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(54) Title: METHODS FOR PREDICTING THE SURVIVAL TIME OF A PATIENT SUFFERING FROM A GLIOBLASTOMA

(57) Abstract: The present invention relates to a method for predicting the survival time of a patient suffering from a glioblastoma comprising the steps consisting of i) determining the level expression of at least one marker selected in the group consisting of FGFR1, $\beta 3$ integrin and ILK in a tumor tissue sample from said patient, ii) comparing said level expression with a predetermined reference value and iii) providing a good prognosis of the survival time when the level expression is lower than the predetermined reference value and a poor prognosis of the survival time when the level expression is higher than the predetermined reference value.



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METHODS FOR PREDICTING THE SURVIVAL TIME OF A PATIENT SUFFERING FROM A GLIOBLASTOMA

5 **FIELD OF THE INVENTION:**

The invention relates to a method for predicting the survival time of a patient suffering from a glioblastoma comprising the steps consisting of i) determining the level expression of at least one marker selected in the group consisting of FGFR1, $\beta 3$ integrin and ILK in a tumor
10 tissue sample from said patient, ii) comparing said level expression with a predetermined reference value and iii) providing a good prognosis of the survival time when the level expression is lower than the predetermined reference value and a poor prognosis of the survival time when the level expression is higher than the predetermined reference value.

15 **BACKGROUND OF THE INVENTION:**

Glioblastomas (GBM) are the most common and malignant primary brain tumors in adults, and account for more than 50% of malignant gliomas. Since 2005, the standard therapy for this tumor includes surgery followed by radiotherapy to 60 Gy in combination
20 with temozolomide (TMZ), allowing an improved survival (Stupp R. et al., 2005). Nevertheless, the prognosis of the patients with a GBM remains very bad, with a median survival of 14.6 months, and a 2-year survival rate of 27.2%, because of a local recurrence mainly due to resistance of the GBM cells to radiotherapy.

The inventors have shown that FGF-2 controls radioresistance through a small GTPase
25 RhoB, whose farnesylated form also modulates GBM cells radioresistance in vitro through the inhibition of mitotic cell death but also controls hypoxia in vitro and in vivo in GBM models (Ader I. et al., 2003). Furthermore inhibition of farnesylation of RhoB led to radiosensitization, vascularization normalization and oxygenation in GBM xenografts (Delmas C. et al., 2003). The inventors recently demonstrated that upstream of RhoB, $\alpha v\beta 3$
30 and $\alpha v\beta 5$ integrins control GBM radioresistance via the integrin linked kinase (ILK) and RhoB. These integrins are also involved in vitro and in vivo through the Focal adhesion kinase (FAK) and RhoB in the control of hypoxia, which plays a crucial role in tumour chemo and radioresistance. Inhibition of this pathway leads to oxygenation and normalization of the vascularization.

The articles Bian XW, 2000, Fukui S, 2003 and Xu BJ 2011 discloses the used of different biomarkers (bFGF or FGF-2, growth hormone, VEGF for example) as having significant prognostic value in terms of survival and time to progression of GBM. However, these articles don't reveal an association between expression of FGFR1 and survival or time to progression of GBM.

Patent application WO97/08549 and CN102147410 discloses test kits comprising means for the detection of the integrin subunit beta-3. However, these patent application don't reveal an association between expression of $\beta 3$ integrin and survival or time to progression of GBM.

Because of the pivotal role of the farnesylated form of RhoB in the GBM radioresistance, the inventors developed a phase I trial associating the farnesyltransferase inhibitor Tipifarnib in continuous administration with radiotherapy in patients with glioblastoma, allowing to defined the recommended dose of 100 mg bid for the development of the phase II, showing encouraging results at this treatment dose (Moyal EC et al., 2007). In this article, they report the results from the bicentric, open-label, single arm, phase II trial of Tipifarnib given concurrently with conformational radiotherapy in adults with newly diagnosed glioblastoma.

Because all the patients treated for a glioblastoma do not have the same sensitivity to radiotherapy and to chemotherapy, some patients will have a rapid relapse after treatment and will die rapidly of their disease, while other patients will relapse several years after treatment, there is a crucial need to find biologic individual predictive factors whose help the physician to intensify or nor the treatment for instance by adding targeted drugs against these biologic factors in the aim to increase the radiosensitivity of the tumors.

SUMMARY OF THE INVENTION:

The inventors analyzed, by immuno-histochemistry performed on the pre-treatment tumor specimens from the patients accrued in the phase I/II study, the expression level of several proteins that they previously demonstrated to be involved in RhoB-mediated glioblastoma radioresistance and hypoxia. They show that FGFR1, $\beta 3$ integrin, ILK or FAK could be used as markers of the survival time or time to progression of patients affected with a glioblastoma.

Thus, the invention relates to a method for predicting the survival time of a patient suffering from a glioblastoma comprising the steps consisting of i) determining the level

expression of at least one marker selected in the group consisting of FGFR1, β 3 integrin and ILK in a tumor tissue sample from said patient, ii) comparing said level expression with a predetermined reference value and iii) providing a good prognosis of the survival time when the level expression is lower than the predetermined reference value and a poor prognosis of the survival time when the level expression is higher than the predetermined reference value.

The invention also relates to a method for predicting the time to progression of a patient affected with a glioblastoma, comprising the steps consisting of i) determining the level expression of at least one marker selected in the group consisting of FGFR1 or FAK in a tumor tissue sample from said patient, ii) comparing said level expression with a predetermined reference value and iii) providing a good prognosis for time to progression when the level expression of FGFR1 is lower than the predetermined reference value or the level expression of FAK is higher than the predetermined reference value and a poor prognosis for time to progression when the level expression of FGFR is higher than the predetermined reference value or the level expression of FAK is lower than the predetermined reference value.

DETAILED DESCRIPTION OF THE INVENTION:

A first object of the invention relates to method for predicting the survival time of a patient suffering from a glioblastoma comprising the steps consisting of i) determining the level expression of at least one marker selected in the group consisting of FGFR1, β 3 integrin and ILK in a tumor tissue sample from said patient, ii) comparing said level expression with a predetermined reference value and iii) providing a good prognosis of the survival time when the level expression is lower than the predetermined reference value and a poor prognosis of the survival time when the level expression is higher than the predetermined reference value.

Another object of the invention relates to method for predicting the sensitivity to treatment of a patient suffering from a glioblastoma, comprising the steps consisting of i) determining the level expression of at least one marker selected in the group consisting of FGFR1, β 3 integrin and ILK in a tumor tissue sample from said patient, ii) comparing said level expression with a predetermined reference value and iii) providing a good prognosis of the survival time when the level expression is lower than the predetermined reference value

and a poor prognosis of the survival time when the level expression is higher than the predetermined reference value.

5 In one embodiment, the invention relates to a method for predicting the survival time or for predicting the sensitivity according to the invention wherein the patient suffering from a glioblastoma is also treated by radio-chemotherapy.

10 As used herein, the term "FGFR1" has its general meaning in the art and refers to a receptor tyrosine kinase whose ligands are specific members of the fibroblast growth factor family. This receptor has a role in mitogenesis, differentiation and angiogenesis.

15 As used herein, the term " β 3 integrin" has its general meaning in the art and refers to a member of the integrin's family which are receptors that mediate attachment between a cell and the tissues surrounding it, which may be other cells or the extracellular matrix (ECM). They also play a role in cell signaling and thereby regulate cellular shape, motility, angiogenesis and the cell cycle.

20 As used herein, the term "ILK" has its general meaning in the art and refers to a 59kDa protein associated with multiple cellular functions including cell migration, cell proliferation, cell-adhesions, signal transduction and angiogenesis; the integrin-linked kinase.

As used herein, the term "patient", is intended for a human affected or likely to be affected with a tumor, preferably a glioblastoma.

25 In one embodiment, the level expression of FGFR1 is determined.

In one embodiment, the level expression of β 3 integrin is determined.

In one embodiment, the level expression of ILK is determined.

30 In one embodiment, the level expression of at least two markers selected in the group consisting of FGFR1, β 3 integrin and ILK are determined together.

In one embodiment, the level expression of FGFR1 and β 3 integrin are determined together.

In one embodiment, the level expression of FGFR1 and ILK are determined together.

In one embodiment, the level expression of $\beta 3$ integrin and ILK are determining together.

In one embodiment, the level expression of FGFR1, $\beta 3$ integrin and ILK are determining together.

5

In another embodiment, the level expression of FAK is determined wherein a good prognosis of the survival time is provided when the level expression is higher than the predetermined reference value and a poor prognosis of the survival time is provided when the level expression is lower than the predetermined reference value.

10

As used herein, the term “FAK” has its general meaning in the art and refers to a protein involved in cellular adhesion and spreading processes named the focal adhesion kinase.

15

As used herein, the term “tumor tissue sample” has its general meaning in the art and encompasses pieces or slices of tissue that have been removed including following a surgical tumor resection or following the collection of a tissue sample for biopsy. The tumor tissue sample can, of course, be subjected to a variety of well-known post-collection preparative and storage techniques (e.g., fixation, storage, freezing, etc.) prior to determining the level expression of the markers. Typically the tumor tissue sample may be paraffin-embedded or frozen.

20

In one embodiment, the tumor tissue sample is selected from the group consisting of a resected glioblastoma, or biopsy from glioblastoma.

25

The method of the invention is particularly suitable for the duration of the disease-free survival (DFS) or the overall survival (OS) and prediction of sensitivity to radiotherapy and could predict sensitivities to targeted drugs against these proteins.

30

In another embodiment, the compound used for the radio-chemotherapy is Tipifarnib, Temozolomide, an anti-FGFR1 compound or an anti-integrin compound such as the Cilengitide.

In another embodiment, the method according to the invention also comprises the determination of the methylation of the gene MGMT (Methylated-DNA-protein-cysteine methyltransferase) wherein a methylation of this gene is associated with longer survival.

5 In another aspect, the invention relates to a method for predicting the time to progression of a patient affected with a glioblastoma, comprising the steps consisting of i) determining the level expression of at least one marker selected in the group consisting of FGFR1 or FAK in a tumor tissue sample from said patient, ii) comparing said level expression with a predetermined reference value and iii) providing a good prognosis for time
10 to progression when the level expression of FGFR1 is lower than the predetermined reference value or when the level expression of FAK is higher than the predetermined reference value and a poor prognosis for time to progression when the level expression of FGFR1 is higher than the predetermined reference value or when the level expression of FAK is lower than the predetermined reference value.

15

In one embodiment, the invention relates to a method for predicting the time to progression according to the invention wherein the patient suffering from a glioblastoma is also treated by radio-chemotherapy.

20 In one embodiment, the level expression of FGFR1 or FAK is determining.

In one embodiment, the level expression of FGFR1 and FAK are determining together.

As used herein, the term "time to progression" denotes the time for a patient to relapse. As used herein, the term "a good prognosis for time to progression" denotes a long
25 time before relapse and the term "a poor prognosis for time to progression" denotes a short time before relapse. For example, a good prognosis for time to progression may be 3 years before the relapse. A poor prognosis for time to progression may be 2 months before relapse.

Determining the level expression of the markers of the invention may be determined
30 by any well known method in the art. Typically, such methods comprise contacting the tumor tissue sample with at least one selective binding agent capable of selectively interacting with the markers of the invention. The selective binding agent may be polyclonal antibody or monoclonal antibody, an antibody fragment, synthetic antibodies, or other protein-specific agents such as nucleic acid or peptide aptamers. Typically, the selective binding agent binds

any of the markers, such as an antibody specific for any of these molecules. Several antibodies have been described in the prior art and many antibodies are also commercially available such as described in the EXAMPLE. For the detection of the antibody that makes the presence of the markers detectable by microscopy or an automated analysis system, the antibodies may be tagged directly with detectable labels such as enzymes, chromogens or fluorescent probes or indirectly detected with a secondary antibody conjugated with detectable labels.

The preferred method according to the present invention is immunohistochemistry.

Such methods comprise contacting a sample with a binding partner capable of selectively interacting with FGFR1, $\beta 3$ integrin, ILK or FAK present in the sample. The binding partner is generally an antibody that may be polyclonal or monoclonal, preferably monoclonal.

One preferred method utilizes immunohistochemistry, a staining method based on immunoenzymatic reactions using monoclonal or polyclonal antibodies to detect cells or specific proteins such as tissue antigens. Typically, immunohistochemistry protocols involve at least some of the following steps:

1) antigen retrieval (eg., by pressure cooking, protease treatment, microwaving, heating in appropriate buffers, etc.);

2) application of primary antibody (i.e. anti-FGFR1, anti- $\beta 3$ integrin, anti-ILK or anti-FAK antibody) and washing;

3) application of a labeled secondary antibody that binds to primary antibody (often a second antibody conjugate that enables the detection in step 5) and wash;

4) an amplification step may be included;

5) application of a detection reagent (e.g. chromagen, fluorescently tagged molecule or any molecule having an appropriate dynamic range to achieve the level of or sensitivity required for the assay);

6) counterstaining may be used and

7) detection using a detection system that makes the presence of the proteins visible (to either the human eye or an automated analysis system), for qualitative or quantitative analyses.

Various immunoenzymatic staining methods are known in the art for detecting a protein of interest. For example, immunoenzymatic interactions can be visualized using different enzymes such as peroxidase, alkaline phosphatase, or different chromogens such as

DAB, AEC, or Fast Red; or fluorescent labels such as FITC, Cy3, Cy5, Cy7, Alexafluors, etc. Counterstains may include H&E, DAPI, Hoechst, so long as such stains are compatible with other detection reagents and the visualization strategy used. As known in the art, amplification reagents may be used to intensify staining signal. For example, tyramide
5 reagents may be used. The staining methods of the present invention may be accomplished using any suitable method or system as would be apparent to one of skill in the art, including automated, semi-automated or manual systems.

Predetermined reference values used for comparison may consist of “cut-of” values
10 that may be determined as described hereunder. Each reference (“cut-off”) value for each biological marker may be predetermined by carrying out a method comprising the steps of

a) providing a collection of tumor tissue samples from cancer patients;

b) providing, for each tumor tissue sample provided at step a), information relating to the actual clinical outcome for the corresponding cancer patient (i.e. the duration of the
15 overall survival (OS));

c) providing a serial of arbitrary quantification values;

d) determining the level expression of the markers of the invention for each tumor tissue sample contained in the collection provided at step a);

e) classifying said tumor tissue samples in two groups for one specific arbitrary
20 quantification value provided at step c), respectively: (i) a first group comprising tumor tissue samples that exhibit a quantification value for said markers that is lower than the said arbitrary quantification value contained in the said serial of quantification values; (ii) a second group comprising tumor tissue samples that exhibit a quantification value for said markers that is higher than the said arbitrary quantification value contained in the said serial of
25 quantification values; whereby two groups of tumor tissue samples are obtained for the said specific quantification value, wherein the tumor tissue samples of each group are separately enumerated;

f) calculating the statistical significance between (i) the quantification value obtained at step e) and (ii) the actual clinical outcome of the patients from which tumor tissue samples
30 contained in the first and second groups defined at step f) derive;

g) reiterating steps f) and g) until every arbitrary quantification value provided at step d) is tested;

h) setting the said predetermined reference value ("cut-off" value) as consisting of the arbitrary quantification value for which the highest statistical significance (most significant) has been calculated at step g).

As it is disclosed above, said method allows the setting of a single "cut-off" value permitting discrimination between poor and good prognosis. Practically, as it is disclosed in the examples herein, high statistical significance values (e.g. low P values) are generally obtained for a range of successive arbitrary quantification values, and not only for a single arbitrary quantification value. Thus, in one alternative embodiment of the method of determining "cut-off" values above, a minimal statistical significance value (minimal threshold of significance, e.g. maximal threshold P value) is arbitrarily set and the range of arbitrary quantification values for which the statistical significance value calculated at step g) is higher (more significant, e.g. lower P value) are retained, whereby a range of quantification values is provided. Said range of quantification values consist of a "cut-off" value according to the invention. According to this specific embodiment of a "cut-off" value, poor or good clinical outcome prognosis can be determined by comparing the level of markers according to the invention determined at step i) with the range of values delimiting the said "cut-off" value. In certain embodiments, a cut-off value consisting of a range of quantification values, consists of a range of values centered on the quantification value for which the highest statistical significance value is found (e.g. generally the minimum P value which is found).

In a particular embodiment, when detection of FGFR1, $\beta 3$ integrin, ILK or FAK is performed by immunochemistry, the method may further comprise a step consisting of determining the amount of cells that express FGFR1, $\beta 3$ integrin, ILK or FAK "FGFR1+ cells", " $\beta 3$ integrin+ cells", "ILK+ cells" or "FAK+ cells").

In a preferred embodiment a percentage of FGFR1+ cells, $\beta 3$ integrin+, cells "ILK+ cells" of at least 20%, preferably at least 21%, more preferably at least 22%, even more preferably at least 23%, at least 24%, at least 25%, at least 26%, at least 27%, preferably at least 28%, more preferably at least 29%, even more preferably 30% and more preferably 50% higher than a predetermined reference values is indicative of a poor prognosis for survival time.

In other words, patients with low percentage of FGFR1+ cells, $\beta 3$ integrin+ cells or "ILK+ cells" are indicative of a good prognosis for survival time.

In another embodiment a percentage of FGFR1+ cells,” of at least 20%, preferably at least 21%, more preferably at least 22%, even more preferably at least 23%, at least 24%, at least 25%, at least 26%, at least 27%, preferably at least 28%, more preferably at least 29%, even more preferably 30% and more preferably 50% higher than predetermined reference values is indicative of a poor prognosis for time to progression.

In still another embodiment a percentage of “FAK+ cells” of at least 20%, preferably at least 21%, more preferably at least 22%, even more preferably at least 23%, at least 24%, at least 25%, at least 26%, at least 27%, preferably at least 28%, more preferably at least 29%, even more preferably 30% and more preferably 50% lower than a predetermined reference values is indicative of a poor prognosis for time to progression.

In a particular embodiment, the step of determining the amount of FGFR1+ cells, β 3 integrin+ cells, “ILK+ cells” or “FAK+ cells” may be combined with a step of determining the staining intensity of the FGFR1+ cells, β 3 integrin+ cells, “ILK+ cells” or “FAK+ cells”.

In one embodiment, an Immuno-Reactive score (IRS) as explained in the examples may be applied.

The markers expression was classified according to the percentage of labelled cells (0 to 100%) and the intensity of the staining, graded from 0 to 3 (0: no staining; 1: weak; 2: medium; 3: strong). The immunoreactive score (IRS) combining both information was then determined as previously published in Remmele W. et al., 1987.

For example and according to the method of the invention, an IRS equal or higher than 4 for the markers FGFR1 or β 3 integrin is indicative of a poor prognosis of the survival time.

For example and according to the method of the invention, an IRS equal or higher than 6 for the marker ILK is indicative of a poor prognosis of the survival time.

For example and according to the method of the invention, an IRS equal or less than 4 for the marker FAK is indicative of a poor prognosis of the survival time or for time to progression.

For example and according to the method of the invention, an IRS equal or higher than 4 for the marker FGFR1 is indicative of a poor prognosis for time to progression.

In one embodiment, the invention relates to FGFR1, β 3 integrin, ILK and FAK as biomarkers for patient suffering from a glioblastoma.

In another embodiment, the invention relates to FGFR1, $\beta 3$ integrin, ILK and FAK as biomarkers for glioblastoma treated by radio-chemotherapy.

In another aspect, the invention relates to a method for selecting a treatment for a patient affected with a glioblastoma wherein a patient with a high level expression of FGFR1, $\beta 3$ integrin or ILK will be treated by anti-FGFR1, anti- $\beta 3$ integrin, or anti-ILK therapy.

In one embodiment, a patient affected with a glioblastoma with a high level expression of $\beta 3$ integrin will be treated by anti- $\beta 3$ integrin therapy selected from the group consisting of Cilengitide, Vitaxin or another anti- $\beta 3$ integrin.

In another aspect, the invention relates to a method for selecting patients who will benefit of anti-FGFR1, anti- $\beta 3$ integrin, or anti-ILK treatment wherein patient with poor prognosis as evaluated by the method according to the invention will benefit of such treatment.

In another aspect, the invention relates to an antagonist of the $\beta 3$ integrin or an inhibitor of the $\beta 3$ integrin expression for use in the treatment of patient suffering from glioblastoma with poor prognosis as evaluated by the method according to the invention.

Typically, the antagonist according to the invention includes but is not limited to a small organic molecule, an antibody, and a polypeptide.

Typically, the inhibitor according to the invention includes but is not limited to siRNAs, Ribozymes.

The methods of the invention are of higher accuracy than currently used staging methods (e.g. UICC-TNM). Accordingly, the methods of the invention can be applied for monitoring the effectiveness of anti-cancer treatments. For example, the present invention provides a method for monitoring the effectiveness of treatment of a subject with an agent comprising the steps of (i) predicting the survival time of the patient before administering said agent by performing the method according to the invention, ii) predicting the survival time of the patient after administering said agent by performing the method according to the invention iii) comparing the survival time of step a) with the survival time of step b) and iv) and providing the conclusion that the agent is effective for the treatment of the cancer when the survival time of step b) is higher than the survival time of step a). In case where the

conclusion is negative then the physician may adapt the treatment by prescribing different dosage or by prescribing another agent to administer. The methods of the invention may also particularly suitable for determining whether the patient will be considered as responder to the treatment (e.g. an immunotherapy agent or a radiotherapy). Typically, when a good prognosis is provided by the methods of the invention the patient may be eligible for the treatment. The methods of the invention may also particularly suitable for determining whether adjuvant therapy (e.g. chemotherapy or radiotherapy) will be required or not. For example, when a good prognosis is provided by the method of the invention, the subsequent anti-cancer treatment may not comprise any adjuvant chemotherapy. However when a poor prognosis is provided by the method of the invention, then the patient may be eligible for the adjuvant chemotherapy.

The present invention includes a kit for performing the method of the present invention comprising means for determining the level of FGFR1, $\beta 3$ integrin, ILK or FAK expression in a tumor tissue sample.

As used herein, the term “means for determining” denotes all physical means which are able to bind to the different markers. For example, means for determining the markers may be an antibody against a marker coupling with a signalling system.

According to the invention, kits of the invention may comprise 2 antibodies directed against FGFR1, $\beta 3$ integrin, ILK or FAK and another molecule coupled with a signalling system which binds to said antibodies.

Typically, the antibodies or combination of antibodies are in the form of solutions ready for use. In one embodiment, kits comprise containers with the solutions ready for use. Any other forms are encompassed by the present invention and the man skilled in the art can routinely adapt the form to the use in immunohistochemistry.

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

FIGURES:

Figure 1. Time to progression and overall survival of the 27 patients treated for a glioblastoma multiforme with radiotherapy and Tipifarnib in the phase II study.

Figure 2. Overall survival in univariate analysis of patients in phase I and II according to : A : ILK expression in tumor cells (ILK low: IRS<6 ; ILK high : IRS $\geq$ 6) ; B : FAK expression in tumor cells (FAK low: IRS<4 ; FAK high : IRS $\geq$ 4) ; C : FGFR1 expression in tumor cells (FGFR1 low: IRS<4 ; FGFR1 high : IRS $\geq$ 4) ; D : α v β 3 expression in tumor cells (α v β 3 low: IRS<4 ; α v β 3 high : IRS $\geq$ 4).

	G0 (N ; %)	G1(N ; %)	G2 (N ; %)	G3 (N ; %)	G4 (N ; %)
Allergy/Immunology	26 ; 96.3	1 ; 3.7	0 ; 0.0	0 ; 0	0 ; 0
Auditory/Ear	25 ; 92.6	1 ; 3.7	1 ; 3.7	0 ; 0	0 ; 0
Blood/Bone Marrow	21 ; 77.8	6 ; 22.2	0 ; 0	0 ; 0	0 ; 0
Cardiac (general, Arrhythmia)	25 ; 92.6	0 ; 0.0	2 ; 7.4	0 ; 0	0 ; 0
Constitutional Symptoms	11 ; 40.7	7 ; 25.9	8 ; 29.6	1 ; 3.7	0 ; 0
Dermatology/Skin	13 ; 48.1	8 ; 29.6	5 ; 18.5	1 ; 3.7	0 ; 0
Gastrointestinal	13 ; 48.1	6 ; 22.2	6 ; 22.2	2 ; 7.4	0 ; 0
Hemorrhage/Bleeding	24 ; 88.9	2 ; 7.4	1 ; 3.7	0 ; 0	0 ; 0
Infection	26 ; 96.3	0 ; 0.0	1 ; 3.7	0 ; 0	0 ; 0
Lymphatics	26 ; 96.3	1 ; 3.7	0 ; 0.0	0 ; 0	0 ; 0
Metabolic/Laboratory	19 ; 70.4	7 ; 25.9	0 ; 0.0	1 ; 3.7	0 ; 0
Musculoskeletal	23 ; 85.2	2 ; 7.4	2 ; 7.4	0 ; 0	0 ; 0

Neurology	4 ; 14.8	5 ; 18.5	16 ; 59.3	2 ; 7.4	0 ; 0
Ocular/Visual	19 ; 70.4	4 ; 14.8	4 ; 14.8	0 ; 0	0 ; 0
Pain	11 ; 40.7	3 ; 11.1	11 ; 40.7	2 ; 7.4	0 ; 0
Pulmonary/Upper Respiratory	25 ; 92.6	1 ; 3.7	1 ; 3.7	0 ; 0	0 ; 0
Renal/Genitourinary	25 ; 92.6	1 ; 3.7	1 ; 3.7	0 ; 0	0 ; 0
Vascular	26 ; 96.3	1 ; 3.7	0 ; 0.0	0 ; 0	0 ; 0

Table 1. Toxicities during the Phase II study, according to the CTCAEv3

5

Markers (n: number of available samples)		Immunoreactive score: N	
Ilk Tumor (n= 32)	Median	4	
	Range	(0 ; 12)	
Ilk Endoth (n= 34)	Median	12	
	Range	(0 ; 12)	
FGFR-1 Tumor (n= 33)	Median	6	
	Range	(0 ; 9)	
FGFR-1 Endoth (n= 31)	Median	6	
	Range	(0 ; 12)	
FGF2 (n= 35)	Median	6	
	Range	(0 ;12)	
$\alpha v\beta 3$ Tumor (n=35)	Media	4	
	Range	(0 ; 12)	
		Median	8

α v β -3 Endoth	(n=35)	Range	(0 ; 12)
α v β 5 Tumor	(n=32)	Median	5
		Range	(1 ; 12)
α v β 5 Endoth	(n=33)	Median	8
		Range	(0 ; 12)
FAK Tumor	(n=32)	Median	2
		Range	(0 ; 12)
FAK Endoth	(n=31)	Median	8
		Range	(0 ;12)

Table 2. Immuno-Reactive Score (IRS) (Tumor = tumor cells; Endoth = Endothelial cells)

5

	IRS	Time To Progression			Overall Survival		
		HR	95%CI	p	HR	95%CI	p
Surgery Biopsy Large surgery		2.66 1	[0.85; 8.34]	0.093	3.98 0.011 1	[1.37;11.56]	
ILK Tumor cells	<6 ≥6	1 1.46 0.396	[0.61; 3.52]		1 0.69 0.530	[0.22;2.17]	
FGFR1 Tumor cells	<4 ≥4	1 4.65 0.047	[1.02;21.21]		1 4.10 0.036	[1.09;15.40]	
α v β 3 Tumor cells	<4 ≥4	NA NA			1 10.38 0.001	[2.70;39.87]	
FAK Tumor cells	<4 ≥4	0.96 0.947 1	[0.33; 2.80]		2.63 0.077 1	[0.90;7.69]	
Age at inclusion							

<=60 ans		3.04	[1.0; 8.82]	NA
>60 ans		0.041		NA
		1		

Table 3. Multivariate analysis (Cox analysis): Time to progression (TTP) and overall survival (OS) for the patients of the phase I and II according to surgical treatment, age and biological markers. The hazard ratios (HR) are presented with 95% confidence interval.

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EXAMPLE:

Material & Methods

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This phase I/II trial was approved by the French Ethics Committee. Thirteen patients were included in the phase I component of this clinical trial. After toxicity observation, the phase II have been opened on December 2005 in two French cancer centers (Claudius Regaud Institute in Toulouse, and Jean Perrin Institute in Clermont Ferrand) and closed to new patient entry on January 2009.

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Patient population

Eligibility criteria for this protocol included the following: >18 years of age, Performans Status (PS) ≤ 2 , and newly diagnosed intracranial GBM confirmed by biopsy or resection no more than 8 weeks before treatment, ≤ 5 cm in case of no resection. No prior treatment was allowed. Patients with the following clinical criteria were excluded: apparent leptomeningeal metastases, patients with uncontrolled seizures despite standard anticonvulsivant therapy, significantly abnormal haematological status as judged by: absolute neutrophil count (ANC) $< 1500/\text{mm}^3$ ($1.5 \times 10^9/\text{l}$), platelet count $< 100,000/\text{mm}^3$ ($100 \times 10^9/\text{l}$), serum bilirubin > 2 mg/dl ($> 34 \mu\text{mol/l}$) or transaminase > 2.5 x the upper limit of institutional normal or creatinine > 1.5 mg/dl ($> 132 \mu\text{mol/l}$), medical history of phlebitis or pulmonary embolism, thrombocytosis, myocardiopathy, or other relevant cardiac pathology (auricular flutter, auricular fibrillation).

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The research was conducted in compliance with the French regulations authorities, including provisions relating to the Public Health Code, laws Bioethics, the Data Protection Act, and the declaration of Helsinki. The terms of this Protocol have been approved by the

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French Agency for Health Products (AFSSAPS) on 02/21/2003 and the Committee to Protect People (CCPRB) of Toulouse I on 01/23/2003. All patients signed informed consent forms.

Treatment plan

According to the result of the Phase I, Tipifarnib ((R115777; Zarnestra), Johnson & Johnson Pharmaceutical Research and Development, Beerse, Belgium), a potent and selective nonpeptidomimetic farnesyltransferase inhibitor (FTI) was daily administered orally at the recommended dose of 100 mg bid every 12 hours since one week before and until the end of the radiation therapy, continuously 7 days/week. No maintenance treatment was conducted.

Radiotherapy was administered with a total dose of 60 Gy in 2 Gy daily fractions delivered 5 days per week given over a 6-week course to the contrast-enhancing tumor or to the surgical bed with a 2-cm margin according to the EORTC protocol. All treatment was delivered with at least 6 MV beams, every day, 5 days per week. No dose reduction was allowed. At progression, all patients received Temozolomide treatment.

The pretreatment evaluation included a complete history, a physical and neurological examination and prestudy laboratory tests obtained within 7 days after accrual, including a CBC count with differential, serum creatinine, total bilirubin, AST, ALT, alkaline phosphatase, blood urea nitrogen, glucose, potassium, sodium. The same laboratory tests were performed weekly during the 6 week-course of radiotherapy associated with Tipifarnib. All patients had a pre-treatment MRI and 3D CSA-MR spectroscopy (Siemens Magnetom Avanto 1.5 T, Erlangen, Germany) within 7 days after inclusion.

Biomarker Study

This analysis was conducted at the pathology department of the Rangueil University Hospital of Toulouse, France. Material for analysis was derived from primary tumor tissue. For all the patients of the phase I treated at the recommended dose and for each patient of the phase II, hematoxylin-eosin-stained sections, routine immunostainings, and paraffin blocks were collected from the archives of the two participating centers. When paraffin blocks were available, additional stainings for FAK, ILK, $\alpha\beta3$ and $\alpha\beta5$ integrins, FGF2 and FGFR1 were performed. Four μm sections were dewaxed, pretreated at 96 °C for 40 minutes to retrieve antigenicity with EDTA (pH 9.0) for ILK, FAK, and FGF2 staining, and with Citrate (pH 6.0) for FGFR1, $\alpha\beta3$ and $\alpha\beta5$ integrins. Treatment with 6% hydrogen peroxide was then performed and saturation of the binding sites was done with 3% PBS. Sections were then

incubated at room temperature with primary antibodies against ILK (mouse monoclonal antibody, clone 65-1, Santa Cruz Biothechnology, 1:100 dilution), FAK (rabbit polyclonal antibody 3285, Cell Signaling Technology, dilution 1:100), FGF2 (mouse monoclonal antibody clone Ab3 3H3, Calbiochem, dilution 1:200), FGFR 1 (mouse monoclonal antibody clone M19B2, Thermo Scientific, dilution 1:100), $\alpha\text{v}\beta 3$ integrin (rabbit monoclonal antibody MAB 2008 clone SAP, Millipore-Chemicon, dilution 1:50), $\alpha\text{v}\beta 5$ integrin (rabbit polyclonal antibody ab15459, Abcam, dilution 1:50). Second anti-bodies against mouse (Dako K0675) or rabbit (Dako K0675) were used. Antibody – antigen complexes were subsequently visualized with a high-sensitivity detection kit (Dako K0675). Peroxidase activity was visualized by use of diaminobenzidine as the chromogen.

Two observers independently and then together scored each sample. Results for each observer were averaged to obtain the final IHC score. The protein expression was classified according to the percentage of labeled cells (0 to 100%) and the intensity of the staining, graded from 0 to 3 (0: no staining; 1: weak; 2: medium; 3: strong). An immunoreactive score (IRS) combining both information was then determined as previously published.

The methylation status of the methyl-guanine methyl transferase (MGMT) was also determined on paraffin blocks when available. The methylation-specific polymerase chain reaction (MS-PCR) described by Hegi was used.

Study Endpoints

The primary objective of this trial was to estimate the time to progression (TTP) and the secondary objectives were to estimate the overall survival, the objective response rate and the radiological time to progression. A safety analysis was also conducted.

MRI was performed every two months from the end of radiation treatment during 6 months, and every 3 months until progression. Response was evaluated by clinical and radiological criteria. Tumor size was measured according a volumetric analysis by using the Sysiphe-Neuroimaging Software Toolbox, delineating tumoral volumes on three dimensional T1 + gadolinium and Flair sequences.

Responses were characterized as follows: complete response (CR): disappearance of all enhancing lesion evaluated by MRI, patient neurologically stable or improved. Partial response (PR): evidence of $\geq 50\%$ reduction in tumor size, patient neurologically stable or improved. Progressive disease (PD): 25% increase of tumor size or any new tumor appearance, patient neurologically worse. Stable disease (SD): all other situations. The cut-off date was on August 30th 2010.

The time to progression, primary end point, was defined from the date of inclusion to the date at which progressive disease was first observed, or to nonreversible neurologic progression or permanently increased corticosteroid requirement. Overall survival was defined from the date of inclusion to the date of death from any cause or date of last news.

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Statistical analysis

The trial was based on the comparison of the data of the current trial with historical control (17). With a median TTP in historical controls of 15 weeks and a target median TTP in this trial of 30 weeks, using a 5% significance level and 80% power, the sample size required was 24 patients. Assuming a 10% drop-out rate, 27 patients were included in the phase II study.

Categorical variables were reported by frequencies and percentages, continuous variables were presented by median and range. Time to progression and overall survival were estimated by the Kaplan-Meier methods. Exact binomial confidence intervals (CIs) were computed for response rates.

In univariate analysis, the significance of various clinical characteristics was performed using logrank test. The minimum p-value approach was used to assess the cut-off for the separation between low and high expression referring to the time to progression endpoint. A multivariate analysis, using Cox proportional hazard modeling was performed to identify whether a factor was an independent predictor of survival in multivariate analysis. Significant variables at univariate analysis ($p < 0.15$) were included in multivariate models. A p value less than 0.05 was considered statistically significant in multivariate analysis. Correlations between two independent continuous variables were investigated using the Spearman's rank correlation coefficients. All statistical analysis were performed were performed using the STATA 11.0 software (STATA Corp, College Station, TX).

Results

Demographic Data

A total of 27 patients were registered in the phase II component between December 2005 and January 2009. Majority of patients were male (n=18, 66.7%). Median age at inclusion was 59 years (Range= 38-79), and PS was 0 or 1 in 96.3% of cases. Twenty two patients (81.5%) had surgery (gross total resection in 17 patients), and 5 patients (18.5%) underwent biopsy. According to the RPA classes, 2 patients were class III (7.4%), 20 patients

were class IV (74.1%) and 5 patients were class V (18.5%). Only 14 / 35 samples were available for evaluating MGMT status. Among them, 2 were methylated (14.3%).

Treatment

The median time of radiation treatment was 6.4 weeks (range= (5.6-7.3)), and all the patients received the full course of radiotherapy, without any interruption. Three patients interrupted the Tipifarnib. One during nine days because of a grade 3 cutaneous rash, the second one missed the treatment for one day, and the last one experienced an urinary infection with elevation of serum creatinin, interrupting the Tipifarnib for three days.

Safety

Toxicity was modest and very tolerable (Table 1). The majority of treatment-specific adverse events were grade 1 or 2. There was no grade 4 event related to treatment and no treatment-related death. No grade 3/4 hematologic adverse event was observed. Five serious adverse events (18.5%) were observed, and only one, a cutaneous rash, was a grade 3 and imputable to Tipifarnib. The other serious adverse events were neurologic (seizure, speech impairment) and vascular (1 thrombosis) not imputable to Tipifarnib.

Response rate and survival

After a median follow up of 80 weeks (range= (21.3;180.1)), 26 patients had progressed according to volumetric and clinical criteria.

The median time to progression was 23.1 weeks (95%CI=[15.4;28.2]). 33.33% (95%CI=[16.77;50.86]) of the patients had no progression at 6 months, and 7.41% (95%CI=[1.30;21.03]) at 1 year (Fig 1). The best radiologic and clinical response was stable disease in 14 patients (51.9%).

At last follow-up news, 21 patients had died and the overall survival was 74.07% at 1 year (95%CI=[53.19;86.70]), and 26.59% at 2 years (95%CI=[10.47;45.96]) (Fig.1) and the median overall survival was 80.3 weeks (95%CI=[57.8;102.7]).

Biological markers studies

Among the 36 patients treated at the recommended dose during the trial (Phase I: n=9, phase II: n=27), the expression of the 6 proteins were studied on available samples of 35 patients: ILK (n=34), FGFR1 (n=33), FGF2 (n=35), $\alpha v \beta 3$ (n=35), $\alpha v \beta 5$ (n=33) and FAK (n=32)..

ILK, FAK, $\alpha v\beta 3$ and $\alpha v\beta 5$ integrins staining were predominantly detected in endothelial cells, but were also present in tumor cells (Table 2). FAK, $\alpha v\beta 3$ integrin and $\alpha v\beta 5$ integrin were often observed in invasion fronts. FGF2 and FGFR1 were more often detected in tumor cells than in endothelial cells (data not shown). We then studied the correlations between the different protein expressions and, then, between the protein expressions and clinical characteristics or clinical outcome.

We did not observe any correlation between protein expression in endothelial cells and clinical outcome.

Correlation between the different protein expressions in tumor cells

ILK expression was statistically linked with $\alpha v\beta 3$ integrin expression ($\rho=0.49$; $P=0.0047$), whereas FGF2 expression was inversely correlated to FAK expression ($\rho=0.3911$; $P=0.0269$).

No correlation but one was observed between protein expressions and clinical characteristics: $\alpha v\beta 3$ integrin expression was statistically correlated with age ($P=0.0146$).

Correlation between protein expressions in tumor cells and clinical outcome

Time To Progression

Thirty three patients progressed during follow-up, the median time to progression was 18.1 weeks (95%CI=[16.9; 25.6]). In univariate analysis, no correlation was found between the time to progression and the following variables: age ($P=0.1072$), gender ($P=0.4731$), PS ($P=0.7326$), type of surgery ($P=0.1267$).

In univariate analysis, no correlation was found between TTP and the following markers: FGF2 (IRS<8 vs ≥ 8 ; $P=0.1692$), $\alpha v\beta 3$ integrin (IRS<4 vs ≥ 4 ; $P=0.9221$) and $\alpha v\beta 5$ integrin (IRS<6 vs ≥ 6 ; $P=0.7943$). There was a tendency for patients with over-expression of ILK (IRS ≥ 6) to have a poorer time to progression ($P=0.0772$), median TTP were estimated to 23.1 weeks (IRS<6) (95%CI=[15.4; 37.1]) and 16.9 weeks (IRS ≥ 6) (95%CI=[13.6; 25.3]). FAK over-expression in tumor cells appeared to be a good prognosis factor for time to progression ($P=0.0488$), while the median TTP was 18.1 weeks (95%CI=[13.6; 25.4]) when IRS was less than 4 and 28.3 weeks (95%CI=[15.4; 43.3]) when IRS was equal or more than 4. Although FGFR1 expression was not statistically correlated with TTP ($p=0.0748$), patients with over-expression of FGFR1 (IRS ≥ 4) seemed to be

associated with a poorer TTP, while the 6 months times to progression were 42.9% when IRS was less than 4 and 30.8% when IRS was equal or more than 4.

Results of multivariate analysis were summarized on table 3. In multivariate analysis, no correlation was found between ILK, FAK $\alpha\text{v}\beta 3$ integrin over-expression and time to progression. The only factors independently associated with TTP were the age (HR=3.04; 95%CI=[1.05; 8.82], P=0.041) and FGFR1 over-expression (HR=4.65; 95%CI=[1.02; 21.21], P=0.047).

Overall Survival

Twenty nine patients were deceased at last follow-up news. The median overall survival was 60.4 weeks (95%CI=[47.3; 97.6]). In univariate analysis, no correlation was identified between overall survival and the following variables: age (P=0.2628), gender (P=0.6090), performance status (P=0.3512). Patients who underwent biopsy (median OS 46.6 weeks, 95%CI=[21.3; 52.4]) were associated with poorer survival compared to patients treated by gross resection (median OS: 74.7 weeks, 95%CI=[57.9; 131.8], P=0.002).

In univariate analysis, no correlation was found between expression of the following markers and the overall survival: $\alpha\text{v}\beta 5$ integrin in tumor cells (IRS <6 vs ≥ 6 , P=0.4260) and FGF2 expression (IRS<8 vs ≥ 8 , p=0.8182). No statistically significant correlation was found between FGFR1 expression and overall survival (P=0.1104), but patients with FGFR1 over-expression tend to have a poorer survival (Median OS=57.9 weeks) compared to those with lower expression (Median OS=102.7 weeks). $\alpha\text{v}\beta 3$ integrin overexpression was highly significantly associated with overall survival, since the 2 years overall survival was 45.8% when IRS was less than 4 and 10% when IRS was equal or higher than 4 (P=0.0014). ILK tumor over-expression was significantly associated with a poorer overall survival (P=0.0284), the median OS being 97.6 weeks (95%CI=[44; 131.8]) for the patients with a low expression of ILK (IRS<6) and 48 weeks (95%CI=[42.8; 74.7]) for the patients with over-expression of ILK (IRS ≥ 6) (Fig.2A). $\alpha\text{v}\beta 3$ integrin over-expression in tumor was statistically associated with an increased risk of death (P= 0.0014), median OS were 102.7 weeks 95%CI=[52.4; Not Reached]) when IRS was less than 4 and 47.3 weeks (95%CI=[38.7; 60.4]) when IRS was equal or higher than 4. FAK over-expression in tumor cells appeared to be correlated with a lower risk of death (P=0.0066) (Fig. 2B), the median OS were 48 weeks (95%CI=[38.7; 64]) for patients with FAK lower-expression and 97.6 weeks (95%CI=[60.4; Not Reached]) for patients who over-expressed FAK.

Results of multivariate analysis were summarized in table 3. Patients who underwent a biopsy were associated with an increased risk of death compared to the patients who underwent a surgical resection (HR=3.98 [1.37; 11.56] ; P=0.011). The adjusted HR of overall survival of patients presenting an over-expression of FGFR1 in tumor cells (IRS $\geq$ 4) compared with patients with low expression of FGFR1 (IRS $<$ 4) was 4.1 (95% CI, 1.09–15.4; P=0.036) (Fig.2C). Moreover, α v β 3 over-expression in tumor cells also appeared statistically significantly associated with an increased risk of death, the adjusted HR was estimated to 10.38 (95%CI [2.70; 39.87], P=0.001) (Fig.2D). These results clearly show that the FGFR1 and α v β 3 integrin over-expression in tumor cells were closely associated with worse survival in patients with a glioblastoma.

The results of the invention confirm the necessity to target α v β 3 and FGFR1 pathway including ILK in the treatment of glioblastoma patients, and suggest that the study of the expression α v β 3 integrin and FGFR1 could be performed in patients included in clinical trials associating inhibitors of these proteins with radiotherapy, in order to better select patients who could benefit from these treatments.

REFERENCES:

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

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15 that aid in the clinical assessment of patients with malignant glioma. *Int J Oncol*. 2012 Jun;40(6):1995-2003. doi: 10.3892/ijo.2012.1355. Epub 2012 Feb 3.

CLAIMS:

1. A method for predicting the survival time of a patient suffering from a glioblastoma comprising the steps consisting of i) determining the level expression of at least one
5 marker selected in the group consisting of FGFR1, β 3 integrin and ILK in a tumor tissue sample from said patient, ii) comparing said level expression with a predetermined reference value and iii) providing a good prognosis of the survival time when the level expression is lower than the predetermined reference value and a poor prognosis of the survival time when the level expression is higher than the
10 predetermined reference value.
2. A method according to the claim 1 wherein the patient suffering from a glioblastoma is treated by radio-chemotherapy.
3. The method according to claims 1 or 2 wherein the level expression of FGFR1, β 3 integrin and ILK are determined.
- 15 4. The method according to claims 1 to 3 wherein tumor tissue sample is selected from the group consisting of a resected glioblastoma, or a biopsy from glioblastoma.
5. The method according to claims 1 to 4 wherein the level expression of the markers is evaluated by immunohistochemistry.
- 20 6. A method for predicting the time to progression of a patient affected with a glioblastoma, comprising the steps consisting of i) determining the level expression of at least one marker selected in the group consisting of FGFR1 or FAK in a tumor tissue sample from said patient, ii) comparing said level expression with a predetermined reference value and iii) providing a good prognosis for time to
25 progression when the level expression of FGFR1 is lower than the predetermined reference value or when the level expression of FAK is higher than the predetermined reference value and a poor prognosis for time to progression when the level expression of FGFR is higher than the predetermined reference value or when the level expression of FAK is lower than the predetermined reference value.
- 30 7. A method according to the claim 6 wherein the patient suffering from a glioblastoma is treated by radio-chemotherapy.

8. A kit for performing the method according to claim 1 or claim 6 which comprises means for determining the level of FGFR1, $\beta 3$ integrin, ILK or FAK expression in a tumor tissue sample.

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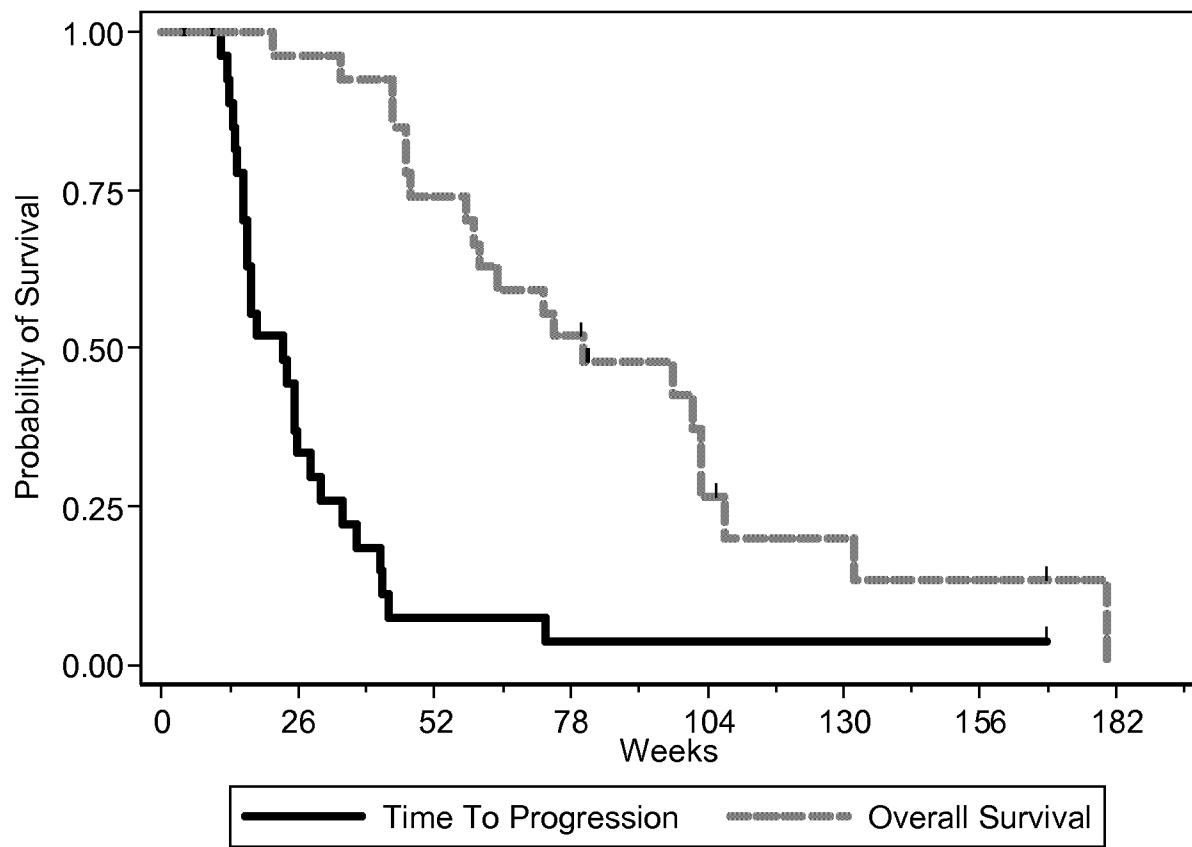


Figure 1

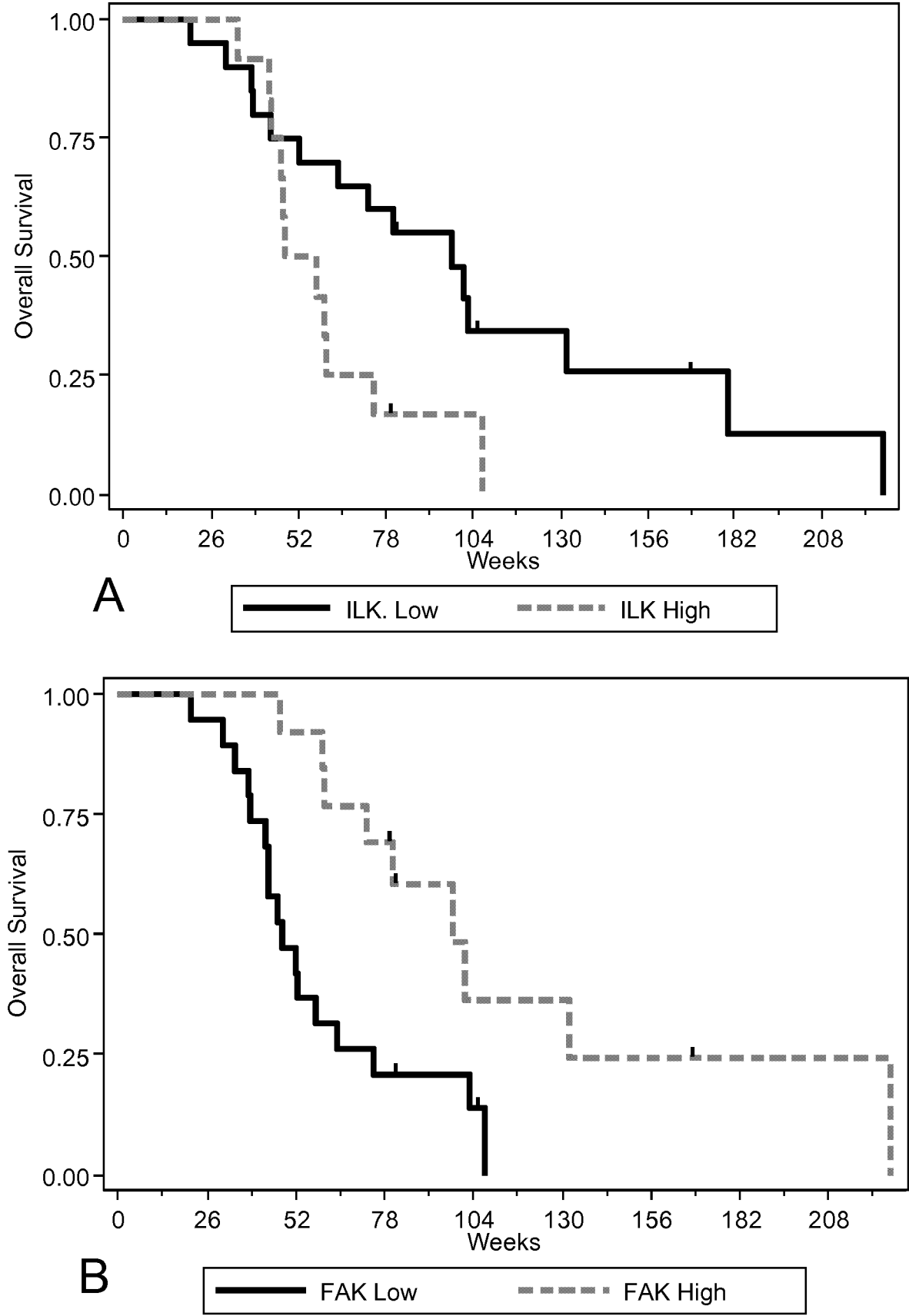


Figure 2 A and B

3/3

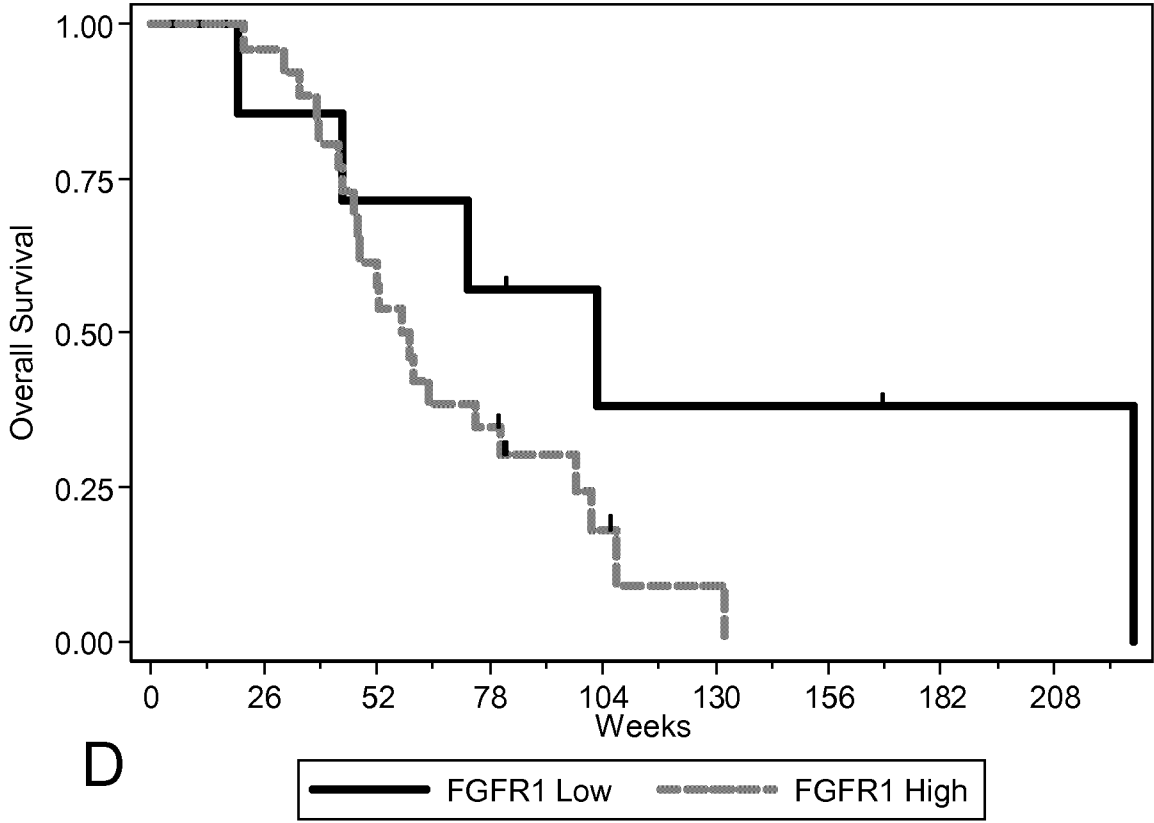
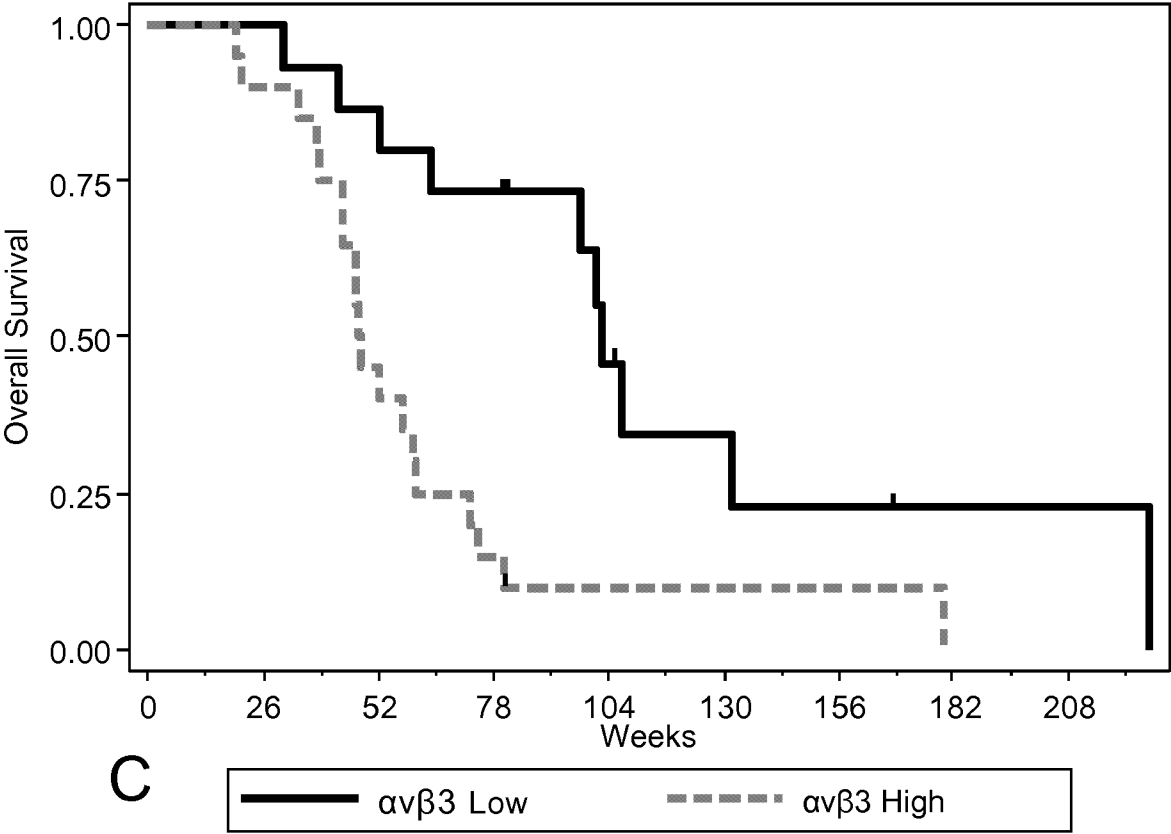


Figure 2 C and D

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2013/066695

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, EMBASE, 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	SANTOSH VANI ET AL: "Grade-Specific Expression of Insulin-like Growth Factor-Binding Proteins-2,-3, and-5 in Astrocytomas: IGFBP-3 Emerges as a Strong Predictor of Survival in Patients with Newly Diagnosed Glioblastoma", CANCER EPIDEMIOLOGY BIOMARKERS & PREVENTION, vol. 19, no. 6, June 2010 (2010-06), pages 1399-1408, XP002684141, ISSN: 1055-9965 p. 1400, col. 1, lines 44 - 52, p. 1407, col. 1, line 13 - col. 2, line 2 -----	1-8
A	EP 2 330 218 A1 (EUROPATH BIOSCIENCES S L [ES]) 8 June 2011 (2011-06-08) par. [0024], claims, fig. 1, 2, 4 ----- -/--	1-8



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

3 September 2013

Date of mailing of the international search report

16/09/2013

Name and mailing address of the ISA/

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Hoesel, Heidi

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2013/066695

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	CN 102 147 410 A (UNIV JILIN) 10 August 2011 (2011-08-10) abstract -----	8
A	US 2011/286923 A1 (SATCHI-FAINARO RONIT [IL] ET AL) 24 November 2011 (2011-11-24) par 19, 20 -----	1-5,8

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INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2013/066695

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GLADSON C L ET AL: "Glioblastoma expression of vitronectin and the alpha v beta 3 integrin. Adhesion mechanism for transformed glial cells.", THE JOURNAL OF CLINICAL INVESTIGATION DEC 1991, vol. 88, no. 6, December 1991 (1991-12), pages 1924-1932, XP002688215, ISSN: 0021-9738 abstract, p. 1926, col. 2, 2nd par., p. 1931, col. 1, lines 14 - 22 -----	1-5,8
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INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2013/066695

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GUTENBERG A ET AL: "Expression of tyrosine kinases FAK and Pyk2 in 331 human astrocytomas", ACTA NEUROPATHOLOGICA, vol. 108, no. 3, September 2004 (2004-09), pages 224-230, XP002688219, ISSN: 0001-6322 abstract, p. 229, lines 3 - 7 -----	8

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2013/066695

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-8(partially)

method for predicting survival time/time to progression of a patient suffering from a glioblastoma by measuring the level of expression of FGFR1, and kit comprising means for determining the level of FGFR1

2-3. claims: 1-5, 8(all partially)

method for predicting survival time of a patient suffering from a glioblastoma by measuring the level of expression of (2) beta-3-integrin, or (3) ILK, and kit comprising means for determining the level of (2) beta-3-integrin, or (3) ILK

4. claims: 6-8(partially)

method for predicting the time to progression of a patient suffering from a glioblastoma by measuring the level of expression of FAK, and kit comprising means for determining the level of FAK

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2013/066695

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