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(57) Abstract: The present disclosure relates to methods of treating cancers, in particular fibrotic cancers such as pancreatic cancer and/or solid cancers such as colorectal cancer. The present disclosure also relates to methods of inhibiting and/or reducing metastasis of primary cancers, in particular fibrotic primary cancers. The methods include administration of a kinase inhibitor.

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"Treatment and/or prevention of cancers"

All documents cited or referenced herein, and all documents cited or referenced in herein cited documents, together with any manufacturer's instructions, descriptions, product specifications, and product sheets for any products mentioned herein or in any document incorporated by reference herein, are hereby incorporated herein by reference in their entirety.

The present application claims priority from AU2020903320 filed 16 September 2020, the entire contents of which are incorporated by reference herein. The present application also claims priority from PCT/AU2020/050994 filed 18 September 2020, the entire contents of which are incorporated by reference herein.

Field

The present disclosure relates to methods of treating cancers, in particular fibrotic cancers such as pancreatic cancer and/or solid cancers such as colorectal cancer. The present disclosure also relates to methods of inhibiting and/or reducing metastasis of primary cancers, in particular fibrotic primary cancers.

Background

Cancer is one of the most common causes of deaths worldwide. However, age-standardized cancer death rates are falling globally. In the US, cancer death rates went down approximately 27% between 1999 and 2019 (CDC, An Update on Cancer Deaths in the United States). This improvement in survival reflects progress in diagnosing at an earlier stage and improvements in treatment. However, there is still a need for methods to treat cancers, in particular those cancers with poorer prognosis and/or which are considered one of the leading causes of cancer death.

Pancreatic ductal adenocarcinoma (PDAC) is an aggressive malignant disease with a 5-year survival rate of less than 10%, and accounts for the 5th most common cause of cancer-related death worldwide (Raimondi et al, Nat Rev Gastroenterol Hepatol, 2009. 6(12): 699-708). The biggest clinical challenge of treating PDAC is the poor response of tumours to therapeutic intervention and the advanced-stage at which patients are diagnosed. In contrast to other cancer types, the survival rate of PDAC patients has not improved substantially over the past 30 years despite advances in conventional therapies (Winter et al, Ann Surg Oncol, 2012. 19(1): 169-75). Two chemotherapy regimens, FOLFIRINOX and Gemcitabine/Abraxane are the standard-of-care therapies for metastatic PDAC; however, response to either of these two regimens is only observed in up to 30% of patients (Conroy et al, New England Journal of Medicine, 2011. 364(19): 1817-1825; Von Hoff et al, N Engl J Med, 2013. 369(18): 1691-703). While surgery offers a potential cure, <10% of cases are operable at diagnosis and more than 90% of patients

that undergo surgery still die of the disease due to local recurrence and/or metastasis (Conroy et al, New England Journal of Medicine, 2011. 364(19): 1817-1825). Thus, new strategies to control the growth and spread of PDAC are urgently required.

Colorectal cancer (colon cancer) is the second most common cause of cancer worldwide. In Australia, colorectal cancer is estimated to be the fourth most commonly diagnosed cancer in 2020 with an estimated 15,494 patients in a population of about 26 million (Cancer Australia Government statistics). Unfortunately, 30-50% of patients have occult or overt metastases at presentation and once tumours have metastasised, prognosis is poor with a five year survival rate dropping to around 14%. In most cases, surgery is required to remove the tumour and depending on the stage of the cancer, further treatment by radiotherapy and/or chemotherapy may be required. However, these methods are invasive and can result in unwanted side effects if radiotherapy or chemotherapy is also involved.

Although aberrant expression of c-SRC and other SRC family kinase members in epithelial cells constitute oncogenic driver events, elevated expression and activation of the myeloid-specific SRC family kinase Hematopoietic Cell Kinase (HCK) in the tumor stroma occurs in many solid malignancies and correlates with poor patient survival (Poh, et al., Cancer Cell, 2017. 31(4): 563-575.e5; Poh et al, Cancer Immunol Res, 2020. 8(4): 428-435). The inventors have previously shown that excessive HCK activity in myeloid cells enhances the growth of gastric and colon adenomas by enhancing the polarization of macrophages towards an immunosuppressive endotype. Meanwhile, genetic ablation or pharmacologic inhibition of HCK impairs tumour growth by reducing the immunosuppressive phenotype of macrophages.

There is a need in the art for methods to treat cancers. There is also a need in the art for methods to treat solid cancers, such as colon cancer. There is also a need in the art for methods to treat fibrotic cancers, such as pancreatic cancer. There is also a need in the art for methods to prevent, inhibit and/or reduce metastasis of primary cancers, for example such as melanoma, breast cancer or pancreatic cancer.

Summary of the disclosure

The present disclosure is based on the finding that HCK expression is elevated in primary cancers, more particularly fibrotic cancers. The present disclosure is based on the finding that inhibition of HCK reduces the growth and progression (i.e. metastasis) of primary and secondary (i.e. metastatic) tumours. Moreover, co-targeting HCK in combination with chemotherapy and/or an immunotherapy agent enhances the anti-tumour activity of either the HCK inhibitor alone or the chemotherapy and/or immunotherapy alone.

In a first aspect there is provided a method of treating and/or preventing a fibrotic cancer in a subject, comprising administering an effective amount of an HCK inhibitor to the subject.

In one example, the present disclosure provides a method of preventing a fibrotic cancer in a subject, comprising administering an effective amount of an HCK inhibitor to the subject.

In some embodiments, the fibrotic cancer is pancreatic cancer.

In some examples, there is provided a method of treating and/or preventing a fibrotic cancer selected from the group consisting of breast cancer, gastric cancer, oesophageal cancer, hepatocellular cancer or melanoma in a subject, comprising administering an effective amount of an HCK inhibitor to the subject.

It has also been found that HCK inhibitors are effective in preventing metastasis of primary cancers, including cancers which do not have a strong fibrotic component. There is also provided a method of inhibiting and/or reducing metastasis of a primary cancer in a subject, comprising administering an effective amount of an HCK inhibitor to the subject.

In some embodiments, the cancer is a fibrotic cancer.

In some embodiments, the cancer is breast cancer, pancreatic cancer, gastric cancer, oesophageal cancer, hepatocellular cancer or melanoma.

In some embodiments the subject is human.

In some embodiments the HCK inhibitor is administered in combination with a further active agent.

In some embodiments, the further active agent is a chemotherapeutic agent.

In some embodiments, the further active agent is an immunotherapy agent or a combination of immunotherapy agents.

In some embodiment, the HCK inhibitor is administered in combination with a chemotherapy and an immunotherapy agent(s). The agents may be administered concurrently or sequentially.

There is also provided a method of treating and/or preventing a fibrotic cancer in a subject, comprising administering an effective amount of a pharmaceutical composition comprising an HCK inhibitor and a pharmaceutically acceptable excipient to the subject. There is also provided a method of preventing a fibrotic cancer in a subject, comprising administering an effective amount of a pharmaceutical composition comprising an HCK inhibitor and a pharmaceutically acceptable excipient to the subject.

There is also provided a method of inhibiting and/or reducing metastasis of a primary cancer in a subject, comprising administering an effective amount of a pharmaceutical composition comprising an HCK inhibitor and a pharmaceutically acceptable excipient to the subject.

There is also provided an HCK inhibitor for use in treating and/or preventing a fibrotic cancer.

In some embodiments, the fibrotic cancer is pancreatic cancer.

There is also provided an HCK inhibitor for use in inhibiting and/or reducing metastasis of a primary cancer.

In some embodiments, the cancer is a fibrotic cancer.

In some embodiments, the cancer is breast cancer, pancreatic cancer, gastric cancer, oesophageal cancer, hepatocellular cancer or melanoma..

There is also provided use of an HCK inhibitor for the manufacture of a medicament for treating and/or preventing a fibrotic cancer.

In some embodiments, the fibrotic cancer is pancreatic cancer.

There is also provided use of an HCK inhibitor for the manufacture of a medicament for inhibiting and/or reducing metastasis of a primary cancer.

In some embodiments, the cancer is a fibrotic cancer.

In some embodiments, the cancer is breast cancer, pancreatic cancer, gastric cancer, oesophageal cancer, hepatocellular cancer or melanoma.

In some examples, the methods of inhibiting or reducing metastasis includes reducing the risk of metastatic disease in a subject compared to a subject who is not treated with the HCK inhibitor. In some examples, the methods of the disclosure reduce the risk of metastasis by 10%, 20%, 30%, 40%, 50%, 60% or greater. In some examples, the metastasis is confined to the lymph nodes.

In some examples, the subject has previously undergone surgery.

The present disclosure is also based on the finding that a tyrosine kinase inhibitor capable of inhibiting HCK improved the anti-tumour activity conferred by anti-PD1.

Accordingly, there is also provided a method of treating a cancer in a subject, comprising:

administering an effective amount of a HCK inhibitor to the subject; and

administering an effective amount of an immune checkpoint inhibitor to the subject.

In some examples, the HCK inhibitor is dasatinib. In some examples, the HCK inhibitor is RK20449. In some examples, the HCK inhibitor is not dasatinib.

In some examples, the immunotherapy or immune checkpoint inhibitor is selected from the group consisting of a PD-1 inhibitor, a PD-L1 inhibitor and a CTLA-4 inhibitor. In some examples, the immunotherapy or immune checkpoint inhibitor is a PD-1 inhibitor. In some examples, the immune checkpoint inhibitor is selected from the group consisting of pembrolizumab, nivolumab, cemiplimab, atezolizumab, avelumab, durvalumab and ipilimumab. In some examples, the immune checkpoint inhibitor is a PD-1 inhibitor selected from the group consisting of pembrolizumab, nivolumab and cemiplimab.

In some examples, the method further comprises administering an effective amount of a CD40 agonist to the subject. In some examples, the CD40 agonist is a agnostic CD40 antibody selected from the group consisting of Sotigalimab (APX005M), and SEA-CD40.

In some examples, the cancer is a solid cancer. In some examples, the cancer is selected from the group consisting of colorectal cancer, gastric cancer, esophageal cancer, lung cancer, breast cancer, melanoma, head and neck squamous cell cancer, cutaneous squamous cell carcinoma, Merkel cell carcinoma, renal cell carcinoma, urothelial carcinoma, cervical

cancer, hepatocellular carcinoma, endometrial carcinoma and tumour mutational burden-high cancer. In some examples, the cancer is colorectal cancer.

In some examples, the cancer is a blood cancer. In some examples, the blood cancer is selected from the group consisting of lymphoma and leukemia. In some examples, the blood cancer is selected from the group consisting of chronic myeloid leukemia, acute lymphoblastic leukemia, classical Hodgkin lymphoma and primary mediastinal large B-cell lymphoma.

In some examples, the subject is human.

In some examples, the immune checkpoint inhibitor is pembrolizumab, which is administered in an amount of about 200mg per 3 weeks, or about 400mg per 6 weeks. In some examples, the immune checkpoint inhibitor is pembrolizumab, which is administered in an amount of up to 150mg per 2 weeks, or in an amount of up to 300mg per 6 weeks.

In some examples, the immune checkpoint inhibitor is nivolumab, which is administered in an amount of up to 3mg/kg per 2 weeks. In some examples, the immune checkpoint inhibitor is nivolumab, which is administered in an amount of up to 2mg/kg per 2 weeks.

In some examples, the immune checkpoint inhibitor is cemiplimab, which is administered in an amount of about 350mg per 3 weeks. In some examples, the immune checkpoint inhibitor is cemiplimab, which is administered in an amount of up to 300mg per 3 weeks.

In some examples, the immune checkpoint inhibitor is ipilimumab, which is administered in an amount of up to 3mg/kg per week. In some examples, the immune checkpoint inhibitor is ipilimumab, which is administered in an amount of up to 2mg/kg per week.

In some examples, the immune checkpoint inhibitor is administered intravenously by infusion or injection.

In some examples, the HCK inhibitor specifically binds a SRC family tyrosine kinase. In some examples, the HCK inhibitor binds the SRC family kinase with an IC_{50} ≤100nM. In some examples, the HCK inhibitor binds non SRC family tyrosine kinases with an IC_{50} >100nM. In some examples, the HCK inhibitor binds the SRC family kinase with an IC_{50} ≤100nM and binds non SRC family tyrosine kinases with an IC_{50} >100nM.

In some examples, there is provided a method of treating a cancer in a subject, comprising:

administering an effective amount of dasatinib to the subject; and

administering an effective amount of an immune checkpoint inhibitor to the subject.

In some examples, dasatinib and the immune checkpoint inhibitor are administered separately, sequentially or simultaneously.

In some examples, dasatinib is administered once per day in an amount of up to 150mg. In some examples, dasatinib is administered once per day in an amount of about 100mg, or about 140mg. In some examples, dasatinib is administered once per day in an amount of up to 80mg.

In some examples, dasatinib is administered orally.

There is also provided dasatinib for use in treating a cancer, wherein dasatinib is administered in combination with an immune checkpoint inhibitor.

There is also provided an immune checkpoint inhibitor for use in treating a cancer, wherein the immune checkpoint inhibitor is administered in combination with dasatinib.

There is also provided use of dasatinib for the manufacture of a medicament for the treatment of a cancer, wherein the medicament is administered in combination with an immune checkpoint inhibitor.

There is also provided use of an immune checkpoint inhibitor for the manufacture of a medicament for the treatment of a cancer, wherein the medicament is administered in combination with dasatinib.

There is also provided a method of treating a cancer in a subject, comprising:

administering an effective amount of a pharmaceutical composition comprising dasatinib and a pharmaceutically acceptable excipient to the subject; and

administering an effective amount of a pharmaceutical composition comprising an immune checkpoint inhibitor and a pharmaceutically acceptable excipient to the subject.

There is also provided a pharmaceutical composition comprising dasatinib and a pharmaceutically acceptable excipient, for use in treating a cancer, wherein the pharmaceutical composition is administered in combination with an immune checkpoint inhibitor.

There is also provided a pharmaceutical composition comprising an immune checkpoint inhibitor and a pharmaceutically acceptable excipient, for use in treating a cancer, wherein the pharmaceutical composition is administered in combination with dasatinib.

There is also provided a method of treating a cancer in a subject, comprising:

administering an effective amount of RK20449 to the subject; and

administering an effective amount of an immune checkpoint inhibitor to the subject.

In some examples, the subject is human.

In some examples, RK20449 and the immune checkpoint inhibitor are administered separately, sequentially or simultaneously.

In some examples, RK20449 is administered orally.

There is also provided RK20449 for use in treating a cancer, wherein RK20449 is administered in combination with an immune checkpoint inhibitor.

There is also provided an immune checkpoint inhibitor for use in treating a cancer, wherein the immune checkpoint inhibitor is administered in combination with RK20449.

There is also provided use of RK20449 for the manufacture of a medicament for the treatment of a cancer, wherein the medicament is administered in combination with an immune checkpoint inhibitor.

There is also provided use of an immune checkpoint inhibitor for the manufacture of a medicament for the treatment of a cancer, wherein the medicament is administered in combination with RK20449.

There is also provided a method of treating a cancer in a subject, comprising:

administering an effective amount of a pharmaceutical composition comprising RK20449 and a pharmaceutically acceptable excipient to the subject; and

administering an effective amount of a pharmaceutical composition comprising an immune checkpoint inhibitor and a pharmaceutically acceptable excipient to the subject.

There is also provided a pharmaceutical composition comprising RK20449 and a pharmaceutically acceptable excipient, for use in treating a cancer, wherein the pharmaceutical composition is administered in combination with an immune checkpoint inhibitor.

There is also provided a pharmaceutical composition comprising an immune checkpoint inhibitor and a pharmaceutically acceptable excipient, for use in treating a cancer, wherein the pharmaceutical composition is administered in combination with RK20449.

There is also provided use of an HCK inhibitor and optionally an immunotherapy in the manufacture of a medicament for preventing a fibrotic cancer or for treating or preventing metastasis of a fibrotic cancer.

Description of the Figures

Figure 1 Genetic ablation of HCK reduces pancreatic cancer metastasis and improves survival. **(A)** shows representative liver images of WT and *Hck*^{KO} mice collected 3 weeks after injection of pancreatic cancer cells into the spleen, which metastasise to the liver. Liver mass in WT and *Hck*^{KO} mice (g per mouse) 3 weeks after intrasplenic injection of pancreatic cancer cells is also shown. **(B)** shows Kaplan-Meier survival analysis of WT and *Hck*^{KO} mice that have been intrasplenically injected with pancreatic tumour cells. **(C)** shows primary pancreatic tumour mass in WT and *Hck*^{KO} mice (g per mouse) 5 weeks following orthotopic tumour cell injection. **(D)** shows H&E sections of secondary organs (liver, spleen, intestine, peritoneum and kidney) from WT and *Hck*^{KO} mice as described in (C). T = tumour. Each symbol represents an individual mouse. Data represents mean ± SEM. P-values from unpaired Student's T-test ***p<0.001.

Figure 2 Therapeutic inhibition of HCK reduces primary tumour growth and pancreatic cancer metastasis in WT mice.

(A) shows primary tumour mass (g per mouse) in WT mice following orthotopic injection of pancreatic cancer cells. WT mice were treated with vehicle (12% Captisol twice daily, i.p.) or RK20449 (30mg/kg twice daily, i.p.) 1 week after tumour cell injection for 4 weeks. **(B)** shows liver mass (g per mouse) in WT mice following intrasplenic injection of pancreatic cancer cells. WT mice were treated with vehicle (12% Captisol twice daily, i.p.) or RK20449 (30mg/kg twice daily, i.p.) 5 days after tumour cell injection for 2 weeks. Each symbol represents an individual mouse. Data represents mean ± SEM, p-values from unpaired Student's T-test ***p<0.001.

Figure 3 Genetic ablation of HCK reduces the desmoplastic tumour microenvironment and abundance of cancer associated fibroblasts.

(A) shows immunohistochemical staining for extracellular matrix proteins and cancer-associated fibroblasts in metastatic PDAC tumours of WT and *Hck*^{KO} mice (intrasplenic model). MT = Massons Trichrome showing collagen secreted by cancer-associated fibroblasts, PDPN = Podoplanin, PDGFRβ = Platelet Derived Growth Factor Receptor Beta, SMA = Smooth muscle actin. **(B)** shows flow cytometry quantification of various cancer-associated fibroblast subtypes shown in (A). iCAF = inflammatory CAFs, apCAF = antigen presenting CAFs, mCAFs = myofibroblasts. **(C)** shows qPCR analysis of CAFs isolated from WT or *Hck*^{KO} mice for genes associated with immune suppression, matrix remodelling, and fibrosis. N=5 mice per group. Each symbol represents an individual mouse. Data represents mean ± SEM, p values from unpaired Student's T-test ***p<0.001.

Figure 4 Genetic ablation of HCK reduces the immunosuppressive tumour microenvironment and enhances the infiltration of cytotoxic effector cells.

(A) shows flow cytometry quantification of myeloid cell populations in metastatic PDAC tumours of WT and *Hck*^{KO} mice (intrasplenic model). TAMs = tumour associated macrophages, AAMs = alternatively-activated macrophages, m-MDSCs = monocytic myeloid derived suppressor cells, g-MDSCs = granulocytic myeloid derived suppressor cells, cDC1s = conventional type 1 dendritic cells. **(B)** shows qPCR analysis on CD45^{+}CD11c^{+}F4/80^{-}MHCII^{+} DCs and CD45^{+}CD11b^{+}F4/80^{High}Ly6c^{-}Ly6g^{-} TAMs isolated from metastatic KPC liver tumors of WT and *Hck*^{KO} mice for genes associated with immune cell activation (*Tnf*, *Il12*, *Ifny*, *Cxcl9*, *Cxcl10*), immune suppression (*Il4*, *Il10*, *Il13*, *Tgfβ*, *Arg1*) and matrix remodeling (*Mmp3, Mmp7, Mmp9*). N≥6 mice per group. **(C)** shows flow cytometry quantification of NK and CD8 T-cells that mediate anti-tumour responses. **(D)** shows representative immunohistochemical staining for CD8 T-cells in WT vs *Hck*^{KO} host mice. Quantification of staining is also shown. **(E)** shows qPCR gene expression analysis of CD8 T-cells and NK cells purified from WT or *Hck*^{KO} mice for Granzyme B and Perforin as markers of immune cell activation. N=7 mice per group. **(F)** shows quantification of immunohistochemical staining for Granzyme B and Perforin in tumours of WT and *Hck*^{KO} mice. Each symbol represents an individual mouse. Data represents mean ± SEM, p-values from unpaired Student's T-test **p<0.01, ***p<0.001.

Figure 5 Genetic ablation of HCK improves response of pancreatic tumours to gemcitabine chemotherapy and extends survival.

(A) shows representative images of WT and *Hck*^{KO} livers following intrasplenic injection of pancreatic cancer cells. Where indicated, mice were treated with vehicle or gemcitabine (120mg/kg twice weekly, i.p.) starting from 5 days post-tumour cell injection for 2 weeks. **(B)** shows liver mass of mice treated as described in (A). Each symbol represents an individual

mouse. **(C)** shows Kaplan-Meier survival analysis of WT and *Hck*^{KO} mice following intrasplenic injection of pancreatic tumour cells. Where indicated, mice were treated with vehicle or gemcitabine (120mg/kg twice weekly, i.p.) starting from 5 days post-tumour cell injection until clinical endpoint. N≥10 mice per group. Data represents mean ± SEM, p-values from unpaired Student's T-test *p<0.05, p**<0.01, ***p<0.001.

Figure 6 Genetic ablation of HCK improves response of pancreatic tumours to anti-CD40 immunotherapy and extends survival.
(A) shows representative images of WT and *Hck*^{KO} livers following intrasplenic injection of pancreatic tumour cells. Where indicated, mice were treated with control IgG or anti-CD40 immunotherapy (200µg once every 3 days, i.p.) starting from 5 days post-tumour cell injection for 2 weeks. **(B)** shows liver mass of mice treated as described in (A). Each symbol represents an individual mouse. **(C)** shows Kaplan-Meier survival analysis of WT and *Hck*^{KO} mice following intrasplenic injection of pancreatic cancer cells. Where indicated, mice were treated with control IgG or anti-CD40 immunotherapy (200µg once every 3 days, i.p.) starting from 5 days post-tumour cell injection until clinical endpoint. N≥10 mice per group. Data represents mean ± SEM, p-values from unpaired Student's T-test *p<0.05, p**<0.01, ***p<0.001.

Figure 7 Genetic ablation of HCK improves response of pancreatic tumours to anti-PD1 immunotherapy and extends survival.
(A) shows representative images of WT and *Hck*^{KO} livers following intrasplenic injection of pancreatic cancer cells. Where indicated, mice were treated with IgG or anti-PD1 immunotherapy (200µg once every 3 days, i.p.) starting from 5 days post-tumour cell injection for 2 weeks. **(B)** shows liver mass of mice treated as described in (A). (Each symbol represents an individual mouse. **(C)** shows Kaplan-Meier survival analysis of WT and *Hck*^{KO} mice following intrasplenic injection of pancreatic cancer cells. Where indicated, mice were treated with IgG or anti-PD1 immunotherapy (200µg once every 3 days, i.p.) starting from 5 days post-tumour cell injection until clinical endpoint. N≥10 mice per group. Data represents mean ± SEM, p-values from unpaired Student's T-test *p<0.05, p**<0.01, ***p<0.001.

Figure 8 Genetic ablation or therapeutic inhibition of HCK improves the efficacy of immunotherapy in colon cancer allografts
(A) shows western blot analysis for the phosphorylated and total protein isoforms of HCK, SRC and LYN in MC38 tumor cell lysates of WT hosts treated with either RK20449 or Dasatinib (30mg/kg, twice daily) for 10 days. Actin was used as a loading control. Each lane represents an individual mouse. **(B)** shows volume of subcutaneous MC38 tumors in WT hosts following treatment with RK20449 (30mg/kg, twice daily), Dasatinib (30mg/kg, twice daily) and/or αPD1 (200µg, once every 3 days). Vehicle control mice were treated with 12% Captisol and an isotype-

matched IgG. **(C)** shows volume of subcutaneous MC38 tumors established in *Hck*^{KO} hosts and treated with Dasatinib (30mg/kg, twice daily) and/or αPD1 (200µg, once every 3 days). **(D)** shows mass of individual subcutaneous MC38 tumors from WT and *Hck*^{KO} hosts treated with αCD40 (100µg, once every 3 days) or an isotype-matched IgG for 10 days. Each symbol represents an individual mouse. Representative tumors are depicted above graphs. Scale bar: 1cm. **(E)** shows mass of individual subcutaneous MC38 from WT hosts. Where indicated, mice were treated with αCD40 (100µg, once every 3 days) in the presence or absence of RK20449 (30mg/kg, twice daily) for 10 days. Vehicle control mice were treated with 12% Captisol and an isotype-matched IgG. Each symbol represents an individual mouse. Data represents mean ± SEM, p-values from unpaired Student's T-test *p<0.05, p**<0.01, ***p<0.001.

Figure 9 Genetic ablation of HCK reduces metastasis in an experimental melanoma model. Metastatic burden to the lung of WT and *Hck*^{KO} hosts is depicted following injection of B16F10 mouse melanoma cells into the tail-vein. Each symbol represents an individual mouse. Where indicated, WT mice were treated with RK20449 (30mg/kg, twice daily, i.p.) four days following tumour cell inoculation for 10 days. Lungs were harvested two weeks following tumour cell injection and fixed in formalin. Data represents mean ± SEM, p-values from unpaired Student's T-test ***p<0.001.

Figure 10 Genetic ablation of HCK reduces metastasis in an experimental breast cancer model. mCherry-labelled E0771 mouse breast cancer cells were orthotopically implanted into the mammary fat pad of WT and *Hck*^{KO} hosts. Lungs were harvested two weeks following primary tumour resection and metastatic burden was quantified by genomic PCR amplification for expression of mCherry positive tumour cells relative to Vimentin (house-keeper control gene). Each symbol represents an individual mouse. Data represents mean ± SEM, p-values from unpaired Student's T-test *p<0.05.

Figure 11 Therapeutic inhibition of HCK reduces PDX tumour burden in mice with a human immune system ("humanized mice").
(A and B) show reduced tumour volume and mass of individual subcutaneous breast cancer patient derived xenografts (PDXs) engrafted in humanized NSG-SGM3 hosts and following treatment with vehicle (12% Captisol, twice daily, i.p.) or RK20449 (30mg/kg, twice daily, i.p.) for 25 days. Each symbol represents an individual mouse. **(C)** shows qPCR analysis on whole PDX tumours from humanized NSG-SGM3 hosts treated as described in Figure 11A for genes associated with human immune cells. N=4 mice per group. **(D)** shows qPCR analysis on whole PDX tumours from humanized NSG-SGM3 hosts treated as described in Figure 11A for genes associated with immune suppression (CD163, MRC1, TGFB, IL10, CXCL12) and immune cell

activation (IL12, IFNG, IL1B, TNF, CXCL9, CXCL10). N=4 mice per group. Data represents mean ± SEM, p-values from unpaired Student's T-test *p<0.05, p**<0.01, ***p<0.001.

Detailed Description

General Techniques and Selected Definitions

The term "and/or", e.g., "X and/or Y" shall be understood to mean either "X and Y" or "X or Y" and shall be taken to provide explicit support for both meanings or for either meaning.

Reference to the singular forms "a", "an" and "the" is also understood to imply the inclusion of plural forms unless the context dictates otherwise.

Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present disclosure as it existed before the priority date of each claim of this application.

Throughout this specification, unless specifically stated otherwise or the context requires otherwise, reference to a single step, composition of matter, group of steps or group of compositions of matter shall be taken to encompass one and a plurality (i.e. one or more) of those steps, compositions of matter, groups of steps or group of compositions of matter.

Each example described herein is to be applied *mutatis mutandis* to each and every other example of the disclosure unless specifically stated otherwise.

Those skilled in the art will appreciate that the disclosure is susceptible to variations and modifications other than those specifically described. It is to be understood that the disclosure includes all such variations and modifications. The disclosure also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations or any two or more of said steps or features.

The present disclosure is not to be limited in scope by the specific examples described herein, which are intended for the purpose of exemplification only. Functionally-equivalent products, compositions and methods are clearly within the scope of the disclosure.

The present invention as described herein can be performed using, unless otherwise indicated, conventional techniques of molecular biology and cellular biology. Such procedures are described, for example, in Sambrook, Fritsch & Maniatis, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratories, New York, Second Edition (1989), whole of vols I, II, and III; DNA Cloning: A Practical Approach, Vols. I and II (D. N. Glover, ed., 1985), IRL Press, Oxford, whole of text; Oligonucleotide Synthesis: A Practical Approach (M. J. Gait, ed, 1984) IRL Press, Oxford, whole of text, and particularly the papers therein by Gait, ppl-22; Atkinson et al, pp35-81; Sproat et al, pp 83-115; and Wu et al, pp 135-151; 4. Nucleic Acid Hybridization: A Practical Approach (B. D. Hames & S. J. Higgins, eds., 1985) IRL Press, Oxford, whole of text; Immobilized Cells and Enzymes: A Practical Approach (1986) IRL Press, Oxford, whole of text; Perbal, B., A Practical Guide to Molecular Cloning (1984); Methods In Enzymology (S. Colowick

and N. Kaplan, eds., Academic Press, Inc.), whole of series, Sakakibara, D., Teichman, J., Lien, E. Land Fenichel, R.L. (1976). Biochem. Biophys. Res. Commun. 73 336-342; Merrifield, R.B. (1963). J. Am. Chem. Soc. 85, 2149-2154; Barany, G. and Merrifield, R.B. (1979) in The Peptides (Gross, E. and Meienhofer, J. eds.), vol. 2, pp. 1-284, Academic Press, New York. 12. Wünsch, E., ed. (1974) Synthese von Peptiden in Houben-Weyls Metoden der Organischen Chemie (Müler, E., ed.), vol. 15, 4th edn., Parts 1 and 2, Thieme, Stuttgart; Bodanszky, M. (1984) Principles of Peptide Synthesis, Springer-Verlag, Heidelberg; Bodanszky, M. & Bodanszky, A. (1984) The Practice of Peptide Synthesis, Springer-Verlag, Heidelberg; Bodanszky, M. (1985) Int. J. Peptide Protein Res. 25, 449-474; Handbook of Experimental Immunology, Vols. I-IV (D. M. Weir and C. C. Blackwell, eds., 1986, Blackwell Scientific Publications); and Animal Cell Culture: Practical Approach, Third Edition (John R. W. Masters, ed., 2000), ISBN 0199637970, whole of text.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated step or element or integer or group of steps or elements or integers but not the exclusion of any other step or element or integer or group of elements or integers.

The term “about”, as used herein when referring to a range is meant to encompass variations of ±20% or ±10%, more preferably ±5%, even more preferably ±1% from the specified amount.

As used herein, the term “treat” or “treatment” or “treating” shall be understood to refer to the medical management of a patient with the intent to cure, ameliorate, stabilize, or prevent a disease, pathological condition or disorder. This term includes active treatment, i.e. treatment directed specifically toward the improvement of a disease, pathological condition, or disorder. In addition, this term includes palliative treatment, i.e. treatment designed for the relief of symptoms rather than curing the disease, pathological condition or disorder; and supportive treatment, i.e. treatment employed to supplement another specific therapy directed towards the improvement of the associated disease, pathological condition or disorder.

As used herein the term “prevention” includes prophylaxis of the specific disorder or condition. For example, as used herein, the term “preventing cancer” refers to preventing the onset or duration of the symptoms associated with cancer. In one embodiment, the term “preventing cancer” refers to slowing or halting the progression of the cancer. In one embodiment, the term “preventing cancer” refers to slowing or preventing metastasis.

The term “metastatic cancer” as used herein refers to a cancer that has spread to a different part of the body from where it started. Typically a “primary” tumour or cancer will start at one site and move to one or more “secondary” sites. For the avoidance of doubt a metastatic cancer refers to advanced cancer or stage 4 cancer. The cancer may have spread via the blood stream or lymphatic system.

The term “subject” as used herein refers to a mammal including human and non-human animals. More particularly, the mammal is a human. Terms such as “subject”, “patient” or “individual” are terms that can, in context, be used interchangeably in the present disclosure.

As used herein, a “PD-1 inhibitor” is any pharmacologic or biologic agent or medicinal product that reduces the activity or expression of PD-1 and/or modulates PD-1 interactions with its ligands and/or other molecules and/or inhibits PD-1 signalling and/or pathway activity.

As used herein "an increase in expression or phosphorylation" refers to an amount of gene expression, protein expression or protein phosphorylation that is at least about 0.05 fold more (for example 0.1, 0.2, 0.3, 0.4, 0.5, 1, 5, 10, 25, 50, 100, 1000, 10,000-fold or more) than the amount of gene expression, protein expression or protein phosphorylation in a subject not undergoing PD-1 inhibition or in a subject prior to undergoing PD-1 inhibition according to the methods described herein. "Increased" as it refers to gene expression, protein expression or protein phosphorylation also means at least about 5% more (for example 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 99 or 100%) than the amount of gene expression, protein expression or protein phosphorylation in a subject not undergoing PD-1 inhibition or in a subject prior to undergoing PD-1 inhibition according to the methods described herein. Amounts can be measured according to methods known in the art for determining amounts of gene expression, protein expression or protein phosphorylation. Ranges provided herein are understood to be shorthand for all of the values within the range. For example, a range of 1 to 50 is understood to include any number, combination of numbers, or sub-range from the group consisting 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 (as well as fractions thereof unless the context clearly dictates otherwise).

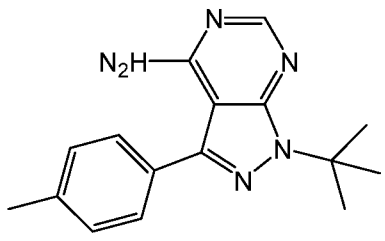
HCK Inhibitors

The present disclosure relates to methods involving the use of HCK inhibitors. HCK (hematopoietic cell kinase) is a member of the SRC family tyrosine kinases. Other examples of SRC family tyrosine kinases include LCK, FYN, LYN and SRC kinases.

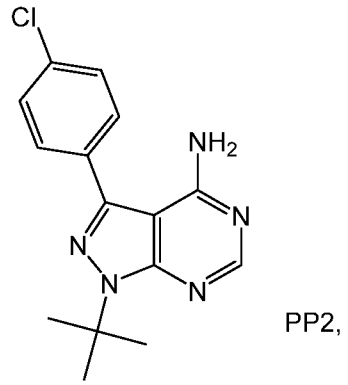
As used herein, a “HCK inhibitor” is a molecule that prevents or reduces, to some extent, the biological activity of HCK. The use of all types of HCK inhibitors are encompassed by the present disclosure including, for example, small molecule inhibitors (e.g. less than 1000Da molecular weight, or more preferably less than 500Da), antibody therapeutics, antibody-drug conjugates, protein/peptide therapeutics and siRNA therapeutic/active agents targeting knockdown of HCK. In some embodiments, the HCK inhibitor is a small molecule. In some embodiments, the HCK inhibitor is a small molecule having a pyrrolopyrimidine or a pyrazolopyrimidine moiety.

HCK inhibitors are described in, for example Saito *et al*, Science Translational Medicine, 2013, Vol 5, Issue 181, 181; Pene-Dumitrescu *et al*, Oncogene, 2008, 27, 7055-7069; Dorman *et al*, Frontiers in Chemistry, 2019, 7, Article 822.

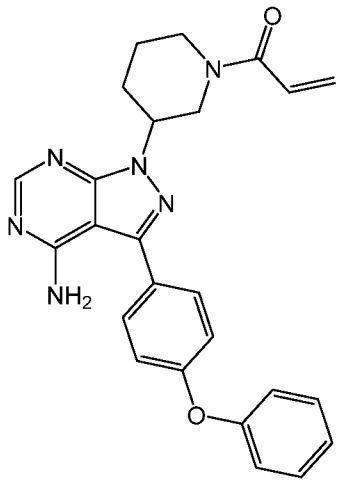
A HCK inhibitor may have broad specificity, for example, may be capable of inhibiting SRC family tyrosine kinases and non-SRC family tyrosine kinases with similar IC_{50}. Examples of HCK inhibitors with broad specificity include dasatanib, bosutinib (SKI-606), PP1, PP2, and ibrutinib (PCI-32765). In some examples, the HCK inhibitor does not have broad specificity. For example, the HCK inhibitor may be selective for SRC family tyrosine kinases. Examples of HCK inhibitors with specificity for SRC-family tyrosine kinases include A-420983, RK-24466, and RK20449 (also referred to as A-419259). The structures of example inhibitors are shown below.



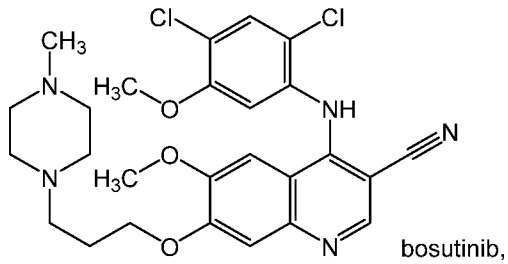
PP1,



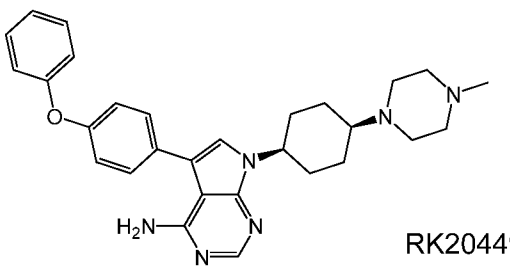
PP2,



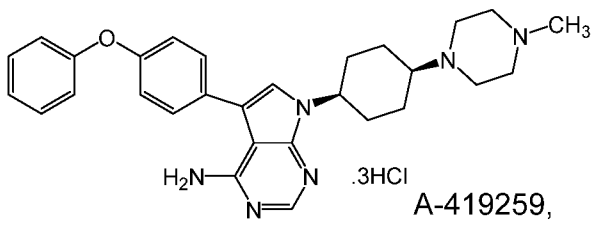
ibrutinib,



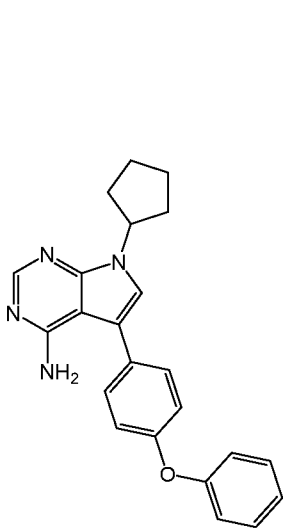
bosutinib,



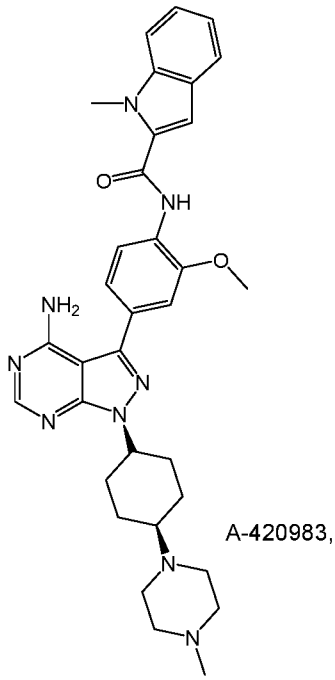
RK20449,



.3HCl A-419259,

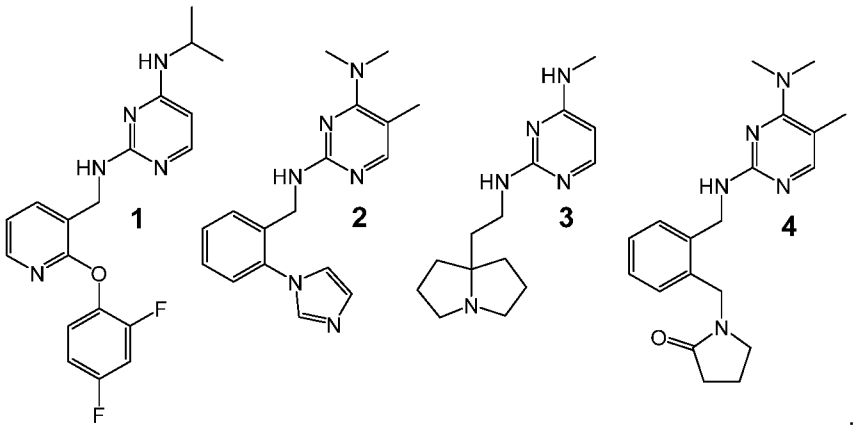


RK-24466,



A-420983,

Further examples of HCK inhibitors include



In some examples, the HCK inhibitors have an IC_{50} of less than 100 nM, less than 50 nM, less than 40 nM, less than 30 nM, less than 20 nM or less than 10 nM for SRC family tyrosine kinases (e.g. HCK, LCK, FYN, LYN and SRC kinases). In some examples, the HCK inhibitors have an IC_{50} of less than 50 nM for SRC family tyrosine kinases. In some examples, the HCK inhibitors have an IC_{50} of less than 20 nM for SRC family tyrosine kinases. In some examples, the HCK inhibitors have an IC_{50} of less than 100 nM, less than 50 nM, less than 40 nM, less than 30 nM, less than 20 nM or less than 10 nM for HCK. In some examples, the HCK inhibitors have an IC_{50} of less than 50 nM for HCK. In some examples, the HCK inhibitors have an IC_{50} of less than 20 nM for HCK. The IC_{50} is measured *in vitro* using techniques known to the person skilled in the art (e.g. Saito *et al*, Science Translational Medicine, 2013, Vol 5, Issue 181, 181).

In some examples, the HCK inhibitors are SRC family specific inhibitors (e.g. RK24466, RK20449), i.e. they are selective for SRC family tyrosine kinases relative to non-SRC family

tyrosine kinases. For example, the HCK inhibitors demonstrate at least 5-fold greater, at least10-fold greater, at least 20-fold greater, at least 30-fold greater, at least 40-fold greater, at least 50-fold greater, at least 100-fold greater or at least 500-fold greater selectivity for SRC family tyrosine kinases (as measured by *in vitro* IC_{50}) relative to non-SRC family kinases. In some examples, the HCK inhibitors demonstrate at least 10-fold greater selectivity for SRC family tyrosine kinases. In some examples, the HCK inhibitor has an IC_{50} of >100 nM for non-SRC family kinases. In some examples, the HCK inhibitor specifically inhibits HCK with an IC_{50} ≤30nM and inhibits non-SRC family kinases with IC_{50} >100nM (Saito *et al*, Science Translational Medicine, 2013, Vol 5, Issue 181, 181ra52).

In some examples, the HCK inhibitors demonstrate at least 10-fold greater, at least 20-fold greater, at least 30-fold greater, at least 40-fold greater, at least 50-fold greater, at least 100-fold greater or at least 500-fold greater selectivity for SRC family tyrosine kinases (as measured by *in vitro* IC_{50}) relative to Abl kinase. In some examples, the HCK inhibitors demonstrate at least 50-fold greater selectivity for SRC family tyrosine kinases relative to Abl kinase (i.e. the HCK inhibitor has a >50-fold lower IC_{50} for inhibition of SRC family kinases *in vitro* relative to Abl kinase). In some examples, the HCK inhibitor has an IC_{50} of >100nM for Abl kinase, or an IC_{50} of >1000nM for Abl kinase. In some examples, the HCK inhibitor specifically inhibits HCK with an IC_{50} ≤30nM and inhibits Abl kinase with an IC_{50} >100nM. In some examples, the HCK inhibitor specifically inhibits HCK with an IC_{50} ≤30nM and inhibits Abl kinase with IC_{50} >1000nM. In some examples, the HCK inhibitor specifically inhibits HCK with an IC_{50} ≤100nM and inhibits BCR kinase with IC_{50} >1000nM. In some examples, the HCK inhibitors demonstrate at least 10-fold greater, at least 20-fold greater, at least 30-fold greater, at least 40-fold greater, at least 50-fold greater, at least 100-fold greater or at least 500-fold greater selectivity for SRC family tyrosine kinases (as measured by *in vitro* IC_{50}) relative to Trk/IGFR1/Ror family kinases (e.g. DDR2). In some examples, the HCK inhibitor has an IC_{50} of >100nM for Trk/IGFR1/Ror family kinases (e.g. DDR2). In some examples, the HCK inhibitor has an IC_{50} of >1000nM for Trk/IGFR1/Ror family kinases (e.g. DDR2). In some examples, the HCK inhibitor specifically inhibits HCK with an IC_{50} ≤100nM and inhibits Trk/IGFR1/Ror family kinases (e.g. DDR2) with IC_{50} >100nM. In some examples, the HCK inhibitor specifically inhibits HCK with an IC_{50} ≤100nM and inhibits Trk/IGFR1/Ror family kinases (e.g. DDR2) with IC_{50} >1000nM.

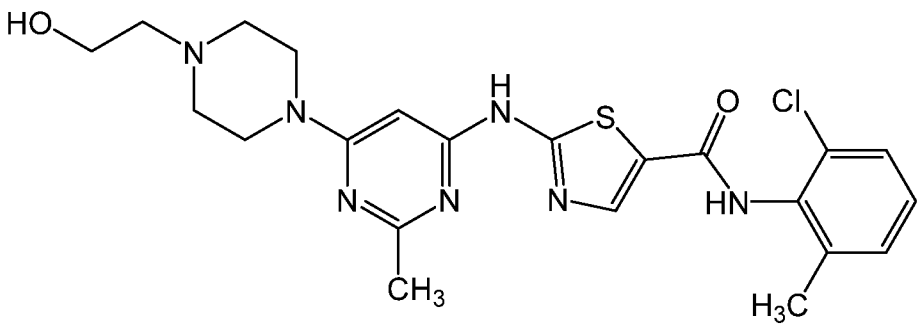
In some embodiments, the HCK inhibitor is not dasatinib. In some embodiments, the HCK inhibitor is not bosutinib. In some embodiments, the cancer is pancreatic cancer and the HCK inhibitor is not dasatinib. In some embodiments, the cancer is pancreatic cancer and the HCK inhibitor is a SRC family tyrosine kinase specific inhibitor. In some embodiments, the cancer is pancreatic cancer and the HCK inhibitor is RK-20449.

The present disclosure encompasses all forms of HCK inhibitors, including the free base and salt forms, and all physical forms, solvates and polymorphs.

In some examples, a salt form of an HCK inhibitor is used. Suitable salts include those formed with organic or inorganic acids or bases. Typically, a pharmaceutically acceptable salt is used. Exemplary acid addition salts include, but are not limited to, sulfate, citrate, acetate, oxalate, chloride, bromide, iodide, nitrate, bisulfate, phosphate, acid phosphate, isonicotinate, lactate, salicylate, acid citrate, tartrate, oleate, tannate, pantothenate, bitartrate, ascorbate, succinate, maleate, gentisinate, fumarate, gluconate, glucuronate, saccharate, formate, benzoate, glutamate, methanesulfonate, ethanesulfonate, benzenesulfonate, p-toluenesulfonate, and pamoate (i.e., 1,1′-methylene-bis-(2-hydroxy-3-naphthoate)) salts. Exemplary base addition salts include, but are not limited to, ammonium salts, alkali metal salts, for example those of potassium and sodium, alkaline earth metal salts, for example those of calcium and magnesium, and salts with organic bases, for example dicyclohexylamine, N-methyl-D-glucomine, morpholine, thiomorpholine, piperidine, pyrrolidine, a mono-, di- or tri-lower alkylamine, for example ethyl-, tert-butyl-, diethyl-, diisopropyl-, triethyl-, tributyl- or dimethyl - propylamine, or a mono-, di- or trihydroxy lower alkylamine, for example mono-, di- or triethanolamine. A pharmaceutically acceptable salt may involve the inclusion of another molecule such as an acetate ion, a succinate ion or other counterion. The counterion may be any organic or inorganic moiety that stabilizes the charge on the parent compound. Furthermore, a pharmaceutically acceptable salt may have more than one charged atom in its structure. Instances where multiple charged atoms are part of the pharmaceutically acceptable salt can have multiple counter ions. Hence, a pharmaceutically acceptable salt can have one or more charged atoms and/or one or more counterion.

Those skilled in the art of medicinal chemistry will appreciate that many organic compounds can form complexes with solvents in which they are reacted or from which they are precipitated or crystallized. These complexes are known as "solvates". For example, a complex with water is known as a "hydrate". As used herein, the phrase “pharmaceutically acceptable solvate” or “solvate” refer to an association of one or more solvent molecules and a compound of the present disclosure. Examples of solvents that form pharmaceutically acceptable solvates include, but are not limited to, water, isopropanol, ethanol, methanol, DMSO, ethyl acetate, acetic acid, and ethanolamine. It will be understood that the present disclosure encompasses solvated forms, including hydrates, of HCK inhibitors, as well as unsolvated forms.

In some embodiments, the HCK inhibitor is dasatinib. Dasatinib is a pan-tyrosine kinase inhibitor having the chemical name: N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide. It has the chemical structure:



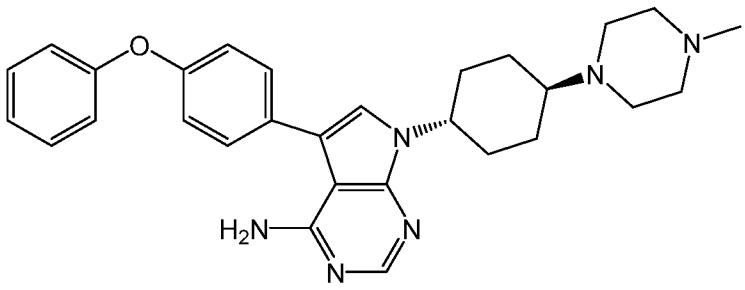
Dasatinib is the active ingredient present in the product SPRYCEL. Dasatinib has been demonstrated to inhibit the following kinases: BCR-ABL, SRC family (SRC, LCK, YES, FYN, HCK), c-KIT, EPHA2, and PDGFRβ.

Dasatinib, its preparation, and uses of the compound are disclosed in, for example, US6,596,746, US7,125,875, US7,153,856, US7,491,725 and US8,680,103, the contents of each of which are incorporated herein by reference.

The present disclosure encompasses all forms of dasatinib, including the free base and salt forms, and all physical forms, solvates and polymorphs. Dasatinib is most commonly used in the form of the free base. In some examples, dasatinib is used in the form of the free base. In some other examples, a salt form of dasatinib is used. Suitable salts include, but are not limited to, those described herein.

Dasatinib is most commonly used in the form of a monohydrate. In some examples, dasatinib is used in the form of a monohydrate.

In some embodiments, the HCK inhibitor is RK20449. RK20449 is an inhibitor of SRC family kinases, including HCK, SRC, LCK and LYN (Wilson et al, Oncogene, 2002. 21(53): 8075-88; Saito *et al*, Science Translational Medicine, 2013, Vol 5, Issue 181, 181ra52). RK20449 is a pyrrolo-pyrimidine derivative having the structure below:



RK20449 has the chemical name 7-[*trans*-4-(4-methyl-1-piperazinyl)cyclohexyl]-5-(4-phenoxy-phenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine. It is also known by the name A-419259, and was described in, for example, Wilson et al, Oncogene, 2002, 21(53) p8075-8088. It is available in salt forms, including as a hydrochloride salt.

The present disclosure encompasses all forms of RK20449, including the free base and salt forms, and all physical forms, solvates and polymorphs. Suitable salts include, but are not limited to, those described herein. In some examples, RK20449 is the hydrochloride salt.

It will be understood that the present disclosure encompasses solvated forms, including hydrates, of RK20449, as well as unsolvated RK20449. In some examples, RK20449 is used in the form of a monohydrate.

In some examples, the HCK inhibitor can be used as a monotherapy (i.e. alone, without combining with one or more additional active agents or therapies). In some examples, the HCK inhibitor can be used or combined with one or more additional active agents or therapies (including, but not limited to, an immune checkpoint inhibitor, radiation therapy or chemotherapy). In some examples, the HCK inhibitor can be used or combined with a chemotherapy. In some examples, the HCK inhibitor can be used or combined with a radiation therapy. In some examples, the HCK inhibitor can be used or combined with an immune checkpoint inhibitor. In some examples, the HCK inhibitor is used without an immune checkpoint inhibitor. The additional active agent may be administered concurrently or sequentially with the HCK inhibitor and the immune checkpoint inhibitor.

Immune checkpoint inhibitors

It is known to those skilled in the art that the immune system provides an inhibitory signal for its components to balance the immune response. Known immune checkpoint proteins include CTLA-4, PD1 and its ligands PD-L1 and PD-L2, and additionally LAG-3, BTLA, B7H3, B7H4, TIM3, KIR. It is recognized in the art that pathways involving LAG3, BTLA, B7H3, B7H4, TIM3 and KIR constitute immune checkpoint pathways similar to those dependent on CTLA-4 and PD-1 (Pardoll, (2012) Nature Rev Cancer 12: 252-264; Mellman et al., (2011) Nature 480: 480-490). In one example, the immune checkpoint protein is a human immune checkpoint protein.

As discussed above, the present disclosure also relates to therapeutic methods and uses involving a HCK inhibitor and an immune checkpoint inhibitor. In particular examples, the present disclosure is directed to the use of an immune checkpoint protein inhibitor, or "immune checkpoint inhibitor". The term "immune checkpoint inhibitor" as used herein refers to any compound that inhibits the function of an immune checkpoint protein. The inhibition includes the spectrum from reduced function to complete blockade. In a particular example, the immune checkpoint protein inhibitor is an inhibitor of a human immune checkpoint protein.

Immune checkpoint proteins have been reported in the art (Pardoll, (2012) Nature Rev. cancer 12:252-264). The term immune checkpoint includes experimental demonstration of stimulation of T lymphocyte responses caused by antigen receptors by inhibition of immune checkpoint protein *in vitro* or *in vivo*, e.g., mice lacking expression of immune checkpoint protein show signs of enhanced antigen-specific T lymphocyte response or autoimmunity (disclosed in Waterhouse et al., (1995) Science 270: 985-988; Nishimura et al., (1999) Immunity 11: 141-151).

It may also include demonstration of inhibition of the CD4+ or CD8+ T cell response caused by antigen receptors due to the intentional stimulation of immune checkpoint proteins *in vitro* or *in vivo* (Zhu et al., (2005) Nature Immunol 6:1245-1252).

Preferred immune checkpoint protein inhibitors are antibodies that specifically recognize immune checkpoint proteins. Examples include CTLA-4, PD1, PDL-1, PD-L2, LAG-3, BTLA, B7H3, B7H4, TIM3 and KIR inhibitors.

Ipilimumab is a fully human CTLA-4 blocking antibody currently sold under the name Yervoy® (Bristol-Myers Squibb). A further CTLA-4 inhibitor is tremelimumab (referenced in Ribas et al., (2013) J. Clin. Oncol. 31: 616-22).

Examples of PD-1 inhibitors include humanized antibodies that block human PD-1, such as lambrolizumab (e.g, WO 2008/156712; Hamid et al., (2013) N. Engl. J. Med. 369:134-144). HPD109A and its humanized derivatives h409A11, h409A16 and h409A17) or pidilizumab (disclosed in Rosenblatt et al., (2011) J Immunother. 34: 409-18), and nivolumab (formerly known as MDX-1106 or BMS-936558, Topalian et al., (2012) N. Eng. J. Med. 366: 2443-3454). Other PD-1 inhibitors include, but are not limited to, the PD-L2 Fc fusion protein also known as B7-DC-Ig or AMP-244 (Mkrticyan M, et al. (2012) J Immunol. 189: 2338-47). Further, immune checkpoint inhibitors include humanized or fully human antibodies that block PD-L1, such as MEDI-4736 (WO2011106389A1), MPDL3280A (US8217149B2) and MIH1 (US8217149B2). Affymetrix (16.59883.82) available through eBioscience and pembrolizumab (sold under the trade name KEYTRUDA) as well as other PD-L1 inhibitors currently under investigation.

In one example, the immune checkpoint inhibitor is selected from the group consisting of a CTLA-4 inhibitor, PD-1 inhibitor, and PD-L1 inhibitor. In one example, the immune checkpoint inhibitor is selected from the group consisting of a CTLA-4 inhibitor, PD-1 inhibitor, and PD-L1 inhibitor. In one example, the inhibitor is selected from the group consisting of ipilimumab, tremelimumab, labrolizumab, nivolumab, pidilizumab, pembrolizumab, spartalizumab, AMP-244, MEDI-4736, MPDL3280A, and MIH1. Lambrolizumab is also known by alternative names MK-3475 and pembrolizumab including analogs, in particular chimerized forms, humanized forms or humanized antibodies.

In some examples, the inhibitor is one that directly or indirectly stimulates or enhances antigen-specific T lymphocytes. Such inhibitors include, but are not limited to, agents that target immune checkpoint proteins and pathways involving PD-L2, LAG3, BTLA, B7H4 and TIM3. For example, human PD-L2 inhibitors known in the art include MIH18 (Pfistershammer et al., (2006) Eur J Immunol. 36: 1104-13). Another example of LAG3 inhibitors known in the art include soluble LAG3 (IMP321 or LAG3-Ig as disclosed in WO20090443273A2 and Brignon et al. (2009) Clin. Cancer Res. 15: 6225-6231) and human LAG3. Mouse antibodies or humanized antibodies (disclosed in WO200832601A1, derived from IMP 701) or fully human antibodies (disclosed in EP 2320940A2) that inhibit human LAG3. Another example is provided by the use of blocking agents against BTLA, including but not limited to antibodies that block the interaction between

human BTLA and its ligands (eg, 4C7 disclosed in WO2011014438). Yet another example includes, but is not limited to, an antibody against human B7H4 (disclosed in WO2013025779A1 and WO2013067492A1) or an antibody against soluble recombinant B7H4 (disclosed in US20120176745A1, or anti-human B7H4 provided by the use of an agent that neutralizes B7H4, including clone H74: eBioscience # 14-5948). Yet another example is provided by agents that neutralize B7-H3, including but not limited to antibodies that neutralize human B7-H3 (e.g, MGA271 disclosed as BRCA84D and a derivative in US2012029496A1). An example includes an antibody that targets human TIM3 (such as those disclosed in WO2013006490A2, or Jones et al., J Exp Med. (2008) Nov 24; 205 (12): Provided by an agent that targets TIM3, including the anti-human TIM3 blocking antibody F38-2E2) disclosed by 2763-79.

Cancers

In some examples, the methods as described herein are useful for treating cancer in a subject. In some examples, the methods as described herein are also useful for preventing cancer in a subject. In some examples, the cancer is a solid cancer, fibrotic cancer, metastatic cancer or blood cancer. In one example, the cancer is a solid cancer. In one example, the cancer is a fibrotic cancer. In one example, the cancer is a metastatic cancer. In one example, the cancer is breast cancer, colon cancer, pancreatic cancer, gastric cancer, oesophageal cancer, hepatocellular cancer or melanoma. In one example, the cancer is pancreatic cancer.

Cancers of various aetiologies frequently contain PD-1 receptor-expressing cancer cell subpopulations. Tumour cell-expressed PD-1 modulates downstream pathways, signalling mediators of which can serve as biomarkers for predicting and monitoring response to therapeutic anti-PD-1 antibodies. In some examples, the cancer is one that expresses PD-1. Expression of PD-1 by a cancer cell can be determined by various means including immunofluorescence, immunohistochemistry, flow cytometry, immunoblot, and *in situ* hybridization.

In some examples, the cancer is one that has previously been identified as not responding or poorly responding to PD-1. The clinician will be able to determine whether a cancer is not responding to PD-1 therapy. Examples include a cancer that does not decrease in size or severity.

Responsiveness to therapy can be assessed by measuring the size of the tumour following therapy or severity/spread of the tumour following therapy (e.g. whether the tumour has increased in stage or metastasised).

Fibrotic Cancers

Fibrosis may be considered the formation of excess connective tissue causing stromal hardening and scar formation. Intratumoural fibrosis can result from the deposition of a collagen matrix by cancer-associated fibroblasts (CAFs). Fibrosis can precede or follow cancer

development and may participate in multiple stages of tumourigenesis and metastasis. Tumours are characterised by extracellular matrix (ECM) deposition, remodelling and cross-linking that drive fibrosis to stiffen the stroma and promote malignancy. The ECM is a highly dynamic structure that is constantly remodelled through enzymatic and non-enzymatic post-translational modification that alter its instructive capacity.

The ECM is broadly classified as either basement membrane (BM) or interstitial matrix. The BM which surrounds cells such as epithelial, endothelial and hepatocytes, is composed of a laminin and collagen IV network that is linked by a perlecan and nidogen network. The BM not only provides structural support but also orchestrates the establishment of cell polarity and binds critical growth factors and cytokines that regulates cell differentiation and maintains tissue homeostasis. Although the basic building blocks are conserved, the BM in each tissue has a specific composition and structure that is specifically tuned to the functional requirement of the organ system.

Interstitial ECMs are composed of proteoglycans and fibrous proteins that maintain tissue hydration and mechanical strength. The proteoglycans in the interstitial ECM (e.g. hyaluronic acid (HA)) bind water through their glycosaminoglycan (GAG) chains. GAGs are un-branched polysaccharide chains composed of repeating disaccharide units that are quite hydrophilic and they adopt highly extended formations that bind water to provide hydration and permit compression resistance in the tissue. Fibrillar collagens are the main structural component of the interstitial ECM that contribute to the tensile strength of the tissue.

Tumour fibrosis can occur in response to chronic inflammation, and elevated numbers of contractile myofibroblasts that secrete abundant ECM proteins and remodeling enzymes that reorganize, cross-link and stiffen the matrix, and cytokines and growth factors that stimulate tumour cell proliferation and invasion yielding a markedly different stroma (Piersma B et al., (2020) Biochim Biophys Acta Rev Cancer 1873(2):188356). The stiffened stroma enhances tumour cell growth, survival and migration and drives mesenchymal transition. A stiff ECM also induces angiogenesis, hypoxia and compromises anti-tumour immunity. Tumour progression and poor patient prognosis correlate with degree of tissue fibrosis and level of stromal stiffness.

Tumour fibrosis is induced by accumulation of transforming growth factor beta, interleukin (IL)-11 and other cytokines (Cook and Schafer, Annu Rev Med, 2020. 71: 263-276). Excessive HCK activity in myeloid cells or macrophages results in excessive production of IL-11, while such cells with genetic inactivation of the HCK gene produce less IL-11 (Poh, et al., Cancer Cell, 2017. 31(4): 563-575.e5; Poh et al, Cancer Immunol Res, 2020. 8(4): 428-435). In some aspects, the methods and uses of the present invention involve therapy of fibrotic cancers, e.g. cancers which are associated with a fibrotic component.

Accordingly, there is provided a method of treating and/or preventing a fibrotic cancer in a subject, comprising administering an effective amount of an HCK inhibitor to the subject. There is also provided a method of preventing a fibrotic cancer in a subject, comprising administering

an effective amount of an HCK inhibitor to the subject. Examples of fibrotic cancers include hepatocellular, liver, gastric, oesophageal, head and neck, colon, pancreatic, lung, cervical and vulvar cancers. In some embodiments, the fibrotic cancer is pancreatic cancer.

In some examples, there is provided use of an HCK inhibitor in the manufacture of a medicament for treating and/or preventing a fibrotic cancer in a subject.

In some examples, there is provided an HCK inhibitor for use in treating and/or preventing a fibrotic cancer in a subject.

In some examples, there is provided a method of preventing a fibrotic cancer in a subject, comprising administering an effective amount of a pharmaceutical composition comprising an HCK inhibitor and a pharmaceutically acceptable excipient to the subject.

In further examples, the size of the tumour (tumour mass) is reduced following treatment with the HCK inhibitor compared to a tumour that is not treated with the HCK inhibitor. In one example, the tumour mass is reduced by at least 50%, at least 40%, at least 30%, at least 20% or at least 10% compared to a tumour that has not been treated with the HCK inhibitor.

In some examples, the fibrotic cancer is pancreatic cancer, gastric cancer, oesophageal cancer, hepatocellular cancer or melanoma. In some examples, the fibrotic cancer is pancreatic cancer.

Metastatic Cancers

The present disclosure is based on the finding that the HCK inhibitor, RK20449, reduced the metastatic growth of certain tumours. This was unexpected as a number of clinical trials have reported that SRC family kinase inhibitors, particularly when used alone, have limited benefits for the treatment of advanced/metastatic disease (Fury et al, Anticancer Res, 2011. 31(1): 249-53; Sharma et al, Invest New Drugs, 2012. 30(3): 1211-5; Gucalp et al, Clinical breast cancer, 2011. 11(5): 306-311). Accordingly, in some aspects, the methods and uses of the present invention involve inhibiting and/or reducing metastasis of a primary cancer. Metastasis is the development of one or more secondary malignant growths at distant sites from a primary site of cancer. Accordingly, there is provided a method of inhibiting and/or reducing metastasis of a primary cancer in a subject, comprising administering an effective amount of an HCK inhibitor to the subject. Inhibition and/or reduction of metastasis may, for example, be a reduced likelihood of developing a secondary cancer following administration of the HCK inhibitor compared with patients who have not been administered an HCK inhibitor. In some embodiments, the primary cancer is a fibrotic cancer. In some embodiments, the primary cancer is selected from the group consisting of pancreatic cancer, melanoma and breast cancer. In some embodiments, the primary cancer is pancreatic cancer.

In a further embodiment, the size of the secondary tumour (tumour mass) is reduced following treatment with the HCK inhibitor compared to a tumour that is not treated with the HCK inhibitor. In one example, the tumour mass is reduced by at least 50%, at least 40%, at least

30%, at least 20% or at least 10% compared to a tumour that has not been treated with the HCK inhibitor.

In some embodiments, a reduction is seen as a reduction in the number of secondary tumours in the subject's body.

Solid Cancers

In one example, the cancer is a solid tumour. A solid tumour is defined herein as a mass of tissue that usually does not contain cysts or liquid areas. In another example, the solid tumour is a sarcoma, carcinoma or lymphoma. The solid tumour may be a tumour selected from the group consisting of colorectal cancer, gastric cancer, oesophageal cancer, lung cancer, breast cancer, melanoma, head and neck squamous cell cancer, cutaneous squamous cell carcinoma, Merkel cell carcinoma, renal cell carcinoma, urothelial carcinoma, cervical cancer, prostate cancer, ovarian cancer, bladder cancer, hepatocellular carcinoma, endometrial carcinoma and tumour mutational burden-high cancer. PD-1 expression, signally or activity can also be indicated by p-S6 expression. In some embodiments, the solid cancer is colorectal cancer or gastric cancer.

Blood Cancers

In one example, the cancer is a blood cancer. In another example, the blood cancer is a cancer selected from the group consisting of chronic myeloid leukemia, acute lymphoblastic leukemia, classical Hodgkin lymphoma and primary mediastinal large B-cell lymphoma.

Pharmaceutical Compositions

The HCK inhibitor may be administered to the subject in any suitable format. Similarly, the immune checkpoint inhibitor may be administered to the subject in any suitable format.

In some examples, dasatinib may be administered to the subject in its approved form according to the product information (PI). In some examples, the immune checkpoint inhibitor (e.g. the PD-1 inhibitor) may be administered to the subject in the format in which it is approved by a regulatory agency (e.g. FDA or TGA). RK20449 may also be administered in any suitable composition.

The pharmaceutical compositions provided herein, may contain pharmaceutical carriers or diluents. The term "composition" as used herein is intended to cover products containing the specified ingredients in the specified amounts, as well as any products produced directly or indirectly from a combination of the specified ingredients in the specified amounts. "Pharmaceutically acceptable" means that the carrier, diluent or excipient must be compatible with the other ingredients of the formulation and not harmful to its recipient. A biological product, such as a compound of the disclosure, may be composed of a pharmaceutical composition containing one or more compounds and a pharmaceutically acceptable carrier. As used herein,

a "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and physiologically compatible similar. Preferably, the carrier is suitable for intravenous, intramuscular, subcutaneous, parenteral, spinal or epidermal administration (e.g., by injection or infusion).

The pharmaceutical composition of the present invention may include one or more pharmaceutically acceptable salts, antioxidants, aqueous and non-aqueous carriers and/or adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Pharmaceutical compositions for administration of the compounds and agents of the present disclosure are suitably presented in unit dosage form and can be prepared by any of the methods well known in pharmacology and drug delivery technology.

In pharmaceutical compositions, the active compound is included in an amount sufficient to effect the desired process or condition of the disease. Pharmaceutical compositions containing active ingredients may be in a form suitable for oral use (e.g. tablet). Compositions intended for oral use may be prepared according to any method known in the art for making pharmaceutical compositions, and such compositions may contain one or more agents selected from the group consisting of sweeteners, flavorings, agents, colorants, antioxidants and preservatives in order to provide a pharmaceutical delicate and palatable formulation. Formulations for oral use can also be presented in the form of hard gelatin capsules in which the active ingredient is mixed with an inert solid diluent such as calcium carbonate, calcium phosphate or kaolin; or in the form of soft gelatin capsules in which the active ingredient is in water or oil (e.g, peanut oil, liquid paraffin, or olive oil).

The pharmaceutical composition of the present invention may also be in the form of an oil-in-water emulsion. The oily phase may be a vegetable oil, such as olive oil or flower oil; or a mineral oil, such as liquid paraffin, or a mixture of these oils. Suitable emulsifiers may be naturally occurring gums, such as acacia or tragacanth; naturally occurring phospholipids, such as soybean, lecithin; and esters or partial esters derived from fatty acids and hexitol anhydrides, such as sorbitan sugar alcohol monooleate; and condensation products of these partial esters with ethylene oxide, such as polyoxyethylene sorbitan monooleate. The emulsion may also contain sweeteners and flavouring agents. Syrups and elixirs can be formulated with sweeteners such as glycerol, propylene glycol, glucose or sucrose. Such formulations may also contain a demulcent, a preservative, a flavoring agent, and a coloring agent. Oral solutions can be prepared in combination with, for example, cyclodextrin, PEG and surfactants.

The compounds of the present disclosure can be formulated for placement in medical devices, which can include a variety of conventional grafts, stents (including stent grafts), catheters, expanders, baskets, or implantable or permanently implanted body cavities. For example, compounds can be delivered to a tumour or the microenvironment surrounding the tumour.

In some examples, for example where the HCK inhibitor is RK20449, it is administered in the form of a pharmaceutical composition which is a tablet, e.g. a coated tablet.

A tablet may be made for example by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may for example be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, lubricating, surface active, or dispersing agent. Moulded tablets may for example be made by moulding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. Tablets may be optionally coated or scored, and may be formulated so as to provide slow or controlled release of the active compound.

The active can, for example, be administered in a form suitable for immediate release or extended release. Immediate release or extended release can be achieved by the use of suitable pharmaceutical compositions comprising the active or, particularly in the case of extended release, by the use of devices such as subcutaneous implants or osmotic pumps. An active may also be administered liposomally.

Exemplary compositions may contain, for example, excipients such as fillers, binders, extenders, disintegrants, diluents, and/or lubricants such as those known in the art. Suitable binders include starch, gelatin, natural sugars such as glucose or beta-lactose, corn sweeteners, natural and synthetic gums such as acacia, tragacanth or sodium alginate, carboxymethylcellulose, polyethylene glycol, waxes, and the like.

Disintegrators include without limitation, starch, methylcellulose, agar, bentonite, xanthan gum, and the like.

Lubricants, glidants, flavours, colouring agents, and stabilisers may also be added for ease of fabrication and use. Lubricants used in these dosage forms include sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride, and the like.

The compositions of the present disclosure may also include polymeric excipients/additives or carriers, e.g., polyvinylpyrrolidones, derivatised celluloses such as hydroxymethylcellulose, hydroxyethylcellulose, and hydroxypropylmethylcellulose, Ficolls (a polymeric sugar), hydroxyethylstarch (HES), dextrates (e.g., cyclodextrins, such as 2-hydroxypropyl-β-cyclodextrin and sulfobutylether-β-cyclodextrin), polyethylene glycols, and pectin. The compositions may further include buffers, thickeners, preservatives (including antioxidants), inorganic salts (e.g., sodium chloride), antimicrobial agents (e.g., benzalkonium chloride), antistatic agents, lipids (e.g., phospholipids such as lecithin and other phosphatidylcholines, phosphatidylethanolamines, fatty acids and fatty esters, steroids (e.g., cholesterol)), and/or chelating agents (e.g., EDTA, zinc and other such suitable cations).

For oral administration in liquid form, the oral drug components can be combined with any oral, non-toxic, pharmaceutically acceptable inert carrier such as ethanol, glycerol, water, and the like.

Other pharmaceutical excipients and/or additives suitable for use in the compositions according to the present disclosure are listed in "Remington: The Science & Practice of Pharmacy", 19.sup.th ed., Williams & Williams, (1995), and in the "Physician's Desk Reference", 52.sup.nd ed., Medical Economics, Montvale, N.J. (1998), and in "Handbook of Pharmaceutical Excipients", Third Ed., Ed. A. H. Kibbe, Pharmaceutical Press, 2000.

As discussed above, in the methods of the present disclosure, where the HCK inhibitor is RK20449, it may for example be administered in the form of a coated tablet. In some examples, RK20449 is administered in the form of a coated tablet containing the active, lactose, microcrystalline cellulose, croscarmellose sodium, hydroxypropyl cellulose and magnesium stearate, and having a coating containing hypromellose, titanium dioxide and polyethylene glycol.

In some examples, RK20449 is administered in the form of coated tablets containing 20mg, 50mg, 70mg or 100mg RK20449.

In some examples, the composition is formulated for parenteral delivery, for example by intravenous infusion or injection.

Compositions for parenteral administration include aqueous and non-aqueous sterile injections, solutions which may contain anti-oxidants, buffers, tonicity modifiers, bacteriostats, and solutes that 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. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials, and may be stored in a freeze-dried condition requiring only the addition of a sterile liquid carrier, for example saline or water-for-injection, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described. Exemplary compositions for parenteral administration include injectable solutions or suspensions which can contain, for example, suitable non-toxic, parenterally acceptable diluents or solvents, such as mannitol, 1.3-butanediol, water, Ringer's solution, an isotonic sodium chloride solution, or other suitable dispersing or wetting an d suspending agents, including synthetic mono- or diglycerides, and fatty acids, including oleic acid, or Cremaphor.

For example, the formulation may be a sterile, lyophilized composition that is suitable for reconstitution in an aqueous vehicle prior to injection. In one example, a formulation suitable for parenteral administration conveniently comprises a sterile aqueous preparation of an active ingredient, which may for example be formulated to be isotonic with the blood of the recipient.

In some examples, the HCK inhibitor is provided in the form of a formulation for intravenous infusión or injection. In some examples, the HCK inhibitor is provided in the form of a concentrate, for admixing with an aqueous diluent such as saline prior to administration. In

some examples, the HCK inhibitor is provided in the form of a solid composition, for admixing with an aqueous diluent such as saline prior to administration.

In some examples, the immune checkpoint inhibitor is provided in the form of a formulation for intravenous infusión or injection. In some examples, the immune checkpoint inhibitor is provided in the form of a concentrate, for admixing with an aqueous diluent such as saline prior to administration. In some examples, the immune checkpoint inhibitor is provided in the form of a solid composition, for admixing with an aqueous diluent such as saline prior to administration.

In some examples, the immune checkpoint inhibitor is provided in the form of an aqueous composition comprising water for injection and one or more of a surfactant, a buffer, an inorganic salt, and a polyol.

In some examples, the immune checkpoint inhibitor is pembrolizumab, and is provided in the form of an aqueous composition for injection comprising wáter for injection, L-histidine, polysorbate 80 and sucrose. In some examples the immune checkpoint inhibitor is pembrolizumab and is provided in a unit dosage form containing about 100mg pembrolizumab.

In some examples, the immune checkpoint inhibitor is nivolumab, and is provided in the form of an aqueous composition for injection which comprises water for injection, manitol, pentetic acid, polysorbate 80, sodium chloride and sodium citrate, and which may contain hydrochloric acid and/or sodium hydroxide to adjust the pH to 6. In some examples, the immune checkpoint inhibitor is nivolumab and is provided in a unit dosage form containing about 40mg, about 100mg, or about 240mg nivolumab.

In some examples, the immune checkpoint inhibitor is cemiplimab, and is provided in the form of an aqueous composition for infusion which contains water for injection, L-histidine, L-proline, sucrose and polysorbate 80. In some examples, the immune checkpoint inhibitor is cemiplimab and is provided in a unit dosage form containing about 350mg cemiplimab.

In some examples, the immune checkpoint inhibitor is atezolizumab, and is provided in the form of an aqueous composition for infusion which contains water for injection, histidine, glacial acetic acid, sucrose and polysorbate 20. In some examples, the immune checkpoint inhibitor is atezolizumab and is provided in a unit dosage form containing about 840mg or about 1200mg atezolizumab.

In some examples, the immune checkpoint inhibitor is avelumab, and is provided in the form of an aqueous composition for infusion which contains water for injection, glacial acetic acid, polysorbate 20 and sodium hydroxide. In some examples, the immune checkpoint inhibitor is avelumab and is provided in a unit dosage form containing about 200mg avelumab.

In some examples, the immune checkpoint inhibitor is durvalumab, and is provided in the form of an aqueous composition for infusion which contains water for injection, histidine, trehalose, and polysorbate 80.

In some examples, the immune checkpoint inhibitor is durvalumab and is provided in a unit dosage form containing about 120mg or about 500mg durvalumab.

In some examples, the immune checkpoint inhibitor is imipililumab, and is provided in the form of an aqueous composition for infusion which comprises water for injection, diethylene triamine pentaacetic acid (DTPA), mannitol, polysorbate 80, sodium chloride, tris hydrochloride, at pH 7. In some examples, the immune checkpoint inhibitor is imipililumab and is provided in a unit dosage form containing about 50mg, or about 200mg imipililumab.

Dosage

The amount of active ingredient that is required to achieve a therapeutic effect may vary with the particular active ingredient, the route of administration, the subject under treatment, including the type, species, age, weight, and sex of the subject, and the particular condition, disorder or disease being treated, as well as its severity.

Dosages of an HCK inhibitor, when used for the indicated effects, will range between, for example, about 0.01 mg per kg of body weight per day (mg/kg/day) to about 1000 mg/kg/day. In some embodiments, the dosage of an HCK inhibitor is between about 0.01 and 1000, 0.1 and 500, 0.1 and 100, 1 and 50 mg/kg/day. In some embodiments, the dosage of an HCK inhibitor is between about 0.1 and 100 mg/kg/day. In some embodiments, the dosage of an HCK inhibitor is between about 0.1 and 10 mg/kg/day. In some embodiments, the dosage of an HCK inhibitor is between about 1 and 5 mg/kg/day. In some embodiments, the dosage of an HCK inhibitor is greater than about 0.01, 0.1, 1, 10, 20, 50, 75, 100, 500, 1000 mg/kg/day.

In some embodiments, the dosage of an HCK inhibitor is less than about 5000, 1000, 75, 50, 20, 10, 1, 0.1 mg/kg/day.

In some embodiments, the HCK inhibitor is administered in an amount in the range of from 5mg per day to 1000mg per day, or from 50 mg per day to 500 mg per day, or from 100mg per day to 400 mg per day, or about 50mg, about 75mg, about 100mg, about 150mg, about 200mg, about 250mg, about 300mg, about 350mg, about 400mg, about 450mg or about 500mg per day.

A HCK inhibitor may for example be administered as a single daily dose, or otherwise the total daily dosage may be administered in divided doses of two, three, or four times daily. In some embodiments, the HCK inhibitor may be dosed less frequently than once per day, for example once per two days, three days, four days, five days, six days, or once per week.

If administered intravenously, an infusion of the compound over a period of time may be used, for example. Furthermore, an HCK inhibitor may be administered in intranasal form via topical use of suitable intranasal vehicles, or via transdermal routes, using those forms of transdermal skin patches well known to those of ordinary skill in the art. To be administered in the form of a transdermal delivery system, the dosage administration will, of course, be continuous rather than intermittent throughout the dosage regimen.

In some examples of the present disclosure, the HCK inhibitor is Dasatinib. Dasatinib has been approved for once daily oral dosing in daily dosage amounts of 100mg and 140mg, depending on the indication.

In some examples of the present disclosure, dasatinib is administered orally. In some examples, dasatinib is administered twice per day or, more preferably, once per day. In some examples dasatinib is administered in an amount of up to 150 mg per day, or up to 140 mg per day, or up to 130mg per day, or up to 120 mg per day, or up to 110 mg per day, or up to 100 mg per day, or up to 90 mg per day, or up to 80 mg per day, or up to 70 mg per day or up to 60 mg per day, or up to 50 mg per day. In some examples, dasatinib is administered in an amount of about 100 mg per day, or in an amount of about 140 mg per day.

In some examples dasatinib is administered once per day in an amount of up to 150 mg per day, or up to 140 mg per day, or up to 130mg per day, or up to 120 mg per day, or up to 110 mg per day, or up to 100 mg per day, or up to 90 mg per day, or up to 80 mg per day, or up to 70 mg per day or up to 60 mg per day, or up to 50 mg per day. In some examples, dasatinib is administered once per day in an amount of about 100 mg per day, or in an amount of about 140 mg per day. In some examples dasatinib is administered orally once per day in an amount of up to 150 mg per day, or up to 140 mg per day, or up to 130mg per day, or up to 120 mg per day, or up to 110 mg per day, or up to 100 mg per day, or up to 90 mg per day, or up to 80 mg per day, or up to 70 mg per day or up to 60 mg per day, or up to 50 mg per day. In some examples, dasatinib is administered orally once per day in an amount of about 100 mg per day, or in an amount of about 140 mg per day.

In some examples, the subject is human. In some examples, the subject is an adult human. In one example, the subject to be treated according to a method described herein is one which has failed first line treatment with chemotherapy. In another example, the subject is one that has previously not responded or poorly responded to PD-1 or another immune checkpoint inhibitor.

Combination Therapy

Whilst an HCK inhibitor may in some cases be used as the sole active agent in the methods of the present disclosure, it is also possible for an HCK inhibitor to be used in combination with one or more further active agents. Accordingly, in some embodiments, an HCK inhibitor is used in combination with one or more further active agents. The present disclosure therefore also provides methods involving use of a combination of an HCK inhibitor and a further active agent. Such one or more further active agents may for example be further anticancer agents, for example further agents useful for treating fibrotic (e.g. pancreatic) cancers, or useful

for treating breast cancer or melanoma. Such one or more further active agents may, for example, be an immune checkpoint inhibitor.

In some embodiments, the further active agent is a chemotherapeutic agent. In some embodiments, the further active agent is selected from the group consisting of gemcitabine, 5-fluorouracil, irinotecan, paclitaxel-albumin (ABRAXANE), everolimus, erlotinib, olaparib, mitomycin, sunitinib, leucovorin, oxaliplatin, cisplatin, carboplatin, radiation therapy, immunotherapy (e.g. an immune checkpoint inhibitor), dabrafenib, encorafenib, vemurafenib, trametinib, cobimetinib, binimetinib, imatinib, nilotinib, larotrectinib, dacarbazine, temozolomide, fotemustine, lomustine, a taxane (e.g. docetaxel, paclitaxel), vinblastine, doxorubicin, epirubicin, cyclophosphamide, capecitabine, eribulin, ixabepilone, mitroxantrone, vinorelbine, adriamycin, cytoxan and methotrexate.

In some embodiments, the further active agent is an immune checkpoint inhibitor.

In some examples, an effective amount of a HCK inhibitor is administered and an effective amount of an immune checkpoint inhibitor is administered to the subject. The HCK inhibitor and the immune checkpoint inhibitor may be administered in any appropriate form to the subject, for example they may be administered separately, sequentially or simultaneously. In some examples, the HCK inhibitor and the immune checkpoint inhibitor are administered separately to the subject; that means that each active is administered as part of a single treatment regime, but they are administered at different times and different dosage intervals, as will be appropriate for the specific ative. In some examples, the HCK inhibitor is RK20449 and an effective amount of RK20449 is administered and an effective amount of an immune checkpoint inhibitor is administered to the subject. RK20449 and the immune checkpoint inhibitor may be administered in any appropriate form to the subject, for example they may be administered separately, sequentially or simultaneously. In some examples, RK20449 and the immune checkpoint inhibitor are administered separately to the subject; that means that each active is administered as part of a single treatment regime, but they are administered at different times and different dosage intervals, as will be appropriate for the specific active.

As shown by the examples below, administration of a combination of HCK inhibitor, such as RK20449, and an immune checkpoint inhibitor also provided effects greater than the properties of each agent as monotherapy. Thus, it is anticipated that the combination therapy of the present disclosure may provide improved therapeutic effects, compared for example with administration of each agent as a single therapy. In other words, in the case, where each active agent is dosed at its currently approved therapeutic dose, improved therapeutic effects (e.g. one or more of reduction of tumour growth, reduced rate of tumour growth, increased survival time) may be observed. Alternatively, use of the combination therapy of the present disclosure may enable the dosage amount of one or both actives to be reduced, e.g. compared with their currently approved dosage levels, and may lead to avoidance of or reduction in the level of side effects experienced by the subject.

Many immune checkpoint inhibitors are typically administered intravenously, e.g. by infusion or injection, at specified dosage intervals, e.g. once every 1, 2 or 3 weeks.

In the case where the immune checkpoint inhibitor is pembrolizumab, it may for example be administered intravenously, e.g. by injection. For example, it may be administered in an amount of about 200mg per 3 weeks, or about 400mg per 6 weeks, or pto 200mg per 3 weeks, or up to 400mg per 3 weeks. In some examples, it may be administered in an amount of up to 150mg per 2 weeks, or in an amount of up to 300mg per 6 weeks.

In the case where the immune checkpoint inhibitor is nivolumab, it may for example be administered intravenously, e.g. by injection. For example, it may be administered in an amount of up to 3mg/kg per 2 weeks, or about 3mg/kg per 2 weeks. In some examples, it may be administered in an amount of up to 2mg/kg per 2 weeks.

In the case where the immune checkpoint inhibitor is cemiplimab, it may for example be administered intravenously, e.g. by infusion. For example, it may be administered in an amount of up to 350mg per 3 weeks, or about 350mg per 3 weeks. In some examples, it may be administered in an amount of up to 300mg per 3 weeks.

In the case where the immune checkpoint inhibitor is ipilimumab, it may for example be administered intravenously, e.g. by injection. For example, it may be administered in an amount of up to 3mg/kg per week, or about 3mg/kg per week. In some examples, it may be administered in an amount of up to 2mg/kg per week.

In the case where the immune checkpoint inhibitor is atezolizumab, it may for example be administered intravenously, e.g. by injection. For example, it may be administered in an amount of up to 840mg per 2 weeks, or up to 1200mg per 3 weeks, or up to 1680 mg per 4 weeks, or about 840mg per 2 weeks, or about 1200 mg per 3 weeks, or about 100mg per 4 weeks. In some examples, it may be administered in an amount of up to 700mg per 2 weeks, or up to 1000mg per 3 weeks, or up to 1400mg per 4 weeks.

In the case where the immune checkpoint inhibitor is avelumab, it may for example be administered intravenously, e.g. by infusion. For example, it may be administered in an amount of up to 10mg/kg per 2 weeks, or about 10mg/kg per 2 weeks, or up to 800mg per 2 weeks, or about 800mg per 2 weeks. In some examples, it may be administered in an amount of up to 600mg per 2 weeks, or up to 8mg/kg per 2 weeks.

In the case where the immune checkpoint inhibitor is durvalumab, it may for example be administered intravenously, e.g. by infusion. For example, it may be administered in an amount of up to 10mg/kg per 2 weeks, or about 10mg/kg per 2 weeks. In some examples, it may be administered in an amount of up to 8mg/kg per 2 weeks.

Whilst in some examples, the HCK inhibitor (e.g. RK20449) and a single immune checkpoint inhibitor may be the only active ingredients administered to the subject, in some other examples, one or more further active ingredients may be administered with the HCK inhibitor and the immune checkpoint inhibitor. For example, more than one immune checkpoint inhibitor may

be administered in combination wih the HCK inhibitor. In some embodiments, a therapeutic regime involving the use of the HCK inhibitor (e.g. RK20449), a PD-L1 or PD-1 inhibitor (e.g. nivolumab, pembrolizumab) and a CTLA4 inhibitor (e.g. ipilimumab) is used, for example for the treatment of cancer (e.g. colon cancer). For DNA mismatch repair (MMR) proficient forms of colon cancer, while these MMR-proficient cancers can remain resistant to single immune checkpoint blockade therapy, it is considered that the overall response rate can be increased by combining a PD-1 or PD-L1 inhibitor plus a CTLA4 inhibitor (Chalabi et al, Nature Medicine, 2020, Vol 26, p566-576). In some embodiments, a combination of nivolumab, ipilimumab and the HCK inhibitor is used. In some embodiments, a combination of nivolumab, ipilimumab and RK20449 is used.

In some examples, more than one immune checkpoint inhibitor may be administered in combination with the HCK inhibitor. In some embodiments, a therapeutic regime involving the use of a HCK inhibitor (e..g. RK20449), a PD-L1 or PD-1 inhibitor (e.g. nivolumab, pembrolizumab) and a CTLA4 inhibitor (e.g. ipilimumab) is used, for example for the treatment of colon cancer. For DNA mismatch repair (MMR) proficient forms of colon cancer, while these MMR-proficient cancers can remain resistant to single immune checkpoint blockade therapy, it is considered that the overall response rate can be increased by combining a PD-1 or PD-L1 inhibitor plus a CTLA4 inhibitor (Chalabi et al, Nature Medicine, 2020, Vol 26, p566-576). In some embodiments, a combination of nivolumab, ipilimumab and a HCK inhibitor is used.

In some embodiments, anticancer agents other than HCK inhibitors and an immune checkpoint inhibitor may also be administered as part of the treatment regime.

In some embodiments, anticancer agents other than HCK inhibitors and an immune checkpoint inhibitor may also be administered as part of the treatment regime.

In some embodiments, the HCK inhibitor is used in combination with an immunotherapy agent. In some examples, the immunotherapy agent is a CD40 agonist. In some examples, the methods and uses described herein further comprise administering an effective amount of a CD40 agonist to the subject. In some examples, the CD40 agonist is agnostic CD40 antibody. Non limiting examples of agnostic CD40 antibody include APX005M, ChiLob7/4, ADC-1013, SEA-CD40, selicrelumab (APX005M), and CDX-1140. In some examples, the agnostic CD40 antibody is selected from the group consisting of Sotigalimab (APX005M), and SEA-CD40.

In some embodiments, the HCK inhibitor is used in combination with a chemotherapeutic agent and an immunotherapy agent.

In some embodiments, the further active agent is an immunotherapy which acts via stimulation of CD40 or OX40. In some embodiments, a combination of HCK inhibitor with a PD-1 or PD-L1 inhibitor and a further active agent which is an immunotherapy which acts via stimulation of CD40 or OX40 is used. In some embodiments, a combination of dasatinib with a PD-1 or PD-L1 inhibitor and a further active agent which is an immunotherapy which acts via stimulation of CD40 or OX40 is used. In some embodiments, a combination of RK20449 with a

PD-1 or PD-L1 inhibitor and a further active agent which is an immunotherapy which acts via stimulation of CD40 or OX40 is used.

The HCK inhibitor and the one or more further pharmaceutically active agents may be administered simultaneously, subsequently or separately. For example, they may be administered as part of the same composition, or by administration of separate compositions.

The further active agents, when employed in combination with an HCK inhbitor, may be used for example in those amounts indicated in the Physicians' Desk Reference or as otherwise determined by one of ordinary skill in the art.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the above-described embodiments, without departing from the broad general scope of the present disclosure. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

EXAMPLES

Materials and Methods

Animals

Age- and sex-matched WT and *Hck*^{KO} (Lowell at al, Genes Dev, 1994. 8(4): 387-98) mice were bred and maintained in specific pathogen–free facilities at La Trobe University, and the Austin Hospital, Australia. All animal studies were approved and conducted in accordance with the Animal Ethics Committee of the Olivia Newton John Cancer Research Institute/Austin Hospital.

Cell lines

The mouse KPC pancreatic adenocarcinoma, MC38 colon cancer, B16F10 melanoma, and E0771 breast cancer cell lines were maintained in DMEM/F12 and 10% fetal calf serum at 37°C. Cell lines were tested negative for mycoplasma.

Antibodies

The antibodies used herein as summarised in Table 1 below.

Table 1: Antibodies

Antibodies	**Source**
Rat anti-mouse CD8a	ThermoFisher
Rabbit anti-mouse PDPN	Abcam
Rabbit anti-mouse αSMA	Abcam
Rabbit anti-mouse PDGFRβ	Abcam

Rabbit anti-mouse Fibronectin	Abcam
Rabbit anti-mouse Granzyme B (D6E9W)	Cell Signalling
Rabbit anti-mouse Perforin (E3W4I)	Cell Signalling
APC-Cy7 rat anti-mouse CD45.2 (30-F11)	BioLegend
FITC rat anti-mouse F4/80 (BM8)	BioLegend
PE rat anti-mouse CD11b (M1/70)	BD Biosciences
PerCP-Cy5.5 rat anti-mouse Ly6G (1A8)	BD Biosciences
eFluor450 rat anti-mouse Ly6C (HK1.4)	ThermoFisher
APC rat anti-mouse CD206 (C068C2)	BioLegend
APC rat anti-mouse NK1.1 (PK136)	eBioscience
PE rat anti-mouse TCRβ (B173150)	BioLegend
PerCP-Cy5.5 rat anti-mouse TCRβ (45-5961-52)	eBioscience
FITC rat anti-mouse CD8a (9150975)	BD Biosciences
PE-Cy7 rat anti-mouse CD8a (9186813)	BD Biosciences
PE rat anti-mouse CD103 (2E7)	ThermoFisher
FITC rat anti-mouse CD11c (3.9)	ThermoFisher
PE rat anti-mouse PDGFRα (APA5)	BioLegend
PE-Cy7 rat anti-mouse CD31 (MEC13.3)	BioLegend
FITC rat anti-mouse EPCAM (G8.8)	BioLegend
PerCP/Cyanine5.5 anti-mouse CD45.2 (104)	BioLegend
APC/Cyanine7 anti-mouse PDPN (8.1.1)	BioLegend
Brilliant Violet 785™ anti-mouse MHCII (M5/114.15.2)	BioLegend
APC anti-mouse Ly6C (HK1.4)	BioLegend
Rabbit anti-mouse pLYN	Cell signalling
Rabbit anti-mouse LYN	Cell signalling
Rabbit anti-mouse pSRC	Cell signalling
Rabbit anti-mouse SRC	Cell signalling
Rabbit anti-mouse pHCK	Abcam
Rabbit anti-mouse HCK	Santa Cruz
Mouse anti-mouse actin	Sigma
IRDye goat anti-rabbit secondary antibody	LI-COR Biosciences
IRDye goat anti-mouse secondary antibody	LI-COR Biosciences
Mouse anti-mouse Actin	Sigma

Immunotherapies

The immunotherapies used herein include anti-CD40 (Clone FGK45) and anti-PD1 (Clone RMP1-14), which be purchased commercially from a number of sources including Bio X Cell.

Dasatinib

Dasatinib was obtained from Selleck Chemicals.

RK20449

RK20449 is available from, for example, Cayman Chemicals and Reagency.

Western Blot

Protein lysates were prepared as previously described and resolved on 10% SDS-polyacrylamide gels (Poh, et al., Cancer Cell, 2017. 31(4): 563-575.e5; Poh et al, Cancer Immunol Res, 2020. 8(4): 428-435). Following dry transfer, PVDF membranes were blocked for 1 hour in Intercept Blocking Buffer (LI-COR Biosciences) and incubated overnight in primary antibodies at 4°C. The next day, blots were incubated with fluorescent-conjugated secondary antibodies for 1 hour. Signals were detected using the Odyssey Infrared Imaging System (LI-COR Biosciences). Actin was used as a loading control.

Orthotopic surgery (pancreatic cancer)

To establish orthotopic pancreas tumours (Nikfarjam et al, J Invest Surg, 2013. 26(6): 352-9), 5 x 10^3 luciferase-labelled KPC pancreatic cancer cells in PBS were implanted into the pancreas tail of WT (C57BL/6) and *Hck*^{KO} mice. The mice were first given an anaesthetic i.p. (Ketamine/Xylazine, 100 mg/kg and 10mg/kg, in 0.1ml). The skin was shaved and cleaned with an aqueous disinfectant. Under sterile conditions, a small subcostal incision was made across the left side of the abdomen. The muscle layer was sutured followed by the skin layer. Wound clips were used to facilitate closure of the skin layer.

Intrasplenic surgery (pancreatic cancer)

To establish pancreatic tumours in the liver via intrasplenic surgery (D'Costa et al, Cancer Research, 2017. 77(21): 5952-5962), WT and *Hck*^{KO} mice were anesthetized with isoflurane. The skin was shaved and cleaned with an aqueous disinfectant. Under sterile conditions, a small incision was made in the midline and the spleen was exposed. 8-10 x 10^5 KPC tumour cells in 50µL PBS were injected into the spleen. After allowing the tumour cells to perfuse to the liver for 1 minute, the spleen was resected and the vessels cauterized. The abdominal cavity was closed with sutures and wound clips were used to facilitate closure of the skin layer.

MC38 colon cancer allograft model

Six-week-old C57BL/6 WT or *Hck*^{KO} mice were subcutaneously injected with $2x10^6$ MC38 cells into the right flank. Once palpable tumors formed, mice were randomized into treatment groups. Tumor volume (mm^3) was measured using digital calipers using the following formula: (length × width^2)/2. Tumor growth was measured by an independent assessor who was blinded to the experimental conditions.

B16F10 melanoma model of experimental metastasis

Experimental lung metastasis was performed by injecting $5x10^5$ B16F10 melanoma cancer cells into the tail vein of WT and *Hck*^{KO} mice. Where indicated, WT mice were treated with RK20449 (30mg/kg, twice daily, i.p.) four days following tumour cell inoculation for 10 days. Lungs were harvested two weeks following tumour cell injection and fixed in formalin. For quantification of metastatic nodules, we obtained three sections from each lung tissue that were 200µM apart. Lung sections were stained with H&E and analyzed with Aperio ImageScope v11.2.0.780 software. Quantification of metastatic tumour burden per μm^2 was performed using an automated cell counter script in FIJI (ImageJ).

E0771 breast cancer metastasis model

1 x 10^5 E0771.LMB mCherry$^+$ breast cancer cells (Cao et al, Cancer Research, 2014. 74(18): 5091) were implanted into the fourth inguinal mammary gland of 10-week-old female WT or *Hck*^{KO} mice. Primary tumours were resected when they reached $800mm^3$ in size to facilitate metastatic spreading of cancer cells to the lung. Lungs were harvested two weeks following tumour resection and metastatic burden was quantified by genomic PCR amplification for expression of mCherry relative to Vimentin. PCR analysis was performed on duplicate samples with Taqman® Real-Time PCR Master mix and probes (Life Technologies) using the Viia7 Real-Time PCR System (Life Technologies). Primer sequences were as follows: *mCherry* fwd: 5′-GACCACCTACAAGGCCAAGAAG-3′, rev: 5′-AGGTGATGTCCAACTTGATGTTGA-3′, hydrolysis probe: 5′FAM-CAGCTGCCCGGCGCCTACA-3′TAMRA and *Vimentin* fwd: 5′-AGCTGCTAACTACCAGGACACTATTG-3′, rev: 5′-CGAAGGTGACGAGCCATCTC-3′, hydrolysis probe: 5′VIC-CCTTCATGTTTTGGATCTCATCCTGCAGG-3′TAMRA.

Humanized mice studies

Three-week-old female NSG-SGM3 mice (NOD.Cg-Prkdcscid Il2rgtm1Wjl Tg(CMV-IL3,CSF2,KITLG)1Eav/MloySzJ) were sub-lethally irradiated and intravenously reconstituted with human CD34+ hematopoietic stem cells (2 donors across N≥6 mice per group). The engraftment levels of mature human CD45+ cells was determined 12 weeks following

transplantation by flow cytometry quantification of peripheral blood. NSG-SGM3 mice that had >25% human CD45+ cells in the peripheral blood were considered engrafted and humanized.

Tumours derived from a triple-negative breast cancer PDX were finely minced and subcutaneously injected into the right flank of humanized NSG-SGM3 mice. Once tumours reached 100mm^3 in volume, mice were randomized into treatment groups. Mice were either administered RK20449 (30mg/kg) or vehicle (12% Captisol) twice daily via i.p. injection for 25 days. Tumour volume (mm^3) was measured using digital calipers using the following formula: (length x width x width)/2.

Drug treatments

Where indicated, mice were either treated with 12% Captisol (vehicle for RK20449, twice daily, i.p.), isotype-matched IgG (200µg once every 3 days, i.p.), RK20449 (30mg/kg, diluted in 12% Captisol, twice daily i.p.), Dasatinib (30mg/kg, diluted in 12% Captisol, twice daily i.p.), anti-CD40 (Clone FGK45, 200µg once every 3 days, i.p.), anti-PD1 (Clone RMP1-14, 200µg once every 3 days, i.p.), or gemcitabine (120mg/kg twice a week, i.p.).

Immunohistochemistry

Slides with paraffin-embedded tissue were de-waxed by being incubated 2 x 10 min in xylene, followed by rehydration in 2 x 5 min in 100 % ethanol (EtOH) and 1 x 5 min in 70 % EtOH. Slides were washed in distilled H_2O (dH_2O). For antigen retrieval, slides were boiled in preheated citrate buffer for 15 min. Slides were then cooled to room temperature and washed with dH_2O. To prevent endogenous peroxidases, the slides were blocked for 20 min in 3 % hydrogen peroxide (H_2O_2) in dH_2O followed by 3 x 5 min wash in dH_2O. To prevent endogenous staining, the slides were blocked for 1 h in 5 % normal goat serum (NGS) in Tris-buffered saline with 0.1 % Tween-20 (TBST) at room temperature. The primary antibodies were diluted in 5% NGS in TBST and incubated overnight at 4°C in a humidified chamber. After 3 x 10 min wash in TBST at room temperature, the secondary antibody, diluted in 5 % NGS in TBST was applied for 1 h at room temperature. When secondary antibodies were biotinylated, slides were washed 3 x 5 min in TBST before ABC mix (1 drop reagent A + 1 drop reagent B in 5 mL TBST) was applied for 30 min at room temperature, followed by 3 x 5min wash in TBST. For visualization, slides were developed with DAB solution (1 drop DAB+, DAKO in 1 mL DAKO substrate buffer). Dependent on the antibody, DAB was applied for 30 – 60 seconds and peroxidase reaction was stopped by transferring the slides into TBST. Slides were then washed in dH_2O to remove excess TBST. Slides were then counterstained in hematoxylin for approximately 20 seconds and washed with dH_2O followed by incubation in Scott's tap water for 20 seconds and another wash in dH_2O. In order to dehydrate the tissue, the slides were placed 1 x 5 min in 70 % EtOH, 2 x 5 minutes in 100 % EtOH and 2 x 10 min in xylene. The tissue was then embedded in DPX Mountant for histology and cover slipped.

Flow cytometry (FACS)

Tumours were cut into 1mm pieces and digested in Collagenase/Dispase (Roche) and DNase I (Roche)in Ca^{2+}-and Mg^{2+}-free Hanks medium plus 10% FCS for 30 minutes at 37°C under continuous rotation. Samples were vortexed for 30 seconds to dissociate immune cells. Subsequently, the cell suspension was filtered and washed in PBS plus 10 % FCS. Samples were incubated in Fc block (ThermoFisher) on ice for 10 min before incubation with fluorophore-conjugated primary antibodies for 20 min on ice in the dark. Following incubation, samples were washed twice and resuspended in PBS with 10% FCS.

Flow cytometry was performed and analysed on the BD FACS Canto. Purification of DCs (identified as CD45+CD11c+F4/80-MHCII+ cells), TAMs (identified as CD45+CD11b+Ly6C–Ly6G-F4/80+ cells), CD8 T-cells (identified as CD45+TCRB+CD8+), NK cells (identified as CD45+NK1.1) and CAFs (identified as EpCAM-CD31-CD45-PDGFRα+PDPN+) cells was performed and analyzed on the Aria cell sorter as previously described (Poh, et al., Cancer Cell, 2017. 31(4): 563-575.e5; Poh et al, Cancer Immunol Res, 2020. 8(4): 428-435). Background fluorescence was estimated by substituting primary antibodies with either their specific isotype controls, and/or fluorescent-minus-one controls, as well as using unstained controls. Dead cells were identified by Sytox Blue (ThermoFisher) staining and excluded from analysis. Analysis of all experiments was performed using compensated data with FlowJo software (Version 10).

qPCR

RNA extraction on FACs purified cells was performed using RNeasy Mini/Micro kit (Qiagen) according to the manufacturer's instructions. cDNA was generated with the SuperScript™ IV First-Strand Synthesis System (ThermoFisher) according to the manufacturer's instructions. RNA extraction on tumour samples was performed using the RN-easy Mini Plus kit (Qiagen) and cDNA was generated using the High-Capacity cDNA Reverse Transcription Kit (ThermoFisher) according to the manufacturer's instructions.

Quantitative RT-PCR analysis was performed on duplicate samples with Taqman® Real-Time PCR Master mix (Life Technologies) using the Viia7 Real-Time PCR System (Life Technologies) over 40 cycles (95ºC for 15s, 60ºC/1min) and following an initial denaturation step at 50°C/2mins, 95ºC/10min. The cDNA concentration of target genes was normalised by amplification of 18S or GAPDH and fold changes in gene expression were obtained using the 2-ΔΔCT method.

Taqman® probes for mouse genes were 18s (Mm04277571_s1), GAPDH (Mm99999915_g1), IL6 (Mm00446190_m1), IL4 (Mm00445259_m1), IL10 (Mm01288386_m1), IL11 (Mm00434162_m1), IL13 (Mm00434204_m1), ARG1 (Mm00475988_m1), TGF-β (Mm01209346_m1), TNF-α (Mm00443258_m1), IL12α (Mm00434169_m1), IFN-γ (Mm01168134_m1), CXCL9 (Mm00434946_m1), CXCL10 (Mm00445235_m1), PRF1

(Mm00812512_m1), GZMB (Mm00442837_m1), MMP3 (Mm00440295_m1), MMP7 (Mm00487724_m1), MMP9 (Mm00442991_m1), and COL1A1 (Mm00801666_g1).

Taqman® probes for human genes were GAPDH (Hs02786624_g1), CD68 (Hs04185218_g1), XCR1 (Hs00245540_s1), NCR2 (Hs00183113_m1), CD4 (Hs01058407_m1), CD8A (Hs00233520_m1), FOXP3 (Hs01085834_m1), TNF (Hs00174128_m1), IL12A (Hs01073447_m1), CXCL9 (Hs00171065_m1), CXCL10 (Hs00171042_m1), IFNG (Hs00989291_m1), IL1B (Hs01555410_m1), CD163 (Hs00174705_m1), MRC1 (Hs07288635_g1) and TGFB (Hs00820148_g1).

Statistics

Unless otherwise stated, comparisons between mean values were performed with a 2-tailed Student's t-test as appropriate using Prism 8 software (GraphPad). A *P* value of less than 0.05 was considered statistically significant. All experiments were performed at least twice with a minimum of three age- and sex-matched mice per group. The specific n (number of animals) used per cohort is indicated in the respective figure legends.

Example 1: Pancreatic tumour growth and metastasis

For the intrasplenic model of "experimental" pancreatic cancer metastasis, mouse KPC pancreatic cells were injected into the spleen of recipient mice to facilitate spreading of cancer cells to the liver via the portal vein and establish tumours in the liver (a common site of metastasis in humans). The KPC cells were originally derived from a endogenous pancreatic tumour in mice Pdx1-Cre;LSL-$Kras^{G12D/+}$;LSL-$Trp53^{R172H/+}$ mice where recombinase activity of a pancreas specific Cre (Pdx1) simultaneously converts the latent (LSL)-$Kras^{G12D/+}$ allele into its active counterpart,and the LSL- $Trp53^{R172H/+}$ allele into one of the 8 most commonly found TP53 mutations in humans. Mice were collected approximately 3 weeks after tumour cell injection. Three weeks after tumour cell injection, it was observed that Hck^{KO} mice developed fewer pancreatic metastasis in the liver compared to WT hosts (Figure 1A). This is consistent with the observation of liver mass, which was also significantly lower in Hck^{KO} hosts compared to WT mice. HCK deficiency also doubled the survival of tumour-bearing Hck^{KO} mice compared to WT hosts (Figure 1B).

For the orthotopic model, mouse KPC pancreatic cancer cells were injected into the tail of the pancreas of WT or Hck^{KO} host mice. In this model, secondary metastases can be detected from 2 weeks onwards. Five weeks after tumour cell injection, Hck^{KO} mice developed smaller primary tumours in the pancreas than WT hosts (Figure 1C). H&E sections of secondary organs from WT and Hck^{KO} mice is shown in Figure 1D, including the absence of metastasis in Hck^{KO} mice. T= tumour. The number of mice with metastasis is shown in Table 2.

Table 2 Mice with metastasis from the orthotopic pancreatic cancer model

	Mice with metastasis	
	WT	***Hck*^{KO}**
Liver	15/15	0/12
Spleen	15/15	0/12
Intestine	7/15	0/12
Peritoneum	4/15	0/12
Kidney	4/15	0/12

Example 2: Therapeutic inhibition of HCK using RK20449 in pancreatic cancer

Orthotopic pancreatic cancer model (primary tumours in pancreas analysed) - WT mice were orthotopically injected with 5 x 10^3 mouse KPC pancreatic cancer cells. Treatment with vehicle (12% Captisol, twice daily, i.p.) or RK20449 (30mg/kg, twice daily, i.p.) commenced 1 week after tumour cell injection and lasted for 4 weeks. RK20449-treated WT mice developed smaller pancreatic tumours compared to vehicle-treated mice (Figure 2A).

Intrasplenic pancreatic cancer metastasis model (liver tumours analysed) - WT mice were intrasplenically injected with 8-10 x 10^5 KPC pancreatic cancer cells. Treatment with vehicle (12% Captisol, twice daily, i.p.) or RK20449 (30mg/kg, twice daily, i.p.) commenced 5 days after tumour cell injection and lasted for 2 weeks. RK20449-treated WT mice developed smaller pancreatic tumours in the liver (liver weight was used as a surrogate measure for tumour burden) compared to vehicle-treated mice (Figure 2B).

Example 3: Loss of HCK signalling reduces the desmoplastic tumour reaction and abundance of cancer-associated fibroblasts (CAFs) in pancreatic cancer

The following data pertains to the intrasplenic mouse pancreatic cancer metastasis model. Figure 3A shows immunohistochemical staining for extracellular matrix proteins and cancer-associated fibroblasts. Figure 3B shows flow cytometry quantification of various cancer-associated fibroblast subtypes shown in Figure 3A. In both figure panels, tumours of *Hck*^{KO} hosts showed an attenuated desmoplastic microenvironment and reduced abundance of cancer-associated fibroblasts compared to WT mice. iCAF = inflammatory CAFs; apCAF = antigen presenting CAFs; mCAFs = myofibroblasts. Figure 3C shows reduced expression of immunosuppressive and fibrotic genes in CAFs purified from tumours of *Hck*^{KO} hosts compared to WT.

Example 4: Genetic ablation of HCK in hosts reduces the infiltration of immunosuppressive immune cells and enhances the infiltration of cytotoxic effector cells in pancreatic cancer

The following data pertains to the intrasplenic pancreatic cancer metastasis model. Figure 4A shows flow cytometry quantification of myeloid cell populations. TAMs = tumour associated macrophages, AAMs = alternatively-activated macrophages, m-MDSCs = monocytic myeloid derived suppressor cells, g-MDSCs = granulocytic myeloid derived suppressor cells, cDC1s = conventional type 1 dendritic cells. Figure 4B shows qPCR analysis on $CD45^{+}CD11c^{+}F4/80^{-}MHCII^{+}$ DCs and $CD45^{+}CD11b^{+}F4/80^{High}Ly6c^{-}Ly6g^{-}$ TAMs isolated from metastatic KPC liver tumors of WT and Hck^{KO} mice for genes associated with immune cell activation (*Tnf*, *Il12*, *Ifny*, *Cxcl9*, *Cxcl10*), immune suppression (*Il4*, *Il10*, *Il13*, *Tgfβ*, *Arg1*) and matrix remodeling (*Mmp3, Mmp7, Mmp9*). Figure 4C shows flow cytometry quantification of cytotoxic CD8 T-cells and NK cells that mediate anti-tumour responses. Figure 4D shows immunohistochemical staining and quantification of tumour infiltrating CD8 T-cells in WT and Hck^{KO} host mice. Figure 4E shows qPCR gene expression analysis of CD8 T-cells and NK cells purified from WT or Hck^{KO} mice for Gzmb (encoding Granzyme B) and Prf1 (encoding Perforin) as markers of immune cell activation. Figure 4F shows immunohistochemical staining of Granzyme B and Perforin in tumours of WT and Hck^{KO} hosts.

Example 5: Genetic ablation of HCK improves the therapeutic activity of gemcitabine chemotherapy in pancreatic cancer tumour-bearing mice

Strategies that target tumour-epithelial cells in pancreatic cancer have failed to reduce tumour burden and improve survival rates for patients. Since PDAC tumours largely consist of stromal cells, namely CAFs and TAMs, these cells may represent a more promising therapeutic target.

A major hallmark of PDAC tumours is its highly immune excluded microenvironment that contains few infiltrated cytotoxic T cells to recognize and fight cancer cells. This immune exclusion is caused by the extremely immunosuppressive tumour microenvironment, which is driven by the desmoplastic reaction and numerous immunosuppressive cell types including MDSCs and TAMs. Thus, drugs that can convert the immunosuppressive tumour microenvironment to an activated and inflammatory endotype may help boost the efficacy of as chemotherapy and immune check-point blockade.

Figure 5 shows that genetic ablation of HCK improves the therapeutic activity of gemcitabine chemotherapy in tumour-bearing mice. These results provide strong rationale for HCK to be considered as an additional therapeutic target to improve the responsiveness PDAC to standard-of-care chemotherapies.

WT and Hck^{KO} mice were injected with KPC cells using the intrasplenic tumour model. Gemcitabine was administered at 120mg/kg twice weekly via i.p. injection starting from 5 days

post-intrasplenic tumour cell injection for two weeks (Figures 5A and 5B). We observed that gemcitabine significantly reduced tumour burden in both genotypes compared to the vehicle-treated controls. However, while gemcitabine-treated WT mice still developed tumours, *Hck*^{KO} mice that received chemotherapy lacked any visible signs of tumour growth (Figures 5A and 5B). In a separate experiment, we also observed that gemcitabine-treated *Hck*^{KO} mice survived significantly longer than all other treatment groups (Figure 5C).

Example 6: Genetic ablation of HCK improves the therapeutic activity of anti-CD40 immunotherapy in pancreatic cancer tumour-bearing mice

The following data pertains to the intrasplenic pancreatic cancer metastasis model. WT and *Hck*^{KO} mice were injected with KPC cells using the intrasplenic tumour model. WT and *Hck*^{KO} mice were treated with anti-CD40 (200µg once every 3 days, i.p.) starting from 5 days post-intrasplenic tumour cell injection for 2 weeks. We observed that anti-CD40 (Figures 6A and 6B) did not reduce tumour burden in WT mice. However, *Hck*^{KO} mice treated with either anti-CD40 lacked any visible signs of tumour growth. In separate experiments, we also observed that anti-CD40-treated *Hck*^{KO} mice survived significantly longer than all other treatment groups (Figure 6C).

Example 7: Genetic ablation of HCK improves the therapeutic activity of anti-PD1 immunotherapy in pancreatic cancer tumour-bearing mice

The following data pertains to the intrasplenic pancreatic cancer metastasis model. WT and *Hck*^{KO} mice were injected with KPC cells using the intrasplenic tumour model. WT and *Hck*^{KO} mice were treated with anti-PD1 (200µg once every 3 days, i.p.) starting from 5 days post-intrasplenic tumour cell injection for 2 weeks. We observed that anti-PD1 (Figures 7A and 7B) did not reduce tumour burden in WT mice. However, *Hck*^{KO} mice treated with anti-PD1 immunotherapy lacked any visible signs of tumour growth. In separate experiments, we also observed that anti-PD1-treated *Hck*^{KO} mice survived significantly longer than all other treatment groups (Figure 7C).

Example 8: Genetic ablation or therapeutic inhibition of HCK improves the efficacy of immunotherapy in colon cancer allografts

The following data pertains to the MC38 colon cancer allograft model. WT mice were treated with RK20449, which showed negligible suppression of the auto-phosphorylated and catalytically active isoform of c-SRC and the related negative regulatory LYN kinase (Figure 8A). RK20449-treatment of tumor-bearing WT mice improved the anti-tumor activity conferred by αPD1 (Figure 8B). This effect was replicated in tumor-bearing WT hosts treated with the pan SRC-kinase inhibitor Dasatinib (Figure 8B); however, this activity was completely lost when Dasatinib was co-administered with αPD1 to tumor-bearing *Hck*^{KO} hosts (Figure 8C). Thus, the

Dasatinib-dependent improvement of αPD1 anti-tumor immune responses is primarily mediated through inhibition of HCK signalling. Both genetic ablation of HCK and pharmacologic inhibition of its activity also improved the therapeutic efficacy of αCD40 in controlling MC38 tumor growth (Figure 8D and 8E).

Example 9: B16F10 model of experimental melanoma metastasis

Experimental melanoma metastasis was performed by injecting $5x10^5$ B16F10 melanoma cancer cells into the tail vein of WT and *Hck*^{KO} mice. Where indicated, WT mice were treated with RK20449 (30mg/kg, twice daily, i.p.) starting from four days following tumour cell injection for 10 days. Lungs were harvested two weeks following tumour cell injection and fixed in formalin. Lung sections were stained with H&E and analysed with Aperio ImageScope v11.2.0.780 software. Quantification of metastatic tumour burden per μm^2 was performed using an automated cell counter script in FIJI (ImageJ). The results are shown in Figure 8. Secondary tumour growth (measured as secondary melanoma lesions) in the lungs of *Hck*^{KO} mice was impaired compared to WT mice.

Example 10: E0771 model of experimental breast cancer metastasis

Experimental breast cancer metastasis was performed by injection of 1 x 10^5 E0771.LMB mCherry$^+$ breast cancer cells into the fourth inguinal mammary gland of 10-week-old female WT or *Hck*^{KO} mice. Primary tumours were resected when they reached $800mm^3$ in size. Lungs were harvested two weeks following tumour resection and metastatic burden was quantified by genomic PCR amplification for expression of mCherry positive tumour cells relative to Vimentin (house keeper control gene). The results are shown in Figure 10. Secondary breast cancer tumour growth (measured as abundance of mCherry signal) was reduced in lungs of *Hck*^{KO} mice compared to WT mice.

Example 11: Therapeutic inhibition of HCK reduces the growth of PDXs in humanized mice

NSG-SGM3 mice were used as hosts following their reconstitution after full-body-radiation with human core-blood derived CD34+ stem cells which gives rise to a human-derived hematopoietic and immune system in the corresponding "humanized mice". Tumours derived from a triple-negative breast cancer PDX were subcutaneously injected into the right flank of "humanized" NSG-SGM3 mice. Once tumours reached $100mm^3$ in volume, mice were randomized into treatment groups. Mice were either administered RK20449 (30mg/kg) or vehicle (12% Captisol) twice daily via i.p. injection for 25 days. The results are shown in Figures 11A and 11B.

We used human-specific probes to interrogate whole PDXs by qPCR analysis for the presence of human immune cells. We noted increased expression of CD8A, NCR2, XCR1 (indicative of CD8$^+$ T-cells, NK cells and cDC1s) in RK20449-treated mice, while transcripts for

human CD4, FOXP3 and CD68 (indicative of $CD4^+$ T-cells, Tregs, and TAMs) remained comparable between tumours recovered from RK20449- and vehicle-treated hosts (Figure 11C). These observations coincided with increased expression of immune cell activation genes including IL12, TNF, IL1B, IFNG, CXCL9 and CXCL10 in PDXs of RK20449-treated mice, and a reduction in genes associated with immunosuppression including CD163, MRC1, TGFB, IL10 and CXCL12 (Figure 11D).

CLAIMS:

1. A method of inhibiting and/or reducing metastasis of a primary cancer in a subject, comprising administering an effective amount of an HCK inhibitor to the subject.

2. A method as claimed in claim 1, wherein the cancer is a fibrotic cancer.

3. A method as claimed in claim 1, wherein the cancer is breast cancer, pancreatic cancer, gastric cancer, oesophageal cancer, hepatocellular cancer or melanoma.

4. A method as claimed in claim 3, wherein the cancer is pancreatic cancer.

5. A method as claimed in any of claims 1 to 4, wherein the subject is human.

6. A method as claimed in any of claims 1 to 5, wherein the HCK inhibitor is administered in combination with a further active agent.

7. A method as claimed in claim 6, wherein the further active agent is a chemotherapeutic agent.

8. A method as claimed in claim 6, wherein the further active agent is an immunotherapy agent.

9. A method of preventing a fibrotic cancer in a subject, comprising administering an effective amount of an HCK inhibitor to the subject.

10. A method of preventing a fibrotic cancer in a subject, comprising administering an effective amount of a pharmaceutical composition comprising an HCK inhibitor and a pharmaceutically acceptable excipient to the subject.

11. A method as claimed in claim 9 or 10, wherein the fibrotic cancer is pancreatic cancer, gastric cancer, oesophageal cancer, hepatocellular cancer or melanoma.

12. A method of inhibiting and/or reducing metastasis of a primary cancer in a subject, comprising administering an effective amount of a pharmaceutical composition comprising an HCK inhibitor and a pharmaceutically acceptable excipient to the subject.

13. A method as claimed in any one of claims 1 to 12, wherein the HCK inhibitor is RK20449.

14. An HCK inhibitor for use in treating and/or preventing a fibrotic cancer.

15. An HCK inhibitor for use as claimed in claim 14, wherein the fibrotic cancer is pancreatic cancer.

16. An HCK inhibitor for use in inhibiting and/or reducing metastasis of a primary cancer.

17. An HCK inhibitor for use as claimed in claim 16, wherein the cancer is a fibrotic cancer.

18. An HCK inhibitor for use as claimed in claim 16, wherein the cancer is breast cancer, pancreatic cancer, gastric cancer, oesophageal cancer, hepatocellular cancer or melanoma.

19. Use of an HCK inhibitor for the manufacture of a medicament for treating and/or preventing a fibrotic cancer.

20. Use as claimed in claim 19, wherein the fibrotic cancer is pancreatic cancer.

21. Use of an HCK inhibitor for the manufacture of a medicament for inhibiting and/or reducing metastasis of a primary cancer.

22. Use as claimed in claim 21, wherein the cancer is a fibrotic cancer.

23. Use as claimed in claim 21, wherein the cancer is breast cancer, pancreatic cancer, gastric cancer, oesophageal cancer, hepatocellular cancer or melanoma.

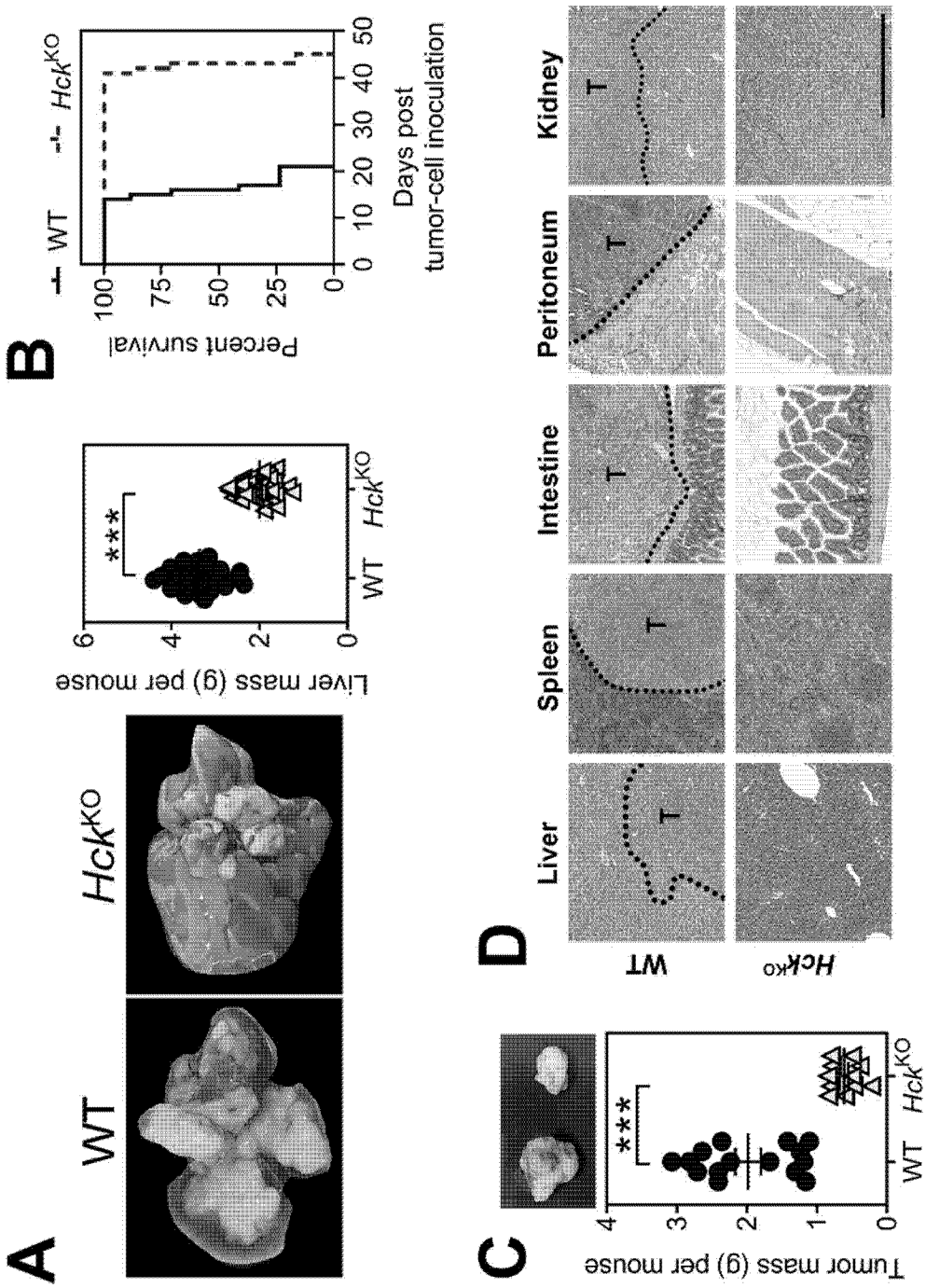

A
WT
Hck^KO
Liver mass (g) per mouse
***
WT
Hck^KO
B
WT
Hck^KO
Percent survival
Days post tumor-cell inoculation
C
Tumor mass (g) per mouse
***
WT
Hck^KO
D
Liver
Spleen
Intestine
Peritoneum
Kidney
WT
Hck^KO
T

FIGURE 1

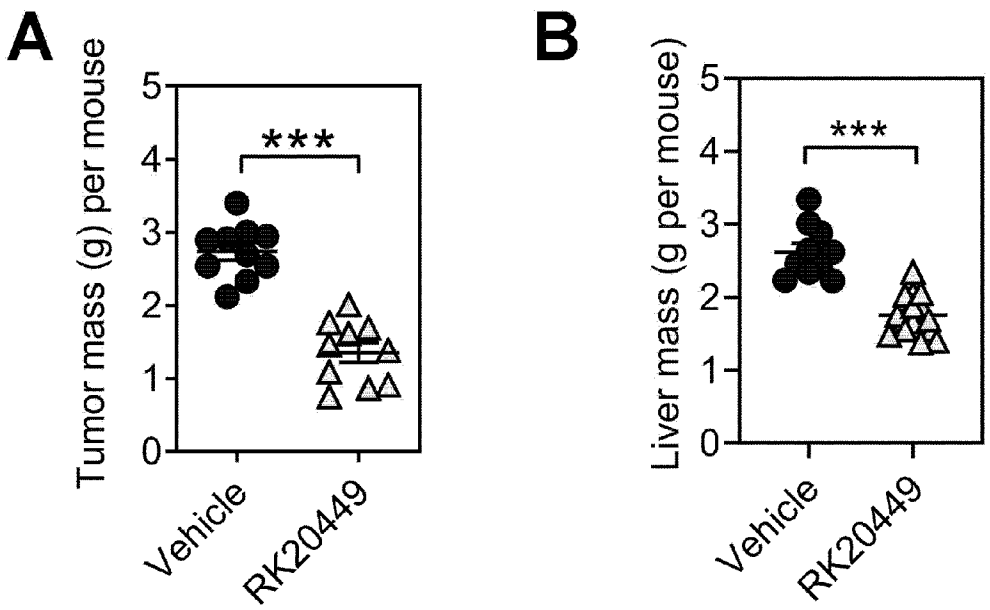

A
Tumor mass (g) per mouse
0
1
2
3
4
5
***
Vehicle
RK20449
B
Liver mass (g per mouse)
0
1
2
3
4
5
***
Vehicle
RK20449

FIGURE 2

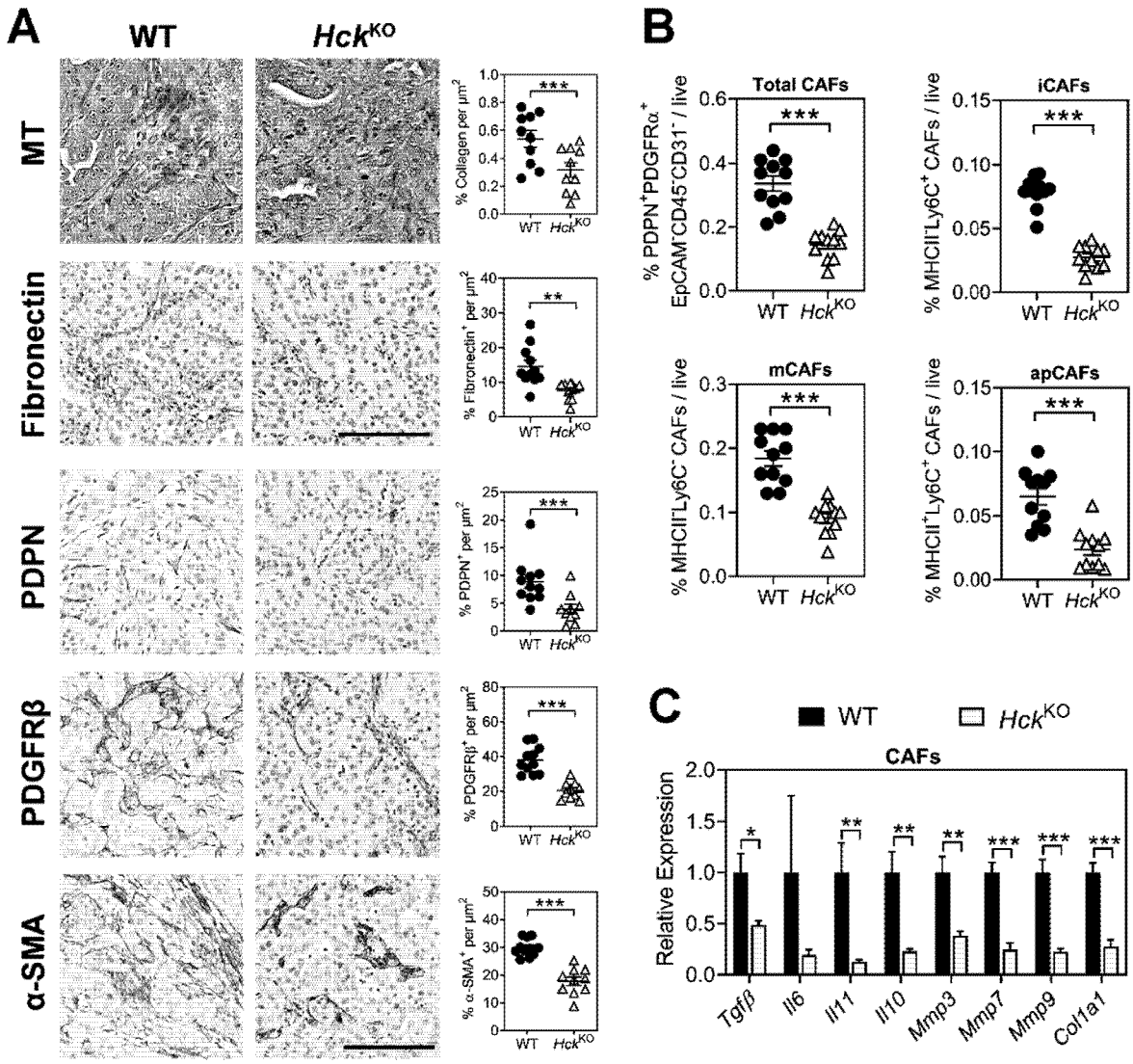

A
WT
Hck^KO
MT
Fibronectin
PDPN
PDGFRβ
α-SMA
% Collagen per µm²
% Fibronectin⁺ per µm²
% PDPN⁺ per µm²
% PDGFRβ⁺ per µm²
% α-SMA⁺ per µm²
B
Total CAFs
% PDPN⁺PDGFRα⁺ EpCAM⁻CD45⁻CD31⁻ / live
iCAFs
% MHCII⁻Ly6C⁺ CAFs / live
mCAFs
% MHCII⁻Ly6C⁻ CAFs / live
apCAFs
% MHCII⁺Ly6C⁺ CAFs / live
C
WT
Hck^KO
CAFs
Relative Expression
Tgfβ
Il6
Il11
Il10
Mmp3
Mmp7
Mmp9
Col1a1

FIGURE 3

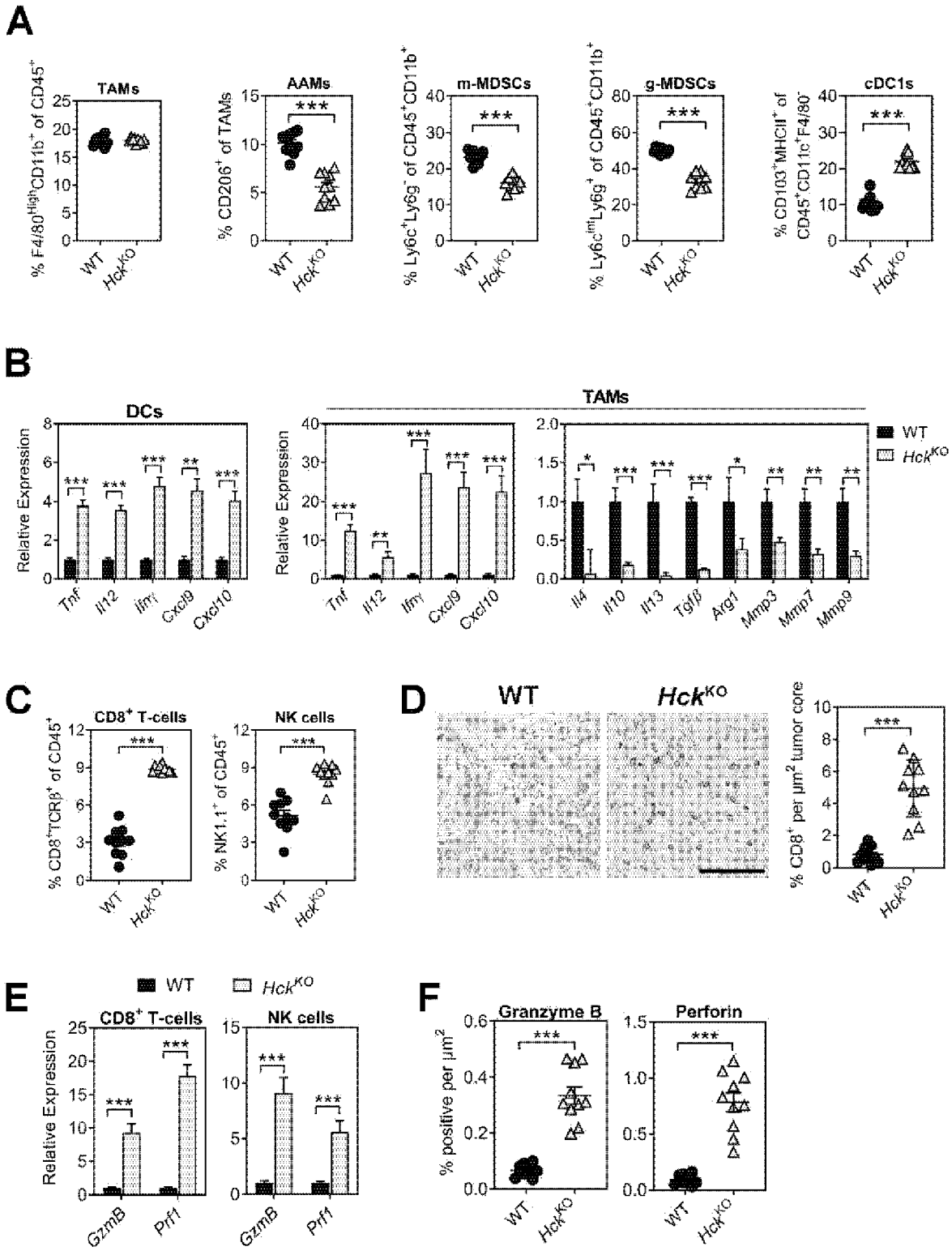

A
TAMs
AAMs
m-MDSCs
g-MDSCs
cDC1s
WT
Hck^KO
B
DCs
TAMs
Relative Expression
WT
Hck^KO
Tnf
Il12
Ifnγ
Cxcl9
Cxcl10
Il4
Il10
Il13
Tgfβ
Arg1
Mmp3
Mmp7
Mmp9
C
CD8+ T-cells
NK cells
D
WT
Hck^KO
E
CD8+ T-cells
NK cells
GzmB
Prf1
F
Granzyme B
Perforin

FIGURE 4

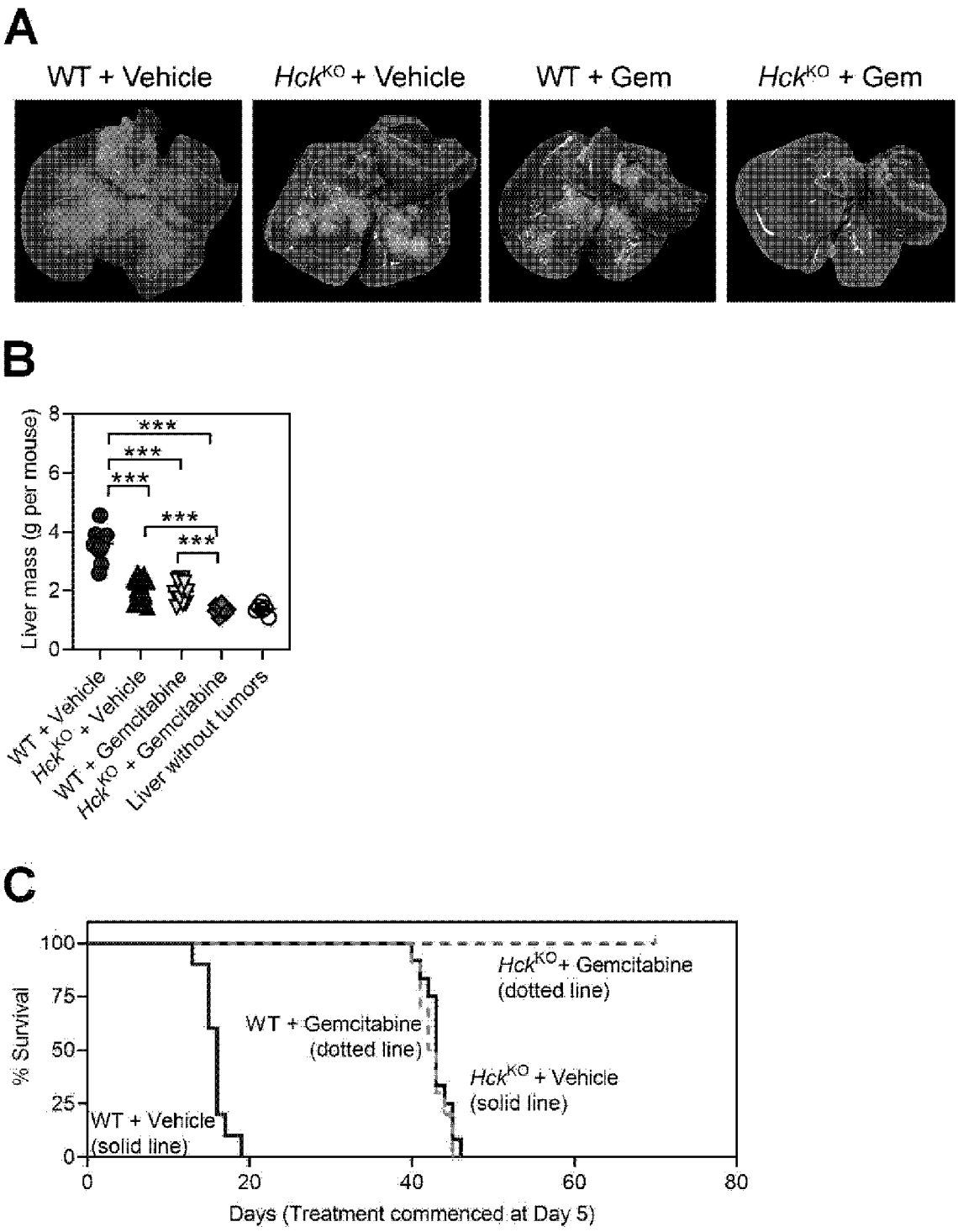

A
WT + Vehicle
Hck^KO + Vehicle
WT + Gem
Hck^KO + Gem
B
Liver mass (g per mouse)
0
2
4
6
8
***
***
***
***
***
WT + Vehicle
Hck^KO + Vehicle
WT + Gemcitabine
Hck^KO + Gemcitabine
Liver without tumors
C
% Survival
0
25
50
75
100
Hck^KO + Gemcitabine
(dotted line)
WT + Gemcitabine
(dotted line)
Hck^KO + Vehicle
(solid line)
WT + Vehicle
(solid line)
0
20
40
60
80
Days (Treatment commenced at Day 5)

FIGURE 5

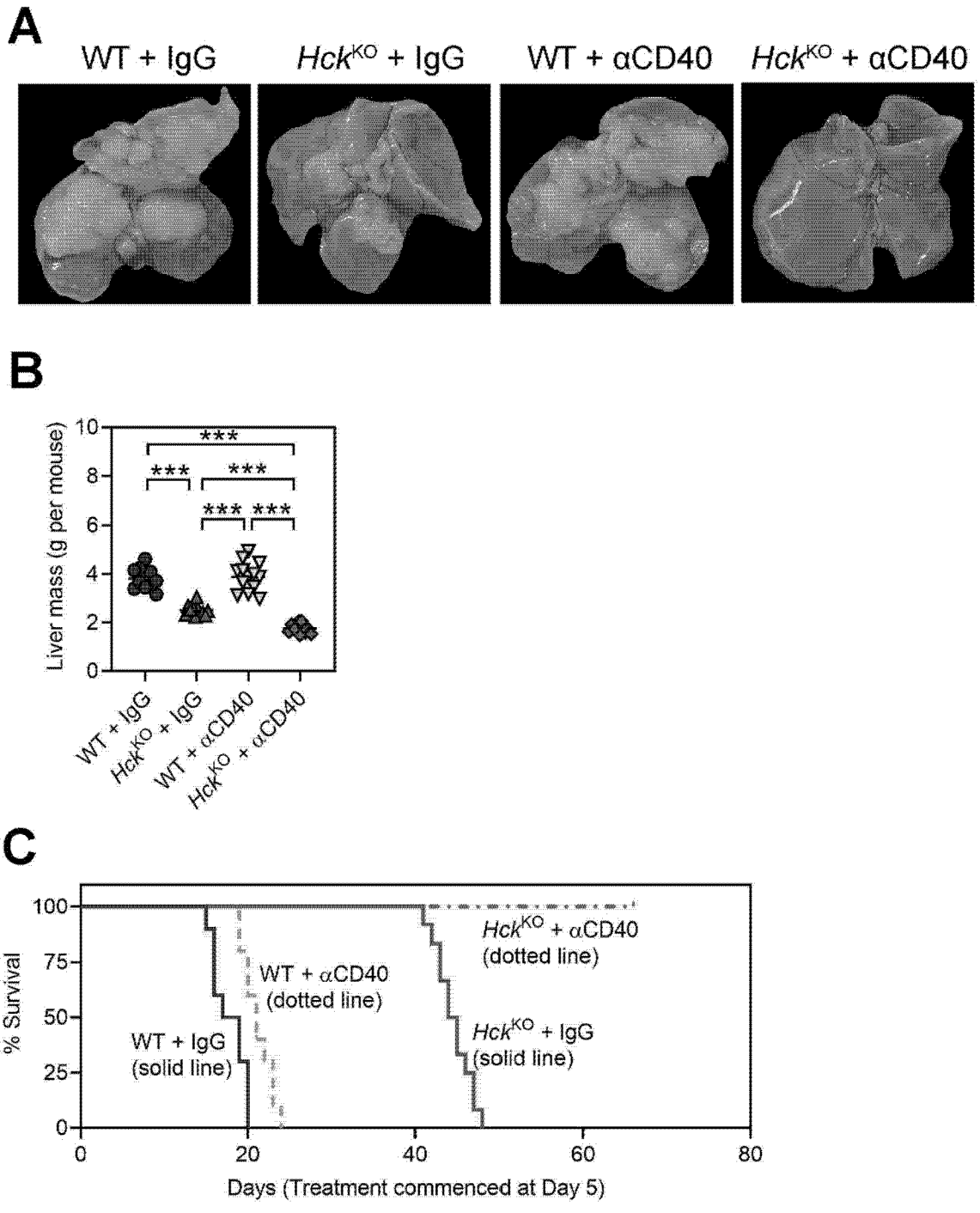

A
WT + IgG
Hck^KO + IgG
WT + αCD40
Hck^KO + αCD40
B
Liver mass (g per mouse)
10
8
6
4
2
0
***
***
***
***
***
WT + IgG
Hck^KO + IgG
WT + αCD40
Hck^KO + αCD40
C
% Survival
100
75
50
25
0
Hck^KO + αCD40
(dotted line)
WT + αCD40
(dotted line)
Hck^KO + IgG
(solid line)
WT + IgG
(solid line)
0
20
40
60
80
Days (Treatment commenced at Day 5)

FIGURE 6

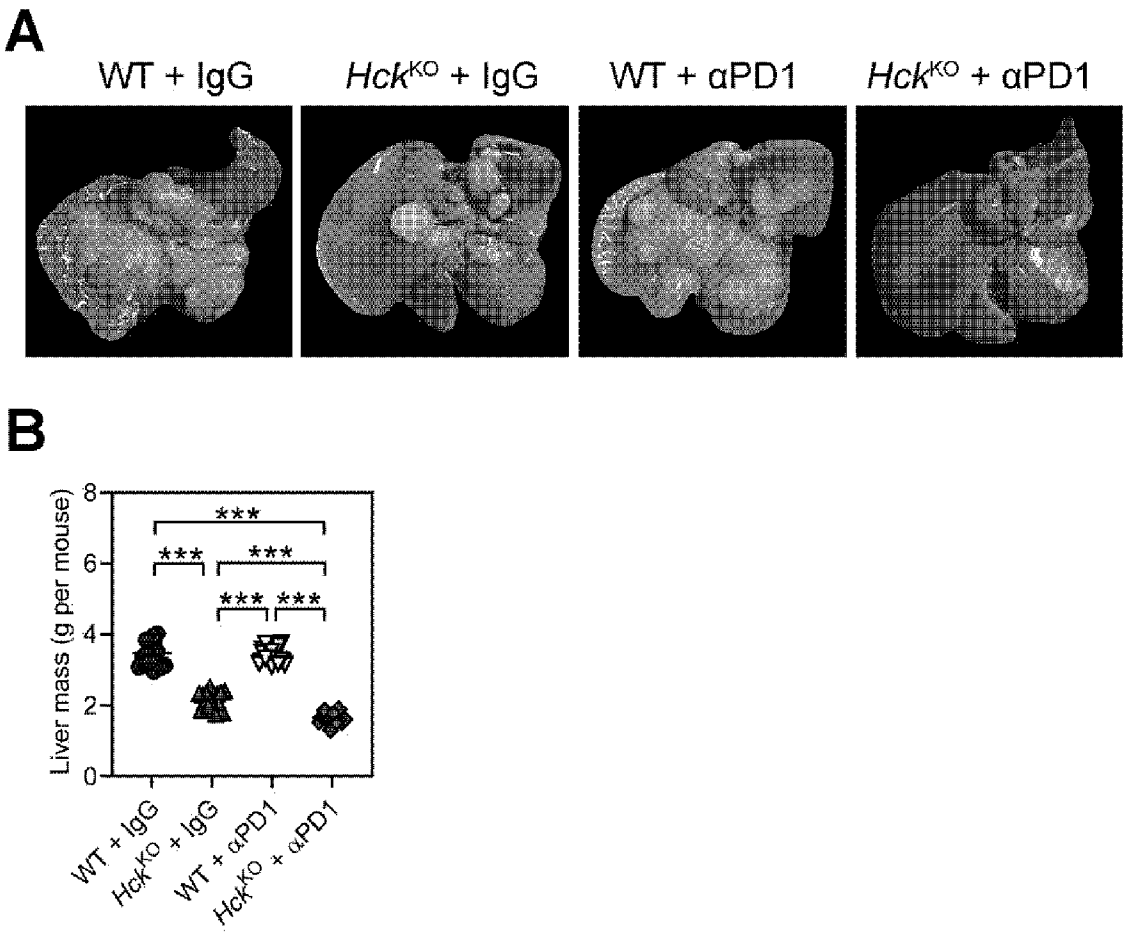

A
WT + IgG
Hck^KO + IgG
WT + αPD1
Hck^KO + αPD1
B
Liver mass (g per mouse)
0
2
4
6
8
***
***
***
***
***
WT + IgG
Hck^KO + IgG
WT + αPD1
Hck^KO + αPD1
C
% Survival
0
25
50
75
100
0
20
40
60
80
Days (Treatment commenced at Day 5)
Hck^KO + αPD1
(dotted line)
WT + αPD1
(dotted line)
WT + IgG
(solid line)
Hck^KO + IgG
(solid line)

FIGURE 7

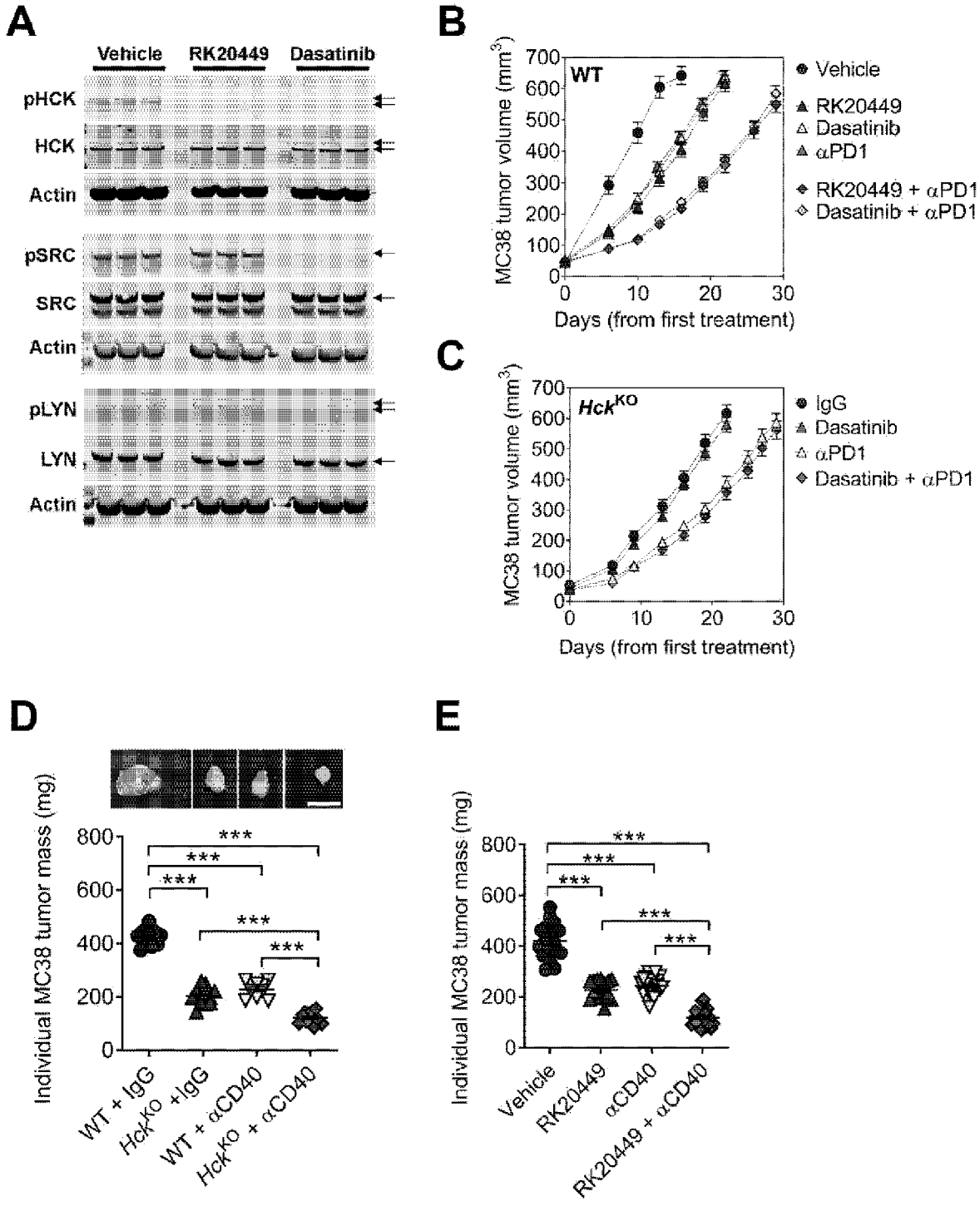

A
Vehicle
RK20449
Dasatinib
pHCK
HCK
Actin
pSRC
SRC
Actin
pLYN
LYN
Actin
B
WT
MC38 tumor volume (mm3)
Days (from first treatment)
Vehicle
RK20449
Dasatinib
αPD1
RK20449 + αPD1
Dasatinib + αPD1
C
HckKO
MC38 tumor volume (mm3)
Days (from first treatment)
IgG
Dasatinib
αPD1
Dasatinib + αPD1
D
Individual MC38 tumor mass (mg)
WT + IgG
HckKO +IgG
WT + αCD40
HckKO + αCD40
E
Individual MC38 tumor mass (mg)
Vehicle
RK20449
αCD40
RK20449 + αCD40

FIGURE 8

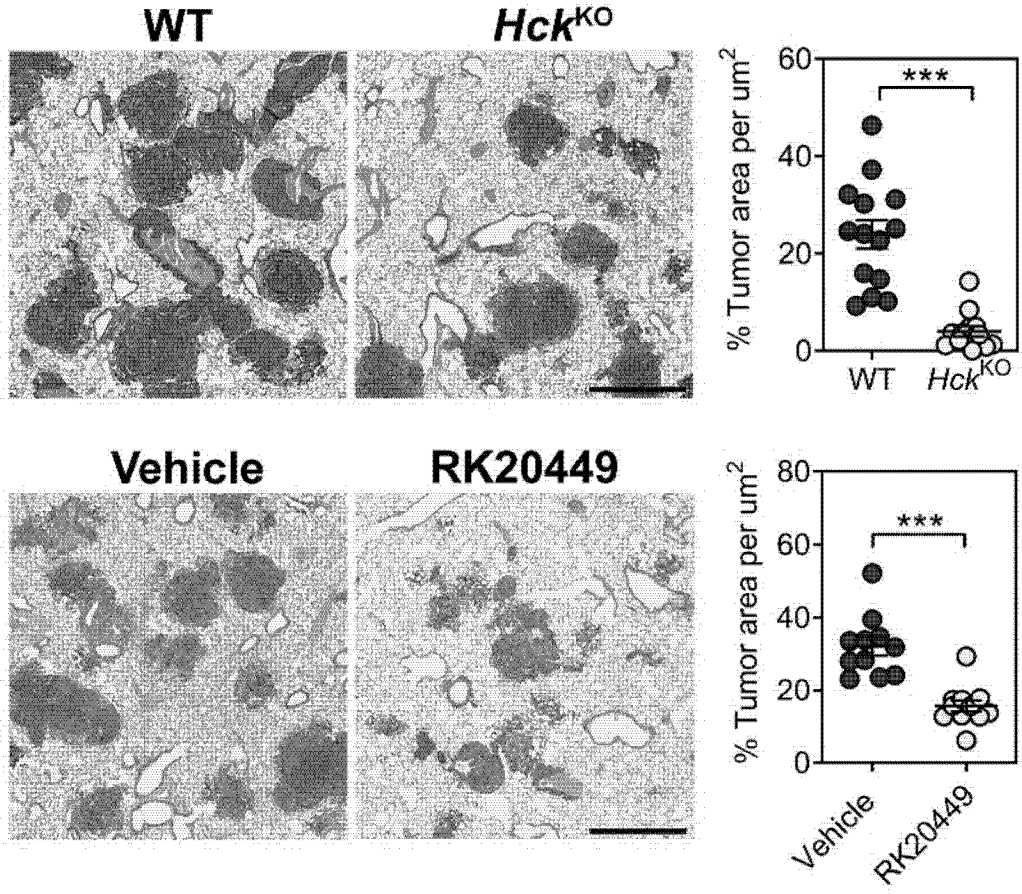

WT
Hck^KO
% Tumor area per um^2
60
40
20
0
***
WT
Hck^KO
Vehicle
RK20449
% Tumor area per um^2
80
60
40
20
0
***
Vehicle
RK20449

FIGURE 9

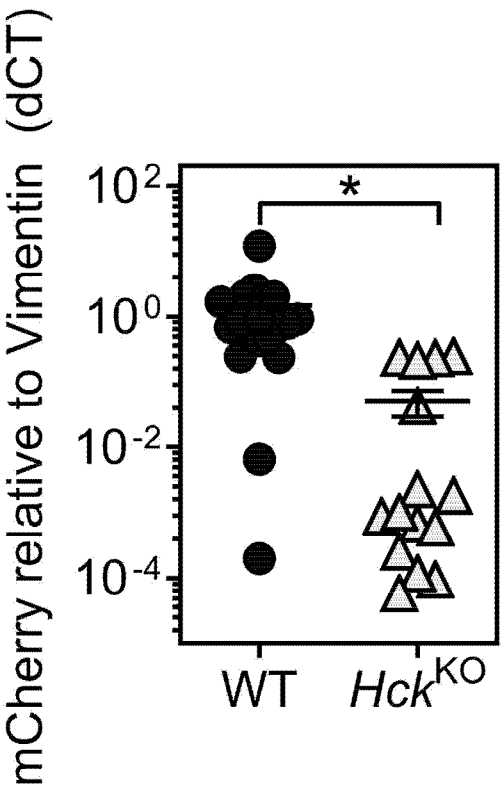


FIGURE 10

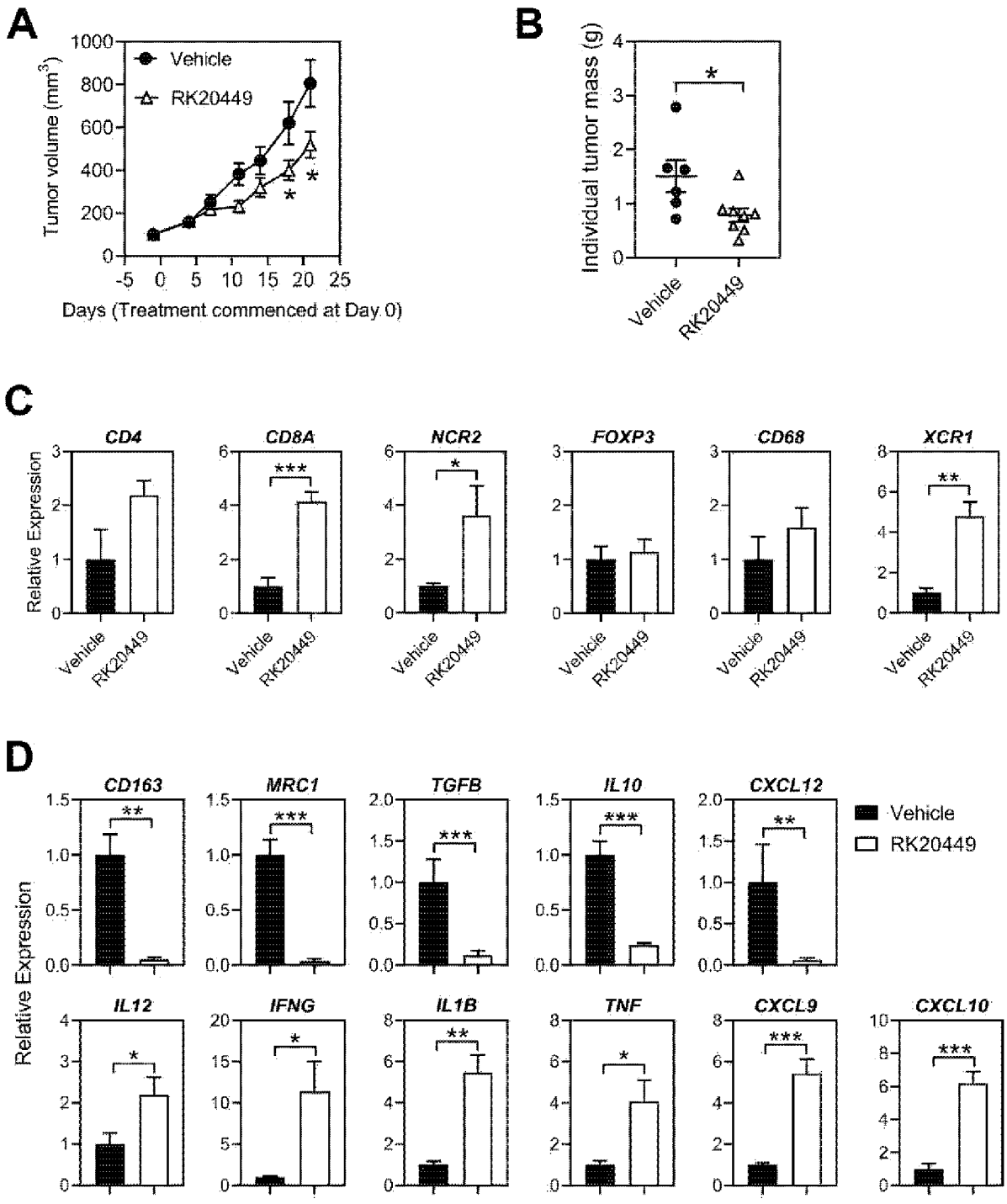

A
Tumor volume (mm3)
Vehicle
RK20449
Days (Treatment commenced at Day 0)
B
Individual tumor mass (g)
Vehicle
RK20449
C
Relative Expression
CD4
CD8A
NCR2
FOXP3
CD68
XCR1
D
CD163
MRC1
TGFB
IL10
CXCL12
IL12
IFNG
IL1B
TNF
CXCL9
CXCL10
Vehicle
RK20449

FIGURE 11

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2021/051073

A. CLASSIFICATION OF SUBJECT MATTER

A61K 31/519 (2006.01) ***A61K 31/506 (2006.01)*** ***A61K 31/7068 (2006.01)*** ***A61K 39/395 (2006.01)***
A61P 35/00 (2006.01) ***A61P 35/04 (2006.01)*** ***C07K 16/28 (2006.01)***

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)

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)

Databases: STN: BIOSIS, BIOTECHNOABS, CABA, CAPLUS, EMBASE, MEDLINE, REGISTRY; EPOQUE:PATENW; PATENTSCOPE; GOOGLE

Search terms: HCK inhibitor, cancer, tumour, RK20449 and similar terms; OLIVIA NEWTON-JOHN CANCER RESEARCH INSTITUTE; POH, Ashleigh Ren-Yi ; ERNST, Matthias Robert Walter

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

[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
"D" document cited by the applicant in the international application
"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
30 November 2021	30 November 2021

Name and mailing address of the ISA/AU

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Authorised officer

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AUSTRALIAN PATENT OFFICE
(ISO 9001 Quality Certified Service)
Telephone No. +61262832745

INTERNATIONAL SEARCH REPORT C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		International application No. **PCT/AU2021/051073**
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2015061752 A1 (PHARMACYCLICS, INC; BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY) 30 April 2015 see whole document, in particular: the abstract, claims and paragraphs [0006] [00162]-[00164]; [00222]-[00245]; [00458]; [00593]-[00688] of D1	1-12 and 14-23
X	Poh et al.:"Inhibition of Hematopoietic Cell Kinase Activity Suppresses Myeloid Cell-Mediated Colon Cancer Progression" 2017, Cancer Cell 31, 563–575 see whole document of D2	1, 2, 9, 10, 12, 13, 14-19 and 21-22
X	US 2015/0044217 A1 (PHARMACYCLICS, INC) 12 February 2015 see whole document, in particular paragraphs [0053]-[0054]and [0158]-[0182] of D3	1-7, 9-12 and 14-23
X	WO 2015/181628 A1 (EISAI R&D MANAGEMENT CO., LTD) 03 December 2015 see whole document, in particular: abstract, claims, figure 1, table 1 and paragraphs [060]; [067] of D4	9, 10, 11 and 13-18
X	WO 2018/052120 A1 (RIKEN) 22 March 2018 see whole document, in particular the abstract, claims and paragraphs: [0007];[0017]-[0019]; [0129] of D5	9, 10, 11 and 13-18
X	Daniel Massó-Vallés, Toni Jauset, Erika Serrano, Nicole M. Sodir, Kim Pedersen, Nesrine I. Affara, Jonathan R. Whitfield, Marie-Eve Beaulieu, Gerard I. Evan, Laurence Elias, Joaquín Arribas, Laura Soucek. "Ibrutinib exerts potent antifibrotic activity in a mouse model of pancreatic adenocarcinoma". [abstract]. In: Proceedings of the 106th Annual Meeting of the American Association for Cancer Research; 2015 Apr 18-22; Philadelphia, PA. Philadelphia (PA): AACR; Cancer Res 2015;75(15 Suppl):Abstract nr 396. doi:10.1158/1538-7445.AM2015-396 [Retrieved from internet on 29 November 2021] <URL: https://cancerres.aacrjournals.org/content/75/15_Supplement/396 > published August 2015 (whole document) see whole document of D6	1-4, 9-12 and 14-23

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/AU2021/051073**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		**Patent Family Member/s**	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
WO 2015061752 A1	30 April 2015	WO 2015061752 A1	30 Apr 2015
		AR 098210 A1	18 May 2016
		AU 2014339816 A1	05 May 2016
		AU 2014339816 B2	28 May 2020
		AU 2020223721 A1	10 Sep 2020
		CA 2927794 A1	30 Apr 2015
		CN 105848680 A	10 Aug 2016
		EA 201690746 A1	30 Dec 2016
		EP 3060251 A1	31 Aug 2016
		JP 2016534157 A	04 Nov 2016
		JP 6508785 B2	08 May 2019
		JP 2019142890 A	29 Aug 2019
		JP 2021063091 A	22 Apr 2021
		KR 20160066554 A	10 Jun 2016
		MX 2016005283 A	20 Feb 2017
		PH 12016500743 A1	20 Jun 2016
		TW 201521728 A	16 Jun 2015
		TW I617309 B	11 Mar 2018
		TW 201801745 A	16 Jan 2018
		TW I660739 B	01 Jun 2019
		US 2015118222 A1	30 Apr 2015
		US 2020397895 A1	24 Dec 2020
		UY 35800 A	31 Dec 2014
US 2015/0044217 A1	12 February 2015	US 2015044217 A1	12 Feb 2015
		US 9415050 B2	16 Aug 2016
		CA 2920534 A1	19 Feb 2015
		EP 3033079 A1	22 Jun 2016
		EP 3033079 B1	31 Oct 2018
		JP 2016528251 A	15 Sep 2016
		JP 6429292 B2	28 Nov 2018
		JP 2019031546 A	28 Feb 2019
		JP 2021008475 A	28 Jan 2021
		US 2017151243 A1	01 Jun 2017
		US 9724349 B2	08 Aug 2017

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/AU2021/051073**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
		US 2018071294 A1	15 Mar 2018
		US 10016434 B2	10 Jul 2018
		US 2019167687 A1	06 Jun 2019
		US 2021145835 A1	20 May 2021
		WO 2015023703 A1	19 Feb 2015
WO 2015/181628 A1	03 December 2015	WO 2015181628 A1	03 Dec 2015
WO 2018/052120 A1	22 March 2018	WO 2018052120 A1	22 Mar 2018
		EP 3512555 A1	24 Jul 2019
		JP 2019529423 A	17 Oct 2019
		US 2019255056 A1	22 Aug 2019

End of Annex

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.