

(51) International Patent Classification:
G01N 33/574 (2006.01)(21) International Application Number:
PCT/EP2016/063390(22) International Filing Date:
10 June 2016 (10.06.2016)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
15305916.7 12 June 2015 (12.06.2015) EP

(71) Applicants: UNIVERSITÉ DE NICE SOPHIA ANTIPOLIS [FR/FR]; Pôle Valorisation Grand Château Parc Valrose 28, Avenue Valrose - BP 2135, 06103 Nice (FR). CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE - CNRS - [FR/FR]; 3, rue Michel Ange, 75016 Paris (FR). INSERM (INSTITUT NATIONAL DE LA SANTÉ ET DE LA RECHERCHE MÉDICALE) [FR/FR]; 101, rue de Tolbiac, 75013 Paris (FR).

(72) Inventors: HUEBER, Anne-Odile; Institut de Biologie Valrose (iBV Bat Sc naturelles-Parc Valrose, 06108 Nice

cedex 2 (FR). CHAKRABANDHU, Krittalak; Institut de Biologie Valrose (IBV), Université de Nice Sophia Antipolis, Parc Valrose, Bâtiment des Sciences Naturelles, 06108 Nice (FR).

(74) Agent: CABINET PLASSERAUD; 66, rue de la Chaussée d'Antin, 75440 Paris Cedex 09 (FR).

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

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE,

[Continued on next page]

(54) Title: METHOD FOR PREDICTING THE RESPONSE TO A TNFRSF6-INFLUENCED CANCER TREATMENT AND FOR PREDICTING RESISTANCE TO A CANCER TREATMENT

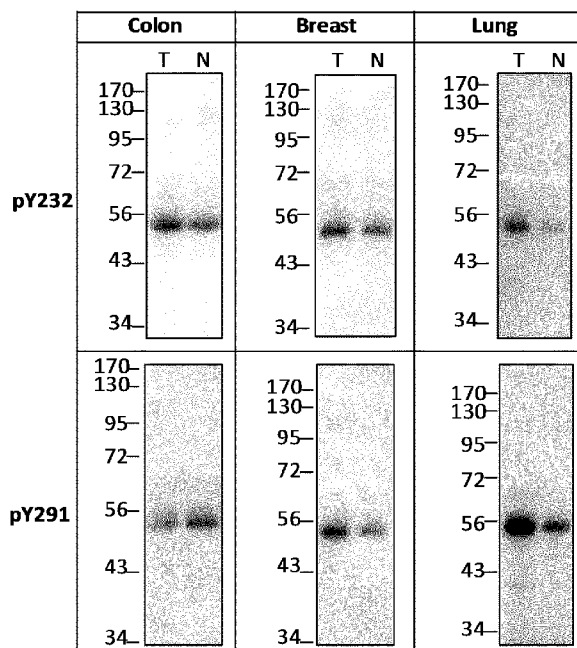


Figure 5

(57) Abstract: The present invention relates to a method based on differential levels of phospho- TNFSFR6, for screening a patient suffering from a cancer influenced by TNFSFR6 signaling, and who might benefit from a given therapy, comprising: a) a step of detecting the phosphorylation of the residues Y232 and Y291 of TNFSFR6, in a biological sample of said patient; and b) comparing the phosphorylation levels of the residues Y232 and Y291 of TNFSFR6 obtained in step a) to their respective reference levels, in which phosphorylation levels of Y232 et Y291 is indicative of the therapy to be given.



DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT,
LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE,
SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA,
GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

- with (an) indication(s) in relation to deposited biological material furnished under Rule 13bis separately from the description (Rules 13bis.4(d)(i) and 48.2(a)(viii))
- with sequence listing part of description (Rule 5.2(a))

Published:

- with international search report (Art. 21(3))

**Method for predicting the response to a TNFRSF6-influenced cancer treatment
and for predicting resistance to a cancer treatment**

The present invention relates to a method for screening a patient suffering from a cancer, particularly a TNFRSF6 signaling-influenced cancer, and who might benefit from a given therapy, comprising:

- a) a step of detecting the phosphorylation of the residues Y232 and Y291 of TNFRSF6, in a biological sample of said patient; and
 - b) comparing the phosphorylation levels of the residues Y232 and Y291 of TNFRSF6 obtained in step a) to their respective reference levels,
- in which phosphorylation levels of Y232 et Y291 is indicative of the therapy to be given.

The present invention also relates to a method for predicting the resistance of a patient suffering from a cancer to a given therapy, comprising:

- a) a step of detecting the phosphorylation of the residues Y232 and Y291 of TNFRSF6, in a biological sample of said patient;
 - b) comparing the phosphorylation levels of the residues Y232 and Y291 of TNFRSF6 obtained in step a) to their respective reference levels,
- in which phosphorylation levels of Y232 and Y291 is indicative of the resistance of said patient to the therapy.

Colorectal cancer (CRC) is a highly prevalent disease that is associated with high mortality and morbidity rates, with > 1,000,000 new cases and 500,000 deaths worldwide every year. The treatment of colorectal cancer can be aimed at curation or palliation. The decision on which aim to adopt depends on various factors, including the patient's health and preferences, as well as the stage of the tumour. When colorectal cancer is caught early, surgery can be curative. However, when it is detected at later stages for which metastases are present, this is less likely and treatment is often directed at palliation, to relieve symptoms caused by the tumour and keep the person as comfortable as possible.

The poor therapeutic outcome of CRC is largely due to the development of resistance to conventional drugs (i.e. chemotherapeutic drugs as well as molecularly targeted agents). The standard chemotherapeutic treatment for metastatic patients for the last 40 years was 5-fluorouracil (5-FU) and leucovorin-based therapy. Major progress has been made
5 by the introduction of regimens containing cytotoxic drugs such as CPT-11/SN-38 (also called camptothecin-11 or irinotecan). However, these combinations remain inactive in about half of the patients (innate or primary resistance), and in addition, resistance to treatment appears in almost all patients who initially responded (acquired or secondary resistance).

10

Drug resistance is a critical setback of cancer management that must be urgently overcome. One way to overcome this problem is designing therapeutic strategies based on multi-pathway targeted therapies and patient stratification based on predictive biomarkers, to increase the chance of therapeutic success. This strategy depends on
15 identification of targets from different pathways and development of reliable molecular biomarkers.

TNFRSF6 (or Fas) signalling is a major pathway that controls cell apoptosis as well as survival pathways. The resistance to TNFRSF6-mediated apoptosis and the emergence of TNFRSF6-mediated proliferation and invasiveness have been demonstrated in
20 oncogenesis and progression of several types of cancers. While therapeutic agents targeting TNFRSF6 have been created and undergone clinical trials, biomarkers of this critical pathway, which could be invaluable in increasing the chance of success of these agents, do not exist to date.

25 There is thus a need to improve the way of choosing the therapy to a given cancer, and particularly colorectal cancer. Particularly, there is a need for a quick and easy way of selecting the best therapy for a given patient, which would be reliable, sensitive and specific, without any laborious experimentation. There is also a need for evaluating, as early as possible, the occurrence of a resistance to a given therapy in a patient suffering
30 from a cancer. This would allow choosing the best therapy for each patient.

The inventors have discovered that tyrosine phosphorylation in the TNFRSF6 death domain is dispensable in TNFRSF6 ligand-induced apoptosis. Tyrosine phosphorylation in the TNFRSF6 death domain rather correlates with the activation of survival signals. The inventors have established that the outcome of TNFRSF6 signaling is determined by its tyrosine phosphorylation status of the death domain. Dephosphorylation of TNFRSF6 tyrosines turns on apoptotic signal, whereas the tyrosine phosphorylation which depends on activities of Src family kinases, turns off the pro-apoptotic signal and turns on the prosurvival signal (Chakrabandhu K, Huault S, Durivault J, Lang K, Ta Ngoc L, Bole A, et al. (2016) An Evolution-Guided Analysis Reveals a Multi-Signaling Regulation of Fas by Tyrosine Phosphorylation and its Implication in Human Cancers. PLoS Biol 14(3)).

The inventors have also discovered that phosphorylated Y232 and phosphorylated Y291 distinctly indicate different roles of TNFRSF6 in cell survival. Namely the phosphorylation of TNFRSF6 at Y232 is important for the increase in entry of G2/M phase of the cell cycle and amphiregulin-induced proliferation signaling of the epidermal growth factor receptor (EGFR) while the phosphorylation of TNFRSF6 at Y291 is important for STAT3-mediated EGFR signaling (Figure 1). They have also discovered that TNFRSF6 plays an important role in the sensitivity of chronic myeloid leukemia (CML) cells to imatinib (Figure 2) and colon cancer cells to cisplatin (Figure 3).

The inventors have also established, notably thanks to the monoclonal antibodies they have created, a method for defining the best therapy for a patient suffering from a cancer, particularly colorectal cancer or breast cancer, based on measuring the phosphorylation of the residues Y232 (tyrosine 232) and Y291 (tyrosine 291) of Fas (or TNFRSF6) in a biological sample of said patient.

As shown in the Example, this approach is reliable, sensitive and specific for a given cancer, and can be easily performed.

The inventors have also established a method for predicting the resistance of a patient suffering from a cancer, particularly colorectal cancer or chronic myeloid leukemia, to a given therapy, particularly platinum complex drug or imatinib, based on measuring the

phosphorylation of the residues Y232 (tyrosine 232) and Y291 (tyrosine 291) of TNFRSF6 in a biological sample of said patient.

The invention thus relates to a method for screening a patient suffering from a cancer and who might benefit from a given therapy, comprising:

- a) a step of detecting the phosphorylation of the residues Y232 and Y291 of TNFRSF6, in a biological sample of said patient; and
- b) comparing the phosphorylation levels of the residues Y232 and Y291 of TNFRSF6 obtained in step a) to their respective reference levels, called Reference 1 and Reference 2,

in which :

- phosphorylation levels of both residues Y232 and Y291 below References 1 and 2 respectively are indicative of the patient to be a good candidate for TNFRSF6-targeting anticancer drugs that activate TNFRSF6-induced cell death signaling,
- a phosphorylation level of residue Y232 below Reference 1 and a phosphorylation level of residue Y291 equal to or higher than Reference 2 are indicative of the patient to be a good candidate for TNFRSF6- or TNFRSF6 ligand-targeting anticancer drugs that inhibit TNFRSF6-induced cell survival signaling, or for the combination of a drug inhibiting STAT3-mediated, EGF-dependent EGFR signaling with a TNFRSF6-targeting anticancer drug activating TNFRSF6-induced cell death signaling,
- a phosphorylation level of residue Y232 equal to or higher than Reference 1 and a phosphorylation level of residue Y291 below Reference 2 are indicative of the patient to be a good candidate for TNFRSF6- or TNFRSF6 ligand-targeting anticancer drugs that inhibit TNFRSF6-induced cell survival signaling, or for drugs inhibiting Amphiregulin-dependent EGFR signaling,
- phosphorylation levels of both residues Y232 and Y291 equal to or higher than References 1 and 2 respectively are indicative of the patient to be a good candidate for TNFRSF6- or TNFRSF6 ligand-targeting anticancer drugs that inhibit TNFRSF6-induced cell survival signaling, or for the combination of a drug inhibiting STAT3-mediated, EGF-dependent EGFR signaling with a

TNFRSF6-targeting anticancer drug activating TNFRSF6-induced cell death signaling, or for drugs inhibiting Amphiregulin-dependent EGFR signaling.

Preferably, the “TNFRSF6-targeting anticancer drug that activates TNFRSF6-induced
5 cell death signaling” is chosen from TNFRSF6-targeting agents such as APO-010, M-
Fas-Ligand (M-FasL), AMF-3d19 or Novotarg.

APO-010 is a recombinant, soluble, hexameric fusion protein consisting of three human Fas ligand (or TNFRSF6 ligand, or TNFRSF6L, or FasL) extracellular domains fused to the dimer-forming collagen domain of human adiponectin.

10 M-FasL is developed by Memgen LLC.

AMF-3d19 is an antibody developed by Amorfis. Said antibody targets disease specific epitopes on misfolded TNFRSF6 of cancer cells.

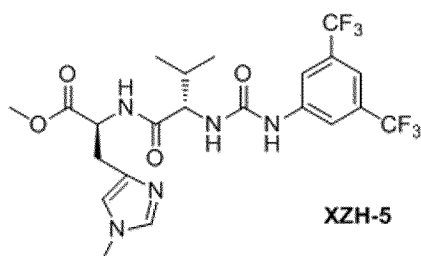
Novotarg is a bispecific antibody targeting CD20 and TNFRSF6. It is developed by Baliopharm.

15 Preferably, the “TNFRSF6- or TNFRSF6 ligand-targeting anticancer drug that inhibits
TNFRSF6-induced cell survival signaling” is chosen from APG101 and MOTI-1001.

APG101 is developed by Apogenix. It is a human soluble fusion protein consisting of the extracellular domain of TNFRSF6 and the Fc portion of IgG1.

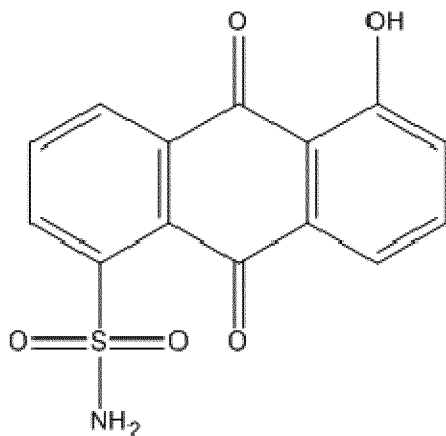
MOTI-1001 is developed by Biomoti. It is a paclitaxel-loaded carrier coated with
20 TNFRSF6.

The “drug inhibiting STAT3-mediated, EGF-dependent EGFR signaling” is preferably chosen from nifuroxazide, morin (i.e. 3,5,7,2',4'-pentahydroxyflavone), XZH-5 of formula (I):



(1)

25 and LLL12 of formula (II):



The drug inhibiting Amphiregulin-dependent EGFR signaling is preferably chosen from monoclonal antibodies against amphiregulin, such as Fsn1006. Fsn1006 is developed by Fusion Antibodies. It is an antibody targeting amphiregulin and heparin-binding EGF-like growth factor.

Indeed, according to the levels of phosphorylation of the residues Y232 and Y291 of TNFRSF6 detected in the samples of many patients, one can define different subgroups: this corresponds to the stratification of patients. Then, one can determine the subgroup of a given patient, and thus his best anti-cancer therapy.

Particularly, said cancer is a TNFRSF6 signaling-influenced cancer, i.e. a cancer in which TNFRSF6 signaling is involved. Preferably, said cancer is chosen from colorectal cancer, leukemia, glioblastoma and breast cancer.

More preferably, said cancer is chosen from colorectal cancer and breast cancer.

Said method is an *in vitro* method, performed with a simple biological sample of a patient.

As used herein, the term “**patient**” refers to an individual with symptoms of and/or suspected of having a cancer. It denotes a mammal, such as a rodent, a feline, a canine, and a primate. Preferably, a patient according to the invention is a human.

As used herein, the term “**biological sample**” of a patient refers to any sample of a patient. Preferably, it refers to a blood sample or a biopsy of a patient. Preferably, said biological sample is a biopsy.

5 As used herein, TNFRSF6, also called CD95, APO-1 or Fas, is a TNF receptor superfamily member, and well known as a key apoptosis activator. TNFRSF6 carries three tyrosine (Y) residues, one in the extracellular domain (Y91) and two in the intracellular domain: Y232 and Y291. TNFRSF6 was mainly considered as a tumor suppressor thanks to its well-known ability to promote cell death (apoptosis). However,
10 it is now well recognized that TNFRSF6, once a well-known apoptosis inducer, promotes and prevents cell growth depending on cellular context (Peter ME (2014) DICE: A novel tumor surveillance mechanism-a new therapy for cancer? Cell Cycle 13, and Peter ME, Budd RC, Desbarats J, Hedrick SM, Hueber AO, et al. (2007) The CD95 receptor: apoptosis revisited. Cell 129: 447-450). Upon binding with its cognate ligand,
15 TNFRSF6 ligand (TNFRSF6L), TNFRSF6 can either: 1. activate programmed cell death (apoptosis) by recruiting adaptor protein, FADD, and procaspase 8 to form death inducing signaling complex (DISC) which leads to the activation of caspase 8, subsequent caspase cascade, and ultimately cell death (Holohan C, Van Schaeybroeck S, Longley DB, Johnston PG (2013) Cancer drug resistance: an evolving paradigm. Nat
20 Rev Cancer 13: 714-726), or 2. activate non-death response including cell proliferation, migration, and invasion (Peter ME, Budd RC, Desbarats J, Hedrick SM, Hueber AO, et al. (2007) The CD95 receptor: apoptosis revisited. Cell 129: 447-450). This multi-modal signaling of TNFRSF6 has been demonstrated in many cancer cell types including colon (Li H, Fan X, Stoicov C, Liu JH, Zubair S, et al. (2009) Human and mouse colon
25 cancer utilizes CD95 signaling for local growth and metastatic spread to liver. Gastroenterology 137: 934-944, 944.e931-934), breast (Malleter M, Tazuin S, Bessede A, Castellano R, Goubard A, et al. (2013) CD95L cell surface cleavage triggers a prometastatic signaling pathway in triple-negative breast cancer. Cancer Res 73: 6711-6721), and glioblastoma (Kleber S, Sancho-Martinez I, Wiestler B, Beisel A, Gieffers
30 C, et al. (2008) Yes and PI3K bind CD95 to signal invasion of glioblastoma. Cancer Cell 13: 235-248). And currently, both pro-apoptotic and pro-survival roles of TNFRSF6 are bases of therapeutic designs that aim to either activate TNFRSF6

signaling (for example APO010 agent targeting extracellular domain of TNFRSF6) (ClinicalTrial.gov, NCT00437736, A Phase I Dose Finding Study of APO010 in Patients With Solid Tumors (AP1001). National Institutes of Health) or inhibit TNFRSF6 signaling triggered by its cognate ligand, TNFRSF6 ligand (TNFRSF6L; for example APG010 targeting TNFRSF6L) (Bendszus M, Debus J, Wick Wea (2012) APG101_CD_002: A phase II, randomized, open-label, multicenter study of weekly APG101 plus reirradiation versus reirradiation in the treatment of patients with recurrent glioblastoma. J Clin Oncol. pp. 2012;2030(Suppl. 2015). Abstract 2034). However, without the information guiding when to activate or inhibit TNFRSF6 signaling, application of TNFRSF6 or TNFRSF6L-targeting agent poses risk of undesirable outcome. Thus, in the context of cancer therapy, there is a need for a means to predict the outcome of TNFRSF6 signalling to properly guide the design of therapies that target TNFRSF6 in an appropriate manner.

15 Indeed, the inventors have discovered that tyrosine phosphorylation in the TNFRSF6 death domain is dispensable in TNFRSF6L-induced apoptosis. Tyrosine phosphorylation in the TNFRSF6 death domain rather correlates with the activation of survival signals.

20 Thus, accordingly, the invention relates to a method for screening a patient suffering from a cancer and who might benefit from a given therapy, by predicting the outcome of TNFRSF6 signaling. This method comprises the first step a) of detecting the phosphorylation of the residues Y232 and Y291 of TNFRSF6, in said sample.

25 Preferably, step a) of detecting the phosphorylation of the residues Y232 and Y291 of TNFRSF6, is performed by any technique known in the art able to reveal the phosphorylation of Y232 and/or Y291 of TNFRSF6. Preferably, said step is performed by immunoblot, immunofluorescence, immunohistochemistry, ELISA, Time-resolved Förster resonance energy transfer (TR-FRET), Amplified Luminescent Proximity

30 Homogenous Assay (ALPHA) or flow cytometry.

More preferably, step a) of detecting the phosphorylation of the residues Y232 and Y291 of TNFRSF6 is performed by using monoclonal antibodies, polyclonal antibodies or probes that are able to bind specifically to phosphorylated Y232 and Y291 respectively. Said monoclonal antibody may be engineered, including being humanized
5 or chimeric. Said probes may be small molecules, such as peptides.

As used herein, the term “human antibody” refers to an antibody in which a substantial portion of the antibody molecule resembles, in amino acid sequence or structure, that of an antibody of human origin. The term “humanized antibody” refers to an antibody
10 which has been modified by genetic engineering or by other means to be similar in structure or amino acid sequence to naturally occurring human antibodies. In a particular embodiment, the antibody of the invention may be a chimeric antibody. Said chimeric antibody of the present invention can be produced by obtaining nucleic sequences encoding VL and VH domains, constructing a chimeric antibody expression
15 vector by inserting them into an expression vector for animal cell having genes encoding human antibody CH and human antibody CL, and expressing the expression vector by introducing it into an animal cell. The CH domain of a chimeric antibody may be any region which belongs to human immunoglobulin, but those of IgG class are suitable and any one of subclasses belonging to IgG class, such as IgG1, IgG2, IgG3
20 and IgG4, can also be used. Also, the CL of a chimeric antibody may be any region which belongs to Ig, and those of kappa class or lambda class can be used. Methods for producing chimeric antibodies involve conventional recombinant DNA and gene transfection techniques are well known in the art (See Morrison SL. et al. (1984) and patent documents US5,202,238 and US5,204, 244).

25

Monoclonal antibodies (mAbs) may be prepared by the following protocol:

1. Coupling of the peptide H-NLSDVDLSK(pY)ITTIAGVMC-OH (SEQ ID NO:1) for Y232, or H-HQLHGKKEA(pY)DTLIKDLKKA-OH (SEQ ID NO:2) for Y291 (pY=phosphotyrosine), to a carrier protein (chicken ovalbumin) using
30 glutaraldehyde and the heterobifunctional agent N-succinimidyl-3-(2-pyridyldithio)-propionate (SPDP)

2. First immunization of Wistar rat with the coupled peptide emulsified in Freund's Complete Adjuvant (CFA) by intraperitoneal (IP) injection (Day 1)
3. Second immunization with the coupled peptide emulsified in by IP injection as emulsions in CFA (Day 15)
- 5 4. Challenge by intravenous (IV) injection (Day 25)
5. Challenge by IV injection (Day 26)
6. Challenge by IV injection (Day 27)
7. Fusion of the rat spleen cells with the myeloma cell line (Day 28)
8. Preclone screening, in particular on 40 multiwell plates, by solid-phase ELISA
10 for antigen specific clones (Day 38)
9. Expansion of fusion positive clones and rescreening by immunoblotting using hybridoma culture supernatant
10. Subcloning of fusion positive clones and screening for antigen specific positive clones by ELISA
- 15 11. Production and purification of the antibody from hybridoma cell culture supernatant. Hybridoma were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum at 37°C, 5% CO₂. Cells were separated from the supernatant by centrifugation. The antibody was purified by two steps of chromatography purification:
20 a. Affinity purification by protein G: the filtered supernatant was loaded onto a HiTrap protein G HP column (GE Healthcare). After washing the column, the bound IgG was eluted from the column by 0.1 M glycine-HCl, pH 2.7 and the buffer was exchanged to 20 mM sodium phosphate, pH 7.0 for application to the next purification step.
25 b. Affinity purification by anti-rat IgG antibody: the IgG purified by protein G column was applied to Hi-Trap NHS-activated HP column (GE Healthcare) conjugated with anti-rat IgG antibody. After washing the column, the bound Rat IgG was eluted from the column by 0.1 M glycine-HCl, pH 2.7 and the buffer was exchanged to phosphate buffered
30 saline (PBS) pH 7.4 and stored at -20°C.

Preferably, the antibody fragments are chosen from Fab (e.g., by papain digestion), Fab' (e.g., by pepsin digestion and partial reduction), F(ab)₂, F(ab')₂ (e.g., by pepsin digestion) and dAb fragments. Such fragments may be produced by enzymatic cleavage, synthetic or recombinant techniques, as known in the art and/or as described herein.

5 Antibody fragments can also be produced in a variety of truncated forms using antibody genes in which one or more stop codons have been introduced upstream of the natural stop site. The various portions of antibodies can be joined together chemically by conventional techniques, or can be prepared as a contiguous protein using genetic engineering techniques.

10

Preferably, the antibody derivatives are chosen from scFv, (scFv)₂, diabodies, multimeric scFv derived from an antibody and fused to a Fc fragment, whole antibodies linked together to reach an aggregated form, and antibodies containing at least two Fabs bound face-to-tail.

15

In a preferred embodiment said antibody is a monoclonal antibody obtainable from the hybridoma deposited at the Collection Nationale de Cultures de Microorganismes (CNCM, Institut Pasteur, 25 rue du Docteur Roux, 75724 Paris Cedex 15, France), in accordance with the terms of Budapest Treaty, on April 17, 2014, under the number
20 CNCM I-4848.

As used herein, the expression “**1C7.3**” refers to an isolated antibody directed against the phosphorylated Y232 of TNFRSF6, which is obtainable from the hybridoma accessible under CNCM deposit number I-4848.

25 In another preferred embodiment said antibody is a monoclonal antibody obtainable from the hybridoma deposited at the Collection Nationale de Cultures de Microorganismes (CNCM, Institut Pasteur, 25 rue du Docteur Roux, 75724 Paris Cedex 15, France), in accordance with the terms of Budapest Treaty, on April 17, 2014, under the number CNCM I-4849.

30 As used herein, the expression “**33A9.2**” refers to an isolated antibody directed against the phosphorylated Y291 of TNFRSF6, which is obtainable from the hybridoma accessible under CNCM deposit number I-4849.

Both antibodies 1C7.3 and 33A9.2 are remarkable in that they allow the direct detection of phosphorylated Y232 and phosphorylated Y291 of TNFRSF6 respectively

(Chakrabandhu K, Huault S, Durivault J, Lang K, Ta Ngoc L, Bole A, et al. (2016) An
5 Evolution-Guided Analysis Reveals a Multi-Signaling Regulation of Fas by Tyrosine Phosphorylation and its Implication in Human Cancers. PLoS Biol 14(3)).

They can detect the phosphorylation in basal condition as well as upon cell activation by TNFRSF6 ligand (Fas ligand) and growth factors such as epidermal growth factor (EGF) (Figure 4). They can also detect tyrosine phosphorylated TNFRSF6 in cancerous
10 and normal tissue lysates from patients suffering from different types of cancers (Figure 5).

Thus, preferably, step a) of detecting the phosphorylation of the residues Y232 and Y291 of TNFRSF6 in said sample of the method of the invention is performed
15 respectively thanks to the monoclonal antibody obtainable from the hybridoma deposited at the Collection Nationale de Cultures de Microorganismes on April 17, 2014, under the number CNCM I-4848, or one of its fragments or derivatives, and thanks to the monoclonal antibody obtainable from the hybridoma deposited at the Collection Nationale de Cultures de Microorganismes on April 17, 2014, under the
20 number CNCM I-4849, or one of its fragments or derivatives.

After step a), the method of the invention further comprises a step b) of comparing the phosphorylation levels of the residues Y232 and Y291 of TNFRSF6 obtained in step a) to their respective reference levels, called Reference 1 and Reference 2.

25 Reference 1 and Reference 2 correspond to calculated values of phosphorylation levels of each residue Y232 or Y291 of TNFRSF6, respectively. Preferably, Reference 1 and 2 are the same and are the score of 1.1.

30 Preferably, step a) is performed according to the following process:

- obtaining the biological samples of a patient, preferably a cancerous tissue and a normal (non-cancerous) tissue of the same organ;

- obtaining lysates of the cancerous and normal tissues of the patient;
- isolating and immunoblotting the proteins of said lysates, thanks to the monoclonal antibody obtainable from the hybridoma deposited at the Collection Nationale de Cultures de Microorganismes on April 17, 2014, under the number CNCM I-4848, or
- 5 one of its fragments or derivatives, and thanks to the monoclonal antibody obtainable from the hybridoma deposited at the Collection Nationale de Cultures de Microorganismes on April 17, 2014, under the number CNCM I-4849, or one of its fragments or derivatives;
- quantifying the immunoblot signal of protein bands corresponding to the canonical
- 10 forms of TNFRSF6, i.e. forms with the size between 45 and 56 kDa; and
- calculating the tyrosine phosphorylation signals.

More preferably, in the case of colon or breast cancer, step a) is performed by (see also figure 6):

15 **Step 1.** SDS-PAGE and immunoblotting of tissue lysates:

This method is based on immunoblotting lysates of biopsies of a cancerous tissue and a normal (non-cancerous) tissue of the same organ of a patient. The tissue lysates are subjected to SDS-PAGE (10% polyacrylamide). Proteins were then transferred on to PVDF membrane using Tris-Glycine buffer (100 mA, overnight at 4°C).

20 Immunoblotting of pY232 and pY291 TNFRSF6 is carried out using the monoclonal anti-pY232 and pY291 TNFRSF6 antibodies. Immunoblotting of the C-terminus of TNFRSF6 is carried out using a polyclonal rabbit anti-TNFRSF6 antibody (e.g. C20 from Santa Cruz). Chemiluminescence detection of the immunoblot bands is carried out using an imager (Odyssey FC, LI-Cor Biotechnology, 2 minutes of exposure).

25 **Step 2.** Densitometric analysis of immunoblot signals:

Perform a densitometric quantification of immunoblot signals of pY232, pY291 and C-terminus of TNFRSF6 protein bands that correspond to the canonical forms of TNFRSF6 (with the size between 45-56 kDa), preferably using Image Studio software Version 2.1 (LI-COR Biotechnology).

30 **Step 3.** Calculation of pY-TNFRSF6 signals:

1. Calculate the normalized values of pY232 and pY291 of TNFRSF6 of the cancerous tissue ($pY232_c^{norm}$ and $pY291_c^{norm}$, respectively) and the normalized values of pY232

and pY291 of TNFRSF6 of the non-cancerous tissue ($pY232_{nc}^{norm}$ and $pY291_{nc}^{norm}$, respectively), using the corresponding signals of the C-terminus (CTer) TNFRSF6 as the reference according to the following equations:

$$pY232_c^{norm} = pY232_c / CTer_c \quad \text{equation 1}$$

$$5 \quad pY291_c^{norm} = pY291_c / CTer_c \quad \text{equation 2}$$

$$pY232_{nc}^{norm} = pY232_{nc} / CTer_{nc} \quad \text{equation 3}$$

$$pY291_{nc}^{norm} = pY291_{nc} / CTer_{nc} \quad \text{equation 4}$$

where c denotes cancerous; nc, non-cancerous; and norm, normalized.

2. From the normalized values pY232 and pY291 of cancerous and non-cancerous tissues, calculate the standardized pY232 ($pY232_c^s$) and pY291 ($pY291_c^s$) values for the cancerous tissue by taking the ratio between the normalized pY232 and pY291 of the cancerous tissue and the normalized pY232 and pY291 of the normal tissue according to the following equations:

$$pY232_c^s = pY232_c^{norm} / pY232_{nc}^{norm} \quad \text{equation 5}$$

$$15 \quad pY291_c^s = pY291_c^{norm} / pY291_{nc}^{norm} \quad \text{equation 6}$$

where s denotes standardized.

In such a manner, the standardized values of pY232 ($pY232_{nc}^s$) and pY291 ($pY291_{nc}^s$) of the normal tissue are the ratio between the normalized pY232 and pY291 of the normal tissue and the normalized pY232 and pY291 of the normal tissue itself and are equal to 1, as shown in the following equations:

$$pY232_{nc}^s = pY232_{nc}^{norm} / pY232_{nc}^{norm} = 1 \quad \text{equation 7}$$

$$pY291_{nc}^s = pY291_{nc}^{norm} / pY291_{nc}^{norm} = 1 \quad \text{equation 8}$$

Then, step b) preferably comprises comparing the respective phosphorylation levels of the residues Y232 and Y291 of TNFRSF6 obtained in step a) to Reference 1 and Reference 2, both of which are equal to 1.1. This value is a reference below which the standardized pY232 or standardized pY291 is considered low when taking into account
 5 the level of pY232 and pY291 of the normal tissue.

More preferably, in the case of colon or breast cancer, step b) is performed by the following steps, performed after step 2. above (see also figure 6):

3. Assign Reference 1 and Reference 2 values, which are the reference pY232
 10 ($pY232^{ref}$) and pY291 ($pY291^{ref}$) values, respectively, to 10% above the corresponding standardized values of pY232 and pY291 of the normal tissue. Thus, Reference 1 and Reference 2 values are both equal to 1.1, as shown in the following equations:

$$\text{Reference 1} = pY232^{ref} = 1.1 \cdot pY232_{nc}^s = 1.1 \quad \text{equation 9}$$

$$15 \quad \text{Reference 2} = pY291^{ref} = 1.1 \cdot pY291_{nc}^s = 1.1 \quad \text{equation 10}$$

where ref denotes reference for TNFRSF6 signaling mode prediction.

4. Perform the diagnosis by comparing the respective standardized values of pY232 ($pY232_c^s$) and pY291 ($pY291_c^s$) of TNFRSF6 obtained in step 2 using equations 5 and
 20 6, respectively, to Reference 1 and Reference 2, respectively, in which :

- standardized phosphorylation levels of both residues Y232 and Y291 below 1.1 are indicative of the patient to be a good candidate for TNFRSF6-targeting anticancer drugs that activate TNFRSF6-induced cell death signaling,
- a standardized phosphorylation level of residue Y232 below 1.1 and a
 25 standardized phosphorylation level of residue Y291 equal to or higher than 1.1 are indicative of the patient to be a good candidate for TNFRSF6 ligand-targeting anticancer drugs that inhibit TNFRSF6-induced cell survival signaling, or for the combination of a drug inhibiting STAT3-mediated EGF-dependent EGFR signaling with a TNFRSF6-targeting anticancer drug activating
 30 TNFRSF6-induced cell death signaling,
- a standardized phosphorylation level of residue Y232 equal to or higher than 1.1 and a standardized phosphorylation level of residue Y291 below 1.1 are

- indicative of the patient to be a good candidate for TNFRSF6- or TNFRSF6 ligand-targeting anticancer drugs that inhibit TNFRSF6-induced cell survival signaling, or for drugs inhibiting amphiregulin-dependent EGFR signaling,
- 5 - standardized phosphorylation levels of both residues Y232 and Y291 equal to or higher than 1.1 are indicative of the patient to be a good candidate for TNFRSF6 ligand-targeting anticancer drugs that inhibit TNFRSF6-induced cell survival signaling, or for the combination of a drug inhibiting STAT3-mediated, EGF-dependent EGFR signaling with a TNFRSF6-targeting anticancer drug activating TNFRSF6-induced cell death signaling, or for drugs inhibiting
- 10 amphiregulin-dependent EGFR signaling.

- Preferably, the method of the invention is for screening a patient suffering from breast cancer or colorectal cancer, who might benefit from a given therapy, comprising:
- a) a step of detecting the phosphorylation of the residues Y232 and Y291 of TNFRSF6, in a biopsy of said patient, said step a) being performed by:
- 15 - obtaining the biological samples of the patient, preferably at least a cancerous tissue and an adjacent normal (non-cancerous) tissue of the same organ;
- obtaining lysates of the cancerous and normal tissues of the patient;
- isolating and immunoblotting the proteins of said lysate, thanks to the monoclonal antibody obtainable from the hybridoma deposited at the Collection Nationale de Cultures de Microorganismes on April 17, 2014, under the number CNCM I-4848, or one of its fragments or derivatives, and thanks to the monoclonal antibody obtainable from the hybridoma deposited at the Collection Nationale de Cultures de Microorganismes on April 17, 2014, under the number CNCM I-4849, or one of its
- 20 fragments or derivatives;
- quantifying the immunoblot signal of protein bands corresponding to the canonical forms of TNFRSF6, i.e. forms with the size between 45 and 56 kDa; and
- b) comparing the respective phosphorylation levels of the residues Y232 and Y291 of TNFRSF6 obtained in step a) to the score of 1.1,
- 25 in which :
- 30

- phosphorylation levels of both residues Y232 and Y291 below 1.1 are indicative of the patient to be a good candidate for TNFRSF6-targeting anticancer drugs that activate TNFRSF6-induced cell death signaling,
- a phosphorylation level of residue Y232 below 1.1 and a phosphorylation level of residue Y291 equal to or higher than 1.1 are indicative of the patient to be a good candidate for TNFRSF6 ligand-targeting anticancer drugs that inhibit TNFRSF6-induced cell survival signaling, or for the combination of a drug inhibiting STAT3-mediated EGF-dependent EGFR signaling with a TNFRSF6-targeting anticancer drug activating TNFRSF6-induced cell death signaling,
- a phosphorylation level of residue Y232 equal to or higher than 1.1 and a phosphorylation level of residue Y291 below 1.1 are indicative of the patient to be a good candidate for TNFRSF6- or TNFRSF6 ligand-targeting anticancer drugs that inhibit TNFRSF6-induced cell survival signaling, or for drugs inhibiting amphiregulin-dependent EGFR signaling,
- phosphorylation levels of both residues Y232 and Y291 equal to or higher than 1.1 are indicative of the patient to be a good candidate for TNFRSF6 ligand-targeting anticancer drugs that inhibit TNFRSF6-induced cell survival signaling, or for the combination of a drug inhibiting STAT3-mediated, EGF-dependent EGFR signaling with a TNFRSF6-targeting anticancer drug activating TNFRSF6-induced cell death signaling, or for drugs inhibiting amphiregulin-dependent EGFR signaling.

The invention also relates to a method for predicting the resistance of a patient suffering from a cancer to a given therapy, comprising:

- a) a step of detecting the phosphorylation of at least one of the residues Y232 and Y291 of TNFRSF6, in a biological sample of said patient;
- b) comparing the phosphorylation levels of at least one of the residues Y232 and Y291 of TNFRSF6 obtained in step a) to at least one reference level.

- The invention also relates to a method for predicting the resistance of a patient suffering from a cancer to a given therapy, comprising:

- a) a step of detecting the phosphorylation of the residues Y232 and Y291 of TNFRSF6, in a biological sample of said patient;
- b) comparing the phosphorylation levels of the residues Y232 and Y291 of TNFRSF6 obtained in step a) to their respective reference levels.

5

Preferably, the given therapy is imatinib or cisplatin; in this case, the method is for predicting the resistance of a patient suffering from a cancer to imatinib or to platinum complexes (like cisplatin), respectively.

- 10 Preferably, according to this method, the cancer is colorectal cancer or a leukemia, preferably a chronic myeloid leukemia (CML).

The biological sample used in this method is as described above. Preferably, it is a biopsy, and preferably a biopsy from CML cells.

15

Preferably, step a) of detecting the phosphorylation of the residues Y232 and Y291 of TNFRSF6, in said sample, is performed by immunoblot, immunofluorescence, immunohistochemistry, ELISA, Time-resolved Förster resonance energy transfer, Amplified Luminescent Proximity Homogenous Assay or flow cytometry.

- 20 Preferably, step a) of detecting the phosphorylation of the residues Y232 and Y291 of TNFRSF6 in said samples, is performed respectively thanks to the monoclonal antibody obtainable from the hybridoma deposited at the Collection Nationale de Cultures de Microorganismes on April 17, 2014, under the number CNCM I-4848, or one of its fragments or derivatives, and thanks to the monoclonal antibody obtainable from the
- 25 hybridoma deposited at the Collection Nationale de Cultures de Microorganismes on April 17, 2014, under the number CNCM I-4849, or one of its fragments or derivatives.

Preferably, the present invention relates to a method for predicting the resistance of a patient suffering from a leukemia to imatinib, comprising:

- 30 a) a step of detecting the phosphorylation of the residues Y232 and Y291 of TNFRSF6, in a biological sample of said patient;

b) comparing the phosphorylation levels of the residues Y232 and Y291 of TNFRSF6 obtained in step a) to their respective reference levels, called Reference 3 and Reference 4,

in which :

- 5 - phosphorylation levels of both residues Y232 and Y291 lower than or equal to References 3 and 4 respectively are indicative of sensibility to imatinib, and
- a phosphorylation level of the residue Y232 lower than or equal to Reference 3 and a phosphorylation level of the residue Y291 higher than Reference 4, or a phosphorylation level of the residue Y232 higher than Reference 3 and a
- 10 phosphorylation level of the residue Y291 lower than or equal to Reference 4, or phosphorylation levels of both residues Y232 and Y291 higher than References 3 and 4 respectively, are indicative of resistance to imatinib.

Preferably, the invention provides a method to predict the resistance of a patient suffering CML to imatinib, notably as explained in Figure 7. Preferably, said method

15 comprises the following steps:

Step 1. SDS-PAGE and immunoblotting of tissue lysates

This method is based on immunoblotting lysate from a biopsied cancerous tissue of the patient to be diagnosed (test lysate) and control lysates, which are obtained from

20 biopsied cancerous tissues or cell lines originated from cancerous tissues of patients (preferably at least 3) known to have a sensitive response to imatinib (control samples). The control samples may be obtained prior to the evaluation for the patient to be diagnosed but the preparation of the control lysates as well as the subsequent steps must be carried out at the same time as the test lysate.

- 25 1. Obtain test lysate and control lysates. The lysates are subjected to SDS-PAGE (10% polyacrylamide). Proteins were then transferred on to PVDF membrane using Tris-Glycine buffer (100 mA, overnight at 4°C). Immunoblotting of pY232 and pY291 TNFRSF6 is carried out using the monoclonal anti-pY232 and pY291 TNFRSF6 antibodies. Immunoblotting of the actin, a reference protein, is carried out using a
- 30 polyclonal rabbit anti- β -actin antibody (e.g. from Sigma). Chemiluminescence detection of the immunoblot bands is carried out using an imager (Odyssey FC, LI-Cor Biotechnology, 2 minutes of exposure).

Step 2. Densitometric analysis of immunoblot signals

Perform a densitometric quantification of immunoblot signals of pY232 and pY291 of TNFRSF6 protein bands that correspond to the canonical forms of TNFRSF6 (with the size between 45-56 kDa) and of the reference protein, actin (the size approximately 43 kDa), using Image Studio software Version 2.1 (LI-COR Biotechnology).

Step 3. Calculation of pY-TNFRSF6 signals and diagnosis

1. Calculate the normalized values of pY232 and pY291 of TNFRSF6 of the cancerous tissue from the patient to be tested ($pY232_t^{norm}$ and $pY291_t^{norm}$, respectively), and of the control samples ($pY232_{ct,x}^{norm}$ and $pY291_{ct,x}^{norm}$, respectively), using the signals of actin as the reference using the following equations:

$$pY232_t^{norm} = pY232_t / Actin_t \quad \text{equation 11}$$

$$pY291_t^{norm} = pY291_t / Actin_t \quad \text{equation 12}$$

$$pY232_{ct,x}^{norm} = pY232_{ct,x} / Actin_{ct,x} \quad \text{equation 13}$$

$$pY291_{ct,x}^{norm} = pY291_{ct,x} / Actin_{ct,x} \quad \text{equation 14}$$

where t denotes test lysate; ct, control; x, control sample number (1, 2, or 3, etc. according to the number of control samples used); and norm, normalized.

2. Calculate the median of the normalized values of pY232 and pY291 of the control samples, $pY232_{ct}^{med}$ and $pY291_{ct}^{med}$, respectively.

3. Calculate the standardized pY232 and pY291 values for the test lysate ($pY232_t^s$ and $pY291_t^s$, respectively) and the control lysates ($pY232_{ct,x}^s$ and $pY291_{ct,x}^s$) by taking the

ratio between normalized values of pY232 and pY291 of TNFRSF6 and the median of the normalized values of pY232 and pY291 of the control samples according to the following equations:

$$pY232_t^s = pY232_t^{norm} / pY232_{ct}^{med} \quad \text{equation 15}$$

$$pY291_t^s = pY291_t^{norm} / pY291_{ct}^{med} \quad \text{equation 16}$$

$$pY232_{ct,x}^s = pY232_{ct,x}^{norm} / pY232_{ct}^{med} \quad \text{equation 17}$$

$$pY291_{ct,x}^s = pY291_{ct,x}^{norm} / pY291_{ct}^{med} \quad \text{equation 18}$$

- 5 where s denotes standardized; t, test lysate; ct, control; x, control sample number (1, 2, or 3, etc. according to the number of control samples used); and med, median.
4. Assign Reference 3, which is the reference pY232 ($pY232^{REF}$), to 10% above the median of standardized pY232 value of all the control samples ($pY232_{ct}^{s,med}$), and Reference 4, which is the reference pY291 ($pY291^{REF}$), to 40% above the median of
- 10 standardized pY291 value of all the control samples ($pY291_{ct}^{s,med}$), by using the following equations:

$$\text{Reference 3} = pY232^{REF} = 1.1 \cdot pY232_{ct}^{s,med} \quad \text{equation 19}$$

$$\text{Reference 4} = pY291^{REF} = 1.4 \cdot pY291_{ct}^{s,med} \quad \text{equation 20}$$

where s denotes standardized; ct, control; and med, median.

- 15 5. Perform the diagnosis by comparing the respective standardized pY232 ($pY232_t^s$) and pY291 ($pY291_t^s$) of the test lysate obtained from equations 15 and 16, respectively, in step 3 to Reference 3 and Reference 4, respectively, in which :
- standardized pY232 and pY291 lower than Reference 3 and Reference 4, respectively are indicative of sensibility to imatinib, and
 - 20 - a phosphorylation level of the residue Y232 lower than Reference 3 and a phosphorylation level of the residue Y291 equal to or higher than Reference 4, or a phosphorylation level of the residue Y232 equal to or higher than Reference 3 and a phosphorylation level of the residue Y291 lower than Reference 4, or phosphorylation levels of both residues Y232 and Y291 equal to higher than
 - 25 Reference 3 and Reference 4, respectively, are indicative of resistance to imatinib.

In case of patients having a resistance to imatinib, alternative therapies may be considered.

These alternative therapies may be chemotherapeutic or molecular targeting agents chosen from temozolomide, fotemustine, dacarbazine, fludarabine, gemcitabine, capecitabine, methotrexate, taxol, taxotere, mercaptopurine, thioguanine, hydroxyurea, cytarabine, cyclophosphamide, ifosfamide, nitrosoureas, platinum complexes such as cisplatin, carboplatin and oxaliplatin, mitomycin, dacarbazine, procarbazine, etoposide, teniposide, campathecins, bleomycin, doxorubicin, idarubicin, daunorubicin, dactinomycin, plicamycin, mitoxantrone, L-casparaginase, epirubicin, 5-fluorouracil, taxanes such as docetaxel and paclitaxel, leucovorin, levamisole, irinotecan (CPT-11), SN-38, estramustine, etoposide, nitrogen mustards, BCNU, nitrosoureas such as carmustine and lomustine, vinca alkaloids such as vinblastine, vincristine and vinorelbine, TNFRSF6-targeting agents such as APO010, TNFRSF6L-targeting agents such as APG101, monoclonal antibodies against EGF receptor or VEGF, such as bevacizumab, cetuximab and panitumumab, kinase inhibitors such as dasatinib, nilotinib, topotecan, genistein, erbstatin, lavendustin and also bortezomib (also called PS341, and sold by Millenium Pharmaceuticals under the name Velcade).

Preferably, alternatively, the method of the invention is for predicting the resistance of a patient suffering from a colorectal cancer to platinum complexes, comprising:

- a) a step of detecting the phosphorylation of the residues Y232 of TNFRSF6, in a biological sample of said patient;
- b) comparing the phosphorylation levels of the residues Y232 of TNFRSF6 obtained in step a) to a reference level.

By “platinum complexes”, it is meant cisplatin, carboplatin or oxaliplatin.

The present invention also relates to a method for predicting the resistance of a patient suffering from a colorectal cancer to platinum complexes, comprising:

- a) a step of detecting the phosphorylation of the residue Y232 of TNFRSF6, in a biological sample of said patient;

b) comparing the phosphorylation level of the residue Y232 of TNFRSF6 obtained in step a) to a reference level, called Reference 5,

in which :

- phosphorylation level of residue Y232 equal to or higher than Reference 5 is indicative of sensibility to platinum complexes, and
- a phosphorylation level of the residue Y232 below Reference 5, is indicative of resistance to platinum complexes.

Preferably, the invention provides a method to predict the resistance of a patient suffering colorectal cancer to platinum complexes, preferably cisplatin, notably as explained in Figure 8. Preferably, said method comprises the following steps:

Step 1. SDS-PAGE and immunoblotting of tissue lysates

This method is based on immunoblotting colorectal cancer cell lysate from a biopsied cancerous tissue of the patient to be diagnosed (test lysate) and control lysates (preferably at least 3), which are obtained from biopsied cancerous tissues of patients known to have sensitive response to cisplatin or cisplatin-sensitive colorectal cancer cell lines originated from cancerous tissues of patients. The control samples may be obtained prior to the evaluation for the patient to be diagnosed but the preparation of the control lysates as well as the subsequent steps must be carried out at the same time as the test lysate.

1. Obtain test lysate and control lysates. The lysates are subjected to SDS-PAGE (10% polyacrylamide). Proteins were then transferred on to PVDF membrane using Tris-Glycine buffer (100 mA, overnight at 4°C). Immunoblotting of pY232 TNFRSF6 is carried out using the monoclonal anti-pY232 TNFRSF6 antibody. Immunoblotting of the C-terminus of TNFRSF6 is carried out using a polyclonal rabbit anti-TNFRSF6 antibody (e.g. C20 from Santa Cruz). Chemiluminescence detection of the immunoblot bands is carried out using an imager (Odyssey FC, LI-Cor Biotechnology, 2 minutes of exposure).

Step 2. Densitometric analysis of immunoblot signals

Perform a densitometric quantification of immunoblot signals of pY232 and C-terminus of TNFRSF6 protein bands that correspond to the canonical forms of TNFRSF6 (with

the size between 45-56 kDa) using Image Studio software Version 2.1 (LI-COR Biotechnology).

Step 3. Calculation of pY-TNFRSF6 signals and diagnosis

1. Calculate the normalized values of pY232 TNFRSF6 of the cancerous tissue from the patient to be tested ($pY232_t^{norm}$), and of the control samples ($pY232_{ct,x}^{norm}$) using the corresponding signals of the C-terminus (CTer) TNFRSF6 as the reference according to the following equations:

$$pY232_t^{norm} = pY232_t / CTer_t \quad \text{equation 21}$$

$$pY232_{ct,x}^{norm} = pY232_{ct,x} / CTer_{ct,x} \quad \text{equation 22}$$

- where t denotes test lysate; ct, control; x, control sample number (1, 2, or 3, etc. according to the number of control samples used); and norm, normalized.

2. Calculate the median of the normalized values of pY232 of the control samples, $pY232_{ct}^{med}$.

3. Calculate the standardized pY232 value for the test lysate ($pY232_t^s$) and the control lysates $pY232_{ct,x}^s$ by taking the ratio between normalized values of pY232 TNFRSF6 and the median of the normalized values of pY232 of the control samples according to the following equations:

$$pY232_t^s = pY232_t^{norm} / pY232_{ct}^{med} \quad \text{equation 23}$$

$$pY232_{ct,x}^s = pY232_{ct,x}^{norm} / pY232_{ct}^{med} \quad \text{equation 24}$$

20

where s denotes standardized; t, test lysate; ct, control; x, control sample number (1, 2, or 3, etc. according to the number of control samples used); and med, median.

4. Assign Reference 5, which is the reference pY232 ($pY232^{REF}$), to 90% of the median of standardized pY232 value of all the control samples ($pY232_{ct}^{s,med}$) by using the following equations:

$$\text{Reference 5} = pY232^{REF} = 0.9 \cdot pY232_{ct}^{s,med} \quad \text{equation 25}$$

where s denotes standardized; ct, control; and med, median.

5. Perform the diagnosis by comparing the respective standardized pY232 of the test lysate ($pY232_t^s$) obtained from equation 23 in step 3 to Reference 5, in which :

- a standardized pY232 value of the test lysate greater than or equal to Reference 5 is indicative of the sensitivity to cisplatin, and
- 5 - a standardized pY232 value of the test lysate lower than Reference 5 is indicative of the resistance to cisplatin.

In case of patients having a resistance to platinum complexes, alternative therapies may be considered.

10 These alternative therapies may be chemotherapeutic agents chosen from temozolomide, fotemustine, dacarbazine, fludarabine, gemcitabine, capecitabine, methotrexate, taxol, taxotere, mercaptopurine, thioguanine, hydroxyurea, cytarabine, cyclophosphamide, ifosfamide, nitrosoureas, mitomycin, dacarbazine, procarbazine, etoposide, teniposide, campathecins, bleomycin, doxorubicin, idarubicin, daunorubicin, 15 dactinomycin, plicamycin, mitoxantrone, L-casparaginase, epirubicin, 5-fluorouracil, taxanes such as docetaxel and paclitaxel, leucovorin, levamisole, irinotecan (CPT-11), SN-38, estramustine, etoposide, nitrogen mustards, BCNU, nitrosoureas such as carmustine and lomustine, vinca alkaloids such as vinblastine, vincristine and vinorelbine, TNFRSF6-targeting agents such as APO010, TNFRSF6L-targeting agents 20 such as APG101, monoclonal antibodies against EGF receptor or VEGF, such as bevacizumab, cetuximab and panitumumab, kinase inhibitors such as imatinib mesylate, dasatinib, nilotinib, topotecan, genistein, erbstatin, lavendustin and also bortezomib (also called PS341, and sold by Millenium Pharmaceuticals under the name Velcade).

25 The invention will now be illustrated thanks to the examples and figures below.

FIGURE LEGENDS

30 **Figure 1: Phosphorylated Y232 and phosphorylated Y291 distinctly indicate different roles of TNFRSF6 in cell survival**

- A.** Phosphorylation of TNFRSF6 at Y232, but not at Y291, is important for the increase in entry of G2/M phase of the cell cycle that is caused by an increase in TNFRSF6 expression. Cell cycle of SW480 cells stably overexpressing control protein (LacZ) or TNFRSF6 protein (wild type, Y232F, or Y291F mutant) was analyzed based on DNA content by flow cytometry. Cells expressing equivalent levels of wild type and dephosphorylated mutant TNFRSF6 were compared. Note the reduction in cells in G2/M cell cycle phase when pY232 of TNFRSF6 was prevented by Y232F mutation.
- B.** Phosphorylation of TNFRSF6 at Y232 but not at Y291 is essential for the activation of cell proliferation induced by EGFR upon the activation with amphiregulin. SW480 cells stably overexpressing control protein (LacZ) or TNFRSF6 protein (wild type, Y232F, or Y291F mutant) were treated with indicated concentration of amphiregulin (ng/ml) for 48 h. The increase in cell viability compared to control cells (untreated) was then quantified using WST-1 assay. Note that CRC cell proliferation triggered by amphiregulin was inhibited when pY232 of TNFRSF6 was prevented by Y232F mutation.
- C.** Phosphorylation of TNFRSF6 at Y291 but not at Y232 is essential for the activation of STAT3 upon the activation EGFR with EGF. SW480 cells stably overexpressing control protein (LacZ) or TNFRSF6 protein (wild type, Y232F, or Y291F mutant) were treated with indicated concentration of EGF at indicated doses for 5 minutes. Cell lysates were collected and subjected to SDS-PAGE and immunoblotting with indicated antibodies. Note that the activation of STAT3 signaling, as observed by its phosphorylation, was increased when Y291 of TNFRSF6 was in the state that mimicked pY291 (Y291D mutation). On the other hand STAT3 activation was inhibited when pY291 of TNFRSF6 was prevented by Y291F mutation.
- D.** In cells whose TNFRSF6 ligand-induced apoptosis is inhibited due to their expression of TNFRSF6 with Y291 constitutively in the phosphorylated state (as mimicked by Y291D mutation), the inhibition of STAT3 using a STAT3-specific inhibitor can partially restore their TNFRSF6 ligand-induced apoptosis. The combination of STAT3 inhibition and TNFRSF6 ligand-induced apoptosis synergistically and significantly causes CRC cell death. SW480 cells stably overexpressing control protein (LacZ) or TNFRSF6 protein (wild type, Y232F, or Y291F mutant) were incubated with STAT3 inhibitor, Stattic, for 30 minutes at 10 ng/ml

before the treatment with TNFRSF6L at 10 ng/ml for 24h. Cell viability was then assessed by WST-1 assay.

Figure 2: Differential levels of pY232 and pY291 TNFRSF6 correlate to imatinib resistance in chronic myeloid leukemia (CML)

A. Apoptosis of CML cells, AR230, induced by crosslinked TNFRSF6 ligand (TNFRSFL, 25 ng/ml) was assessed based on the quantification of cells having DNA content at sub-G1 level by FACS analysis. Note that the CML cells were resistant to TNFRSF6L-induced apoptosis.

B. TNFRSF6 is a factor involved in the level of sensitivity of CML cells to imatinib. Level of apoptosis of AR230 cells infected with control plasmid or short-hairpin RNAi against TNFRSF6 (sh-TNFRSF6) induced by imatinib (1 μ M) was analysed. Note that the suppression of TNFRSF6 by RNAi increased the sensitivity of the CML cells to imatinib.

C. The levels of tyrosine phosphorylation of TNFRSF6 is indicative of the resistance of CML cells to imatinib. Lysate from different CML cells lines, sensitive (S, parental) or resistant (R) to imatinib were subjected to SDS-PAGE and immunoblotting followed by densitometric analysis of the intensity of pY232 and pY291 of TNFRSF6 and actin. The intensity values of pY232 and pY291 TNFRSF6 were normalized by actin intensity and the normalized levels of pY232 and pY291 TNFRSF6 are presented as folds compared to parental cells. Note that all imatinib-resistant CML cells had a higher level(s) of pY-TNFRSF6 at Y232 and/or Y291 when compared to their corresponding sensitive CML cells.

Figure 3: Levels of pY232 TNFRSF6 correlate to cisplatin resistance in colorectal cancer cells

Cisplatin-resistant CRC cells have a reduced level of pY232 TNFRSF6 when compared to cisplatin-sensitive cells. Lysates from different CRC cell lines, sensitive (S, parental) or resistant (R) to cisplatin were subjected to SDS-PAGE and immunoblotting followed by densitometric analysis of the intensity of pY232 and pY291 of TNFRSF6 and TNFRSF6. The intensity values of pY232 and pY291 TNFRSF6 were normalized by

the TNFRSF6 intensity and the normalized levels of pY232 and pY291 TNFRSF6 are presented as folds compared to parental cells.

Figure 4: Phosphorylation at Y232 and Y291 correlates to activation of proteins in survival pathways

Activating proliferative signals by TNFRSF6L or EGF in SW480 cells leads to an activation TNFRSF6 as seen by its increased pY232 and pY291 levels along with the activation of EGFR survival signaling as seen by a significant increase in EGFR, Erk and/or Akt phosphorylation. SW480 cells were synchronized by serum deprivation and treated with indicated doses of uncrosslinked soluble TNFRSF6L (A) or EGF (B) for 5 min. Cell lysates were collected in hot SDS buffer and subjected to SDS-PAGE and immunoblotting with indicated antibodies.

Figure 5: pY232 and pY291 TNFRSF6 can be detected in normal and cancerous tissues

Phosphorylation of TNFRSF6 at Y232 and Y291 can be detected in human normal (N) and cancerous (T) tissues from colon, breast, and lungs. Tissue lysates from patients suffering from colon, breast, or lung cancer were subjected to SDS-PAGE followed by immunoblotting with indicated antibodies.

Figure 6: A method for tumor stratification based on the levels of pY232 and pY291 TNFRSF6 detected by the anti-pY232 and anti-pY291 monoclonal antibodies

A. Schematic diagram of the method for tumor stratification based on the detection and scoring of pY232 and pY291 TNFRSF6. Colon tumor tissue lysates were collected in modified RIPA buffer and subjected to immunoblotting with anti-pY232, anti-pY291 TNFRSF6 and C-terminus of TNFRSF6 antibodies. The intensity of pY232 and pY291 TNFRSF6 bands (43-56 kDa) and C-terminus of TNFRSF6 band (43-56 kDa) were analysed by densitometric analysis. The pY232 and pY291 signals normalized with the signal of C-terminus of TNFRSF6 were scored (see method). The tumors were then classified into groups based on their pY232 and pY291 scoring.

B. Table 1, stratification of 12 different colon cancer patients based on the pY232 and pY291 TNFRSF6 scores of their tumors. Table 2, stratification of 10 different breast cancer patients based on the pY232 and pY291 TNFRSF6 scores of their tumors. TNM, TNM Classification of malignant tumors.

5

Figure 7: A method for predicting sensitivity of CML cells toward imatinib based on the levels of pY232 and pY291 TNFRSF6 detected by anti-pY232 and anti-pY291 TNFRSF6 antibodies

A. Schematic diagram of the method for CML cells stratification based on the detection and scoring of pY232 and pY291 TNFRSF6. CML cell lysates were collected in lysis buffer and subjected to immunoblotting with anti-pY232, anti-pY291 TNFRSF6 and anti-actin antibodies. The intensity of pY232 and pY291 TNFRSF6 bands (43-56 kDa) and actin band (43 kDa) were analysed by densitometric analysis. The pY232 and pY291 signals normalized with the signal of actin were scored (see method). The tumors were then classified into groups based on their pY232 and pY291 scoring. The control lysates 1, 2 and 3 were obtained from imatinib-sensitive cells (AR230, K562, and LAMA84 cell lines, respectively) obtained from CML patients. The example test lysate shown was obtained from LAMA84 cells with known imatinib resistance (LAMA84R, R denotes resistance to imatinib).

20

B. Table 1, stratification of 3 imatinib-sensitive and 3 imatinib-resistant cells based on the pY232 and pY291 TNFRSF6 scores to predict sensitivity/resistance to imatinib (see method). Test lysates 1, 2, and 3 were obtained from imatinib-sensitive cells (AR230, K562, and LAMA84 cell lines, respectively) obtained from CML patients. Test lysates 4, 5, and 6 were obtained from imatinib-resistant cells (AR230R, K562R, and LAMA84R cell lines, respectively) obtained from the parental cells (AR230, K562, and LAMA84 cell lines) whose imatinib resistance was selected based on their ability to survive permanent imatinib treatment.

Figure 8: A method for predicting sensitivity of colon cancer cells toward cisplatin based on the level of pY232 TNFRSF6 detected by anti-pY232 and anti-pY291 TNFRSF6 antibodies

30

A. Schematic diagram of the method for colon cancer cells stratification based on the detection and scoring of pY232 TNFRSF6. Colon tumor cell lysates were collected in lysis buffer and subjected to immunoblotting with anti-pY232 TNFRSF6 and anti-C-terminus TNFRSF6 antibodies. The intensity of pY232 bands (43-56 kDa) and C-terminus of TNFRSF6 bands (43-56 kDa) were analysed by densitometric analysis. The pY232 signals normalized with the signal of C-terminus of TNFRSF6 were scored (see method). The tumors were then classified into groups based on their pY232 scoring. The control lysates 1, 2, and 3 were obtained from cisplatin-sensitive colon cancer cells (HT29, SW480, and HCT15 cell lines, respectively) obtained from colon cancer patients. The example test lysate shown was obtained from SW480 cells with known cisplatin resistance (SW480R, R denotes resistance to cisplatin).

B. Table 1, identification of cisplatin-sensitive and cisplatin-resistant cell lines based on the pY232 TNFRSF6 scores to predict sensitivity to cisplatin (see method) Test lysates 1, 2, and 3 were obtained from cisplatin-sensitive cells (HT29, SW480, and HCT15 cells, respectively) obtained from colon cancer patients. Test lysates 4, 5, and 6 were obtained from cisplatin-resistant cells (HT29R, SW480R, and HCT15R cells, respectively) obtained from the parental cells (HT29, SW480, and HCT15 cells, respectively) whose cisplatin resistance was selected based on their ability to survive permanent cisplatin treatment.

Example:

MATERIALS AND METHODS

Cell lines

Colon cell lines SW480 were purchased from the American Type Culture Collection (ATCC), HT29 and HCT15 from Leibniz-Institut, German collection of microorganisms and cell culture (DSMZ). The cells were kept in PRMI 1640 supplemented with 10% fetal bovine serum (FBS) and maintained at 37°C, 5% CO₂. Unless otherwise stated, stable cell lines were established by transducing cells with

lentivirus vector (pLenti6) expressing C-terminally V5-tagged wild type TNFRSF6 protein or TNFRSF6 carrying one of the following mutations: Y232F, Y291D, and Y291F. As controls, cells were transduced with pLenti6-LacZ.V5. Cells stably expressing the proteins of interest were selected by blasticidin resistance.

5

Cell lysate preparation for the detection of pY232 and pY291 TNFRSF6 by immunoblotting

- Cell lines: Cells were seeded in 10-cm dishes at 4×10^6 cells/dish (colon cell lines) or seeded at 10^6 cells/ml in T25 flask (CML cells) for 24h. Cells were collected in the lysis buffer (120 mM Tris-HCl pH 6.8, 4% SDS, 10 mM NaP-P, 10 mM NaF, 25 mM β -glycerol phosphate, 5 mM Na_2VO_4 , protease inhibitor cocktail), preheated at 95°C ('hot SDS' buffer). The lysate was stored at -80°C until subjected to SDS-PAGE and immunoblotting by indicated antibodies.
- Normal and tumor tissues: The normal and tumor tissue lysates were obtained from Protein Biotechnologies (USA). They were prepared by homogenizing tissue specimens in modified RIPA buffer (PBS, pH7.4, 1 $\mu\text{g/ml}$ aprotinin, 1 $\mu\text{g/ml}$ pepstatin, 1 $\mu\text{g/ml}$ leupeptin, mM NaF, 1 mM EDTA, 1 mM PMSF, 0.1% SDS, 0.25% Na deoxycholate, 1 mM Na_2VO_4) to obtain the soluble proteins and centrifuging to clarify. The lysate were stored at -80°C until subjected to SDS-PAGE and immunoblotting by indicated antibodies.

10

15

20

DNA quantification assay for cell cycle analyses

- FACS-based DNA quantification method was used to analyze the cell cycle phases as well as apoptosis (based on DNA fragmentation by quantifying sub-G1 cell population): Cells were plated in RPMI+10% FBS at 5×10^5 cells/well in 6-well plate 24 h before the treatment. Cell death triggered by crosslinked-TNFRSF6L was done by incubating cells with Flag-tagged recombinant human TNFRSF6L (rhTNFRSF6L; Alexis) plus 1 $\mu\text{g/ml}$ anti-Flag Ab (M2), at 37°C , 5% CO_2 and incubated for a specified time. Floating cells were collected and adherent cells were detached by trypsinization. Both cell populations were pooled and collected following centrifugation. Cells were then fixed with 70% ethanol (-20°C), washed in 38 mM sodium citrate (pH 7.4), stained with

25

30

propidium iodide with RNase A, and analyzed with a flow cytometer (LSRFortessa or FACSCalibur, Becton Dickinson). For apoptosis analysis the proportion of apoptotic cells represented by the subG1 peak was determined and represented as percent of cell death[36]. For cell cycle analysis, given that the fluorescence of cells in the G₂/M phase was twice as high as that of cells in the G₀/G₁ phase, the relative amount of cells in the G₀ phase and G₁ phase (before S phase), in the S phase, and in the G₂ phase and M phase (after S phase) was determined.

- WST-1 Viability assay: For cell death assessment, cells were seeded in RPMI+10% FCS at 10⁴ cells/well in 96-well plate 24 h before the treatment. Cell death triggered by crosslinked-TNFRSF6 ligand (TNFRSF6L) was done by incubating cells with Flag-tagged recombinant human TNFRSF6L (Alexis) plus 1 µg/ml anti-Flag antibody (M2), at 37°C, 5% CO₂ and incubated for 24h. Where indicated, cells were pretreated with inhibitors 30 minutes prior to the addition of TNFRSF6L. Following the incubation WST-1 reagent was added to each well. Cells were incubated for 4h before measurement at 450 nm and 690 nm (reference) with a spectrometer (Biotek). After subtracting blank control, absorbance at 690 nm was subtracted from that obtained at 450 nm. Viability of the cells was calculate as percentage of cell viability compared to control (untreated cells) using the following equation: % Viability = 100x (A₄₅₀-A₆₉₀)_{treated}/(A₄₅₀-A₆₉₀)_{control}, A, absorbance.

RESULTS

25

Phosphorylated Y232 and phosphorylated Y291 distinctly indicate different roles of TNFRSF6 in cell survival

1. pY232 TNFRSF6, but not pY291, is important for the increase in the entry to G₂/M phase of cell cycle caused by the increase in expression of TNFRSF6, as demonstrated in Figure 1A that this effect was lost in cells carrying unphosphorylable Y232 mutant (Y232F, upper panel) but not in cells carrying unphosphorylable Y291 mutant (Y291F, lower panel).

2. pY232 TNFRSF6, but not pY291, is important for cell proliferation triggered by amphiregulin (a ligand of EGFR), as demonstrated in Figure 1B that amphiregulin-induced proliferation was only inhibited in cells carrying Y232F mutant but not Y291F mutant.

5 3. pY291 TNFRSF6, but not pY232, is important for the activation of STAT3 transcription factor which is triggered by EGF (a ligand of EGFR), as demonstrated in Figure 1C.

4. The cellular protection from TNFRSF6 ligand induced apoptosis conferred by constitutive phosphorylation of Y291 (as simulated by Y291D mutation which is the proxy for pY291) was reversed by inhibiting STAT3 activity using a STAT3 inhibitor, STATIC (Figure 1D).

These data demonstrate that pY232 and pY291 play different roles in cancer cell survival and their differential levels can be used to indicate different situation in the tumors:

1. Elevated pY232 is indicative of cell survival advantage conferred by signaling pathways that involve cell cycle entry and amphiregulin-dependent EGFR proliferative signaling;

2. Elevated pY291 is indicative of cell survival advantage conferred by signaling pathways that involve STAT3 mediated EGF-dependent EGFR proliferative signaling and the inhibition of STAT3 pathway can synergize with the activation of TNFRSF6 apoptotic signaling in cancer that exhibits elevated pY291 TNFRSF6.

This emphasizes the importance of differential measurement of pY232 and pY291 in the diagnosis of cancers that are influenced by TNFRSF6 survival signaling, which will thus allows the stratification of patients based on TNFRSF6 different signaling modes.

pY232 and pY291 TNFRSF6 can be detected in normal and cancerous tissues As shown in Figure 5, phosphorylation of TNFRSF6 at Y232 and Y291 can be detected in human normal (N) and cancerous (T) tissues of colon, breast and lung.

Method to diagnose colon and breast cancer based on TNFRSF6 survival signaling prediction using differential levels of pY232 and pY291 TNFRSF6

Summary of the following method is found in Figure 6.

Step 1. SDS-PAGE and immunoblotting of tissue lysates

This method is based on immunoblotting lysates of biopsies of a cancerous tissue and a normal (non-cancerous) tissue of the same organ of a patient. The tissue lysates are
 5 subjected to SDS-PAGE (10% polyacrylamide). Proteins were then transferred on to PVDF membrane using Tris-Glycine buffer (100 mA, overnight at 4C). Immunoblotting of pY232 and pY291 TNFRSF6 is carried out using the monoclonal anti-pY232 and pY291 TNFRSF6 antibodies. Immunoblotting of the C-terminus of TNFRSF6 is carried out using a polyclonal rabbit anti-TNFRSF6 antibody (e.g. C20
 10 from Santa Cruz). Chemiluminescence detection of the immunoblot bands is carried out using an imager (Odyssey FC, LI-Cor Biotechnology, 2 minutes of exposure).

Step 2. Densitometric analysis of immunoblot signals

Perform a densitometric quantification of immunoblot signals of pY232, pY291 and C-terminus of TNFRSF6 protein bands that correspond to the canonical forms of
 15 TNFRSF6 (with the size between 45-56 kDa) using Image Studio software Version 2.1 (LI-COR Biotechnology).

Step 3. Calculation of pY-TNFRSF6 signals and diagnosis

1. Calculate the normalized values of pY232 and pY291 of TNFRSF6 of the cancerous tissue ($pY232_c^{norm}$ and $pY291_c^{norm}$, respectively) and the normalized values of pY232
 20 and pY291 of TNFRSF6 the non-cancerous tissue ($pY232_{nc}^{norm}$ and $pY291_{nc}^{norm}$, respectively), using the corresponding signals of the C-terminus (CTer) TNFRSF6 as the reference according to the following equations:

$$pY232_c^{norm} = pY232_c / CTer_c \quad \text{equation 1}$$

$$pY291_c^{norm} = pY291_c / CTer_c \quad \text{equation 2}$$

$$25 \quad pY232_{nc}^{norm} = pY232_{nc} / CTer_{nc} \quad \text{equation 3}$$

$$pY291_{nc}^{norm} = pY291_{nc} / CTer_{nc} \quad \text{equation 4}$$

, where c denotes cancerous; nc, non-cancerous; and norm, normalized.

2. From the normalized values pY232 and pY291 of cancerous and non-cancerous tissues, calculate the standardized pY232 ($pY232_c^s$) and pY291 ($pY291_c^s$) values for the cancerous tissue by taking the ratio between the normalized pY232 and pY291 of the cancerous tissue and the normalized pY232 and pY291 of the normal tissue, respectively according to the following equations:

$$pY232_c^s = pY232_c^{norm} / pY232_{nc}^{norm} \quad \text{equation 5}$$

$$pY291_c^s = pY291_c^{norm} / pY291_{nc}^{norm} \quad \text{equation 6}$$

, where s denotes standardized.

- In such manner, the standardized values of pY232 ($pY232_{nc}^s$) and pY291 ($pY291_{nc}^s$) of the normal tissue are the ratios between the normalized pY232 and pY291 of the normal tissue and the normalized pY232 and pY291 of the normal tissue itself, respectively, and are equal to 1, as shown in the following equations:

$$pY232_{nc}^s = pY232_{nc}^{norm} / pY232_{nc}^{norm} = 1 \quad \text{equation 7}$$

$$pY291_{nc}^s = pY291_{nc}^{norm} / pY291_{nc}^{norm} = 1 \quad \text{equation 8}$$

3. Assign Reference 1 and Reference 2 values, which are the reference pY232 ($pY232^{ref}$) and pY291 ($pY291^{ref}$) values, respectively, to 10% above the corresponding standardized values of pY232 and pY291 of the normal tissue. Thus, Reference 1 and Reference 2 values are both equal to 1.1, as shown in the following equations:

$$\text{Reference 1} = pY232^{ref} = 1.1 \cdot pY232_{nc}^s = 1.1 \quad \text{equation 9}$$

$$\text{Reference 2} = pY291^{ref} = 1.1 \cdot pY291_{nc}^s = 1.1 \quad \text{equation 10}$$

where ref denotes reference for TNFRSF6 signaling mode prediction.

- The level of pY232 or pY291 of TNFRSF6 is considered low when the standardized pY232 or pY291 of TNFRSF6 value is below the Reference 1 or Reference 2, respectively. The level of pY232 or pY291 of TNFRSF6 is considered high when the

standardized pY232 or pY291 of TNFRSF6 value is equal to or above the Reference 1 or Reference 2, respectively.

Based on the assigned 'high' and 'low' pYTNFRSF6 levels, the tumors were classified into one of the following 4 groups:

- Group I Low pY232 and low pY291. A patient with tumor classified to this group is diagnosed as having cancer with low influence of TNFRSF6 survival signal. The patient may be a candidate for TNFRSF6-targeting anticancer drugs that aim to activate TNFRSF6-induced cell death signaling in order to trigger cancer cell apoptosis.
- Group II Low pY232 and high pY291. A patient with tumor classified to this group is diagnosed as having cancer with high TNFRSF6 survival signal which involves STAT3-mediated EGF-dependent EGFR survival signaling. The patient may be a candidate for TNFRSF6 ligand-targeting anticancer drugs that aim to inhibit TNFRSF6-induced cell survival signaling in order to prevent TNFRSF6-mediated cancer cell survival and proliferation. Alternatively, for this patient, a regime that involves inhibition of STAT3-mediated EGF-dependent EGFR signaling may also be considered in combination with TNFRSF6-targeting anticancer drugs that aim to activate TNFRSF6-induced cell death signaling in order to activate TNFRSF6-mediated apoptosis. Since the inhibition of STAT3 activity abolish survival signaling conferred by pY291 (Figure 1D), this approach leads to a synergic increase in cancer cell death.
- Group III High pY232 and low pY291. Since Phosphorylation of TNFRSF6 at Y232 is essential for G2/M phase cell cycle entry and the activation of cell proliferation induced by EGFR upon the activation with amphiregulin, a patient with tumor classified to this group is diagnosed as having cancer with high TNFRSF6 survival signal which mediates G2/M phase cell cycle entry and amphiregulin-dependent EGFR survival signaling. The patient may be a candidate for TNFRSF6 ligand-targeting anticancer drugs that aim to inhibit TNFRSF6-induced cell survival signaling in order to prevent TNFRSF6-mediated cancer cell survival and proliferation. In this patient, a regime that

targets cell cycle and/or involves inhibition of amphiregulin-dependent EGFR signaling may also be considered.

- *Group IV* High pY232 and high pY291. A patient with tumor classified to this group is diagnosed as having cancer with high TNFRSF6 survival signal which mediates G2/M phase cell cycle entry, STAT3-mediated EGF-dependent, and amphiregulin-dependent EGFR survival signaling. The patient may be a candidate for TNFRSF6 ligand-targeting anticancer drugs that aim to inhibit TNFRSF6-induced cell survival signaling in order to prevent TNFRSF6-mediated cancer cell survival and proliferation. In this patient, a regime that targets cell cycle and/or involves inhibition of amphiregulin-dependent EGFR signaling, or the combination of a drug inhibiting STAT3-mediated, EGF-dependent EGFR signaling with a TNFRSF6-targeting anticancer drug activating TNFRSF6-induced cell death signaling may also be considered.

Stratifications were obtained for colon cancer (Figure 6B, Table 1), and for breast cancer (Figure 6B, Table 2).

Method to diagnose imatinib resistance in CML based on differential levels of pY232 and pY291 TNFRSF6

Summary of the following method is found in Figure 7.

Step 1. SDS-PAGE and immunoblotting of tissue lysates

This method is based on immunoblotting lysate from a biopsied cancerous tissue of the patient to be diagnose (test lysate) and control lysates, which are obtained from biopsied cancerous tissues or cell lines originated from cancerous tissues of patients (preferably at least 3) known have sensitive response to imatinib (control samples). The control samples may be obtained prior to the evaluation for the patient to be diagnose but the preparation of the control lysates as well as the subsequent steps must be carried out at the same time as the test lysate.

1. Obtain lysate of the cancerous tissue of the patient (test lysate) and control lysates. The tissue lysates are subjected to SDS-PAGE (10% polyacrylamide). Proteins were then transferred on to PVDF membrane using Tris-Glycine buffer (100 mA, overnight at 4C). Immunoblotting of pY232 and pY291 TNFRSF6 is carried out using the monoclonal anti-pY232 and pY291 TNFRSF6 antibodies. Immunoblotting of the actin,

a reference protein, is carried out using a polyclonal rabbit anti- β -actin antibody (e.g. from Sigma). Chemiluminescence detection of the immunoblot bands is carried out using an imager (Odyssey FC, LI-Cor Biotechnology, 2 minutes of exposure).

Step 2. Densitometric analysis of immunoblot signals

- 5 Perform a densitometric quantification of immunoblot signals of pY232 and pY291 of TNFRSF6 protein bands that correspond to the canonical forms of TNFRSF6 (with the size between 45-56 kDa) and of the reference protein, actin (the size approximately 43 kDa), using Image Studio software Version 2.1 (LI-COR Biotechnology).

Step 3. Calculation of pY-TNFRSF6 signals and diagnosis

- 10 1. Calculate the normalized values of pY232 and pY291 of TNFRSF6 of the cancerous tissue from the patient to be tested ($pY232_t^{norm}$ and $pY291_t^{norm}$, respectively), and of the control samples ($pY232_{ct,x}^{norm}$ and $pY291_{ct,x}^{norm}$, respectively), using the signals of actin as the reference using the following equations:

$$pY232_t^{norm} = pY232_t / Actin_t \quad \text{equation 11}$$

$$15 \quad pY291_t^{norm} = pY291_t / Actin_t \quad \text{equation 12}$$

$$pY232_{ct,x}^{norm} = pY232_{ct,x} / Actin_{ct,x} \quad \text{equation 13}$$

$$pY291_{ct,x}^{norm} = pY291_{ct,x} / Actin_{ct,x} \quad \text{equation 14}$$

where t denotes test lysate; ct, control; x, control sample number (1, 2, or 3, etc. according to the number of control samples used); and norm, normalized.

- 20 2. Calculate the median of the normalized values of pY232 and pY291 of the control samples, $pY232_{ct}^{med}$ and $pY291_{ct}^{med}$, respectively.

3. Calculate the standardized pY232 and pY291 values for the test lysate ($pY232_t^s$ and $pY291_t^s$, respectively) and the control lysates by taking the ratio between normalized values of pY232 and pY291 of TNFRSF6 and the median of the normalized values of pY232 and pY291 of the control samples according to the following equations:

$$25 \quad pY232_t^s = pY232_t^{norm} / pY232_{ct}^{med} \quad \text{equation 15}$$

$$pY291_t^s = pY291_t^{norm} / pY291_{ct}^{med} \quad \text{equation 16}$$

$$pY232_{ct,x}^s = pY232_{ct,x}^{norm} / pY232_{ct}^{med} \quad \text{equation 17}$$

$$pY291_{ct,x}^s = pY291_{ct,x}^{norm} / pY291_{ct}^{med} \quad \text{equation 18}$$

where s denotes standardized; t, test lysate; ct, control; x, control sample number (1, 2,
5 or 3, etc. according to the number of control samples used); and med, median.

4. Assign Reference 3, which is the reference pY232 ($pY232^{REF}$), to 10% above the
median of standardized pY232 value of all the control samples ($pY232_{ct}^{s,med}$), and
Reference 4, which is the reference pY291 ($pY291^{REF}$), to 40% above the median of
standardized pY291 value of all the control samples ($pY291_{ct}^{s,med}$), by using the

10 following equations:

$$\text{Reference 3} = pY232^{REF} = 1.1 \cdot pY232_{ct}^{s,med} \quad \text{equation 19}$$

$$\text{Reference 4} = pY291^{REF} = 1.4 \cdot pY291_{ct}^{s,med} \quad \text{equation 20}$$

where s denotes standardized; ct, control; and med, median.

5. Perform the diagnosis by comparing the respective standardized pY232 ($pY232_t^s$)

15 and pY291 ($pY291_t^s$) of the test lysate obtained in step 3, from equations 15 and 16,

respectively, to Reference 3 and Reference 4, respectively.

The level of pY232 or pY291 of TNFRSF6 is considered low when the standardized
pY232 or pY291 of TNFRSF6 value is below the Reference 3 or Reference 4,
respectively. The level of pY232 or pY291 of TNFRSF6 is considered high when the
20 standardized pY232 or pY291 of TNFRSF6 value is equal to or above the Reference 3
or Reference 4, respectively.

Based on the assigned 'high' and 'low' pYTNFRSF6 levels, the tested CML samples
were classified into one of the following 2 groups:

Group I Low pY232 and low pY291. A patient with CML sample classified to this
25 group is diagnosed as having CML that is sensitive to imatinib, and

Group II Low pY232 and high pY291, or high pY232 and low pY291, or high pY232 and high pY291. A patient with CML sample classified to this group is diagnosed as having CML that is resistant to imatinib.

- 5 Classification of sensitivity toward imatinib was obtained for CML cells based on the levels of pY232 and pY291 of TNFRSF6 (Figure 7B, Table 1)

Method to diagnose cisplatin resistance in colorectal cancer (CRC) based on differential level of pY232

- 10 Summary of the following method is found in Figure 8.

Step 1. SDS-PAGE and immunoblotting of tissue lysates

- This method is based on immunoblotting colorectal cancer cell lysate from a biopsied cancerous tissue of the patient to be diagnosed (test lysate) and control lysates (preferably at least 3), which are obtained from biopsied cancerous tissues of patients
15 known to have sensitive response to cisplatin or cisplatin-sensitive colorectal cancer cell lines originated from cancerous tissues of patients . The control samples may be obtained prior to the evaluation for the patient to be diagnosed but the preparation of the control lysates as well as the subsequent steps must be carried out at the same time as the test lysate.

- 20 1. Obtain test lysate and control lysates. The lysates are subjected to SDS-PAGE (10% polyacrylamide). Proteins were then transferred on to PVDF membrane using Tris-Glycine buffer (100 mA, overnight at 4C). Immunoblotting of pY232 TNFRSF6 is carried out using the monoclonal anti-pY232 TNFRSF6 antibody. Immunoblotting of the C-terminus of TNFRSF6 is carried out using a polyclonal rabbit anti-TNFRSF6
25 antibody (e.g. C20 from Santa Cruz). Chemiluminescence detection of the immunoblot bands is carried out using an imager (Odyssey FC, LI-Cor Biotechnology, 2 minutes of exposure).

Step 2. Densitometric analysis of immunoblot signals

- Perform a densitometric quantification of immunoblot signals of pY232 and C-terminus
30 of TNFRSF6 protein bands that correspond to the canonical forms of TNFRSF6 (with the size between 45-56 kDa) using Image Studio software Version 2.1 (LI-COR Biotechnology).

Step 3. Calculation of pY-TNFRSF6 signals and diagnosis

1. Calculate the normalized values of pY232 TNFRSF6 of the cancerous tissue from the patient to be tested ($pY232_t^{norm}$), and of the control samples ($pY232_{ct,x}^{norm}$) using the corresponding signals of the C-terminus (CTer) TNFRSF6 as the reference according to the following equations:

$$pY232_t^{norm} = pY232_t / CTer_t \quad \text{equation 21}$$

$$pY232_{ct,x}^{norm} = pY232_{ct,x} / CTer_{ct,x} \quad \text{equation 22}$$

where t denotes test lysate; ct, control; x, control sample number (1, 2, or 3, etc. according to the number of control samples used); and norm, normalized.

2. Calculate the median of the normalized values of pY232 of the control samples, $pY232_{ct}^{med}$.

3. Calculate the standardized pY232 value for the test lysate ($pY232_t^s$) and the control lysates $pY232_{ct,x}^s$ by taking the ratio between normalized values of pY232 TNFRSF6 and the median of the normalized values of pY232 of the control samples according to the following equations:

$$pY232_t^s = pY232_t^{norm} / pY232_{ct}^{med} \quad \text{equation 23}$$

$$pY232_{ct,x}^s = pY232_{ct,x}^{norm} / pY232_{ct}^{med} \quad \text{equation 24}$$

where s denotes standardized; t, test lysate; ct, control; x, control sample number (1, 2, or 3, etc. according to the number of control samples used); and med, median.

4. Assign Reference 5, which is the reference pY232 ($pY232^{REF}$), to 90% of the median of standardized pY232 value of all the control samples ($pY232_{ct}^{s,med}$) by using the following equations:

$$\text{Reference 5} = pY232^{REF} = 0.9 \cdot pY232_{ct}^{s,med} \quad \text{equation 25}$$

where s denotes standardized; ct, control; and med, median.

5. Perform the diagnosis by comparing the respective standardized pY232 ($pY232_t^s$) of the test lysate obtained in step 3, from equation 23, to Reference 5.

The level of pY232 of TNFRSF6 is considered low when the standardized pY232 of TNFRSF6 value is below the Reference 5. The level of pY232 is considered high when the standardized pY232 of TNFRSF6 value is equal to or above the Reference 5.

Based on the assigned 'high' and 'low' pY232 of TNFRSF6 level, the tested CRC samples were classified into one of the following 2 groups:

10 Group I High pY232. A patient with tumor classified to this group is diagnosed as having cancer with sensitivity to cisplatin.

Group II Low pY232 A patient with tumor classified to this group is diagnosed as having cancer with resistance to cisplatin.

15 Classification of sensitivity toward cisplatin was obtained for CRC cells based on the level of pY232 of TNFRSF6 (Figure 8B, Table 1).

CLAIMS

1. Method for screening a patient suffering from a cancer, particularly a TNFRSF6 signaling-influenced cancer, and who might benefit from a given therapy, comprising:

- a) a step of detecting the phosphorylation of the residues Y232 and Y291 of TNFRSF6, in a biological sample of said patient; and
- b) comparing the phosphorylation levels of the residues Y232 and Y291 of TNFRSF6 obtained in step a) to their respective reference levels, called Reference 1 and Reference 2,

in which :

- phosphorylation levels of both residues Y232 and Y291 below References 1 and 2 respectively are indicative of the patient to be a good candidate for TNFRSF6-targeting anticancer drugs that activate TNFRSF6-induced cell death signaling,
- a phosphorylation level of residue Y232 below Reference 1 and a phosphorylation level of residue Y291 equal to or higher than Reference 2 are indicative of the patient to be a good candidate for TNFRSF6- or TNFRSF6 ligand-targeting anticancer drugs that inhibit TNFRSF6-induced cell survival signaling, or for the combination of a drug inhibiting STAT3-mediated EGF-dependent EGFR signaling with a TNFRSF6-targeting anticancer drug activating TNFRSF6-induced cell death signaling,
- a phosphorylation level of residue Y232 equal to or higher than Reference 1 and a phosphorylation level of residue Y291 below Reference 2 are indicative of the patient to be a good candidate for TNFRSF6- or TNFRSF6 ligand-targeting anticancer drugs that inhibit TNFRSF6-induced cell survival signaling, or for drugs inhibiting Amphiregulin-dependent EGFR signaling,
- phosphorylation levels of both residues Y232 and Y291 equal to or higher than References 1 and 2 respectively are indicative of the patient to be a good candidate for TNFRSF6- or TNFRSF6 ligand-targeting anticancer drugs that inhibit TNFRSF6-induced cell survival signaling, or for drugs inhibiting Amphiregulin-dependent EGFR signaling, or for the combination of a drug inhibiting STAT3-mediated EGF-dependent EGFR signaling with a TNFRSF6-targeting anticancer drug activating TNFRSF6-induced cell death signaling.

2. Method according to claim 1, characterized in that the cancer is colorectal cancer or breast cancer.

3. Method according to any one of claims 1 to 2, characterized in that the biological sample is a biopsy.

4. Method according to any one of claims 1 to 3, characterized in that step a) of detecting the phosphorylation of the residues Y232 and Y291 of TNFRSF6 in said sample, is performed by immunoblot, immunofluorescence, immunohistochemistry, ELISA, Time-resolved Förster resonance energy transfer, Amplified Luminescent Proximity Homogenous Assay or flow cytometry.

5. Method according to any one of claims 1 to 4, characterized in that step a) of detecting the phosphorylation of the residues Y232 and Y291 of TNFRSF6 in said sample, is performed respectively thanks to the monoclonal antibodies obtainable from the hybridoma deposited at the Collection Nationale de Cultures de Microorganismes on April 17, 2014, under the number CNCM I-4848, or one of its fragments or derivatives, and thanks to the monoclonal antibody obtainable from the hybridoma deposited at the Collection Nationale de Cultures de Microorganismes on April 17, 2014, under the number CNCM I-4849, or one of its fragments or derivatives.

6. Method according to any one of claims 1 to 5, characterized in that Reference 1 and Reference 2 correspond to calculated values of phosphorylation levels of each residue Y232 or Y291 of TNFRSF6, respectively.

7. Method according to any one of claims 1 to 6, characterized in that Reference 1 and 2 are the same and are the score of 1.1, and wherein step a) is performed according to the following process:

- obtaining a lysate of the biological sample of the patient;
- isolating and immunoblotting the proteins of said lysate, thanks to the monoclonal antibodies obtainable from the hybridoma deposited at the Collection Nationale de Cultures de Microorganismes on April 17, 2014, under the number CNCM I-4848, or

one of its fragments or derivatives, and thanks to the monoclonal antibody obtainable from the hybridoma deposited at the Collection Nationale de Cultures de Microorganismes on April 17, 2014, under the number CNCM I-4849, or one of its fragments or derivatives;

- quantifying the immunoblot signal of protein bands corresponding to the canonical forms of TNFRSF6; and
- calculating the tyrosine phosphorylation signals.

8. Method for predicting the resistance of a patient suffering from a cancer to a given therapy, comprising:

- a) a step of detecting the phosphorylation of at least one of the residues Y232 and Y291 of TNFRSF6, in a biological sample of said patient;
- b) comparing the phosphorylation levels of at least one of the residues Y232 and Y291 of TNFRSF6 obtained in step a) to at least one reference level.

9. Method according to claim 8, characterized in that the cancer is colorectal cancer or a leukemia, preferably a chronic myeloid leukemia.

10. Method according to any one of claims 8 to 9, characterized in that the biological sample is a biopsy.

11. Method according to any one of claims 8 to 10, characterized in that step a) of detecting the phosphorylation of the residues Y232 and Y291 of TNFRSF6, in said sample, is performed by immunoblot, immunofluorescence, immunohistochemistry, ELISA, Time-resolved Förster resonance energy transfer, Amplified Luminescent Proximity Homogenous Assay or flow cytometry.

12. Method according to any one of claims 8 to 11, characterized in that step a) of detecting the phosphorylation of the residues Y232 and Y291 of TNFRSF6 in said sample, is performed respectively thanks to the monoclonal antibody obtainable from the hybridoma deposited at the Collection Nationale de Cultures de Microorganismes on April 17, 2014, under the number CNCM I-4848, or one of its fragments or derivatives,

and thanks to the monoclonal antibody obtainable from the hybridoma deposited at the Collection Nationale de Cultures de Microorganismes on April 17, 2014, under the number CNCM I-4849, or one of its fragments or derivatives.

13. Method according to any one of claims 8 to 12, characterized in that it is for predicting the resistance of a patient suffering from a leukemia to imatinib, comprising:

- a) a step of detecting the phosphorylation of the residues Y232 and Y291 of TNFRSF6, in a biological sample of said patient;
- b) comparing the phosphorylation levels of the residues Y232 and Y291 of TNFRSF6 obtained in step a) to their respective reference levels, called Reference 3 and Reference 4,

in which :

- phosphorylation levels of both residues Y232 and Y291 less than or equal to References 3 and 4 respectively are indicative of sensibility to imatinib, and
- a phosphorylation level of the residue Y232 less than or equal to Reference 3 and a phosphorylation level of the residue Y291 higher than Reference 4, or a phosphorylation level of the residue Y232 higher than Reference 3 and a phosphorylation level of the residue Y291 less than or equal to Reference 4, or phosphorylation levels of both residues Y232 and Y291 higher than References 3 and 4 respectively, are indicative of resistance to imatinib.

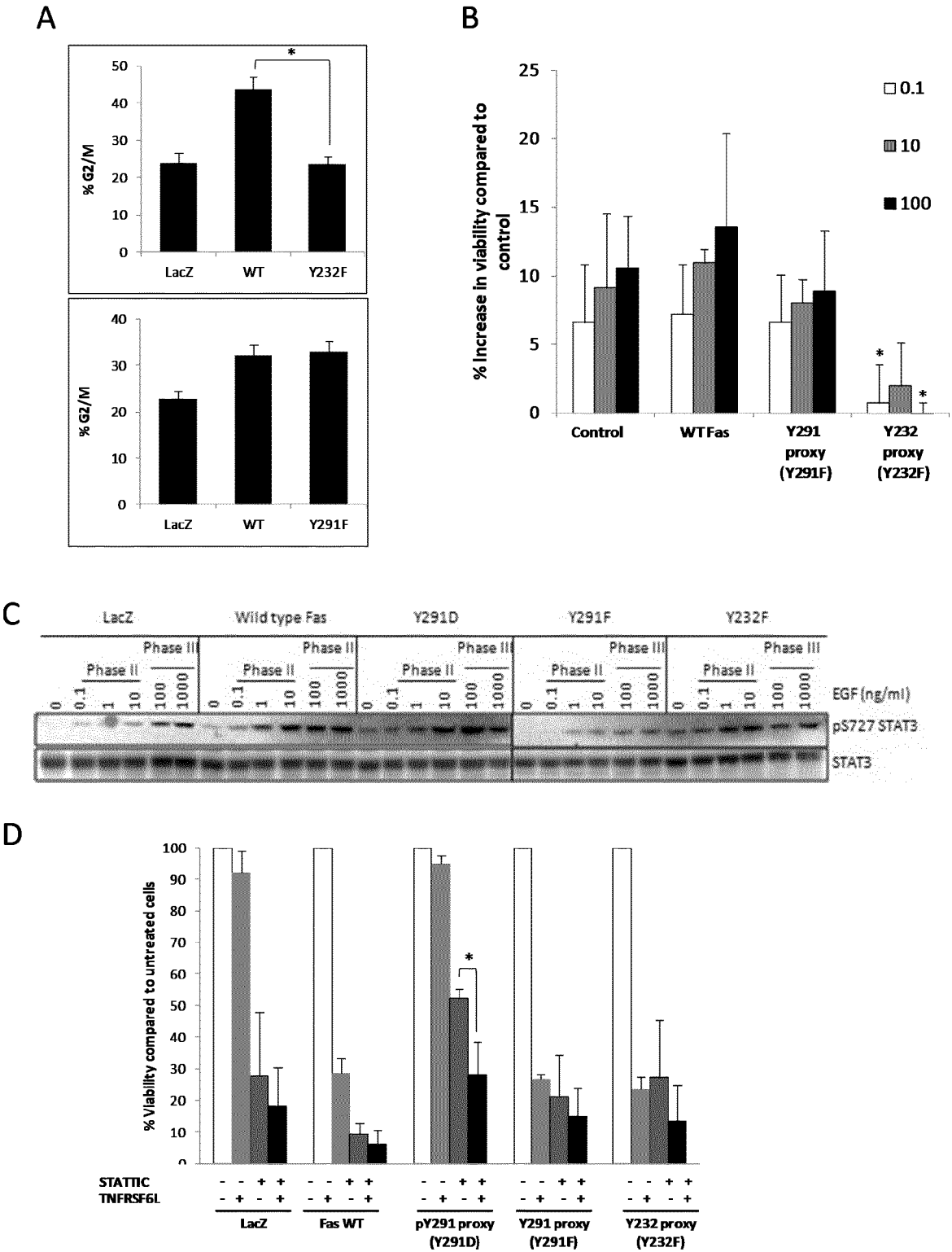
14. Method according to any one of claims 8 to 13, characterized in that it is for predicting the resistance of a patient suffering from a colorectal cancer to platinum complexes, comprising:

- a) a step of detecting the phosphorylation of the residue Y232 of TNFRSF6, in a biological sample of said patient;
- b) comparing the phosphorylation level of the residue Y232 of TNFRSF6 obtained in step a) to a reference level, called Reference 5,

in which :

- phosphorylation level of residue Y232 equal to or higher than Reference 5 is indicative of sensibility to platinum complexes, and

- a phosphorylation level of the residue Y232 below Reference 5, is indicative of resistance to platinum complexes.



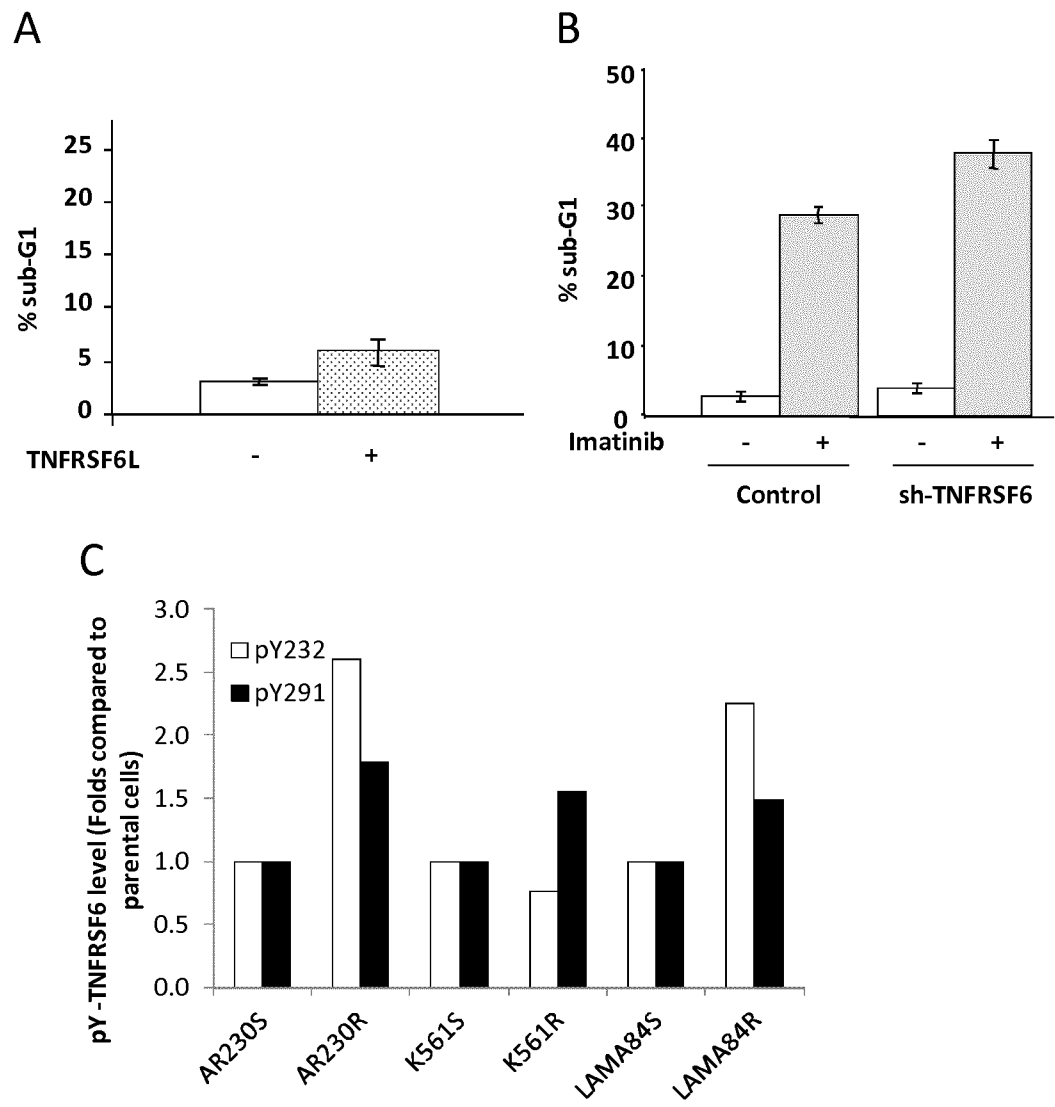


Figure 2

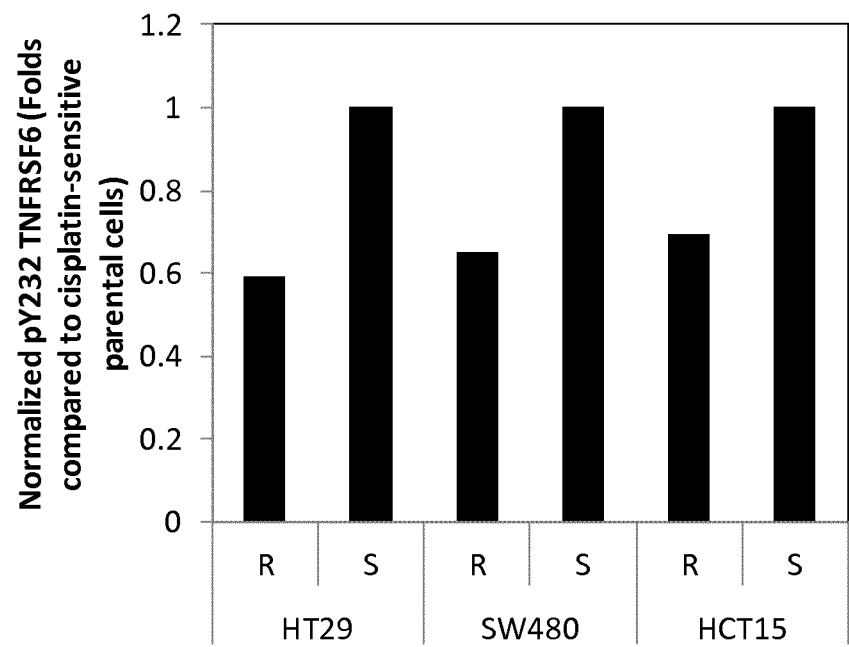


Figure 3

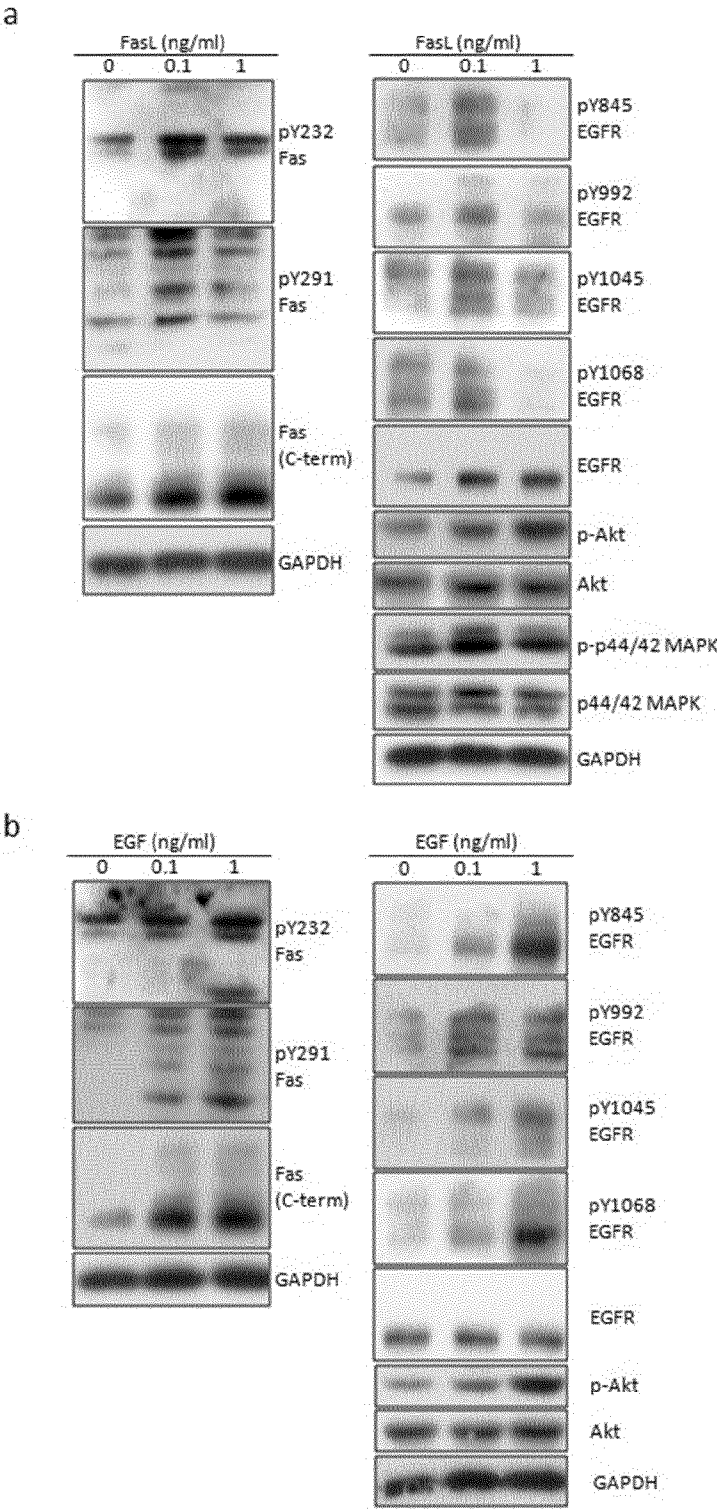


Figure 4

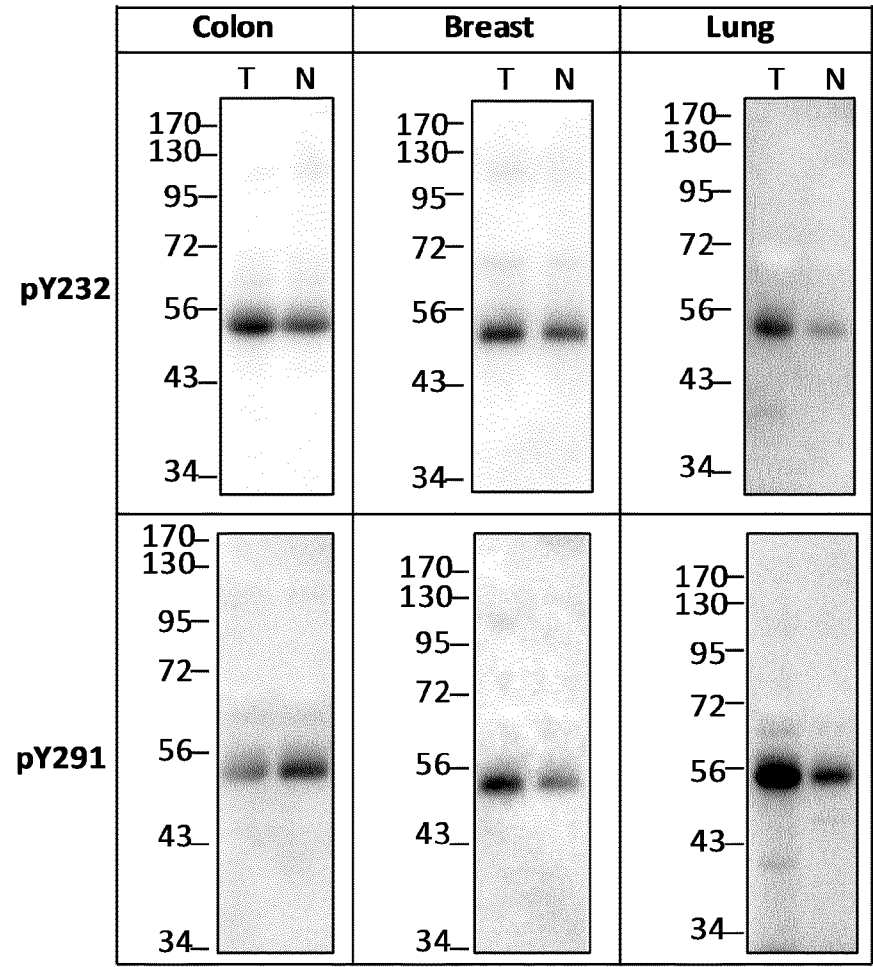
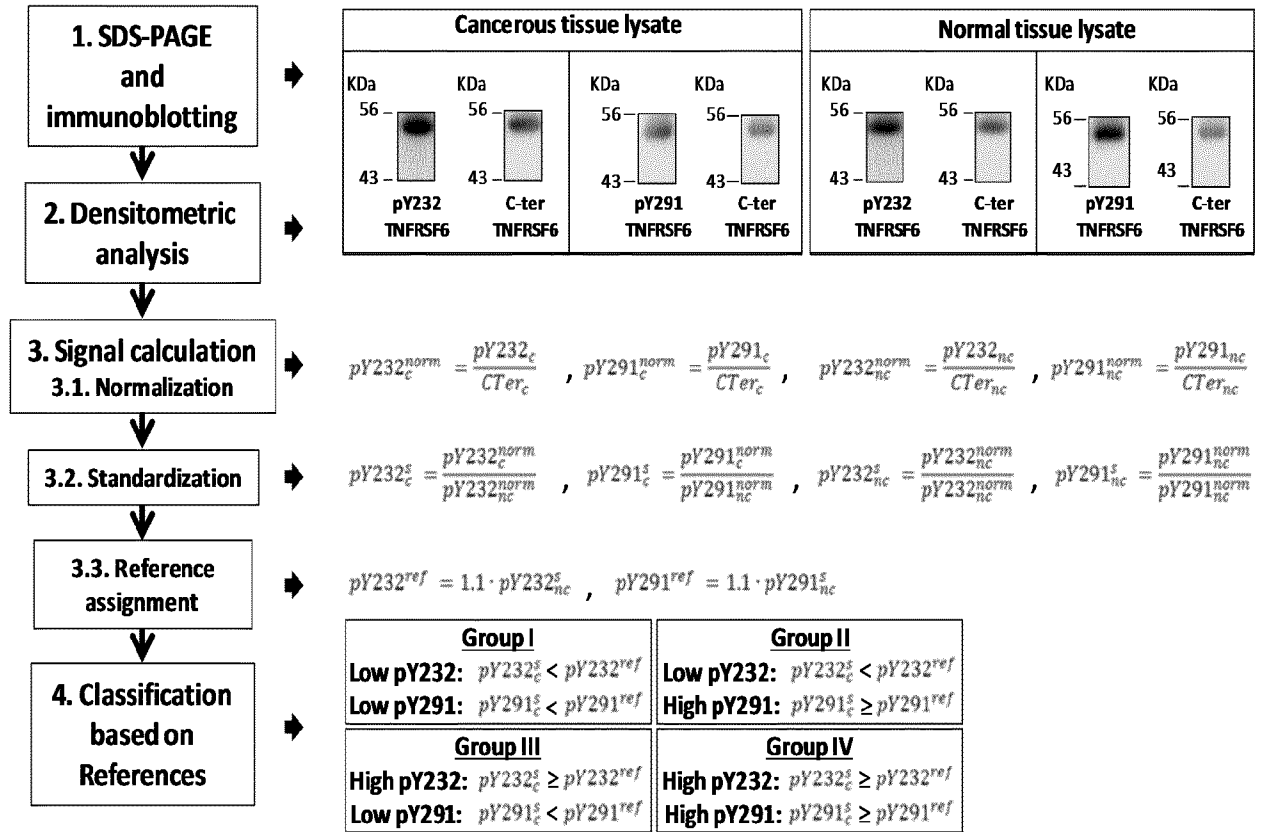


Figure 5

A. Summary of a method to predict sensitivity to TNFRSF6- and TNFSF6-targeted treatment against colon and breast cancer based on levels of pY232 and pY291 TNFRSF6



B. Stratification of colorectal and breast cancer patients based on predicted responses to TNFRSF6- and TNFSF6-targeted treatment

Table 1. Colorectal cancer classification based on Y232 and Y291 TNFRSF6 phosphorylation

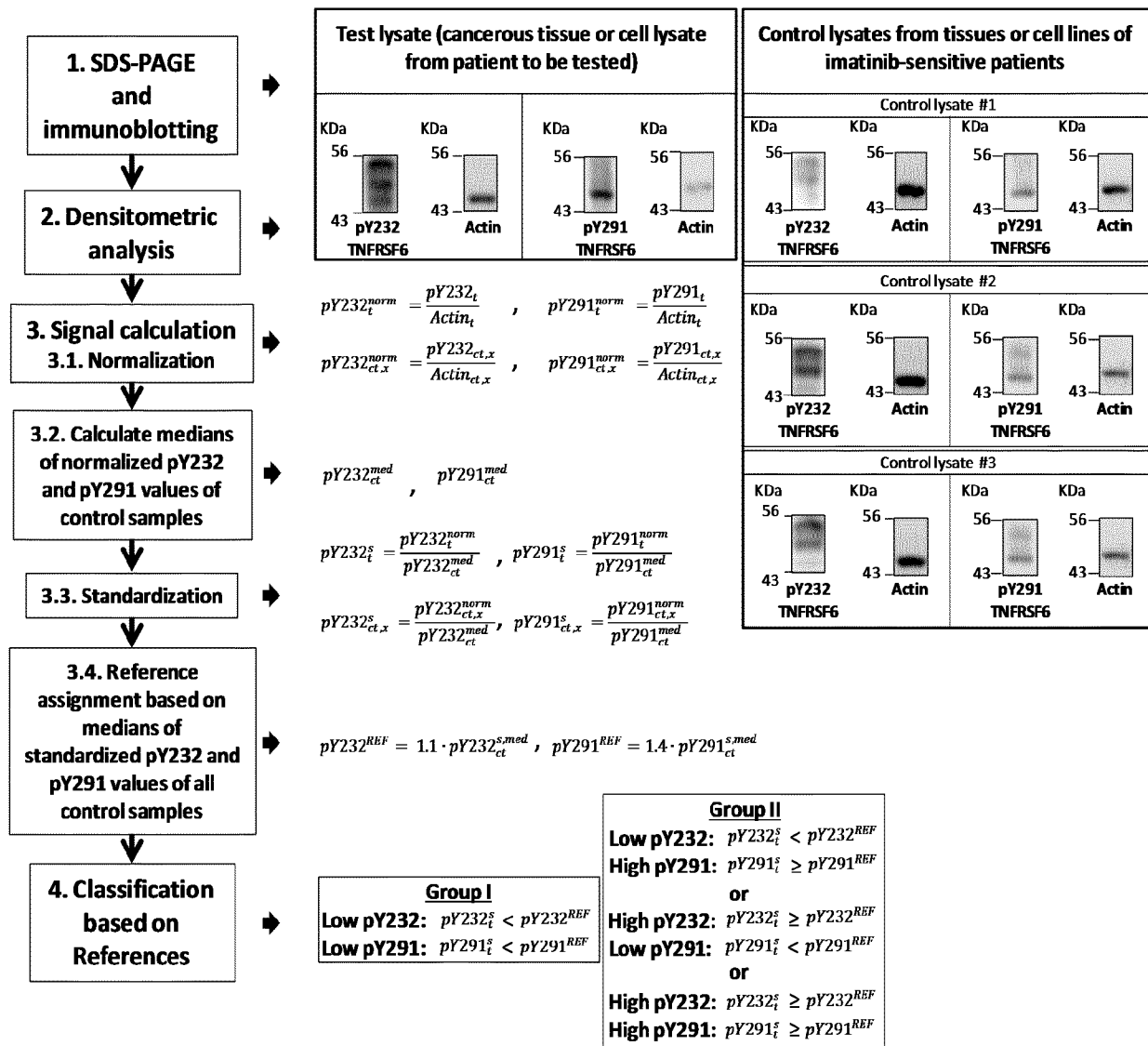
pYFas classification			Patient	Score		TNM	Stage
Group	pY232	pY291		pY232	pY291		
I	Low	Low	1	0.9	0.6	T2N0M0	I
			2	0.6	1.0	T4N2M0	n/a
			3	0.2	0.6	T3N1M0	IV
II	Low	High	4	0.9	2.1	T2N1M0	IIIA
			5	0.8	2.0	T2N0M0	n/a
			6	0.9	1.3	T3N0M0	III
III	High	Low	7	1.1	1.0	T3N0M0	II
			8	1.5	1.0	T2N1M0	II
			9	1.1	0.9	T2N0M0	I
			10	1.4	0.7	T2N1M0	IIIA
IV	High	High	11	1.3	1.4	T2N1M0	IIIA
			12	1.7	2.4	T3N0M1	IV

Table 2. Breast cancer classification based on Y232 and Y291 TNFRSF6 phosphorylation

pYFas classification			Patient	Score		TNM	Stage
Group	pY232	pY291		pY232	pY291		
I	Low	Low	1	1.0	0.7	T4N2Mx	IV
			2	0.7	0.5	T2N0M0	IIA
II	Low	High	3	0.9	1.5	T3N1M0	IIIA
			4	0.8	1.3	T2N0M0	IIB
III	High	Low	5	2.0	0.6	T4N1Mx	IV
			6	1.1	0.6	T4N1M0	IV
			7	1.3	0.5	T3N2M0	III
IV	High	High	8	3.7	2.6	T4N0M0	IV
			9	1.5	1.5	T4N2Mx	IV
			10	1.1	1.3	T2N1M0	IIB

Figure 6

A. Summary of a method to predict the resistance of chronic myeloid leukemia to imatinib



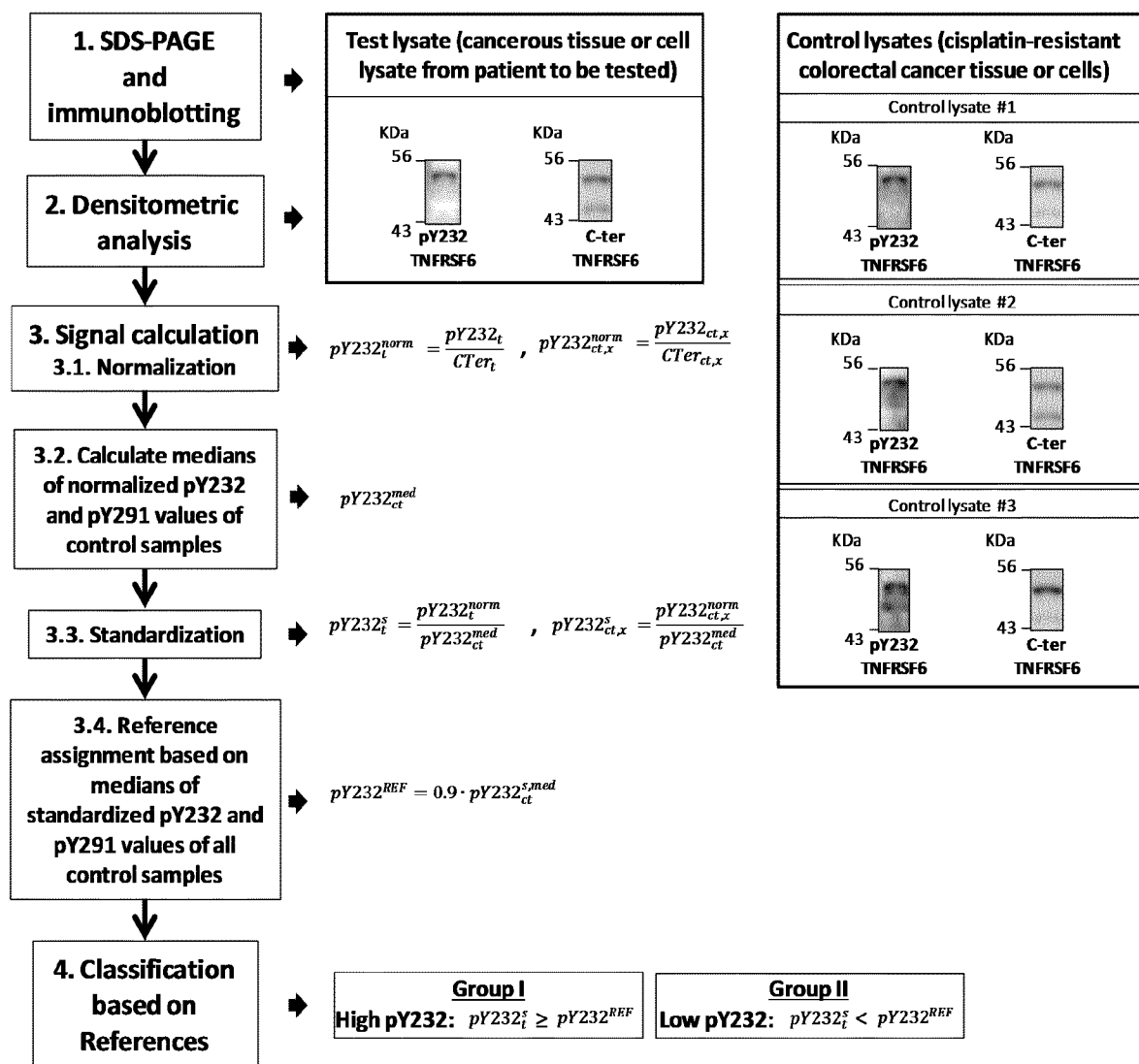
B. Prediction of CML cell sensitivity to imatinib

Table 1. Classification of CML cell sensitivity to imatinib based on Y232 and Y291 TNFRSF6 phosphorylation

pYFas classification			Test lysate	Score	
Group	pY232	pY291		pY232	pY291
I (Imatinib sensitive)	Low	Low	1	0.5	0.6
			2	1.0	1.0
			3	1.1	1.3
II (Imatinib resistant)	Low	High	4	0.8	1.5
	High	Low	5	1.3	1.1
	High	High	6	2.4	2.0

Figure 7

A. Summary of a method to predict the resistance of colorectal cancer to cisplatin



B. Prediction of colorectal cancer cell sensitivity to cisplatin

Table 1. Classification of colorectal cancer cell sensitivity to cisplatin based on Y232 and Y291 TNFRSF6 phosphorylation

pYFas classification		Test lysate	Score pY232
Group	pY232		
I (Cisplatin sensitive)	High	1	1.0
		2	1.0
		3	1.1
II (Cisplatin resistant)	Low	4	0.6
		5	0.6
		6	0.8

Figure 8

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2016/063390

A. CLASSIFICATION OF SUBJECT MATTER

INV. G01N33/574

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)

G01N

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, BIOSIS, COMPENDEX, EMBASE, INSPEC, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JOHNSON NATHALIE ET AL: "FAS Mutations Are Associated with Therapeutic Resistance in Follicular Lymphoma", BLOOD, vol. 120, no. 21, November 2012 (2012-11), page 1549, XP008178188, & 54TH ANNUAL MEETING AND EXPOSITION OF THE AMERICAN-SOCIETY-OF-HEMATOLOGY (ASH); ATLANTA, GA, USA; DECEMBER 08 -11, 2012 abstract ----- -/--	1-14



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

8 August 2016

Date of mailing of the international search report

17/08/2016

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Tuytman, Antonin

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2016/063390

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	M. A. PARK ET AL: "Sorafenib Activates CD95 and Promotes Autophagy and Cell Death via Src Family Kinases in Gastrointestinal Tumor Cells", MOLECULAR CANCER THERAPEUTICS, vol. 9, no. 8, 1 August 2010 (2010-08-01), pages 2220-2231, XP055130568, ISSN: 1535-7163, DOI: 10.1158/1535-7163.MCT-10-0274 abstract -----	1-14
A	RODRIGUEZ-PINILLA M ET AL: "beta-Catenin, Nf-kappaB and FAS protein expression are independent events in head and neck cancer: study of their association with clinical parameters", CANCER LETTERS, NEW YORK, NY, US, vol. 230, no. 1, 8 December 2005 (2005-12-08), pages 141-148, XP027607805, ISSN: 0304-3835 [retrieved on 2005-12-08] abstract -----	1-14
X,P	KRITTALAK CHAKRABANDHU ET AL: "An Evolution-Guided Analysis Reveals a Multi-Signaling Regulation of Fas by Tyrosine Phosphorylation and its Implication in Human Cancers", PLOS BIOLOGY, vol. 14, no. 3, 4 March 2016 (2016-03-04), page e1002401, XP055294180, US ISSN: 1544-9173, DOI: 10.1371/journal.pbio.1002401 cited in the application the whole document -----	1-14