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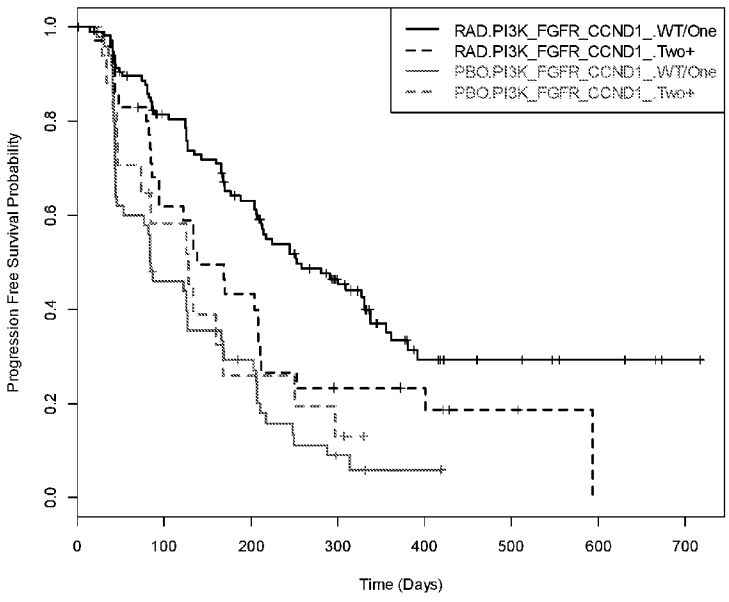
[Continued on next page]

(54) Title: MARKERS ASSOCIATED WITH MTOR INHIBITION

(57) Abstract: The present invention relates to methods for predicting sensitivity of a cancer patient for treatment with an mTOR inhibitor wherein minimal genetic alterations in the PI3K pathway and FGFR pathways and CCND1 are indicative of a higher benefit from mTOR therapy.

FIGURE 1



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MARKERS ASSOCIATED WITH MTOR INHIBITION

[001] Cancer is a highly heterogeneous disease. Until the last decade, most therapies have been developed based on disease, not on underlying molecular causes. However, development of targeted therapies requires testing in targeted populations matched to a drug`s mechanism of action. There is a need for identification of biomarkers that enable prediction of responses to cancer drugs.

[002] The present invention provides biomarkers that help identify patients that respond well to treatment with an mTOR inhibitor such as everolimus and thus enable patient derive higher benefit from therapy with an mTOR inhibitor such as everolimus.

[003] In accordance with one aspect of the present invention, it has now been found that patients which have minimal genetic alterations in the PI3K pathway and FGFR genes and CCND1 gene or activities, such as e.g. expression, of said genes show a good response to everolimus therapy and derive higher benefit from everolimus therapy. Furthermore, it has now been found in accordance with the present invention that patients with no genetic alterations in the PI3K pathway and FGFR genes or PI3K pathway and CCND1 gene show a good response to everolimus therapy and derive higher benefit from everolimus therapy.

[004] In one aspect, the present invention provides a method of predicting the sensitivity of a cancer patient for treatment with an mTOR inhibitor, the method comprising: a) obtaining a cancer sample from a cancer patient, b) analyzing genetic alterations of PI3K and FGFR pathways and CCND1 for genetic alterations in the cancer sample obtained from the patient.

[005] The terms "cancer sample" can be used generally to refer to a sample of any type which contains cells or products that have been secreted from cells to be evaluated by the method of the invention, including but not limited to, a sample of isolated cells, a tissue sample or a bodily fluid sample. A sample of isolated cells is a specimen of cells, typically in suspension or separated from connective tissue which may have connected the cells within a tissue in vivo, which have been collected from an organ, tissue or fluid by any suitable method which results in the collection of a suitable number of cells for evaluation by the method of the invention. The cells in the cell sample are not necessarily of the same type, although purification methods can be used to enrich for the type of cells that are preferably evaluated. Cells can be obtained, for example, by scraping of a tissue, processing of a tissue sample to release individual cells, or isolation from a bodily fluid.

[006] A “tissue sample”, although similar to a sample of isolated cells, is defined herein as a section of an organ or tissue of the body which typically includes several cell types, optionally with cytoskeletal structures that hold the cells together. The term "tissue sample" may be used, in some instances, interchangeably with a "cell sample", although term "tissue sample" may more often used to designate a more complex structure than a cell sample. A tissue sample can be obtained by a biopsy, for example, including by cutting, slicing, or a punch.

[007] A “bodily fluid sample”, like the tissue sample, contains the cells to be evaluated, and is a fluid obtained by any method suitable for the particular bodily fluid to be sampled. Bodily fluids suitable for sampling include, but are not limited to, blood, mucous, seminal fluid, saliva, sputum, bronchial lavage, breast milk, bile and urine.

[008] Minimal genetic variations in the PI3K pathway and FGFR genes and CCND1 gene or no genetic variations in the PI3K pathway and FGFR genes or in the PI3K pathway and CCND1 gene, are indicative of higher benefit from therapy with mTOR inhibitor, such as everolimus. Such benefit is for instance seen in higher clinical efficacy such as e.g. longer PFS or longer OS. The term “minimal genetic variations” refers to few genetic variations in the mentioned genes or the genes of the mentioned pathways, for instance 3 or 2 or 1 or no genetic variations, as compared to the corresponding wt genes. Genetic variations include for instance alterations or mutations as described below. Typically, patients with “minimal genetic variations” have either no variation (i.e. wild-type) or only one genetic variation in the mentioned gene as compared to the two or more genetic variations in these genes.

[009] Preferably, patients with minimal genetic variations have 1 or no genetic alteration in the genes of the PI3K and FGFR pathways and CCND1. In one preferred embodiment, the patient has no PI3K pathway activation and normal PI3K activity. In one preferred embodiment, the patients have no genetic alterations of PI3K pathway and CCND1 or no genetic alterations of PI3K pathway and FGFR genes. In another preferred embodiment of the present invention, patients with minimal genetic variations have no mutation in PI3K pathway and no amplification/mutations in FGFR1/2 genes and no amplification in CCND1 gene.

[0010] In another embodiment, patients with minimal genetic variation have a single mutation in one of the following: PI3K pathway or FGFR1/2 genes or CCND1 gene. In one preferred embodiment, patients with minimal genetic alterations have a wt CCND1 gene. In another preferred embodiment, patients with minimal genetic alterations have no alterations in the PI3K pathway and a wt CCND1 gene. In another embodiment, patients with minimal genetic alterations have no alterations in the PI3K pathway and no alterations in the FGFR1/2 genes. In

another preferred embodiment, patients with minimal genetic alterations have one genetic alteration in the PI3K pathway and a wt CCND1 gene. The genetic alteration is preferably a mutation in any exon of the PIK3C2A gene, in particular in exons 1, 5, 7, 9, 20 or a loss of function mutation of the PTEN gene (particularly exons 5 or 8). Such patients preferably have wt FGFR genes. In another preferred embodiment, patients with minimal genetic alterations have one genetic alteration in the PI3K pathway and no genetic alterations in the FGFR1/2 genes. The genetic alteration is preferably a mutation in any exon of the PIK3C2A gene, in particular in exons 1, 5, 7, 9, 20 or a loss of function mutation of the PTEN gene (particular exons 5 or 8). Such patients preferably have wt CCND1 gene. In another preferred embodiment, patients with minimal genetic alterations have no genetic alterations in the PI3K pathway and the FGFR genes and the CCND1 gene.

[0011] In one preferred embodiment, the genetic alteration in the PI3K pathway does not lead to an activated PI3K pathway. An activated PI3K pathway in accordance with the present invention is can be judged by the presence of oncogenic PIK3C2A mutations and low PTEN activity. In a preferred embodiment, normal PI3K pathway activity (i.e. not activated PI3K pathway) refers to PI3K pathway (normal) is defined as no mutation in any exons of PIK3Ca gene, in particular in exons 1, 5, 7, 9, 20, and no loss of function mutations in PTEN gene.

[0012] In one embodiment, patients with minimal genetic alterations have a mutation/amplification in the FGFR1 or FGFR2 gene and no genetic alteration in the PI3K pathway, in particular no mutation in any exon of the PIK3C2A gene, in particular in exons 1, 5, 7, 9, 20 and no a loss of function mutation of the PTEN gene (particular exons 5 or 8). Such patients preferably have wt CCND1 gene.

[0013] In another embodiment, patients with minimal genetic alterations have a mutation/amplification in the CCND1 gene and no genetic alteration in the PI3K pathway, in particular no mutation in any exon of the PIK3C2A gene, in particular in exons 1, 5, 7, 9, 20 and no a loss of function mutation of the PTEN gene (particular exons 5 or 8). Such patients preferably have wt FGFR1/2 genes.

[0014] The PI3K pathway is well known in the art and described in the literature (see for instance Hemmings and Restuccia: Cold Spring Harb Perspect Biol 2012; doi: 10.1101/cshperspect.a011189). The PI3K/AKT/mTOR pathway is an intracellular signalling pathway important in apoptosis and hence cancer e.g. breast cancer. It is activated by IGF-1 and has a number of downstream effects which either promote protein synthesis or inhibit protein breakdown. In the context of the present application, genetic alterations or mutations in the PI3K pathway refer in particular to mutations or alterations (i.e. changes as compared to the

wild-type form) in any of the exons of the PIK3C2A (HGNC:8971) gene, in particular in exons 1, 5, 7, 9, 20, and no loss of function mutations in the PTEN (HGNC:9588) gene (in particular PTEN mutations in exons 5 to 8). Mutations and alterations in the genes PI3K genes and loss of function mutations PTEN genes are known in the art (for instance in the COSMIC database at http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/) and/or can be readily detected by the skilled person, for instance by sequencing technologies.

[0015] A “loss of function” (LOF) mutation is a mutation or allele of a gene, the result of which is that the gene product (such as the encoded protein) has less than normal or no function in a cell or organism (including a human cell or human being). When the allele has a complete loss of function (null allele) it is often called an amorphic mutation. Phenotypes associated with loss of function mutations are often recessive.

[0016] A “substitution” is a mutation that exchanges one base for another e.g. a point mutation (for instance a missense mutation i.e., a change in a single "chemical letter" such as switching an A to a G). Such a substitution could (1) change a codon to one that encodes a different amino acid and cause a small change in the protein produced (for example, sickle cell anemia is caused by a substitution in the beta-hemoglobin gene, which alters a single amino acid in the protein produced; (2) change a codon to one that encodes the same amino acid and causes no change in the protein produced (“silent mutations”); or (3) change an amino-acid-coding codon to a single "stop" codon and cause an incomplete protein (an incomplete protein is usually nonfunctional).

[0017] An “insertion” is a mutation in which extra base pairs are inserted into a place in the DNA.

[0018] An “gene duplication” or “gene amplification” is a mutation or alteration characterized by the production of multiple copies of a particular gene or genes. Gene amplification often occurs in cancer cells.

[0019] A “deletion” is a mutation in which a section of DNA is lost, or deleted.

[0020] A “frameshift” is a mutation caused by insertions or deletions) of a number of nucleotides that is not evenly divisible by three from a DNA sequence. Due to the triplet nature of gene expression by codons, the insertion or deletion can change the reading frame (the grouping of the codons), resulting in a completely different translation from the original. This often generates truncated proteins that result in loss of function.

[0021] It has been found in accordance with the present invention that adding AKT1, mTOR, TSC 1 or 2 mutations to the analysis of the PI3K pathway has negligible effect.

[0022] The FGFR pathway is well known in the art and described in the literature. In the context of the present invention refers, the term FGFR pathway refers in particular to amplifications/mutations of the FGFR1 (HGNC:3688) or FGFR2 (HGNC:3689) genes. Such mutations include known mutations (for instance in the COSMIC database) and/or can be readily detected by the skilled person, for instance by sequencing technologies. Mutations, amplification and overexpression of the CCND1 gene (CyclinD1, HGNC:1582), which alter cell cycle progression, are observed frequently in a variety of tumors and may contribute to tumorigenesis. Such mutations include mutations that are known (see e.g. in the COSMIC database or "Entrez Gene: CCND1 cyclin D1") and/or can be readily detected by the skilled person, for instance by sequencing technologies. In a preferred embodiment, patients have no mutations or amplifications of the FGFR1 or FGFR2 gene. In a preferred emobiment, patients have no amplifications of the FGFR1 or FGFR2 gene. In a preferred embodiment, patients have wild type FGFR genes. In another preferred embodiment, patients have no mutations or amplifications of the CCND1 gene. In a preferred embodiment, patients have no amplifications of the CCND1 gene.

[0023] It has been found in accordance with the present invention that the therapeutic benefit of an mTOR inhibitor such as everolimus in patients with minimal genetic alterations is observed in patients regardless their FGFR1 or FGFR2 genotypes.

[0024] The term “genetic alteration” or “mutation” of genes is well known in the art and includes for instance point mutations, deletions, insertions, duplications, amplifications. Genetic alterations or mutations are for instance described in The Catalogue of Somatic Mutations in Cancer (COSMIC) and the Single Nucleotide Polymorphism Database (dbSNP) at NCBI.

[0025] In a further embodiment, the present invention provides a method of treating a cancer patient with an mTOR inhibitor such as rapamycin or a rapamcine derivative (e.g. everolimus) comprising: a) obtaining a cancer sample from a cancer patient, b) analyzing the cancer samples obtained from the patient for genetic alterations in the PI3K pathway and FGFR genes and CCND1 gene, c) administering an effective amount of the mTOR inhibitor to a patient who has minimal genetic alterations, such as no or 1 genetic alteration, in the PI3K and FGFR pathways and CCND1.

[0026] In one embodiment, the present invention provides a method of treating a cancer patient with an mTOR inhibitor such as rapamycin or a rapamcine derivative (e.g. everolimus) comprising: a) obtaining a cancer sample from a cancer patient, b) analyzing the cancer samples obtained from the patient for genetic alterations in the PI3K pathway and FGFR genes or PI3K pathway and CCND1 gene, c) administering an effective amount of the mTOR inhibitor

to a patient who has no genetic alterations in the PI3K pathway and FGFR genes or PI3K pathway and CCND1 gene.

[0027] In another embodiment, the present invention provides a method of treating a cancer patient with an mTOR inhibitor such as rapamycin or a rapamcine derivative (e.g. everolimus) comprising: a) obtaining a cancer sample from a cancer patient, b) analyzing the cancer samples obtained from the patient for genetic alterations in the PI3K and FGFR genes or PI3K pathway and CCND1 gene, c) administering an effective amount of the mTOR inhibitor to a patient who has no genetic alterations in the PI3K pathway and FGFR genes or PI3K pathway and CCND1 gene.

[0028] In one embodiment, the present invention provides an mTOR inhibitor such as rapamycin or a rapamcine derivative (e.g. everolimus) for use in the treatment of a cancer patient with an mTOR inhibitor wherein the patient is selected on the basis of: a) analyzing the cancer samples obtained from the patient for genetic alterations in the PI3K pathway and FGFR genes and CCND1 gene, b) selecting the patient who has minimal genetic alterations, such as no or 1 genetic alteration, in the PI3K pathway and FGFR genes and CCND1 gene.

[0029] In another embodiment the present invention provides an mTOR inhibitor such as rapamycin or a rapamycine derivative (e.g. everolimus) for use in the treatment of a cancer in a patient, wherein the patient is selected on the basis of: a) analyzing the cancer samples obtained from the patient for genetic alterations in the PI3K pathway and FGFR genes or PI3K pathway and CCND1 gene; b) selecting the patient who has no genetic alteration in the PI3K pathway and FGFR genes or in the PI3K pathway and CCND1 gene.

[0030] The cancer patients in the above embodiments of the present invention are preferably breast cancer patients, more preferably hormone receptor-positive (HR+) HER2-negative (HER2-) advanced breast cancer patient.

[0031] In one embodiment the present invention provides pharmaceutical compositions comprising an mTOR inhibitor for use in the treatment of cancer in a patient, wherein the patient is selected on the basis of showing minimal genetic alterations, such as no or 1 genetic alteration, in the PI3K pathway and FGFR genes and CCND1, and wherein the minimal genetic alterations correlates with the patient having higher benefit from mTOR therapy, such as for instance longer PFS or longer OS.

[0032] In another embodiment the present invention provides a pharmaceutical composition comprising an mTOR inhibitor for use in the treatment of cancer in a patient, wherein the patient is selected on the basis of no genetic alteration in the PI3K pathway and FGFR genes or in the PI3K pathway and CCND1 gene, and wherein the absence of genetic

alterations correlates with the patient having higher benefit from mTOR therapy, such as longer PFS or longer OS.

[0033] In a preferred embodiment, the pharmaceutical compositions are for use in a breast cancer patient, preferably a hormone receptor-positive (HR+) HER2-negative (HER2-) advanced breast cancer patient.

[0034] In a preferred embodiment, the mTOR inhibitor of the pharmaceutical compositions is rapamycin or a rapamycin derivative, preferably temsirolimus or everolimus, most preferably everolimus. In another preferred embodiment, the mTOR inhibitor is everolimus wherein everolimus is administered in a dose from 2 to 20 mg daily, preferably 2.5 mg, 5 mg, 7.5 mg or 10 mg daily.

[0035] In one embodiment, the present invention provides a kit for predicting the sensitivity of a cancer patient for treatment with an mTOR inhibitor comprising
i) means for detecting minimal genetic alterations, such as no or 1 genetic alteration, in the PI3K and FGFR pathways and CCND1; and ii) instructions how to use said kit.

[0036] In another embodiment, the present invention provides a kit for predicting the sensitivity of a cancer patient for treatment with an mTOR inhibitor comprising i) means for detecting no genetic alteration in PI3K pathway and FGFR genes or no genetic alteration in PI3K pathway and CCND1 gene; and ii) instructions how to use said kit.

[0037] Kits in accordance with the present invention are for instance useful for diagnostic methods (e.g. as companion diagnostic) to select patients, e.g. breast cancer patients, that have better responses to treatments with an mTOR inhibitor such as rapamycin or a rapamycin derivative, preferably temsirolimus or everolimus, more everolimus.

[0038] A kit according to the present invention is preferably used for breast cancer patients, more preferably for hormone receptor-positive (HR+) HER2-negative (HER2-) advanced breast cancer patients. The cancer patients are treated with an mTOR inhibitor, preferably rapamycin or a rapamycin derivative, more preferably temsirolimus or everolimus, most preferably everolimus.

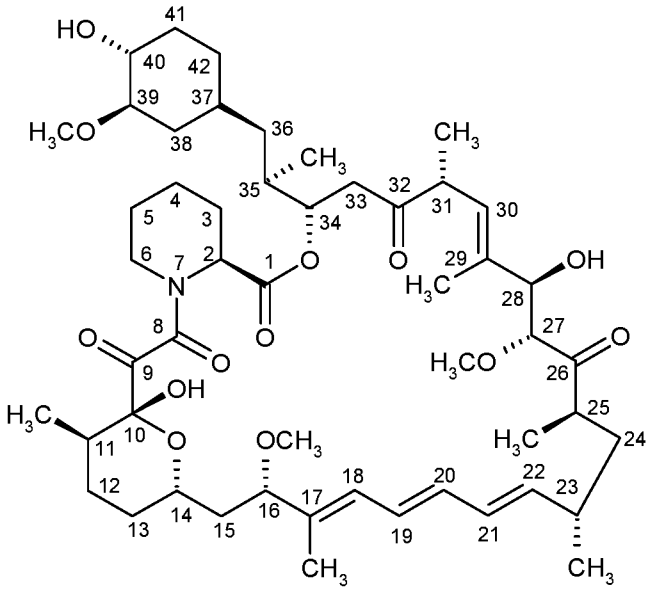
[0039] In one embodiment, the present invention provides an mTOR inhibitor for use in the treatment of a hormone receptor-positive (HR+) HER2-negative (HER2-) advanced breast cancer patient, wherein the patient has minimal genetic alterations, such as no or 1 genetic alteration, in the PI3K pathway and FGFR genes and CCND1. In another embodiment, the present invention provides an mTOR inhibitor for use in the treatment of a hormone receptor-positive (HR+) HER2-negative (HER2-) advanced breast cancer patient, wherein the patient has no genetic alteration in the PI3K pathway and FGFR genes or in the PI3K pathway and CCND1

gene. Patients with minimal genetic alterations in the PI3K pathway and FGFR genes and CCND1 gene or patients with no genetic alterations in the PI3K pathway and FGFR genes or in the PI3K pathway and CCND1 gene for instance show a good response to everolimus therapy and derive higher benefit from everolimus therapy, such as for instance longer PFS or OS.

[0040] In one embodiment, the present invention provides uses of PI3K pathway and FGFR genes and CCND1 gene as a biomarker for prediction of sensitivity of a breast cancer patient, preferably a hormone receptor-positive (HR+) HER2-negative (HER2-) advanced breast cancer patient, to treatment with an mTOR inhibitor such as rapamycin or a rapamycin derivative, preferably temsirolimus or everolimus, most preferably everolimus. Minimal genetic alterations in the PI3K pathway and FGFR genes and CCND1 gene or no genetic alterations in the PI3K pathway and FGFR genes or in the PI3K pathway and CCND1 gene are predictive of a good response to everolimus therapy and derive higher benefit from everolimus therapy, such as for instance longer PFS or OS.

[0041] An mTOR inhibitor as used herein is a compound which targets intracellular mTOR ("mammalian Target of rapamycin"). mTOR is a family member of phosphatidylinositol 3-kinase(P13-kinase) related kinase. The compound rapamycin and other mTOR inhibitors inhibit mTOR activity via a complex with its intracellular receptor FKBP12 (FK506-binding protein 12). mTOR modulates translation of specific mRNAs via the regulation of the phosphorylation state of several different translation proteins, mainly 4E-PB1, P70S6K (p70S6 kinase 1) and eEF2.

[0042] Rapamycin (sirolimus) is a known macrolide antibiotic produced by Streptomyces hygroscopicus of formula



[0043] Other mTOR inhibitors include rapamycin derivatives, for example including rapamycin substituted in position 40 and/or 16 and/or 32. Examples of other mTOR inhibitors include 40-O-alkyl-rapamycin derivatives, e.g. 40-O-hydroxyalkyl-rapamycin derivatives, for example 40-O-(2-hydroxy)-ethyl-rapamycin (everolimus), rapamycin derivatives which are substituted in 40 position by heterocyclyl, e.g. 40-epi-(tetrazolyl)-rapamycin (also known as ABT578), 32-deoxo-rapamycin derivatives and 32-hydroxy-rapamycin derivatives, such as 32-deoxorapamycin, 16-O-substituted rapamycin derivatives such as 16-pent-2-ynyloxy-32-deoxorapamycin, 16-pent-2-ynyloxy-32(S or R) -dihydro-rapamycin, or 16-pent-2-ynyloxy-32(S or R)-dihydro-40-O-(2-hydroxyethyl)-rapamycin, rapamycin derivatives which are acylated at the oxygen in position 40, e.g. 40-[3-hydroxy-2-(hydroxy-methyl)-2-methylpropanoate]-rapamycin (also known as CCI779 or temsirolimus), rapamycin derivatives (also sometimes designated as rapalogs) as disclosed in WO9802441 or WO0114387, e.g. including AP23573, such as 40-O-dimethylphosphinyl-rapamycin, compounds disclosed under the name biolimus (biolimus A9), including 40-O-(2-ethoxy)ethyl-rapamycin, and compounds disclosed under the name TAFA-93, AP23464, AP23675 or AP23841; or mTOR inhibitors as e.g. disclosed in WO2004101583, WO9205179, WO9402136, WO9402385 and WO9613273.

[0044] Preferred mTOR inhibitors include rapamycin, and/or 40-O-(2-hydroxyethyl)-rapamycin, and/or 32-deoxorapamycin, and/or 16-pent-2-ynyloxy-32-deoxorapamycin, and/or 16-pent-2-ynyloxy-32 (S or R) -dihydro-rapamycin, and/or 16-pent-2- ynyloxy-32 (S orR)-dihydro-40-0- (2-hydroxyethyl)-rapamycin, and/or 40- [3-hydroxy-2- (hydroxy- methyl)-2-methylpropanoate]-rapamycin (also known as CCI779 or temsirolimus) and/or 40-epi-(tetrazolyl)- rapamycin (also known as ABT578, Zotarolimus), and/or the so-called rapalogs, e. g. as disclosed in WO9802441, WO0114387 and WO0364383, AP23573 (Ridaforolimus), AP23464, AP23675 or AP23841, e.g. AP23573, and/or compounds disclosed under the name TAFA-93, and/or compounds disclosed under the name biolimus. Particularly preferred are rapamycin and rapamycin derivatives which are approved as cancer treatments such as temsirolimus and everolimus. The most preferred mTOR inhibitor is everolimus.

Examples

[0045] In a large pivotal clinical trial, it has been found that everolimus (EVE) plus exemestane (EXE) more than doubled progression-free survival (PFS) while maintaining quality

of life vs EXE alone in postmenopausal women with hormone receptor–positive (HR+), HER2-negative (HER2–) advanced breast cancer (BOLERO-2 phase 3; NCT00863655). PFS benefit was seen in all clinically defined subgroups.

[0046] Definition of genetic alterations as used for the present analysis: for both oncogene (OG) and tumor suppressor gene (TSG), any somatic variations with known or inferred functional impacts on OG or TSG activities, regardless their allele frequencies, are qualified. Selection of covariates as used for the present analysis: Clinical and demographic features that are associated with PFS within a treatment arm. Selections of genes/cluster of genes for correlative analysis as used for the present analysis: Single gene: alteration rate>10% in the NGS population; Cluster: by functional similarities or gene class. Statistical methods as used for the present analysis: Cox proportional hazards models applied to variant and pathway info; Multigene models assessed using backwards elimination Cox models - Kaplan – Meier curves and Forest plots used to visualize data.

[0047] Methods: Exon sequence and gene copy number variations are analyzed in 182 cancer-related genes by next-generation sequencing (NGS). Correlations with PFS are evaluated using univariate and multivariate Cox models: No match in COSMIC and dbSNP.

[0048] Results: NGS data (>250x coverage) are successfully generated from archival tumor specimens from 227 patients (NGS population, 157 in EVE+EXE arm and 70 in EXE arm) whose baseline characteristics and clinical outcome are comparable to the trial population (PFS HR = 0.40 and 0.45, respectively). The treatment benefit of EVE+EXE over EXE is maintained in the subgroups defined by each of the 9 genes with a mutation rate >10% (eg, PIK3CA, FGFR1, CCND1) or when less frequently mutated genes (eg, PTEN, AKT1) are included in their respective pathways. Patients with 0 or 1 genetic alteration in PI3K or FGFR pathways or CCND1 have a greater treatment effect from EVE (HR = 0.27, 95% CI 0.18-0.41, adjusted by covariates, in 76% of the NGS population), indicating the value of these pathways for predicting sensitivity to EVE in this setting.

[0049] Conclusions: This is the first global registration trial in which efficacy-predictive biomarkers were explored by correlating broad genetic variations with clinical efficacy. The results suggest that a large subgroup of patients (76%), defined by minimal genetic variations in the PI3K or FGFR pathways or CCND1, derives the most benefit from EVE therapy (HR = 0.27 vs 0.40 for the full NGS population).

Summary of Preferred Embodiments

[0050] Certain aspects, features and embodiments of the present disclosure are summarized in the following items and can be used respectively alone or in combination:

A method of predicting sensitivity of a cancer patient for treatment with an mTOR inhibitor, the method comprising the steps of:
a) obtaining a cancer sample from a cancer patient
b) analyzing the cancer samples obtained from the patient for genetic alterations in the PI3K pathway and FGFR genes and CCND1 gene wherein minimal genetic alterations, such as no or 1 genetic alteration, in the PI3K pathway and FGFR pathways and CCND1 is indicative of higher benefit from mTOR therapy, such as longer PFS or longer OS.

A method according to claim 1wherein the patient has a single mutation or amplification in the PI3K pathway or FGFR1/2 genes or CCND1 gene.

A method according to claims 1 or 2 wherein the patient has 1 genetic alteration in any of the exons of PIK3C2A gene selected from exons 1, 5, 7, 9, 20 and combinations thereof and no loss of function mutations in PTEN gene or no genetic alteration in any of the exons of the PIK3C2A gene, selected from exons 1, 5, 7, 9, 20 and combination thereof and a loss of function mutation in the PTEN gene.

A method according to claims 1 or 2 wherein the patient has a genetic alteration selected from a mutation or amplification in the FGFR genes.

A method according to claims 1 or 2 wherein the patient has a genetic alteration selected from a mutation or amplification in the CCND1 gene.

A method according to claim 1 wherein the patient has no genetic alteration in the PI3K pathway, in particular no genetic alteration in any of the exons of the PIK3C2A gene, selected from any of exons 1, 5, 7, 9, 20 and combinations thereof and no loss of function mutations in PTEN gene, and no genetic alteration in the FGFR1/2 genes and no genetic alteration in the CCND1 gene.

A method of predicting sensitivity of a cancer patient for treatment with an mTOR inhibitor, the method comprising the steps of:

a) obtaining a cancer sample from a cancer patient
b) analyzing the cancer samples obtained from the patient for genetic alterations in the PI3K pathway and FGFR genes or PI3K pathway and CCND1 gene wherein no genetic alteration in PI3K pathway and CCND1 gene or no genetic alteration in PI3K pathway and FGFR genes is indicative of higher benefit from mTOR therapy, such as longer PFS or longer OS.

A method according to claims 1 or 7wherein the cancer patient is a breast cancer patient, preferably a hormone receptor-positive (HR+) HER2-negative (HER2-) advanced breast cancer patient.

A method according to claims 1 or 7wherein the mTOR inhibitor is rapamycin or a rapamycin derivative, preferably temsirolimus or everolimus, most preferably everolimus.

A method of treating a cancer patient with an mTOR inhibitor comprising the steps of:
a) obtaining a cancer sample from a cancer patient
b) analyzing the cancer samples obtained from the patient for genetic alterations in the PI3K pathway and FGFR genes and CCND1 gene
c) administering an effective amount of the mTOR inhibitor to a patient who has minimal genetic alterations selected from no or 1 genetic alteration, in the PI3K pathway and FGFR genes and CCND1 gene.

A method of treating a cancer patient with an mTOR inhibitor comprising the steps of:
a) obtaining a cancer sample from a cancer patient
b) analyzing the cancer samples obtained from the patient for genetic alterations in the PI3K and FGFR genes or PI3K pathway and CCND1 gene
c) administering an effective amount of the mTOR inhibitor to a patient who has no genetic alterations in the PI3K pathway and FGFR genes or PI3K pathway and CCND1 gene.

A mTOR inhibitor for use in treatinga cancer in a patient, wherein the patient is selected for treating on the basis of:
a) analyzing cancer samples obtained from apatient for genetic alterations in the PI3K pathway and FGFR genes and CCND1 gene;
b) selecting the patient who has minimal genetic alterations selected from no or 1 genetic alteration, in the PI3K pathway and FGFR genes and CCND1 gene.

A mTOR inhibitor for use in treatinga cancer in a patient, wherein the patient is selected for treating on the basis of:
a) analyzing the cancer samples obtained from apatient for genetic alterations in the PI3K pathway and FGFR genes or PI3K pathway and CCND1 gene;
b) selecting the patient who exhibits no genetic alteration in the PI3K pathway and FGFR genes or in the CCND1 gene.

A method according to claims10, 11, 12 or 13 wherein the cancer patient is a breast cancer patient, preferably a hormone receptor-positive (HR+) HER2-negative (HER2-) advanced breast cancer patient.

A method according to claims10, 11, 12, 13 or 14 wherein the mTOR inhibitor is rapamycin or a rapamycin derivative, preferably temsirolimus or everolimus, most preferably everolimus.

A pharmaceutical composition comprising an mTOR inhibitor for use in treatingcancer in a patient, wherein the patient is selected on the basis of showing minimal genetic alterations selected from no or 1 genetic alteration, in the PI3K pathway and FGFR genes and CCND1, and wherein the minimal genetic alterations correlates with the patient having higher benefit from mTOR therapy, such as longer PFS or longer OS.

A pharmaceutical composition comprising an mTOR inhibitor for use in treatingcancer in a patient, wherein the patient is selected on the basis of no genetic alteration in the PI3K pathway and FGFR genes or PI3K pathway and CCND1 gene, and wherein the absence of genetic alterations correlates with the patient having higher benefit from mTOR therapy, such as longer PFS or longer OS.

A pharmaceutical composition according to claims16 or 17 wherein the cancer patient is a breast cancer patient, preferably a hormone receptor-positive (HR+) HER2-negative (HER2-) advanced breast cancer patient.

A pharmaceutical composition according to claims16, 17 or 18 wherein the mTOR inhibitor is rapamycin or a rapamycin derivative, preferably temsirolimus or everolimus, most preferably everolimus.

A pharmaceutical composition according to claims16, 17, 18 or 19 wherein everolimus is administered in a dose from 2 to 20 mg daily, preferably 2.5 mg, 5 mg, 7.5 mg or 10 mg daily.

A kit for predicting the sensitivity of a cancer patient for treatment with an mTOR inhibitor comprising:
i) means for detecting minimal genetic alterations, such as no or 1 genetic alteration, in the PI3K and FGFR pathways and CCND1; and
ii) instructions how to use said kit.

A kit for predicting the sensitivity of a cancer patient for treatment with an mTOR inhibitor comprising:
i) means for detecting no genetic alteration in PI3K pathway and FGFR genes or no genetic alteration in PI3K pathway and CCND1 gene; and
ii) instructions how to use said kit.

A kit according to claims21 or 22 wherein the kit comprises means for detecting genetic alterations in any of the exons of the PIK3C2A gene, selected fromexons 1, 5, 7, 9, 20 and combinations thereof and means for detecting loss of function mutations in PTEN gene.

A kit according to claims21 or 22 wherein the cancer patient is a breast cancer patient, preferably a hormone receptor-positive (HR+) HER2-negative (HER2-) advanced breast cancer patient.

Use of a kit according to claims21 or 22 for the prediction of the response to an mTOR therapy of a breast cancer patient, preferably a hormone receptor-positive (HR+) HER2-negative (HER2-) advanced breast cancer patient.

A kit or use of a kit according to claims 21 or 22 wherein the mTOR inhibitor is inhibitor is rapamycin or a rapamycin derivative, preferably temsirolimus or everolimus, most preferably everolimus.

An mTOR inhibitor for use in treatinga hormone receptor-positive (HR+) HER2-negative (HER2-) advanced breast cancer patient according to claims 1 or 7, wherein the patient has minimal

genetic alterationsselected from no or 1 genetic alteration, in the PI3K pathway and FGFR genes and CCND1.

An mTOR inhibitor for use in treatinga hormone receptor-positive (HR+) HER2-negative (HER2-) advanced breast cancer patient according to claims 1 or 7, wherein the patient has no genetic alteration in the PI3K pathway and FGFR genes or in the PI3K pathway and CCND1 gene.

An mTOR for use according to claims 27 or 28 wherein the the mTOR inhibitor is inhibitor is rapamycin or a rapamycin derivative, preferably temsirolimus or everolimus, most preferably everolimus.

Use of PI3K pathway and FGFR genes and CCND1 gene as a biomarker for prediction of sensitivity of a breast cancer patient, preferably a hormone receptor-positive (HR+) HER2-negative (HER2-) advanced breast cancer patient, to treatment with an mTOR inhibitor such as rapamycin or a rapamycin derivative, preferably temsirolimus or everolimus, most preferably everolimus.

Use of PI3K pathway and FGFR genes or PI3K pathway and CCND1 gene as a biomarker for prediction of sensitivity of a breast cancer patient, preferably a hormone receptor-positive (HR+) HER2-negative (HER2-) advanced breast cancer patient, to treatment with an mTOR inhibitor such as rapamycin or a rapamycin derivative, preferably temsirolimus or everolimus, most preferably everolimus, wherein the absence of genetic alterations in PI3K pathway and CCND1 gene or in PI3K pathway and FGFR genes is indicative of higher benefit from mTOR inhibition, such as rapamycin or a rapamycin derivative, preferably temsirolimus or everolimus, most preferably everolimus, therapy.

Description of the Figures

[0051] **Figure 1:** Superior RAD Therapeutic Benefit in Patients with Minimal Genetic Variation in FGFR/PI3K/CCND1 Pathways. WT/One: No mutation in PI3K AND no amplification/mutations in FGFR1/2 AND no amplification in CCND1 OR A single mutation or amplification in PI3K OR FGFR1/2 OR CCND1. Two+: Two or more mutations or amplification in PI3K OR FGFR1/2 OR CCND1.

[0052] **Figure 2:** Superior RAD Therapeutic Benefit in Patients with Minimal Genetic Variation in FGFR/PI3K/CCND1 Pathways

[0053] **Figure 3** illustrate longer PFS in RAD arm patients with normal PI3K activity

[0054] **Figure 4** illustrates longer PFS in RAD arm patients with wild type FGFR genes

[0055] **Figure 5** illustrates longer PFS in RAD arm patients with wild type CCND1 gene

[0056] **Figure 6** illustrates the association of Common Genetic Alteration with PFS by Treatment-Univariate Analysis

[0057] **Figure 7** shows correlative results: summary of MVA

[0058] **Figure 8:** Genetic Alterations in NGS Population. Overall, the genetic landscape of tumors in BOLERO-2 is largely similar to that previously observed in patients with HR+ breast cancer observed (Cancer Genome Atlas Network. *Nature*. 2012;490:61-70.) In the top table, missense mutations and gene amplifications (ie, 6 or more copies of a genetic sequence) are observed with relatively high frequency In addition, sequence alterations that might lead to altered expression of gene products were observed in a number of samples Rearrangements and bi-allelic deletions were observed infrequently In the lower table, patients have a mean 4.1 known somatic alterations/sample and 219 patients have at least 1 known somatic mutation 104 genes having at least 1 known somatic mutation are identified. Known somatic alterations: genetic alterations matched in COSMIC, but not in dbSNP. Novel alterations: No match in both COSMIC and dbSNP.

[0059] **Figure 9:** BOLERO-2 NGS Subset: Frequency of Genetic Alterations in Key Pathways. Genetic landscape of tumors in BOLERO-2 (with focus on genes used in the correlative analysis and genes altered in at least 4% of the tumors analyzed by NGS). This figure shows a subset of the genetic alterations

observed in the NGS population and includes the most frequently mutated genes used in the correlative analyses and other somatic changes observed in at least 4% of patients PIK3CA was the most frequently altered gene (48.5% of samples) and is an example of a druggable target (ie, drugs targeting the pathway this gene functions within are either already available or in clinical trials) Cell cycle (Cyclin D1), checkpoint genes (TP53), and the surface receptor FGFR1 were among the most frequently altered genes. Other potentially druggable targets/pathways include MDM4, ARID1A, MAP2K4, AKT, and the estrogen receptor (ESR1).

CLAIMS

1. A method for predicting sensitivity of a cancer patient for treatment with an mTOR inhibitor, the method comprising the steps of:
a) obtaining a cancer sample from a cancer patient
b) analyzing the cancer samples obtained from the patient for genetic alterations in the PI3K pathway and FGFR genes and CCND1 gene, wherein minimal genetic alterations selected from no or 1 genetic alteration, in the PI3K pathway and FGFR pathways and CCND1 is indicative of higher benefit from mTOR therapy, such as longer PFS or longer OS.

2. A method according to claim 1 wherein the patient has a single mutation or amplification in the PI3K pathway or FGFR1/2 genes or CCND1 gene.

3. A method according to claim 1 or 2 wherein the patient has 1 genetic alteration in any of the exons of PIK3C2A gene, selected from any of exons 1, 5, 7, 9, 20 and combinations thereof and no loss of function mutations in PTEN gene or no genetic alteration in any of the exons of the PIK3C2A gene, selected from any of exons 1, 5, 7, 9, 20 and combinations thereof and a loss of function mutation in the PTEN gene.

4. A method according to claim 1 or 2 wherein the patient has a mutation or amplification in the FGFR genes.

5. A method according to claim 1 or 2 wherein the patient has a genetic alteration such as a mutation or amplification in the CCND1 gene.

6. A method according to claim 1 wherein the patient has no genetic alteration in the PI3K pathway, in particular no genetic alteration in any of the exons of the PIK3C2A gene, selected from any of exons 1, 5, 7, 9, 20 and combinations thereof and no loss of function mutations in PTEN gene, and no genetic alteration in the FGFR1/2 genes and no genetic alteration in the CCND1 gene.

7. A method for predicting sensitivity of a cancer patient for treatment with an mTOR inhibitor, the method comprising the steps of:

a) obtaining a cancer sample from a cancer patient; and
b) analyzing the cancer samples obtained from the patient for genetic alterations in the PI3K pathway and FGFR genes or PI3K pathway and CCND1 gene, wherein no genetic alteration in PI3K pathway and CCND1 gene or no genetic alteration in PI3K pathway and FGFR genes is indicative of higher benefit from mTOR therapy, such as longer PFS or longer OS.

8. A method according to claim 1 or 7 wherein the cancer patient is a breast cancer patient, preferably a hormone receptor-positive (HR+) HER2-negative (HER2-) breast cancer patient, more preferably a hormone receptor-positive (HR+) HER2-negative (HER2-) advanced breast cancer patient.

9. A method according to claim 1 or 7 wherein the mTOR inhibitor is rapamycin or a rapamycin derivative, preferably temsirolimus or everolimus, most preferably everolimus.

10. A method for treating a cancer patient with an mTOR inhibitor comprising the steps of:
a) obtaining a cancer sample from a cancer patient
b) analyzing the cancer samples obtained from the patient for genetic alterations in the PI3K pathway and FGFR genes and CCND1 gene; and
c) administering an effective amount of the mTOR inhibitor to a patient who has minimal genetic alterations selected from no or 1 genetic alteration, in the PI3K pathway and FGFR genes and CCND1 gene.

11. A method for treating a cancer patient with an mTOR inhibitor comprising:
a) obtaining a cancer sample from a cancer patient
b) analyzing the cancer samples obtained from the patient for genetic alterations in the PI3K and FGFR genes or PI3K pathway and CCND1 gene; and
c) administering an effective amount of the mTOR inhibitor to a patient who has no genetic alterations in the PI3K pathway and FGFR genes or PI3K pathway and CCND1 gene.

12. A method for treating a cancer in a patient using an mTor inhibitor and selected the patient for treating comprising the steps of:
a) analyzing the cancer samples obtained from the patient for genetic alterations in the PI3K pathway and FGFR genes and CCND1 gene; and

b) selecting the patient who has minimal genetic alterations selected from no or 1 genetic alteration, in the PI3K pathway and FGFR genes and CCND1 gene.

13. A method for treating a cancer in a patient using an mTor inhibitor and selected the patient for treating comprising the steps of:
a) analyzing the cancer samples obtained from the patient for genetic alterations in the PI3K pathway and FGFR genes or PI3K pathway and CCND1 gene; and
b) selecting the patient who no genetic alteration in the PI3K pathway and FGFR genes or in the CCND1 gene.

14.The method according to any one of claims 10-13, wherein the patient has no or 1 genetic alteration in the PI3K pathway and FGFR genes or PI3K pathway and CCND1 gene.

15. The method according to any one of claims 10-13 wherein the cancer patient is a breast cancer patient, preferably a hormone receptor-positive (HR+) HER2-negative (HER2-) advanced breast cancer patient.

16. The method according to any one of claims 10-13 wherein the mTOR inhibitor is rapamycin or a rapamycin derivative, preferably temsirolimus or everolimus, most preferably everolimus.

17. A pharmaceutical composition comprising an mTOR inhibitor for use in the treatment of cancer in a patient, wherein the patient is selected on the basis of showing minimal genetic alterations selected from no or 1 genetic alteration, in the PI3K pathway and FGFR genes and CCND1, and wherein the minimal genetic alterations correlates with the patient having higher benefit from mTOR therapy, such as longer PFS or longer OS.

18. A pharmaceutical composition comprising an mTOR inhibitor for use in the treatment of a cancer in a patient, wherein the patient has no or 1 genetic alteration in the PI3K pathway and FGFR genes or PI3K pathway and CCND1 gene.

19. A pharmaceutical composition comprising an mTOR inhibitor for use in the treatment of cancer in a patient, wherein the patient is selected on the basis of no genetic alteration in the

PI3K pathway and FGFR genes or PI3K pathway and CCND1 gene, and wherein the absence of genetic alterations correlates with the patient having higher benefit from mTOR therapy, such as longer PFS or longer OS.

20. A pharmaceutical composition according to claim 17 to 19 wherein the cancer patient is a breast cancer patient, preferably a hormone receptor-positive (HR+) HER2-negative (HER2-) advanced breast cancer patient.

21. A pharmaceutical composition according to claim 17 to 19 wherein the mTOR inhibitor is rapamycin or a rapamycin derivative, preferably temsirolimus or everolimus, most preferably everolimus.

22. A pharmaceutical composition according to claim 17 to 19 wherein everolimus is administered in a dose from 2 to 20 mg daily, preferably 2.5 mg, 5 mg, 7.5 mg or 10 mg daily.

23. A kit for predicting the sensitivity of a cancer patient for treatment with an mTOR inhibitor comprising
i) means for detecting minimal genetic alterations, such as no or 1 genetic alteration, in the PI3K and FGFR pathways and CCND1; and
ii) instructions how to use said kit.

24. A kit for predicting the sensitivity of a cancer patient for treatment with an mTOR inhibitor comprising
i) means for detecting no genetic alteration in PI3K pathway and FGFR genes or no genetic alteration in PI3K pathway and CCND1 gene; and
ii) instructions how to use said kit.

25. A kit according to claim 23 or 24 wherein the kit comprises means for detecting genetic alterations in any of the exons of the PIK3C2A gene, in particular in any of exons 1, 5, 7, 9, 20 and means for detecting loss of function mutations in PTEN gene.

26. A kit according to claim 23 or 24 wherein the cancer patient is a breast cancer patient, preferably a hormone receptor-positive (HR+) HER2-negative (HER2-) advanced breast cancer patient.

27. Use of a kit according to claim 23 or 24 for the prediction of the response to an mTOR therapy of a breast cancer patient, preferably a hormone receptor-positive (HR+) HER2-negative (HER2-) advanced breast cancer patient.

28. A kit or use of a kit according to claim 23 or 24 wherein the mTOR inhibitor is inhibitor is rapamycin or a rapamycin derivative, preferably temsirolimus or everolimus, most preferably everolimus.

29. An mTOR inhibitor for use in the treatment of a hormone receptor-positive (HR+) HER2-negative (HER2-) advanced breast cancer patient, wherein the patient has minimal genetic alterations selected from no or 1 genetic alteration, in the PI3K pathway and FGFR genes and CCND1.

30. An mTOR inhibitor for use in the treatment of a hormone receptor-positive (HR+) HER2-negative (HER2-) advanced breast cancer patient, wherein the patient has no genetic alteration in the PI3K pathway and FGFR genes or in the PI3K pathway and CCND1 gene.

31. An mTOR for use according to claim 29 or 30 wherein the the mTOR inhibitor is inhibitor is rapamycin or a rapamycin derivative, preferably temsirolimus or everolimus, most preferably everolimus.

32. Use of PI3K pathway and FGFR genes and CCND1 gene as a biomarker for prediction of sensitivity of a breast cancer patient, preferably a hormone receptor-positive (HR+) HER2-negative (HER2-) advanced breast cancer patient, to treatment with an mTOR inhibitor such as rapamycin or a rapamycin derivative, preferably temsirolimus or everolimus, most preferably everolimus.

33. Use of PI3K pathway and FGFR genes or PI3K pathway and CCND1 gene as a biomarker for prediction of sensitivity of a breast cancer patient, preferably a hormone receptor-positive (HR+) HER2-negative (HER2-) advanced breast cancer patient, to treatment with an mTOR inhibitor such as rapamycin or a rapamycin derivative, preferably temsirolimus or everolimus, most preferably everolimus, wherein the absence of genetic alterations in PI3K pathway and CCND1 gene or in PI3K pathway and FGFR genes is indicative of higher benefit

from mTOR inhibition, such as rapamycin or a rapamycin derivative, preferably temsirolimus or everolimus, most preferably everolimus, therapy.

FIGURE 1

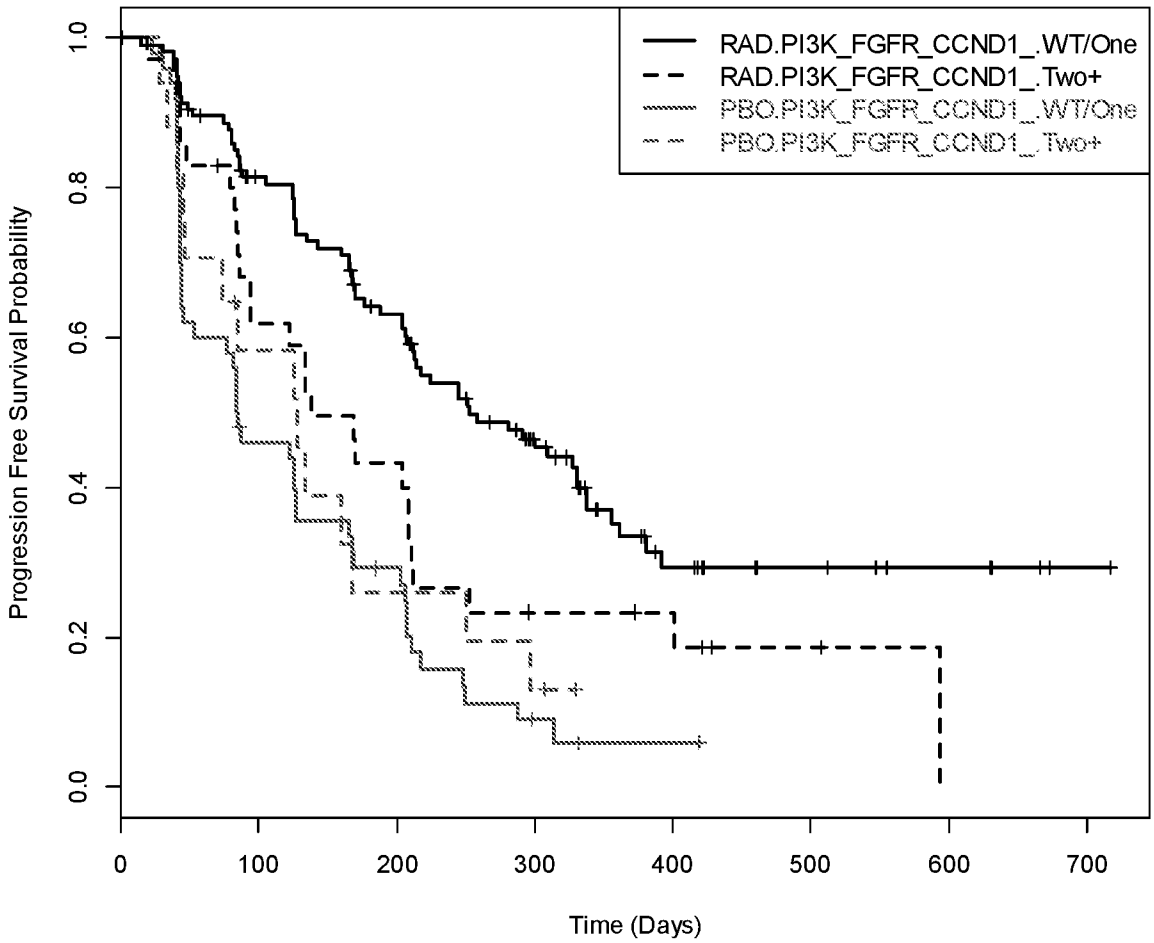

RAD.PI3K_FGFR_CCND1_.WT/One
RAD.PI3K_FGFR_CCND1_.Two+
PBO.PI3K_FGFR_CCND1_.WT/One
PBO.PI3K_FGFR_CCND1_.Two+
Progression Free Survival Probability
0.0
0.2
0.4
0.6
0.8
1.0
0
100
200
300
400
500
600
700
Time (Days)

FIGURE 2

Sub-group	N (% in subgroup)	Events (%)	Median PFS (95%CI)	HR (95%CI)
RAD-WT/One	119 (69%)	67 (56%)	253 (207 - 331)	0.26 (0.17 - 0.40)
PBO-WT/One	53 (31%)	45 (85%)	84.5 (41 - 127)	
RAD-Two+	38 (69%)	27 (71%)	138 (86 - 208)	0.78 (0.39 - 1.56)
PBO-Two+	17 (31%)	14 (82%)	128 (45 - 168)	

- WT/One: No mutation in PI3K AND no amplification/mutations in FGFR1/2 AND no amplification in CCND1 (27% of NGS) OR A single mutation or amplification in PI3K OR FGFR1/2 OR CCND1 (49% of NGS)
- Two+: Two or more mutations or amplification in PI3K OR FGFR1/2 OR CCND1 (24% of NGS)

- Analysis adjusted for Race and Tumor Sample Type (Primary or Metastasis)
- HR for RAD/PBO in ITT is 0.45 (0.38-0.54); HR for NGS is 0.40 (0.28-0.55)
- Total 6 patients with mutations in all 3 genes/pathways, 5 in the RAD arm

FIGURE 3

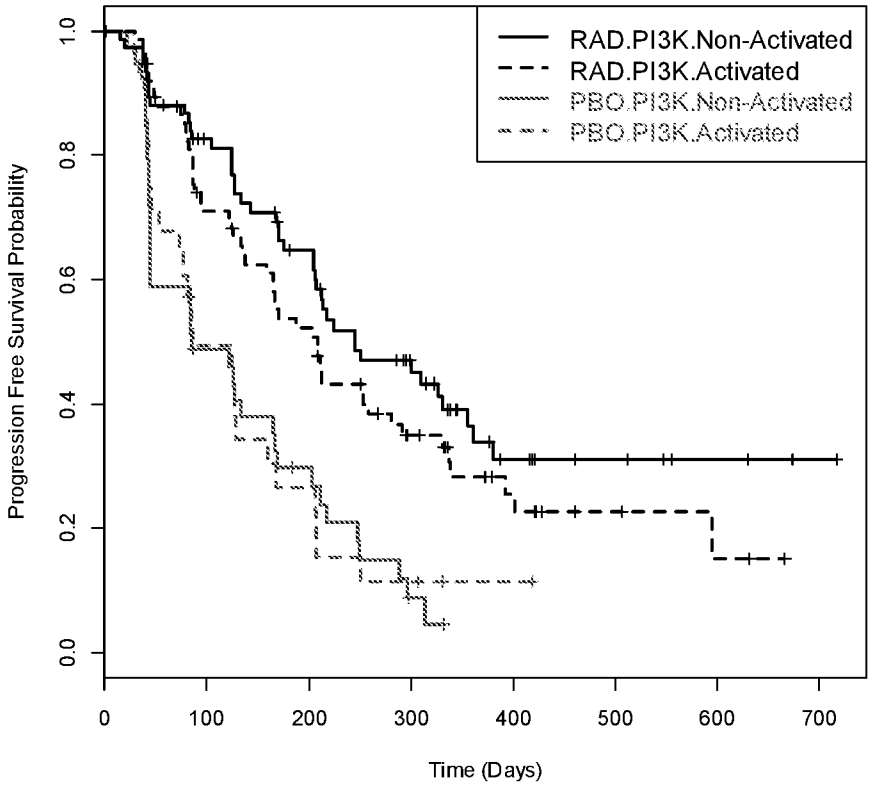

RAD.PI3K.Non-Activated
RAD.PI3K.Activated
PBO.PI3K.Non-Activated
PBO.PI3K.Activated
Progression Free Survival Probability
0.0
0.2
0.4
0.6
0.8
1.0
0
100
200
300
400
500
600
700
Time (Days)

FIGURE 4

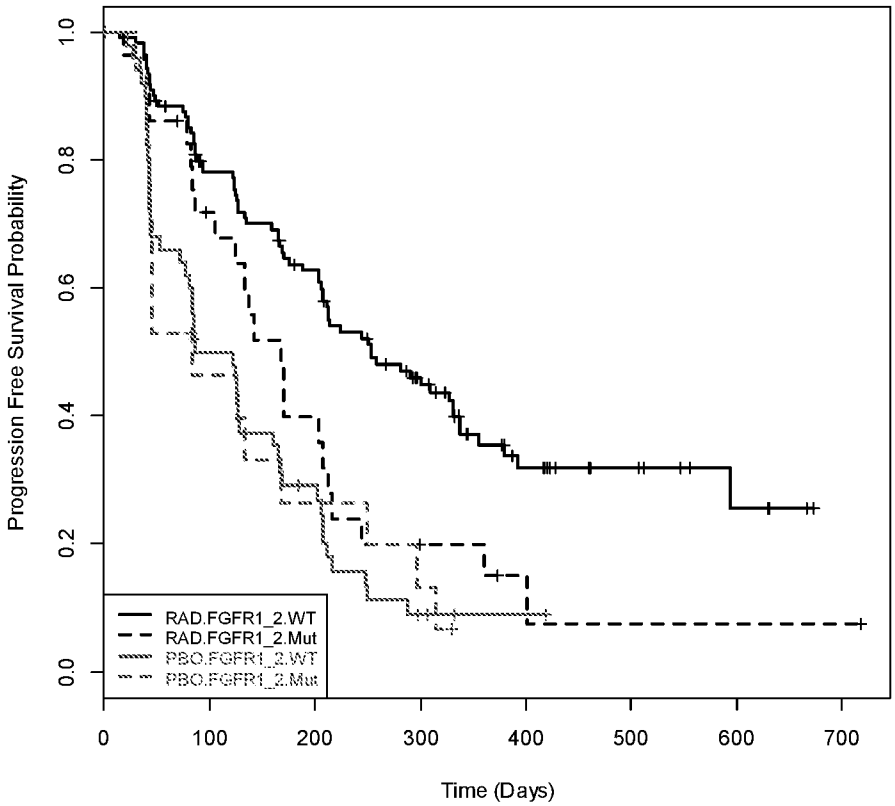

Progression Free Survival Probability
0.0
0.2
0.4
0.6
0.8
1.0
0
100
200
300
400
500
600
700
Time (Days)
RAD.FGFR1_2.WT
RAD.FGFR1_2.Mut
PBO.FGFR1_2.WT
PBO.FGFR1_2.Mut

FIGURE 5

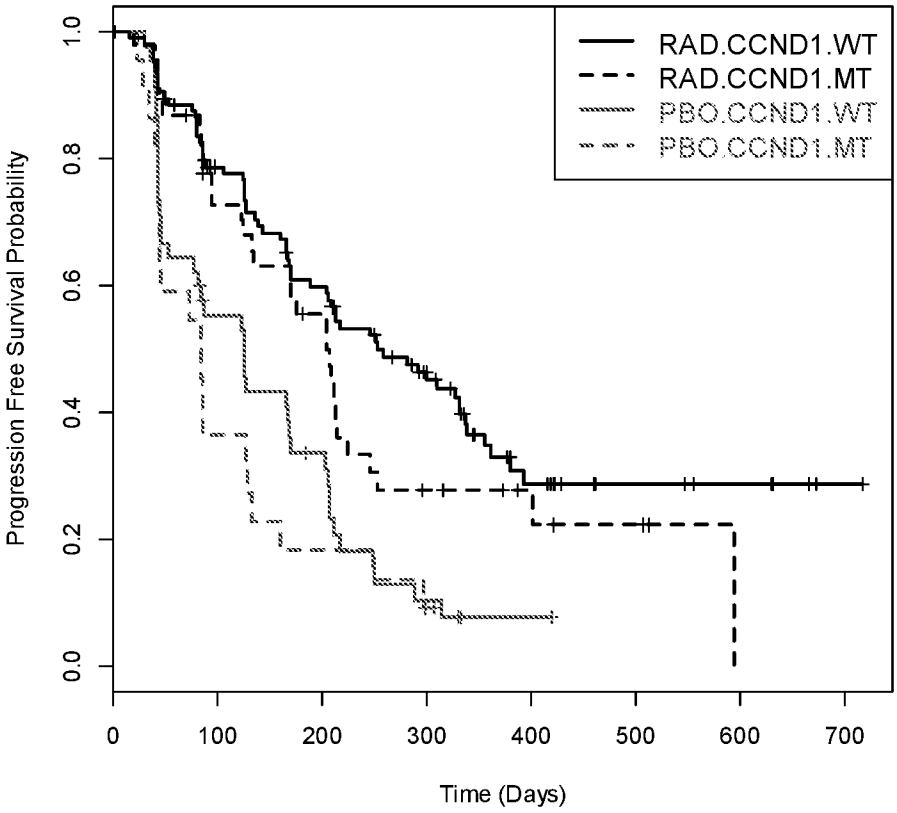
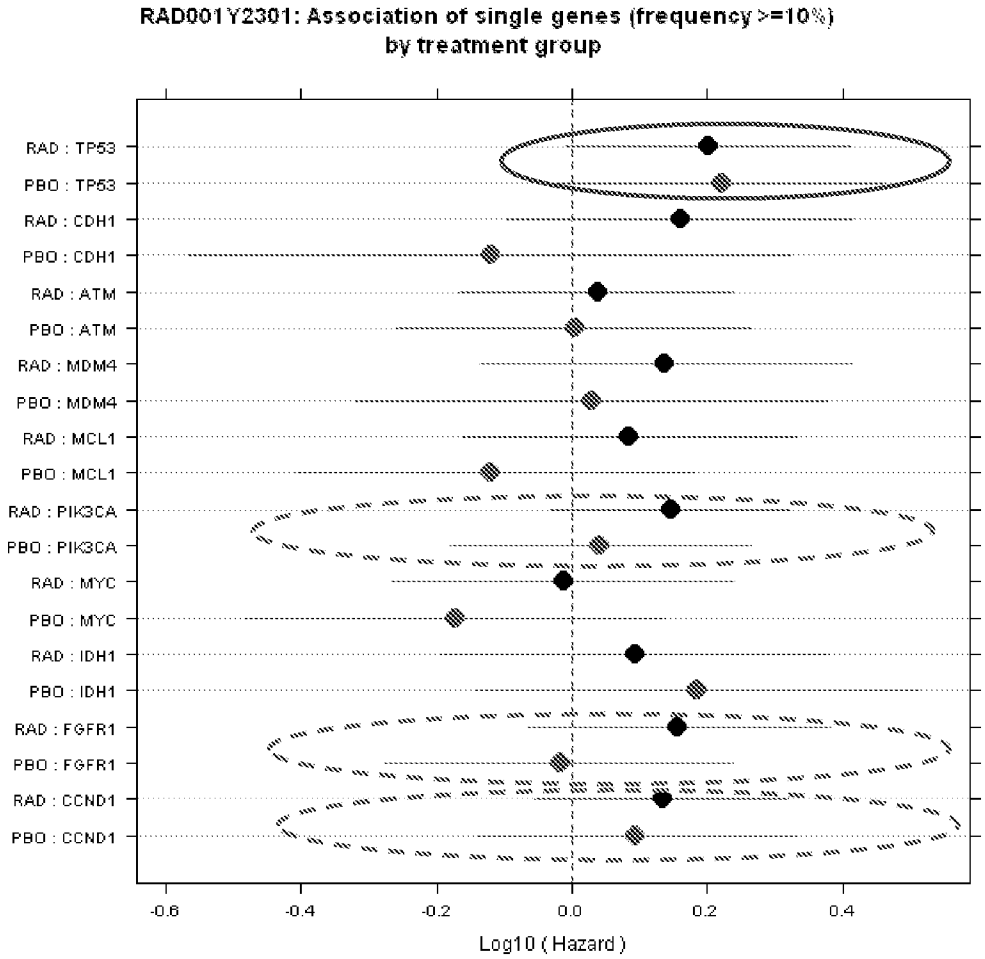

RAD.CCND1.WT
RAD.CCND1.MT
PBO.CCND1.WT
PBO.CCND1.MT
Progression Free Survival Probability
0.0
0.2
0.4
0.6
0.8
1.0
0
100
200
300
400
500
600
700
Time (Days)

FIGURE 6



- 10 single gene with mutation rate > 10% in overall NGS population
  - Previously reported single gene highlighted by red ovals
- Patients with TP53 mutations have shorter PFS, regardless of the treatment (border-line significant)
- No other estimates was statistically significant at the 5% level of significant
- High vs. low pS6 or Ki67 levels showed similar HRs in both arms (back-up)

FIGURE 7

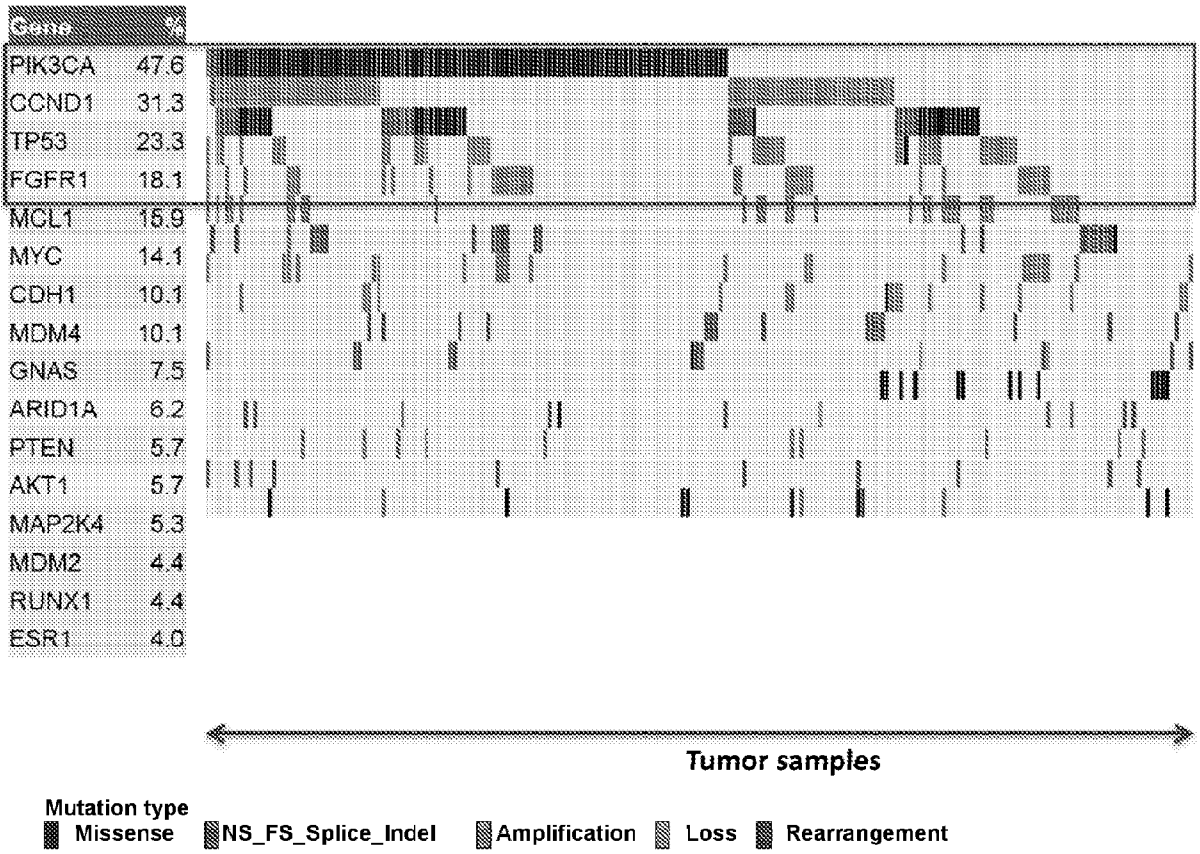
		Treatment Arm					
		RAD			Placebo		
Biomarker	status	N	Events	Within Arm HR (95%CI)	N	Events	Within Arm HR (95%CI)
PI3K pathway	Active	78	51	1.32 (0.88 - 1.97)	30	24	0.97 (0.57 - 1.63)
	Non	79	43	--	40	35	--
PI3K Pathway + AKT	Active	84	55	1.33 (0.88 - 2.01)	36	29	0.88 (0.53 – 1.47)
	Non	73	39	--	34	30	--
PIK3CA	mut	68	46	1.49 (0.99 - 2.24)	27	22	0.95 (0.56 – 1.61)
	wt	89	48	--	43	37	--
PTEN	normal	14	7	0.84 (0.39, 1.82)	7	6	1.42 (0.60, 3.33)
	null	143	87	--	63	53	--
FGFR1+2	amp	29	23	1.51 (1.15 - 2.97)	17	15	0.96 (0.53 – 1.73)
	normal	128	71	--	53	44	--
FGFR1	amp	24	18	1.43 (0.85, 2.40)	17	15	0.96 (0.53 – 1.73)
	normal	133	76	--	53	44	--
Cyclin D1	amp	49	31	1.36 (0.88 - 2.09)	22	20	1.24 (0.72 – 2.14)
	normal	108	63	--	48	39	--

FIGURE 8

Alteration types	Sub-type	Number of alterations	Somatic alterations	Novel alterations
Sequence alteration	Missense	1,234	216	1,018
	Nonsense/Frameshift/ Splice variant/ Insertion/Deletion	256	128	128
Rearrangement		24	3	21
Copy number variations (CNV)	Amplification (≥6 copies)	516		
	Bi-allelic deletion	26		

Distribution of known genetic alterations	
Known somatic alteration/sample, mean (range)	4.1 (0-15)
Patients with at least one known somatic alteration, n	219
Genes with at least one known somatic alteration, n	104

FIGURE 9


Gene %
PIK3CA 47.6
CCND1 31.3
TP53 23.3
FGFR1 18.1
MCL1 15.9
MYC 14.1
CDH1 10.1
MDM4 10.1
GNAS 7.5
ARID1A 6.2
PTEN 5.7
AKT1 5.7
MAP2K4 5.3
MDM2 4.4
RUNX1 4.4
ESR1 4.0
Tumor samples
Mutation type
Missense
NS_FS_Splice_Indel
Amplification
Loss
Rearrangement

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2014/061396

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

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

B. FIELDS SEARCHED

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

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2006/122053 A2 (ARIAD GENE THERAPEUTICS INC [US]; BEDROSIAN CAMILLE L [US]; CLACKSON T) 16 November 2006 (2006-11-16) abstract; claims 33, 56, 68-78	23-28, 32,33
X	GB 2 488 028 A (ASTRAZENECA AB [SE]) 15 August 2012 (2012-08-15) abstract; claims 6, 18	23-29,31

-/--

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

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 August 2014	02/09/2014

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

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2014/061396

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ELI R ZUNDER ET AL: "Discovery of Drug-Resistant and Drug-Sensitizing Mutations in the Oncogenic PI3K Isoform p110 alpha", CANCER CELL, CELL PRESS, US, [Online] vol. 14, no. 2, 12 August 2008 (2008-08-12), pages 180-192, XP008159215, ISSN: 1535-6108 Retrieved from the Internet: URL:http://www.sciencedirect.com/science/article/pii/S1535610808002250> abstract Significance on page 180 Discussion	1-22,30
A	B WEIGELT ET AL: "PIK3CA mutation, but not PTEN loss of function, determines the sensitivity of breast cancer cells to mTOR inhibitory drugs", ONCOGENE, vol. 30, no. 29, 21 July 2011 (2011-07-21), pages 3222-3233, XP055080671, ISSN: 0950-9232, DOI: 10.1038/onc.2011.42 abstract; figures 1-5	1-33
A	F. JANKU ET AL: "PIK3CA Mutations in Patients with Advanced Cancers Treated with PI3K/AKT/mTOR Axis Inhibitors", MOLECULAR CANCER THERAPEUTICS, vol. 10, no. 3, 1 March 2011 (2011-03-01), pages 558-565, XP055033927, ISSN: 1535-7163, DOI: 10.1158/1535-7163.MCT-10-0994 abstract; table 1 discussion	1-33
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