains a need for selecting, by means of a few markers, a particular
30 subgroup of patients in order to attain more specific and efficient cancer treatments and, in particular, PDAC treatments.

SUMMARY OF THE INVENTION

In accordance with a first aspect, the invention relates to an *in vitro* or *ex vivo* method for determining the efficiency of a compound modulating the c-Myc oncogene activity for treating cancer, preferentially pancreatic adenocarcinoma, of a human in need thereof, comprising the steps of:

- a) measuring, in a biological sample from said human, the expression level of at least one marker gene selected from a group consisting of CDC20, KPNA2, PLK1, SRM, RFC4, MCM2, RUVBL2, MAD2L1, CCT4, CAD, VSIG2, BCL2L15, RAB25, TXNIP, CTSE and ERN2; and
- b) determining the efficiency of said compound on the c-Myc oncogene activity from the measurement obtained in step a).

According to a second aspect, the invention relates to an *in vitro* or *ex vivo* method for prognosticating a cancer, preferentially pancreatic adenocarcinoma, of a human, comprising the steps of:

- a1) measuring, in a biological sample from said human, the expression level of at least one marker gene selected from a group consisting of CDC20, KPNA2, PLK1, SRM, RFC4, MCM2, RUVBL2, MAD2L1, CCT4, CAD, VSIG2, BCL2L15, RAB25, TXNIP, CTSE and ERN2; and
- a2) determining, from said measurement, if the MYC pathway is activated or not in said cancer; and
- b) prognosticating said cancer from the determination and measurement obtained in steps a1) and a2).

According to a third aspect, the invention relates to a composition comprising at least one probe for

quantitative measuring the expression level of at least one cancer marker gene, preferentially pancreatic adenocarcinoma marker gene, involved by the c-Myc oncogene and selected from a group consisting of CDC20, KPNA2, PLK1, SRM, RFC4, MCM2, RUVBL2, MAD2L1, CCT4, CAD, VSIG2, BCL2L15, RAB25, TXNIP, CTSE and ERN2.

According to a fourth aspect, the invention relates to a kit comprising at least one probe for quantitative measuring the expression level of at least one cancer marker gene, preferentially pancreatic adenocarcinoma marker gene, involved by the c-Myc oncogene and selected from a group consisting of CDC20, KPNA2, PLK1, SRM, RFC4, MCM2, RUVBL2, MAD2L1, CCT4, CAD, VSIG2, BCL2L15, RAB25, TXNIP, CTSE and ERN2.

According to a fifth aspect, the present invention relates to a solid support comprising at least one probe for quantitative measuring the expression level of at least one cancer marker gene, preferentially pancreatic adenocarcinoma marker gene, involved by the c-Myc oncogene and selected from a group consisting of CDC20, KPNA2, PLK1, SRM, RFC4, MCM2, RUVBL2, MAD2L1, CCT4, CAD, VSIG2, BCL2L15, RAB25, TXNIP, CTSE and ERN2.

25

Then, according to a sixth aspect, the invention relates to a method for selecting cancer markers, according to which:

transcriptomes of tumor cells are provided for a plurality of patients;

a specific expression pathway of one or a plurality of specific genes that are involved in said cancer are identified;

an expression profile of said specific genes is established for said specific expression pathway;

the transcriptomes are analyzed for the plurality of patients;

5 some reference genes are selected for the considered specific pathway, for said cancer ; and

a signature characterizing said pathway for said cancer is defined.

10 Advantageously, the *in vitro* or *ex vivo* method for determining the efficiency of a compound modulating the c-Myc oncogene expression for treating cancer, preferentially pancreatic adenocarcinoma, of human in need thereof, is characterized in that: - in step a) it
15 is measured the expression level of at least one marker gene selected from a group consisting of CDC20, KPNA2, PLK1, SRM, RFC4, MCM2, RUVBL2, MAD2L1, CCT4 and CAD; - in step a) it is measured the expression level of at least one marker gene selected from a group consisting of
20 VSIG2, BCL2L15, RAB25, TXNIP, CTSE and ERN2; - in step a) it is measured the expression level of at least one marker gene selected from a first group consisting of CDC20, KPNA2, PLK1, SRM, RFC4, MCM2, RUVBL2, MAD2L1, CCT4 and CAD, and the expression level of at least one marker
25 gene selected from a second group consisting of VSIG2, BCL2L15, RAB25, TXNIP, CTSE and ERN2; - in step a) it is measured the expression level of CDC20, KPNA2, PLK1, SRM, RFC4, MCM2, RUVBL2, MAD2L1, CCT4 and CAD; - in step a) it is measured the expression level of VSIG2, BCL2L15,
30 RAB25, TXNIP, CTSE and ERN2; - it is determined the efficiency of a compound for treating cancer of human in need thereof in which a Myc-high pathway is involved; - it is determined the efficiency of a compound for

treating cancer of human in need thereof in which a Myc-low pathway is involved; - the compound is a BET proteins inhibitor; - the compound is selected from a group consisting of (*S*)-*tert*-butyl 2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-
5 a][1,4]diazepin-6-yl)acetate, OTX015/MK-8628 (Merck™), TEN-010 (Tensha Therapeutics™), ZEN-3365 (Zenith Epigenetics™), ABBV-075 (AbbVie™), INCB-54329 (Incyte™), GS-5829 (Gilead Sciences™); - the biological sample is
10 selected from the group consisting of a tumor sample, a xenograft, a primary culture and an organoid derived from biopsy.

BRIEF DESCRIPTION OF THE FIGURES

15

Other features and aspects of the present invention will be apparent from the following description and the accompanying drawings, in which:

20 Fig. 1A illustrates the hierarchical clustering and expression heatmap for the top significantly high-expressed genes in MYC-high patients, and includes a dendrogram illustrating the genetic distance between patients of two major subgroups, which are defined as the
25 MYC-high and MYC-low subgroups;

Fig. 1B provides the enrichment scores obtained for the MYC-high vs. MYC-low subgroups, for various biological processes including the cell cycle process, the
30 digestion, the DNA replication and the glycoprotein metabolism, and using Gene Set Enrichment Analysis (GSEA);

Fig. 1C illustrates (i) a semi-quantitative Ki67 scoring, and (ii) a differentiation scoring between the MYC-high and MYC-low subgroups, wherein "***" corresponds to $p < 0.01$ and "****" corresponds to $p < 0.001$;

5

Fig. 1D illustrates the relapse free and the overall survival of the MYC-high and the MYC-low subgroups along time;

10 Fig. 2A represents the hierarchical clustering and expression heatmap for the top significantly low-expressed genes in MYC-high patients;

Fig. 2B provides the transcripts of the 6 top-score
15 upregulated marker genes in the MYC-low subgroup;

Fig. 2C provides the transcripts of the 10 top-score upregulated marker genes in the MYC-high subgroup;

20 Fig. 2D shows the box plots representing the normalized expression ratios for the sixteen selected transcripts in the MYC-associated signature, wherein expression ratios > 1 correspond to the MYC-high profile, expression ratios < 1 correspond to the MYC-low profile, and the \$ symbol
25 points out 4 false positives that were detected in the signature;

Fig. 3A are box plots representing normalized expression ratios from the transcriptomic data (Affimetrix™) as in
30 Fig. 2D for 4 Patient Derived Xenografts (PDX) of each MYC-high and MYC-low subgroup;

Fig. 3B are box plots representing normalized expression ratios by RT-qPCR for the same 4 PDX as in Fig. 3A;

Fig. 3C are box plots representing normalized expression ratios by RT-qPCR for the same eight PDX (as in Fig. 3A) derived primary cultures, wherein "****" corresponds to $p < 0.001$;

Fig. 4A are chemograms obtained for the 8 PDX derived cell lines showing the viability of cells treated by JQ1 (i) for four patients presenting the upregulated marker genes in MYC-high, and (ii) for four patients presenting the upregulated marker genes in MYC-low;

Fig. 4B provides box plots representing the JQ1 sensitivity for the 6 highest concentrations used in chemograms;

Fig. 4C are histograms representing the IC_{50} for JQ1 for 4 MYC-high and 4 MYC-low cell lines;

Fig. 4D are histograms representing spheroids volumes from 3 derived cell lines in each group treated with JQ1 at 2 μ M during 72h or with DMSO (0.05%), wherein "****" corresponds to $p < 0.001$;

Fig. 4E illustrates the effect of the JQ1 treatment on MYC-high primary cell CRCM16;

Fig. 5A provides box plots representing the normalized expression ratios for the MYC signature in 16 new PDX, used as validation or test cohort, by RT-qPCR.

Fig. 5B provides chemograms for 8 PDX derived cells lines: the viability of cells which is treated by JQ1 (i) for 4 patients presenting the upregulated marker genes in MYC-high pathway, and (ii) for 4 patients presenting the upregulated marker genes in MYC-low pathway;

Fig. 5C shows a graph representing JQ1 sensitivity for the 6 highest concentrations used in chemograms, and wherein "*" corresponds to $p < 0.05$ and "***" corresponds to $p < 0.01$;

Fig. 5D contains histograms that are representing the IC_{50} for JQ1 for the 3 MYC-high and the 3 MYC-low cell lines taken from the validation cohort, and wherein "*" corresponds to $p < 0.05$; and

Figs. 6A and 6B are images illustrating the Ki67 and differentiation scores.

Fig. 7 shows representative microphotographies (on top) and Hematoxylin & Eosin staining (on bottom) of different organoid cultures established from EUS-FNA biopsies. White scale bars (on top) represent $1000\mu m$ and black scale bars (on bottom) represent $200\mu m$.

DETAILED DESCRIPTION OF THE INVENTION

Current treatments for patients with a cancer and, in particular, PDAC, are not always effective primarily due to the recently discovered fact that their tumors can be both molecularly and clinically heterogeneous. The variability in the treatment responses appears to be due, on one hand, to the difficulty for drugs to reach the

transformed cells, which is the case for PDAC because of the compact PDAC stroma, resulting in hypovascularization, and, on the other hand, to the marked differences in cellular susceptibility to drugs into the tumors due to their molecular heterogeneity.

Therefore, it is important to develop methods to stratify patients in a manner that allows to predict their susceptibility to the treatments so as to increase their therapeutic responses which will results in increased survival expectancies.

The invention focuses on a subgroup of cancers which are characterized by a deregulation of the c-MYC pathway. A specific molecular signature is established, allowing to identify tumors and, in particular, PDAC, wherein it is possible to specifically inhibit the expression of BET-proteins.

As used herein:

the terms "c-MYC pathway", "c-MYC" and grammatical variations thereof, refer to a pathway wherein the c-Myc oncogene is involved;

the terms "MYC-high", "MYC-high subtype", "MYC-high subgroup" and grammatical variations thereof, refer to a pathway wherein the c-Myc oncogene is involved, and notably by overexpressed or upregulated specific marker genes involved in the particular MYC-high pathway;

the terms "MYC-low", "MYC-low subtype", "MYC-low subgroup" and a grammatical variations thereof, refer to

a pathway wherein the c-MYC oncogene is involved, and notably by overexpressed or upregulated specific marker genes involved in the particular MYC-low pathway;

5 the acronym "BET" means "bromodomain and extra-terminal". It can be used in the below description the terms "BET" or "BET-proteins" which mean "bromodomain and extra-terminal proteins". The terms "BET-inhibitors" may also been used, which mean "bromodomain and extra-
10 terminal proteins inhibitors";

 the acronym "JQ1" mean "(S)-tert-butyl 2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetate". For
15 "JQ1", it can also be used in the below description the term "thienotriazolodiazepine".

c-Myc controls more than 15% of genes responsible for proliferation, differentiation, and cellular metabolism
20 in pancreatic adenocarcinoma as well as other cancers making this transcription factor a prime target for treating patients.

Consistent with these observations it is defined a MYC
25 transcriptional program as a restricted signature that allowed the selection of distinct subtype of cancer, notably PDAC tumors. This MYC-associated signature is established by using selected MYC target genes available from the molecular signature database and which are
30 reported to be directly regulated by this transcription factor. From the 239 putative MYC-dependent genes (see the following table 1), 16 targets were selected

according to the fold change between c-MYC-high and c-MYC-low PDAC (see the following table 2).

Rank	Gene symbol	Affymetrix probeset ID's	T-Test (median)	p-value	FDR (BH)	Fold Change
1	KPNA2	16837270	8,98	0.001996	0.004458	1,74
2	SNRPD2	16873391	7,76	0.001996	0.004458	1,31
3	YWHAQ	16894361	7,64	0.001996	0.004458	1,2
4	RUVBL2	16863946	7,56	0.001996	0.004458	1,48
5	PLK1	16817017	7,03	0.001996	0.004458	1,6
6	ERH	16794256	6,84	0.001996	0.004458	1,36
7	MCM2	16945101	6,7	0.001996	0.004458	1,51
8	CCNA2	16979515	6,6	0.001996	0.004458	1,61
9	CDC20	16663514	6,58	0.001996	0.004458	1,73
10	XPO1	16898110	6,57	0.001996	0.004458	1,33
11	PSMA2	17057035	6,5	0.001996	0.004458	1,34
12	TCOF1	16991078	6,31	0.001996	0.004458	1,37
13	PSMD7	16821021	6,22	0.001996	0.004458	1,4
14	RFC4	16962493	6,21	0.001996	0.004458	1,65
15	EIF4H	17047073	6	0.001996	0.004458	1,28
16	KARS	16828514	5,95	0.001996	0.004458	1,38
17	MAD2L1	16979389	5,75	0.001996	0.004458	1,68
18	PHB2	16760649	5,7	0.001996	0.004458	1,56
19	CCT4	16898175	5,6	0.001996	0.004458	1,42
20	CDK4	16766683	5,53	0.001996	0.004458	1,58
21	PSMD14	16886919	5,49	0.001996	0.004458	1,33
22	TRIM28	16866337	5,44	0.001996	0.004458	1,36
23	HSPD1	16906962	5,43	0.001996	0.004458	1,39
24	MCM7	17060412	5,31	0.001996	0.004458	1,55
25	PLK4	16970563	5,26	0.001996	0.004458	1,95
26	C1QBP	16840284	5,04	0.001996	0.004458	1,34
27	MCM4	17068782	5,02	0.001996	0.004458	1,61
28	CCT5	16983172	4,98	0.001996	0.004458	1,37

29	PRDX4	17102111	4, 97	0.001996	0.004458	1, 31
30	MRT04	16660199	4, 93	0.001996	0.004458	1, 36
31	PRPS2	17101517	4, 92	0.001996	0.004458	1, 36
32	NDUFAB1	16825097	4, 92	0.001996	0.004458	1, 32
33	TBRG4	17057433	4, 83	0.001996	0.004458	1, 21
34	PRPF31	16865193	4, 77	0.001996	0.004458	1, 28
35	RRM1	16721126	4, 74	0.001996	0.004458	1, 47
36	EIF2S2	16918485	4, 69	0.001996	0.004458	1, 29
37	MCM6	16903090	4, 68	0.001996	0.004458	1, 55
38	PCNA	16916958	4, 61	0.001996	0.004458	1, 44
39	SSB	16887334	4, 58	0.001996	0.004458	1, 25
40	SRM	16681611	4, 54	0.001996	0.004458	1, 7
41	PRDX3	16718922	4, 54	0.001996	0.004458	1, 42
42	LAS1L	17111688	4, 5	0.001996	0.004458	1, 33
43	HNRNPR	16683271	4, 44	0.001996	0.004458	1, 25
44	EIF1AX	17109706	4, 36	0.001996	0.004458	1, 37
45	GSPT1	16824004	4, 35	0.001996	0.004458	1, 34
46	KPNB1	16835272	4, 28	0.001996	0.004458	1, 23
47	PPM1G	16895848	4, 27	0.001996	0.004458	1, 29
48	EIF3M	16723294	4, 24	0.001996	0.004458	1, 29
49	CANX	16993397	4, 23	0.003992	0.007633	1, 15
50	PSMA1	16736049	4, 21	0.001996	0.004458	1, 25
51	RNPS1	16823097	4, 14	0.001996	0.004458	1, 25
52	PTGES3	16766283	4, 14	0.001996	0.004458	1, 21
53	RPL6	16770445	4, 12	0.001996	0.004458	1, 33
54	H2AFZ	16978334	4, 06	0.001996	0.004458	1, 29
55	UTP20	16755750	4, 05	0.001996	0.004458	1, 71
56	TRA2B	16962359	4, 05	0.007984	0.01344	1, 18
57	RANBP1	16927198	4, 02	0.003992	0.007633	1, 24
58	TYMS	16850477	4, 01	0.001996	0.004458	1, 7
59	NHP2	17003479	4, 01	0.001996	0.004458	1, 4
60	ETF1	17000465	4	0.001996	0.004458	1, 31
61	USP1	16665447	3, 99	0.001996	0.004458	1, 39
62	CNBP	16958953	3, 93	0.001996	0.004458	1, 18

63	GLO1	17018812	3, 9	0.003992	0.007633	1, 35
64	PSMA7	16920910	3, 9	0.001996	0.004458	1, 32
65	RAN	16759202	3, 84	0.001996	0.004458	1, 45
66	VDAC3	17068541	3, 82	0.001996	0.004458	1, 31
67	SF3B3	16820787	3, 81	0.001996	0.004458	1, 36
68	STARD7	16900450	3, 8	0.001996	0.004458	1, 26
69	PABPC4	16685669	3, 79	0.001996	0.004458	1, 27
70	CDK2	16752305	3, 76	0.001996	0.004458	1, 31
71	GOT2	16826985	3, 71	0.001996	0.004458	1, 46
72	SNRPB2	16911605	3, 71	0.001996	0.004458	1, 26
73	SSBP1	17052394	3, 68	0.01198	0.01947	1, 18
74	PSMB2	16685144	3, 67	0.001996	0.004458	1, 22
75	DHX15	16974873	3, 65	0.005988	0.0106	1, 13
76	SRSF1	16846993	3, 65	0.007984	0.01344	1, 15
77	CAD	16878137	3, 63	0.001996	0.004458	1, 51
78	TCP1	17025297	3, 62	0.001996	0.004458	1, 45
79	NOC4L	16759500	3, 62	0.001996	0.004458	1, 34
80	G3BP1	16991261	3, 61	0.001996	0.004458	1, 21
81	CCT7	16881485	3, 59	0.001996	0.004458	1, 33
82	NOP16	17003050	3, 57	0.001996	0.004458	1, 35
83	DDX21	16705474	3, 57	0.001996	0.004458	1, 56
84	CCT2	16753964	3, 54	0.001996	0.004458	1, 41
85	VDAC1	16999985	3, 54	0.001996	0.004458	1, 22
86	CLNS1A	16742491	3, 53	0.001996	0.004458	1, 23
87	MYBBP1A	16840018	3, 51	0.005988	0.0106	1, 27
88	SUPV3L1	16705531	3, 51	0.001996	0.004458	1, 75
89	PGK1	17105047	3, 49	0.003992	0.007633	1, 18
90	WDR74	16739608	3, 48	0.001996	0.004458	1, 44
91	RPLP0	16771216	3, 47	0.005988	0.0106	1, 28
92	TXNL4A	16856153	3, 44	0.003992	0.007633	1, 55
93	PSMC6	16784228	3, 42	0.005988	0.0106	1, 17
94	GNB2L1	17004092	3, 41	0.001996	0.004458	1, 22
95	RABEPK	17089037	3, 39	0.001996	0.004458	1, 23
96	EIF2S1	16785631	3, 37	0.003992	0.007633	1, 43

97	WDR43	16878556	3, 36	0.001996	0.004458	1, 44
98	DCTPP1	16825794	3, 28	0.001996	0.004458	1, 36
99	SNRPG	16898764	3, 27	0.001996	0.004458	1, 33
100	MCM5	16929573	3, 26	0.003992	0.007633	1, 43
101	UNG	16756627	3, 24	0.001996	0.004458	1, 42
102	HSP90AB1	17009217	3, 23	0.003992	0.007633	1, 13
103	EIF3B	17042946	3, 22	0.003992	0.007633	1, 28
104	PSMA6	16783389	3, 16	0.003992	0.007633	1, 27
105	PSMA4	16803540	3, 16	0.03792	0.0546	1, 18
106	NIP7	16820584	3, 15	0.001996	0.004458	1, 42
107	NOP56	16910825	3, 15	0.001996	0.004458	1, 46
108	NDUFAF4	17021837	3, 11	0.003992	0.007633	1, 44
109	XRCC6	16930678	3, 06	0.02595	0.039	1, 18
110	VBP1	17108562	3, 04	0.001996	0.004458	1, 22
111	SNRPA	16862333	3, 03	0.003992	0.007633	1, 36
112	TOMM70A	16956773	3, 03	0.005988	0.0106	1, 19
113	TUFM	16825410	3	0.001996	0.004458	1, 26
114	ABCE1	16971139	2, 97	0.003992	0.007633	1, 47
115	IARS	17095775	2, 97	0.001996	0.004458	1, 32
116	PA2G4	16752453	2, 95	0.003992	0.007633	1, 24
117	NPM1	16992324	2, 94	0.001996	0.004458	1, 37
118	SNRPA1	16813900	2, 94	0.02595	0.039	1, 14
119	YWHAE	16839331	2, 91	0.001996	0.004458	1, 17
120	PUS1	16759395	2, 9	0.001996	0.004458	1, 18
121	ODC1	16894402	2, 9	0.005988	0.0106	1, 82
122	FBL	16872267	2, 89	0.001996	0.004458	1, 48
123	SRSF2	16849321	2, 88	0.005988	0.0106	1, 16
124	NAP1L1	16767756	2, 87	0.001996	0.004458	2, 12
125	NOP2	16760437	2, 87	0.01198	0.01947	1, 43
126	POLD2	17057174	2, 86	0.001996	0.004458	1, 27
127	TFDP1	16776905	2, 85	0.06387	0.08674	1, 11
128	LSM14B	16915570	2, 83	0.007984	0.01344	1, 15
129	SET	17089798	2, 83	0.007984	0.01344	1, 25
130	FARSA	16869372	2, 83	0.001996	0.004458	1, 3

131	UBA2	16860746	2, 8	0.001996	0.004458	1, 23
132	EXOSC5	16872567	2, 78	0.003992	0.007633	1, 16
133	CDC45	16927052	2, 77	0.003992	0.007633	1, 21
134	IMP4	16885526	2, 76	0.001996	0.004458	1, 26
135	RPS3	16729103	2, 76	0.005988	0.0106	1, 21
136	XPOT	16753465	2, 72	0.001996	0.004458	1, 66
137	HDAC2	17022854	2, 71	0.003992	0.007633	1, 19
138	RPS5	16866282	2, 71	0.01198	0.01947	1, 2
139	PHB	16846416	2, 7	0.02994	0.04417	1, 12
140	SLC25A3	16755520	2, 7	0.001996	0.004458	1, 33
141	CBX3	17044423	2, 7	0.007984	0.01344	1, 2
142	LDHA	16722603	2, 7	0.04192	0.05963	1, 17
143	IPO4	16791086	2, 69	0.001996	0.004458	1, 31
144	RPL14	16939495	2, 67	0.07585	0.09906	1, 13
145	DUT	16800839	2, 66	0.03593	0.05204	1, 18
146	ACP1	16876440	2, 65	0.01996	0.03098	1, 13
147	HNRNPA1	16751993	2, 64	0.03992	0.05713	1, 2
148	AIMP2	17043355	2, 61	0.005988	0.0106	1, 19
149	SNRPD1	16851344	2, 57	0.007984	0.01344	1, 47
150	PSMC4	16862145	2, 47	0.01397	0.02226	1, 23
151	APEX1	16781516	2, 45	0.003992	0.007633	1, 31
152	PWP1	16756475	2, 44	0.001996	0.004458	1, 59
153	BYSL	17008544	2, 42	0.02395	0.0367	1, 17
154	RPL18	16873987	2, 42	0.00998	0.01656	1, 12
155	EEF1B2	16889938	2, 41	0.02794	0.04148	1, 2
156	GRWD1	16863783	2, 38	0.02794	0.04148	1, 1
157	IFRD1	17050381	2, 34	0.01597	0.02527	1, 24
158	SRSF7	16896650	2, 33	0.06387	0.08674	1, 13
159	COX5A	16811739	2, 33	0.007984	0.01344	1, 14
160	BUB3	16710271	2, 31	0.01796	0.02806	1, 38
161	COPS5	17077888	2, 3	0.02196	0.03385	1, 15
162	RRP12	16717106	2, 28	0.001996	0.004458	1, 31
163	POLE3	17097495	2, 27	0.05389	0.07532	1, 12
164	SF3A1	16933818	2, 26	0.06786	0.09061	1, 1

165	EIF3D	16934583	2, 23	0.08583	0.1109	1, 13
166	HNRNPU	16701324	2, 22	0.03194	0.04683	1, 12
167	PSMD3	16833934	2, 22	0.005988	0.0106	1, 3
168	HDDC2	17023308	2, 19	0.09182	0.1161	1, 27
169	RSL1D1	16823991	2, 16	0.02595	0.039	1, 41
170	AP3S1	16988174	2, 15	0.001996	0.004458	1, 26
171	SRPK1	17018530	2, 08	0.0499	0.07015	1, 06
172	RRP9	16954623	2, 07	0.09381	0.1174	1, 1
173	PSMD8	16861704	2, 01	0.07585	0.09906	1, 14
174	PCBP1	16881153	2, 01	0.1557	0.1833	1, 12
175	DDX18	16884877	2	0.01796	0.02806	1, 2
176	ORC2	16907173	1, 96	0.07385	0.09752	1, 17
177	ILF2	16693515	1, 86	0.1078	0.1321	1, 11
178	CSTF2	17105347	1, 86	0.03593	0.05204	1, 16
179	PPIA	17045738	1, 85	0.1098	0.1339	1, 22
180	RAD23B	17087933	1, 84	0.07385	0.09752	1, 18
181	MRPL9	16693173	1, 84	0.05988	0.08225	1, 2
182	CUL1	17053019	1, 83	0.01397	0.02226	1, 23
183	IMPDH2	16953813	1, 8	0.04591	0.06492	1, 3
184	NMD3	16947626	1, 75	0.08383	0.1089	1, 23
185	SYNCRIP	17021385	1, 71	0.1357	0.1614	1, 12
186	EIF4E	16978142	1, 68	0.1597	0.1871	1, 09
187	CCT3	16694529	1, 66	0.09381	0.1174	1, 07
188	SRSF3	17007996	1, 63	0.05788	0.07997	1, 06
189	EXOSC7	16940036	1, 58	0.1697	0.1978	1, 15
190	PSMB3	16833698	1, 57	0.1178	0.1422	1, 21
191	RPL22	16681064	1, 54	0.1058	0.1303	1, 09
192	PRMT3	16722799	1, 53	0.06786	0.09061	1, 39
193	RCL1	17083295	1, 53	0.0978	0.1217	1, 15
194	CYC1	17073565	1, 5	0.01397	0.02226	1, 29
195	CTNS	16829738	1, 48	0.1018	0.1261	1, 12
196	NOLC1	16708552	1, 43	0.08782	0.1122	1, 51
197	PPRC1	16708533	1, 4	0.06587	0.08894	1, 31
198	UBE2E1	16938344	1, 38	0.2754	0.3105	1, 06

199	EIF3J	16800431	1,38	0.4092	0.4445	1,12
200	TARDBP	16658926	1,33	0.00998	0.01656	1,31
201	RPS6	17092737	1,28	0.1717	0.1992	1,17
202	MYC	17072669	1,24	0.1138	0.138	1,4
203	EIF4G2	16735801	1,24	0.3852	0.4204	1,01
204	HK2	16881838	1,22	0.4331	0.4642	1,04
205	HNRNPD	16977340	1,18	0.6108	0.6402	1,02
206	MPHOSPH10	16881274	1,17	0.08782	0.1122	1,15
207	HPRT1	17107045	1,16	0.3214	0.3572	1,16
208	NCBP2	16963402	1,15	0.1277	0.1534	1,15
209	U2AF1	16926148	1,15	0.3713	0.4089	1,09
210	DEK	17015987	1,14	0.2236	0.2569	1,13
211	EPRS	16699392	1,13	0.3832	0.4202	1,05
212	TFB2M	16701407	1,11	0.2635	0.2984	1,05
213	PES1	16933931	1,07	0.1756	0.2028	1,19
214	GNL3	16941661	1,04	0.5349	0.5657	1,11
215	PSMD1	16892039	1,03	0.6906	0.7145	1,04
216	HNRNPA2B1	17056031	0.9459	0.1437	0.17	1,06
217	SNRPD3	16928282	0.8702	0.3074	0.3433	1,05
218	SMARCC1	16953199	0.8071	0.2275	0.2602	1,01
219	MRPL23	16720820	0.8037	0.3453	0.3821	1,06
220	HDGF	16694742	0.7125	0.4132	0.4448	1,02
221	SERBP1	16688308	0.5663	0.6467	0.6749	1,06
222	MAP3K6	16683960	0.558	0.2834	0.318	1,03
223	LSM7	16866940	0.5212	0.6647	0.6907	1,05
224	SLC29A2	16740778	0.4233	0.5349	0.5657	1,01
225	UBE2L3	16927615	0.3884	0.7505	0.7731	1,03
226	RPS19BP1	16935264	0.3745	0.9321	0.9321	1,02
227	TMEM97	16832429	0.3107	0.8723	0.8833	1,04
228	RPL34	16969624	0.3099	0.9142	0.918	1,02
229	NCBP1	17087343	0.2708	0.7745	0.791	1,08
230	HNRNPA3	16888031	-0.2364	0.8244	0.8384	-1,08
231	MRPS18A	17019516	-0.2544	0.8902	0.8977	-1,01
232	RPRML	16846035	-0.4361	0.5788	0.6094	-1,04

233	PABPC1	17079672	-0.4857	0.7605	0.7801	1,12
234	SLC19A1	16926595	-0.5267	0.525	0.5601	-1,02
235	HNRNPC	16790233	-1,3	0.1357	0.1614	-1,1
236	PPA2	16978661	-1,32	0.4132	0.4448	-1,03
237	DUSP2	16900441	-1,83	0.2335	0.2658	-1,07
238	SORD	16800506	-1,94	0.09182	0.1161	-1,2
239	FAM120A	17086921	-2,12	0.05788	0.07997	-1,11

Table 1 : Rank-listed transcripts

	Gene Symbol	Affimetrix ID	Ref Seq	mRNA assignment
Up- regulated transcripts in Myc- high cohort	CDC20	16663514	NM 001255	<i>Homo sapiens cell division cycle 20</i>
	KPNA2	16837270	NM 002266	<i>Homo sapiens karyopherin alpha 2</i>
	PLK1	16817017	ENST0000030009 3	<i>Homo sapiens polo-like kinase 1</i>
	SRM	16681611	NM 003132	<i>Homo sapiens spermidine synthase</i>
	RFC4	16962493	NM 002916	<i>Homo sapiens replication factor C (activator 1) 4</i>
	MCM2	16945101	ENST0000026505 6	<i>Homo sapiens minichromosome maintenance complex component 2</i>
	RUVBL2	16863946	NM 006666	<i>Homo sapiens</i>

				<i>RuvB-like 2 (E. coli)</i>
	MAD2L1	16979389	ENST00000296509	<i>Homo sapiens MAD2 mitotic arrest deficient-like 1</i>
	CCT4	16898175	NM 006430	<i>Homo sapiens chaperonin containing TCP1, subunit 4 (delta)</i>
	CAD	16878137	NM 004341	<i>Homo sapiens carbamoyl-phosphate synthetase 2</i>
Up-regulated transcripts in Myc-low cohort	VSIG2	16745683	NM 014312	<i>Homo sapiens V-set and immunoglobulin domain containing 2</i>
	BCL2L15	16691121	NM 001010922	<i>Homo sapiens BCL2-like 15 (BCL2L15)</i>
	RAB25	16671901	NM 020387	<i>Homo sapiens RAB25, member RAS oncogene family</i>
	TXNIP	16669796	NM 006472	<i>Homo sapiens thioredoxin interacting protein</i>
	CTSE	16676547	NM 001910	<i>Homo sapiens cathepsin E, transcript</i>

				<i>variant 1</i>
	ERN2	16825120	NM 033266	<i>Homo sapiens endoplasmic reticulum to nucleus signaling 2</i>

Table 2: List of biomarkers used in the transcriptomic signature in PDAC

5 Those targets usefully identify highly proliferative PDAC with low degree of differentiation and patient with poor clinical outcome as shown in Figure 1C and 1D. An efficient signature, like the one reported here, which is easily applicable and of low cost, for detecting patients
10 having a MYC-high PDAC for example is of clear clinical interest, particularly in non-operable patients which represents about 85% of PDAC.

The present invention relates to an *in vitro* or *ex vivo*
15 method for determining the efficiency of a compound modulating the c-Myc oncogene activity for treating cancer, preferentially pancreatic adenocarcinoma, of human in need thereof, comprising the steps of: a) measuring, in a biological sample from said human, the
20 expression level of at least one marker gene selected from a group consisting of CDC20, KPNA2, PLK1, SRM, RFC4, MCM2, RUVBL2, MAD2L1, CCT4, CAD, VSIG2, BCL2L15, RAB25, TXNIP, CTSE and ERN2; and, b) determining the efficiency of said compound on the c-Myc oncogene activity from the
25 measurement obtained in step a).

According to the invention, "measuring the expression of at least one gene" encompasses measuring the expression of at least one nucleic acid encoded by said at least one gene, which includes RNA molecules encoded by said at least one gene. In a non-limitative manner, the product of one gene may comprise RNA molecules including mRNA, tRNA, rRNA, small-interfering RNAs (siRNA), non-coding RNAs and microRNAs, which can be modified or not.

10 The expression of at least one gene may also encompass, in a more indirect manner, measuring the expression of at least one protein encoded by said at least one gene.

Methods for measuring the expression of at least one gene in a biological sample are known from the art. In a non-limitative manner, the expression of a protein may be achieved using Western blot, Slot blot, Dot blot, ELISA (Enzyme Linked Immuno-Sorbent Assay), immunofluorescence, electronic or confocal microscopy FRET (fluorescence resonance energy transfer), TR-FRET (time resolved FRET/FRET), FLIM (fluorescence lifetime imaging microscopy), FSPIM (fluorescence spectral imaging microscopy), FRAP (fluorescence recovery after photobleaching), flux cytometry, enzymatic tests.

25 Preferably, measuring the expression of at least one gene includes measuring the expression of at least one nucleic acid encoded by said at least one gene.

30 In a non-limitative manner, the expression of a nucleic acid may be achieved using polymerase-chain reaction (PCR), reverse transcriptase polymerase-chain reaction

(RT-PCR), Northern Blot, Ribonuclease protection assays, microarrays, *in situ* hybridization.

In particular, the expression of a nucleic acid may be
5 advantageously achieved by using the nCounter® DX
Analysis System with Flare configuration commercialized
by NanoString Technologies® company. The nCounter® Gene
Expression Assay is designed to provide a sensitive,
reproducible and highly multiplexed method for detecting
10 nucleic acid such as mRNA with molecular barcodes called
nCounter Reporter probes without the use of reverse
transcription or amplification. The probe pair consists
of the Reporter Probe, which carries the signal on its 5'
end, and the Capture Probe which carries a biotin on the
15 3' end. The color codes carry six positions and each
position can be one of four colors, thus allowing for a
large diversity of tags that can be mixed together in a
single well for direct hybridization to targets and yet
still be individually resolved and identified during data
20 collection. Unlike microarrays or PCR-based gene
expression analysis technologies, such system does not
rely on synthesis of a cDNA strand or PCR amplification.
No enzymes are used in this procedure. Instead, the
barcode-labeled probes anneal directly to mRNAs in
25 solution, and the hybrid molecule is then immobilized,
detected, and counted.

The method according to the present invention is
advantageously characterized in that, in step a) it is
30 measured the expression level of at least one marker gene
selected from a group consisting of CDC20, KPNA2, PLK1,
SRM, RFC4, MCM2, RUVBL2, MAD2L1, CCT4 and CAD. In a
preferred embodiment, in step a) it is measured the

expression level of at least one marker gene selected from a group consisting of VSIG2, BCL2L15, RAB25, TXNIP, CTSE and ERN2. Advantageously, in step a) it is measured the expression level of at least one marker gene selected from a first group consisting of CDC20, KPNA2, PLK1, SRM, RFC4, MCM2, RUVBL2, MAD2L1, CCT4 and CAD, and the expression level of at least one marker gene selected from a second group consisting of VSIG2, BCL2L15, RAB25, TXNIP, CTSE and ERN2. Advantageously, in step a) it is measured the expression level of CDC20, KPNA2, PLK1, SRM, RFC4, MCM2, RUVBL2, MAD2L1, CCT4 and CAD. Advantageously, in step a) it is measured the expression level of VSIG2, BCL2L15, RAB25, TXNIP, CTSE and ERN2. Advantageously, in step a) it is measured the expression level of CDC20, KPNA2, PLK1, SRM, RFC4, MCM2, RUVBL2, MAD2L1, CCT4, CAD, VSIG2, BCL2L15, RAB25, TXNIP, CTSE and ERN2.

According to the invention, the efficiency of a compound for treating cancer and, in particular, pancreatic adenocarcinoma, of human in need thereof, and in which a Myc-high pathway and/or a Myc-low pathway is involved, is determined.

Treatments of cancer patients are preceded by the molecular characterization of their tumor. This specific characterization is allowing to select the most appropriate treatments towards an individualized medicine approach.

The compounds that are tested are notably BET proteins inhibitors. The BET family of proteins includes BRD2, BRD3, BRD4 and BRDT (Asangani IA, Dommeti VL, Wang X, Malik R, Cieslik M, Yang R, Escara-Wilke J, Wilder-Romans

K, Dhanireddy S, Engelke C et al (2014) *Therapeutic targeting of BET bromodomain proteins in castration-resistant prostate cancer*. Nature 510: 278-282).

5 According to the invention, the word "compound" is to be understood as any compound which may have a potential therapeutic effect for treating cancer and, in particular, PDAC, or alternatively for improving or maintaining the prognosis of a human. A "derivative" of
10 said compound may comprise any active metabolite that is susceptible to be obtained *in vivo* from said compound after its administration, and which thus includes any active metabolite of said compound, and/or salt thereof. It is noted that the compounds and derivatives thereof
15 may be considered either alone or in combination, without departing from the scope of the invention. In particular, any pharmaceutical composition comprising said compounds, either alone or in combination is to be considered within the scope of the invention. When the aforementioned
20 compounds are considered in combination, they may be administered either individually or sequentially.

The compounds are more particularly selected from a group consisting of (*S*)-*tert*-butyl 2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-
25 *a*][1,4]diazepin-6-yl)acetate, OTX015/MK-8628 (Merck™), TEN-010 (Tensha Therapeutics™), ZEN-3365 (Zenith Epigenetics™), ABBV-075 (AbbVie™), INCB-54329 (Incyte™), GS-5829 (Gilead Sciences™).

30 Mechanistically, (*S*)-*tert*-butyl 2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)acetate competitively inhibits the

BET proteins from binding to acetylated lysines residues of histones. This process prevents the association of transcriptional complexes with chromatin and thus decreases expression of RNA species that are dependent of this mechanism of transcription. Many studies suggest that the main mechanism by which BET inhibitors affect tumoral growth is by their effects on c-MYC expression and activity.

10 The efficiency of the compound as determined in step b) is considered to be good if the condition of the patient is expected to improve after administration of said compound or, alternatively, if the life expectancy of said patient is expected to improve after administration of the compound. In other words, according to the invention, "determining the efficiency of a compound for treating cancer in said human" encompasses determining if the compound is suitable for killing cells, in particular primary cells or xenografts, that derive from said cancer.

20 The method according to the present invention is advantageously characterized in that it is applied for treating pancreatic adenocarcinoma of human in need thereof.

According to the present invention, the biological samples are tumor samples derived from cancer, notably tumor samples derived from pancreatic adenocarcinoma, and more preferably tumor samples derived from the head or the tail of the pancreas.

The words "tumor sample" and "tumor tissue sample" encompass (i) a global primary tumor (as a whole), (ii) a tissue sample from the center of the tumor, (iii) a tissue sample from the tissue directly surrounding the tumor which tissue may be more specifically named the "invasive margin" of the tumor, (iv) lymphoid islets in close proximity with the tumor, (v) the lymph nodes located at the closest proximity of the tumor, (vi) a tumor tissue sample collected prior surgery (for follow-up of patients after treatment for example), (vii) a tumor tissue sample collected after surgery, (viii) a tumor tissue sample derived from the head of the pancreas, (viii) a tumor tissue sample derived from the tail of the pancreas, and (ix) a distant metastasis.

15

A tumor tissue sample, irrespective of whether it is derived from the tail or the head of the pancreas, the center of the tumor, from the invasive margin of the tumor, or from the closest lymph nodes, encompasses pieces or slices of tissue, or even cell samples, that have been removed from the tumor, including following a surgical tumor resection or following the collection of a tissue sample for biopsy, for further quantification of one or several biological markers, notably through histology or immunohistochemistry methods, through flow cytometry methods and through methods of gene or protein expression analysis, including genomic and proteomic analysis. The tumor sample derived from PDAC can be a sample collected by endoscopic aspiration, including Endoscopic Ultrasound-Guided Fine-Needle Aspiration, or by surgery. It is worth noting that only 15 to 20% of patients are operated whereas virtually all the patients with a PDAC are submitted to a biopsy under Endoscopic

UltraSound-guided Fine-Needle Aspiration (EUS-FNA). These biopsies can become an inestimable source of living material from each PDAC. However, said biopsies, when obtained, are strongly contaminated by blood and stromal components, which impedes their direct utilization for the macromolecules extractions. To bypass this major inconvenient the inventors set up the production of organoids directly from PDAC biopsies and after a few days of growth the material become almost without contamination.

These organoids are a well-cleaned source of materials for extracting, in small quantities but pure, RNA, DNA, proteins etc. by using standards approaches developed for small amounts of material (such as the nCounter® Dx Analysis Sytem with Flex configuration).

Moreover, organoid cultures enable various molecular studies due to the high purity to epithelial tumor compartment. Indeed, RNA-, DNA- and protein-based assays are readily and quickly accomplishable with organoid. Unlike the PDX procedure, which take at least two months to grow, organoids allow to obtain biological exploitable material in a window of time in which an optimized therapeutic strategy may be efficiently applied to the patients. Moreover, organoids conserves the degree of intratumoral characteristics of the primary tumor and is able to be amplifiable conserving these characteristics for at least several passages.

Thus, in another preferred embodiment, the biological sample is an organoid derived from a tumor tissue sample

obtained from patient undergoing surgery or, preferably, from biopsy.

Organoids are preferably derived from pancreatic tumor sample such as tumor tissue sample from patients undergoing surgery or from pancreatic biopsies of patients suffering from Pancreatic Ductal Adenocarcinoma (PDAC). More preferably organoid are derived from pancreatic biopsy of patients suffering from PDAC.

The words "organoid" refers to miniaturized and simplified version of an organ produced in vitro in three dimensions that shows realistic micro-anatomy. It is derived from one or a few cells from a tumor tissue sample, preferably from a biopsy from patients with tumor.

In another embodiment, the biological sample is selected from the group consisting of a xenograft and a primary culture, notably a primary culture of epithelial cells. The present invention also relates to an *in vitro* or *ex vivo* method for prognosticating cancer of a human, comprising the steps of: a1) measuring, in a biological sample from said human, the expression level of at least one marker gene selected from a group consisting of CDC20, KPNA2, PLK1, SRM, RFC4, MCM2, RUVBL2, MAD2L1, CCT4, CAD, VSIG2, BCL2L15, RAB25, TXNIP, CTSE and ERN2; a2) determining, from said measurement, if the MYC pathway is activated or not in said cancer; and b) prognosticating said cancer from the measurement and determination obtained in steps a1) and a2). More precisely, the *in vitro* or *ex vivo* method of present invention is advantageously for prognosticating

pancreatic adenocarcinoma. Nevertheless, all cancers wherein the MYC pathway is activated are generally known to have poor clinical outcome, and aggressiveness. Thus, the measure of the expression levels of the above markers genes, and the determination of the activation or of the non-activation of the MYC pathway, would permit to determine if a cancer is MYC-high or MYC-low, and thus to prognosticate such cancer.

As used herein, the expression "prognosticating", "prognosis" and "prognosis of progression of cancer" encompasses the prognosis, in a patient wherein the occurrence of cancer has already been diagnosed, of various events, including: (i) the chances of occurrence of metastasis; (ii) the chances of occurrence of loco-regional recurrence of cancer, including PDAC; and (iii) the chances of occurrence of "short-term" or "long-term" survival following testing with the *in vitro* or *ex vivo* prognosis method according to the invention.

According to the invention, "short-term" and "long-term" survival relate to the life expectancy of one given human having cancer, notably PDAC. In particular, a "short-term survival" may refer to a life expectancy of one given human having cancer, notably PDAC, which does not exceed 8 months. A "long-term survival" may refer to a life expectancy of one given human having cancer, notably PDAC, which exceeds 8 months.

In even more preferred embodiment, the *in vitro* or *ex vivo* method for prognosticating a cancer, notably pancreatic adenocarcinoma, of a human in which the c-MYC oncogene is involved, comprising the steps of a)

measuring, in a biological sample from said human, the expression level of at least one marker gene selected from a group consisting of CDC20, KPNA2, PLK1, SRM, RFC4, MCM2, RUVBL2, MAD2L1, CCT4, CAD, VSIG2, BCL2L15, RAB25, TXNIP, CTSE and ERN2; and b) prognosticating said cancer from the measurement obtained in step a).

This method is advantageously characterized in that, in step a), it is measured the expression level of at least one marker gene selected from a group consisting of CDC20, KPNA2, PLK1, SRM, RFC4, MCM2, RUVBL2, MAD2L1, CCT4 and CAD. In a preferred embodiment, in step a), it is measured the expression level of at least one marker gene selected from a group consisting of VSIG2, BCL2L15, RAB25, TXNIP, CTSE and ERN2. In the method for prognosticating according to the invention, and in particular in step a) it is measured the expression level of at least one marker gene selected from a first group consisting of CDC20, KPNA2, PLK1, SRM, RFC4, MCM2, RUVBL2, MAD2L1, CCT4 and CAD, and the expression level of at least one marker gene selected from a second group consisting of VSIG2, BCL2L15, RAB25, TXNIP, CTSE and ERN2. Advantageously, in step a) it is measured the expression level of CDC20, KPNA2, PLK1, SRM, RFC4, MCM2, RUVBL2, MAD2L1, CCT4 and CAD. Advantageously, in step a) it is measured the expression level of VSIG2, BCL2L15, RAB25, TXNIP, CTSE and ERN2. Advantageously, in step a) it is measured the expression level of CDC20, KPNA2, PLK1, SRM, RFC4, MCM2, RUVBL2, MAD2L1, CCT4, CAD, VSIG2, BCL2L15, RAB25, TXNIP, CTSE and ERN2.

The invention also relates to a composition comprising at least one probe for quantitative measuring the expression

level of at least one cancer marker gene, notably pancreatic adenocarcinoma marker gene, involved by the c-Myc oncogene and selected from a group consisting of CDC20, KPNA2, PLK1, SRM, RFC4, MCM2, RUVBL2, MAD2L1, 5 CCT4, CAD, VSIG2, BCL2L15, RAB25, TXNIP, CTSE and ERN2.

This composition is advantageously characterized in that, at least one probe measures the expression level of at least one marker gene selected from a group consisting of 10 CDC20, KPNA2, PLK1, SRM, RFC4, MCM2, RUVBL2, MAD2L1, CCT4 and CAD. In another embodiment, the probe measures the expression level of at least one marker gene selected from a group consisting of VSIG2, BCL2L15, RAB25, TXNIP, CTSE and ERN2. Preferentially, a probe measures the 15 expression level of at least one marker gene selected from a first group consisting of CDC20, KPNA2, PLK1, SRM, RFC4, MCM2, RUVBL2, MAD2L1, CCT4 and CAD, and a probe measures the expression level of at least one marker gene selected from a second group consisting of VSIG2, 20 BCL2L15, RAB25, TXNIP, CTSE and ERN2. Advantageously, the at least one probe measure the expression level of CDC20, KPNA2, PLK1, SRM, RFC4, MCM2, RUVBL2, MAD2L1, CCT4 and CAD. at least one probe measures the expression level of VSIG2, BCL2L15, RAB25, TXNIP, CTSE and ERN2. 25 Advantageously, at least one probe measured the expression level of CDC20, KPNA2, PLK1, SRM, RFC4, MCM2, RUVBL2, MAD2L1, CCT4, CAD, VSIG2, BCL2L15, RAB25, TXNIP, CTSE and ERN2.

30 The present invention also concerns a kit comprising at least one probe for quantitative measuring the expression level of at least one cancer marker gene, notably pancreatic adenocarcinoma marker gene, involved by the c-

Myc oncogene and selected from a group consisting of CDC20, KPNA2, PLK1, SRM, RFC4, MCM2, RUVBL2, MAD2L1, CCT4, CAD, VSIG2, BCL2L15, RAB25, TXNIP, CTSE and ERN2.

5 The kit is advantageously characterized in that, at least one probe measured the expression level of at least one marker gene selected from a group consisting of CDC20, KPNA2, PLK1, SRM, RFC4, MCM2, RUVBL2, MAD2L1, CCT4 and CAD. In a preferred embodiment, at least one probe
10 measured the expression level of at least one marker gene selected from a group consisting of VSIG2, BCL2L15, RAB25, TXNIP, CTSE and ERN2. In the kit according to the invention, at least one probe measured the expression level of at least one marker gene selected from a first
15 group consisting of CDC20, KPNA2, PLK1, SRM, RFC4, MCM2, RUVBL2, MAD2L1, CCT4 and CAD, and at least one probe measured the expression level of at least one marker gene selected from a second group consisting of VSIG2, BCL2L15, RAB25, TXNIP, CTSE and ERN2. Advantageously, at
20 least one probe measured the expression level of CDC20, KPNA2, PLK1, SRM, RFC4, MCM2, RUVBL2, MAD2L1, CCT4 and CAD. Advantageously, at least one probe measured the expression level of VSIG2, BCL2L15, RAB25, TXNIP, CTSE and ERN2. Advantageously, at least one probe measured the
25 expression level of CDC20, KPNA2, PLK1, SRM, RFC4, MCM2, RUVBL2, MAD2L1, CCT4, CAD, VSIG2, BCL2L15, RAB25, TXNIP, CTSE and ERN2.

Another aspect of the present invention relates to a
30 solid support comprising a probe for quantitative measuring the expression level of at least one cancer marker gene, notably pancreatic adenocarcinoma marker gene, involved by the c-Myc oncogene and selected from a

group consisting of CDC20, KPNA2, PLK1, SRM, RFC4, MCM2, RUVBL2, MAD2L1, CCT4, CAD, VSIG2, BCL2L15, RAB25, TXNIP, CTSE and ERN2.

5 Again, the solid support is advantageously characterized in that, at least one probe measured the expression level of at least one marker gene selected from a group consisting of CDC20, KPNA2, PLK1, SRM, RFC4, MCM2, RUVBL2, MAD2L1, CCT4 and CAD. In a preferred embodiment,
10 at least one probe measured the expression level of at least one marker gene selected from a group consisting of VSIG2, BCL2L15, RAB25, TXNIP, CTSE and ERN2. In the solid support according to the invention, at least one probe measured the expression level of at least one marker gene
15 selected from a first group consisting of CDC20, KPNA2, PLK1, SRM, RFC4, MCM2, RUVBL2, MAD2L1, CCT4 and CAD, and at least one probe measured the expression level of at least one marker gene selected from a second group consisting of VSIG2, BCL2L15, RAB25, TXNIP, CTSE and
20 ERN2. Advantageously, at least one probe measured the expression level of CDC20, KPNA2, PLK1, SRM, RFC4, MCM2, RUVBL2, MAD2L1, CCT4 and CAD. Advantageously, at least one probe measured the expression level of VSIG2, BCL2L15, RAB25, TXNIP, CTSE and ERN2. Advantageously, at
25 least one probe measured the expression level of CDC20, KPNA2, PLK1, SRM, RFC4, MCM2, RUVBL2, MAD2L1, CCT4, CAD, VSIG2, BCL2L15, RAB25, TXNIP, CTSE and ERN2.

This work was focused on c-MYC but a similar approach can
30 be applied to other pathways for other cancer types.

EXAMPLE 1

Methods

The following methods were used for the implementation of the invention, and in the examples:

5

PDAC samples and cell culture

Two types of samples were obtained from patient having PDAC: endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) biopsies from patients with unresectable tumors and tumor tissue samples from patients undergoing surgery. All the samples were anonymized, and postsurgical anatomopathology reports were provided. Histopathologic evaluation was performed on 5 μ m hematoxylin and eosin stained sections of patient tumors and xenografts, which were examined under a light microscope. These sections were compared with the original human tumor when available. Samples from EUS-FNA were mixed with 100 μ l of Matrigel™ (BD Biosciences™, Franklin Lakes, NJ) and injected in the upper right flank of a nude mouse (Swiss Nude Mouse Crl: NU(lco)-Foxnlnu; Charles River Laboratories, Wilmington, MA). Samples from surgery were fragmented, mixed with 100 μ l of Matrigel™, and implanted with a trocar (10 gauge; Innovative Research of America, Sarasota, FL) in the subcutaneous right upper flank of an anesthetized and disinfected mouse. When the tumors reached 1 cm³, the mice were sacrificed, and the tumors were removed. Xenografts that failed to develop within 6 months were discontinued.

30 Primary cell cultures were obtained from xenografts. Tissues were splited into several small pieces and processed in a biosafety chamber. After a fine mincing, they were treated with collagenase type V (ref C9263;

Sigma™) and trypsin/EDTA (ref 25200-056; Gibco, Life Technologies™) and suspended in DMEM supplemented with 1% w/w Penicillin/Streptomycin (Gibco, Life Technologies™) and 10% Fetal Bovine Serum (Lonza). After
5 centrifugation, cells were re-suspended in Serum Free Ductal Media (SFDM) adapted from the method of Schreiber et al. (Schreiber FS, Deramaudt TB, Brunner TB, Boretti MI, Gooch KJ, Stoffers DA, Bernhard EJ, Rustgi AK (2004) Successful growth and characterization of mouse
10 pancreatic ductal cells: functional properties of the Ki-RAS(G12V) oncogene. Gastroenterology 127: 250-260) without antibiotic and incubated at 37°C in a 5% CO2 incubator.

15 Gene Expression Microarrays

Total RNA was purified from xenograft using TRIzol® Reagent (Gibco, Life Technologies™). 50 to 100 mg of fresh frozen tissue per ml of TRIzol® was disrupted using a homogenizer followed by a single step of
20 phenol/chloroform purification. Total RNA was quantified using the Nanodrop spectrophotometer (NanoDrop Technologies™, Inc) and RNA Integrity Number (RIN) was calculated using the Agilent 2100 Bioanalyzer (Agilent Technologies™, Santa Clara, CA). RNA samples that
25 reached a RIN between 8 and 10 were used for microarray hybridization (GeneChip; Affymetrix™ Inc., Santa Clara, CA). The Genechip Human Gene 2.0 ST Arrays were washed and stained using the Affymetrix GeneChip fluidic station 450 (protocol EukGE-WS2v5_450) and were scanned using a
30 GeneChip scanner 3000 G7 (Affymetrix™ Inc., Santa Clara, CA). GeneChip operating software version 1.4 (Affymetrix™ Inc., Santa Clara, CA) was used to obtain chip images and for quality control. Background

subtraction and normalization of probe set intensities were performed using the method of Robust Multi-array Analysis (RMA) (Irizarry RA, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, Scherf U, Speed TP (2003) Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics* 4: 249-264). Microarray analysis was performed by the CHU de Québec Research Center Gene Expression Platform (Quebec City, Quebec, Canada). 17 samples of the cohort were previously published (Duconseil P, Gilabert M, Gayet O, Loncle C, Moutardier V, Turrini O, Calvo E, Ewald J, Giovannini M, Gasmi M et al (2015) *Transcriptomic analysis predicts survival and sensitivity to anticancer drugs of patients with a pancreatic adenocarcinoma*).

15

Bioinformatics analysis

RMA normalized data from microarrays were imported into GENE-E (version 3.0.204; Broad Institute, Cambridge, MA, USA) to generate heatmaps. The color intensity on the heatmap reflects global expression within a minimum (25%) in blue and a maximum (75%) in red. Cluster analysis, using Euclidian distance correlation of samples only, and distance for the clustering were calculated using a complete linkage algorithm. Differentially expressed genes were identified using t-test ratio and false discovery rate were estimated using Benjamini & Hochberg method (Benjamini Y, Drai D, Elmer G, Kafkafi N, Golani I (2001) *Controlling the false discovery rate in behavior genetics research*. *Behavioural brain research* 125: 279-284). Gene set enrichment analysis (GSEA) was performed using the Broad Institute platform and statistical significance (false discovery rate) was seated at 0.05.

GSEA Analysis

Two categories of pre-defined gene sets in the Molecular Signatures Database (MSigDB, Broad Institute, Cambridge, MA, USA) were selected for analysis named the Hallmarks set, and the C5 set, a Gene Ontology (GO) molecular function gene set derived from the Molecular Function Ontology database (Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES et al (2005) *Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles*, Proc Natl Acad Sci U S A 102: 15545-15550). The gene sets included in the analysis were limited to those that contained between 15 and 500 genes. Permutation was conducted 1,000 times according to default-weighted enrichment statistics and by using a t-test ratio metric to rank genes according to their differential expression levels across the MYC-high and MYC-low subgroups. Significant gene sets were defined as those with a nominal p-value <0.05. Calculation of the false discovery rate (FDR) was used to correct for multiple comparisons and gene set sizes (Benjamini Y, Drai D, Elmer G, Kafkafi N, Golani I (2001) *Controlling the false discovery rate in behavior genetics research*, Behavioural brain research 125: 279-284).

25

Chemograms

Cells were screened for their chemosensitivity to JQ1 compound (Sigma-Aldrich™, St Louis, MO, USA). Five thousand cells per well were plated in 96-wells plates in SFDM medium. Twenty four hours later the media was supplemented with increasing concentrations of JQ1 and incubated for an additional 72 h period. Each experiment was done in triplicate and repeated at least three times.

30

10 increasing concentrations of JQ1 were used ranging from 0 to 30 μ M.

Spheroids outgrowth assay

5 Fifteen thousand cells per well were seeded in 96-well round bottom plates with medium containing 20% methylcellulose (Sigma-Aldrich™, St Louis, MO, USA). After 48 h incubation, cells with spheroids of uniform size and shape were incubated with 2 μ M JQ1 during 72h.
10 Images were captured every day with an Evos microscope, equipped with a 4X/N.A. (0.13) objective lens (Thermo Fisher Scientific™, Waltham, MA, USA). Spheroids volumes were determined by the following equation: $V_{\text{spheroids}} = 4/3 \pi r^3$. Results were expressed as a
15 percentage of spheroid growth compared with no treatment condition (DMSO 0.05%).

Viability assays

Cell viability was estimated after addition of
20 PrestoBlue™ reagent (Life Technologies™, Carlsbad, CA, USA) for 3h, following the supplier protocol.

Proteins extraction and western-blotting

Cells were washed with ice-cold PBS and lysed in Laemmli
25 sodium dodecyl sulfate-sample buffer (90mM Tris-HCl [pH 6.8], 2.5% sodium dodecyl sulfate, 15% glycerol). Samples were then boiled, sonicated, and protein concentrations were determined using the bicinchoninic acid (BCA) assay (Biorad™, Hercules, CA, USA) with bovine serum albumin
30 as standard. B-mercaptoethanol and bromophenol blue were then added to a final concentration of 1% and 0.005%, respectively. Proteins (20 μ g) were separated by SDS-PAGE in 10% or 12.5% gels and were detected immunologically

following electro-transfer onto equilibrated PVDF (Imobilon-P membranes, Millipore™, Billerica, MA, USA). PVDF membranes were stained with Ponceau Red to assure a correct transfer of proteins and molecular weight markers. Membranes were blocked in PBS containing 5% powdered milk and 0.05% Tween® 20 for 1h at 25°C. Membranes were then incubated overnight at 4°C with primary antibodies in blocking solution and thereafter with horseradish peroxidase-conjugated IgG for 1h. Blots were visualized using the Amersham ECL system. The MYC antibody was purified from hybridomas clone 9E10 and used at 1/500 (ATCC® CRL-1729, ATCC France). The p27kip1 antibody (C19) was purchased from Santa Cruz™ and used at 1/1000. The cleaved caspase 3 (Asp175) antibodies was purchased from Cell Signaling™ (#9661) and used at 1/500. The β -actin antibody (AC-74) was purchased from Sigma-Aldrich™ and used at 1/10000.

Real Time quantitative PCR

Total RNA (1 μ g) was used as a template for cDNA synthesis, using the GoScript™ reverse transcription kit (Promega™, Madison, WI, USA). The GoTaq® qPCR 2X Master Mix (Promega™, Madison, WI, USA) that include all components for quantitative PCR, except sample DNA, primers and water, was used to quantified the sixteen MYC-high signature markers. Primers list for each transcript is available in the following Table 3.

Transcripts	Forward primer sequence (5'-3')	F primer position	Reverse primer sequence (5'-3')	R primer position
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CDC20	GCACAGTTCGCGTTCGAGA GG	4-22	CTGGATTGCCAGGAGTTC GG	191- 171
KPNA2	CTGCCCCGTCTTCACAGATT CA	32-52	GCGGAGAAGTAGCATCATC AGG	190- 169
PLK1	AAAGAGATCCCGGAGGTCC TA	112- 132	GGCTGCGGTGAATGGATAT TTC	322- 301
SRM	GTGGTGGCCTATGCCTACT G	688- 707	CTCCTGGAAGTTCGTGCTC G	783- 764
RFC4	CCGCTGACCAAGGATCGAG	43-61	AGGGAACGGGTTTGGCTTT C	118-99
MCM2	ATGGCGGAATCATCGGAAT CC	1-21	GGTGAGGGCATCAGTACGC	117-99
RUVBL2	GTCGGGCAGTCCTTATTGC T	209- 228	TGGTCGATCAATCTGGATC TCC	450- 429
MAD2L1	GTTCTTCTCATTCGGCATC AACA	66-88	GAGTCCGTATTTCTGCACT CG	153- 133
CCT4	ATGCCCCGAGAATGTGGCAC	1-19	GCTTGTCGCGGTCTTGATA G	88-69
CAD	AGTGGTGTTTCAAACCGGC AT	78-98	CAGAGGATAGGTGAGCACT AAGA	168- 146
VSIG2	CATCTCTGAGTCCCATCCA ATCC	201- 223	TGACCCGCTTTGACTTAGA AC	277- 257
BCL2L15	TTGCCAGCCGGAACCTATG	86-104	CGAAGGCGACCAGCAATGA TA	173- 153
RAB25	ATCGGCGAATCAGGTGTGG	52-70	TAGGTCTGGTGCTTGGTTA GG	299- 279
TXNIP	GGTCTTTAACGACCCTGAA AAGG	36-58	ACACGAGTAACTTCACACA CCT	122- 101
CTSE	AGGCATCCGTCCCTCAAGA A	82-101	CCTTGGCACTCTGGTCCAT TG	202- 182
ERN2	GAGTCCACAGGTTTCATACT CTCA	228- 250	CTGTCTGCTTGCTTAGTGC G	316- 297
28S subunit	AACGAGATTCCCACTGTCC C	3824- 3843	CCGTAAAACGACGGCCAG	3943- 3925

Table 3: Primers list for each transcript

Reaction conditions were denaturation at 95°C for 2 min; 40 cycles of 15s at 95°C, 45s at 60°C. Reactions were carried out using the AryaMx real-time PCR system and analyzed using the AriaMx software v1.1 (Agilent Technologies™, Santa Clara, CA, USA).

Ki67 staining and quantification

Full-thickness, 5-µm sections were cut from formalin-fixed, paraffin-embedded blocks from all 55 PDX. The samples were then stained with the Ki67 antibody (MIB-1 clone, 1:160; Dako™, France) using tonsillar tissue as a positive control. Negative controls were run simultaneously with the primary antibody replaced with a buffer. Antigen retrieval was conducted in citrate buffer at pH 6.0 under pressure for 3 min. Envision Dual Link Kit (Dako™) was used for detection, with diaminobenzidine (DAB) as the chromogen and hematoxylin as the counterstain. Staining was considered positive when brown nuclear labeling was observed in epithelial compartment. A standard Olympus BX41 microscope was used to identify tumor hot spots in each case. The percentage of tumor cell staining was independently counted by three reviewers (BB, MB, and ND) and with « eyeballing » methodology.

Differentiation scoring

Formalin-fixed tumors were submitted to an hospital histology core facility, and paraffin-embedded sections were cut for hematoxylin and eosin (H&E) staining. For histopathological scoring, H&E-stained slides were scored for the penetrance of each histological hallmark on a

scale of 0 to 2. The predominant tumor phenotype gave the pathological score for the whole tumor (0 = poorly differentiated, 1 = moderately differentiated, 2 = well differentiated).

5

Method for normalizing expression ratios

Considering the expression of upregulated gene i in patient α as $u_{i\alpha}$ and the expression of downregulated gene j in patient α as $d_{j\alpha}$, the sum of expression of each
10 marker in all patients was calculated:

$$U_i = \sum_{\alpha} u_{i\alpha}, \quad 1 < i < 10 \quad \text{and} \quad D_j = \sum_{\alpha} d_{j\alpha}, \quad 1 < j < 6$$

The mean centered normalized expression was then
15 calculated for the two set of markers:

$$\widetilde{U}_{i\alpha} = \frac{u_{i\alpha} \cdot (100)}{U_i} \quad \text{and} \quad \widetilde{D}_{j\alpha} = \frac{d_{j\alpha} \cdot (100)}{D_j}$$

The normalized ratio between upregulated gene U_i and
20 downregulated gene D_j is:

$$r_{ij\alpha} = \left(\frac{\widetilde{U}_{i\alpha}}{\widetilde{D}_{j\alpha}} \right), \quad 1 < i < 10 \text{ and } 1 < j < 6$$

The median of the 60 normalized ratios is then
25 calculated:

$$\widetilde{m}_{\alpha} = \text{median}(r_{ij\alpha}, \forall 1 < i < 10 \text{ and } 1 < j < 6)$$

$\widetilde{m}_{\alpha} > 1$ show a MYC-high profile and $\widetilde{m}_{\alpha} < 1$ show a MYC-low profile.

30

Statistical analysis

Overall survival and relapse free survival were analyzed using the Kaplan-Meier log-rank test to assess differences in survival. Box-and-whiskers plots show the medians, quartiles and range of continuous data to demonstrate the variability of data and the degree of normality. For continuous variables, non-parametric unpaired two-tailed t-test were perform under the assumption of equal variance.

10 Results

Selection of PDAC patients with MYC-high or MYC-low activity by using a gene expression profile signature.

15 In order to stratify a cohort of 55 PDAC patients, 30 primary tumors obtained from surgery and 25 biopsy samples taken by EUS-FNA were implanted subcutaneously into mice and preserved as PDX. The histopathologic and clinical characteristics of the patients from the learning cohort are displayed in the following Table 4.

Patients Distribution (learning cohort)				
		All	Resectable	Unresectable
n		55	30	25
Sex	Male	34 (62%)	18	16
	Female	21 (38%)	12	9
Age	Mean	64	66	61
	(Min-Max)	(41-86)	(45-86)	(41-83)
Other Cancers	No	44 (80%)	20	24
	Yes	11 (20%)	10	1
Tumor Location	Head	32 (58%)	19	13
	Undefined	6 (11%)	0	6

	Body	3 (5.5%)	2	1
	Tail	14 (25.5%)	9	5
Specimen Type	Primary tumor	46 (84%)	30	16
	Hepatic Metastasis	5 (9%)	0	5
	Carcinomatosis	4 (7%)	0	4
Tumor Status at Diagnosis	Localized	29 (53%)	28	1
	Locally Advanced	8 (14,5%)	2	6
	Metastatic	13 (23.5%)	0	13
	Carcinomatosis	5 (9%)	0	5

Table 4: Clinicopathological parameters from the learning cohort of patients

5 The main anatomopathological characteristics of the patients primary tumors were preserved in xenografts after successive passages. Growth rates to reach a tumor volume of 1 cm³ ranged from 2 to 6 months in most of the PDX. Total RNA was obtained from the 55 PDX, and the gene
10 expression profiling was performed using an Affymetrix™ platform. Subsequently, a selection was achieved of a panel of 239 RNAs regulated by MYC in accordance to the MYC targets v1 and v2 list from Molecular Signatures Database (MSigDB) (see the rank-listed transcripts
15 available in Table 1). Figure 1A represent the hierarchical clustering and expression heatmap for the top significantly high-expressed genes in MYC-high patients. The dendrogram showing the genetic distance between patients indicates the presence of two major
20 subgroups defined as MYC-high and MYC-low. Interestingly, it is observed that 17/55 (30.9%) patients are

characterized by an increase in the expression of 134/239 MYC targets RNAs (p-values <0.03 and q-values (FDR) <0.05).

5 In order to gain insight into the potential biological processes enriched in MYC-high *versus* MYC-low subgroups it a Gene Set Enrichment Analysis (GSEA) was performed. As shown in Figure 1B, the MYC-high subgroup is characterized by a low differentiated phenotype and their
10 two more significant associated biological processes are cell cycle process (Normalized Enrichment Score = 3.60 and FDR <0.25) and DNA replication and genome maintenance (Normalized Enrichment Score = 3.24 and FDR <0.25). In contrast, the MYC-low subgroup is characterized by
15 biological processes that reflect a more differentiated state of pancreatic tumors such as digestion (Normalized Enrichment Score = -2.23 and FDR <0.25) and glycoproteins metabolism (Normalized Enrichment Score = -1.76 and FDR <0.25). To confirm that MYC-high patients give rise to
20 PDX with high proliferative index, it is performed an IHC-based Ki67 staining scoring on the epithelial compartment of the 55 PDXs. As shown in Figure 1C (left part), this semi-quantitative scoring reveals that MYC-high patient-derived PDXs proliferate more than MYC-low
25 subgroup (Ki67 mean score 2.88 ± 0.25 N=17 vs. 2.06 ± 0.12 N=38, $p=0.0018$). In addition, it is determined the degree of differentiation for both subgroups on H&E staining on paraffin embedded tissues sections. As shown in Figure 1C (right part), MYC-high subgroup shows lower
30 differentiation state than MYC-low subgroup (differentiation mean score 0.77 ± 0.2 N=17 vs. 1.82 ± 0.08 N=38, $p<0.0001$). The Ki67 and differentiation scores are shown in Figures 6A and 6B. Moreover, we analyzed the

clinical outcome of both MYC-high and MYC-low patients using a Kaplan-Meier analysis and considering both the overall and the relapsing free survival time for the 55 patient cohort. As shown in Figure 1D, the overall survival median is 9.2 months for the MYC-high vs. 18.8 months for the MYC-low subgroup (HR=2.43 [1.1 to 5.1]). The relapse free survival median is 5.6 months and 11.5 months for MYC-high and MYC-low subgroup respectively (HR=2.7 [1.3 to 5.8]). Altogether, these observations indicate that it can be identified patients with MYC-high and MYC-low activity. Moreover, PDAC with MYC-high activity are characterized by increased proliferation, lower differentiation status and they have poor survival expectancy. Combined, these observations constitute a solid characterization of the molecular, biological, and medical features of the c-Myc status in patient derived xenograft, which is necessary to build the trajectory toward the testing of novel therapies aimed at treating this distinct subgroup of tumors.

20

MYC-dependent RNAs signatures can be used for classifying distinct PDAC subtypes

To define a specific MYC signature, that can be used to classify tumor subtypes, it is selected a total of 16 genes. The first 10 (Figure 2C) were identified from the gene set corresponding to the upregulated in the MYC-high group of patients. To obtain the genes upregulated in the MYC-low subgroup it is identified the 6 top-score upregulated genes in the MYC-low patients by a t-test analysis from the whole gene expression profiles (Figure 2A and Figure 2B). The list of these 16 biomarkers used

in the transcriptomic signature is shown in the above Table 2.

For each patient, 60 ratios were computed after mean
5 centered normalization of the 16 markers revealing the
MYC-high or MYC-low profiles. As shown in Figure 2D, it
is possible to detect the 17 MYC-high patients with
medians of expression ratios up to 1 with an excellent
specificity and accuracy. Affymetrix™ data was then
10 confirmed by RT-qPCR on 4 putative MYC-high and 4 MYC-low
patients. Each transcript was normalized with the 28S
ribosomal RNA, the relative quantity was calculated by
the $\Delta\Delta C_t$ method and the ratios were calculated after
normalization. The signature was able to definitively
15 detect all MYC-high and MYC-low profiles by RT-qPCR as
shown in Figures 3A and 3B. It is then assessed the MYC
signature on primary cultures derived from the same 8
xenografts (Figure 3C) and found that the corresponding
MYC-high or MYC-low profiles were correctly detected.
20 Therefore, it is concluded that the signature based on
these transcripts is a reliable for identifying tumor
subtypes based on their c-MYC status.

MYC-high PDX are sensitive to growth inhibition by the
25 BET inhibitor JQ1

It was hypothesized that the subgroup of PDX belonging to
the MYC-high phenotype should be more sensitives to
pharmacological inhibition of MYC activity, which
30 currently cannot be targeted directly but instead through
the inactivation of BET proteins. To test this
hypothesis, a panel of pancreatic PDX-derived primary
cultures was treated with the well-characterized BET

inhibitor JQ1. According to their MYC signature, it was selected 4 MYC-high PDX (CRCM16, CRCM17, CRCM04, and CRCM08) and 4 MYC-low patients (CRCM05, CRCM11, CRCM10, and CRCM109) (Figure 2D). It was then assessed the viability of cells with increasing dose of drug (chemograms) for 72h. As shown in Figures 4A and 4B, MYC-high cells exhibit higher sensitivity to JQ1 treatment compared to the MYC-low ones. The mean of IC_{50} for the MYC-high cells is $2.3 \mu M \pm 0.8$ whereas the one corresponding to MYC-low primary cells was of $39.22 \mu M \pm 16$ (Figure 4C). It was also evaluated the effect of JQ1 in patient derived cells grown in 3D culture conditions. As shown in Figure 4D, MYC-high spheroids were more sensitive to the JQ1 treatment for 72h (50% reduction in volume) than their MYC-low counterpart (25% reduction in volume). As a positive control, the effect of JQ1 treatment on the MYC-high primary cell CRCM16 was analyzed and a significant decrease of MYC protein level was determined (Figure 4E). Importantly, MYC depletion is accompanied with an increase of p27^{Kip1} level and an increase in the cleavage of caspase 3 which may explain the antitumor effect of the compound. Thus, these experimental therapeutic experiments suggest that inhibition of BET proteins by small drugs like JQ1 will be beneficial to antagonize the growth of pancreatic cells which carry the c-MYC-high status.

MYC-dependent RNA signature identify MYC-high patients on an independent validation cohort

30

Sixteen new PDAC patients were included in the study as an independent validation cohort. The histopathologic and

clinical characteristics of patients from the test cohort are displayed in Table 5.

Patients Distribution (validation cohort)				
		All	Resectable	Unresectable
n		16	7	9
Sex	Male	10 (62.5%)	3	7
	Female	6 (37.5%)	4	2
Age	Mean	69	67	70
	(Min-Max)	(53-83)	(58-71)	(53-83)
Other Cancers	No	11 (68.75%)	4	7
	Yes	5 (31.25%)	3	2
Tumor Location	Head	5 (31.25%)	3	2
	Undefined	2 (12.5%)	0	2
	Body	2 (12.5%)	1	1
	Tail	7 (43.75%)	3	4
Specimen Type	Primary tumor	13 (81.25%)	7	6
	Hepatic Metastasis	2 (12.5%)	0	2
	Carcinomatosis	1 (6.25%)	0	1
Tumor Status at Diagnosis	Localized	7 (43.75%)	7	0
	Locally advanced	1 (6,25%)	0	1
	Metastasis	6 (37.5%)	0	6
	Carcinomatosis	2 (12.5%)	0	2

5 Table 5: Clinicopathological parameters from the validation cohort of patients.

16 PDX were obtained and, from them, 6 PDX-derived cells. The expression of 16 MYC-associated marker genes by RT-qPCR was measured and it was determined that 8 patients

present a MYC-high profile (CRCM43, CRCM26, CRCM50, CRCM19, CRCM30, CRCM114, CRCM116 and CRCM34) and 8 shows a MYC-low profile (CRCM23, CRCM21, CRCM108, CRCM25, CRCM28, CRCM112, CRCM29, CRCM42) as described in Figure 5A. Of the 6 primary cultures available, 3 presented a MYC-high (CRCM116, CRCM114 and CRCM34) and 3 a MYC-low profile (CRCM112, CRCM21 and CRCM28). To test their sensitivity to BET inhibitors, cells were treated with increasing concentrations of JQ1 and as expected the 3 MYC-high cells showed to be more sensitive than the MYC-low cells as shown in Figure 5C. The mean of IC₅₀ for the MYC-high cells is 5.65 μ M $\pm$ 2.4 whereas it is of 223.71 μ M $\pm$ 191.5 for the MYC-low primary cultures (Figure 5D) which are close to the study cohort presented in Figure 5C. These results confirm that cells from PDAC presenting a MYC-high profile are more sensitive to JQ1 treatment compared to the cell presenting a MYC-low profile.

EXAMPLE 2: Use of organoid as biological sample

Contrary to example 1 wherein Patient Derived Xenografts (PDX) is used in order to obtain a quantity of nucleic acid sufficient to perform molecular analyses (see chapter "PDAC samples and cell culture" above), the inventor have also demonstrated that organoids can be used alternatively. Unlike the PDX procedure, which take at least two months to grow, organoids allow to obtain biological exploitable material in a window of time in which an optimized therapeutic strategy may be efficiently applied to the patients.

Then, after RNA extraction, the inventors proceeded to Signature validation with nCounter® Dx Analysis System

with FLEX configuration from NanoString® Technologies Companies.

Methods:

5

Obtaining EUS-FNA biopsy

The samples were obtained by Endoscopic UltraSound-guided Fine-Needle Aspiration (EUS-FNA) biopsies from patients
10 with unresectable tumors who represent up to 85% of all PDAC patients as part of the standard care of patients having a PDAC.

Methodology to obtain an maintain Biopsy Derived 15 Pancreatic Organoids (BDPO)

BDPO are generated from EUS-FNA biopsies that were rapidly digested using Tumor dissociation kit, human (Miltenyi Biotec). After centrifugation, samples were re-
20 suspended in Red Blood Cell (RBC) lysis buffer (eBioscience). Mixtures were filtered on cell EASY strainer 100µm (Greiner Bio One) and re-suspended into Pancreatic Organoids Feeding Medium (POFM) [DMEM:F12 supplemented with HEPES (1x Invitrogen) + Glutamax (1x,
25 Invitrogen), penicillin/streptomycin (1x, Invitrogen) + EGF (0.05 µg/ml) + A83-01 (0.5 µM) + FGF (0.1 µg/ml) + Gastrin (1nM) + mNoggin (0.1µg/ml) + N-Acetylcystein 1.25 mM + Nicotinamide (10mM) + human R-spondin (1µg/ml) + Wnt3a (0.1 µg/ml) + Growth Factor Reduced (GFR) MatriGel®
30 (5%)].

BDPO suspensions were then placed into 12-well plate coated with 150µl GFR MatriGel® (Corning). Media were replaced every 2-3 days. For the BDPO passaging, media

were harvested and keep on ice during the digestion step. For the digestion: 0.5ml/well Digestion Media [DMEM supplemented with 1% collagenase/dispase (100mg/ml, Sigma)] were add to the well and incubate at 37°C for 1 to 1.5 hours. The incubation was then stopped with Resuspension Media [DMEM supplemented with 1% BSA and 1% penicillin/streptomycin]. Digested pulps were gently spin and supernatants were discard and replaced with 2ml of StemPro Accutase (Life Technology) cell dissociation reagent for an additional 30 min period at 37°C. BDPO can be re-seeding at a density of 150.000 to 200.000 cells per well in a 12-well plate or used for direct macromolecules extraction. Figure 7 shows the result of different organoids cultures established from EUS-FNA biopsies.

RNA extraction from BDPO

For RNA isolation, organoids were harvested by dissolving Matrigel including organoids with ice-cold PBS. Following centrifugation at 700rpm for 5min at 4 °C, supernatant was removed and pelleted organoids were carefully resuspended and homogenized in 350µl of RLT buffer (Qiagen). Total RNA was isolated using the RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions. RNA concentration and purity (A260/A280 ratio) was determined by spectrophotometric analysis (Epoch Microplate Spectrophotometer, Bioteck Instruments). RNA Integrity Number (RIN) was calculated using the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). RNA samples that reached a RIN between 8 and 10 were used for Nanostring hybridization.

Signature validation with nCounter Dx analysis system
with FLEX configuration from Nanostring technology

Briefly, for each BDPO sample, 100 nanograms of total RNA
5 was hybridized to a custom probeset relative to specific
molecular signatures and according to the manufacturer's
instructions. Raw count data were normalized by: (1)
Background correction (2) positive control correction and
(3) housekeeping gene correction.

10

The codeset is composed by sequences of a reporter probe
and a capture probe that hybridize 10 c-Myc targets
(CDC20, KPNA2, PLK1, SRM, RFC4, MCM2, RUVBL2, MAD2L1,
CCT4, CAD), 6 PDAC expressed genes down-regulated in PDAC
15 with high c-Myc activity (VSIG2, BCL2L15, RAB25, TXNIP,
CTSE, ERN2) and 6 housekeeping genes (RBM47, AP1G2,
GRIPAP1, HNRNPA3, NDUFS1, CDV3) used for normalization
procedure. This signature is able to detect poor outcome
patients with an aggressive PDAC sensitive to BET
20 inhibitors.

Each Capture Probe and Reporter probe are designed in
order to hybridize a target sequence for each gene of
interest. The target sequences are the following:

25

BCL2L15:

TATTTTAAGAGACTCTATCTTAGGAGAGCTTAAGTGATTGGGCTGCAGGAAGAAGAC
ATTGTAACCCAGGAATTAAAAATGGATTCAGATTGCCTGATTT

30

CAD:

TTCCTCGATGGGACCTTAGCAAGTTCCTGCGAGTCAGCACAAAGATTGGGAGCTGCA
TGAAGAGCGTTGGTGAAGTCATGGGCATTGGGCGTTCATTGA

CCT4:GCGTTCGTGCTTTTGCAGATGCTATGGAGGTCATTCCATCTACACTAGCTGA
AAATGCCGGCCTGAATCCCATTCTACAGTAACAGAACTAAGAAACCG

CDC20:

5 GGAACATCAGAAAGCCTGGGCTTTGAACCTGAACGGTTTTGATGTAGAGGAAGCCAA
GATCCTTCGGCTCAGTGGAAAACCACAAAATGCGCCAGAGGGT

CTSE:

10 TTTGTGGCAAAAATACTTCCTAGGTGGTGCTGGGTACTTCTTGTTGCATCCTGTCAG
GAGGCAGATAATGCTGGTGCCTCTCTATTGGTAATGTTAAGAC

ERN2:

15 ATCGAAGGACCAATGTACGTCACAGAAATGGCCTTTCTCTCTGACCCAGCAGATGGC
AGCCTGTACATCTTGGGGACCCAAAAACAACAGGGATTAATGA

KPNA2NM:

TGATGATCCAGAAGTATTAGCAGATACCTGCTGGGCTATTTCTCTACCTTACTGATGG
TCCAAATGAACGAATTGGCATGGTGGTGAAAACAGGAGTTGTG

20 MAD2L1

TGGCCGAGTTCTTCTCATTCGGCATCAACAGCATTTTATATCAGCGTGGCATATATC
CATCTGAAACCTTTACTCGAGTGCAGAAATACGGACTCACCTT

MCM2:

25 CCATTCTCCTCGCAGATCTGGTGGACAGCTGCAAGCCAGGAGACGAGATAGAGCTGA
CTGGCATCTATCACAACAACCTATGATGGCTCCCTCAACACTGC

PLK1:

30 GCTTGGCTGCCAGTACCTGCACCGAAACCGAGTTATTCATCGAGACCTCAAGCTGGG
CAACCTTTTCCTGAATGAAGATCTGGAGGTGAAAATAGGGGAT

RAB25:

GGCCCGAATGTTGCTGAAAACAATGGACTGCTCTTCCTGGAGACCTCAGCCCTGGA
CTCTACCAATGTTGAGCTAGCCTTTGAGACTGTCCTGAAAGAA

RFC4:

5 ACAGGTGGAAAGGAGATCACAGAGAAAGTGATTACAGACATTGCCGGGGTAATACCA
GCTGAGAAAATTGATGGAGTATTTGCTGCCTGTCAGAGTGGCT

RUVBL2:

10 GACGCAGGCCTTCCGGCGGTCCATCGGCGTTCGCATCAAGGAGGAGACGGAGATCAT
CGAAGGGGAGGTGGTGGAGATCCAGATTGATCGACCAGCAACA

SRM:

GCAGTAAGACCTATGGCAACGTGCTGGTGTGGACGGTGTTCATCCAGTGCACGGAGA
GAGACGAGTTCTCCTACCAGGAGATGATCGCCAACCTGCCTCT
15

TXNIPNM:

CCCCCTTCCTGCCCTGTGTTAGGAGATAGGGATATTGGCCCCCTCACTGCAGCTGCCAG
CACTTGGTCAGTCACTCTCAGCCATAGCACTTTGTTCACTGTC

20 VSIG2

GAGTGGCCGGAGCTCTGATTGGGGTGCTCCTGGGCGTGCTGTTGCTGTCAGTTGCTG
CGTTCTGCCTGGTCAGGTTCCAGAAAGAGAGGGGGAAGAAGCC

CLAIMS

1. An *in vitro* or *ex vivo* method for determining the efficiency of a compound modulating the c-Myc oncogene expression for treating cancer, preferentially pancreatic adenocarcinoma, of a human in need thereof, comprising
5 the steps of:
a) measuring, in a biological sample from said human, the expression level of at least one marker gene selected from a group consisting of CDC20, KPNA2, PLK1, SRM, RFC4, MCM2, RUVBL2, MAD2L1, CCT4, CAD, VSIG2, BCL2L15, RAB25,
10 TXNIP, CTSE and ERN2; and
b) determining the efficiency of said compound on the c-Myc oncogene expression from the measurement obtained in step a).
- 15 2. The method according to claim 1, wherein in step a) it is measured the expression level of at least one marker gene selected from a group consisting of CDC20, KPNA2, PLK1, SRM, RFC4, MCM2, RUVBL2, MAD2L1, CCT4 and CAD.
- 20 3. The method according to anyone of preceding claims, wherein in step a) it is measured the expression level of at least one marker gene selected from a group consisting of VSIG2, BCL2L15, RAB25, TXNIP, CTSE and ERN2.
- 25 4. The method according to anyone of the preceding claims, wherein in step a) it is measured the expression level of at least one marker gene selected from a first group consisting of CDC20, KPNA2, PLK1, SRM, RFC4, MCM2, RUVBL2, MAD2L1, CCT4 and CAD, and the expression level of
30 at least one marker gene selected from a second group

consisting of VSIG2, BCL2L15, RAB25, TXNIP, CTSE and ERN2.

5 5. The method according to anyone of the preceding claims, wherein in step a) it is measured the expression level of CDC20, KPNA2, PLK1, SRM, RFC4, MCM2, RUVBL2, MAD2L1, CCT4 and CAD.

10 6. The method according to anyone of the preceding claims, wherein in step a) it is measured the expression level of VSIG2, BCL2L15, RAB25, TXNIP, CTSE and ERN2.

15 7. The method according to anyone of the preceding claims, wherein it is determined the efficiency of a compound for treating cancer of human in need thereof in which a Myc-high pathway is involved.

20 8. The method according to anyone of the preceding claims, wherein it is determined the efficiency of a compound for treating cancer of human in need thereof in which a Myc-low pathway is involved.

25 9. The method according to anyone of preceding claims, wherein the compound is a BET proteins inhibitor.

10. The method according to anyone of the preceding claims, wherein the compound is selected from a group consisting of (*S*)-*tert*-butyl 2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)acetate, OTX015/MK-8628, TEN-010, ZEN-3365, ABBV-075, INCB-54329, GS-5829.

11. The method according to anyone of the preceding claims, wherein the biological sample is selected from the group consisting of a tumor sample, a xenograft, a primary culture and an organoid.

5

12. An *in vitro* or *ex vivo* method for prognosticating a cancer, preferentially pancreatic adenocarcinoma, of a human, comprising the steps of:

- 10 a1) measuring, in a biological sample from said human, the expression level of at least one marker gene selected from a group consisting of CDC20, KPNA2, PLK1, SRM, RFC4, MCM2, RUVBL2, MAD2L1, CCT4, CAD, VSIG2, BCL2L15, RAB25, TXNIP, CTSE and ERN2;
- 15 a2) determining, from said measurement, if the MYC pathway is activated or not in said cancer; and
- b) prognosticating said cancer from the measurement and determination obtained in steps a1) and a2).

13. A composition comprising at least one probe for quantitative measuring the expression level of at least one cancer marker gene, preferentially pancreatic adenocarcinoma marker gene, involved by the c-Myc oncogene and selected from a group consisting of CDC20, KPNA2, PLK1, SRM, RFC4, MCM2, RUVBL2, MAD2L1, CCT4, CAD, VSIG2, BCL2L15, RAB25, TXNIP, CTSE and ERN2.

20

25

14. A kit comprising at least one probe for quantitative measuring the expression level of at least one cancer marker gene, preferentially pancreatic adenocarcinoma marker gene, involved by the c-Myc oncogene and selected from a group consisting of CDC20, KPNA2, PLK1, SRM, RFC4, MCM2, RUVBL2, MAD2L1, CCT4, CAD, VSIG2, BCL2L15, RAB25, TXNIP, CTSE and ERN2.

30

15. A solid support comprising at least one probe for
quantitative measuring the expression level of at least
one cancer marker gene, preferentially pancreatic
5 adenocarcinoma marker gene, involved by the c-Myc
oncogene and selected from a group consisting of CDC20,
KPNA2, PLK1, SRM, RFC4, MCM2, RUVBL2, MAD2L1, CCT4, CAD,
VSIG2, BCL2L15, RAB25, TXNIP, CTSE and ERN2.

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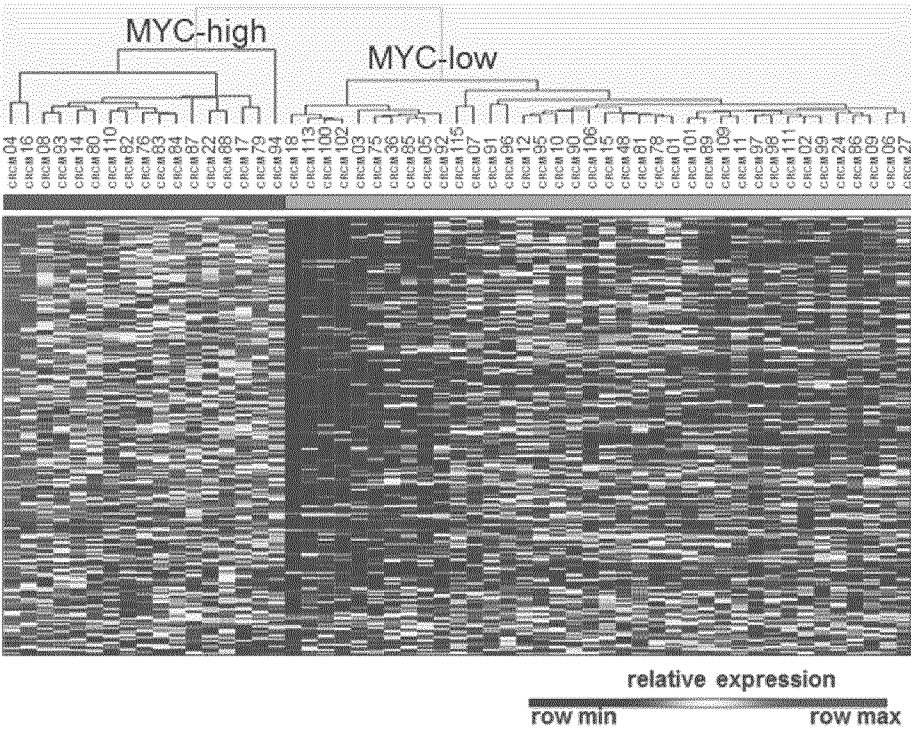


Fig. 1A

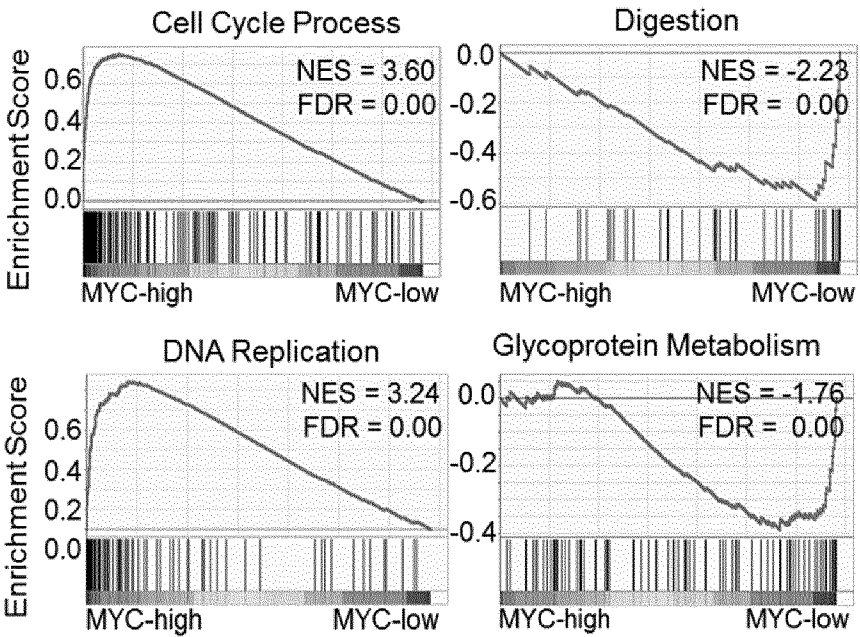


Fig. 1B

2/13

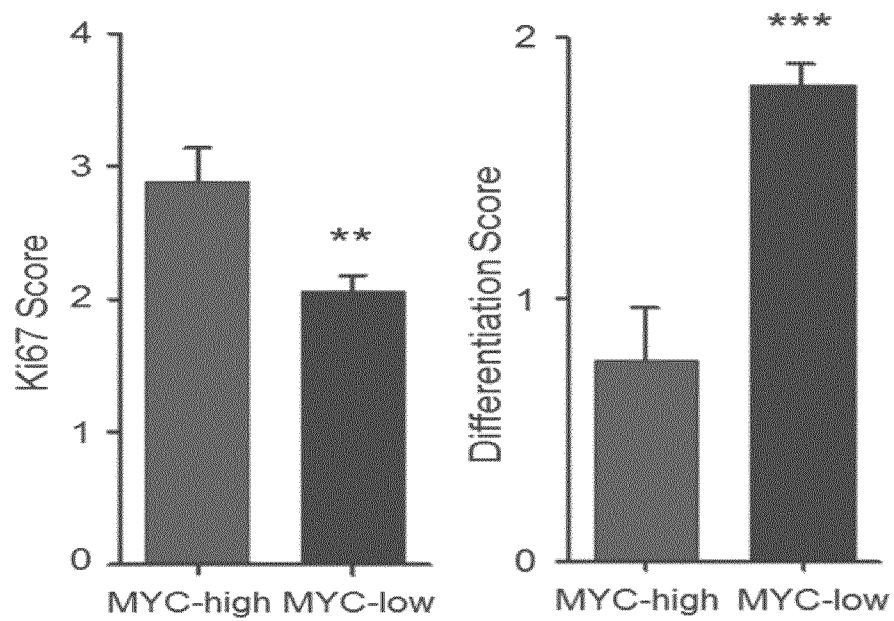


Fig. 1C

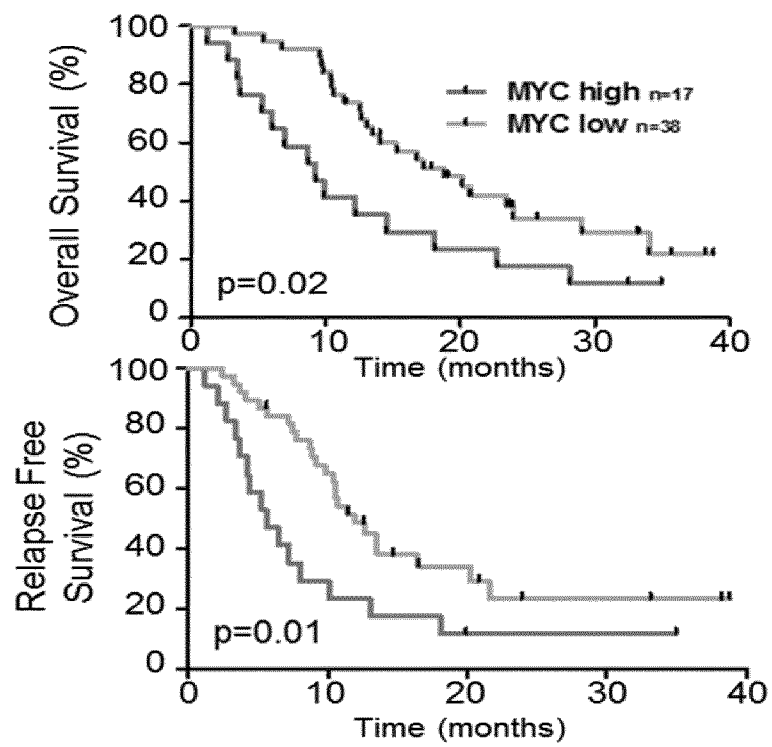


Fig. 1D

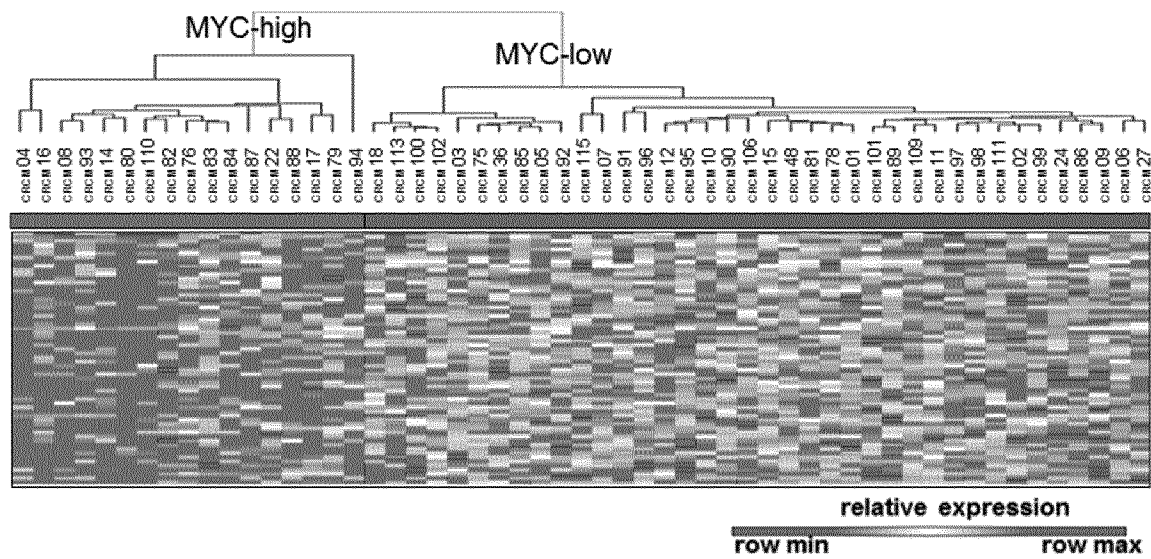


Fig. 2A

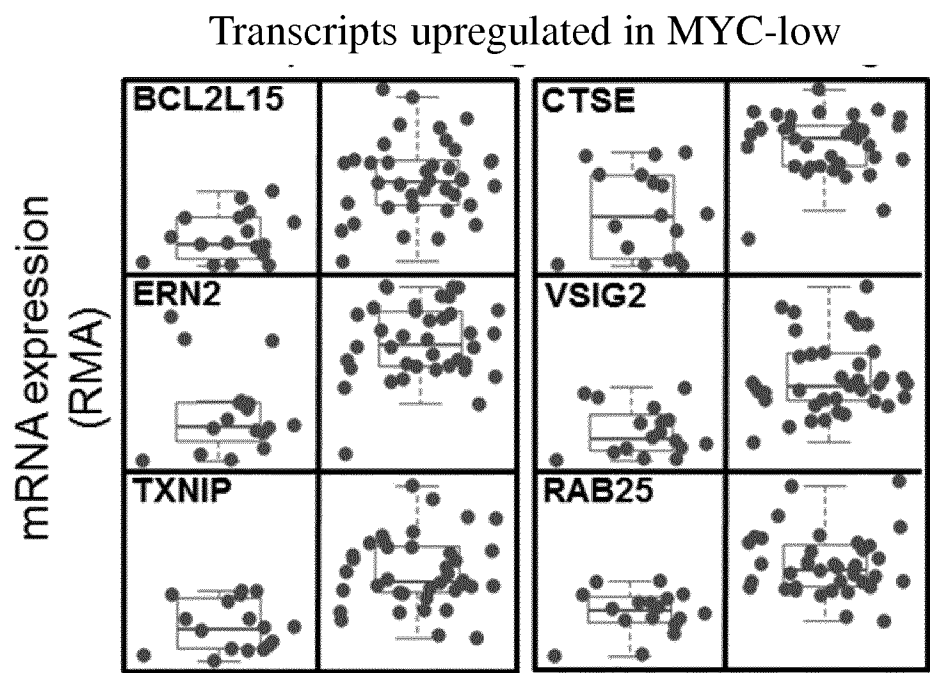


Fig. 2B

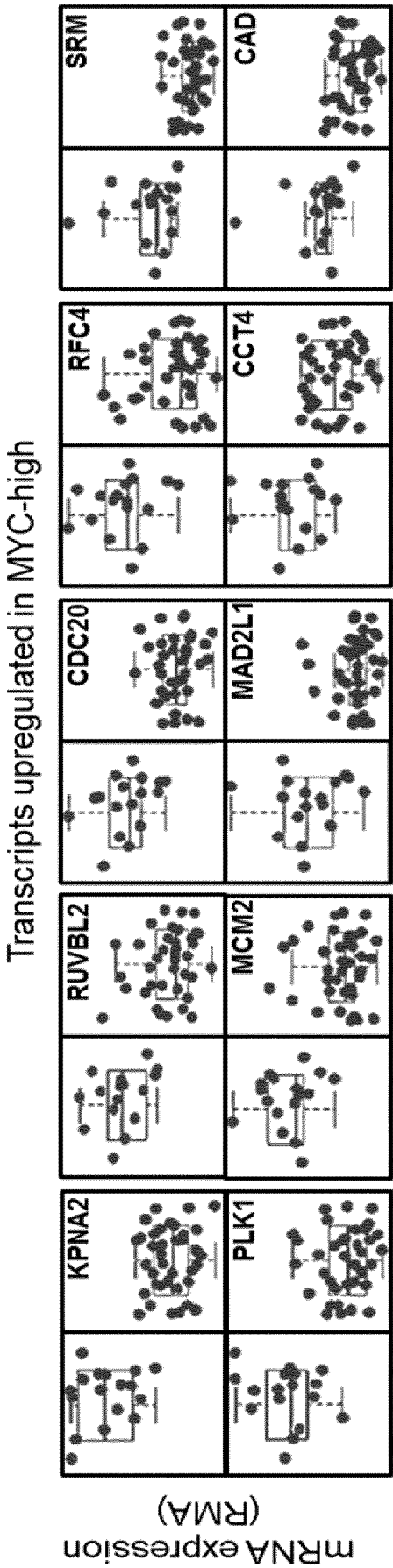


Fig. 2C

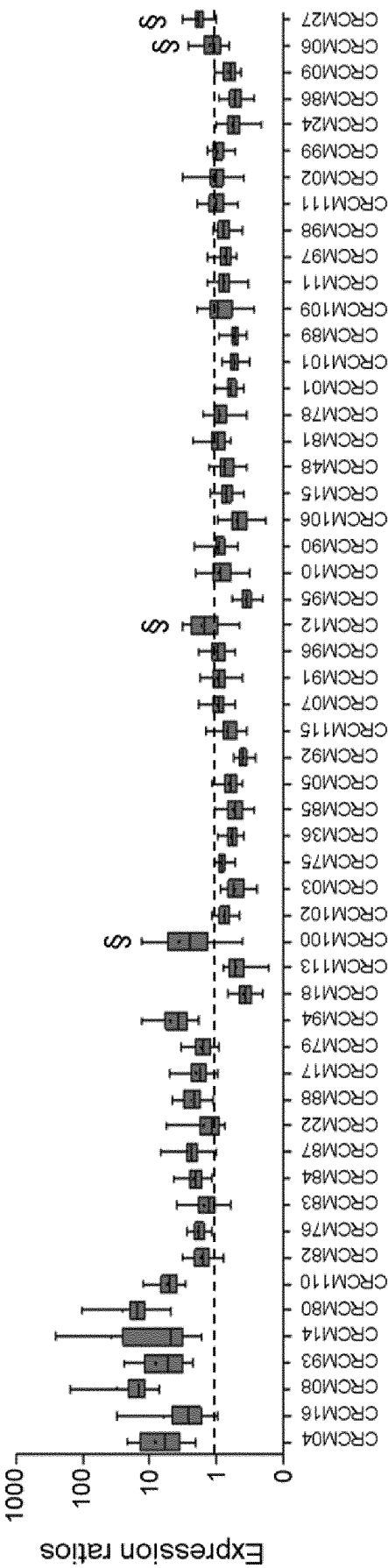


Fig. 2D

Affymetrix dataset on PDX

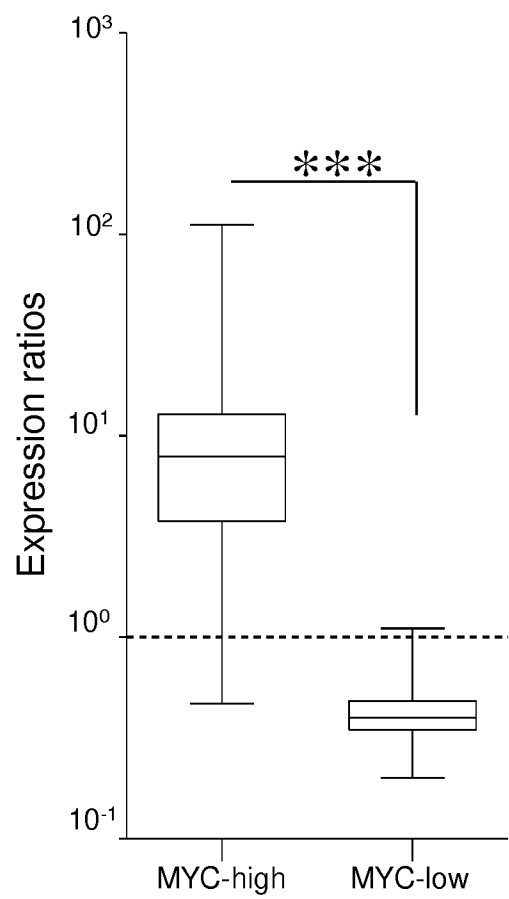


Fig. 3A

RT-qPCR dataset on PDX

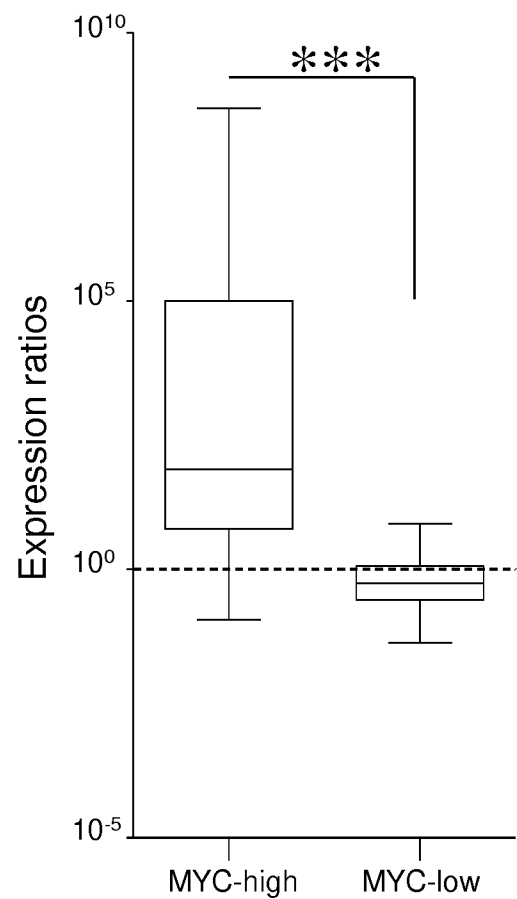


Fig. 3B

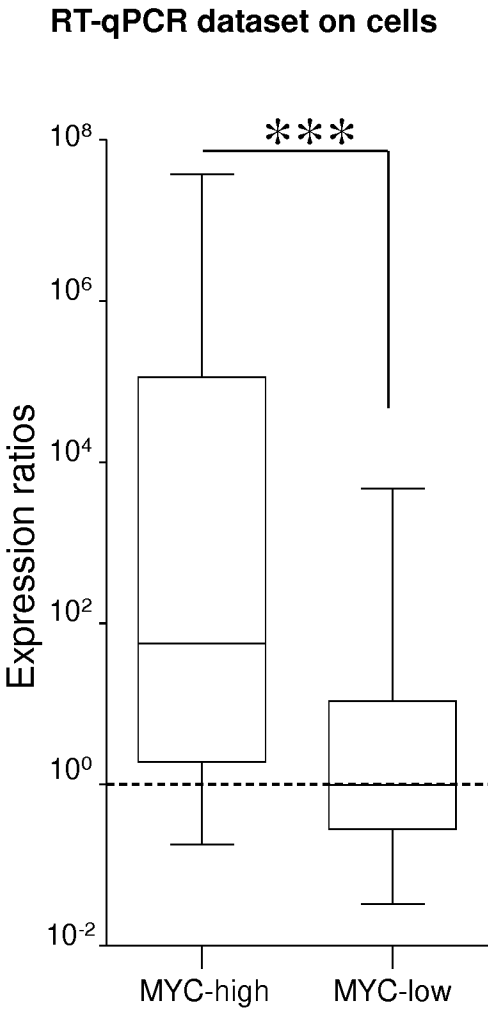


Fig. 3C

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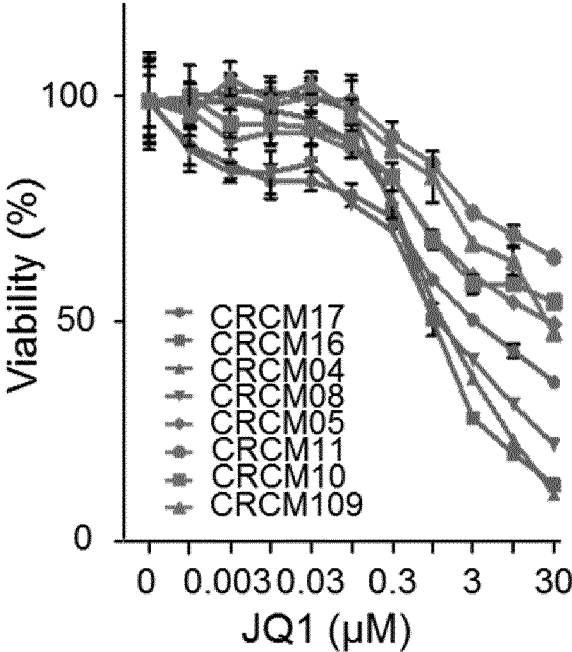


Fig. 4A

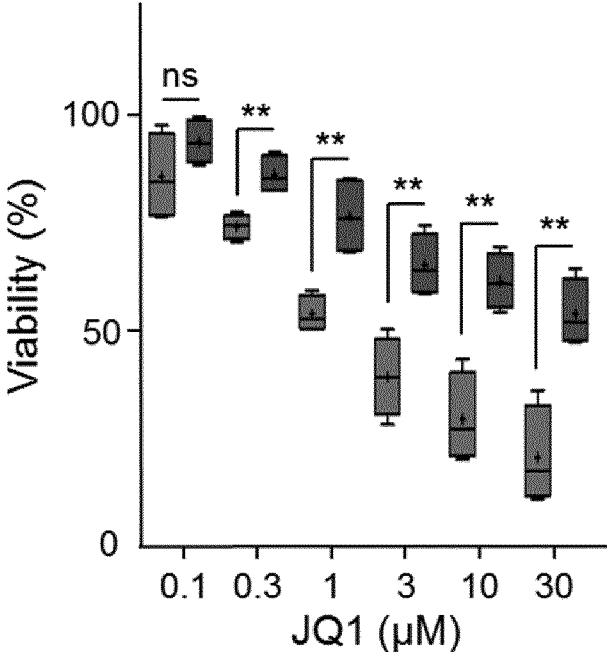


Fig. 4B

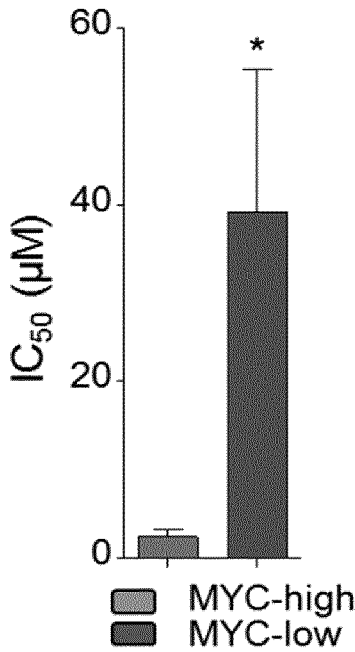


Fig. 4C

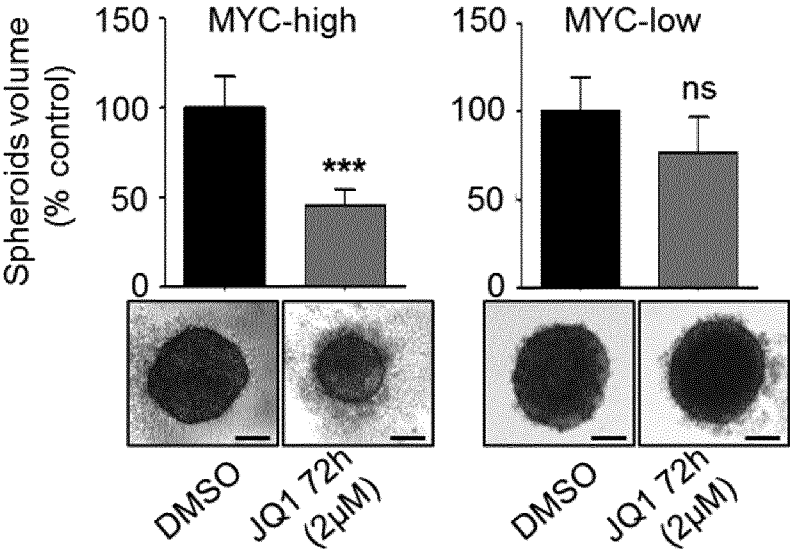


Fig. 4D

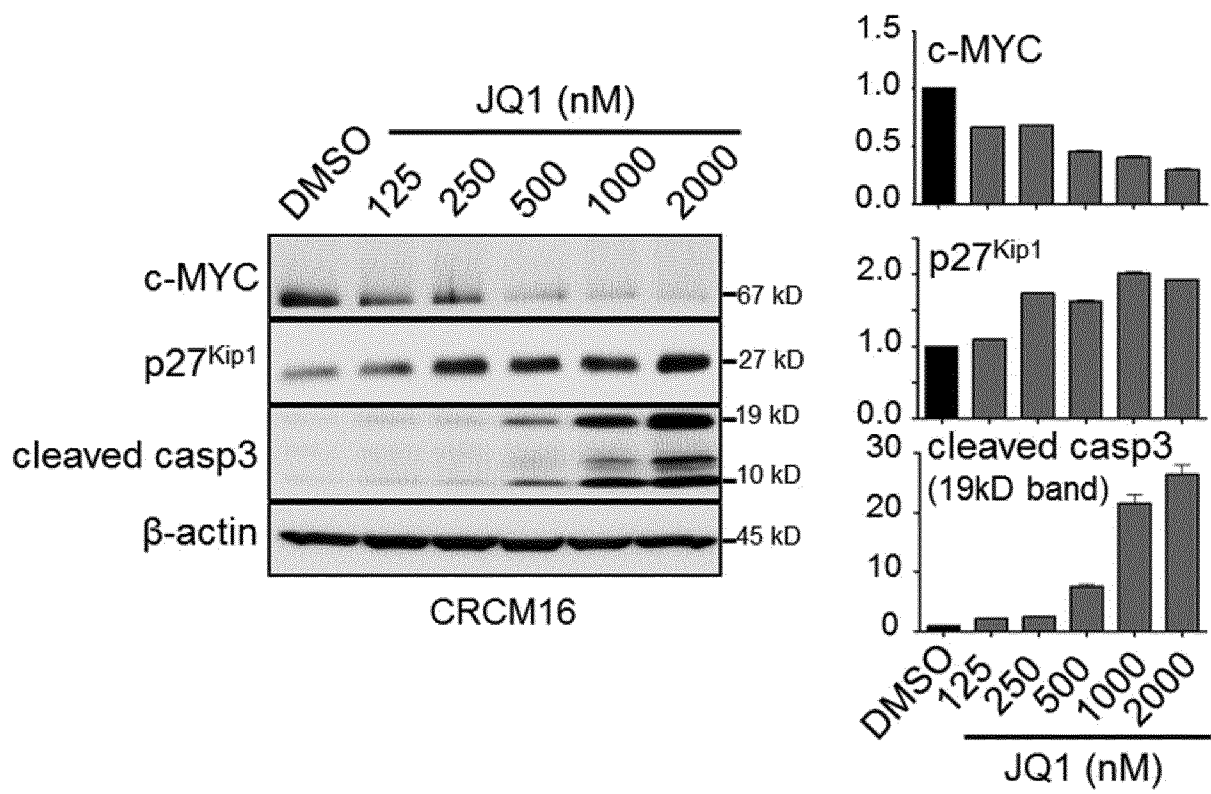


Fig. 4E

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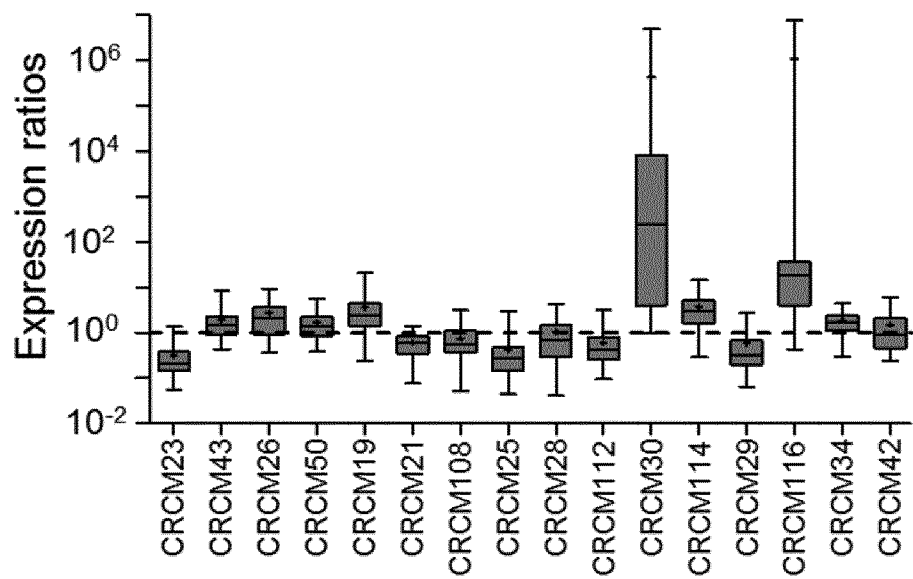


Fig.5A

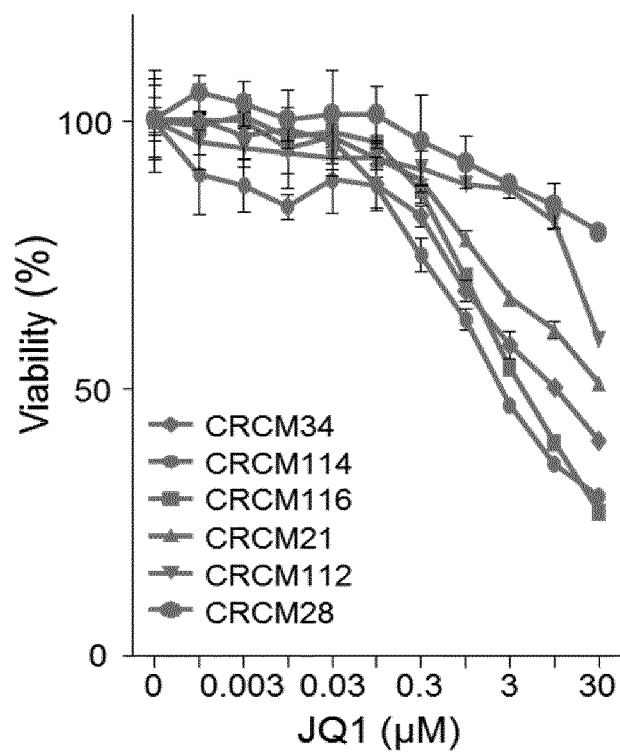


Fig.5B

10/13

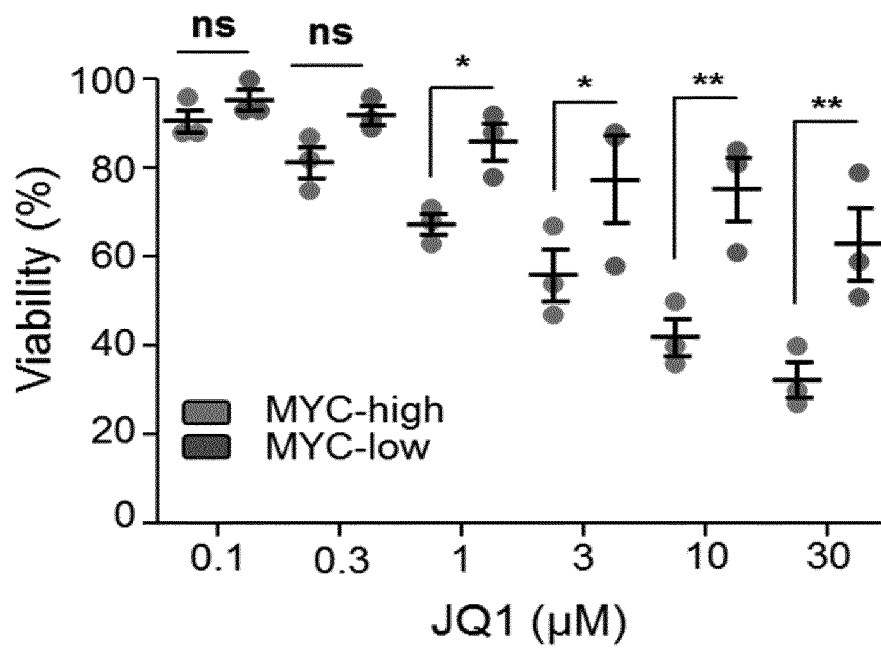


Fig. 5C

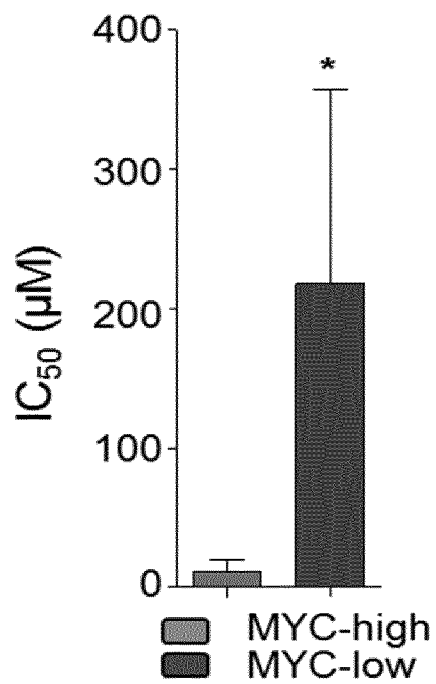


Fig. 5D

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Ki67 staining (IHC)

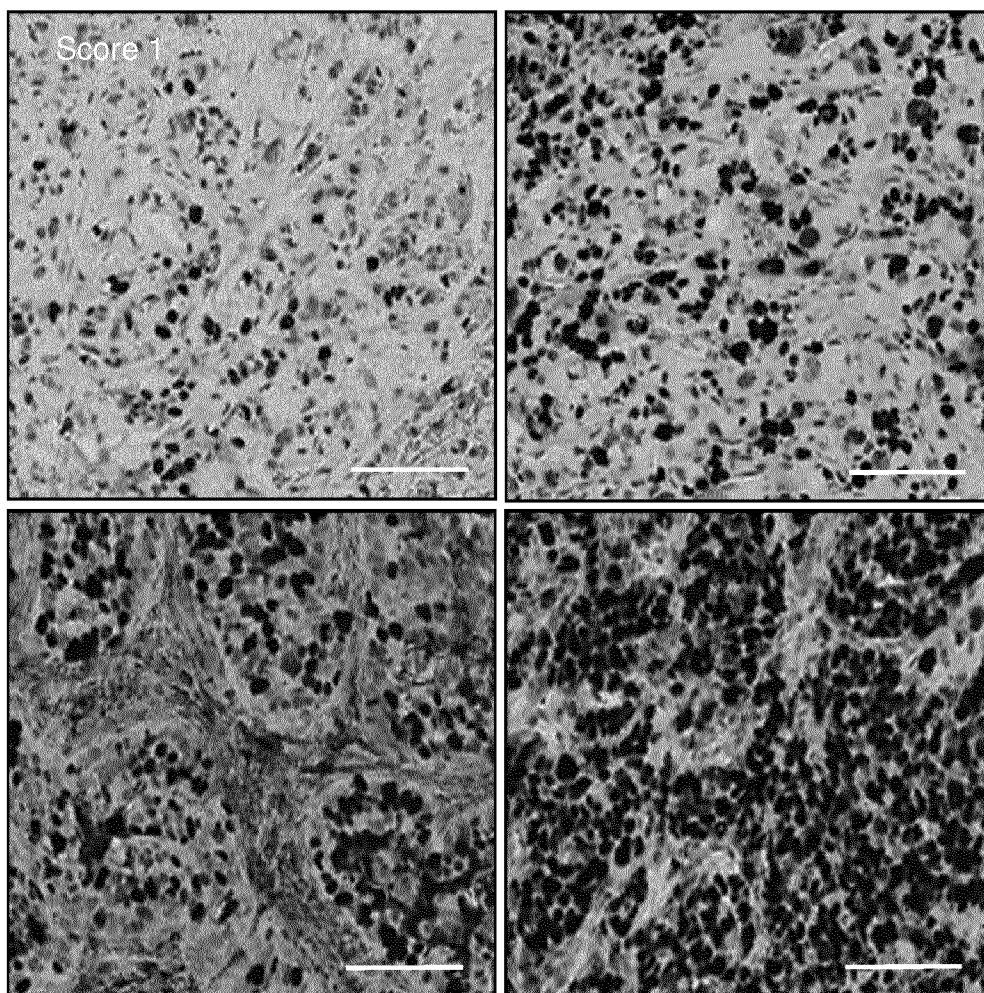


Fig. 6A

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Differentiation (H&E)

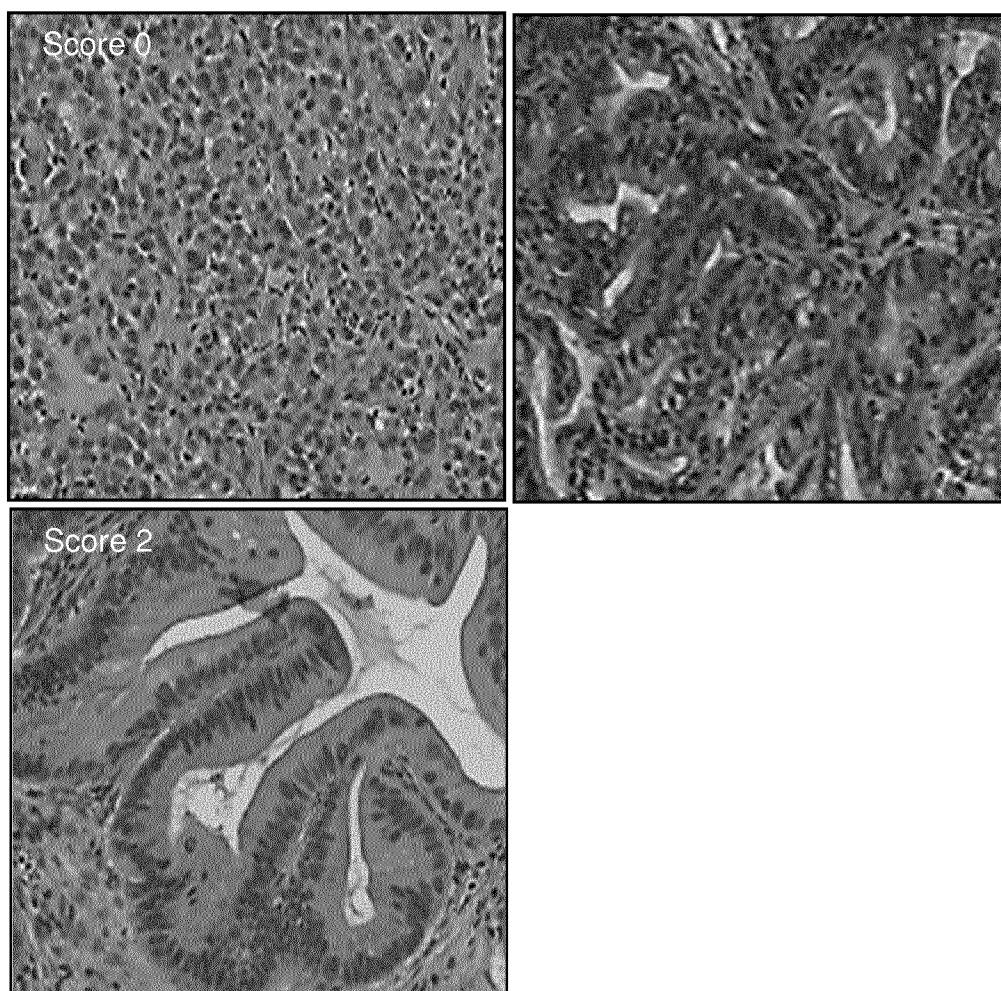


Fig. 6B

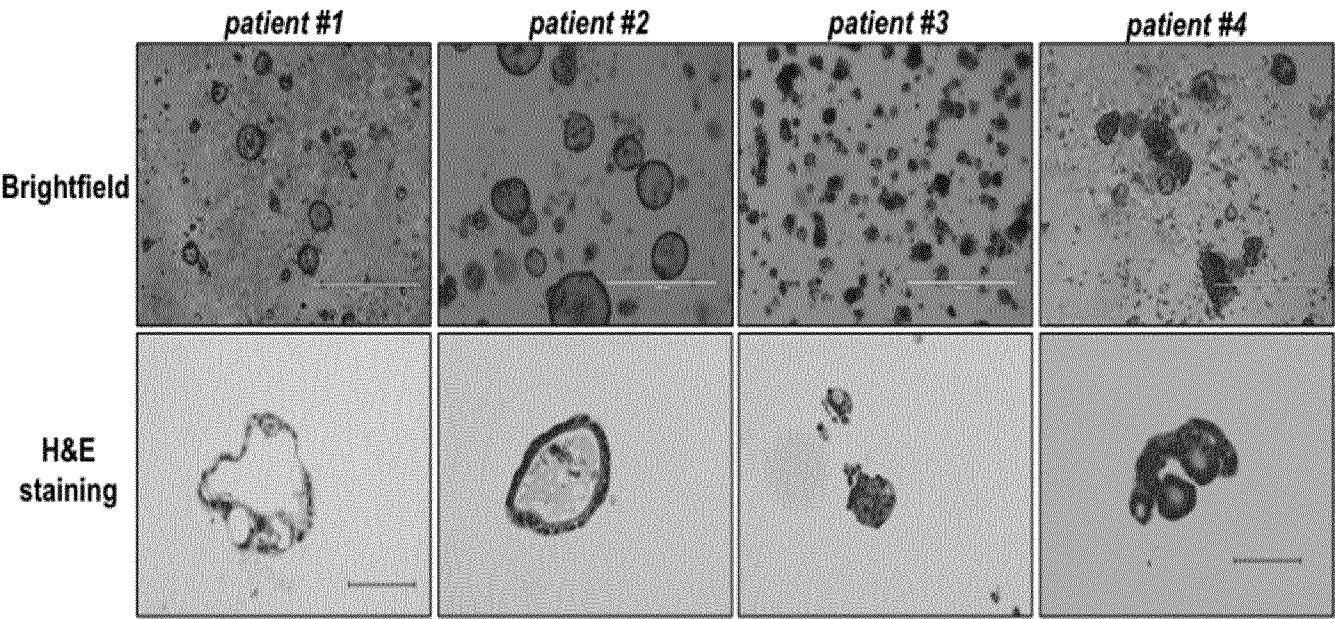


Fig. 7

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2017/079977

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, BIOSIS, Sequence Search, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 2 105 511 A1 (FRAUNHOFER GES FORSCHUNG [DE]) 30 September 2009 (2009-09-30) paragraph [[0009]] - paragraph [[0022]] -----	1-15
A	Chengzhi He ET AL: "Original Article Expression and prognostic value of c-Myc and Fas (CD95/AP01) in patients with pancreatic cancer", Int J Clin Exp Pathol, 1 January 2014 (2014-01-01), XP055379473, Retrieved from the Internet: URL:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3925922/pdf/ijcep0007-0742.pdf the whole document ----- -/--	1



Further documents are listed in the continuation of Box C.



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

19 February 2018

Date of mailing of the international search report

07/03/2018

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
 NL - 2280 HV Rijswijk
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 Fax: (+31-70) 340-3016

Authorized officer

Gabriels, Jan

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2017/079977

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KRISHAN KUMAR ET AL: "GLI2-dependent c-MYC upregulation mediates resistance of pancreatic cancer cells to the BET bromodomain inhibitor JQ1", SCIENTIFIC REPORTS, vol. 5, no. 1, 25 March 2015 (2015-03-25), pages 1-9, XP055363330, DOI: 10.1038/srep09489 the whole document -----	1
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