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(54) Title: MATERIALS AND METHODS FOR MONITORING THE DEVELOPMENT OF RESISTANCE OF CANCERS TO TREATMENT

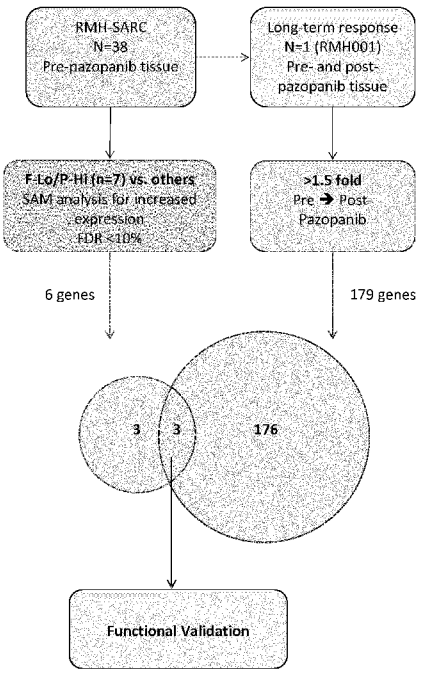

Figure 1

(57) Abstract: Materials and methods for monitoring the development of resistance of cancers to treatment The present invention relates to materials and methods for monitoring and treating cancers and to methods of identifying/detecting/monitoring the development of resistance of cancers to tyrosine kinase inhibitors.

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[Continued on next page]

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Materials and methods for monitoring the development of resistance of cancers to treatment

Field of the Invention

The present invention relates to materials and methods for monitoring and treating cancers and to methods of identifying/detecting/monitoring the development of resistance of cancers to tyrosine kinase inhibitors.

Background

Cancer is a complex and dynamic disease, and many different ways of analysing and classifying tumours have been developed with the aims of determining the prognosis for the patient, and informing treatment decisions.

Pazopanib is an oral multi-target tyrosine kinase inhibitor (TKI) that operates through the inhibition of multiple tyrosine kinases that mediate angiogenesis and cell growth, such as vascular endothelial growth factor receptors (VEGFRs), platelet-derived growth factor receptors (PDGFRs), fibroblast growth factor receptors (FGFRs) and c-Kit. Based on the results of the PALETTE phase III trial[1], wherein patients with advanced soft tissue sarcoma (STS) randomised to receive pazopanib showed significantly longer progression-free survival (PFS) compared to those randomised to receive placebo (median PFS 4.6 v. 1.6 months; HR 0.31; 95%CI 0.24-0.40; $p<0.0001$), pazopanib was approved for the treatment of advanced non-adipocytic STS following failure of previous chemotherapy. However, no difference in overall survival was seen between treatment arms in the PALETTE trial, whilst clinical efficacy of pazopanib varies widely on a patient-by-patient basis in a manner that is not predicted by routinely assessed clinicopathological disease characteristics.

A significant proportion of patients demonstrate primary pazopanib resistance, with disease that continues to progress despite initiation of drug therapy. A minority of patients

attain objective tumour response to pazopanib, although the eventual development of acquired resistance and tumour progression is universal in such cases. A third pattern of disease response involves a significant proportion of patients whose tumours are seen to have neither significantly progressed nor responded to pazopanib at first radiological assessment after drug commencement. This group will encompass a range of phenotypes that include intrinsically slow-growing tumours whose clinical course is unaffected by pazopanib exposure (and thus possess intrinsic resistance), and those who harbour aggressive, fast-growing tumours which are stabilised by pazopanib effect and thus reflect therapeutic sensitivity (although will inevitably develop acquired resistance).

Currently, little is understood regarding the underlying aspects of tumour biology that determines which of these patterns of response any given sarcoma will exhibit on pazopanib exposure, nor the underlying molecular mechanisms that underpin pre-existing or emergent pazopanib resistance. Improved understanding of mechanisms of disease sensitivity and resistance to pazopanib would have clear clinical relevance in terms of the development of biomarkers that are able to predict initial pazopanib sensitivity and/or the emergence of eventual resistance, and therapeutic approaches for overcoming pazopanib resistance.

Summary of the Invention

The present invention is based on research to identify biomarkers associated with the development of resistance of cancer to treatment with tyrosine kinase inhibitors (TKI) such as Pazopanib.

Although the role of inflammatory cytokines and chemokines has previously been investigated in the context of intrinsic resistance to pazopanib treatment, these markers have not been investigated in the context of cancers that are initially sensitive to TKI treatment.

For instance, Tran *et al.* previously showed that patients with higher circulating IL-8 levels, detected in peripheral blood samples taken prior to commencement of pazopanib treatment within two prospective clinical studies in patients with advanced renal cell carcinoma (RCC), was associated with lower levels of tumour response and shorter PFS compared to patients with lower circulating IL-8[2]. Importantly, in this study, when the initial prognostic association of various cytokines detected in a single arm, majority TKI-naïve, phase II cohort were tested in an entirely TKI-naïve validation cohort derived from a randomised phase III trial, high IL-8 was shown to behave as a negative prognostic but not predictive biomarker for pazopanib therapy in RCC, whilst IL-6 showed both negative prognostic but positive predictive value.

In a later study by Pal *et al.* serial sampling of peripheral blood was performed and circulating cytokine levels measured at pre-treatment baseline and then during treatment in a prospective cohort of RCC patients treated with pazopanib[3]. Here, having been low at pre-treatment baseline, levels of a number of circulating factors including IL-8 were significantly elevated at 6 and 12 months post-commencement of pazopanib in patients whose tumours did not show objective radiological response to drug. Meanwhile, in patients whose tumours significantly shrank with pazopanib treatment, IL-8 levels remained suppressed up to 12 months.

Whilst the two previous studies have investigated IL-8 association with intrinsic pazopanib resistance in patients, they have not described IL-8's association within patients that are initially sensitive to pazopanib treatment but go on to develop an acquired resistance. In other words, the data presented by Tran *et al.* and Pal *et al.* for patients that are intrinsically resistance (i.e. high IL-8 levels infer an intrinsic resistance that results in poorer pazopanib patient response) can help predict initial pazopanib sensitivity in patients. However, it does not give information on the emergence of acquired pazopanib

resistance in initially sensitive patients and whether or not IL-8 and other markers are associated with this type of resistance.

Understanding and being able to predict the emergence of acquired pazopanib resistance through tracking of IL-8 (and other cytokines) levels in patients that initially respond well to pazopanib but relapse due to an acquired resistance to the drug, could help in the development of therapeutic approaches to overcome this acquired resistance phenomenon.

The present inventors show an association between high gene expression levels of IL-8 and other genes in cytokine signalling pathways and intrinsic and acquired resistance in tumour specimens from a cohort of sarcoma patients. Experimental data presented herein provides evidence that IL-8 induces pazopanib resistance in cancer cell line models of soft tissue malignancy. Evidence of a role for IL-8 (and other cytokines) in acquired resistance is provided by analysis of serial tumour samples from a patient who exhibited significant and durable objective response to pazopanib, followed by eventual disease progression.

In matched tumour specimens taken prior to start of pazopanib (and thus can be seen as pazopanib sensitive, in view of subsequent clinical response) and then from a progressing lesion sampled in the context of multifocal progression, we have shown marked increase in intratumoral IL-8 (and other cytokines) gene expression between sensitive baseline and progressing, drug-resistant lesions; which indicates that monitoring levels of IL-8 (and other cytokines) is a way of monitoring the evolution and development of pazopanib resistance.

This finding also suggests that increased IL-8 (and other cytokine) expression may induce pazopanib resistance in a previously sensitive system, and that inhibition of IL-8 can prevent onset of resistance. To validate this, we carried out in vitro functional validation experiments using two pazopanib sensitive cell lines to determine the association of IL-8 with

pazopanib resistance. The aim was to assess whether we could induce specific IL-8 driven resistance to pazopanib in these cell lines and to determine the effects IL-8 has on cell viability and apoptosis.

Accordingly, the inventors have identified several markers (including IL-8) which can be used independently or in combination to identify the development of resistance to TKI treatment.

Additionally, the inventors have identified IL-8 as a target for the treatment of cancer, which can be targeted in combination with TKI treatment, for example to prevent the development of resistance to the TKI, or which could be used independently as a target, for example, in TKI resistant cancers.

In particular, a group of cytokine-cytokine receptor interaction-related genes have been identified as markers for the development of TKI resistance, and as targets for cancer treatment. Specifically, the upregulation of these genes after TKI treatment has been shown to be linked to the development of resistance to TKI treatment.

Accordingly, the invention relates to the use of the expression levels of cytokine-cytokine receptor interaction-related genes for identifying the development of resistance, and as targets for cancer treatment.

In a first aspect, the invention relates to a method of monitoring cancer in an individual for the development of resistance to treatment with a TKI. Specifically, provided is a method of monitoring cancer in an individual for the development of resistance to treatment with a TKI, the method comprising:

comparing the expression level of a marker before treatment with the TKI, to expression levels of the marker after treatment with the TKI,

wherein an at least 1.5-fold increase in expression of the marker after treatment with the TKI as compared to before treatment with the TKI is indicative of the development of resistance to the TKI,

wherein the marker is selected from:

IL8, ACVR1C, ACVR2A, ALK, ASXL1, B2M, BCL2A1, BID, BIRC3, BMP8A, BNIP3, CACNA2D2, CACNG4, CALML5, CALML6, CAPN2, CASP12, CASP9, CBLC, CCND2, CD19, CDC14A, CDC14B, CDH1, CDKN2A, CEBPE, CLCF1, CNTFR, COL2A1, COL3A1, COL5A1, CRLF2, CTNNB1, DDIT3, DUSP4, E2F5, EFNA2, EGFR, EPHA2, FAS, FGF1, FGF10, FGF2, FGF23, FGF6, FIGF, FLNA, FLT1, FLT3, FN1, FUT8, GATA3, GNG12, GRIA3, GTF2H3, H2AFX, HES5, HOXA9, HPGD, HSP90B1, IBSP, ID4, IGFBP3, IL12A, IL13RA2, IL19, IL1A, IL1B, IL1R1, IL22RA1, IL23R, IL24, IL2RA, IL6, IL6R, INHBA, IRAK2, IRS1, ITGA2, ITGA3, ITGA6, ITGA8, ITGA9, ITGB3, ITGB6, ITGB7, JAG1, KAT2B, KITLG, LAMA1, LAMA3, LAMB3, LAMC3, LEFTY2, LEPR, LIF, LTBP1, MAD2L2, MAML2, MAP2K4, MAP3K1, MAP3K5, MAPK1, MAPK10, MDM2, MED12, MMP3, MMP7, MMP9, MNAT1, MPL, NBN, NFATC1, NFE2L2, NGF, NOG, NOS3, NUPR1, PAX8, PBRM1, PDGFD, PGF, PIK3R1, PLA1A, PLA2G4A, PLAU, POLE2, POLR2D, PPARGC1A, PPP2CB, PPP3CA, PPP3CC, PRKACB, PRKAR2A, PRKAR2B, PTEN, PTPN11, RAC1, RASAL1, RB1, RELN, RET, RIN1, RPS6KA5, RRAS2, SF3B1, SFN, SFRP1, SFRP2, SHC3, SIRT4, SKP1, SOCS1, SOCS2, SOST, SPOP, SPP1, STAG2, TBL1XR1, TCL1B, TET2, TLR2, TMPRSS2, TNFRSF10A, TNFRSF10D, TNN, TSHR, TSPAN7, TTK, UTY, VEGFC, WNT10A, WNT10B, WNT11, WNT16, WNT4, WNT5A, WNT5B, and WNT7A.

In particular, the marker may be selected from IL-8, IL-6 and LIF. In particular, the marker may be IL-8.

The expression levels of a plurality of markers before treatment with the TKI may be compared with the expression level of each of the plurality of markers after treatment with the TKI, wherein an at least 1.5-fold increase in expression of at least one of the markers after treatment with the TKI as compared to before treatment with the TKI is indicative of the development of resistance to the TKI, and wherein the plurality of markers is selected from: IL8, ACVR1C, ACVR2A, ALK, ASXL1, B2M, BCL2A1, BID,

BIRC3, BMP8A, BNIP3, CACNA2D2, CACNG4, CALML5, CALML6, CAPN2, CASP12, CASP9, CBLC, CCND2, CD19, CDC14A, CDC14B, CDH1, CDKN2A, CEBPE, CLCF1, CNTFR, COL2A1, COL3A1, COL5A1, CRLF2, CTNNB1, DDIT3, DUSP4, E2F5, EFNA2, EGFR, EPHA2, FAS, FGF1, FGF10, FGF2, FGF23, FGF6, FIGF, FLNA, FLT1, FLT3, FN1, FUT8, GATA3, GNG12, GRIA3, GTF2H3, H2AFX, HES5, HOXA9, HPGD, HSP90B1, IBSP, ID4, IGFBP3, IL12A, IL13RA2, IL19, IL1A, IL1B, IL1R1, IL22RA1, IL23R, IL24, IL2RA, IL6, IL6R, INHBA, IRAK2, IRS1, ITGA2, ITGA3, ITGA6, ITGA8, ITGA9, ITGB3, ITGB6, ITGB7, JAG1, KAT2B, KITLG, LAMA1, LAMA3, LAMB3, LAMC3, LEFTY2, LEPR, LIF, LTBP1, MAD2L2, MAML2, MAP2K4, MAP3K1, MAP3K5, MAPK1, MAPK10, MDM2, MED12, MMP3, MMP7, MMP9, MNAT1, MPL, NBN, NFATC1, NFE2L2, NGF, NOG, NOS3, NUPR1, PAX8, PBRM1, PDGFD, PGF, PIK3R1, PLA1A, PLA2G4A, PLAU, POLE2, POLR2D, PPARGC1A, PPP2CB, PPP3CA, PPP3CC, PRKACB, PRKAR2A, PRKAR2B, PTEN, PTPN11, RAC1, RASAL1, RB1, RELN, RET, RIN1, RPS6KA5, RRAS2, SF3B1, SFN, SFRP1, SFRP2, SHC3, SIRT4, SKP1, SOCS1, SOCS2, SOST, SPOP, SPP1, STAG2, TBL1XR1, TCL1B, TET2, TLR2, TMPRSS2, TNFRSF10A, TNFRSF10D, TNN, TSHR, TSPAN7, TTK, UTY, VEGFC, WNT10A, WNT10B, WNT11, WNT16, WNT4, WNT5A, WNT5B, and WNT7A.

The method may comprise the steps of:

determining the expression level of the at least one marker prior to treatment; and

determining the expression level of the at least one marker after TKI treatment,

wherein the comparing step compares the expression level as determined in the prior to treatment with the expression level determined after treatment. The determining steps may be conducted *ex vivo*.

The determining steps may be carried out on samples obtained from the individual. Accordingly, the methods may comprise the steps of:

determining the expression level of the at least one marker in a sample obtained from the patient prior to treatment with the TKI; and

determining the expression level of the at least one marker in a sample obtained from the patient after treatment with the TKI,

wherein the comparing step compares the expression level as determined in the sample obtained prior to treatment with the TKI with the expression level determined in the sample obtained after treatment with the TKI.

The samples may be blood samples, urine samples or samples of cancer cells.

The cancer may be initially sensitive to the TKI.

The methods may comprise additional steps of delivering a prognosis, wherein the increase in expression of one or more markers is indicative of a poor prognosis. The methods may comprise additional steps of treatment with an inhibitor of IL-8, IL-6, LIF or one of their receptors as described elsewhere herein.

The TKI may be selected from: Pazopanib, Regorafenib, Sorafenib, Sunitinib, Lenvatinib, Axitinib, Nintedanib, and Ponatinib, and pharmaceutically acceptable salts thereof. In particular, the TKI may be Pazopanib or a pharmaceutically acceptable salt thereof.

The cancer may be selected from: soft tissues sarcoma (STS), metastatic renal cell carcinomas (mRCC), gastrointestinal stromal tumour (GIST), hepatocellular carcinoma (HCC), neuroendocrine tumour (NET), medullary thyroid cancer (MTC), non-squamous non-small cell lung cancer (non-squamous NSCLC), and chronic myeloid leukaemia (CML). In particular, the cancer may be STS, for example advanced soft tissue sarcoma and/or spindle cell sarcoma.

For example, the TKI may be Pazopanib or a pharmaceutically acceptable salt thereof and the cancer may be soft tissue sarcoma. In particular, the TKI may be Pazopanib or a

pharmaceutically acceptable salt thereof and the cancer may be advanced soft tissue sarcoma.

An increase in expression of the marker of at least 1.6-fold, at least 1.7-fold, at least 1.8-fold, at least 1.9-fold, at least 2.0-fold, at least 2.1-fold, at least 2.2-fold, at least 2.3-fold, at least 2.4-fold, at least 2.5-fold, at least 2.6-fold, at least 2.7-fold, at least 2.8-fold, at least 2.9-fold, at least 3.0-fold, at least 3.1-fold, at least 3.2-fold, at least 3.3-fold, at least 3.4-fold, at least 3.5-fold, at least 3.6-fold, at least 3.7-fold, at least 3.8-fold, at least 3.9-fold, or at least 4.0-fold may be indicative of the development of resistance to the TKI.

In these aspects, the method may also be described as a method of identifying the development of resistance to a TKI, or as a method of detecting the development of resistance to a TKI. Alternatively the methods may be described as methods of monitoring/identifying/detecting resistance to treatment with a TKI. Alternatively the methods may be described as methods of monitoring/identifying/detecting acquired resistance to treatment with a TKI.

In a further aspect, the invention provides a cytokine inhibitor and a TKI for use in a method of treating cancer in an individual, wherein the cytokine inhibitor is:

a) an inhibitor of IL-8 and/or an inhibitor of an IL-8 receptor; and/or

b) an inhibitor of IL-6 and/or an inhibitor of an IL-6 receptor; and/or

c) an inhibitor of LIF and/or an inhibitor of a LIF receptor.

Also provided is a cytokine inhibitor for use in a method of treating cancer in an individual that has developed resistance to treatment with a TKI, wherein the cytokine inhibitor is an inhibitor of IL-8 and/or an

inhibitor of an IL-8 receptor; and/or an inhibitor of IL-6 and/or an inhibitor of an IL-6 receptor; and/or an inhibitor of LIF and/or an inhibitor of a LIF receptor. The cancer may have been indicated as having developed resistance to the TKI using the methods described herein.

Also provided is a cytokine inhibitor for use in a method of preventing the development of resistance of a cancer to a TKI in the treatment of cancer in an individual, wherein the cytokine inhibitor is an inhibitor of IL-8 and/or an inhibitor of an IL-8 receptor; and/or an inhibitor of IL-6 and/or an inhibitor of an IL-6 receptor; and/or an inhibitor of LIF and/or an inhibitor of a LIF receptor.

According to these aspects the cytokine inhibitor may be used in combination with a TKI for the treatment of cancer.

The TKI may be selected from: Pazopanib, Regorafenib, Sorafenib, Sunitinib, Lenvatinib, Axitinib, Nintedanib, and Ponatinib, and pharmaceutically acceptable salts thereof. In particular, the TKI may be Pazopanib or a pharmaceutically acceptable salt thereof.

The cancer may be selected from: soft tissues sarcoma (STS), metastatic renal cell carcinomas (mRCC), gastrointestinal stromal tumour (GIST), hepatocellular carcinoma (HCC), neuroendocrine tumour (NET), medullary thyroid cancer (MTC), non-squamous non-small cell lung cancer (non-squamous NSCLC), and chronic myeloid leukaemia (CML). In particular, the cancer may be STS, for example advanced soft tissue sarcoma and/or spindle cell sarcoma.

For example, the TKI may be Pazopanib or a pharmaceutically acceptable salt thereof and the cancer may be soft tissue sarcoma. In particular, the TKI may be Pazopanib or a pharmaceutically acceptable salt thereof and the cancer may be advanced soft tissue sarcoma.

The cytokine inhibitor may be a small molecule inhibitor, an antibody, a ligand trap, a peptide fragment or a nucleic acid inhibitor.

The cytokine inhibitor, for example the IL-8 inhibitor or IL-8 receptor inhibitor, may be a small molecule inhibitor, an antibody, for example an IL-8 neutralising antibody, a ligand trap, a peptide fragment or a nucleic acid inhibitor, for example an antisense nucleic acid targeting IL-8 or an IL-8 receptor, siRNA, shRNA, CRISPR or miRNA.

Also provided is a pharmaceutical composition comprising a cytokine inhibitor and a TKI, wherein the cytokine inhibitor is an inhibitor of IL-8 and/or an inhibitor of an IL-8 receptor; and/or an inhibitor of IL-6 and/or an inhibitor of an IL-6 receptor; and/or an inhibitor of LIF and/or an inhibitor of a LIF receptor.

The markers, methods of measuring them, cancers, tyrosine kinase inhibitors and other details of the invention are described below. These details are applicable to all of the aspects of the invention.

Markers

In the context of the present invention the 'markers' or 'biomarkers' allow monitoring of development of resistance to TKI, and allow identification of cancers/tumour cells that are developing resistance to a TKI. The biomarkers include expression levels of particular genes or proteins.

The markers may be detected by conventional means in a sample containing cancer cells or cancer cell material or components (e.g. nucleic acids and/or proteins), obtained from an individual, or in a tumour cell line.

Accordingly, the methods and uses disclosed herein may involve the step of determining the expression level of a (bio)marker.

The methods and uses may base a prognostic, diagnostic or treatment decision on the expression level of a biomarker as already determined.

The methods and uses may involve the step of determining the expression level of a marker in a sample obtained from an individual, for example, a sample of cancer cells, or a blood sample or a urine sample.

The markers include those generally involved in cytokine-cytokine receptor interactions. The markers are shown in table 2. The markers are:

IL-8, ACVR1C, ACVR2A, ALK, ASXL1, B2M, BCL2A1, BID, BIRC3, BMP8A, BNIP3, CACNA2D2, CACNG4, CALML5, CALML6, CAPN2, CASP12, CASP9, CBLC, CCND2, CD19, CDC14A, CDC14B, CDH1, CDKN2A, CEBPE, CLCF1, CNTFR, COL2A1, COL3A1, COL5A1, CRLF2, CTNNB1, DDIT3, DUSP4, E2F5, EFNA2, EGFR, EPHA2, FAS, FGF1, FGF10, FGF2, FGF23, FGF6, FIGF, FLNA, FLT1, FLT3, FN1, FUT8, GATA3, GNG12, GRIA3, GTF2H3, H2AFX, HES5, HOXA9, HPGD, HSP90B1, IBSP, ID4, IGFBP3, IL12A, IL13RA2, IL19, IL1A, IL1B, IL1R1, IL22RA1, IL23R, IL24, IL2RA, IL6, IL6R, INHBA, IRAK2, IRS1, ITGA2, ITGA3, ITGA6, ITGA8, ITGA9, ITGB3, ITGB6, ITGB7, JAG1, KAT2B, KITLG, LAMA1, LAMA3, LAMB3, LAMC3, LEFTY2, LEPR, LIF, LTBP1, MAD2L2, MAML2, MAP2K4, MAP3K1, MAP3K5, MAPK1, MAPK10, MDM2, MED12, MMP3, MMP7, MMP9, MNAT1, MPL, NBN, NFATC1, NFE2L2, NGF, NOG, NOS3, NUPR1, PAX8, PBRM1, PDGFD, PGF, PIK3R1, PLA1A, PLA2G4A, PLAU, POLE2, POLR2D, PPARGC1A, PPP2CB, PPP3CA, PPP3CC, PRKACB, PRKAR2A, PRKAR2B, PTEN, PTPN11, RAC1, RASAL1, RB1, RELN, RET, RIN1, RPS6KA5, RRAS2, SF3B1, SFN, SFRP1, SFRP2, SHC3, SIRT4, SKP1, SOCS1, SOCS2, SOST, SPOP, SPP1, STAG2, TBL1XR1, TCL1B, TET2, TLR2, TMPRSS2, TNFRSF10A, TNFRSF10D, TNN, TSHR, TSPAN7, TTK, UTY, VEGFC, WNT10A, WNT10B, WNT11, WNT16, WNT4, WNT5A, WNT5B, WNT7A.

1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 155 or all of the

markers maybe used. Where a plurality of markers are used the expression level of each marker after treatment with the TKI is compared to the expression level of the same marker after treatment with the TKI.

Particularly preferred markers are markers which can be identified in the circulation. Any of the markers may be measured in the circulation at the nucleic acid level. IL-8, FGF1, FGF10, FGF2, FGF23, FGF6, FIGF, IL12A, IL19, IL1A, IL1B, IL24, IL6, LIF, NGF, VEGFC, WNT10A, WNT10B, WNT11, WNT16, WNT4, WNT5A, WNT5B, and WNT7A may be measured in the circulation at the protein level.

For example, the markers may include IL-8, IL-6 and/or LIF. For example, the markers may include all three of IL-8, IL-6 and LIF. Alternatively the markers may be IL-8 and IL-6. Alternatively the marker may be IL-8 or IL-6.

Reference to determining the expression level of a marker refers to determination of the expression level of an expression product of the gene. Expression level may be determined at the nucleic acid level or the protein level.

The gene expression levels determined may be considered to provide an expression profile. By “expression profile” is meant a set of data relating to the level of expression of one or more of the relevant genes in an individual, in a form which allows comparison with comparable expression profiles (e.g. from individuals for whom the prognosis is already known), in order to assist in the determination of prognosis and in the selection of an individual for treatment with a TKI.

The determination of gene expression levels may involve determining the presence or amount of mRNA in a sample of cancer cells or blood sample. Methods for doing this are well known to the skilled person. Gene expression levels may be determined in a sample using any conventional method, for example using

labelled nucleic acid probes, nucleic acid microarrays or using nucleic acid synthesis (such as quantitative PCR, e.g. quantitative real time RT-PCR). For example, gene expression levels may be determined using RNA microarrays or cDNA microarrays.

Alternatively or additionally, the determination of gene expression levels may involve determining the protein levels expressed from the genes in a sample containing cancer cells obtained from an individual, or in a blood sample or urine sample from the individual. Protein expression levels may be determined by any available means, including using immunological assays. For example, expression levels may be determined by immunohistochemistry (IHC), Western blotting, ELISA, immunoelectrophoresis, immunoprecipitation and immunostaining.

For example, any of the anti-IL8 antibodies: Abcam Ab7747 and Ab18672, Creative BioLabs ABX-IL8, Genmab HuMax-IL8, CST #94853, Sigma I8026 and HPA057179, Santa Cruz Biotechnology Sc-376750, HuMab 10F8 may be used to detect the levels of IL-8.

For example, any of the anti-IL6 antibodies: Invitrogen #701028 IL-6 Rabbit Monoclonal Antibody (4H16L21); Invitrogen #AHC0762 IL-6 Monoclonal Antibody (8H12); Invitrogen #P620 IL-6 Polyclonal Antibody; R&D Systems MAB206 Human/Primate IL-6 Antibody; R&D Systems MAB2061 Human IL-6 Antibody; Sigma Anti-IL6 (human) Antibody MABF342; Sigma Anti-IL-6 Antibody CBL2117; Sigma Anti-IL6 Antibody MABF41; Abcam Anti-IL6 Antibody (ab6672); Abcam Anti-IL6 Antibody (ab9324) may be used to detect the levels of IL-6.

For example, any of the anti-LIF antibodies: Abcam Anti-LIF Antibody (ab113262); Abcam Anti-LIF Antibody (ab138002); R&D Systems Human LIF Antibody AF-250-NA; Invitrogen LIF Polyclonal Antibody PA5-47337; Invitrogen LIF Polyclonal Antibody PA5-21122; Santa Cruz Biotechnology LIF Antibody E-10 sc-515931; Sigma Anti-LIF Antibody produced in Rabbit HPA018844; Sigma Anti-LIF

antibody produced in Goat SAB2502145; Sigma Anti-LIF antibody produced in Mouse SAB1406083; Sigma Anti-LIF antibody produced in Rabbit SAB2101344 may be used to detect the levels of LIF.

Marker expression levels may be compared with the expression levels of the same genes prior to treatment with a TKI. The same test may be used for determining the expression level of a marker prior to and post treatment with a TKI.

The expression levels of markers may be measured/determined at any point prior to (before) or post (after) TKI treatment. The determination of expression level prior to TKI treatment may be carried out after the cancer is diagnosed. The determination of expression level prior to TKI treatment may be carried out after surgery or other treatments (but before any TKI is administered). The determination of expression level prior to treatment with the TKI may be carried out after treatment with agents other than the particular TKI.

The determination of expression level of a marker after TKI treatment may be carried out at several points after the start of TKI treatment. As such, the marker level may be used to monitor the cancer. For example, the expression level of the marker may be measured on a regular basis after treatment with a TKI has begun. For example between daily and annually. For example, the expression level may be determined weekly to annually. For example, the expression level may be determined every 2-30 weeks, for example every 2-12 weeks. At the point where the marker increases in expression, acquired resistance to the TKI is indicated.

Generally, an at least 1.5 fold increase in expression of a marker is indicative of the development of TKI resistance. In other words, an at least 1.5 fold increase in expression of a marker is indicative of acquired TKI resistance.

An increase in expression of at least one of the markers of at least 1.6-fold, 1.7-fold, 1.8-fold, 1.9-fold, 2.0-fold, 2.1-fold, 2.2-fold, 2.3-fold, 2.4-fold, 2.5-fold, 2.6-fold, 2.7-fold, 2.8-fold, 2.9-fold, 3.0-fold, 3.1-fold, 3.2-fold, 3.3-fold, 3.4-fold, 3.5-fold, 3.6-fold, 3.7-fold, 3.8-fold, 3.9-fold, or 4.0-fold is indicative of the development of resistance/acquired resistance to the TKI.

Where more than one marker is used, an increase in expression of at least 1.5-fold of at least one of the markers is indicative of acquired resistance. Additionally, at least 50% at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, of the markers may show an increase in expression. At least 50% at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, substantially all of, or all of the markers may show an increase in expression of at least 1.5-fold.

Samples

A "test sample" as used herein may, in some cases, be a cell or tissue sample (e.g. a biopsy), a biological fluid, an extract (e.g. a protein or DNA extract obtained from the subject). In particular, the sample may be a tumour sample, a blood sample (including plasma or serum sample), a cerebrospinal fluid sample, a urine sample or a non-tumour tissue sample. The sample may be one which has been freshly obtained from the subject or may be one which has been processed and/or stored prior to making a determination (e.g. frozen, fixed or subjected to one or more purification, enrichment or extractions steps). In some cases, the sample may be obtained directly from the tumour, obtained from circulating cancer cells and/or circulating tumour DNA.

The methods and uses may involve the step of determining the expression level of a marker in a sample obtained from an individual, for example, a sample of cancer cells (e.g. a tumour

sample), or a blood sample (e.g. a blood serum or plasma sample), or a urine sample.

In some embodiments the methods may comprise the step of obtaining a sample of cancer cells, a blood sample (e.g. a blood serum or plasma sample), or a urine sample from an individual. Specifically, the methods may comprise a step of obtaining a sample before treatment with the TKI, and obtaining a sample after treatment with a TKI. The obtained samples may be tested as described to determine expression levels of a marker.

As described above, the determination of expression level of a marker after TKI treatment may be carried out at several points after the start of TKI treatment. As such, the multiple samples may be taken after the start of TKI treatment. These samples may be obtained on a regular basis after treatment with a TKI has begun. For example between daily and annually. For example, the samples may be obtained weekly to annually. For example, the samples may be obtained every 2-30 weeks, for example every 2-12 weeks.

In particular a blood, e.g serum or plasma, sample may be used. This has the advantage of not requiring a more invasive biopsy. Circulating nucleic acids from all of the markers can be used to determine gene expression levels. Accordingly, the expression levels may be determined at the nucleic acid level in a blood sample.

The levels of many cytokine-cytokine interaction protein may also be monitored in the circulation. In particular, the levels of IL-8, FGF1, FGF10, FGF2, FGF23, FGF6, FIGF, IL12A, IL19, IL1A, IL1B, IL24, IL6, LIF, NGF, VEGFC, WNT10A, WNT10B, WNT11, WNT16, WNT4, WNT5A, WNT5B, and WNT7A may be measured in the circulation at the protein level. For example, the expression level of these markers may be detected in a blood sample.

These markers may also be detected in a urine sample.

Tyrosine Kinase Inhibitors

Tyrosine kinase inhibitors which can be used for the treatment of cancer find use in the present invention, in particular TKIs with a similar activity profile to Pazopanib.

These include the small molecule inhibitors Pazopanib (CAS number 444731-52-6), Regorafenib (CAS number 755037-03-7), Sorafenib (CAS number 284461-73-0), Sunitinib (CAS number 341031-54-7), Lenvatinib (CAS number 417716-92-8), Axitinib (CAS number 319460-85-0), Nintedanib (CAS number 656247-18-6), and Ponatinib (CAS number 943319-70-8), and pharmaceutically acceptable salts thereof.

Any one of these TKIs may be used in accordance with the present invention. In a preferred embodiment the TKI is Pazopanib.

While any pharmaceutically acceptable salt is contemplated herein, particular examples of salt forms of TKIs, which are contemplated in accordance with the present invention, include: for Pazopanib: hydrochloride, 5-(4-chloropyrimidin-2ylamino)-2-methylbenzenesulfonamide, N,2,3-trimethyl-2H-indazol-6-amine; for Regorafenib: Isethionate, Ethylsulfonate, Hydrochloride, Mesylate, Phenylsulfonate; for Sunitinib: Hydrochloride, Malate, Fumurate, D-tartrate, L-tartrate, Citrate; for Lenvatinib: Mesylate (main form), P-toluenesulfonate; for Nindetanib: Esiliate (main form); for Pontanib: Hydrochloride.

Salts or derivatives of the exemplary inhibitors may be used for the treatment of cancer. As used herein "derivatives" of the therapeutic agents includes salts, coordination complexes, esters such as in vivo hydrolysable esters, free acids or bases, hydrates, prodrugs or lipids, coupling partners.

Salts of the compounds of the invention are preferably physiologically well tolerated and non-toxic. Many examples of salts are known to those skilled in the art. Compounds having acidic groups, such as phosphates or sulfates, can form salts

with alkaline or alkaline earth metals such as Na, K, Mg and Ca, and with organic amines such as triethylamine and Tris (2-hydroxyethyl) amine. Salts can be formed between compounds with basic groups, e.g., amines, with inorganic acids such as hydrochloric acid, phosphoric acid or sulfuric acid, or organic acids such as acetic acid, citric acid, benzoic acid, fumaric acid, or tartaric acid. Compounds having both acidic and basic groups can form internal salts.

Esters can be formed between hydroxyl or carboxylic acid groups present in the compound and an appropriate carboxylic acid or alcohol reaction partner, using techniques well known in the art.

Derivatives which as prodrugs of the compounds are convertible in vivo or in vitro into one of the parent compounds. Typically, at least one of the biological activities of compound will be reduced in the prodrug form of the compound, and can be activated by conversion of the prodrug to release the compound or a metabolite of it.

Other derivatives include coupling partners of the compounds in which the compounds is linked to a coupling partner, e.g. by being chemically coupled to the compound or physically associated with it. Examples of coupling partners include a label or reporter molecule, a supporting substrate, a carrier or transport molecule, an effector, a drug, an antibody or an inhibitor. Coupling partners can be covalently linked to compounds of the invention via an appropriate functional group on the compound such as a hydroxyl group, a carboxyl group or an amino group. Other derivatives include formulating the compounds with liposomes.

Cancers

The cancers which are monitored and treated according to the present invention are any of the cancers treatable using the TKIs. In particular, the cancers and cancer cell lines are initially susceptible to treatment with the TKI. In other words,

the cancers are initially sensitive to treatment with a TKI. These cancer do not have an inherent resistance to the TKI.

In other words, for a sensitive cancer, treatment with the TKI will initially result in either the reduction in size of a cancer, or in the delay or prevention of growth of the cancer. Shrinking or stable tumours are thus indicative of sensitivity to a TKI. An initially sensitive tumour may show some initial increase in size due to inflammation related to tumour cell death, which is later followed by of tumour shrinkage. This occurrence may be referred to as pseudoprogression. Accordingly, initially sensitive cancers may show some increase in size before reducing or stabilising.

The terms “developed resistance” and “acquired resistance” are used interchangeably herein to refer to cancers or cell lines which were initially sensitive to a TKI, but have developed resistance. In these instances, although the cancer or cells shows initial sensitivity, they later acquire resistance and the cancer progresses. In particular, tumour growth and metastases may be signs of developed resistance. Acquired resistance may be indicated by initial shrinking or stabilisation of tumour size, followed by increase in tumour size or metastasis over the course of treatment with a TKI.

Tumour/cancer size may be monitored for example, using imaging.

The cancer may a cancer which has not previously been treated with a TKI.

Cancers to be treated or monitored according to the present invention include:
Soft tissues sarcomas (STS), for example advanced soft tissue sarcomas, metastatic renal cell carcinomas (mRCC), gastrointestinal stromal tumour (GIST), hepatocellular carcinoma (HCC), neuroendocrine tumour (NET), medullary thyroid cancer (MTC; also known as medullary thyroid carcinoma), non-squamous

non-small cell lung cancer (NSCLC), and chronic myeloid leukaemia (CML).

In particular the cancer may be STS, for example advanced STS. For example, the cancer may be spindle cell sarcoma.

If the cancer is a soft tissues sarcoma (STS), the methods disclosed herein may be employed to monitor resistance to treatment with Pazopanib or Regorafenib, in particular Pazopanib.

If the cancer is a metastatic renal cell carcinoma (mRCC), the methods disclosed herein may be employed to monitor resistance to Pazopanib, Sorafenib, Sunitinib, Lenvatinib or Axitinib. In particular the methods may be used to monitor resistance to Pazopanib for treatment of metastatic renal cell carcinoma.

If the cancer is a gastrointestinal stromal tumour (GIST), the methods disclosed herein may be employed to monitor resistance to treatment with Regorafenib or Sunitinib. The methods may be used to monitor resistance to Regorafenib as a third-line treatment for GIST.

If the cancer is a hepatocellular carcinoma (HCC), the methods disclosed herein may be employed to monitor resistance to treatment with Sorafenib.

If the cancer is a neuroendocrine tumour (NET), the methods disclosed herein may be employed to monitor resistance to treatment with Sunitinib.

If the cancer is a medullary thyroid cancer (MTC), the methods disclosed herein may be employed to monitor resistance to treatment with Lenvatinib.

If the cancer is a non-squamous non-small cell lung cancer (non-squamous NSCLC), the methods disclosed herein may be employed to monitor resistance to treatment with Nintedanib. The methods may

be used to monitor resistance to Nintedanib as a second-line treatment for non-squamous NSCLC.

If the cancer is a chronic myeloid leukaemia (CML), the methods disclosed herein may be employed to monitor resistance to treatment with Ponatinib. The methods may be used to monitor resistance to of Ponatinib to treat solid tumour CML.

In particular the cancer may be a soft-tissue sarcoma and the TKI may be Pazopanib. For example the cancer may be an advanced soft tissue sarcoma.

Methods of monitoring the development of resistance and treatments disclosed herein particularly apply to soft-tissue sarcomas and treatment with pazopanib. In particular the soft-tissue sarcoma is advanced STS and the TKI is pazopanib.

The methods disclosed herein may be applied to advanced cancers. Generally 'advanced' cancers are not amenable to curative surgery, because they are locally advanced, locally recurrent or metastatic. For example, advanced STS is STS that is not amenable to curative surgery.

It is also contemplated that the methods disclosed herein would be useful in early stage disease, for example pre-operatively.

Individuals

The individuals to be monitored in accordance with the present invention may not have previously been treated with the TKI, e.g. pazopanib.

The individual to be treated is an animal, preferably a mammal, in particular a human.

The individuals to be treated in accordance with the present invention may have a cancer that has acquired resistance to a TKI.

Prognoses

The methods described herein may also be prognostic methods, accordingly, they may include a step of determining or providing a prognosis based on the change in expression levels of a marker.

For example, the indication of development of resistance to a TKI (e.g. pazopanib) is indicative of a poor prognosis. For example, the increase in expression of may indicate overall survival of fewer than 18 months, for example fewer than 12 months or fewer than 6 months.

For example, the increase in expression of may indicate survival after onset of resistance of fewer than 18 months, for example fewer than 12 months or fewer than 6 months.

Medical treatments

The indication of development of resistance to a TKI may also inform treatment choices. For example, a patient indicated to have developed of resistance to a TKI may be selected for an alternative treatment course, for example a combination therapy, an alternative TKI, surgery, or radiotherapy. A patient indicated to have developed of resistance to a TKI may be selected for treatment with a cytokine inhibitor, or a combination of a cytokine inhibitor and the TKI as described elsewhere herein. The cytokine inhibitor is an inhibitor of IL-8, IL-6, LIF or their receptors.

The methods may include a step of treating the individual. The aspects of the invention relating to medical uses are discussed in more detail below. The details about particular TKIs, cancers (including particular combinations of TKIs and cancers) as described above apply to all of these aspects.

In one aspect the invention provides methods and medical uses for the treatment of cancers with a cytokine inhibitor. The cytokine inhibitor is an inhibitor of IL-8, IL-6, LIF or their receptors.

According to these methods and uses, the cytokine inhibitor may be used in combination with a TKI. The invention thus provides a cytokine inhibitor and a TKI for use in a method of treating cancer in an individual, the method comprising administering the cytokine inhibitor and the TKI to an individual in need thereof. The invention also provides a method treating cancer with a combination of a cytokine inhibitor and a TKI. Also provided is use of a cytokine inhibitor and a TKI in the manufacture of a medicament for treating cancer.

In another aspect, a cytokine inhibitor for use in a method of treating cancer in an individual that has developed resistance to treatment with a TKI is provided. Similarly, a method of treating cancer with a cytokine inhibitor in an individual that has developed resistance to treatment with a TKI is provided, the method comprising administering the cytokine inhibitor to an individual in need thereof. Further, the use of an cytokine inhibitor in the manufacture of a medicament for treating cancer that has developed resistance to treatment with a TKI is provided.

According to this aspect, the individual may have been determined to or indicated as having developed resistance to a TKI using the methods of monitoring cancer described herein. In particular, the individual may be treated in the event that the increase in marker expression, as described elsewhere herein, is indicative of development of resistance to a TKI. Accordingly, the methods may include the steps of:

i) obtaining a sample from an individual prior to TKI treatment

ii) determining the expression level of a marker in the sample

iii) treating the cancer with a TKI

iv) obtaining a sample from an individual after TKI treatment

v) determining the expression level of the marker in the sample

vi) comparing the expression level as determined in the sample obtained prior to treatment with the TKI with the expression level determined in the sample obtained after treatment with the TKI, wherein an at least 1.5-fold increase in expression of the marker after treatment with the TKI as compared to before treatment with the TKI is indicative of the development of resistance to the TKI; and, where the development of resistance to the TKI is indicated:

vii) administering the cytokine inhibitor, optionally in combination with the TKI, to the individual.

According to these aspects, the cytokine inhibitor for may be used in combination with a TKI for the treatment of cancer.

In another aspect, a cytokine inhibitor for use in a method of preventing the development of resistance of a cancer to a TKI in the treatment of cancer in an individual. Similarly provided is a method of preventing the development of resistance of a cancer is provided, the method comprising administering the cytokine inhibitor to an individual in need thereof, wherein the individual has developed resistance to treatment with a TKI. Further provided is the use of a cytokine inhibitor in the manufacture of a medicament for treating cancer, wherein the cytokine inhibitor is for preventing the development of resistance of the cancer to a TKI.

In the above aspects, the cytokine inhibitor may be used in combination with a TKI for the treatment of cancer.

An “individual” in need of treatment may also be described as a “patient” in need of treatment.

The cytokine inhibitor is selected from:

- an IL-8 inhibitor or IL-8 receptor inhibitor (i.e. a CXCR1 or CXCR2 inhibitor)
- an IL-6 inhibitor or IL-6 receptor inhibitor (i.e. a IL-6RA or GP130 inhibitor)

- a LIF inhibitor or LIF receptor inhibitor (i.e. a LIFRA or GP130 inhibitor)

In some preferred embodiments the cytokine inhibitor is an IL-8 or IL-8 receptor inhibitor.

Suitable TKI are described elsewhere herein and include Pazopanib, Regorafenib, Sorafenib, Sunitinib, Lenvatinib, Axitinib, Nintedanib, and Ponatinib, and pharmaceutically acceptable salts thereof. In particular the TKI may be pazopanib or a pharmaceutically acceptable salt thereof.

Types of cancer to be treated are disclosed elsewhere herein and include soft tissues sarcoma (STS), metastatic renal cell carcinomas (mRCC), gastrointestinal stromal tumour (GIST), hepatocellular carcinoma (HCC), neuroendocrine tumour (NET), medullary thyroid cancer (MTC), non-squamous non-small cell lung cancer (non-squamous NSCLC), and chronic myeloid leukaemia (CML). In particular the cancer may be STS, for example advanced soft tissue sarcoma and/or spindle cell sarcoma.

For example, the TKI is Pazopanib (or a pharmaceutically acceptable salt thereof) and the cancer may be soft tissue sarcoma. For example, the TKI may be Pazopanib (or a pharmaceutically acceptable salt thereof) and the cancer may be advanced soft tissue sarcoma.

IL-8, IL-6, LIF inhibitors

The terms "IL-8 inhibitor" and "inhibitor of IL-8" are used interchangeably herein. Similarly, the terms "IL-6 inhibitor" and "inhibitor of IL-6" are used interchangeably herein, and the terms "LIF inhibitor" and "inhibitor of LIF" are used interchangeably herein. Inhibitors of IL-8, IL-6, LIF and their receptors

The cytokine inhibitor may be a small molecule inhibitor, an antibody, for example, an IL-8 neutralising antibody, a ligand

trap, a peptide fragment or a nucleic acid inhibitor, for example an antisense nucleic acid targeting the cytokine or its receptor, siRNA, shRNA, CRISPR or miRNA. The inhibitor may be a genome editing system.

Inhibitors of interleukin 8 (IL-8) and IL-8 receptors are known in the art and are characterised by significantly inhibiting the activity of IL-8 or its receptor, or specifically decreasing the amount of IL-8.

In particular, the inhibitors may bind to IL-8 or an IL-8 receptor and inhibit the activity of the IL-8 or IL-8 receptor. In particular, an inhibitor may specifically bind to IL-8 or an IL-8 receptor. The IL-8 receptors are CXCR1 (human Entrez 3577) and CXCR2 (human Entrez 3579).

Exemplary inhibitors include small molecule inhibitors, antibodies, for example, an IL-8 neutralising antibody, ligand traps, peptide fragments and nucleic acid inhibitors, such as siRNA and antisense molecule targeting IL-8 RNA, shRNA, CRISPR or miRNA.

IL-8 receptor, CXCR1/2 inhibitors include: Reparixin (CAS 266359-93-7), Ladarixin (CAS 849776-05-2), Repertaxin (CAS 266359-83-5), Danirixin (CAS 954126-98-8), AZD-5069 (CAS 878385-84-3), AZD-8309 (CAS 333742-48-6), SB-656933 (CAS 688763-65-7), DF2755A (sodium (2S)-2-(4-{[4-(trifluoromethyl)-1,3-thiazol-2-yl]amino}phenyl) propanoate; Lopes et al., Volume 103, 2016, 69-79, ISSN 1043-6618; DF2156A (Ladarixin; CAS 849776-05-2), DF2162 ({4-[(1R)-2-amino-1-methyl-2-oxoethyl]phenyl trifluoromethanesulphonate}; Barsante et al. British Journal of Pharmacology (2008) 153, 992–1002), SCH563705 (CAS 473728-58-4), SCH527123 (Navarixin; CAS 473727-83-2).

Exemplary IL-8 neutralising antibodies include ABX-IL8, HuMax-IL8 and HuMab-10F8.

Inhibitors of interleukin 6 (IL-6) and IL-6 receptors are known in the art and are characterised by significantly inhibiting the activity of IL-6 or its receptor, or specifically decreasing the amount of IL-6.

In particular, the inhibitors may bind to IL-6 or an IL-6 receptor and inhibit the activity of the IL-6 or IL-6 receptor. In particular, an inhibitor may specifically bind to IL-6 or an IL-6 receptor. The IL-6 receptors are IL-6Rα (human Entrez 3570) and GP130 (human Entrez 3572).

IL-6 and IL-6 receptor inhibitors are known in the art, for example as disclosed in Heo et al. Oncotarget. 2016; 7:15460-15473. Examples of such inhibitors are given below, with their target given in parentheses.

Small molecule IL-6 and IL-6 receptor inhibitors include: Diacerein (IL6R) (CAS 1379-02-1; Bharti et al. Oncogene volume 35, pages 3965–3975); Bazedoxifene (GP130) (CAS 198481-32-2; Wu et al. Molecular cancer therapeutics 2016;15(11):2609-2619); Madindoline A (GP130) (CAS 184877-64-3); SC144 (GP130) (CAS 917497-70-2); Raloxifene (GP130) (CAS 84449-90-1); LMT-28 (GP130) (CAS 1239600-18-0); ERBF and ERBA (IL6R).

Antibody inhibitors of IL-6 and the IL-6 receptor include: B-R3 and B-P4 (GP130), Tocilizumab (IL6R), Sarilumab (IL6R), ALX-0061 (IL6R), NRI (IL6R), SANT-7 (IL6R), Siltuximab (IL6), Sirukumab (IL6), Olokizumab (IL6), mAb 1339 (IL6), Clazakizumab (IL6), PF-04236921 (IL6), C326 (IL6), MEDI 5117 (IL6), 6a (IL6), Sgp130Fc (IL6). These antibodies are disclosed in Heo et al. Oncotarget. 2016; 7:15460-15473.

Inhibitors of leukaemia inhibitory factor (LIF) and LIF receptors are known in the art and are characterised by significantly inhibiting the activity of LIF or its receptor, or specifically decreasing the amount of LIF.

In particular, the inhibitors may bind to LIF or a LIF receptor and inhibit the activity of the LIF or LIF receptor. In particular, an inhibitor may specifically bind to LIF or a LIF receptor. The LIF receptors are LIFRα (human Entrez 3977) and GP130 (human Entrez 3572).

LIF and LIF receptor inhibitors are known in the art, for example as disclosed in Heo et al. Oncotarget. 2016; 7:15460-15473. Examples of such inhibitors are given below, with their target given in parentheses.

Small molecule IL-6 and IL-6 receptor inhibitors include: EC330 (LIFR) (CAS 2016795-77-8; Nair et al. Mol Cancer Ther January 1 2018 (17) (1 Supplement) LB-B04; **DOI**: 10.1158/1535-7163.TARG-17-LB-B04), EC359 (LIFR) (Nair et al. Mol Cancer Ther January 1 2018 (17) (1 Supplement) LB-B04; **DOI**: 10.1158/1535-7163.TARG-17-LB-B04), Bazedoxifene (GP130) (CAS 198481-32-2; Wu et al. Molecular cancer therapeutics 2016;15(11):2609-2619); Madindoline A (GP130) (CAS 184877-64-3); SC144 (GP130) (CAS 917497-70-2); Raloxifene (GP130) (CAS 84449-90-1); LMT-28 (GP130) (CAS 1239600-18-0).

Antibody inhibitors of IL-6 and the IL-6 receptor include: B-R3 and B-P4 (GP130) (Heo et al. Oncotarget. 2016; 7:15460-15473).
The inhibitors may be used in a therapeutically effective amount. In the context of the treatment of cancer, the cytokine inhibitors may be used in an amount which induces apoptosis of cancer cells and/or induces sensitivity to TKI inhibitors (that have acquired resistance to TKI inhibitors) and/or inhibits resistance to a TKI inhibitor.

Salts or derivatives of small molecule inhibitors may be used for the treatment of cancer. As used herein "derivatives" of the therapeutic agents includes salts, coordination complexes, esters such as in vivo hydrolysable esters, free acids or bases, hydrates, prodrugs or lipids, coupling partners.

Salts of the compounds of the invention are preferably

physiologically well tolerated and non-toxic. Many examples of salts are known to those skilled in the art. Compounds having acidic groups, such as phosphates or sulfates, can form salts with alkaline or alkaline earth metals such as Na, K, Mg and Ca, and with organic amines such as triethylamine and Tris (2-hydroxyethyl) amine. Salts can be formed between compounds with basic groups, e.g., amines, with inorganic acids such as hydrochloric acid, phosphoric acid or sulfuric acid, or organic acids such as acetic acid, citric acid, benzoic acid, fumaric acid, or tartaric acid. Compounds having both acidic and basic groups can form internal salts.

Esters can be formed between hydroxyl or carboxylic acid groups present in the compound and an appropriate carboxylic acid or alcohol reaction partner, using techniques well known in the art.

Derivatives which as prodrugs of the compounds are convertible in vivo or in vitro into one of the parent compounds. Typically, at least one of the biological activities of compound will be reduced in the prodrug form of the compound, and can be activated by conversion of the prodrug to release the compound or a metabolite of it.

Other derivatives include coupling partners of the compounds in which the compounds is linked to a coupling partner, e.g. by being chemically coupled to the compound or physically associated with it. Examples of coupling partners include a label or reporter molecule, a supporting substrate, a carrier or transport molecule, an effector, a drug, an antibody or an inhibitor. Coupling partners can be covalently linked to compounds of the invention via an appropriate functional group on the compound such as a hydroxyl group, a carboxyl group or an amino group. Other derivatives include formulating the compounds with liposomes.

As used herein, the term "antibody" includes an immunoglobulin whether natural or partly or wholly synthetically produced. The

term also covers any polypeptide or protein comprising an antibody binding domain. Antibody fragments which comprise an antigen binding domain include Fab, scFv, Fv, dAb, Fd, and diabodies. It is possible to take monoclonal and other antibodies and use techniques of recombinant DNA technology to produce other antibodies or chimeric molecules which retain the specificity of the original antibody. Such techniques may involve introducing DNA encoding the immunoglobulin variable region, or the complementarity determining regions (CDRs), of an antibody to the constant regions, or constant regions plus framework regions, of a different immunoglobulin. See, for instance, EP 0 184 187 A, GB 2,188,638 A or EP 0 239 400 A.

Antibodies can be modified in a number of ways and the term "antibody molecule" should be construed as covering any specific binding member or substance having an antibody antigen-binding domain with the required specificity. Thus, this term covers antibody fragments and derivatives, including any polypeptide comprising an immunoglobulin binding domain, whether natural or wholly or partially synthetic. Chimeric molecules comprising an immunoglobulin binding domain, or equivalent, fused to another polypeptide are therefore included. Cloning and expression of chimeric antibodies are described in EP 0 120 694 A and EP 0 125 023 A.

It has been shown that fragments of a whole antibody can perform the function of binding antigens. Examples of binding fragments are (i) the Fab fragment consisting of VL, VH, CL and CH1 domains; (ii) the Fd fragment consisting of the VH and CH1 domains; (iii) the Fv fragment consisting of the VL and VH domains of a single antibody; (iv) the dAb fragment (Ward, E.S. et al., Nature 341, 544-546 (1989)) which consists of a VH domain; (v) isolated CDR regions; (vi) F(ab')2 fragments, a bivalent fragment comprising two linked Fab fragments (vii) single chain Fv molecules (scFv), wherein a VH domain and a VL domain are linked by a peptide linker which allows the two domains to associate to form an antigen binding site (Bird et al,

Science, 242; 423-426, 1988; Huston et al, PNAS USA, 85: 5879-5883, 1988); (viii) bispecific single chain Fv dimers (WO 93/11161) and (ix) "diabodies", multivalent or multispecific fragments constructed by gene fusion (WO 94/13804; Holliger et al, P.N.A.S. USA, 90: 6444-6448, 1993); (x) immunoadhesins (WO 98/50431). Fv, scFv or diabody molecules may be stabilised by the incorporation of disulphide bridges linking the VH and VL domains (Reiter et al, Nature Biotech, 14: 1239-1245, 1996). Minibodies comprising a scFv joined to a CH3 domain may also be made (Hu et al, Cancer Res., 56: 3055-3061, 1996).

Preferred antibodies used in accordance with the present invention are isolated, in the sense of being free from contaminants such as antibodies able to bind other polypeptides and/or free of serum components. Monoclonal antibodies are preferred for some purposes, though polyclonal antibodies are within the scope of the present invention.

The reactivities of antibodies on a sample may be determined by any appropriate means. Tagging with individual reporter molecules is one possibility. The reporter molecules may directly or indirectly generate detectable, and preferably measurable, signals. The linkage of reporter molecules may be directly or indirectly, covalently, e.g. via a peptide bond or non-covalently. Linkage via a peptide bond may be as a result of recombinant expression of a gene fusion encoding antibody and reporter molecule. One favoured mode is by covalent linkage of each antibody with an individual fluorochrome, phosphor or laser exciting dye with spectrally isolated absorption or emission characteristics. Suitable fluorochromes include fluorescein, rhodamine, phycoerythrin and Texas Red. Suitable chromogenic dyes include diaminobenzidine.

Other reporters include macromolecular colloidal particles or particulate material such as latex beads that are coloured, magnetic or paramagnetic, and biologically or chemically active agents that can directly or indirectly cause detectable signals

to be visually observed, electronically detected or otherwise recorded. These molecules may be enzymes which catalyse reactions that develop or change colours or cause changes in electrical properties, for example. They may be molecularly excitable, such that electronic transitions between energy states result in characteristic spectral absorptions or emissions. They may include chemical entities used in conjunction with biosensors. Biotin/avidin or biotin/streptavidin and alkaline phosphatase detection systems may be employed.

Antibodies according to the present invention may be used in screening for the presence of a polypeptide, for example in a test sample containing cells or cell lysate as discussed, and may be used in purifying and/or isolating a polypeptide according to the present invention, for instance following production of the polypeptide by expression from encoding nucleic acid. Antibodies may modulate the activity of the polypeptide to which they bind and so, if that polypeptide has a deleterious effect in an individual, may be useful in a therapeutic context (which may include prophylaxis).

Another class of inhibitors useful for treating cancer according to the present invention is ligand traps. Ligand traps comprise an antibody regions (e.g. the Fc region) and a ligand binding domain of another protein.

A ligand trap may act as a free form of the target receptor to be inhibited, thus preventing binding of a ligand to the native receptor.

In the context of the present invention, the ligand trap may bind to IL-8, IL-6 or LIF. In other words, the ligand trap may comprise the ligand binding domain of an IL-8, IL-6 or LIF receptor, or a variant thereof which binds to IL-8, IL-6 or LIF.

Another class of inhibitors useful for treating cancer in accordance with the invention is peptide fragments that interfere

with the activity of the IL-8, IL-6 or LIF receptors. Peptide fragments may be generated wholly or partly by chemical synthesis that block the catalytic sites of the IL-8, IL-6 or LIF receptors. A peptide fragment may interfere with receptor dimerization, for example.

Peptide fragments can be readily prepared according to well-established, standard liquid or, preferably, solid-phase peptide synthesis methods, general descriptions of which are broadly available (see, for example, in J.M. Stewart and J.D. Young, Solid Phase Peptide Synthesis, 2nd edition, Pierce Chemical Company, Rockford, Illinois (1984), in M. Bodanzsky and A. Bodanzsky, The Practice of Peptide Synthesis, Springer Verlag, New York (1984); and Applied Biosystems 430A Users Manual, ABI Inc., Foster City, California), or they may be prepared in solution, by the liquid phase method or by any combination of solid-phase, liquid phase and solution chemistry, e.g. by first completing the respective peptide portion and then, if desired and appropriate, after removal of any protecting groups being present, by introduction of the residue X by reaction of the respective carbonic or sulfonic acid or a reactive derivative thereof.

Other candidate compounds for inhibiting IL-8, IL-6 or LIF receptors may be based on modelling the 3-dimensional structure of these receptors and using rational drug design to provide candidate compounds with particular molecular shape, size and charge characteristics. A candidate inhibitor, for example, may be a "functional analogue" of a peptide fragment or other compound which inhibits the component. A functional analogue has the same functional activity as the peptide or other compound in question. Examples of such analogues include chemical compounds which are modelled to resemble the three dimensional structure of the component in an area which contacts another component, and in particular the arrangement of the key amino acid residues as they appear.

Another class of inhibitors useful for treatment of cancer in accordance with the invention includes nucleic acid inhibitors of IL-8, IL-6 or LIF or their receptors, or the complements thereof, which inhibit activity or function by down-regulating production of active polypeptide. This can be monitored using conventional methods well known in the art, for example by screening using real time PCR.

Expression of IL-8, IL-6 or LIF or their receptors may be inhibited using anti-sense or RNAi technology. The use of these approaches to down-regulate gene expression is now well-established in the art.

Anti-sense oligonucleotides may be designed to hybridise to the complementary sequence of nucleic acid, pre-mRNA or mature mRNA, interfering with the production of the base excision repair pathway component so that its expression is reduced or completely or substantially completely prevented. In addition to targeting coding sequence, anti-sense techniques may be used to target control sequences of a gene, e.g. in the 5' flanking sequence, whereby the anti-sense oligonucleotides can interfere with expression control sequences. The construction of anti-sense sequences and their use is described for example in Peyman & Ulman, Chemical Reviews, 90:543-584, 1990 and Crooke, Ann. Rev. Pharmacol. Toxicol., 32:329-376, 1992.

Oligonucleotides may be generated *in vitro* or *ex vivo* for administration or anti-sense RNA may be generated *in vivo* within cells in which down-regulation is desired. Thus, double-stranded DNA may be placed under the control of a promoter in a "reverse orientation" such that transcription of the anti-sense strand of the DNA yields RNA which is complementary to normal mRNA transcribed from the sense strand of the target gene. The complementary anti-sense RNA sequence is thought then to bind with mRNA to form a duplex, inhibiting translation of the endogenous mRNA from the target gene into protein. Whether or not this is the actual mode of action is still uncertain.

However, it is established fact that the technique works.

The complete sequence corresponding to the coding sequence in reverse orientation need not be used. For example fragments of sufficient length may be used. It is a routine matter for the person skilled in the art to screen fragments of various sizes and from various parts of the coding or flanking sequences of a gene to optimise the level of anti-sense inhibition. It may be advantageous to include the initiating methionine ATG codon, and perhaps one or more nucleotides upstream of the initiating codon. A suitable fragment may have about 14-23 nucleotides, e.g., about 15, 16 or 17 nucleotides.

An alternative to anti-sense is to use a copy of all or part of the target gene inserted in sense, that is the same orientation as the target gene, to achieve reduction in expression of the target gene by co-suppression (Angell & Baulcombe, The EMBO Journal 16(12):3675-3684, 1997 and Voinnet & Baulcombe, Nature, 389: 553, 1997). Double stranded RNA (dsRNA) has been found to be even more effective in gene silencing than both sense or antisense strands alone (Fire et al, Nature 391, 806-811, 1998). dsRNA mediated silencing is gene specific and is often termed RNA interference (RNAi). Methods relating to the use of RNAi to silence genes in *C. elegans*, Drosophila, plants, and mammals are known in the art (Fire, Trends Genet., 15: 358-363, 19999; Sharp, RNA interference, Genes Dev. 15: 485-490 2001; Hammond et al., Nature Rev. Genet. 2: 110-1119, 2001; Tuschl, Chem. Biochem. 2: 239-245, 2001; Hamilton et al., Science 286: 950-952, 1999; Hammond, et al., Nature 404: 293-296, 2000; Zamore et al., Cell, 101: 25-33, 2000; Bernstein, Nature, 409: 363-366, 2001; Elbashir et al, Genes Dev., 15: 188-200, 2001; WO01/29058; WO99/32619, and Elbashir et al, Nature, 411: 494-498, 2001).

RNA interference is a two-step process. First, dsRNA is cleaved within the cell to yield short interfering RNAs (siRNAs) of about 21-23nt length with 5' terminal phosphate and 3' short overhangs (~2nt). The siRNAs target the corresponding mRNA sequence

specifically for destruction (Zamore, Nature Structural Biology, 8, 9, 746-750, 2001.

RNAi may also be efficiently induced using chemically synthesized siRNA duplexes of the same structure with 3'-overhang ends (Zamore et al, Cell, 101: 25-33, 2000). Synthetic siRNA duplexes have been shown to specifically suppress expression of endogenous and heterologeous genes in a wide range of mammalian cell lines (Elbashir et al, Nature, 411: 494-498, 2001).

Another possibility is that nucleic acid is used which on transcription produces a ribozyme, able to cut nucleic acid at a specific site and therefore also useful in influencing gene expression, e.g., see Kashani-Sabet & Scanlon, Cancer Gene Therapy, 2(3): 213-223, 1995 and Mercola & Cohen, Cancer Gene Therapy, 2(1): 47-59, 1995.

Small RNA molecules may be employed to regulate gene expression. These include targeted degradation of mRNAs by small interfering RNAs (siRNAs), post transcriptional gene silencing (PTGs), developmentally regulated sequence-specific translational repression of mRNA by micro-RNAs (miRNAs) and targeted transcriptional gene silencing.

A role for the RNAi machinery and small RNAs in targeting of heterochromatin complexes and epigenetic gene silencing at specific chromosomal loci has also been demonstrated. Double-stranded RNA (dsRNA)-dependent post transcriptional silencing, also known as RNA interference (RNAi), is a phenomenon in which dsRNA complexes can target specific genes of homology for silencing in a short period of time. It acts as a signal to promote degradation of mRNA with sequence identity. A 20-nt siRNA is generally long enough to induce gene-specific silencing, but short enough to evade host response. The decrease in expression of targeted gene products can be extensive with 90% silencing induced by a few molecules of siRNA.

In the art, these RNA sequences are termed "short or small interfering RNAs" (siRNAs) or "microRNAs" (miRNAs) depending on their origin. Both types of sequence may be used to down-regulate gene expression by binding to complimentary RNAs and either triggering mRNA elimination (RNAi) or arresting mRNA translation into protein. siRNA are derived by processing of long double stranded RNAs and when found in nature are typically of exogenous origin. Micro-interfering RNAs (miRNA) are endogenously encoded small non-coding RNAs, derived by processing of short hairpins. Both siRNA and miRNA can inhibit the translation of mRNAs bearing partially complimentary target sequences without RNA cleavage and degrade mRNAs bearing fully complementary sequences.

The siRNA ligands are typically double stranded and, in order to optimise the effectiveness of RNA mediated down-regulation of the function of a target gene, it is preferred that the length of the siRNA molecule is chosen to ensure correct recognition of the siRNA by the RISC complex that mediates the recognition by the siRNA of the mRNA target and so that the siRNA is short enough to reduce a host response.

miRNA ligands are typically single stranded and have regions that are partially complementary enabling the ligands to form a hairpin. miRNAs are RNA genes which are transcribed from DNA, but are not translated into protein. A DNA sequence that codes for a miRNA gene is longer than the miRNA. This DNA sequence includes the miRNA sequence and an approximate reverse complement. When this DNA sequence is transcribed into a single-stranded RNA molecule, the miRNA sequence and its reverse-complement base pair to form a partially double stranded RNA segment. The design of microRNA sequences is discussed in John et al, PLoS Biology, 11(2), 1862-1879, 2004.

Typically, the RNA ligands intended to mimic the effects of siRNA or miRNA have between 10 and 40 ribonucleotides (or synthetic analogues thereof), more preferably between 17 and 30

ribonucleotides, more preferably between 19 and 25 ribonucleotides and most preferably between 21 and 23 ribonucleotides. In some embodiments of the invention employing double-stranded siRNA, the molecule may have symmetric 3' overhangs, e.g. of one or two (ribo)nucleotides, typically a UU of dTdT 3' overhang. Based on the disclosure provided herein, the skilled person can readily design suitable siRNA and miRNA sequences, for example using resources such as Ambion's siRNA finder, see http://www.ambion.com/techlib/misc/siRNA_finder.html. siRNA and miRNA sequences can be synthetically produced and added exogenously to cause gene downregulation or produced using expression systems (e.g. vectors). In a preferred embodiment the siRNA is synthesized synthetically.

Longer double stranded RNAs may be processed in the cell to produce siRNAs (e.g. see Myers, Nature Biotechnology, 21: 324-328, 2003). The longer dsRNA molecule may have symmetric 3' or 5' overhangs, e.g. of one or two (ribo)nucleotides, or may have blunt ends. The longer dsRNA molecules may be 25 nucleotides or longer. Preferably, the longer dsRNA molecules are between 25 and 30 nucleotides long. More preferably, the longer dsRNA molecules are between 25 and 27 nucleotides long. Most preferably, the longer dsRNA molecules are 27 nucleotides in length. dsRNAs 30 nucleotides or more in length may be expressed using the vector pDECAP (Shinagawa et al., Genes and Dev., 17: 1340-5, 2003).

Another alternative is the expression of a short hairpin RNA molecule (shRNA) in the cell. shRNAs are more stable than synthetic siRNAs. A shRNA consists of short inverted repeats separated by a small loop sequence. One inverted repeat is complimentary to the gene target. In the cell the shRNA is processed by DICER into a siRNA which degrades the target gene mRNA and suppresses expression. In a preferred embodiment the shRNA is produced endogenously (within a cell) by transcription from a vector. shRNAs may be produced within a cell by transfecting the cell with a vector encoding the shRNA sequence

under control of a RNA polymerase III promoter such as the human H1 or 7SK promoter or a RNA polymerase II promoter. Alternatively, the shRNA may be synthesised exogenously (in vitro) by transcription from a vector. The shRNA may then be introduced directly into the cell. Preferably, the shRNA sequence is between 40 and 100 bases in length, more preferably between 40 and 70 bases in length. The stem of the hairpin is preferably between 19 and 30 base pairs in length. The stem may contain G-U pairings to stabilise the hairpin structure.

In one embodiment, the siRNA, longer dsRNA or miRNA is produced endogenously (within a cell) by transcription from a vector. The vector may be introduced into the cell in any of the ways known in the art. Optionally, expression of the RNA sequence can be regulated using a tissue specific promoter. In a further embodiment, the siRNA, longer dsRNA or miRNA is produced exogenously (*in vitro*) by transcription from a vector.

Alternatively, siRNA molecules may be synthesized using standard solid or solution phase synthesis techniques, which are known in the art. Linkages between nucleotides may be phosphodiester bonds or alternatives, e.g., linking groups of the formula P(O)S, (thioate); P(S)S, (dithioate); P(O)NR'2; P(O)R'; P(O)OR6; CO; or CONR'2 wherein R is H (or a salt) or alkyl (1-12C) and R6 is alkyl (1-9C) is joined to adjacent nucleotides through-O-or-S-.

Modified nucleotide bases can be used in addition to the naturally occurring bases, and may confer advantageous properties on siRNA molecules containing them.

For example, modified bases may increase the stability of the siRNA molecule, thereby reducing the amount required for silencing. The provision of modified bases may also provide siRNA molecules, which are more, or less, stable than unmodified siRNA.

The term 'modified nucleotide base' encompasses nucleotides with

a covalently modified base and/or sugar. For example, modified nucleotides include nucleotides having sugars, which are covalently attached to low molecular weight organic groups other than a hydroxyl group at the 3'position and other than a phosphate group at the 5'position. Thus modified nucleotides may also include 2'substituted sugars such as 2'-O-methyl- ; 2-O-alkyl ; 2-O-allyl ; 2'-S-alkyl; 2'-S-allyl; 2'-fluoro- ; 2'-halo or 2; azido-ribose, carbocyclic sugar analogues a-anomeric sugars; epimeric sugars such as arabinose, xyloses or lyxoses, pyranose sugars, furanose sugars and sedoheptulose.

Modified nucleotides are known in the art and include alkylated purines and pyrimidines, acylated purines and pyrimidines, and other heterocycles. These classes of pyrimidines and purines are known in the art and include pseudoisocytosine, N4,N4-ethanocytosine, 8-hydroxy-N6-methyladenine, 4-acetylcytosine,5-(carboxyhydroxylmethyl) uracil, 5 fluorouracil, 5-bromouracil, 5-carboxymethylaminomethyl-2-thiouracil, 5-carboxymethylaminomethyl uracil, dihydrouracil, inosine, N6-isopentyl-adenine, 1-methyladenine, 1-methylpseudouracil, 1-methylguanine, 2,2-dimethylguanine, 2methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-methyladenine, 7-methylguanine, 5-methylaminomethyl uracil, 5-methoxy amino methyl-2-thiouracil, -D-mannosylqueosine, 5-methoxycarbonylmethyluracil, 5methoxyuracil, 2 methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid methyl ester, psueouracil, 2-thiocytosine, 5-methyl-2 thiouracil, 2-thiouracil, 4-thiouracil, 5methyluracil, N-uracil-5-oxyacetic acid methylester, uracil 5-oxyacetic acid, queosine, 2-thiocytosine, 5-propyluracil, 5-propylcytosine, 5-ethyluracil, 5ethylcytosine, 5-butyluracil, 5-pentyluracil, 5-pentylcytosine, and 2,6,diaminopurine, methylpsuedouracil, 1-methylguanine, 1-methylcytosine.

Other inhibitors of IL-8, IL-6 or LIF or their receptors include genome editing systems, for example Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 systems, zinc

finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), as well as systems using other nucleases that can cause DNA breaks or bind to DNA. These systems can be used to prevent the expression of functioning IL-8, IL-6 or LIF or their receptors in target cells. Such genome editing systems are also inhibitors within the scope of the present invention.

Administration and pharmaceutical compositions

The active agents disclosed herein for the treatment of cancer, such as IL-8 inhibitors and TKI, may be administered alone, but it is generally preferable to provide them in pharmaceutical compositions that additionally comprise with one or more pharmaceutically acceptable carriers, adjuvants, excipients, diluents, fillers, buffers, stabilisers, preservatives, lubricants, or other materials well known to those skilled in the art and optionally other therapeutic or prophylactic agents. Examples of components of pharmaceutical compositions are provided in Remington's Pharmaceutical Sciences, 20th Edition, 2000, pub. Lippincott, Williams & Wilkins.

The term "pharmaceutically acceptable" as used herein includes compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgement, suitable for use in contact with the tissues of a subject (e.g. human) without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio. Each carrier, excipient, etc. must also be "acceptable" in the sense of being compatible with the other ingredients of the formulation.

The active agents disclosed herein for the treatment of cancer are preferably for administration to an individual in a "prophylactically effective amount" or a "therapeutically effective amount" (as the case may be, although prophylaxis may be considered therapy), this being sufficient to show benefit to the individual. For example, the agents (inhibitors) may be administered in amount sufficient to delay tumour progression, or

prevent tumour growth and/or metastasis or to shrink tumours. For example, the agents may be administered in an amount sufficient to induce apoptosis of cancer cells.

The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated. Prescription of treatment, e.g. decisions on dosage etc., is within the responsibility of general practitioners and other medical doctors, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Examples of the techniques and protocols mentioned above can be found in Remington's Pharmaceutical Sciences, 20th Edition, 2000, Lippincott, Williams & Wilkins. A composition may be administered alone or in combination with other treatments, either simultaneously or sequentially, dependent upon the condition to be treated.

The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. Such methods include the step of bringing the active compound into association with a carrier, which may constitute one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association the active compound with liquid carriers or finely divided solid carriers or both, and then if necessary shaping the product.

The agents disclosed herein for the treatment of cancer may be administered to a subject by any convenient route of administration, whether systemically/peripherally or at the site of desired action, including but not limited to, oral (e.g. by ingestion); topical (including e.g. transdermal, intranasal, ocular, buccal, and sublingual); pulmonary (e.g. by inhalation or insufflation therapy using, e.g. an aerosol, e.g. through mouth or nose); rectal; vaginal; parenteral, for example, by injection,

including subcutaneous, intradermal, intramuscular, intravenous, intraarterial, intracardiac, intrathecal, intraspinal, intracapsular, subcapsular, intraorbital, intraperitoneal, intratracheal, subcuticular, intraarticular, subarachnoid, and intrasternal; by implant of a depot, for example, subcutaneously or intramuscularly.

Formulations suitable for oral administration (e.g., by ingestion) may be presented as discrete units such as capsules, cachets or tablets, each containing a predetermined amount of the active compound; as a powder or granules; as a solution or suspension in an aqueous or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion; as a bolus; as an electuary; or as a paste.

Formulations suitable for parenteral administration (e.g., by injection, including cutaneous, subcutaneous, intramuscular, intravenous and intradermal), include aqueous and non-aqueous isotonic, pyrogen-free, sterile injection solutions which may contain anti-oxidants, buffers, preservatives, stabilisers, bacteriostats, and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents, and liposomes or other microparticulate systems which are designed to target the compound to blood components or one or more organs. Examples of suitable isotonic vehicles for use in such formulations include Sodium Chloride Injection, Ringer's Solution, or Lactated Ringer's Injection. Typically, the concentration of the active compound in the solution is from about 1 ng/ml to about 10 μg/ml, for example from about 10 ng/ml to about 1 μg/ml. The formulations may be presented in unit-dose or multi-dose sealed containers, for example, ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules, and

tablets. Formulations may be in the form of liposomes or other microparticulate systems which are designed to target the active compound to blood components or one or more organs.

Compositions comprising agents disclosed herein for the treatment of cancer may be used in the methods described herein in combination with standard chemotherapeutic regimes or in conjunction with radiotherapy. Examples of other chemotherapeutic agents include Amsacrine (Amsidine), Bleomycin, Busulfan, Capecitabine (Xeloda), Carboplatin, Carmustine (BCNU), Chlorambucil(Leukeran), Cisplatin, Cladribine(Leustat), Clofarabine (Evoltra), Crisantaspase (Erwinase), Cyclophosphamide, Cytarabine (ARA-C), Dacarbazine (DTIC), Dactinomycin (Actinomycin D),Daunorubicin, Docetaxel (Taxotere), Doxorubicin, Epirubicin, Etoposide (Vepesid, VP-16), Fludarabine (Fludara), Fluorouracil (5-FU), Gemcitabine (Gemzar), Hydroxyurea (Hydroxycarbamide, Hydrea) ,Idarubicin (Zavedos). Ifosfamide (Mitoxana), Irinotecan (CPT-11, Campto), Leucovorin (folinic acid), Liposomal doxorubicin (Caelyx, Myocet), Liposomal daunorubicin (DaunoXome®) Lomustine, Melphalan, Mercaptopurine, Mesna, Methotrexate, Mitomycin, Mitoxantrone, Oxaliplatin (Eloxatin), Paclitaxel (Taxol), Pemetrexed (Alimta), Pentostatin (Nipent), Procarbazine, Raltitrexed (Tomudex®), Streptozocin (Zanosar®), Tegafur-uracil (Uftoral), Temozolomide (Temodal), Teniposide (Vumon), Thiotepa, Tioguanine (6-TG) (Lanvis), Topotecan (Hycamtin), Treosulfan, Vinblastine (Velbe), Vincristine (Oncovin), Vindesine (Eldisine) and Vinorelbine (Navelbine).

Methods of determining the most effective means and dosage of administration are well known to those of skill in the art and will vary with the formulation used for therapy, the purpose of the therapy, the target cell being treated, and the subject being treated. Single or multiple administrations can be carried out with the dose level and pattern being selected by the treating physician. Where the active compound is a salt, an ester, prodrug, or the like, the amount administered is calculated on

the basis of the parent compound, and so the actual weight to be used is increased proportionately.

Where both an IL-8 inhibitor and a TKI are administered, this may be referred to as a combination therapy or combined treatment. Their use "in combination" denotes any form of concurrent or parallel treatment with am IL-8 inhibitor and a TKI.

Administration of the IL-8 inhibitor and the TKI may be in the same composition or in separate compositions. In one aspect a pharmaceutical composition comprising IL-8 and a TKI is provided. The pharmaceutical composition for use in a method of treatment is contemplated.

Where the IL-8 inhibitor and the TKI are in the same composition, administration of the two inhibitors is simultaneous.

In other embodiments, the IL-8 inhibitor and the TKI are in separate compositions and may be administered simultaneously or sequentially. Sequential administration means that the IL-8 inhibitor is administered prior to or after administration of the TKI.

Figures

Figure 1. IL8 and other cytokine signalling-related gene expression in clinical tumour samples are associated with pazopanib resistance.

Expression of 730 cancer signalling pathway-related genes in pre-pazopanib tumour samples was measured for each patient in n=38 cohort. Expression of these genes was also measured in post-progression tumour for one of these patients (RMH001).

In 38 pre-treatment samples, SAM analysis was used to identify 6 genes that had significantly increased expression in the 7 cases with a poor-prognosis related IHC pattern of low FGFR1 and high PDGFRA expression (F-Lo/P-Hi)vs 31 other cases (blue) When comparing pre- and post-pazopanib samples from RMH001, 179 genes

were found have at least 1.5 fold increase in expression (red; right-hand side of figure).

Figure 2. Clinical overview, pazopanib response and pre-and post-treatment tumour gene expression in a patient with long-term response to pazopanib.

Patient RMH001 underwent radical resection of a high grade spindle cell sarcoma of the uterus at month 0, followed by early relapse with disseminated abdomino-pelvic disease. Site and burden of relapsed disease is graphically summarised (NB exponential cm^3 scale) over clinical course up to point post-pazopanib tumour sampling.

Therapeutic exposure to radiotherapy and systemic agents is superimposed. 3rd line systemic therapy with pazopanib was commenced for progressive disease during month 15, prior to which an excision biopsy of abdominal wall metastasis had been sampled and serves as pre-pazopanib tumour specimen. After good initial response to pazopanib (see disease burden graphic and PET-CT images from Month 14 and Month 20), the patient underwent consolidation radiotherapy, followed by eventual disease progression and discontinuation of pazopanib at month 27. A core needle biopsy of a progressing abdominal wall metastasis was taken during month 29 and serves as post-progression tumour specimen.

Targeted gene expression measurement was performed on pre-pazopanib and post-progression sample. Shown are fold change in expression levels of the 3 genes overlapping between red and blue gene lists in Figure 1.

Figure 3. Rhabdoid tumour cell lines show sensitivity to pazopanib.

Dose-response curves of pazopanib-resistant (black; top 12 lines) and –sensitive (red/blue; bottom two lines) cell lines. A panel of 14 sarcoma cell lines was treated with a range of drug

concentrations to determine IC_{50} values. Cell viability is normalised to DMSO control (n=2 or 3).

Figure 4. Pazopanib-sensitive cell lines develop acquired resistance with chronic and prolonged drug treatment

Dose-response curves of pazopanib-resistant PazR variants (red; top line) and parental cells (black; bottom line) – **A**; A204 and A204PazR, **B**; G402 and G402PazR. Cell viability is normalised to DMSO control (n=3).

Figure 5. Addition of IL-8 leads to an increase in cell viability in the presence of pazopanib.

Dose-response curves of parental cells with pazopanib treatment only (black; bottom lines) and parental cells treated concomitantly with pazopanib and 100 ng/µL IL-8 (red; top lines) – **A**; A204 and A204PazR, **B**; G402 and G402PazR. Cell viability is normalised to DMSO control (n=3). **C**; Bar plots showing the IC_{50} values derived from Figures 5A-B. Statistical analysis of the concomitant pazopanib/IL-8 treatment versus pazopanib only was performed by paired Student's t test (Paz only left-hand bar, Paz +IL8 right-hand bar).

Figure 6. Addition of IL-8 leads to a decrease in apoptosis resulting from pazopanib treatment.

Bar plots showing the normalised fold change in caspase 3/7 activity in cells treated with pazopanib only or cells treated concomitantly with pazopanib and 100 ng/µL IL-8 (n=3) – **A**; A204, **B**: G402. Data are normalised to DMSO control. Statistical analysis between the concomitant pazopanib/IL-8 treatment versus pazopanib only was performed by ANOVA with Tukey's multiple comparison test (Paz only left-hand bar, Paz +IL8 right-hand bar).

Figure 7. siRNA IL-8 knockdown in PazR variants decreases cell viability

Bar plots showing cell viability of cells upon siRNA silencing of IL-8 – **A**; A204PazR, **B**; G402PazR. Cell viability data are

normalised to RISC-Free transfection (n=3). Statistical analysis of siIL-8 versus RISC-Free was performed by paired Student's t test.

Figure 8. Addition of IL-8, IL-6 or LIF leads to an increase in cell viability in the presence of pazopanib.
Bar plots showing the IC_{50} values resulting from dose-response curves to pazopanib or pazopanib plus cytokine (IL8, IL-6 or LIF). Statistical analysis of the concomitant pazopanib + IL-8/IL-6/LIF treatment versus pazopanib only was performed by paired Student's t test.

Examples

Experimental procedures

Patient selection and treatment

Collection and analysis of anonymised archival FFPE tissue and associated clinical data was approved as a sub-study protocol amendment to the Royal Marsden-sponsored Elucidation of a Molecular signature of Pazopanib Response in Advanced soft tissue Sarcoma including Solitary fibrous tumours (EMPRASS) study. Patients were retrospectively identified for inclusion by search of institutional database and electronic patient records complied during routine clinical practice. Eligibility criteria for inclusion were: i) histopathological diagnosis of soft tissue tumour as confirmed by specialist sarcoma histopathologist; ii) received at least one dose of pazopanib for treatment of unresectable or advanced STS; iii) available FFPE tumour specimen, obtained from patient prior to first dose of pazopanib. Treatment and response monitoring was as per standard institutional practice, with pazopanib at 800 mg once daily until disease progression, intolerable toxicity or significant clinical deterioration. Dose interruption and/or reduction were instigated based on standard institutional guidelines and the discretion of the treating physician. Baseline clinicopathological characteristics and survival data were collected on retrospective review of contemporaneous electronic medical records. All related radiological imaging was retrospectively reviewed and disease

response assed on computerised tomography (CT) images according to RECIST 1.1.

Tissue selection and processing

Available pre-pazopanib FFPE tumour specimens were identified and retrieved from our institutional diagnostic archive. Where more than one pre-treatment specimen was identified, the one taken closest to pazopanib start date was processed. For RMH001, who exhibited good initial pazopanib response, both pre-pazopanib and post-progression FFPE tumour specimens were obtained. Newly sectioned H&E slides were reviewed to confirm viable tumour content. With reference to H&E, blocks containing tumour material of sufficient size were marked in three spatially discrete areas of representative viable tumour tissue. Sections were then used for total RNA extraction using All Prep DNA/RNA FFPE kit (Qiagen, Hilden, Germany) following vendor's standard protocol. RNA concentrations were measured using Qubit fluorometric quantitation (Thermo Fisher Scientific, Waltham, MA, USA). RNA integrity number and percentage of tRNA <300 bp in size was measured using 2100 Bioanalyzer system (Agilent, Santa Clara, CA, USA). RNA was stored at -80 °C until use in downstream analyses.

Gene Expression Analysis

Expression of 730 genes, representing 13 major cancer pathways including key driver genes was assessed using nCounter PanCancer Pathways panel (NanoString Technologies, Seattle, WA, USA). 150 ng total RNA was used as input for hybridisation and digital analysis as per the manufacturer's instructions using nCounter Dx analysis system (NanoString Technologies). In cases with high RNA degradation, loading adjustments of up to 300 ng were made. Expression data was processed as follows: a) background correction was performed by subtracting the geometric mean of the negative control probes, b) normalised by positive control normalisation factor calculated as geometric mean of the positive controls followed by normalisation with the housekeeping genes. Expression values were then Log2 transformed and subjected to gene-based centring

Computation of Pazopanib resistant Module score

In order to find genes involved in intrinsic resistance to pazopanib, we performed SAM analysis[5] on gene expression data from the RMH-SARC cohort to identify genes with significantly difference in expression when comparing tumours from cases with F-Lo/P-Hi IHC status versus the rest of the cohort. For each sample, a gene module score was calculated according to the expression of the relevant genes as described below:

$$\text{Module score(s)} = \sum_{i=1}^{n} W_i X_i / \sum |W_i|$$

where n is the number of genes in a module, X_i represents the normalised gene expression in the new sample, and gene-specific weights W_i are equal to their associated weights as shown in **Table 1**, according to the direction (+/-) of their association with the phenotype in the RMH-SARC cohort (n=38), as determined using SAM analysis.

Cell culture and derivation of acquired resistant sublines

A204 and G402 cells were obtained from ATCC. Cells were cultured in DMEM media supplemented with 10% FBS and 0.5% penicillin/streptomycin in 95% air, 5% CO_2 atmosphere at 37 °C. Media replenished twice weekly. Cells were stored in 20% DMSO-FBS freezing media in liquid nitrogen.

Pazopanib (LC Laboratories, Woburn, MA, USA) was used to induce resistance in A204/G402 cells. Cells were grown initially in media containing drug concentration at the determined IC_{50} values from cell viability assays. The drug was incremented when cells had proliferated to near confluency alongside minimal visible cell death. Drug concentration was incremented to 1 µM, 2 µM, 3 µM and 5 µM in a stepwise manner. A final drug concentration of 5 µM was maintained in resistant cells. Resistance was determined using cell viability assays. Media and drug replenished twice weekly.

Cell Viability and Apoptosis Assays

Cells were seeded (2,000/well) into 96-well plates. After 24 hrs, cells were treated with inhibitors/ligands at the indicated concentration and/or combination and incubated for 24 hrs for apoptosis measurement by Caspase-Glo 3/7 Assay (Promega, Madison, WI, USA) or for 72 hrs for cell viability measurement by CellTiter-Glo Assay (Promega), following the manufacturer's recommendations. IC_{50} data were generated from dose response curves using three/four-parameter regression fit in PRISM 7 software (GraphPad, La Jolla, CA, USA). Drugs and ligands used include Pazopanib (LC Laboratories), and Recombinant Human IL-8, LIF and IL-6 (Peprotech, Rocky Hill, NJ, USA).

siRNA Transfection

Cells (2,000/well) were reverse transfected into 96 well plates with SMARTpool siGENOME IL-8 siRNA (Dharmacon, Lafayette, CO, USA) using Lullaby transfection reagent (Oz Biosciences, San Diego, CA, USA) and OptiMEM (Gibco, Waltham, MA, USA). Transfected cells were incubated for 72 hrs post-transfection for cell viability CellTiter-Glo assay (Promega), following the manufacturer's recommendations. Results were normalised to cells transfected with a non-targeting siRNA pool. siRNA transfection controls included untransfected cells, cells transfected with UBB siRNA (Dharmacon), and cells transfected with siGlo RISC-Free siRNA (Dharmacon).

Results

IL-8 is associated with intrinsic and acquired clinical resistance to pazopanib

To identify genes with a potential role in conferring tumour resistance to pazopanib, we took a multi-pronged approach outlined in **Figure 1.** In order to find genes involved in intrinsic resistance to pazopanib, we performed SAM analysis of gene expression data from pre-treatment specimens from a cohort of sarcoma patients that have been treated with pazopanib - RMH-SARC (n=38). This analysis identified genes with significantly increased expression in cases with FGFR1-low/PDGFRA-high (F-Lo/P-

Hi) IHC status versus the rest of the cohort. We have previously identified that sarcoma patients with F-Lo/P-Hi IHC status harbour intrinsic pazopanib resistance and have very poor outcomes when treated with pazopanib (see, UK1712871.1).

To assess which genes may be involved in acquired resistance to pazopanib, we also analysed a single patient case study within RMH-SARC (RMH001) who had experienced a long-term response of almost 12 months of disease control with pazopanib therapy (**Figure 2**). We generated and analysed gene expression data derived from tumour samples taken 4 months prior to commencing pazopanib therapy (pre-treatment sample) and 2 months after tumour progression and discontinuation of pazopanib (post-pazopanib sample). (**Figure 1-2**). From the long-term responder single patient tissue series, we focused on all genes with >1.5 fold increase in expression in post-pazopanib compared to the pre-treatment specimen (**Figure 1-2**).

SAM analysis identified 6 genes with significantly increased expression in F-Lo/P-Hi compared to IHC negative tumours (**Figure 1-2; Table 1**).

Table 1. 6 genes identified by SAM analysis with significantly increased expression in F-Lo/P-Hi patients vs 31 IHC negative cases.

Gene name	ENTREZ ID	Module Score(d)	Fold Change	q-value (%)
LIF	3976	2.724	6.400	0
IL-6	3569	2.577	6.403	0
IL-8	3576	3.108	14.960	0
ETV4	2118	2.563	4.806	0
IL-11	3589	3.242	6.364	0
SSX1	6756	2.661	8.664	0

179 genes had an at least 1.5 fold increase in expression between pre-treatment and post-progression samples in RMH001 (**Figure 2; Table 2**).

Table 2. 179 genes identified as >1.5 fold increase in expression between pre- and post-pazopanib tumour samples in RMH001.

Gene name	ENTREZ ID
ACVR1C	130399
ACVR2A	92
ALK	238
ASXL1	171023
B2M	567
BCL2A1	597
BID	637
BIRC3	330
BMP8A	353500
BNIP3	664
CACNA2D2	9254
CACNG4	27092
CALML5	51806
CALML6	163688
CAPN2	824
CASP12	120329
CASP9	842
CBLC	23624
CCND2	894
CD19	930
CDC14A	8556
CDC14B	8555
CDH1	999

Gene name	ENTREZ ID
IGFBP3	3486
IL12A	3592
IL13RA2	3598
IL19	29949
IL1A	3552
IL1B	3553
IL1R1	3554
IL22RA1	58985
IL23R	149233
IL24	11009
IL2RA	3559
IL6	3569
IL6R	3570
IL8	3576
INHBA	3624
IRAK2	3656
IRS1	3667
ITGA2	3673
ITGA3	3675
ITGA6	3655
ITGA8	8516
ITGA9	3680
ITGB3	3690

Gene name	ENTREZ ID
PIK3R1	5295
PLA1A	51365
PLA2G4A	5321
PLAU	5328
POLE2	5427
POLR2D	5433
PPARGC1A	10891
PPP2CB	5516
PPP3CA	5530
PPP3CC	5533
PRKACB	5567
PRKAR2A	5576
PRKAR2B	5577
PTEN	5728
PTPN11	5781
RAC1	5879
RASAL1	8437
RB1	5925
RELN	5649
RET	5979
RIN1	9610
RPS6KA5	9252
RRAS2	22800

Gene name	ENTREZ ID
CDKN2A	1029
CEBPE	1053
CLCF1	23529
CNTFR	1271
COL2A1	1280
COL3A1	1281
COL5A1	1289
CRLF2	64109
CTNNB1	1499
DDIT3	1649
DUSP4	1846
E2F5	1875
EFNA2	1943
EGFR	1956
EPHA2	1969
FAS	355
FGF1	2246
FGF10	2255
FGF2	2247
FGF23	8074
FGF6	2251
FIGF	2277
FLNA	2316
FLT1	2321
FLT3	2322
FN1	2335
FUT8	2530
GATA3	2625
GNG12	55970

Gene name	ENTREZ ID
ITGB6	3694
ITGB7	3695
JAG1	182
KAT2B	8850
KITLG	4254
LAMA1	284217
LAMA3	3909
LAMB3	3914
LAMC3	10319
LEFTY2	7044
LEPR	3953
LIF	3976
LTBP1	4052
MAD2L2	10459
MAML2	84441
MAP2K4	6416
MAP3K1	4214
MAP3K5	4217
MAPK1	5594
MAPK10	5602
MDM2	4193
MED12	9968
MMP3	4314
MMP7	4316
MMP9	4318
MNAT1	4331
MPL	4352
NBN	4683
NFATC1	4772

Gene name	ENTREZ ID
SF3B1	23451
SFN	2810
SFRP1	6422
SFRP2	6423
SHC3	53358
SIRT4	23409
SKP1	6500
SOCS1	8651
SOCS2	8835
SOST	50964
SPOP	8405
SPP1	6696
STAG2	10735
TBL1XR1	79718
TCL1B	9623
TET2	54790
TLR2	7097
TMPRSS2	7113
TNFRSF10A	8797
TNFRSF10D	8793
TNN	63923
TSHR	7253
TSPAN7	7102
TTK	7272
UTY	7404
VEGFC	7424
WNT10A	80326
WNT10B	7480
WNT11	7481

Gene name	ENTREZ ID
GRIA3	2892
GTF2H3	2967
H2AFX	3014
HES5	388585
HOXA9	3205
HPGD	3248
HSP90B1	7184
IBSP	3381
ID4	3400

Gene name	ENTREZ ID
NFE2L2	4780
NGF	4803
NOG	9241
NOS3	4846
NUPR1	26471
PAX8	7849
PBRM1	55193
PDGFD	80310
PGF	5228

Gene name	ENTREZ ID
WNT16	51384
WNT4	54361
WNT5A	7474
WNT5B	81029
WNT7A	7476

3 genes overlapped between these two gene sets (**Figure 1: Table 3**).

Table 3. 3 overlapping genes between Tables 1 and 2.

Gene name	ENTREZ ID
IL-6	3569
IL-8	3576
LIF	3976

IL-8 was one of the three overlapping genes across the two gene sets. The data demonstrates that an increase in IL-8 gene expression is associated with acquired clinical resistance to pazopanib in a system that was previously sensitive and responded well to treatment. IL-8 was therefore the focus of our subsequent validation studies.

Functional validation demonstrates that IL-8 mediates acquired resistance to pazopanib

We and others have shown that across multiple sarcoma cell line models that the rhabdoid tumour cell lines A204 and G402 harbour exquisite sensitivity to pazopanib treatment[4] (**Figure 3**). We first sought to assess if IL-8 was capable of inducing resistance to pazopanib in these cell lines. As a positive control, we used

previously described acquired pazopanib resistant (PazR) variants of these cells which have been subjected to long-term escalating dose treatment with pazopanib (**Figure 4A-B**). Addition of exogenous IL-8 led to an increase in cell viability in the presence of pazopanib in both A204 and G402 (**Figure 5A-C**). We also assessed the impact of IL-8 on the apoptosis caused by pazopanib treatment. IL-8 was capable of reducing apoptosis resulting from pazopanib treatment (**Figure 6A-B**). In addition, we performed siRNA-mediated knockdown of IL-8 in the A204PazR and G402PazR variants which resulted in a significant decrease in cell viability compared to the RISC-free controls showing that these sub-lines have growth dependency on IL-8 (**Figure 7A-B**). This data demonstrates that IL-8 induces pazopanib resistance via an increase in cell viability and a reduction in apoptosis in two distinct cell line models.

Functional validation demonstrates that IL-6 and LIF mediates acquired resistance to pazopanib.

Similar to the results shown for IL-8 above, addition of exogenous IL-6 or LIF led to an increase in cell viability in the presence of pazopanib in both A204 and G402 (Figure 8).

Statements of disclosure

Numbered statements relating to the disclosure are presented below:

1. A method of monitoring cancer in an individual for the development of resistance to treatment with a TKI, the method comprising:

comparing the expression level of a marker before treatment with the TKI, to expression levels of the marker after treatment with the TKI,

wherein an at least 1.5-fold increase in expression of the marker after treatment with the TKI as compared to before treatment with the TKI is indicative of the development of resistance to the TKI,

wherein the marker is selected from:

IL8, ACVR1C, ACVR2A, ALK, ASXL1, B2M, BCL2A1, BID, BIRC3, BMP8A, BNIP3, CACNA2D2, CACNG4, CALML5, CALML6, CAPN2, CASP12, CASP9, CBLC, CCND2, CD19, CDC14A, CDC14B, CDH1, CDKN2A, CEBPE, CLCF1, CNTFR, COL2A1, COL3A1, COL5A1, CRLF2, CTNNB1, DDIT3, DUSP4, E2F5, EFNA2, EGFR, EPHA2, FAS, FGF1, FGF10, FGF2, FGF23, FGF6, FIGF, FLNA, FLT1, FLT3, FN1, FUT8, GATA3, GNG12, GRIA3, GTF2H3, H2AFX, HES5, HOXA9, HPGD, HSP90B1, IBSP, ID4, IGFBP3, IL12A, IL13RA2, IL19, IL1A, IL1B, IL1R1, IL22RA1, IL23R, IL24, IL2RA, IL6, IL6R, INHBA, IRAK2, IRS1, ITGA2, ITGA3, ITGA6, ITGA8, ITGA9, ITGB3, ITGB6, ITGB7, JAG1, KAT2B, KITLG, LAMA1, LAMA3, LAMB3, LAMC3, LEFTY2, LEPR, LIF, LTBP1, MAD2L2, MAML2, MAP2K4, MAP3K1, MAP3K5, MAPK1, MAPK10, MDM2, MED12, MMP3, MMP7, MMP9, MNAT1, MPL, NBN, NFATC1, NFE2L2, NGF, NOG, NOS3, NUPR1, PAX8, PBRM1, PDGFD, PGF, PIK3R1, PLA1A, PLA2G4A, PLAU, POLE2, POLR2D, PPARGC1A, PPP2CB, PPP3CA, PPP3CC, PRKACB, PRKAR2A, PRKAR2B, PTEN, PTPN11, RAC1, RASAL1, RB1, RELN, RET, RIN1, RPS6KA5, RRAS2, SF3B1, SFN, SFRP1, SFRP2, SHC3, SIRT4, SKP1, SOCS1, SOCS2, SOST, SPOP, SPP1, STAG2, TBL1XR1, TCL1B, TET2, TLR2, TMPRSS2, TNFRSF10A, TNFRSF10D, TNN, TSHR, TSPAN7, TTK, UTY, VEGFC, WNT10A, WNT10B, WNT11, WNT16, WNT4, WNT5A, WNT5B, and WNT7A.

2. The method of statement 1, wherein the marker is selected from IL-8, IL-6 and LIF.

3. The method of statement 1, wherein the marker is IL-8.

4. The method of any one of statements 1-3, wherein the expression levels of a plurality of markers before treatment with the TKI is compared with the expression level of each of the plurality of markers after treatment with the TKI,

wherein an at least 1.5-fold increase in expression of at least one of the markers after treatment with the TKI as compared to before treatment with the TKI is indicative of the development of resistance to the TKI,

wherein the plurality of markers is selected from: IL8, ACVR1C, ACVR2A, ALK, ASXL1, B2M, BCL2A1, BID, BIRC3, BMP8A, BNIP3, CACNA2D2, CACNG4, CALML5, CALML6, CAPN2, CASP12, CASP9, CBLC, CCND2, CD19, CDC14A, CDC14B, CDH1, CDKN2A, CEBPE, CLCF1, CNTFR, COL2A1, COL3A1, COL5A1, CRLF2, CTNNB1, DDIT3, DUSP4, E2F5, EFNA2, EGFR, EPHA2, FAS, FGF1, FGF10, FGF2, FGF23, FGF6, FIGF, FLNA, FLT1, FLT3, FN1, FUT8, GATA3, GNG12, GRIA3, GTF2H3, H2AFX, HES5, HOXA9, HPGD, HSP90B1, IBSP, ID4, IGFBP3, IL12A, IL13RA2, IL19, IL1A, IL1B, IL1R1, IL22RA1, IL23R, IL24, IL2RA, IL6, IL6R, INHBA, IRAK2, IRS1, ITGA2, ITGA3, ITGA6, ITGA8, ITGA9, ITGB3, ITGB6, ITGB7, JAG1, KAT2B, KITLG, LAMA1, LAMA3, LAMB3, LAMC3, LEFTY2, LEPR, LIF, LTBP1, MAD2L2, MAML2, MAP2K4, MAP3K1, MAP3K5, MAPK1, MAPK10, MDM2, MED12, MMP3, MMP7, MMP9, MNAT1, MPL, NBN, NFATC1, NFE2L2, NGF, NOG, NOS3, NUPR1, PAX8, PBRM1, PDGFD, PGF, PIK3R1, PLA1A, PLA2G4A, PLAU, POLE2, POLR2D, PPARGC1A, PPP2CB, PPP3CA, PPP3CC, PRKACB, PRKAR2A, PRKAR2B, PTEN, PTPN11, RAC1, RASAL1, RB1, RELN, RET, RIN1, RPS6KA5, RRAS2, SF3B1, SFN, SFRP1, SFRP2, SHC3, SIRT4, SKP1, SOCS1, SOCS2, SOST, SPOP, SPP1, STAG2, TBL1XR1, TCL1B, TET2, TLR2, TMPRSS2, TNFRSF10A, TNFRSF10D, TNN, TSHR, TSPAN7, TTK, UTY, VEGFC, WNT10A, WNT10B, WNT11, WNT16, WNT4, WNT5A, WNT5B, and WNT7A.

5. The method of any one of the preceding statements, comprising a step of:

determining the expression level of the at least one marker prior to treatment; and

determining the expression level of the at least one marker after TKI treatment,

wherein the comparing step compares the expression level as determined in the prior to treatment with the expression level determined after treatment.

6. The method of statements 5, wherein the determining steps are conducted *ex vivo*.

7. The method of any one of the preceding statements, comprising a step of:

determining the expression level of the at least one marker in a sample obtained from the patient prior to treatment with the TKI; and

determining the expression level of the at least one marker in a sample obtained from the patient after treatment with the TKI,

wherein the comparing step compares the expression level as determined in the sample obtained prior to treatment with the TKI with the expression level determined in the sample obtained after treatment with the TKI.

8. The method of statement 7, wherein the samples are blood samples.

9. The method of statement 7, wherein the samples are samples of cancer cells.

10. The method of any one of the preceding statements wherein the cancer is initially sensitive to the TKI.

11. The method of any one of the preceding statements, further comprising the step of delivering a prognosis, wherein the increase in expression of one or more markers is indicative of a poor prognosis.

12. The method of any one of the preceding statements wherein the TKI is selected from: Pazopanib, Regorafenib, Sorafenib, Sunitinib, Lenvatinib, Axitinib, Nintedanib, and Ponatinib, and pharmaceutically acceptable salts thereof.

13. The method of any one of the preceding statements wherein the TKI is Pazopanib or a pharmaceutically acceptable salt thereof.

14. The method of any one of the preceding statements wherein the cancer is selected from: soft tissues sarcoma (STS), metastatic renal cell carcinomas (mRCC), gastrointestinal stromal tumour (GIST), hepatocellular carcinoma (HCC), neuroendocrine tumour (NET), medullary thyroid cancer (MTC), non-squamous non-small cell lung cancer (non-squamous NSCLC), and chronic myeloid leukaemia (CML).

15. The method of any one of the preceding statements wherein the cancer is STS, for example advanced soft tissue sarcoma and/or spindle cell sarcoma.

16. The method of any one of the preceding statements wherein the TKI is Pazopanib or a pharmaceutically acceptable salt thereof and the cancer is soft tissue sarcoma.

17. The method of any one of the preceding statements wherein the TKI is Pazopanib or a pharmaceutically acceptable salt thereof and the cancer is advanced soft tissue sarcoma.

18. The method of any one of the preceding statements wherein an increase in expression of the marker of at least 1.6-fold, 1.7-fold, 1.8-fold, 1.9-fold, 2.0-fold, 2.1-fold, 2.2-fold, 2.3-fold, 2.4-fold, 2.5-fold, 2.6-fold, 2.7-fold, 2.8-fold, 2.9-fold, 3.0-fold, 3.1-fold, 3.2-fold, 3.3-fold, 3.4-fold, 3.5-fold, 3.6-fold, 3.7-fold, 3.8-fold, 3.9-fold, or 4.0-fold is indicative of the development of resistance to the TKI.

19. A cytokine inhibitor and a TKI for use in a method of treating cancer in an individual, wherein the cytokine inhibitor is

a) an inhibitor of IL-8 and/or an inhibitor of an IL-8 receptor; and/or

b) an inhibitor of IL-6 and/or an inhibitor of an IL-6 receptor; and/or

c) an inhibitor of LIF and/or an inhibitor of a LIF receptor.

20. A cytokine inhibitor for use in a method of treating cancer in an individual that has developed resistance to treatment with a TKI, wherein the cytokine inhibitor is

a) an inhibitor of IL-8 and/or an inhibitor of an IL-8 receptor; and/or

b) an inhibitor of IL-6 and/or an inhibitor of an IL-6 receptor; and/or

c) an inhibitor of LIF and/or an inhibitor of a LIF receptor.

21. The cytokine inhibitor for use according to statement 20, wherein the individual has been determined to have developed resistance to a TKI using the method of any one of statements 1-18.

22. A cytokine inhibitor for use in a method of preventing the development of resistance of a cancer to a TKI in the treatment of cancer in an individual, wherein the cytokine inhibitor is

a) an inhibitor of IL-8 and/or an inhibitor of an IL-8 receptor; and/or

b) an inhibitor of IL-6 and/or an inhibitor of an IL-6 receptor; and/or

c) an inhibitor of LIF and/or an inhibitor of a LIF receptor.

23. The cytokine inhibitor for use according to any one of statements 20-22, wherein the cytokine inhibitor is used in combination with a TKI for the treatment of cancer.

24. The cytokine inhibitor, or the cytokine inhibitor and a TKI for use according to any one of statements 19-23, wherein the TKI is selected from: Pazopanib, Regorafenib, Sorafenib, Sunitinib, Lenvatinib, Axitinib, Nintedanib, and Ponatinib, and pharmaceutically acceptable salts thereof.

25. The cytokine inhibitor, or the cytokine inhibitor and a TKI for use according to any one of statements 19-23, wherein the TKI is Pazopanib or a pharmaceutically acceptable salt thereof.

26. The cytokine inhibitor, or the cytokine inhibitor and a TKI for use according to any one of statements 19-25, wherein the cancer is selected from: soft tissues sarcoma (STS), metastatic renal cell carcinomas (mRCC), gastrointestinal stromal tumour (GIST), hepatocellular carcinoma (HCC), neuroendocrine tumour (NET), medullary thyroid cancer (MTC), non-squamous non-small cell lung cancer (non-squamous NSCLC), and chronic myeloid leukaemia (CML).

27. The cytokine inhibitor, or the cytokine inhibitor and a TKI for use according to statement 26, wherein the cancer is STS, for example advanced soft tissue sarcoma and/or spindle cell sarcoma.

28. The cytokine inhibitor, or the cytokine inhibitor and a TKI for use according to any one of statements 19-27, wherein the TKI is Pazopanib or a pharmaceutically acceptable salt thereof and the cancer is soft tissue sarcoma.

29. The cytokine inhibitor, or the cytokine inhibitor and a TKI for use according to any one of statements 19-28 wherein the TKI is Pazopanib or a pharmaceutically acceptable salt thereof and the cancer is advanced soft tissue sarcoma.

30. The cytokine inhibitor, or the cytokine inhibitor and a TKI for use according to any one of statements 19-29, wherein the cytokine inhibitor is a small molecule inhibitor, an antibody, a ligand trap, a peptide fragment or a nucleic acid inhibitor.

31. The cytokine inhibitor, or the cytokine inhibitor and a TKI for use according to any one of statements 19-29, wherein the cytokine inhibitor is an IL-8 or IL-8 receptor inhibitor selected from: Reparixin (CAS 266359-93-7), Ladarixin (CAS 849776-05-2), Repertaxin (CAS 266359-83-5), Danirixin (CAS 954126-98-8), AZD-5069 (CAS 878385-84-3), AZD-8309 (CAS 333742-48-6), SB-656933 (CAS 688763-65-7), DF2755A (sodium (2S)-2-(4-{[4-(trifluoromethyl)-1,3-thiazol-2-yl]amino}phenyl) propanoate); DF2156A (Ladarixin; CAS 849776-05-2), DF2162 ({4-[(1R)-2-amino-1-methyl-2-oxoethyl]phenyl trifluoromethanesulphonate}), SCH563705 (CAS 473728-58-4), SCH527123 (Navarixin; CAS 473727-83-2), ABX-IL8, HuMax-IL8 and HuMab-10F8.

32. A pharmaceutical composition comprising a cytokine inhibitor and a TKI, wherein the cytokine inhibitor is

a) an inhibitor of IL-8 and/or an inhibitor of an IL-8 receptor; and/or

b) an inhibitor of IL-6 and/or an inhibitor of an IL-6 receptor; and/or

c) an inhibitor of LIF and/or an inhibitor of a LIF receptor.

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4. Wong, J.P., Todd, J.R., Finetti, M.A., McCarthy, F., Broncel, M., Vyse, S., Luczynski, M.T., Crosier, S., Ryall, K.A., Holmes, K., Payne, L.S., Daley, F., Wai, P., Jenks, A., Tanos, B., Tan, A.C., Natrajan, R.C., Williamson, D., and Huang, P.H., 2016, Dual Targeting of PDGFRα and FGFR1 Displays Synergistic Efficacy in Malignant Rhabdoid Tumors, Cell Rep., 17 (5), Pg. 1265-1275

5. Tusher, V.G., Tibshirani, R., and Chu, G., 2001, Significance analysis of microarrays applied to the ionizing radiation response, Proc Natl Acad Sci USA, 98 (9), Pg. 5116-5121

***

All references cited herein are incorporated herein by reference in their entirety and for all purposes to the same extent as if each individual publication or patent or patent application was

specifically and individually indicated to be incorporated by reference in its entirety.

The specific embodiments described herein are offered by way of example, not by way of limitation. Any sub-titles herein are included for convenience only, and are not to be construed as limiting the disclosure in any way.

Claims

1. A method of monitoring cancer in an individual for the development of resistance to treatment with a TKI, the method comprising:

comparing the expression level of a marker before treatment with the TKI, to expression levels of the marker after treatment with the TKI,

wherein an at least 1.5-fold increase in expression of the marker after treatment with the TKI as compared to before treatment with the TKI is indicative of the development of resistance to the TKI,

wherein the marker is selected from:

IL8, ACVR1C, ACVR2A, ALK, ASXL1, B2M, BCL2A1, BID, BIRC3, BMP8A, BNIP3, CACNA2D2, CACNG4, CALML5, CALML6, CAPN2, CASP12, CASP9, CBLC, CCND2, CD19, CDC14A, CDC14B, CDH1, CDKN2A, CEBPE, CLCF1, CNTFR, COL2A1, COL3A1, COL5A1, CRLF2, CTNNB1, DDIT3, DUSP4, E2F5, EFNA2, EGFR, EPHA2, FAS, FGF1, FGF10, FGF2, FGF23, FGF6, FIGF, FLNA, FLT1, FLT3, FN1, FUT8, GATA3, GNG12, GRIA3, GTF2H3, H2AFX, HES5, HOXA9, HPGD, HSP90B1, IBSP, ID4, IGFBP3, IL12A, IL13RA2, IL19, IL1A, IL1B, IL1R1, IL22RA1, IL23R, IL24, IL2RA, IL6, IL6R, INHBA, IRAK2, IRS1, ITGA2, ITGA3, ITGA6, ITGA8, ITGA9, ITGB3, ITGB6, ITGB7, JAG1, KAT2B, KITLG, LAMA1, LAMA3, LAMB3, LAMC3, LEFTY2, LEPR, LIF, LTBP1, MAD2L2, MAML2, MAP2K4, MAP3K1, MAP3K5, MAPK1, MAPK10, MDM2, MED12, MMP3, MMP7, MMP9, MNAT1, MPL, NBN, NFATC1, NFE2L2, NGF, NOG, NOS3, NUPR1, PAX8, PBRM1, PDGFD, PGF, PIK3R1, PLA1A, PLA2G4A, PLAU, POLE2, POLR2D, PPARGC1A, PPP2CB, PPP3CA, PPP3CC, PRKACB, PRKAR2A, PRKAR2B, PTEN, PTPN11, RAC1, RASAL1, RB1, RELN, RET, RIN1, RPS6KA5, RRAS2, SF3B1, SFN, SFRP1, SFRP2, SHC3, SIRT4, SKP1, SOCS1, SOCS2, SOST, SPOP, SPP1, STAG2, TBL1XR1, TCL1B, TET2, TLR2, TMPRSS2, TNFRSF10A, TNFRSF10D, TNN, TSHR, TSPAN7, TTK, UTY, VEGFC, WNT10A, WNT10B, WNT11, WNT16, WNT4, WNT5A, WNT5B, and WNT7A.

2. The method of claim 1, wherein the marker is selected from IL-8, IL-6 and LIF.

3. The method of claim 1, wherein the marker is IL-8.

4. The method of any one of claims 1-3, wherein the expression levels of a plurality of markers before treatment with the TKI is compared with the expression level of each of the plurality of markers after treatment with the TKI,

wherein an at least 1.5-fold increase in expression of at least one of the markers after treatment with the TKI as compared to before treatment with the TKI is indicative of the development of resistance to the TKI,

wherein the plurality of markers is selected from: IL8, ACVR1C, ACVR2A, ALK, ASXL1, B2M, BCL2A1, BID, BIRC3, BMP8A, BNIP3, CACNA2D2, CACNG4, CALML5, CALML6, CAPN2, CASP12, CASP9, CBLC, CCND2, CD19, CDC14A, CDC14B, CDH1, CDKN2A, CEBPE, CLCF1, CNTFR, COL2A1, COL3A1, COL5A1, CRLF2, CTNNB1, DDIT3, DUSP4, E2F5, EFNA2, EGFR, EPHA2, FAS, FGF1, FGF10, FGF2, FGF23, FGF6, FIGF, FLNA, FLT1, FLT3, FN1, FUT8, GATA3, GNG12, GRIA3, GTF2H3, H2AFX, HES5, HOXA9, HPGD, HSP90B1, IBSP, ID4, IGFBP3, IL12A, IL13RA2, IL19, IL1A, IL1B, IL1R1, IL22RA1, IL23R, IL24, IL2RA, IL6, IL6R, INHBA, IRAK2, IRS1, ITGA2, ITGA3, ITGA6, ITGA8, ITGA9, ITGB3, ITGB6, ITGB7, JAG1, KAT2B, KITLG, LAMA1, LAMA3, LAMB3, LAMC3, LEFTY2, LEPR, LIF, LTBP1, MAD2L2, MAML2, MAP2K4, MAP3K1, MAP3K5, MAPK1, MAPK10, MDM2, MED12, MMP3, MMP7, MMP9, MNAT1, MPL, NBN, NFATC1, NFE2L2, NGF, NOG, NOS3, NUPR1, PAX8, PBRM1, PDGFD, PGF, PIK3R1, PLA1A, PLA2G4A, PLAU, POLE2, POLR2D, PPARGC1A, PPP2CB, PPP3CA, PPP3CC, PRKACB, PRKAR2A, PRKAR2B, PTEN, PTPN11, RAC1, RASAL1, RB1, RELN, RET, RIN1, RPS6KA5, RRAS2, SF3B1, SFN, SFRP1, SFRP2, SHC3, SIRT4, SKP1, SOCS1, SOCS2, SOST, SPOP, SPP1, STAG2, TBL1XR1, TCL1B, TET2, TLR2, TMPRSS2, TNFRSF10A, TNFRSF10D, TNN, TSHR, TSPAN7, TTK, UTY, VEGFC, WNT10A, WNT10B, WNT11, WNT16, WNT4, WNT5A, WNT5B, and WNT7A.

5. The method of any one of the preceding claims, comprising a step of:

determining the expression level of the at least one marker prior to treatment; and

determining the expression level of the at least one marker after TKI treatment,

wherein the comparing step compares the expression level as determined in the prior to treatment with the expression level determined after treatment.

6. The method of claim 5, wherein the determining steps are conducted *ex vivo*.

7. The method of any one of the preceding claims, comprising a step of:

determining the expression level of the at least one marker in a sample obtained from the patient prior to treatment with the TKI; and

determining the expression level of the at least one marker in a sample obtained from the patient after treatment with the TKI,

wherein the comparing step compares the expression level as determined in the sample obtained prior to treatment with the TKI with the expression level determined in the sample obtained after treatment with the TKI.

8. The method of claim 7, wherein the samples are blood samples.

9. The method of claim 7, wherein the samples are samples of cancer cells.

10. The method of any one of the preceding claims wherein the cancer is initially sensitive to the TKI.

11. The method of any one of the preceding claims, further comprising the step of delivering a prognosis, wherein the increase in expression of one or more markers is indicative of a poor prognosis.

12. The method of any one of the preceding claims wherein the TKI is selected from: Pazopanib, Regorafenib, Sorafenib, Sunitinib, Lenvatinib, Axitinib, Nintedanib, and Ponatinib, and pharmaceutically acceptable salts thereof.

13. The method of any one of the preceding claims wherein the TKI is Pazopanib or a pharmaceutically acceptable salt thereof.

14. The method of any one of the preceding claims wherein the cancer is selected from: soft tissues sarcoma (STS), metastatic renal cell carcinomas (mRCC), gastrointestinal stromal tumour (GIST), hepatocellular carcinoma (HCC), neuroendocrine tumour (NET), medullary thyroid cancer (MTC), non-squamous non-small cell lung cancer (non-squamous NSCLC), and chronic myeloid leukaemia (CML).

15. The method of any one of the preceding claims wherein the cancer is STS, for example advanced soft tissue sarcoma and/or spindle cell sarcoma.

16. The method of any one of the preceding claims wherein the TKI is Pazopanib or a pharmaceutically acceptable salt thereof and the cancer is soft tissue sarcoma.

17. The method of any one of the preceding claims wherein the TKI is Pazopanib or a pharmaceutically acceptable salt thereof and the cancer is advanced soft tissue sarcoma.

18. The method of any one of the preceding claims wherein an increase in expression of the marker of at least 1.6-fold, 1.7-fold, 1.8-fold, 1.9-fold, 2.0-fold, 2.1-fold, 2.2-fold, 2.3-fold, 2.4-fold, 2.5-fold, 2.6-fold, 2.7-fold, 2.8-fold, 2.9-fold, 3.0-fold, 3.1-fold, 3.2-fold, 3.3-fold, 3.4-fold, 3.5-fold, 3.6-fold, 3.7-fold, 3.8-fold, 3.9-fold, or 4.0-fold is indicative of the development of resistance to the TKI.

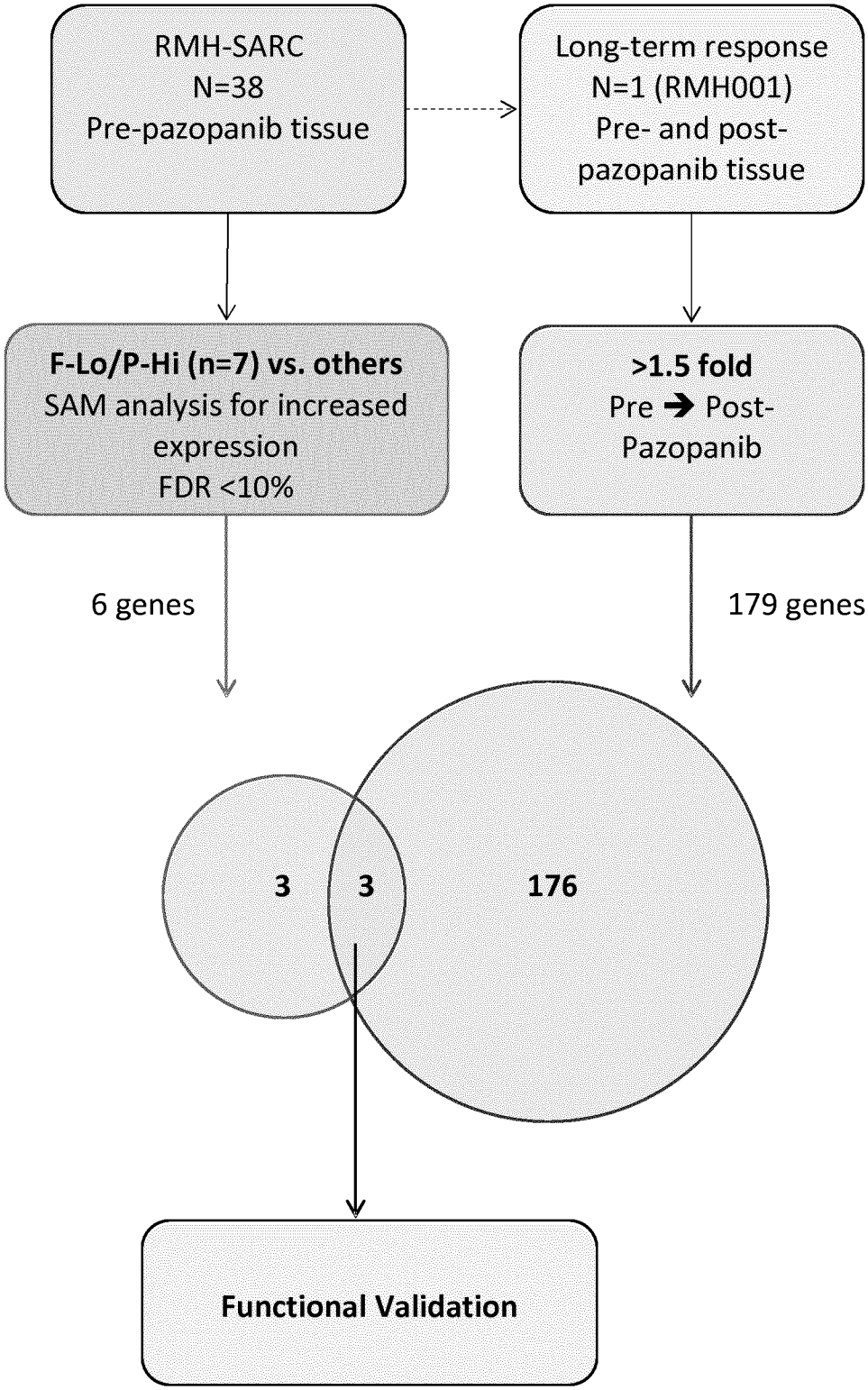
RMH-SARC
N=38
Pre-pazopanib tissue
Long-term response
N=1 (RMH001)
Pre- and post-pazopanib tissue
F-Lo/P-Hi (n=7) vs. others
SAM analysis for increased expression
FDR <10%
>1.5 fold
Pre → Post-Pazopanib
6 genes
179 genes
3
3
176
Functional Validation

Figure 1

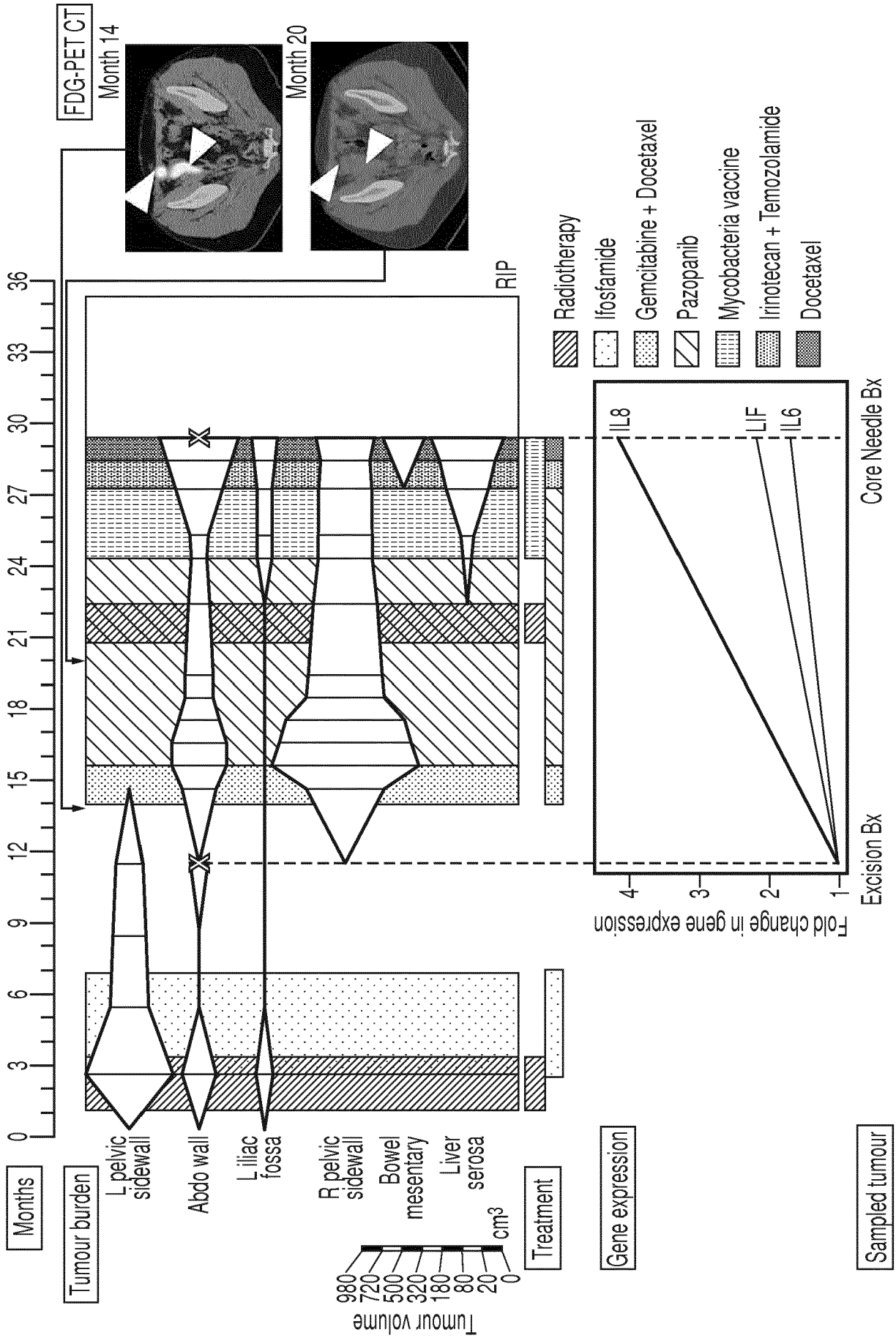
FDG-PET CT
Month 14
Month 20
RIP
Radiotherapy
Ifosfamide
Gemcitabine + Docetaxel
Pazopanib
Mycobacteria vaccine
Irinotecan + Temozolamide
Docetaxel
IL8
LIF
IL6
Core Needle Bx
Excision Bx
Fold change in gene expression
Months
Tumour burden
L pelvic sidewall
Abdo wall
L iliac fossa
R pelvic sidewall
Bowel mesentary
Liver serosa
Tumour volume
980
720
500
320
180
80
20
0
cm3
Treatment
Gene expression
Sampled tumour

FIG. 2

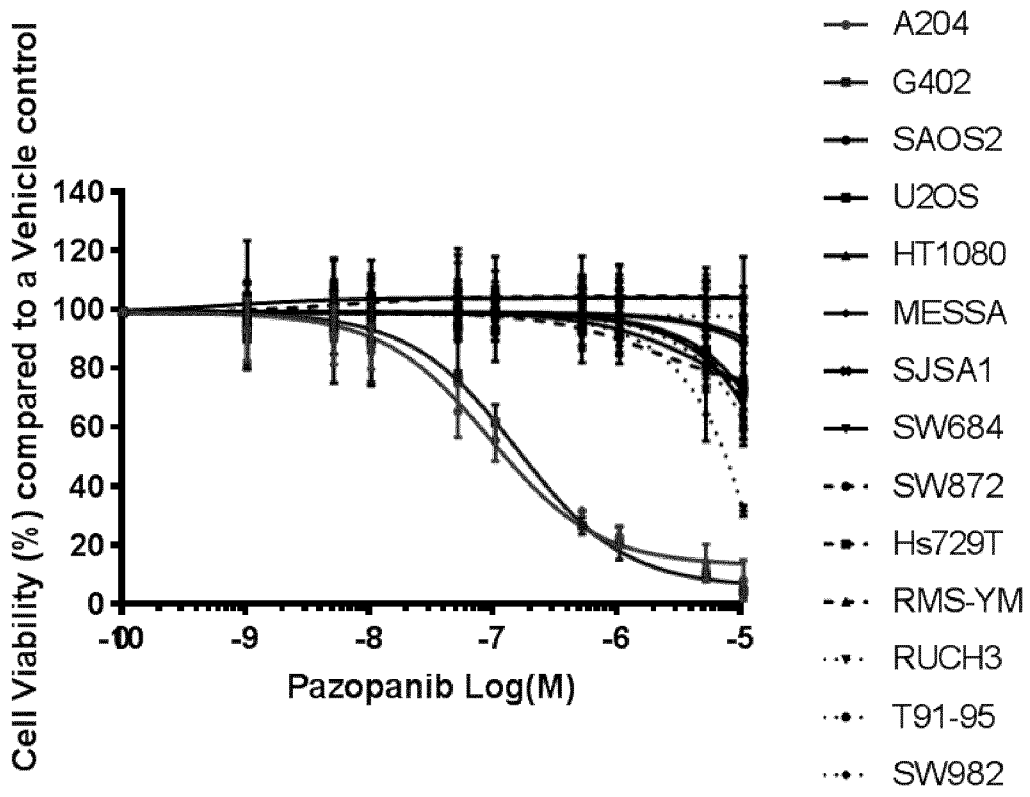
Cell Viability (%) compared to a Vehicle control
140
120
100
80
60
40
20
0
-10
-9
-8
-7
-6
-5
Pazopanib Log(M)
A204
G402
SAOS2
U2OS
HT1080
MESSA
SJSA1
SW684
SW872
Hs729T
RMS-YM
RUCH3
T91-95
SW982

Figure 3

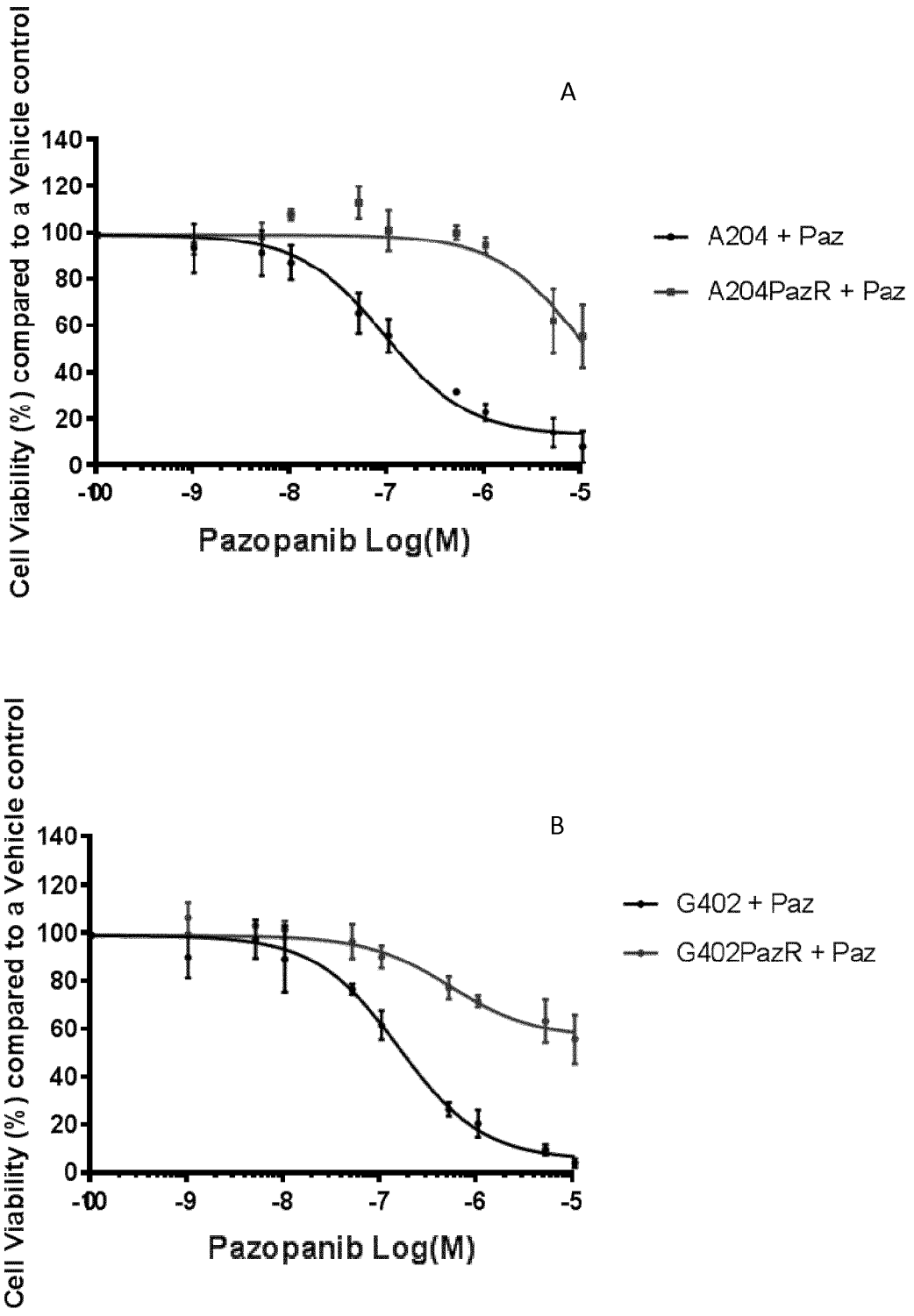
A
Cell Viability (%) compared to a Vehicle control
140
120
100
80
60
40
20
0
-10
-9
-8
-7
-6
-5
Pazopanib Log(M)
A204 + Paz
A204PazR + Paz
B
Cell Viability (%) compared to a Vehicle control
140
120
100
80
60
40
20
0
-10
-9
-8
-7
-6
-5
Pazopanib Log(M)
G402 + Paz
G402PazR + Paz

Figure 4

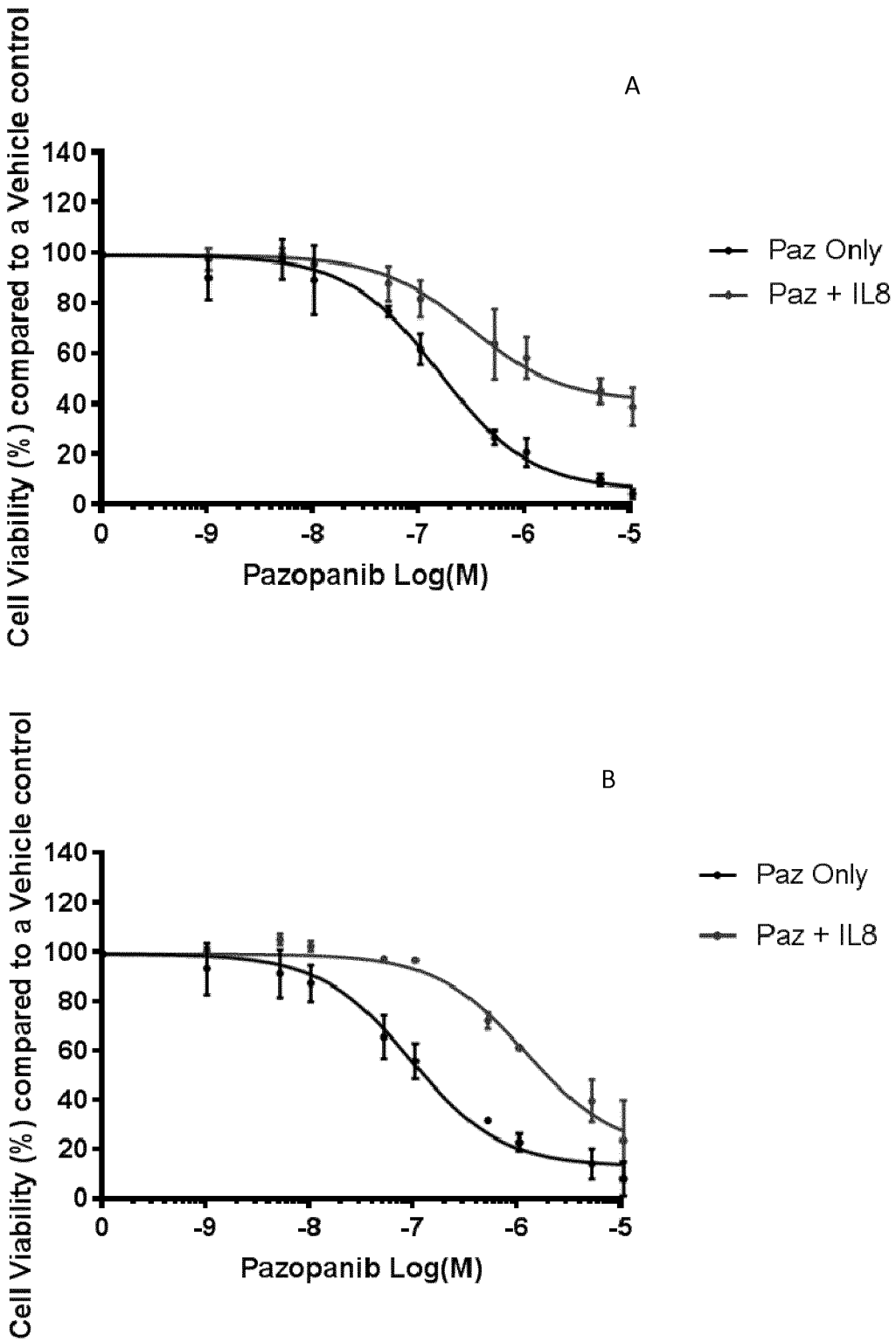
A
Cell Viability (%) compared to a Vehicle control
140
120
100
80
60
40
20
0
0
-9
-8
-7
-6
-5
Pazopanib Log(M)
Paz Only
Paz + IL8
B
Cell Viability (%) compared to a Vehicle control
140
120
100
80
60
40
20
0
0
-9
-8
-7
-6
-5
Pazopanib Log(M)
Paz Only
Paz + IL8

Figure 5

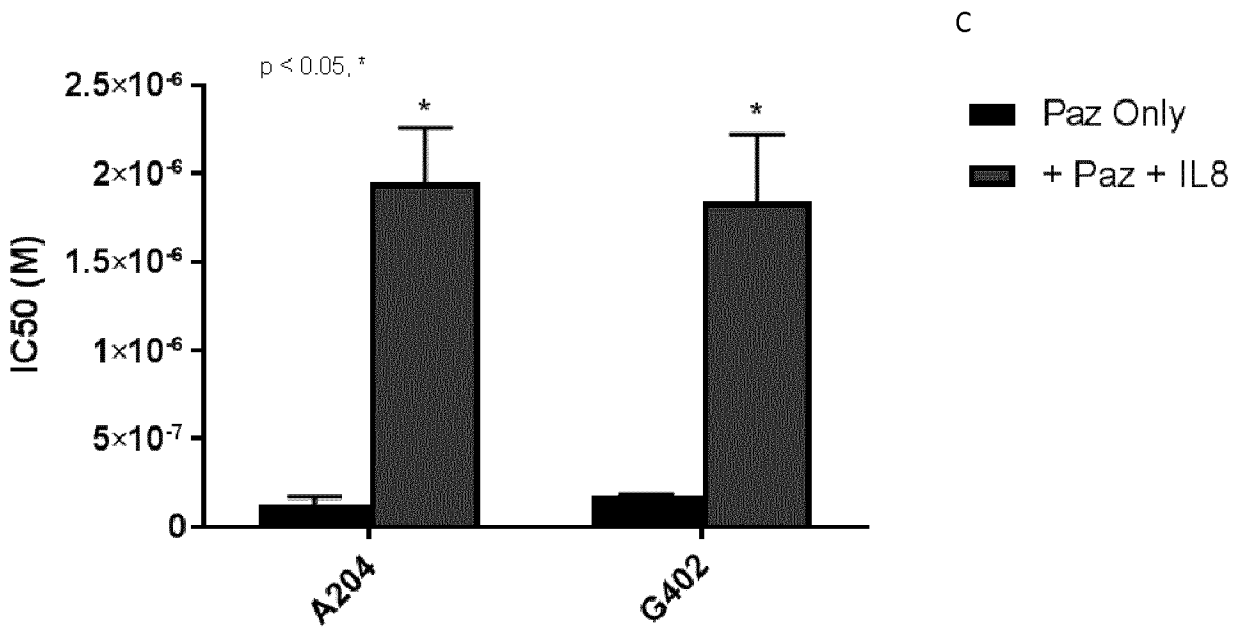

Figure 5 (continued)

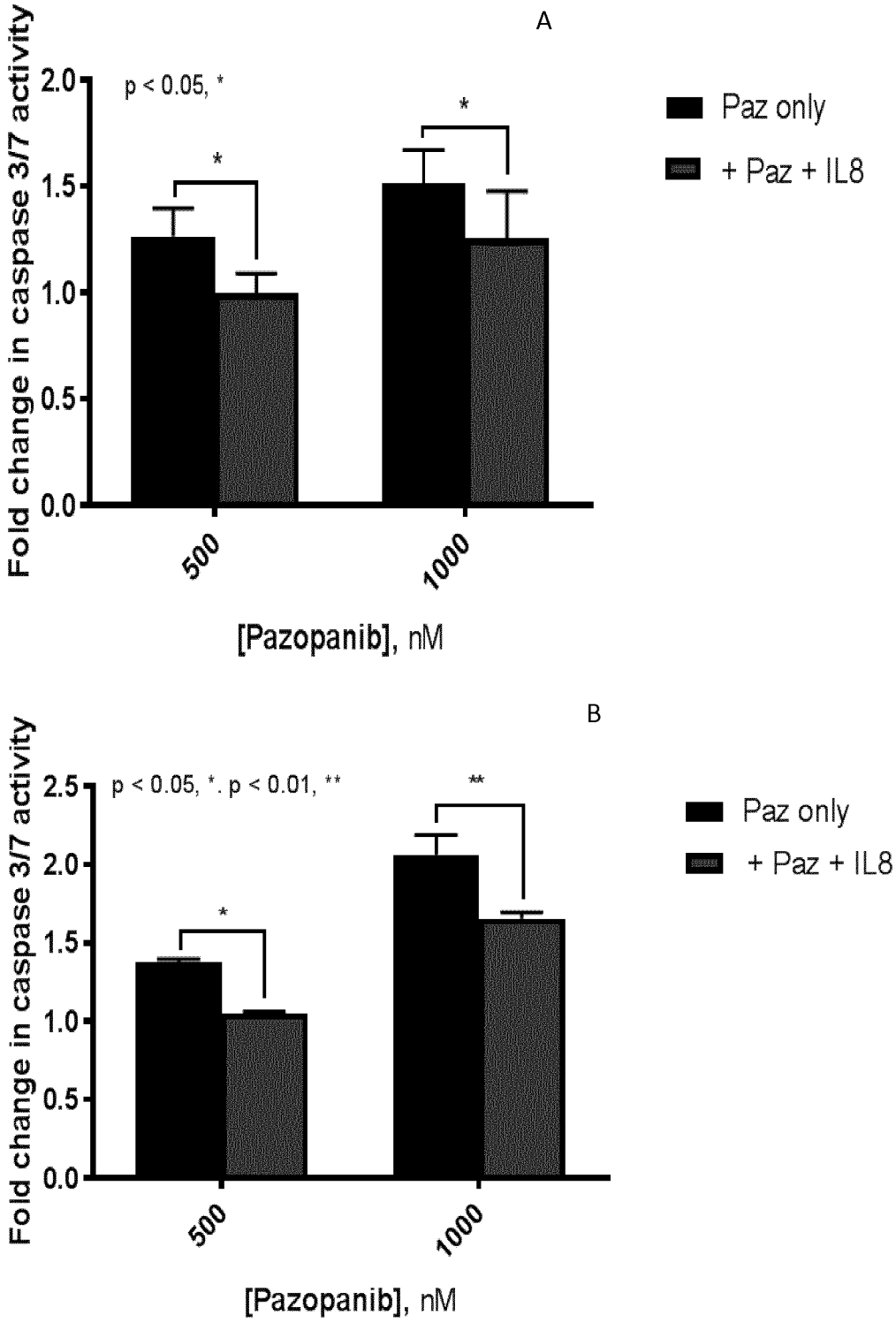
A
p < 0.05, *
*
*
Fold change in caspase 3/7 activity
2.0
1.5
1.0
0.5
0.0
500
1000
[Pazopanib], nM
Paz only
+ Paz + IL8
B
p < 0.05, *. p < 0.01, **
**
*
Fold change in caspase 3/7 activity
2.5
2.0
1.5
1.0
0.5
0.0
500
1000
[Pazopanib], nM
Paz only
+ Paz + IL8

Figure 6

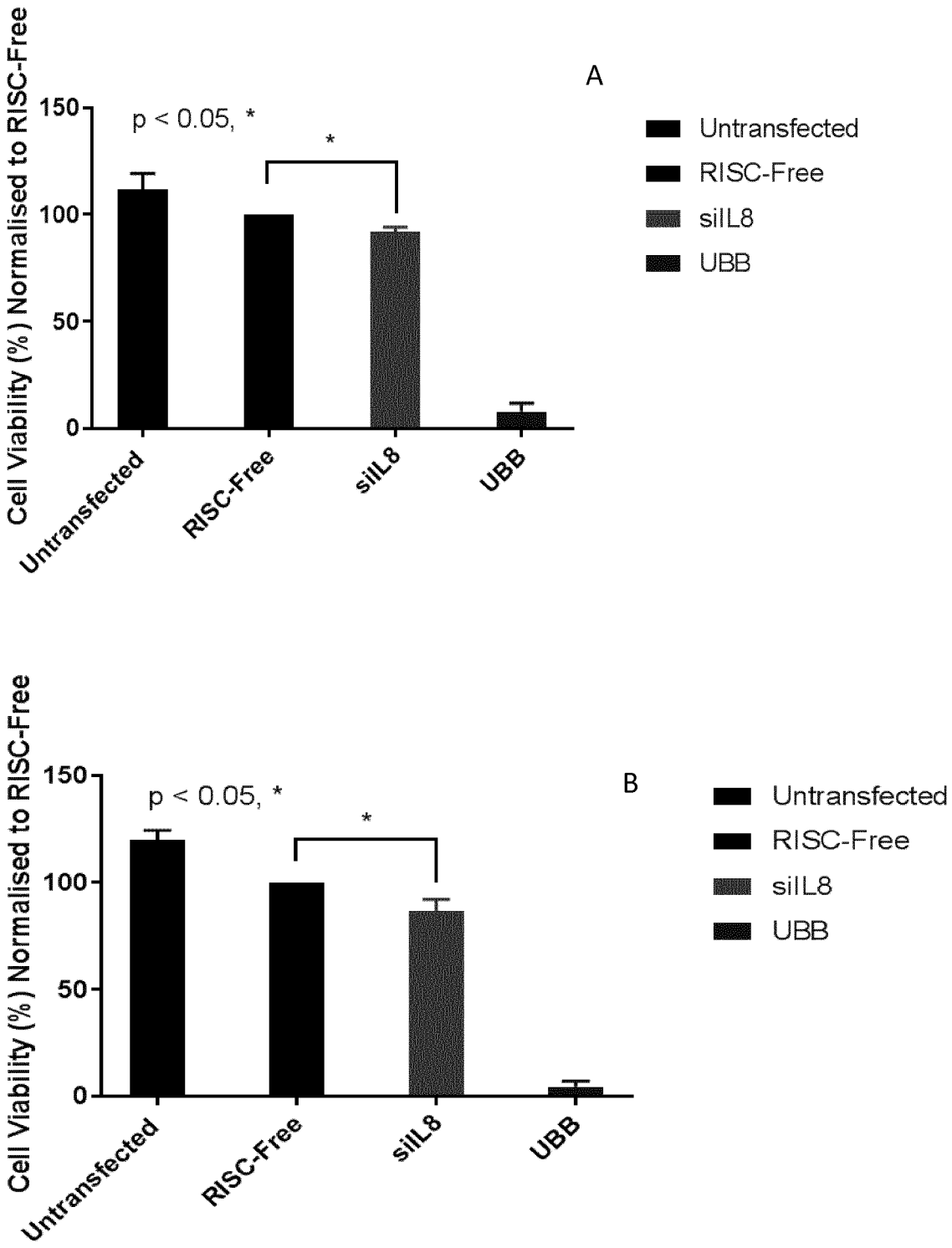
A
Cell Viability (%) Normalised to RISC-Free
150
100
50
0
p < 0.05, *
*
Untransfected
RISC-Free
siIL8
UBB
Untransfected
RISC-Free
siIL8
UBB
B
Cell Viability (%) Normalised to RISC-Free
150
100
50
0
p < 0.05, *
*
Untransfected
RISC-Free
siIL8
UBB
Untransfected
RISC-Free
siIL8
UBB

Figure 7

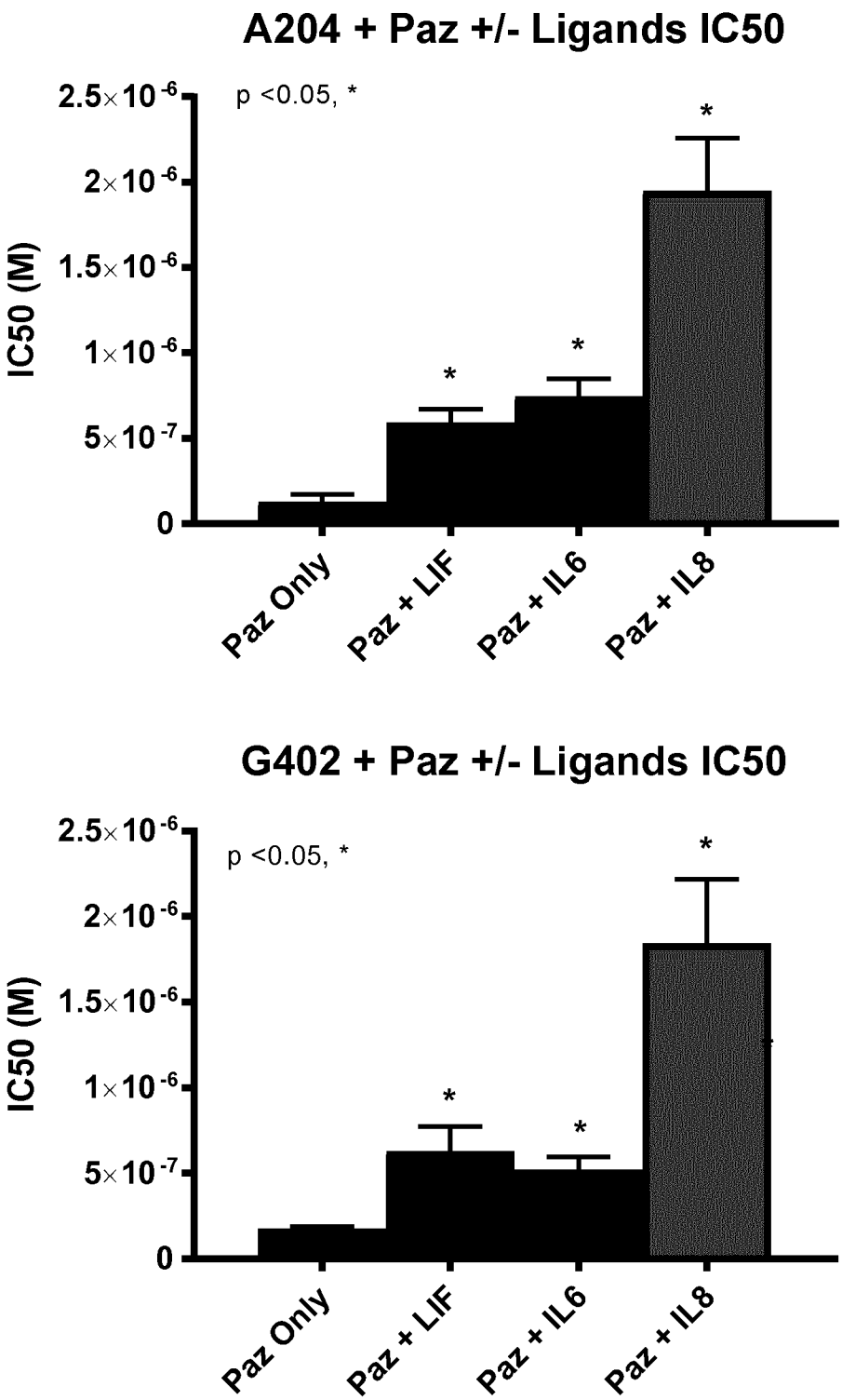
A204 + Paz +/- Ligands IC50
p <0.05, *
IC50 (M)
2.5×10-6
2×10-6
1.5×10-6
1×10-6
5×10-7
0
*
*
*
Paz Only
Paz + LIF
Paz + IL6
Paz + IL8
G402 + Paz +/- Ligands IC50
p <0.05, *
IC50 (M)
2.5×10-6
2×10-6
1.5×10-6
1×10-6
5×10-7
0
*
*
*
Paz Only
Paz + LIF
Paz + IL6
Paz + IL8

Figure 8

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2019/064231

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/6886
ADD.

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

B. FIELDS SEARCHED

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

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2010/233680 A1 (TAYLOR IAN [US] ET AL) 16 September 2010 (2010-09-16) abstract; claims 1-8 paragraphs [0013], [0193]; claims 1-8	1-18
A	US 2016/032403 A1 (SINGH SHARAT [US] ET AL) 4 February 2016 (2016-02-04) claim 19; example 1	1-18

-/--

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

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 August 2019	07/11/2019

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	Aguilera, Miguel

1

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

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2019/064231

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HUANG DAN ET AL: "Interleukin-8 mediates resistance to antiangiogenic agent sunitinib in renal cell carcinoma", CANCER RESEARCH, vol. 70, no. 3, 1 February 2010 (2010-02-01), pages 1063-1071, XP002583999, ISSN: 1538-7445, DOI: 10.1158/0008-5472.CAN-09-3965 [retrieved on 2010-01-26] page 1064, column 1, paragraph 3-4 page 1065, column 1, paragraph 1 page 1065, column 2, paragraph 1 figures 2B, 3B -----	1-18
X	SUMANTA KUMAR PAL ET AL: "Pazopanib as Third Line Therapy for Metastatic Renal Cell Carcinoma: Clinical Efficacy and Temporal Analysis of Cytokine Profile", JOURNAL OF UROLOGY., vol. 193, no. 4, 1 April 2015 (2015-04-01) , pages 1114-1121, XP055614616, BALTIMORE, MD, US ISSN: 0022-5347, DOI: 10.1016/j.juro.2014.09.110 abstract; figure 2 page 1116, column 2, paragraph 2 page 1120, column 1, paragraph 2 -----	1-18

1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2019/064231

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.:

1-18(partially)

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: 1-18(partially)

Methods of monitoring cancer in an individual for the development of resistance to treatment with a TKI, the method comprising coparing the expression level of IL-8 before and after treatment.

2-179. claims: 1-18(partially)

Methods of monitoring cancer in an individual for the development of resistance to treatment with a TKI, the method comprising coparing the expression level of ACVR1C before and after treatment.

-

[idem for each one of the genes listed in claim 1]

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2019/064231

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2010233680 A1	16-09-2010	CA 2637369 A1	24-05-2007
		EP 1945819 A2	23-07-2008
		JP 2009515526 A	16-04-2009
		KR 20080080531 A	04-09-2008
		US 2010233680 A1	16-09-2010
		US 2013143762 A1	06-06-2013
		WO 2007058968 A2	24-05-2007
US 2016032403 A1	04-02-2016	EP 2954068 A1	16-12-2015
		HK 1218939 A1	17-03-2017
		US 2016032403 A1	04-02-2016
		WO 2014122582 A1	14-08-2014