

SEPTEMBER 19, 2023

THE CONTROLLER OF PATENTS  
THE PATENT OFFICE  
NEW DELHI

**KIND ATTN.: MS. ANITA JATAV**  
**[CONTROLLER OF PATENTS & DESIGNS]**

**RE: OPPOSITION U/S 25(1) OF THE PATENT ACT – BY RAHUL LAXMAN  
GAJBHIYE AGAINST INDIAN PATENT APPLICATION NO. 201617025251  
FILED ON 22/07/2016  
APPLICANT: 1. PFIZER INC; 2. MERCK SHARP & DOHME CORP.  
R&A REF.: OPP0427**

Respected Madam,

We are filing this representation by way of Pre-Grant Opposition along with annexures u/s 25 (1) of the Patents Act, 1970 and Rule 55 of the Patent Rules, 2003 in Form 7A.

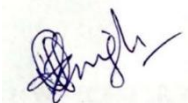
The Learned Controller is requested to take said opposition along with annexures on record and proceed further in the matter and keep the Opponent advised of each and every step taken in the matter.

We crave the leave of the Learned Controller to submit additional documents and/or evidence to support any of the averments in the representation as may be necessitated during the future proceeding.

Lastly, we request the Learned Controller to grant an opportunity of being heard before the present Opposition is finally decided

Thanking you,

Yours faithfully,



PRAGYA SINGH THAKUR (IN /PA – 3329)  
OF RAJESHWARI AND ASSOCIATES  
AGENT FOR THE OPPONENT

Encl.: As stated

C.C: LAKSHMI KUMARAN & SRIDHARAN  
E-mail: [iprdel@lakshmisri.com](mailto:iprdel@lakshmisri.com);

**Also at:** A - 202, First Floor, Shivalik Enclave, Malviya Nagar, New Delhi-110017

**BEFORE THE CONTROLLER OF PATENTS, THE PATENT OFFICE, NEW DELHI**

**IN THE MATTER OF:**

The Patents Act, 1970 as amended by the Patents (Amendment) Act 2005, and The Patents Rules, 2003, as amended by The Patents (Amendment) Rules, 2006

**AND**

IN THE MATTER of Pre-grant opposition under Section 25(1)

**AND**

IN THE MATTER of Indian Patent Application No. 201617025251

**IN THE MATTER OF:**

**RAHUL LAXMAN GAJBHIYE**

**.....OPPONENT**

**VS.**

**1. PFIZER INC; 2. MERCK SHARP & DOHME CORP.**

**.....APPLICANT**

**PRE-GRANT OPPOSITION BY RAHUL LAXMAN GAJBHIYE**

**INDEX**

<b>S. No.</b>	<b>PARTICULARS</b>	<b>Page Nos.</b>
1.	Form 7A	1-2
2.	Opposition u/s 25(1) by the Opponent	3-28
3.	<b>Annexure - 1:</b> Copy of claims currently on record.	29-31
4.	<b>Annexure – 2:</b> Copy of Article David J McEwan, “Emerging therapeutic aspects in oncology”; British Journal of Pharmacology; (2013) 169 1647–1651 – published August 2013.	32-36
5.	<b>Annexure – 3:</b> Copy of Article Anna Azvolinsky; “Evolution of Care in Advanced Kidney Cancer”; Targeted Therapies in Oncology, September 2013, Volume 2, Issue 5 – published September 19, 2013.	37-40
6.	<b>Annexure – 4:</b> Copy of Article Rini et al, “Comparative effectiveness of axitinib versus sorafenib in advanced renal cell carcinoma (AXIS): a randomised phase 3 trial”; The Lancet; Vol 378 December 3, 2011 – published November 04, 2011.	41-49

7.	<b>Annexure – 5:</b> Copy of Article Yasuda et al., “Simultaneous blockade of programmed death 1 and vascular endothelial growth factor receptor 2 (VEGFR2) induces synergistic anti-tumour effect in vivo”; Clinical and Experimental Immunology, 172: 500–506 – published June 2013.	50-56
8.	<b>Annexure – 6:</b> Copy of WO2012135408A1 – published 04 October 2012	57-123
9.	<b>Annexure – 7:</b> Copy of Article vi. INLYTA (axitinib) FDA Label – published 2012	124-145
10.	Power of Attorney	Will follow

Dated this 19<sup>th</sup> day of September, 2023



PRAGYA SINGH THAKUR (IN /PA – 3329)  
OF RAJESHWARI AND ASSOCIATES  
AGENT FOR THE OPPONENT

TO  
THE CONTROLLER OF PATENTS  
THE PATENT OFFICE, NEW DELHI

**FORM 7A**  
**THE PATENTS ACT,**  
**1970 (39 OF 1970)**  
**AND**  
**THE PATENTS RULES, 2003**  
**REPRESENTATION FOR OPPOSITION TO GRANT OF PATENT**  
**[See Rule 55]**

I, **RAHUL LAXMAN GAJBHIYE**, having his address at 220 Misal layout, near shiv mandir, Bada Indora, Jaripatka, Nagpur, Maharashtra Pin Code 440014, India, hereby give representation by way of opposition to the grant of patent in respect of application No: **201617025251** filed on **22/07/2016** made by **1. PFIZER INC; 2. MERCK SHARP & DOHME CORP.** on the grounds:

- (a) Section 25(1)(e)– that the subject-matter claimed in the impugned application is obvious and clearly does not involve any inventive step.
- b) Section 25(1)(f)– that the subject of any claim of the complete specification, is not an invention within the meaning of this act or is not patentable under this act.
- c) Section 25(1)(g)– that the complete specification of the impugned application does not sufficiently and clearly describe the invention or the method by which it is to be performed.
- d) Section 25(1)(h) - Failure to disclose the information required by section 8 of the Patents Act.

**(Detailed grounds are set out in the Opposition as attached)**

My address in India is:

**RAJESHWARI & ASSOCIATES**  
**S-357, FIRST FLOOR**  
**NEAR HDFC BANK**  
**PANCHSEEL PARK**  
**NEW DELHI-110017, INDIA**  
**TEL +91-11-41038911**  
**MOBILE NO: 8368982401**  
**Email: [pragya@ralegal.co.in](mailto:pragya@ralegal.co.in);**



Dated this 19<sup>th</sup> day of September, 2023

A handwritten signature in blue ink, appearing to read 'Pragya Singh', is written over a light green rectangular background.

PRAGYA SINGH THAKUR (IN /PA – 3329)  
OF RAJESHWARI AND ASSOCIATES  
AGENT FOR THE OPPONENT

TO  
THE CONTROLLER OF PATENTS  
THE PATENT OFFICE, NEW DELHI

**BEFORE THE CONTROLLER OF PATENTS, THE PATENT OFFICE, NEW DELHI**

In the matter of Section 25(1) of The Patents Act, 1970 as amended by The Patents (Amendment) Act 2005;

And

In the matter of Rule 55 of The Patents Rules 2003 as amended by the Patent (Amendment) Rules, 2006

And

IN THE MATTER of Indian Patent Application 201617025251 filed on 22/07/2016 in the name of PFIZER INC; 2. MERCK SHARP & DOHME CORP.

REPRESENTATION BY:

RAHUL LAXMAN GAJBHIYE .....OPPONENT

VS.

1. PFIZER INC; 2. MERCK SHARP & DOHME CORP. ....APPLICANT

**REPRESENTATION BY WAY OF PRE-GRANT OPPOSITION UNDER  
SECTION 25(1) OF THE PATENTS ACT, 1970**

I, Rahul Laxman Gajbhiye, hereby submit my representation by way of opposition to the grant of patent in respect of Indian Patent Application 201617025251 dated 22/07/2016 in the name of 1. PFIZER INC; 2. MERCK SHARP & DOHME CORP. titled "Combination of a PD 1 antagonist and a VEGFR inhibitor for treating cancer"

**STATEMENT OF CASE OF OPPONENT**

1. The Opponent has learnt that the Applicant has filed an Indian Patent Application 201617025251 (hereinafter "the Impugned Patent Application") on 22/07/2016. The impugned patent application was published in the official journal of the patent office on 31/08/2016, which is currently pending before the Patent Office. The Impugned Patent Application is the national phase application of PCT/US2015/014212 and draws its priority from US application

61/935,809 dated 04 February 2014

2. The Impugned Patent Application is entitled “Combination of a PD 1 Antagonist and a VEGFR Inhibitor for Treating Cancer”.
3. The Opponent by way of this present pre-grant opposition submits that the claims currently pending on record are not patentable under the provisions provided in this Act. The claims as filed and currently on record are annexed herewith as **Annexure-1** and reproduced herein below for ready reference:
  1. A medicament comprising an antagonist of a Programmed Death 1 protein (PD-1) for use in combination with a vascular endothelial growth factor receptor (VEGFR) inhibitor for treating a cancer in an individual, wherein the PD-1 antagonist is an anti-PD-1 monoclonal antibody which comprises a heavy chain and a light chain, wherein the heavy and light chains comprise SEQ ID NO:21 and SEQ ID NO:22, respectively, and further wherein the VEGFR inhibitor is N-methyl-2-[3-((E)-2-pyridin-2-yl-vinyl)-1H-indazol-6-ylsulfanyl]-benzamide or a pharmaceutically acceptable salt thereof.
  2. A medicament comprising a vascular endothelial growth factor receptor (VEGFR) inhibitor for use in combination with an antagonist of a Programmed Death 1 protein (PD-1) for treating a cancer in an individual, wherein the VEGFR inhibitor is N-methyl-2-[3-((E)-2-pyridin-2-yl-vinyl)-1H-indazol-6-ylsulfanyl]-benzamide or a pharmaceutically acceptable salt thereof, and further wherein the PD-1 antagonist is an anti-PD-1 monoclonal antibody which comprises a heavy chain and a light chain, wherein the heavy and light chains comprise SEQ ID NO:21 and SEQ ID NO:22.
  3. The medicament as claimed in claim 1 or 2, wherein the individual is a human.
  4. The medicament as claimed in any one of claims 1 to 3, wherein the cancer is a solid tumor that tests positive for Programmed Death-Ligand 1 (PD-L1) expression by an immunohistochemical (IHC) assay.
  5. The medicament of any as claimed in claims 1 to 3, wherein the cancer is renal cell carcinoma.
  6. The medicament as claimed in any one of claims 1 to 5, wherein the PD-1 antagonist is pembrolizumab and the VEGFR inhibitor is axitinib.

7. The medicament as claimed in claim 6, wherein the pembrolizumab is formulated as a liquid medicament which comprises 25 mg/ml pembrolizumab, 7% (w/v) sucrose, 0.02% (w/v) polysorbate 80 in 10 mM histidine buffer pH 5.5 and axitinib is formulated as a 1 mg tablet or a 5 mg tablet.
8. A kit which comprises a first container, a second container and a package insert, wherein the first container comprises at least one dose of a medicament comprising an antagonist of a Programmed Death 1 protein (PD-1), the second container comprises at least one dose of a medicament comprising a vascular endothelial growth factor receptor (VEGFR) inhibitor, and the package insert comprises instructions for treating an individual for cancer using the medicaments, wherein the PD-1 antagonist is an anti-PD-1 monoclonal antibody which comprises a heavy chain and a light chain, wherein the heavy and light chains comprise SEQ ID NO:21 and SEQ ID NO:22, respectively, and further wherein the VEGFR inhibitor is N-methyl-2-[3-((E)-2-pyridin-2-yl-vinyl)-1H-indazol-6-ylsulfanyl]-benzamide or a pharmaceutically acceptable salt thereof.
9. The kit as claimed in claim 8, wherein the instructions state that the medicaments are intended for use in treating an individual having a cancer that tests positive for Programmed Death-Ligand 1 (PD-L1) expression by an immunohistochemical (IHC) assay.
10. The kit as claimed in claim 8 or 9, wherein the individual is a human.
11. The kit as claimed in any one of claims 8 to 10, wherein the PD-1 antagonist is pembrolizumab formulated as a liquid medicament and the VEGFR inhibitor is axitinib formulated as a 1 mg tablet or a 5 mg tablet.
12. The use or kit as claimed in any one of claims 1-4 or 6-10, wherein the cancer is bladder cancer, breast cancer, clear cell kidney cancer, head/neck squamous cell carcinoma, lung squamous cell carcinoma, malignant melanoma, non-small-cell lung cancer (NSCLC), ovarian cancer, pancreatic cancer, prostate cancer, renal cell cancer, small-cell lung cancer (SCLC), triple negative breast cancer, acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myeloid leukemia (CML), diffuse large B-cell lymphoma (DLBCL), follicular lymphoma, Hodgkin's lymphoma (HL), mantle cell lymphoma (MCL), multiple myeloma (MM), myeloid cell leukemia-1 protein (Mcl-1), myelodysplastic syndrome (MDS), non-Hodgkin's lymphoma (NHL), or small lymphocytic lymphoma (SLL).

13. The use or kit as claimed in any one of the above claims, wherein the cancer is advanced renal cell carcinoma.

14. A medicament comprising:

- a) pembrolizumab for use in combination with axitinib for treating a cancer in a human individual by a method comprising administering to the individual (i) axitinib formulated as a 1 mg tablet or a 5 mg tablet and pembrolizumab formulated as a liquid medicament which comprises 25 mg/ml pembrolizumab or (ii) axitinib formulated as a 1 mg tablet or a 5 mg tablet and pembrolizumab formulated as a liquid medicament which comprises 25 mg/ml pembrolizumab; or
- b) axitinib for use in combination with pembrolizumab for treating a cancer in a human individual by a method comprising administering to the individual (i) axitinib formulated as a 1 mg tablet or a 5 mg tablet and pembrolizumab formulated as a liquid medicament which comprises 25 mg/ml pembrolizumab or (ii) axitinib formulated as a 1 mg tablet or a 5 mg tablet and pembrolizumab formulated as a liquid medicament which comprises 25 mg/ml pembrolizumab.

4. Impugned Patent Application: The present pre-grant opposition is against Indian Patent Application 201617025251 dated 22/07/2016 in the name of PFIZER INC. and MERCK SHARP & DOHME CORP. titled “COMBINATION OF A PD 1 ANTAGONIST AND A VEGFR INHIBITOR FOR TREATING CANCER” and is drawn towards a medicament comprising an antagonist of a Programmed Death 1 protein (PD-1) for use in combination with a vascular endothelial growth factor receptor (VEGFR) inhibitor for treating a cancer.

5. The claimed medicament for cancer treatment incorporates PD-1 inhibitor which is Pembrolizumab, and the VEGFR inhibitor is N-methyl-2-[3-((E)-2-pyridin-2-yl-vinyl)-1H-indazol-6-ylsulfanyl]-benzamide or a pharmaceutically acceptable salt thereof, which is also well known as Axitinib.

The structure of Pembrolizumab is as shown below:

## Heavy chain

QVQLVQSGVE	VKKPGASVKV	SCKASGYTFT	NYMYWVRQA	PGQGLEWMGG	50
INPSNGGTNF	NEKFKNRVTL	TTDSSTTTAY	MELKSLQFDD	TAVYYCARRD	100
YRFDMGFDYW	GQGTTVTVSS	ASTKGPSVFP	LAPCSRSTSE	STAALGCLVK	150
DYFPEPVTVS	WNSGALTSGV	HTFPAVLQSS	GLYSLSSVVT	VPSSSLGTKT	200
YTCNVDHKPS	NTKVDKRVES	KYGPPCPPCP	APEFLGGPSV	FLFPPKPKDT	250
LMISRTPEVT	CVVVDVSQED	PEVQFNWYVD	GVEVHNAKTK	PREEQFNSTY	300
RVVSVLTVLH	QDWLNGKEYK	CKVSNKGLPS	SIEKTISKAK	GQPREPQVYT	350
LPPSQEEMTK	NQVSLTCLVK	GFYPSDIAVE	WESNGQPENN	YKTTTPVLDS	400
DGSFFLYSRL	TVDKSRWQEG	NVFSCSVMHE	ALHNHYTQKS	LSLSLGK	447

## Light chain

EIVLTQSPAT	LSLSPGERAT	LSCRASKGVS	TSGYSYLHWY	QQKPGQAPRL	50'
LIYLA SYLES	GVPARFSGSG	SGTDFTLTIS	SLEPEDFAVY	YCQHSRDLPL	100'
TFGGGTKVEI	KRTVAAPSVF	IFPPSDEQLK	SGTASVCLL	NNFYPREAKV	150'
QWKVDNALQS	GNSQESVTEQ	DSKDSTYSL	STLTLSKADY	EKHKVYACEV	200'
THQGLSSPVT	KSFNRGEC				218'

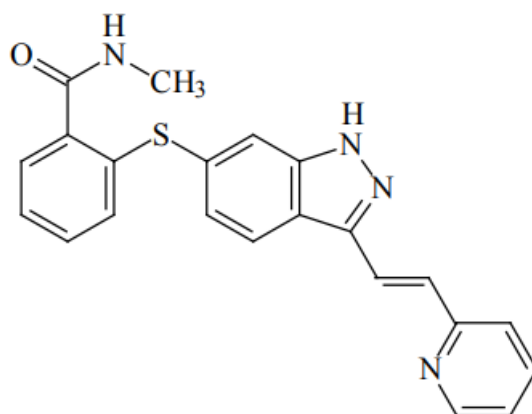
## Disulfide bridges

22-96	22"-96"	23'-92'	23'''-92'''	134-218'	134"-218'''	138'-198'	138'''-198'''
147-203	147"-203"	226-226"	229-229"	261-321	261"-321"	367-425	367'''-425'''

## Glycosylation sites (N)

Asn-297	Asn-297"
---------	----------

The structure of Axitinib is as shown below:



6. Pembrolizumab also has alternative names MK-3475 and Lambrolizumab. Pembrolizumab is Immunoglobulin G4, anti-(human protein PDCD1 (programmed cell death 1)) (human-*Musmusculus* monoclonal heavy chain), disulfide with human-*Musmusculus* monoclonal light chain, dimer

Axitinib is also known as (E)-N-Methyl-2-((3-(2-(pyridin-2-yl)vinyl)-1H-indazol-6-yl)thio)benzamide

7. **PRIOR ARTS:** The opponent wishes to rely on the following prior arts as evidence in support of the grounds of opposition.

- i. David J McEwan, “Emerging therapeutic aspects in oncology”; British Journal of Pharmacology; (2013) 169 1647–1651 – published August 2013 (**Annexed herewith as Annexure 2**)
- ii. Anna Azvolinsky; “Evolution of Care in Advanced Kidney Cancer”; Targeted Therapies in Oncology, September 2013, Volume 2, Issue 5 – published September 19, 2013 (**Annexed herewith as Annexure 3**)
- iii. Rini et al, “Comparative effectiveness of axitinib versus sorafenib in advanced renal cell carcinoma (AXIS): a randomised phase 3 trial”; The Lancet; Vol 378 December 3, 2011 – published November 04, 2011 (**Annexed herewith as Annexure 4**)
- iv. Yasuda et al., “Simultaneous blockade of programmed death 1 and vascular endothelial growth factor receptor 2 (VEGFR2) induces synergistic anti-tumour effect in vivo”; Clinical and Experimental Immunology, 172: 500–506 – published June 2013 (**Annexed herewith as Annexure 5**)
- v. WO2012135408A1 – published 04 October 2012 (**Annexed herewith as Annexure 6**)
- vi. INLYTA (axitinib) FDA Label – published 2012 (**Annexed herewith as Annexure 7**)

8. It is submitted that the claims of impugned patent application are liable to be refused on following grounds as below, which are without prejudice to each other:

- a) **Section 25(1)(e)**– that the subject-matter claimed in the impugned application is obvious and clearly does not involve any inventive step.
- b) **Section 25(1)(f)**– that the subject of any claim of the complete specification, is not an invention within the meaning of this act or is not patentable under this act.

- c) **Section 25(1)(g)**– that the complete specification of the impugned application does not sufficiently and clearly describe the invention or the method by which it is to be performed.
- d) **Section 25(1)(h)** - Failure to disclose the information required by section 8 of the Patents Act.

**GROUND 1: Section 25(1)(e) Lack of Inventive step**

9. The Opponent states that the subject-matter of all the claims 1 to 14 of the impugned application lacks inventive merit and is obvious to a person skilled in the art in view of the prior art documents annexed in the instant pre-grant opposition.
10. It is submitted that the invention as claimed is obvious and does not involve any inventive step, in view of the disclosures published prior to the earliest priority date of the impugned patent application i.e. prior to 04/02/2014.
11. It is submitted that claims 1-14 of the impugned application lack inventive step and are obvious in view of common general knowledge in art and combined with teachings of the following:
  - David J McEwan, “Emerging therapeutic aspects in oncology”; British Journal of Pharmacology; (2013) 169 1647–1651
  - Anna Azvolinsky; “Evolution of Care in Advanced Kidney Cancer”; Targeted Therapies in Oncology, September 2013, Volume 2, Issue 5
  - Rini et al, “Comparative effectiveness of axitinib versus sorafenib in advanced renal cell carcinoma (AXIS): a randomised phase 3 trial”; The Lancet; Vol 378 December 3, 2011
  - Yasuda et al., “Simultaneous blockade of programmed death 1 and vascular endothelial growth factor receptor 2 (VEGFR2) induces synergistic anti-tumour effect in vivo”; Clinical and Experimental Immunology, 172: 500–506
  - WO2012135408A1 (WO’408)
  - INLYTA (axitinib) FDA Label



12. It is submitted that McEwan discloses an overview of the therapeutic options available and being explored for the treatment of various types of cancers at the time of publication i.e. till 2013 which predates the priority date of the impugned application. The evolution of cancer therapies from an earlier time period till the present time is charted (Table 1, internal page 1648) with an emphasis on the fact that in present times, treatments are more targeted towards the individual subtype or phenotype of cancer (section entitled “Today” Table 1, internal page 1648).
13. McEwan (Annexure 2) further discloses (Table 2, internal page 1649) discloses the examples of recent specifically targeted drugs used for cancer. Among the enlisted monoclonal antibodies in aforementioned table, the two antibodies specifically indicated for renal cell carcinoma is the antibody lambrolizumab (which is another name for Pembrolizumab), with the other being nivolumab. The target of both is disclosed to be PD-1 receptor. It is notable that no other monoclonal antibodies except those targeting PD-1 receptor are enlisted to be used for specific targeting of renal cell carcinoma. Thus, a preference for PD-1 targeting antibodies such as specifically listed Pembrolizumab is disclosed.
14. Further, among the enlisted tyrosine kinase small molecule inhibitors, the only drug specifically indicated for renal cell carcinoma are the TKI Axitinib and sorafenib, with one of the target for both the aforementioned TKI's being listed as VEGFR.
15. Thus, McEwan clearly discloses and teaches that predating the priority date of the impugned application, a very limited number of options were available for treatment renal cell carcinoma, among which the two categories indicated were PD-1 receptor targeting monoclonal antibodies and tyrosine kinase small molecule inhibitors, among them VEGFR targeting TKI such as axitinib and sorafenib. Thus, a person skilled in the art would be motivated to consider the above combination for targeted treatment of cancer such as renal cell carcinoma.
16. Azvolinsky (Annexure 3) discloses the clinical options that were being considered for the treatment of renal cell carcinoma before the priority date of the impugned application. It is disclosed that an effort was undertaken to sequence these therapies for the aforementioned cancer (para 1, page 1/ 4).

17. It is disclosed that among the targeted agent sequencing efforts, it has become apparent that although second-line and subsequent therapies become gradually less effective, axitinib, sorafenib, and everolimus are all options for second-line therapy (para 3, internal page 2/4).
18. It is further disclosed that the only drug that has been tested exclusively in a second-line setting is axitinib, a VEGF inhibitor approved for metastatic RCC after failure of one systemic therapy (para 3, internal page 2/4).
19. Significantly, it is disclosed that for patients who had previously failed sunitinib, axitinib significantly delayed PFS for 2.6 months longer compared with sorafenib (para 3, internal page 2/4).
20. Further, Azvolinsky also discloses that immunotherapy checkpoint agents, including lambrolizumab (synonym for pembrolizumab) is are currently in phase I trials for solid tumors (last para, internal page 2/ 4).
21. Thus, Azvolinsky clearly teaches that among sunitinib and axitinib (which both target VEGFR), axitinib is clinical candidate of choice considered for those individuals who have failed sunitinib. Azvolinsky also importantly teaches that in such individuals, axitinib is indeed more effective as compared to another drug (sorafenib). Thus, even among TKI's targeting VEGFR, Axitinib would be the preferred agent based on such clinical data. Further, azvolinsky also discloses that pembrolizumab was already in phase I trials for solid tumors and was thus known to be effective enough to be tested in such a trial.
22. Rini et al. (Annexure 4) discloses the results of a randomised phase 3 study comparing axitinib, describes as a potent and selective second generation inhibitor of vascular endothelial growth factor (VEGF) receptors, with sorafenib, described as an approved VEGF receptor inhibitor, as second-line therapy in patients with metastatic renal cell cancer (Background, Summary, internal page 1931). It is disclosed that the trial included patients coming from 175 sites (hospitals and outpatient clinics) in 22 countries aged 18 years or older with confirmed renal clear-cell carcinoma who progressed despite first-line therapy containing sunitinib, bevacizumab plus interferon-alfa, temsirolimus, or cytokines (Methods, Summary, internal page 1931).

23. It is disclosed, among the findings of the trial, that in patients previously treated with sunitinib, median PFS was 4·8 months for axitinib and 3·4 months for sorafenib (internal page 1935, sentence bridging para 1 and 2).
24. Further, it is disclosed that the preplanned subgroup analyses showed a significant superiority of axitinib over sorafenib in both previous sunitinib and previous cytokine treatment subgroups (discussion, second para, internal page, 1936).
25. Rini et al. concludes that results from this phase 3 study of axitinib showed a statistically significant and clinically meaningful improvement in median PFS compared with sorafenib in patients with advanced renal cell carcinoma.
26. Thus, it is evident from the disclosure of Rini et al. that Axitinib is clearly the superior therapeutic agent for the treatment of renal cell carcinoma in comparison to sunitinib and sorafenib. Rini et al. teaches a preference for choosing Axitinib over other agents for treatment of RCC.
27. Yasuda et al., (Annexure 5) discloses the results of evaluated combining the blockade of PD-1 and vascular endothelial growth factor receptor 2 (VEGFR2) in a murine cancer model using Colon-26 adenocarcinoma (Summary, internal page 500).
28. The results of this study showed that treatment with anti-VEGFR2 mAb or combination therapy inhibited the development of tumourmicrovessels significantly compared with control (last para, internal page 502)
29. Further, it is reported that treatment with anti-PD-1 mAb or combination therapy induced a significant increase in the expression of interferon (IFN)- $\gamma$ , tumour necrosis factor (TNF)- $\alpha$  and granzyme B in comparison with control (Fig. 5). Thus, PD-1 blockade enhanced T cell recruitment and activated local immune status, thereby resulting in tumour reduction (para bridging left and right columns, internal page 503)
30. It is concluded that the study shows, for the first time, that the combination of PD-1 and VEGFR2 had induced a synergistic in-vivo anti-tumour effect without overt toxicity (last para, internal

page 505).

31. Thus, Yasuda et al. clearly discloses and teaches that a combination of PD-1 and VEGFR displays synergistic in-vivo anti-tumor effect without overt toxicity and this approach warrants further investigation.
32. Thus, a combined teaching of the prior arts (Annexure 2-5) teaches that for the treatment of cancer (especially renal cell carcinoma) targeted therapies were investigated and the options included PD-1 targeting monoclonal antibodies and VEGFR targeting tyrosine kinase inhibitors. Annexure 2 teaches that among the monoclonal antibodies, PD-1 targeting antibody such as pembrolizumab was available and among VEGFR inhibiting TKI inhibitors molecule such as axitinib was available. Annexure 3 and Annexure 4 teach that among the tyrosine kinase inhibitors that target VEGFR, the drug axitinib is the second line therapy for individuals who have not had success with sunitinib. The drug axitinib was found to be clearly superior to another TKI sorafenib, thus indicating preference for axitinib. The prior art Annexure 5 teaches that combination of PD-1 and VEGFR displays synergistic in-vivo anti-tumor effect without overt toxicity. Thus, a combined teaching of all of the above prior art very clearly teaches that a combination of PD-1 inhibitor (such as pembrolizumab) and VEGFR inhibitor (axitinib) would be effective for targeted treatment of cancer such as renal cell carcinoma. Thus, claim 1, which claims a medicament comprising an antagonist of a Programmed Death 1 protein (PD-1) for use in combination with a vascular endothelial growth factor receptor (VEGFR) inhibitor for treating a cancer in an individual, is clearly obvious in view of Annexures 2-5.

**Claim 2 lacks inventive step in view of Annexures 2-5**

33. Claim 2 claims a medicament comprising a VEGFR inhibitor, which is axitinib, for use in combination with PD-1 antagonist monoclonal antibody for treating cancer. As discussed in preceding paragraph, the combination of VEGFR inhibitor (preferentially axitinib) and monoclonal antibody (pembrolizumab) is clearly taught by a combined reading of prior arts Annexures 2-5. Thus, claim 2 is also obvious in view of Annexures 2-5.

**Claims 3-6 lack inventive step in view of Annexures 2-5**

34. Claims 3 depends on claim 1 or 2, where the individual is a human. Since the Annexures 2-4 describe clinical outcomes of studies in humans, it is more than apparent that pembrolizumab and axitinib are used to treat cancer in humans. Their combination, administered to treat humans is thus clearly obvious.
35. Claims 4-5 do not recite any technical features of the invention. Thus, the features of these claims are not pertinent to working of invention. Dependent claim 6 claims a medicament wherein the PD-1 antagonist is pembrolizumab and the VEGFR inhibitor is axitinib. In view of Annexures 2-5, it is clearly obvious to preferentially combine PD-1 antagonist pembrolizumab and VEGFR inhibitor axitinib. Hence claim 6 also lacks inventive step.

**Claim 7 lacks inventive step in view of WO2012135408A1 and INLYTA (axitinib) FDA Label**

36. Claim 7 claims the pembrolizumab is formulated as a liquid medicament which comprises 25 mg/ml pembrolizumab, 7% (w/v) sucrose, 0.02% (w/v) polysorbate 80 in 10 mM histidine buffer pH 5.5 and axitinib is formulated as a 1 mg tablet or a 5 mg tablet.
37. The prior art WO'408 pertains to stable formulations of antibodies against human programmed death receptor PD-1. WO' discloses the formulation of antibody h409A11 including final liquid formulation comprising 7% sucrose, 0.02% polysorbate 80, and 25 mg/mL h409A11 (para [00119], internal page 27). This antibody (h409A11) is pembrolizumab as evidenced from the data presented in paras [00161]-[00163], (internal page 37-38). There, the formulation of pembrolizumab disclosed in WO'408 is identical as the one claimed in claim 7.
38. Further, the FDA label of INLYTA (axitinib), published 2012, discloses the dose of axitinib to be 1 mg and 5 mg tablets (description, internal page 11), which is the same as that claimed in claim 7. Thus, all features of claim 7 are disclosed in Annexure 6 and Annexure 7. Since it is already established that it would be obvious to combine the PD-1 antagonist pembrolizumab and VEGFR inhibitor axitinib, it would be obvious to arrive at a combination of the formulation of pembrolizumab with well-known tablet dose form of axitinib. Hence claim 7 lacks inventive

step.

**Claim 8-13 lack inventive step in view of Annexures 2-7**

39. Claim 8 relates to a kit which comprises a first container, a second container and a package insert with the first container comprising PD-1 protein, second container comprising VEGFR inhibitor and package insert comprising instructions for treating an individual for cancer. Claims 9-13 depend on claim 8. Neither independent claim 8, nor claims 9-13 that depend from it, defines any technical features. Further, in view of the combination of a PD-1 targeting monoclonal antibody and VEGFR inhibitor being obvious as established in preceding paragraphs, the claims directed to a kit are obvious and do not involve any inventive step.

**Claim 14 lacks inventive step in view of Annexures 2-7**

40. Claim 14 is directed to a medicament comprising both drugs (pembrolizumab and axitinib) for use in combination and where the pembrolizumab is in the form of a liquid medicament which comprises 25 mg/ml pembrolizumab and axitinib is in the form of 1 mg tablet or a 5 mg tablet. It is humbly submitted that the motivation to combine a PD-1 targeting monoclonal antibody (such as pembrolizumab) with a VEGFR inhibitor (axitinib) is already established from annexures 2-5. Further, the annexures 6-7 disclose the formulation of PD-1 antibody (pembrolizumab) and the tablet form with specific dose 1 mg and 5 mg of axitinib respectively. Thus, all features of claim 14 are disclosed from prior art annexures 2-7. Claim 14 is thus obvious in view of annexures 2-7.
41. In view of the above, the subject matter of the present application is obvious and lacks inventive merit, hence the application ought to be rejected on this ground alone.
42. It is humbly submitted that the claimed invention is directed to a medicament comprising an antagonist of a Programmed Death 1 protein (PD-1) for use in combination with a vascular endothelial growth factor receptor (VEGFR) inhibitor for treating a cancer in an individual.
43. The complete specification of the impugned application includes example 1, which is a study to evaluate the efficacy of a combination of axitinib and MK-3475 in human patients with RCC (para [00159]-[00169], internal page 36-37). However, it is evident that said no actual outcome

or conclusions about the efficacy of the aforementioned combination from the said study are demonstrated in the specification. The only data demonstrated by Applicant is through post-priority publications submitted with the response to the FER. Since the Applicant is required to demonstrate the possession of the invention at the time of filing, such data should have been included in the specification as filed. Further, it is evident that both pembrolizumab and axitinib do not exist as a single product as per the specification, but simply as two separate products which are administered to individuals with cancer. Since both drugs were already known to efficacious for treatment of cancer, it was incumbent upon the Applicant to demonstrate enhanced efficacy data of this combination over the individual effect of both therapeutic agents. Such data is completely absent from the specification.

44. Without prejudice to the above, the Opponent knows that the Applicant has submitted the following documents in their FER response by which the Applicant is trying to establish that the claimed combination provides new and unexpected results:
45. Atkins et. al., "Axitinib in Combination with Pembrolizumab in Patients with Advanced Renal Cell Carcinoma," presented at the European Society of Medical Oncology (ESM), October 7 to 11, 2016, Copenhagen Denmark. This publication relates to preliminary safety and efficacy results for the combination of axitinib with pembrolizumab in patients with advanced renal cell carcinoma. According to the Applicant, the data presented therein, showing that 37 (71.2%) patients had confirmed objective response and 10 (19.2%) patients had stable disease after treatment with axitinib and pembrolizumab, is a demonstration of efficacy in humans, of claimed combination. However, this publication does not present any comparative data of claimed combination against other anti-cancer agents, let alone a combination of other PD-1 agents and VEGFR inhibitors. Hence there is no way to ascertain whether the combination is more efficacious when compared to other agents.
46. Procopio et. al., "Combination therapies for patients with metastatic renal cell carcinoma," *Lancet*, 19:281-283 (2018). This document was published as a comment on various combination therapies for patients with metastatic renal cell carcinoma. It reports on the results of a phase Ib trial published in *The Lancet Oncology* (Author: Michael B Atkins) primarily designed to assess the feasibility of the combination of pembrolizumab and axitinib as first-line treatment in

patients with metastatic renal cell carcinoma (para 3, internal page 282). The Applicant has stated in its FER response that Procopio et. al. describes the clinical data as “impressive” and note the “synergistic and additive effect of the study combination.” However, the Applicant has misleadingly shown a truncated statement from the publication. The actual statement reads as *“Are the impressive clinical data from this trial related to the synergistic and additive effect of the study combination or would the results be the same with a sequential use of the two drugs? This question cannot be answered at present; however, the 8% of patients reported in Atkins and colleagues” study who had a complete response suggests that this combination could be very promising.”* Thus, the purported synergistic and additive effect highlighted by Applicant is not definitive as per the document itself. Further, the study which is referenced in Procopio et al. is again not a comparative study. Therefore, no conclusion can be drawn about whether this claimed combination is more efficacious than other anti-cancer drugs in combination.

47. Joshi et. al., “ASCO GU 2018: Safety and Efficacy of Axitinib in Combination with Pembrolizumab in Patients with Advanced Renal Cell Cancer”. This publication references the open-label, phase 1b trial studying the safety/efficacy of axitinib in combination with pembrolizumab in patients with metastatic renal cell carcinoma by Michael B. Atkins. The Applicant has stated in its FER response that “Joshi et. al. describes the results of Atkins et. al. as presenting “impressive statistics with promisingly durable treatment responses. The waterfall plot below shows the distribution of responses, with most exhibiting some effect.” (refer, page 2 of Joshi et.al.). The results are both surprising and encouraging.”. However, the publication Joshi et al. also states (para 2, internal page 2/ 2), while discussing the positive results, that “Yet to be answered is whether this response requires co-administration of axi+pembro, or if sequential administration may also lead to similar response rates”. Therefore, even a post-priority document does not rule out the possibility that both drugs may not in fact need to be given together to individuals suffering from cancer. This is in clear contradiction to the impugned application, which is claiming both drugs for use in combination. Further, other PD-1 targeting monoclonal antibody (nivolumab) was known in the prior art (refer document Annexure 2). It is not known whether the claimed combination is better than the combination of other PD-1 targeting antibody (such as nivolumab) in combination with with a VEGFR targeting TKI, as the Atkins trial does not test the claimed combination against other such combinations. Therefore, in absence of such data, no technical advancement can be established.



48. Rini et al., (“Pembrolizumab plus Axitinib versus Sunitinib for Advanced Renal-Cell Carcinoma,” N. Engl. J. Med. (2019) e-published February 16, 2019. This publication describes reports the results of an open-label, phase 3 trial. The FER reply includes a statement from Rini et al. that “[a]mong patients with previously untreated advanced renal-cell carcinoma, treatment with pembrolizumab plus axitinib resulted in significantly longer overall survival and progressionfree survival, as well as a higher objective response rate, than treatment with sunitinib.” However, the prior art already Azvolinsky already discloses thatfor patients who had previously failed sunitinib, axitinib significantly delayed PFS for 2.6 months longer compared with sorafenib (para 3, internal page 2/4). Further the prior art Rini et al. cited herein (Annexure 4) discloses that the preplanned subgroup analyses showed a significant superiority of axitinib over sorafenib in both previous sunitinib and previous cytokine treatment subgroups (discussion, second para, internal page, 1936). This clearly indicates that axitinib was known to be indicated for individuals unresponsive to sunitinib. It was already a known choice for such individuals and thus, a trial evaluating the effectiveness of a combination incorporating axitinib against sunitinib would anyway have a higher likelihood of success given the teaching of prior art in this regard. Further, the Rini et al. trial evaluated a combination of two agents against one other therapeutic agent and not against another combination of drugs, let alone against a combination of a PD-1 targeting monoclonal antibody and a VEGFR inhibitor.
49. Domblides et al., “Emerging antiangiogenics for renal cancer,” Expert Opinion on Emerging Drugs 18(4):495-511 (2013). This document has a publication date pre-dating the priority date of the impugned application and was submitted along with the FER response, purportedly to show that selection of axitinib was not definitively taught in prior art and that the person skilled in the art had a huge range of TKI’s to choose from with no clear pointer towards axitinib. However, this document itself discloses (internal page 499) that, in the AGILE trial, the primary end point was a strict superiority of axitinib, with an HR of 0.78. It is reported that even though this study did not reach statistical significance, in the pre-planned subgroupof patients with good performance status (PS = 0), the median PFS reported was significantly superior in the axitinib group of patients with 13.7 months (95% CI: 10.1 - 19.4) and 6.6 months (95% CI: 4.7 - 9.9) in the sorafenib group (HR = 0.64, 95% CI:0.42 - 0.99, p = 0.022). Thus, even this publication (and the prior arts cited herein) indicate that axitinib demonstrated superiority as compared to other

TKI's and would be chosen for investigating a combination with other drugs. As per Annexure 2, one of only two preferred VEGFR targeting TKI's drugs for renal cell carcinoma was axitinib. Thus there was a clear preference for axitinib already known in prior art. Thus, this publication does not invalidate the fact that a preference for axitinib in RCC was known in the art.

50. Dorff et al., "Novel tyrosine kinase inhibitors for renal cell carcinoma," Expert Rev. Clin. Pharmacol. 7(1):67-73 (2014). This review publication discusses novel tyrosine kinase inhibitors for renal cell carcinoma, and especially five TKI's in development for RCC which are tivozanib; dovitinib; regorafenib; cabozantinib; and tivantinib. This publication, purportedly cited by Applicant to show the other TKI's being considered for RCC does not invalidate the prior art cited herein by opponent showing a clear preference for axitinib, especially for renal cell carcinoma. As already contended above, Annexure 2 shows that, one of only two preferred VEGFR targeting TKI's drugs for renal cell carcinoma was axitinib. Thus there was a clear preference for axitinib already known in prior art. Thus, this publication does not invalidate the fact that a preference for axitinib in RCC was known in the art.
51. In light of all the publications discussed above, it is apparent that the Applicant has failed to establish that this particular invention has any technical advancement or provides any technical contribution to the field of the invention.

## **GROUND 2: Claims not patentable under Section 25(1)(f)**

### **The claimed subject matter is not patentable under Section 3(e) of the Act**

52. It is submitted that the impugned patent application falls within the purview of section 3(e) of the Patents Act, 1970 which states that "*a substance obtained by a mere admixture resulting only in the aggregation of the properties of the components thereof or a process for producing such substance*".
53. The two therapeutic agents, pembrolizumab (MK-3475) and axitinib, were well-established as agents used in the treatment of cancer predating the priority date of the impugned application, as evidenced from the prior art. Since the claims are directed to a combination of the aforementioned agents, evidence for synergistic effect of the above combination in treatment of

cancer should have been demonstrated in the specification itself.

54. The impugned application contains no experimental data to establish any such synergistic effect with the combined use of pembrolizumab (MK-3475) and axitinib. The example included in the impugned application is study to evaluate the efficacy of a combination of axitinib and MK-3475 in human patients with RCC (para [00159]-[00169], internal page 36-37). However, the outcome of said study are absent from the specification. Thus, the impugned application fails to establish any synergy resulting from the combination of pembrolizumab and axitinib. The claimed combination therapy is thus exclusively based on the well-known facts that pembrolizumab (MK-3475) is an effective anti-PD-1 antibody and axitinib is an effective VEGFR inhibitor.
55. It is submitted that, as contended previously, the data for a synergistic effect of a claimed combination ought to be clearly demonstrated in the specification as-filed. The post-priority data submitted by the Applicant ought not to be considered for establishing said effect which is absent from the specification.
56. Further, the Applicant has submitted various documents in support of the claimed combination of the impugned application. These documents do not demonstrate a synergistic effect as will be discussed below:
57. Atkins et. al., “Axitinib in Combination with Pembrolizumab in Patients with Advanced Renal Cell Carcinoma,” presented at the European Society of Medical Oncology (ESM), October 7 to 11, 2016, Copenhagen Denmark. This publication relates to preliminary safety and efficacy results for the combination of axitinib with pembrolizumab in patients with advanced renal cell carcinoma. According to the Applicant, the data presented therein, showing that 37 (71.2%) patients had confirmed objective response and 10 (19.2%) patients had stable disease after treatment with axitinib and pembrolizumab, is a demonstration of efficacy in humans, of claimed combination. However, this publication does not present any comparative data of claimed combination against other anti-cancer agents, let alone a combination of other PD-1 agents and VEGFR inhibitors. Hence there is no way to ascertain whether the combination is more efficacious when compared to other agents.

58. Rothermundt et al., “Successful treatment with an anti-PD-1 antibody for progressing brain metastases in renal cell cancer,” *Annals of Oncology*, 25:544-552 (2016). This publication pertains to case report of an individual clear-cell renal cell cancer (ccRCC) with metastases, who underwent several treatments in the course of her illness (para 1, 2 and 3, internal page 544). It is disclosed that Upon systemic and central nervous system (CNS) progression 5 months later following treatment with another monoclonal antibody (bevacizumab), treatment with axitinib was started—achieving partial response (PR). However, after 4 months, in March 2015, further systemic and CNS progression was documented. Subsequent to this, treatment with pembrolizumab, was initiated. After four infusions, the patient experienced complete resolution of lung metastases, stabilization of other metastases. Importantly, regression of all brain metastases was documented on magnetic resonance imaging. It is important to note here (a point which the Applicant has omitted from its FER reply) that axitinib treatment and pembrolizumab treatment were not given simultaneously to this individual. The pembrolizumab treatment started four (4) whole months after the individual was already on a regimen of axitinib alone. In fact, the pembrolizumab was initiated when the patient exhibited further systemic and CNS progression after being on axitinib alone for 4 months. Thus, no synergy is demonstrated as there is no concurrent administration. Thus, this publication is not an evidence supporting the claimed combination.

59. Procopio et al., “Combination therapies for patients with metastatic renal cell carcinoma,” *Lancet*, 19:281-283 (2018). This document was published as a comment on various combination therapies for patients with metastatic renal cell carcinoma. It reports on the results of a phase Ib trial published in *The Lancet Oncology* (Author: Michael B Atkins) primarily designed to assess the feasibility of the combination of pembrolizumab and axitinib as first-line treatment in patients with metastatic renal cell carcinoma (para 3, internal page 282). The Applicant has stated in its FER response that Procopio et al. describes the clinical data as “impressive” and note the “synergistic and additive effect of the study combination.” However, the Applicant has misleadingly shown a truncated statement from the publication. The actual statement reads as *“Are the impressive clinical data from this trial related to the synergistic and additive effect of the study combination or would the results be the same with a sequential use of the two drugs? This question cannot be answered at present; however, the 8% of patients reported in Atkins and colleagues’ study who had a complete response suggests that this combination could be very*

*promising.*” Thus, the purported synergistic and additive effect highlighted by Applicant is not definitive as per the document itself. Further, the study which is referenced in Procopio et al. is again not a comparative study. Therefore, no conclusion can be drawn about whether this claimed combination is more efficacious than other anti-cancer drugs in combination.

60. Joshi et. al., “ASCO GU 2018: Safety and Efficacy of Axitinib in Combination with Pembrolizumab in Patients with Advanced Renal Cell Cancer”. This publication references the open-label, phase 1b trial studying the safety/efficacy of axitinib in combination with pembrolizumab in patients with metastatic renal cell carcinoma by Michael B. Atkins. The Applicant has stated in its FER response that “Joshi et. al. describes the results of Atkins et. al. as presenting “impressive statistics with promisingly durable treatment responses. The waterfall plot below shows the distribution of responses, with most exhibiting some effect.” (refer, page 2 of Joshi et.al.). The results are both surprising and encouraging.” However, the publication Joshi et al. also states (para 2, internal page 2/ 2), while discussing the positive results, that “Yet to be answered is whether this response requires co-administration of axi+pembro, or if sequential administration may also lead to similar response rates”. Therefore, even a post-priority document does not rule out the possibility that both drugs may not in fact need to be given together to individuals suffering from cancer. This is in clear contradiction to the impugned application, which is claiming both drugs for use in combination. Further, other PD-1 targeting monoclonal antibody (nivolumab) was known in the prior art (refer document Annexure 2). It is not known whether the claimed combination is better than the combination of other PD-1 targeting antibody (such as nivolumab) in combination with with a VEGFR targeting TKI, as the Atkins trial does not test the claimed combination against other such combinations. Therefore, in absence of such data, no technical advancement can be established.

61. Rini et. al., (“Pembrolizumab plus Axitinib versus Sunitinib for Advanced Renal-Cell Carcinoma,” N. Engl. J. Med. (2019) e-published February 16, 2019. This publication describes reports the results of an open-label, phase 3 trial. The FER reply includes a statement from Rini et al. that “[a]mong patients with previously untreated advanced renal-cell carcinoma, treatment with pembrolizumab plus axitinib resulted in significantly longer overall survival and progression free survival, as well as a higher objective response rate, than treatment with sunitinib.” However, the prior art already Azvolinsky already discloses that for patients who had

previously failed sunitinib, axitinib significantly delayed PFS for 2.6 months longer compared with sorafenib (para 3, internal page 2/4). Further the prior art Rini et al. cited herein (Annexure 4) discloses that the preplanned subgroup analyses showed a significant superiority of axitinib over sorafenib in both previous sunitinib and previous cytokine treatment subgroups (discussion, second para, internal page, 1936). This clearly indicates that axitinib was known to be indicated for individuals unresponsive to sunitinib and the superiority of axitinib over sunitinib. It was already a known choice for such individuals and thus, a trial evaluating the effectiveness of a combination incorporating axitinib against sunitinib would anyway have a higher likelihood of success given the teaching of prior art in this regard. Further, the Rini et al. trial evaluated a combination of two agents against one other therapeutic agent and not against another combination of drugs, let alone against a combination of a PD-1 targeting monoclonal antibody and a VEGFR inhibitor.

62. Therefore, the alleged invention is nothing but an admixture of the two active components with aggregation of their known anticancer properties.

63. Thus, it is submitted that the impugned application falls within the purview of section 3(e) of the Patents Act, 1970 and should be refused on the said ground.

**The claimed subject matter in not patentable under Section 3(i) of the Act**

64. It is submitted that the impugned patent application falls within the purview of section 3(i) of the Patents Act, 1970 which states that “*any process for the medicinal, surgical, curative, prophylactic [diagnostic, therapeutic] or other treatment of human beings or any process for a similar treatment of animals to render them free of disease or to increase their economic value or that of their products.*”

65. It is submitted that the claimed invention relates to a combination of PD-1 targeting monoclonal antibody pembrolizumab and VEGFR targeting small molecule axitinib. However, as per the alleged invention, both drugs do not exist as part of a single product. The pembrolizumab is formulated as a liquid, while the axitinib is present in the form of a tablet. The two agents are administered separately to cancer patients for treatment. The invention is therefore nothing more

than a method of treatment.

66. Further, the Applicant has cited Rothermundt et. al., “Successful treatment with an anti-PD-1 antibody for progressing brain metastases in renal cell cancer,” *Annals of Oncology*, 25:544-552 (2016) in support of their application. This publication pertains to case report of an individual clear-cell renal cell cancer (ccRCC) with metastases, who underwent several treatments in the course of her illness (para 1, 2 and 3, internal page 544). It is disclosed that Upon systemic and central nervous system (CNS) progression 5 months later following treatment with another monoclonal antibody (bevacizumab), treatment with axitinib was started—achieving partial response (PR). However, after 4 months, in March 2015, further systemic and CNS progression was documented. Subsequent to this, treatment with pembrolizumab, was initiated. After four infusions, the patient experienced complete resolution of lung metastases, stabilization of other metastases. Importantly, regression of all brain metastases was documented on magnetic resonance imaging. It is important to note here (a point which the Applicant has omitted from its FER reply) that axitinib treatment and pembrolizumab treatment were not given simultaneously to this individual. The pembrolizumab treatment started four (4) whole months after the individual was already on a regimen of axitinib alone. In fact, the pembrolizumab was initiated when the patient exhibited further systemic and CNS progression after being on axitinib alone for 4 months. Thus, it is clear that there is no concurrent administration of the two agents axitinib and pembrolizumab. The combination is this only a treatment protocol. Therefore, the claimed combination is nothing but a method of treatment. This publication is therefore not an evidence supporting the claimed combination.

67. Thus, it is humbly submitted that the impugned application falls within the purview of section 3(i) of the Patents Act, 1970 and should be refused on the said ground.

### **Ground 3: Claims not patentable under Section 25(1)(g)**

68. It is submitted that complete specification does not sufficiently and clearly describe the invention or the method by which it is to be performed.

69. It is submitted that it is a well settled law that the specification should clearly and fairly describe

the invention and disclose the best mode of working the invention so that the person skilled in the art could perform the invention without any undue efforts. Further, it is submitted that claims of impugned application are not fairly based on the specification and the complete specification does not fairly describe the invention and the method by which it is to be performed.

70. **Lack of sufficiency of disclosure in relation to Claims 1-14:** The claims of the impugned application are directed to combination therapies comprising two agents, pembrolizumab (MK-3475) and axitinib, that were known as anti-cancer agents prior to the impugned application. As noted above, the impugned application does not contain any data to demonstrate efficacy of the claimed combination in any experimental setting and certainly not in the treatment of human cancer. The impugned application comprises a single Example 1, which merely describes the design of a study protocol for the treatment of patients with renal cell carcinoma (RCC) with a combination of axitinib and MK-3475. Example 1 of the impugned application describes nothing more than a study design on paper. It follows that the claims must be found insufficient.
71. During prosecution, the Applicant sought to rely on post-published data for evidence of a technical effect. The Applicant submitted this post-published data to remedy the deficiencies in the as-filed specification, which is not permissible under the Act. It is important to take notice of the fact that a Patentee or an Applicant is required to show workability of an invention at the time of filing of the complete specification. Having failed to provide such disclosure/information raises serious doubts on whether or not the Applicant actually possessed the invention at the time of filing the complete specification. It is established case law that post-filed data cannot be used in order to establish plausibility of claimed therapeutic effect. Instead, post-published data can only be used to back-up and confirm a therapeutic effect that is already plausibly disclosed in the application as filed. In the instant case, there are absolutely no data in the impugned application demonstrating therapeutic efficacy for the claimed combination. The only example (Example 1) included in the impugned application is a study protocol for the testing of a combination of MK-3475 and axitinib in renal cell carcinoma patients. No results are presented. Therefore, such insufficiency of the impugned application could not be cured by filing post-published evidence/clinical data.



72. Hence, the claimed subject-matter is not sufficiently disclosed by the specification of the impugned application, which is a defect that cannot be fixed by post-published evidence.
73. **Claims 1, 2, 8, 12 and 14 are not enabled:** The independent claims 1, 2, 8 and 14 extend to the treatment of all cancers. As evidenced by dependent claim 12, treatment extends to a variety of solid tumors. Claim 12 provides a long list of various, very different cancers. Accordingly, to sufficiently disclose the therapeutic effect in the treatment of all these specific, very different cancers, it would need to be plausible for the skilled person that the claimed combination therapy achieves a therapeutic effect in the treatment of **all** of these specific cancers. However, there is no evidence in the impugned application to render it credible that a therapeutic efficacy will be achieved in any cancer type. As noted above, there are absolutely no data in the impugned application demonstrating therapeutic efficacy for the claimed combination in any cancer type. As discussed extensively above, the examples of the impugned application include nothing more than a study protocol. It is thus not plausible that any cancer can be treated with the claimed combination therapy. Therefore, the subject-matter of claims 1, 2, 8, 12 and 14 is insufficiently disclosed, which is a defect that, as already noted above, cannot be fixed by post-published evidence.

**Ground 5: Information Relating To Corresponding Applications Under Section 8 section 25(1)(h)**

74. The Applicant has failed to disclose to the Patent Office the information required under Section 8. The Applicant is required to provide all the information regarding the prosecution of the equivalent applications till the grant of the Indian application to the Patent Office in writing from time to time and also within the prescribed time.
75. It is observed that Applicant has not provided information about updated the status of corresponding application in the Form-3 which information has not been provided to the learned Controller.
76. Therefore, the applicant has failed to comply with the requirements of the section 8 of the act and the opponent demands rejection on this ground also.

77. It is submitted that the Applicant has failed to disclose the details of corresponding foreign applications and impugned patent application to be refused.

78. The opponents crave leave to file further submissions and evidence with respect to this ground.

### **Conclusion**

79. In view of the above, the claims are not novel, inventive and not patentable and insufficient. The pre-grant opposition as filed may be allowed and the subject patent application may be refused.

### **Hearing Requested**

80. The Opponent hereby requests a hearing under section 25(1) of the Patents Act, 1970 (hereinafter referred to as “the Patents Act”) and Rule 55 of the Patents Rules (hereinafter referred to as “the Rules”).

## **P R A Y E R**

In the fact and circumstances of the case, the Opponent prays as follows:

- i. that the Controller take the present Opposition on record; that the Indian application 201617025251, be rejected under Section 25(1) of the Patents (Amendment) Act, 2005;
- ii. that the Opponent may be allowed to file further documents and evidence if necessary to support their averments;
- iii. that the Opponent may be allowed to file rejoinder and affidavit if necessary to support their averments;
- iv. that the Opponent may be granted an opportunity of being heard in the matter before any final orders are passed;
- v. that the Opponent may be allowed to make further submissions in case the Patentee makes any amendments in the claims;

- vi. any other reliefs considering the facts and circumstances may be granted in favour of the Opponent in the interest of justice.

Dated this 19<sup>th</sup> day of September, 2023



PRAGYA SINGH THAKUR (IN /PA – 3329)  
OF RAJESHWARI AND ASSOCIATES  
AGENT FOR THE OPPONENT

TO,  
THE CONTROLLER OF PATENTS  
THE PATENT OFFICE, NEW DELHI

# Annexure - 1

## **I/We Claim:**

1. A medicament comprising an antagonist of a Programmed Death 1 protein (PD-1) for use in combination with a vascular endothelial growth factor receptor (VEGFR) inhibitor for treating a cancer in an individual, wherein the PD-1 antagonist is an anti-PD-1 monoclonal antibody which comprises a heavy chain and a light chain, wherein the heavy and light chains comprise SEQ ID NO:21 and SEQ ID NO:22, respectively, and further wherein the VEGFR inhibitor is N-methyl-2-[3-((E)-2-pyridin-2-yl-vinyl)-1H-indazol-6-ylsulfanyl]-benzamide or a pharmaceutically acceptable salt thereof.
2. A medicament comprising a vascular endothelial growth factor receptor (VEGFR) inhibitor for use in combination with an antagonist of a Programmed Death 1 protein (PD-1) for treating a cancer in an individual, wherein the VEGFR inhibitor is N-methyl-2-[3-((E)-2-pyridin-2-yl-vinyl)-1H-indazol-6-ylsulfanyl]-benzamide or a pharmaceutically acceptable salt thereof, and further wherein the PD-1 antagonist is an anti-PD-1 monoclonal antibody which comprises a heavy chain and a light chain, wherein the heavy and light chains comprise SEQ ID NO:21 and SEQ ID NO:22.
3. The medicament as claimed in claim 1 or 2, wherein the individual is a human.
4. The medicament as claimed in any one of claims 1 to 3, wherein the cancer is a solid tumor that tests positive for Programmed Death-Ligand 1 (PD-L1) expression by an immunohistochemical (IHC) assay.
5. The medicament of any as claimed in claims 1 to 3, wherein the cancer is renal cell carcinoma.
6. The medicament as claimed in any one of claims 1 to 5, wherein the PD-1 antagonist is pembrolizumab and the VEGFR inhibitor is axitinib.

7. The medicament as claimed in claim 6, wherein the pembrolizumab is formulated as a liquid medicament which comprises 25 mg/ml pembrolizumab, 7% (w/v) sucrose, 0.02% (w/v) polysorbate 80 in 10 mM histidine buffer pH 5.5 and axitinib is formulated as a 1 mg tablet or a 5 mg tablet.

5

8. A kit which comprises a first container, a second container and a package insert, wherein the first container comprises at least one dose of a medicament comprising an antagonist of a Programmed Death 1 protein (PD-1), the second container comprises at least one dose of a medicament comprising a vascular endothelial growth factor receptor (VEGFR) inhibitor, and the package insert comprises instructions for treating an individual for cancer using the medicaments, wherein the PD-1 antagonist is an anti-PD-1 monoclonal antibody which comprises a heavy chain and a light chain, wherein the heavy and light chains comprise SEQ ID NO:21 and SEQ ID NO:22, respectively, and further wherein the VEGFR inhibitor is N-methyl-2-[3-((E)-2-pyridin-2-yl-vinyl)-1H-indazol-6-ylsulfanyl]-benzamide or a pharmaceutically acceptable salt thereof.

10

15

9. The kit as claimed in claim 8, wherein the instructions state that the medicaments are intended for use in treating an individual having a cancer that tests positive for Programmed Death-Ligand 1 (PD-L1) expression by an immunohistochemical (IHC) assay.

20

10. The kit as claimed in claim 8 or 9, wherein the individual is a human.

11. The kit as claimed in any one of claims 8 to 10, wherein the PD-1 antagonist is pembrolizumab formulated as a liquid medicament and the VEGFR inhibitor is axitinib formulated as a 1 mg tablet or a 5 mg tablet.

25

12. The use or kit as claimed in any one of claims 1-4 or 6-10, wherein the cancer is bladder cancer, breast cancer, clear cell kidney cancer, head/neck squamous cell carcinoma, lung squamous cell carcinoma, malignant melanoma, non-small-cell lung cancer (NSCLC), ovarian cancer, pancreatic cancer, prostate cancer, renal cell cancer, small-cell lung cancer

30

(SCLC), triple negative breast cancer, acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myeloid leukemia (CML), diffuse large B-cell lymphoma (DLBCL), follicular lymphoma, Hodgkin's lymphoma (HL), mantle cell lymphoma (MCL), multiple myeloma (MM), myeloid cell leukemia-1 protein (Mcl-1), myelodysplastic syndrome (MDS), non-Hodgkin's lymphoma (NHL), or small lymphocytic lymphoma (SLL).

13. The use or kit as claimed in any one of the above claims, wherein the cancer is advanced renal cell carcinoma.

14. A medicament comprising:

- a) pembrolizumab for use in combination with axitinib for treating a cancer in a human individual by a method comprising administering to the individual (i) axitinib formulated as a 1 mg tablet or a 5 mg tablet and pembrolizumab formulated as a liquid medicament which comprises 25 mg/ml pembrolizumab or (ii) axitinib formulated as a 1 mg tablet or a 5 mg tablet and pembrolizumab formulated as a liquid medicament which comprises 25 mg/ml pembrolizumab; or
- b) axitinib for use in combination with pembrolizumab for treating a cancer in a human individual by a method comprising administering to the individual (i) axitinib formulated as a 1 mg tablet or a 5 mg tablet and pembrolizumab formulated as a liquid medicament which comprises 25 mg/ml pembrolizumab or (ii) axitinib formulated as a 1 mg tablet or a 5 mg tablet and pembrolizumab formulated as a liquid medicament which comprises 25 mg/ml pembrolizumab.

**Date 22 July 2016**

**MALATHI LAKSHMIKUMARAN**  
**IN/PA- 1433**  
**AGENT FOR THE APPLICANTS**

**To**  
**The Controller of Patents**  
**The Patent Office, at New Delhi**

Theme Section: Emerging Therapeutic Aspects in Oncology

## EDITORIAL

# Emerging therapeutic aspects in oncology

David J MacEwan

*Department of Molecular and Clinical Pharmacology, Institute of Translational Medicine, University of Liverpool, Liverpool, UK*

### Correspondence

David J MacEwan, Department of Molecular and Clinical Pharmacology, Institute of Translational Medicine, University of Liverpool, Liverpool L69 3GE, UK. E-mail: macewan@liverpool.ac.uk

### Keywords

chemotherapy; TKI; biologics; tumor; drug resistance

Cancer remains a peculiarly stubborn disease to treat. Some forms of cancer have seen tremendous advances in the effectiveness of their treatments, whereas other forms have remained resistant to pharmacological control. This lack of hope for success is in part due to the types of drugs that are used in the clinic, and the targeted biological system being based purely on cellular growth rates. However, recent drugs designed to affect specific signalling pathways or proteins have been showing much success. Thanks to the ingenuity of pharmacologists in understanding and targeting these processes, there have been real improvements in treatment. Here we are presented with some of the research into such critical systems that have to be understood, so that they can be conquered. We will also look at the challenges facing cancer pharmacologists and what the field may present to us all in the future.

### LINKED ARTICLES

This article is part of a themed section on Emerging Therapeutic Aspects in Oncology. To view the other articles in this section visit <http://dx.doi.org/10.1111/bph.2013.169.issue-8>

## Basis of chemotherapy

'Dad, how do antibiotics work?' My wife groans, as she knows my 9-year-old daughter has just asked a question that will clear the dinner table fast. 'Well, sweetheart', I begin, 'a bacterial cell wall has different chemicals in its body, that allow antibiotics to inhibit the bug from making more of its cell membranes, which it needs to grow'. 'And importantly', I continue, getting into my stride, 'humans don't have those same chemicals in their cell membranes, so antibiotics are much less poisonous to us, but will kill bugs dead! When the doctor gives us antibiotics, our cells still grow with them, but bugs' cells can't'. All five of them are disappearing fast now, so I bring out the big guns, the utterly memorable take-home message they'll all remember for the rest of their lives: 'antibiotics are poisonous to humans and bugs, but just a bit more poisonous to bugs'. At this point I'm talking to the dog who is the only other animal in the room, and all she cares about are scraps of food. However boring these answers are to children (and dogs), I have effectively described the basis of cancer chemotherapy over the last century.

There are only three treatments that can be used for cancer patients: surgery, radiotherapy and drugs. As can be seen from Table 1, before the 1940s and before radiotherapy was possible, if your tumour surgery failed, then as a cancer patient, your fate was in the hands of whatever drugs your

physician had even heard about, never mind what agents they had at their disposal. Much of the cancer research and chemotherapy testing in the early 20th century was based on administering a range of toxic compounds to the cancer patient, then waiting to see what (if anything) happened. Many of these early trials were not successful, with effects of toxic compounds on seriously ill patients having variable effects, as we can imagine. However, there was some good pioneering science (Nicholls, 1936) that paved the way for scientists to systematically identify and develop cytotoxic agents that could be just tolerated by cancer patients, but with significantly greater toxicity towards their tumours.

## Dawn of cancer therapeutics

As with the antibiotics, these 'new age' chemotherapeutic agents were slightly more toxic to cancer cells than they were to our normal cells. And just like antibiotics, some of them were actually antibiotics. From the mustard gas-based agents that emerged from the experiences in the two World Wars, to the antimetabolites, there was a range of compounds that were remarkably able to treat cancers in most patients with some success. Most of the notable successes were in the treatment of blood cancers, such as leukaemia, lymphomas and myeloma, with solid tumours being less susceptible to these

Table 1

The development of cancer therapies

	Cancer is a major cause of death. Over 22 million people suffer worldwide and there are over 6 million deaths per year. In many cases there are no effective treatments.
Pre-18th century	Cancer is a death sentence, medically untreatable. Often not classified as cancer.
Eighteenth century	Certain cancers treatable by surgery. Cancer as a definition of a type of disease is improving.
Nineteenth century	Development of anaesthesia means radical, surgical solutions become more mainstream. Mastectomy developed. Excision of specific localized tumours more possible, improving survival rates.
Twentieth century	Surgeons become more skilled in excision of tumours. Bone and soft tissue operations developed, often avoiding amputation. Definition of cancer types and subtypes improves. William Blair-Bell treats breast cancer patients with colloidal lead.
1940s	The field of chemotherapy born following an accident where mustard gas, a chemical weapon used throughout World Wars I and II, is discovered to lower white blood cell counts. Louis Goodman and Alfred Gilman Sr (father of the Nobel Prize-winning Alfred Gilman) begin to test nitrogen mustard gas-like compounds on lymphoma patients, with apparent success. Sidney Farber tests antifolates, such as methotrexate, on childhood leukaemia patients with success.
1950s onwards	Jane Cooke Wright uses the antifolate methotrexate in the treatment of a solid tumour type in breast cancer patients. Expansion of the arsenal of cancer chemotherapeutics when drug screening identifies the <i>Vinca</i> alkaloids (from the Madagascar periwinkle) as useful agents. As found with microbes, cytotoxic antibiotics (e.g. doxorubicin) are more toxic to cancer cells than to normal non-cancerous cells. Serendipity favours the discovery of platinum-containing chemicals (cisplatin, carboplatin), as bacteria growing around platinum electrodes grow but have no cell division. Combination therapy (combining several chemotherapeutic agents to improve cancer treatments) is refined – this refining of a combination of drugs to reduce patient morbidity rates, is continuously being trialled, and has led to vast improvements in cancer survival rates over the years.
1980s onwards	Significant advances in conventional treatments – surgery, radiotherapy, hormone therapy and chemotherapy. The use of computers improves not only the machines used in the clinic, but the machines and software used to research cancer treatment discoveries, leading to faster assessments of potential breakthroughs. Taxane microtubule poisons (e.g. taxol) isolated from the bark of the Pacific Yew tree, are found to be significantly effective in ovarian cancer. Steroid hormone antagonists (e.g. tamoxifen) are developed and used to treat oestrogen receptor-positive breast cancer patients.
2000s	Some cancers – testicular, prostate, ovarian, endometrial and breast – become more treatable with more selective anti-hormone medicines such as aromatase inhibitors, gonadotrophin analogues, anti-androgens and improving anti-oestrogen drugs. Hormone-sensitive cancers are better treated with these newer agents. <i>Rational Drug Design</i> : Nicholas Lydon and Brian Druker took a more rational approach to designing an inhibitor of the Bcr-Abl Philadelphia chromosome found in CML. Their drug, Gleevec, was effective in those patients and its use has improved survival rates in CML cancer patients from 50 to 95% of all cases. This design of a more specifically targeted drug has encouraged the development of many similar agents aimed at more specific subtypes of cancers. <i>Stem Cell Transplants</i> : Autologous- (from self) or allogenic- (from a healthy donor) transplants of haematopoietic stem cells from bone marrow, greatly improves survival chances of many patients with blood cancers that do not respond well to conventional chemotherapies.
2010s	Success in the removal of primary tumours. Hormone therapies increasingly promising. Treatments such as chemotherapy and radiotherapy still have chronic side effects, but improvements are sought in the co-administration of drugs to improve the management of unwanted side-effects. <i>TKIs</i> : The success of Gleevec, an inhibitor of the Abelson tyrosine kinase, leads to a rapid expansion of knowledge of the effectiveness of TKIs in certain types of cancers. Because of the widespread nature of tyrosine phosphorylation, many of the side-effects of these drugs can be extreme. Inhibitors designed against growth factor receptors (EGF, FGF, PDGF, VEGF) prove useful in the clinic. Much of the success comes through refinement of the use of these agents in combination therapies. <i>Biological agents</i> : The use of 'humanized' monoclonal antibodies against TNF was hugely successful in treating of inflammatory diseases such as rheumatoid arthritis. The synthesis of 'humanized' monoclonal antibodies such as Herceptin (against the HER2 protein found in many breast cancer cases) or Rituximab (against the CD20 found in non-Hodgkin's lymphoma cases) revolutionize the treatment of those types of cancer, with morbidity rates falling dramatically after their introduction.
Today	The race to understand cancer, improve diagnosis and genotyping, as well as developing more selective and effective pharmacological treatments continues. A combination of clinical, genomic, and proteomic data may herald a new era of smart pharmacogenomics, where treatments are more targeted towards the individual subtype or phenotype of cancer that you have. Virtually all cancers have improved their overall survival rates as a result of these treatments. Some cancers (such as testicular cancer and CML) are nearly 100% 'curable'; whereas other cancers such as Hodgkin's lymphoma and childhood leukaemia, which were previously universally fatal, now have markedly better odds of survival, because of these improvements in treatment.
Tomorrow	The refining of cryosurgery – using liquid nitrogen to freeze, lasers to cut and vaporize cancers – and radiofrequency to heat cancer cells into submission. Better genotyping and proteomic methods for smarter screening of cancer subtypes. Personalized medicine with tumour cell genomic sequencing to define the exact drugs needed for each individual patient's disease. Development of more targeted therapies (nanotechnology) with fewer side effects. Increased use of monoclonal antibodies as immunoconjugates or radio-labelled immunoconjugates, or glycoconjugates to target specific cancer cells to improve the selective delivery of the cytotoxic payloads, only to the cancer cells. Keyhole surgery has saved the lives of many who could not survive the trauma of major surgery and, similarly, robotic surgery will allow effective excision of tumours with less surgical trauma.



**Table 2**

Some examples of recent, specifically targeted drugs used for cancer. Note that currently there are over 300 mAb-based potential therapeutic agents

Name	Target	Comment
<b>Monoclonal antibodies</b>		
Alemtuzumab	CD52	Mature B-cells (CLL), B-cell CLL
Belimumab	BAFF/BLyS	B-cell hyperactivity disorders/systemic lupus erythematosus
Bevacizumab	VEGF-A	Angiogenesis inhibitor
Catumaxomab	EpCAM	Tumour marker
Cetuximab, panitumumab	EGFR/ErbB/HER1	Colorectal cancer
Daratumumab	CD38	Multiple myeloma
Dupilumab	IL-4 receptor $\alpha$ -subunit	Asthma
Elotuzumab	CS-1	Myeloma
Gemtuzumab	CD33	Multi-lineage B-cells (AML).
Infliximab, adalimumab	TNF	Autoimmune diseases, e.g. rheumatoid arthritis, Crohn's disease, ankylosing spondylitis.
Ipilimumab	CTLA-4	Melanoma
MPDL320A	PD-1 ligand (CD274)	Kidney, lung, colorectal, gastric cancers
Nivolumab, pembrolizumab	PD-1 receptor (CD279)	NSCLC, renal cell carcinoma, melanoma
Ramucirumab	VEGFR-2	Anti-angiogenesis, colon cancer
Rituximab, ibritumomab, obinutuzumab	CD20. Yttrium-90/indium-111-labelled, glycosylation-engineered variants	Lymphoid B-cells (non-Hodgkin's lymphoma), CLL, diffuse large B-cell lymphoma
Trastuzumab, tositumomab	HER2/neu receptor	Breast cancer
<b>Tyrosine kinase small molecule inhibitors</b>		
Axitinib	PDGFR/VEGFR/c-kit	Renal cell carcinoma
Bosutinib	Src	Leukaemia
Brivanib	VEGF and FGF	Colon cancer
Crizotinib, LDK378	ALK, ROS1	Anaplastic large cell lymphoma, non-small cell lung carcinoma, neuroblastoma,
Erlotinib, gefitinib	EGFR/ErbB/HER1	NSCLC
Ibrutinib	BTK	CLL, multiple myeloma, lymphomas
Imatinib, nilotinib	BCR-Abl	CML
Lapatinib	HER2/neu	Breast cancer
Lestaurtinib	FLT3, JAK2, TrkA/B/C	AML, myeloproliferative disorders
Ruxolitinib	JAK 1 and 2	JAK2 V617F MDS, AML, for polycythemia vera, myelofibrosis
Sorafenib	Raf kinases/PDGFR/VEGFR	Renal cell carcinoma, hepatocellular carcinoma
Vandetanib	VEGFR	
Vemurafenib, regorafenib	BRAF	Melanoma, colon cancer
<b>Other</b>		
Abiraterone	CYP17	Castration-resistant prostate cancer
Aflibercept	VEGF-A/B, placental growth factor	Fusion protein for colorectal cancer and macular degeneration
BIND-014	Prostate-specific membrane antigen	Docetaxel loaded nanoparticle for treatment of prostate, lung and bladder cancers
Bortezomib	Proteasome inhibitor	Multiple myeloma
Enzalutamide	Androgen receptor antagonist	Prostate cancer
Etanercept	TNF	TNFR2–TNFR2 fusion protein
Idelalisib	PI-3-K $\delta$	CLL, non-Hodgkin's lymphoma
Omacetaxine	Proteasome inhibitor	Imatinib-resistant T315I mutant BCR-Abl CML
Palbociclib	Cyclin-dependent kinases –4 and –6	Breast cancer
Perifosine	Akt and JNK pathways	
Talimogene laherparepvec	Oncolytic virus	Melanoma
Vismodegib	Sonic Hedgehog pathway	Basal cell carcinomas

ALK, anaplastic lymphoma kinase; AML, acute myeloid leukaemia; BAFF, B cell activating factor; BLyS, B lymphocyte stimulator; BTK, Bruton's tyrosine kinase; CLL, chronic lymphocytic leukaemia; CML, chronic myeloid leukaemia; CS-1, CD2 subset-1; CTLA, cytotoxic T lymphocyte antigen; DLBCL, diffuse large B-cell lymphoma; EpCAM, epithelial cell adhesion molecule; ErbB, epidermal growth factor receptor; FGF, fibroblast growth factor; FLT, Fms-like tyrosine kinase; HER, human epidermal growth factor receptor; JAK, Janus-activated kinase; JNK, c-Jun N-terminal-kinase; MDS, myelodysplastic syndrome; NSCLC, non-small cell lung cancer; PD, programmed cell death protein; PDGFR, platelet-derived growth factor receptor; PI-3-K, phosphatidylinositol-3-kinase; ROS1, c-ros oncogene 1; VEGFR, vascular endothelial growth factor receptor.

agents. Breast cancer was once again at the forefront of early chemotherapy towards solid tumours. It is interesting that these cancer types are again showing the greatest success in cancer research.

One outcome was clear from these range of compounds that constituted the entire arsenal for cancer therapy – although they may have had different molecular mechanisms of action, their cellular basis of action was to kill the rapidly dividing cancer cells rather than their slower-growing, non-cancerous counterparts. And for many decades, the pharmaceutical industry tried in vain to generate more targeted ‘magic bullets’.

## Targeted design

Brian Druker is credited with the dawn of rational drug design in pharmacology. His drug imatinib was certainly at the forefront of drug design in this new shiny era in pharmacology. Anything was possible. It is fortunate that this tyrosine kinase inhibitor (TKI) has good selectivity towards the Abelson TK, and also towards the BCR-Abl (breakpoint cluster region-Abelson chimaera or the Philadelphia chromosome or translocation t (9;22)(q34.1;q11.2)) chimeric TK of the Philadelphia chromosome found in many chronic myeloid leukaemia (CML) patients. This ‘wonder drug’ has sustained the majority of CML patients, turning the diagnosis from a death sentence 20 years ago into something to live with. Now, many of the challenges in this form of cancer are about the best way to treat those who are resistant to imatinib (Heasman *et al.* 2011).

This drug did infuse a new vigour into the pharmaceutical industry. If their TKI can work, maybe we can tailor our TKI

into something effective too. Indeed, a significant part of the recent advances in oncology therapeutics has been just such refinement of TKI use (Table 2). The invention of humanized anti-TNG- $\alpha$  monoclonal antibodies (mAb) revolutionized the treatment of autoimmune diseases, especially rheumatoid arthritis. This success also encouraged industry to pursue the mAb approach and come up with rituximab for the treatment of non-Hodgkin’s lymphoma (to which my mother-in-law owes her longevity). It is now pleasing to see the development of a wide range of mAbs that are increasingly used in the treatment of an equally wide range of cancer types (Table 2). As well as the more traditional small-molecule inhibitors, many of these cancer agents are mAbs, radiolabelled-mAbs, or chimeric proteins. Other exciting cancer agents include oncolytic viruses, or even nanoparticles loaded with chemotherapeutic agents. Both these types of therapy are able to direct the drug to more selectively to cancer cells and thus to deliver more effectively their payload of cytotoxic chemicals. As well as improvements in drug delivery systems, it should also be noted that much of the recent improvements in the treatment of cancers and in survival rates has come about by clinical trials to refine such therapies used either as monotherapies at various doses, or used in combination therapy. Although we are heading in the right direction, as summarized in Table 3, the overall success of these cancer drugs is dwarfed by many more treatments for much less life-threatening conditions.

## Challenges

As shown by some of the reviews in this issue, there are still many challenges within oncology. For example, targeting the

**Table 3**

The top selling drugs of 2012

Rank	Name	Trade name	Target	Treating
1	Adalimumab	Humira	TNF $\alpha$	Rheumatoid arthritis
2	Etanercept	Enbrel	TNF $\alpha$	Rheumatoid arthritis
3	Fluticasone/salmeterol	Advair/Seretide	Glucocorticoid and $\beta_2$ -adrenoceptor	Asthma
4	Infliximab	Remicade	TNF $\alpha$	Rheumatoid arthritis
<b>5</b>	<b><i>Rituximab</i></b>	<b><i>Rituxan</i></b>	<b><i>CD20 mAb</i></b>	<b><i>Non-Hodgkin’s lymphoma</i></b>
6	Rosuvastatin	Crestor	HMG-CoA reductase (inhibitor)	Atherosclerosis
7	Insulin glargine	Lantus	Insulin analogue	Type 1 diabetes
<b>8</b>	<b><i>Trastuzumab</i></b>	<b><i>Herceptin</i></b>	<b><i>Her2/Neu mAb</i></b>	<b><i>Breast cancer</i></b>
<b>9</b>	<b><i>Bevacizumab</i></b>	<b><i>Avastin</i></b>	<b><i>VEGF-A mAb</i></b>	<b><i>Tumour angiogenesis</i></b>
10	Atorvastatin	Lipitor	HMG-CoA reductase (inhibitor)	Atherosclerosis
11	Aripiprazole	Abilify	D <sub>2</sub> /5-HT <sub>1A</sub> receptor partial agonist	Antipsychotic/ antidepressant
12	Clopidogrel	Plavix	P2Y <sub>12</sub> receptor antagonist	Antiplatelet/ anticoagulant
13	Duloxetine	Cymbalta	Serotonin/noradrenaline re-uptake inhibitor	Antidepressant/ anti-anxiety
<b>14</b>	<b><i>Imatinib</i></b>	<b><i>Gleevec</i></b>	<b><i>BCR-Abl tyrosine kinase</i></b>	<b><i>Philadelphia Chromosome positive CML</i></b>
15	Tiotropium	Spiriva	M <sub>3</sub> receptor antagonist	Asthma/COPD

Cancer drugs are in bold and italics.

stem cells is necessary to eradicate the cancer (Piccoli *et al.*, 2013). Cell maintenance and conditioning may require other cytokine co-factors that could be pharmacological targets of synthetic or naturally occurring compounds (Aggarwal *et al.*, 2013). Also a cancer stem cell may not behave the same in its native microenvironment (Sinclair *et al.*, 2013) as it would *in vitro*. Much of the way in which we attempt to treat cancers may require simultaneous targeting of several pathways to defeat the overlapping resistance mechanisms that can be created in tumours (Sale and Cook, 2013). We may also have to utilize nature's own attack systems, such as the death receptors (Micheau *et al.*, 2013) that belong to the TNF receptor superfamily (MacEwan, 2002). Cancer researchers may also need to look towards the death mechanisms within cells, given that no matter how a cell creates a cancerous phenotype, there are only a handful of ways that the cell can be killed (Kvinlaug *et al.*, 2011). Furthermore, there may be an absolute need to block the cancer cell's own repair mechanisms (Curtin, 2013) for therapy to be fully effective.

As can be seen from this issue, there is still a lot of work that needs to be done to understand the biology of each individual type of cancer. In this regard, patient-specific genomic sequencing of tumour biopsies is likely and this information will be translated into a personalized drug regime for each patient. The question is, do we have the right drugs to cope with such an ideal approach to treatment? Many questions remain to be solved. For example, one of the most successful cancer drugs in trial is ibrutinib, an inhibitor of Bruton's TK (BTK), in the treatment of B cell blood cancers (MacEwan *et al.*, 2013). The clinical success of this drug is clear. What is less clear is what makes this compound such a successful candidate with limited side effects, relative to other similar chemotherapeutic agents. It may be related to its physicochemical properties, affecting its pharmacodistribution and thus limiting adverse reactions to the drug in the body. It may be related to the selectivity of the BTK-signalling machinery in cellular processes such as apoptosis, adhesion, doubling time, differentiation or even platelet aggregation. Whatever the reason, this drug, along with the other successes outlined here, are a source of optimism for the eventual control and eradication of cancer. And with good

science, we will prevail. To finish with my earlier analogy – unlike antibiotics, with cancer drugs, there have been enormous advances made over the last century. Long may it continue.

## References

- Aggarwal BB, Gupta SC, Sung B (2013). Curcumin: an orally bioavailable blocker of TNF and other pro-inflammatory biomarkers. *Br J Pharmacol* 169: 1672–1692.
- Curtin NJ (2013). Inhibiting the DNA damage response as a therapeutic manoeuvre in cancer. *Br J Pharmacol* 169: 1745–1765.
- Heasman S, Small M, MacEwan DJ (2011). Targeted CML treatments. *Br Oncol Pharm Assoc J* 3: 11–15.
- Kvinlaug BT, Chan WI, Bullinger L, Ramaswami M, Sears C, Foster D *et al.* (2011). Common and overlapping oncogenic pathways contribute to the evolution of acute myeloid leukemias. *Cancer Res* 71: 4117–4129.
- MacEwan DJ (2002). TNF ligands and receptors: a matter of life and death. *Br J Pharmacol* 135: 855–875.
- MacEwan DJ, Bowles KM, Rushworth SA (2013). Emerging therapy targeting Bruton's tyrosine kinase (BTK): potential for ibrutinib and other BTK inhibitors. *Drugs Future*. (in press).
- Micheau O, Shirley S, Dufour F (2013). Death receptors as targets in cancer. *Br J Pharmacol* 169: 1723–1744.
- Nicholls AG (1936). The late William Blair-Bell, M.D., F.R.C.S. *Can Med Assoc J* 34: 683–684.
- Piccoli C, Agriesti F, Scrima R, Falzetti F, Di Ianni M, Capitanio N (2013). To breathe or not to breathe: the haematopoietic stem/progenitor cells dilemma. *Br J Pharmacol* 169: 1652–1671.
- Sale MJ, Cook SJ (2013). That which does not kill me makes me stronger; combining ERK1/2 pathway inhibitors and BH3 mimetics to kill tumour cells and prevent acquired resistance. *Br J Pharmacol* 169: 1708–1722.
- Sinclair A, Latif AL, Holyoake TL (2013). Targeting survival pathways in chronic myeloid leukaemia stem cells. *Br J Pharmacol* 169: 1693–1707.



Choose Specialty ▼

Advertisement

## Evolution of Care in Advanced Kidney Cancer

Sep 19, 2013

Anna Azvolinsky, PhD

Publication Article

### Targeted Therapies in Oncology

September 2013

Volume 2 Issue 5



*With seven targeted therapies approved for metastatic renal cell carcinoma (RCC), researchers and drug developers are now focusing on understanding the best way to sequence these therapies and on identifying predictive biomarkers of response.*

Brian Rini, MD

### Which Agent First-Line?

With seven targeted therapies approved for metastatic renal cell carcinoma (RCC), researchers and drug developers are now focusing on understanding the best way to sequence these therapies and on identifying predictive biomarkers of response. Six of these agents are oral and fall into two categories: multi-tyrosine kinase inhibitors against the vascular endothelial growth factor (VEGF) or inhibitors of the mammalian target of rapamycin (mTOR). Based on recent phase III randomized, large clinical trials, two VEGF inhibitors are now the standard of care for first-line RCC.

Sunitinib was the first VEGF inhibitor approved for RCC and is still widely used as a result of strong clinical data showing greater progression-free survival (PFS) and overall survival (OS) in treatment-naïve patients compared to interferon alfa.<sup>1</sup> The second widely used oral VEGF inhibitor is pazopanib; studies have demonstrated that pazopanib improved PFS in treatment-naïve metastatic RCC compared to placebo.<sup>2</sup>

The recent phase II RECORD-3 trial,<sup>3</sup> comparing first-line everolimus, an mTOR inhibitor, followed by sunitinib to the reverse sequence, showed that treatment with sunitinib first produced longer PFS for patients.

The COMPARZ trial,<sup>4</sup> reported at the 2012 annual European Society for Medical Oncology (ESMO) Congress in Vienna, Austria, directly compared the efficacy of pazopanib to sunitinib in patients with metastatic, treatment-naïve RCC. While both drugs demonstrated similar efficacy (31% overall response rate in the pazopanib arm vs 25% in the sunitinib arm; 28.4 months OS in the pazopanib arm vs 29.3 months in the sunitinib arm), patients perceived pazopanib to have a better side-effect profile compared with sunitinib. Patients on pazopanib had less hematological toxicity, hand-foot syndrome, rash, fatigue, and peripheral edema.

From their experience at the Johns Hopkins Sidney Kimmel Comprehensive Cancer Center, Jenny J. Kim, MD, assistant professor of Oncology, said that both sunitinib and pazopanib are being used in the frontline setting for these patients, although these agents have slightly different side-effect profiles. Kim and her colleagues have noticed that in their experience, patients tend to have more



hand-foot syndrome and stomatitis with sunitinib and more liver toxicity with pazopanib. With the approval of pazopanib in 2009, it has been well incorporated along with sunitinib into the gastroenterology practice at Johns Hopkins in the treatment of metastatic RCC.

“There is a trend towards favoring pazopanib recently,” said Kim. “This is most likely due to a lack of handfoot syndrome, which seems to affect patients’ day-to-day quality of life.” This observation in clinical practice is consistent with what was seen in the COMPARZ trial.

It is important to mention, Kim noted, that discontinuation rates were similar for both sunitinib and pazopanib in the study, although patient subjective toxicity experience was better with pazopanib. One potential explanation may be that there were higher liver toxicities with pazopanib compared with sunitinib, which led to discontinuation of pazopanib in the trial.

### Targeted Agent Sequencing

Another small, 168-patient study, the PISCES trial,<sup>5</sup> reinforced the COMPARZ trial data, showing that 70% of patients on the trial preferred pazopanib while 22% preferred sunitinib. “While these trials are not perfect, they will likely have an impact on the oncologic community as more ‘tangible’ data comparing the two agents,” said Kim. Although second-line and subsequent therapies become gradually less effective, axitinib, sorafenib, and everolimus are all options for second-line therapy. “The only drug that has been tested exclusively in a second-line setting is axitinib,” said Brian Rini, MD, professor of Medicine at Case Western Reserve University and a practicing oncologist at the Cleveland Clinic Taussig Cancer Institute in Cleveland, Ohio. Axitinib is a VEGF inhibitor approved for metastatic RCC after failure of one systemic therapy; approval was based on the AXIS trial.<sup>6</sup> For patients who had previously failed sunitinib, axitinib significantly delayed PFS for 2.6 months longer compared with sorafenib.

“Clinical trials point to the importance of clinicians to maximize each oral targeted agent and to expose patients to multiple drugs over the course of metastatic disease,” said Rini. When to switch a patient’s therapy in the clinical setting remains a challenge, as the timing of the switch from one therapy to another has not been formally tested. A specific and potent sequencing regimen probably does not exist with the current drugs we have, unfortunately, said Rini.

### PFS versus OS?

According to Kim, all of these agents are being used in the second-line setting because there is a lack of complete cross-resistance among them. “At this point, without robust biomarkers of response, exposure to all different agents is probably key for the patients’ best clinical outcome,” said Kim. The recently announced phase III INTORSECT trial compared temsirolimus, an mTOR inhibitor, to sorafenib, a VEGF inhibitor, in patients previously treated with sunitinib.<sup>7</sup> Although there was no difference in PFS, patients on sorafenib had a longer OS compared with those on temsirolimus.

### The Next Wave of Therapies

“This result raises a major question,” said Kim. “Is PFS a valid endpoint for these trials or should we be using OS as a better measure of efficacy?” The checkpoint inhibitors currently in development are clearly promising, said Rini. “These are the next wave of effective kidney cancer therapies.” According to Rini, effectiveness of anti-VEGF therapy may have reached a plateau and the development of the immunotherapy checkpoint antibodies are revitalizing kidney cancer research, providing patients with a chance at durable, potentially longterm responses.

Results from a phase I trial with the anti-PD-1 antibody nivolumab (BMS- 936558) show that the immunotherapy can produce responses in approximately 30% of heavily pretreated patients, and that the responses are durable.<sup>8</sup> Nivolumab is now being tested in a phase III trial in pretreated advanced or metastatic RCC.<sup>9</sup> Two other immunotherapy checkpoint agents, MPDL3280A and lambrolizumab, are currently in phase I trials for solid tumors. “There have been great results so far with nivolumab,” said Kim.

Although most combinations of targeted agents have proven to be disappointing either due to toxicity or lack of improved efficacy, checkpoint inhibitors may provide renewed promise in the combinatorial efforts, according to Kim.

“Within the last decade, we have almost tripled the lifespan of metastatic RCC patients,” said Kim. The checkpoint inhibitors now have the potential to turn kidney cancer into a chronic disease, she said.

The next questions for both the newer immunotherapies and targeted oral drugs is how to best identify patients who are most likely to respond. There are no biomarkers yet, but intensive research to identify them is under way.

## References

1. Motzer RJ, Hutson TE, Tomczak P, et al. Overall survival and updated results for sunitinib compared with interferon alfa in patients with metastatic renal cell carcinoma. *J Clin Oncol*. 2009;27(22):3584-3590.
2. Sternberg CN, Davis ID, Mardiak J, et al. Pazopanib in locally advanced or metastatic renal cell carcinoma: results of a randomized phase III trial. *J Clin Oncol*. 2010;28(6):1061-1068.
3. Motzer RJ, Barrios CH, Kim TM, et al. Record-3: phase II randomized trial comparing sequential first-line everolimus (EVE) and second-line sunitinib (SUN) versus first-line SUN and second-line EVE in patients with metastatic renal cell carcinoma (mRCC). *J Clin Oncol*. 2013;31(suppl, abstract 4504).
4. Motzer R, Hudson TE, Reeves J, et al. Randomized, open-label, phase III trial of pazopanib versus sunitinib in first-line treatment of patients with metastatic renal cell carcinoma: results of the COMPARZ trial. Presented at: the 2012 European Society for Medical Oncology Congress; September 28-October 2, 2012; Vienna, Austria. *Ann Oncol*. 2012;23(suppl 9; abstr LBA 8).
5. Escudier BJ, Porta C, Bono P, et al. Patient preference between pazopanib (Paz) and sunitinib (Sun): results of a randomized double-blind, placebo- controlled, cross-over study in patients with metastatic renal cell carcinoma (mRCC) – PISCES study. Presented at: the 2012 American Society of Clinical Oncology Annual Meeting; June 1-5, 2012; Chicago, IL. *J Clin Oncol*. 2012;30(suppl; abstr CRA4502).
6. Motzer RJ, Escudier B, Tomczak P, et al. Axitinib versus sorafenib as second-line treatment for advanced renal cell carcinoma: overall survival analysis and updated results from a randomised phase 3 trial. *Lancet Oncol*. 2013;14(6):552-562.
7. Hutson T, Escudier B, Esteban E, et al. Temsirolimus vs sorafenib as second line therapy in metastatic renal cell carcinoma: results from the INTORSECT trial. Presented at: the 2012 European Society for Medical Oncology Congress; September 28-October 2, 2012; Vienna, Austria. *Ann Oncol*. 2012;23(suppl 9; abstr LBA22).
8. Drake CG, McDermott DF, Sznol M, et al. Survival, safety, and response duration results of nivolumab (anti-PD-1; BMS-936558; ONO- 4538) in a phase I trial in patients with previously treated metastatic renal cell carcinoma (mRCC): long-term patient follow-up. *J Clin Oncol*. 2013;31(suppl, abstr 4514).
9. Study of BMS-936558 vs everolimus in pretreated advanced or metastatic clear-cell RCC. Clinicaltrials.gov ID: NCT01668784.

## Articles in this issue



Sequester Forcing Chemo Treatments Into Hospitals Costing Taxpayers More

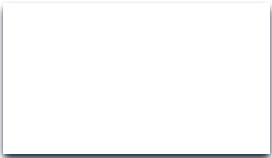


Drug Shortages Adversely Affect Treatment Decisions

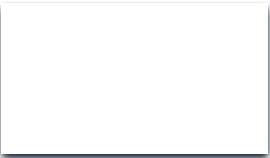
## Related Videos



Clear Cell RCC: Strategies for Selecting Appropriate Frontline Treatment



Clinical Trial Data Updates in Advanced Renal Cell Carcinoma



Recent Data on Lenvatinib Plus Pembrolizumab in Advanced RCC



CLEAR  
Pembrolizumab in Advanced Renal Cell Carcinoma

Related Content





Roundtable Roundup: Graft-vs-Host Disease  
August 31st 2023

About Us  
CureToday.com  
OncNursingNews.com  
Terms & Conditions

Advertise  
CancerNetwork.com  
Do Not Sell My Information

Contact Us  
OncLive.com  
Privacy

Contact Info  
 2 Clarke Drive  
Cranbury, NJ 08512  
 609-716-7777



© 2023 MJH Life Sciences  
All rights reserved.



# Comparative effectiveness of axitinib versus sorafenib in advanced renal cell carcinoma (AXIS): a randomised phase 3 trial



Brian I Rini, Bernard Escudier, Piotr Tomczak, Andrey Kaprin, Cezary Szczylik, Thomas E Hutson, M Dror Michaelson, Vera A Gorbunova, Martin E Gore, Igor G Rusakov, Sylvie Negrier, Yen-Chuan Ou, Daniel Castellano, Ho Yeong Lim, Hirotsugu Uemura, Jamal Tarazi, David Cella, Connie Chen, Brad Rosbrook, Sinil Kim, Robert J Motzer

## Summary

**Background** The treatment of advanced renal cell carcinoma has been revolutionised by targeted therapy with drugs that block angiogenesis. So far, no phase 3 randomised trials comparing the effectiveness of one targeted agent against another have been reported. We did a randomised phase 3 study comparing axitinib, a potent and selective second-generation inhibitor of vascular endothelial growth factor (VEGF) receptors, with sorafenib, an approved VEGF receptor inhibitor, as second-line therapy in patients with metastatic renal cell cancer.

**Methods** We included patients coming from 175 sites (hospitals and outpatient clinics) in 22 countries aged 18 years or older with confirmed renal clear-cell carcinoma who progressed despite first-line therapy containing sunitinib, bevacizumab plus interferon-alfa, temsirolimus, or cytokines. Patients were stratified according to Eastern Cooperative Oncology Group performance status and type of previous treatment and then randomly assigned (1:1) to either axitinib (5 mg twice daily) or sorafenib (400 mg twice daily). Axitinib dose increases to 7 mg and then to 10 mg, twice daily, were allowed for those patients without hypertension or adverse reactions above grade 2. Participants were not masked to study treatment. The primary endpoint was progression-free survival (PFS) and was assessed by a masked, independent radiology review and analysed by intention to treat. This trial was registered on ClinicalTrials.gov, number NCT00678392.

**Findings** A total of 723 patients were enrolled and randomly assigned to receive axitinib (n=361) or sorafenib (n=362). The median PFS was 6·7 months with axitinib compared to 4·7 months with sorafenib (hazard ratio 0·665; 95% CI 0·544–0·812; one-sided p<0·0001). Treatment was discontinued because of toxic effects in 14 (4%) of 359 patients treated with axitinib and 29 (8%) of 355 patients treated with sorafenib. The most common adverse events were diarrhoea, hypertension, and fatigue in the axitinib arm, and diarrhoea, palmar-plantar erythrodysesthesia, and alopecia in the sorafenib arm.

**Interpretation** Axitinib resulted in significantly longer PFS compared with sorafenib. Axitinib is a treatment option for second-line therapy of advanced renal cell carcinoma.

**Funding** Pfizer Inc.

## Introduction

Renal cell carcinoma is diagnosed in about 170 000 people worldwide annually, resulting in 72 000 deaths.<sup>1</sup> Many patients present with advanced or unresectable disease, and up to 30% of patients who have initially localised disease will eventually relapse.<sup>2</sup> Renal cell carcinoma is often resistant to chemotherapy and cytokines such as interleukin 2 and interferon-alfa.<sup>3</sup>

Loss of function of the von Hippel-Lindau (VHL) gene in renal cell carcinoma leads to dysregulation of the vascular endothelial growth factor (VEGF) pathway, VEGF protein overexpression, and increased tumour angiogenesis. Antiangiogenic drugs targeting the VEGF pathway with proven benefit in renal cell carcinoma include inhibitors of VEGF receptor (VEGFR; sunitinib, sorafenib, and pazopanib) and bevacizumab.<sup>4–7</sup> All these drugs have been approved on the basis of randomised trials comparing the targeted drug to either cytokine

therapy or placebo. To date, no phase 3 study comparing two targeted drugs has been reported.

Axitinib is a potent, selective, second-generation inhibitor of VEGFR 1, 2, and 3 that blocks VEGFRs at sub-nanomolar drug concentrations.<sup>8</sup> Relative potency of axitinib is 50–450 times greater than that of the first-generation VEGFR inhibitors.<sup>9</sup> Additionally, first-generation inhibitors block other targets, such as platelet-derived growth factor receptors (PDGFR), b-RAF, KIT, and FLT-3, which are not substantially inhibited by axitinib.<sup>10–12</sup> These off-target activities might contribute to the adverse effects of the first-generation inhibitors, suggesting that more specific inhibitors of VEGFR such as axitinib might have an enhanced therapeutic window.

In a phase 2 study<sup>13</sup> of patients with cytokine-refractory renal cell carcinoma, the objective response rate with axitinib was 44%, with a median time to progression of

*Lancet* 2011; 378: 1931–39

Published Online

November 4, 2011

DOI:10.1016/S0140-

6736(11)61613-9

See [Comment](#) page 1898

Cleveland Clinic Taussig Cancer Institute, Main Campus, Euclid Avenue, Cleveland, OH, USA (B I Rini MD); Institut Gustave

Roussy/Service d'Immunothérapie, Villejuif, France (B Escudier, MD); Klinika

Onkologii, Uniwersytet

Medyczny, Poznań, Poland (P Tomczak MD); Russian

Research Centre of

Roentgenology and

Radiology, Moscow, Russia (Prof A Kaprin MD); Central

Clinical Hospital of Military

Medical Institute, Klinika

Onkologii, Warsaw, Poland (C Szczylik MD);

Baylor-Sammons/Texas

Oncology Physician's

Association, Sammons Cancer

Center, Dallas, TX, USA (T E Hutson MD); Massachusetts

General Hospital Cancer

Center, Boston, MA, USA (M D Michaelson MD); State

Institution National

Cancer Research Center,

Chemotherapy Department,

Moscow, Russia (Prof V A Gorbunova MD); Royal

Marsden Hospital, London, UK (Prof M E Gore MD); Herten

Research Institute of Oncology,

Moscow, Russia (Prof I G Rusakov MD); Centre

Léon Bérard, Service

d'Oncologie Médicale, Lyon,

France (Prof S Negrier MD);

Taichung Veterans General

Hospital, Department of

Surgery 160, Section 3,

Taichung, Taiwan (Prof Y-C Ou MD); Hospital

Universitario 12 de Octubre,

Servicio Oncología Médica,

Madrid, Spain (D Castellano MD); Samsung

Medical Center,

Hematology-Oncology,



Gangnam-Gu, Seoul, South Korea (Prof H Y Lim MD); Kinki University Hospital, Department of Urology, Osaka-Sayama, Osaka, Japan (Prof H Uemura MD); Pfizer Inc, Clinical Oncology, San Diego, CA, USA (J Tarazi MD, B Rosbrook MS, S Kim MD); Northwestern University, Medical Social Sciences, Chicago, IL, USA (D Cella PhD); Pfizer Inc, Global Outcomes Research, New York, NY, USA (C Chen PharmD); and Memorial Sloan-Kettering Cancer Center, New York, NY, USA (R J Motzer MD)

Correspondence to: Dr Brian I Rini, Cleveland Clinic Taussig Cancer Institute, Main Campus, Mail Code R35, 9500 Euclid Avenue, Cleveland, OH 44195, USA  
riniib2@ccf.org

15·7 months and an overall survival of 29·9 months.<sup>13</sup> The 5-year survival rate was 20·6% (95% CI 10·9–32·4).<sup>14</sup> In Japanese patients with cytokine-refractory renal cell carcinoma, the objective response rate with axitinib was 55%, with a median progression-free survival (PFS) of 12 months.<sup>15</sup> In patients with sorafenib-refractory renal cell carcinoma, the objective response rate with axitinib was 23%, with a median PFS of 7·4 months.<sup>16</sup> These phase 2 data suggested that axitinib could be an effective second-line treatment in patients with advanced renal cell carcinoma. This randomised phase 3 trial (AXIS) was designed to directly compare the efficacy and safety of axitinib versus sorafenib in patients with advanced renal cell carcinoma who had disease progression after initial systemic therapy.

	Axitinib (n=361)	Sorafenib (n=362)
<b>Age (years)</b>		
Median (range)	61 (20–82)	61 (22–80)
<b>Sex</b>		
Male	265 (73%)	258 (71%)
Female	96 (27%)	104 (29%)
<b>Ethnic origin</b>		
White	278 (77%)	269 (74%)
Black	1 (<1%)	4 (1%)
Asian	77 (21%)	81 (22%)
Other	5 (1%)	8 (2%)
<b>ECOG performance status</b>		
0	195 (54%)	200 (55%)
1	162 (45%)	160 (44%)
>1	1 (<1%)*	0
<b>MSKCC risk subgroup†‡</b>		
Favourable	100 (28%)	101 (28%)
Intermediate	134 (37%)	130 (36%)
Poor	118 (33%)	120 (33%)
NA	9 (2%)	11 (3%)
<b>Heng et al<sup>22</sup> risk factors‡</b>		
Favourable	66 (18%)	79 (22%)
Intermediate	236 (65%)	225 (62%)
Poor	37 (10%)	34 (9%)
NA	22 (6%)	24 (7%)
<b>Previous systemic therapy</b>		
Sunitinib	194 (54%)	195 (54%)
Cytokines	126 (35%)	125 (35%)
Bevacizumab	29 (8%)	30 (8%)
Temsirolimus	12 (3%)	12 (3%)

Data are n (%) unless otherwise stated. ECOG=Eastern Cooperative Oncology Group. MSKCC=Memorial Sloan-Kettering Cancer Center. NA=not available. \*Protocol violation. †MSKCC risk groups were derived with three risk factors: haemoglobin ( $\leq 130$  g/L vs  $>130$  g/L for men, and  $\leq 115$  g/L vs  $>115$  g/L for women), corrected calcium ( $<2.5$  mmol/L vs  $\geq 2.5$  mmol/L), and ECOG performance status (0 vs 1). MSKCC risk groups were defined as favourable (0 factors), intermediate (1 factor), or poor (2–3 factors). ‡Heng risk groups were defined as favourable (0 factors), intermediate (1–2 factors), or poor ( $\geq 3$  factors).

**Table 1: Baseline demographics and clinical characteristics**

## Methods

### Study design and participants

In this multicentre phase 3 randomised controlled trial, we enrolled patients 18 years or older with histologically or cytologically confirmed renal cell carcinoma with a clear-cell component. All patients had measurable disease by Response Evaluation Criteria in Solid Tumours (RECIST, version 1.0)<sup>17</sup> and RECIST-defined progressive disease as assessed by investigators after one previous systemic first-line regimen with a sunitinib-based, bevacizumab plus interferon-alfa-based, temsirolimus-based, or cytokine-based regimen, which reflected all regimens with regulatory approvals at the time of study design. Eligibility criteria were 2 weeks or more since end of previous systemic treatment (4 weeks or more for bevacizumab plus interferon-alfa); Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1; life expectancy of 12 weeks or more; and adequate renal, hepatic, and haematological organ function. Exclusion criteria were history of malignancy other than renal cell carcinoma; present use or anticipated need for cytochrome P450 (CYP)3A4-inhibiting, CYP3A4-inducing, or CYP1A2-inducing drugs; known HIV or acquired immunodeficiency syndrome-related disease; CNS metastasis; uncontrolled hypertension; myocardial infarction, uncontrolled angina, congestive heart failure, or cerebrovascular accident within previous 12 months; and deep vein thrombosis or pulmonary embolism within previous 6 months.

The trial was approved by the institutional review board or ethics committee at every centre and complied with Good Clinical Practice guidelines, the Declaration of Helsinki, and local laws. All patients provided written informed consent before any trial procedure.

### Randomisation and masking

We stratified patients according to ECOG performance status (0 or 1) and type of previous treatment (ie, regimen containing sunitinib, bevacizumab, temsirolimus, or cytokine), and then randomly assigned them (1:1) to receive either axitinib or sorafenib. Randomisation lists were generated from an independent randomisation group using a permuted block design of size four (two to axitinib and two to sorafenib) within each stratum. A web-enabled centralised registration system concealed treatment allocation before registration and allowed centres to enrol patients directly. Patients and investigators were not masked to study treatment. Progression-free survival and objective response rate were assessed by a masked independent radiology review.

### Procedures

Axitinib was given orally at a starting dose of 5 mg twice daily. Patients who tolerated the starting dose with no adverse reactions above grade 2 of Common Terminology Criteria for Adverse Events (CTCAE) for at least 2 weeks were allowed to have their dose increased, at the discretion

of the treating physician, to 7 mg twice daily, unless the patient's blood pressure was higher than 150/90 mm Hg or the patient was receiving antihypertensive medication. Subsequently, with the same criteria, patients who tolerated the axitinib dose of 7 mg had their dose increased to a maximum of 10 mg twice daily. The axitinib dose could be reduced to 3 mg twice daily and then further to 2 mg twice daily, if needed.

The starting dose of sorafenib was 400 mg twice daily, which could be decreased to 400 mg once daily, and then to 400 mg every other day if dose reduction was needed because of toxic effects. Patients were treated until progression of disease (RECIST version 1.0<sup>17</sup>), occurrence of unacceptable toxic effects, death, or withdrawal of patient consent. Crossover between study drugs was not allowed.

We did clinical assessments for safety, including medical history and physical examination, vital signs, clinical laboratory assessment, and ECOG performance status, at baseline, week 2, week 4, and every 4 weeks thereafter. We did tumour assessments, including CT, MRI and bone scans, at screening, after 6 and 12 weeks of therapy, and every 8 weeks thereafter. We assessed safety throughout the study, and graded severity of adverse events using CTCAE version 3.0. We assessed symptom deterioration using the validated Functional Assessment of Cancer Therapy Kidney Symptom Index (FKSI) questionnaire<sup>18</sup> and the FKSI-Disease-Related Symptoms (FKSI-DRS)<sup>19</sup> subscale that specifically measures symptoms associated with advanced renal cell carcinoma. We obtained assessments at baseline and day 1 of every 4-week cycle. High scores on these scales represent better health status (less severe symptoms or concerns) than low scores. We predefined the minimally important difference for the FKSI-15 subscale as five points and for the FKSI-DRS subscale as three points, as previously established.<sup>18,19</sup>

### Statistical analyses

The primary endpoint was PFS, defined as time from randomisation to either first documentation of RECIST-defined disease progression (per independent radiology review of images) or death due to any cause, whichever came first. Secondary endpoints were overall survival, objective response rate, duration of response, and time to deterioration, a composite endpoint consisting of time to death, disease progression, or worsening of symptoms. Symptom deterioration was defined as two consecutive available decreases of at least 5 points from baseline using FKSI-15 ( $\geq 3$  points using FKSI-DRS), unless it was the final score, for which one decrease was sufficient.

We assessed efficacy in the intention-to-treat population on the basis of assessments by a blinded independent radiology review committee. This study was designed to test the hypothesis that treatment would result in an improvement in median PFS from 5 months with sorafenib, based on previous clinical data,<sup>5,20</sup> to 7 months

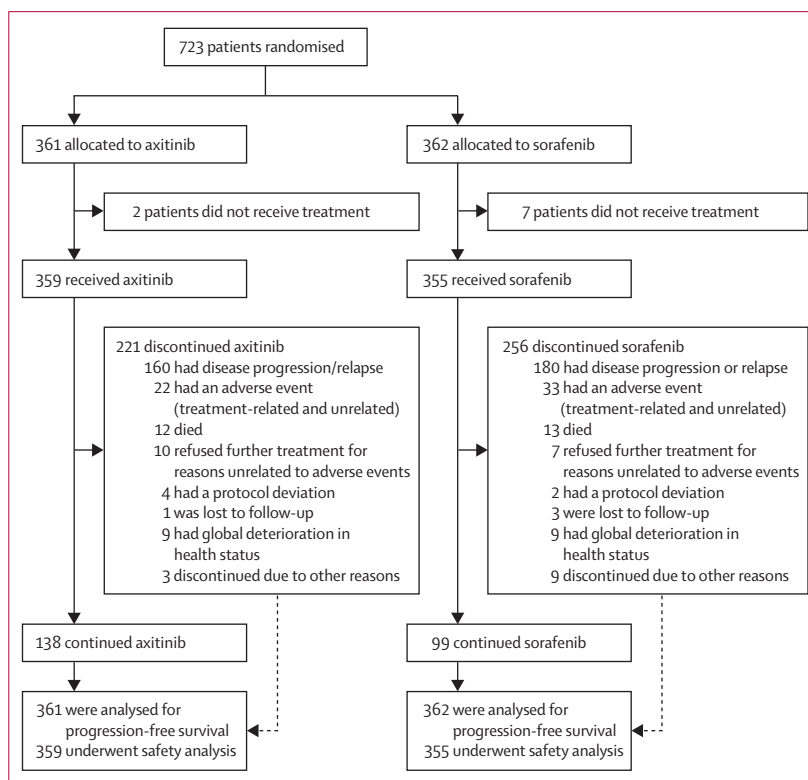


Figure 1: Trial profile

with axitinib. The initial target sample size was calculated based on 90% power to show the improvement in PFS using a one-sided log-rank test at a significance level of 0.025. It was estimated that about 650 patients would need to be enrolled to observe 409 patients with progressive disease or death. The significance for efficacy analysis was calculated with the Lan-DeMets procedure with an O'Brien-Fleming stopping rule. For the secondary endpoint of overall survival, a total of 417 events were required for a log-rank test with an overall one-sided significance level of 0.025 to have power of 0.80, assuming a 31.67% improvement in median overall survival and a follow-up period of about 37 months. We used Kaplan-Meier methods to obtain estimates of median PFS. In accordance with the statistical design, a stratified, one-sided, log-rank test adjusting for ECOG performance status and previous treatment was used to compare PFS between the two treatment groups. Cox proportional-hazards models were used to explore potential effects of baseline stratification factors. A one-sided Cochran-Mantel-Haenszel test stratified by ECOG performance status and previous treatment was used to compare objective response rates between the two treatment groups. We analysed duration of response using descriptive statistics. We assessed symptom deterioration in the intention-to-treat population using the pre-specified time to deterioration composite endpoint. We used survival analysis methods, including

Kaplan-Meier plots and log-rank tests, to compare time to deterioration between the two treatment groups. East version 5 was used to calculate the sample size and stopping boundaries; all other statistical analyses were done with SAS version 9.2.

This trial was registered on ClinicalTrials.gov, number NCT00678392.

### Role of the funding source

Pfizer Inc was involved in design of the study, and collection and analysis of the data. All co-authors made the decision to publish these data and agreed upon the final content of the report. All authors had access to the primary data and vouch for the accuracy and completeness of the data reported. The corresponding author had full access to all data and final responsibility to submit the report for publication.

### Results

Between Sept 15, 2008, and July 23, 2010, 723 patients with advanced renal cell carcinoma coming from 175 sites (hospitals and outpatient clinics) in 22 countries were randomly assigned to axitinib or sorafenib. Of these, 389 (54%) patients had previously received sunitinib, 251 (35%) cytokines, 59 (8%) bevacizumab, and 24 (3%) temsirolimus. Demographics and baseline characteristics were typical of a population with advanced renal cell

carcinoma and were well balanced between the axitinib and sorafenib groups (table 1).

As of the data cutoff date of Aug 31, 2010, 221 (61%) of 361 patients in the axitinib treatment group and 256 (71%) of 362 patients in the sorafenib group had discontinued study treatment (figure 1). Patients received axitinib for a median duration of 6.4 months (range 0.03–22), and sorafenib for 5.0 months (range 0.03–20). Reasons for discontinuation included disease progression (figure 1) and treatment-related adverse events (14 [4%] of 359 in axitinib and 29 [8%] of 355 in sorafenib groups). The most common adverse events leading to discontinuation in the axitinib group were fatigue (4 [1%]) and transient ischaemic attack (3 [ $<1\%$ ]) and in the sorafenib group were palmar-plantar erythrodysesthesia (4 [1%]), diarrhoea (3 [ $<1\%$ ]), and asthenia (3 [ $<1\%$ ]).

The mean relative dose intensity (defined as the actual total dose/intended total dose $\times 100$ ) was 99% in the axitinib group and 92% in the sorafenib group. One or more dose interruptions (due to missed dose or toxic effects) were reported in 276 (77%) of 359 patients given axitinib and 285 (80%) of 355 patients given sorafenib. At least one dose reduction was reported in 110 (31%) of 359 patients given axitinib and 185 (52%) of 355 patients given sorafenib. Dose was increased above 5 mg twice daily in 132 (37%) patients receiving axitinib.

	Independent Review Committee Assessment				Investigator Assessment			
	Axitinib (n=361)	Sorafenib (n=362)	HR for progression or death (95% CI)	p value	Axitinib (n=361)	Sorafenib (n=362)	HR for progression or death (95% CI)	p value
<b>Progression-free survival</b>								
Overall estimated median progression-free survival (months)	6.7 (6.3–8.6)	4.7 (4.6–5.6)	0.665 (0.544–0.812)*	<0.0001	8.3 (6.6–9.0)	5.6 (4.7–6.5)	0.658 (0.543–0.798)*	<0.0001
Stratified estimated median progression-free survival (months)								
Previous cytokine regimen	12.1 (10.1–3.9)	6.5 (6.3–8.3)	0.464 (0.318–0.676)†	<0.0001	12.0 (10.1–13.8)	8.3 (6.6–9.9)	0.636 (0.449–0.900)†	0.0049
Previous sunitinib regimen	4.8 (4.5–6.4)	3.4 (2.8–4.7)	0.741 (0.573–0.958)†	0.0107	6.5 (4.8–7.6)	4.5 (3.0–4.7)	0.636 (0.494–0.818)†	0.0002
Previous bevacizumab regimen	4.2 (2.8–6.5)	4.7 (2.8–6.7)	1.147 (0.568–2.317)†	0.6366	6.5 (2.8–8.3)	4.5 (3.0–6.5)	0.753 (0.365–1.553)†	0.2126
Previous temsirolimus regimen	10.1 (1.5–10.2)	5.3 (1.5–10.1)	0.511 (0.140–1.865)†	0.1425	2.6 (1.5–17.1)	5.7 (2.6–8.3)	1.210 (0.433–3.382)†	0.6342
<b>Objective tumour response</b>								
Best observed RECIST response, n (%)								
Complete response	0	0	..	..	0	1 (<1%)	..	..
Partial response	70 (19%)	34 (9%)	..	..	70 (19%)	39 (11%)	..	..
Stable disease $\geq 20$ weeks	96 (27%)	77 (21%)	..	..	122 (34%)	96 (27%)	..	..
Stable disease <20 weeks	84 (23%)	120 (33%)	..	..	85 (24%)	117 (32%)	..	..
Progressive disease	78 (22%)	76 (21%)	..	..	60 (17%)	66 (18%)	..	..
Not assessed	0	0	..	..	13 (4%)	32 (9%)	..	..
Indeterminate§	22 (6%)	42 (12%)	..	..	11 (3%)	8 (2%)	..	..
Objective response rate, n (%)‡	70 (19%)	34 (9%)	..	0.0001	70 (19%)	40 (11%)	..	0.0007
95% CI	15.4–23.9	6.6–12.9	..	..	15.4–23.9	8.0–14.7	..	..

Numbers in brackets are 95% CIs unless otherwise stated. RECIST=Response Evaluation Criteria In Solid Tumors. ECOG=Eastern Cooperative Oncology Group. \*One-sided log-rank test stratified by ECOG performance status and previous treatment. †One-sided log-rank test stratified by ECOG performance status. ‡One-sided Cochran-Mantel-Haenszel test of treatment stratified by ECOG performance status and previous treatment. §Indeterminate included patients with no post-baseline scans, target lesions that were indeterminate at subsequent timepoints, or patients randomised and not treated.

Table 2: Summary of efficacy measures in intention-to-treat population

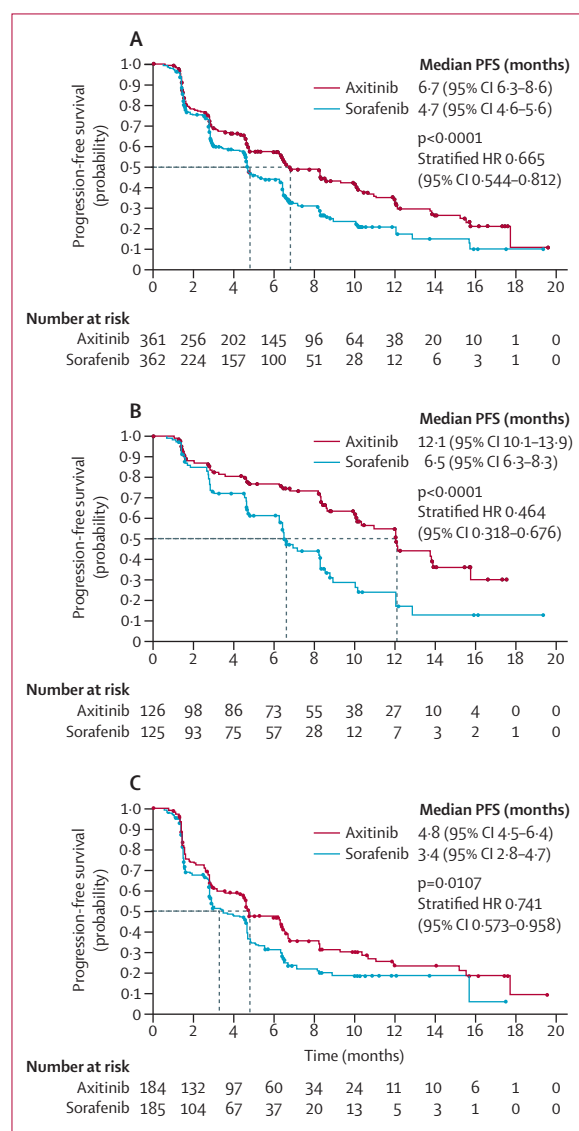
In the 723 patients randomised, 402 primary outcome events (death or disease progression as assessed by blinded independent radiology review committee) were reported. The median PFS was 6.7 months for axitinib versus 4.7 months for sorafenib (hazard ratio [HR] for disease progression or death of 0.665 [95% CI 0.544–0.812];  $p<0.0001$ , stratified one-sided log-rank test; table 2; figure 2A).

In patients who had previously received cytokines, median PFS was 12.1 months for axitinib and 6.5 months for sorafenib (HR 0.464 [95% CI 0.318–0.676];  $p<0.0001$ ) (table 2; figure 2B). In patients previously treated

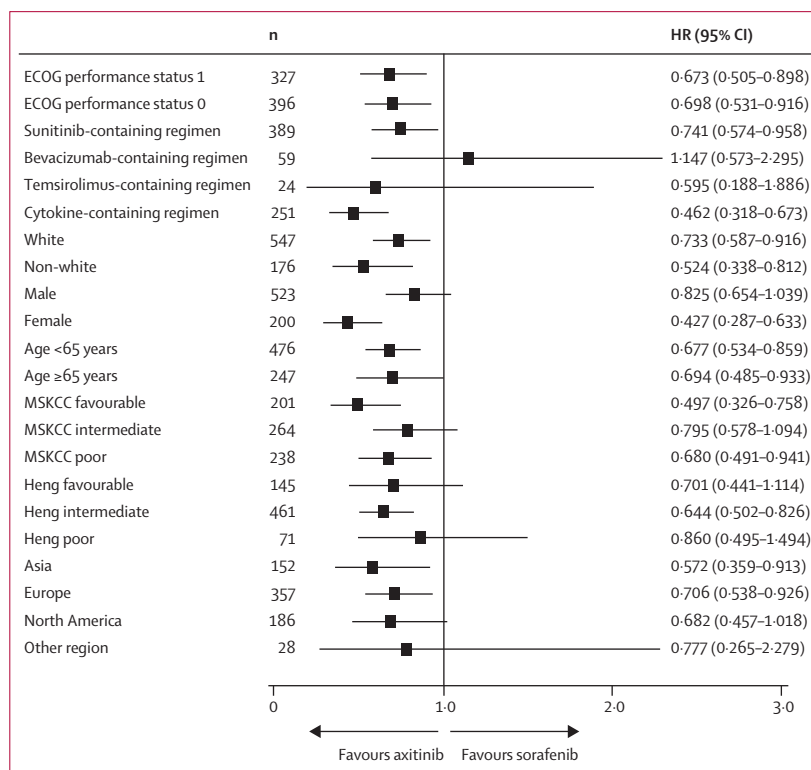
with sunitinib, median PFS was 4.8 months for axitinib and 3.4 months for sorafenib (HR 0.741 [95% CI 0.573–0.958];  $p=0.0107$ ; table 2; figure 2C). Additional subgroup analyses of PFS based on age, sex, risk categories of the Memorial Sloan-Kettering Cancer Center (MSKCC), and region showed a consistent advantage with axitinib (figure 3).

The objective response rate as assessed by masked independent radiology review committee was 19% for axitinib and 9% for sorafenib ( $p=0.0001$ ), with a median duration of response of 11 months (95% CI 7.4–not estimable) for axitinib and 10.6 months (8.8–11.5) for sorafenib (table 2). Of the 417 events required for overall survival analysis, only 223 events occurred. The data of overall survival will be reported separately when mature.

The most frequent adverse events associated with axitinib were diarrhoea, hypertension, fatigue, decreased appetite, nausea, and dysphonia, each occurring in more than 30% of patients (table 3). Hypertension, nausea, dysphonia, and hypothyroidism were more common with axitinib, whereas palmar-plantar erythrodysesthesia, alopecia, and rash were more common with sorafenib. The most common adverse event of grade 3 or higher or laboratory abnormalities were hypertension, diarrhoea, and fatigue with axitinib, and palmar-plantar erythrodysesthesia, hypophosphataemia, lipase elevation, and hypertension with sorafenib (table 3).



**Figure 2:** Kaplan-Meier estimated median PFS in patients who received axitinib or sorafenib as second-line therapy for metastatic renal cell cancer HR=hazard ratio. PFS=progression-free survival. (A) all patients, (B) patients previously treated with cytokine-based regimen, and (C) patients previously treated with sunitinib-based regimen (full analysis set, by independent review committee assessments).  $p$  values based on one-sided, stratified log-rank test.



**Figure 3:** Cox proportional-hazards analysis of progression-free survival by various patient's baseline and prognostic factors

ECOG=Eastern Cooperative Oncology Group. MSKCC=Memorial Sloan-Kettering Cancer Center.

	Axitinib (n=359)		Sorafenib (n=355)	
	All grades	≥Grade 3	All grades	≥Grade 3
<b>Adverse events</b>				
Diarrhoea	197 (55%)	38 (11%)	189 (53%)	26 (7%)
Hypertension	145 (40%)	56 (16%)	103 (29%)	39 (11%)
Fatigue	140 (39%)	41 (11%)	112 (32%)	18 (5%)
Decreased appetite	123 (34%)	18 (5%)	101 (29%)	13 (4%)
Nausea	116 (32%)	9 (3%)	77 (22%)	4 (1%)
Dysphonia	111 (31%)	0	48 (14%)	0
Palmar-plantar erythrodysesthesia	98 (27%)	18 (5%)	181 (51%)	57 (16%)
Weight decreased	89 (25%)	8 (2%)	74 (21%)	5 (1%)
Vomiting	85 (24%)	12 (3%)	61 (17%)	3 (1%)
Asthenia	74 (21%)	19 (5%)	50 (14%)	9 (3%)
Constipation	73 (20%)	4 (1%)	72 (20%)	3 (1%)
Hypothyroidism	69 (19%)	1 (<1%)	29 (8%)	0
Cough	55 (15%)	3 (1%)	59 (17%)	2 (1%)
Mucosal inflammation	55 (15%)	5 (1%)	44 (12%)	2 (1%)
Arthralgia	54 (15%)	5 (1%)	39 (11%)	5 (1%)
Stomatitis	54 (15%)	5 (1%)	44 (12%)	1 (<1%)
Rash	45 (13%)	1 (<1%)	112 (32%)	14 (4%)
Alopecia	14 (4%)	0	115 (32%)	0
<b>Laboratory abnormalities*</b>				
Anaemia	113/320 (35%)	1/320 (<1%)	165/316 (52%)	12/316 (4%)
Haemoglobin elevation	31/320 (10%)	NA	3/316 (1%)	NA
Neutropenia	19/316 (6%)	2/316 (1%)	26/308 (8%)	2/308 (1%)
Thrombocytopenia	48/312 (15%)	1/312 (<1%)	44/310 (14%)	0
Lymphopenia	106/317 (33%)	10/317 (3%)	111/309 (36%)	11/309 (4%)
Creatinine elevation	185/336 (55%)	0	131/318 (41%)	1/318 (<1%)
Hypophosphataemia	43/336 (13%)	6/336 (2%)	158/318 (50%)	51/318 (16%)
Hypercalcaemia	19/336 (6%)	0	5/319 (2%)	0
Hypocalcaemia	132/336 (39%)	4/336 (1%)	188/319 (59%)	5/319 (2%)
Lipase elevation	91/338 (27%)	16/338 (5%)	148/319 (46%)	47/319 (15%)

Data are n (%). NA=not available. \*Denominator for each laboratory abnormality differed depending on the availability of baseline and at least one on-study test result.

**Table 3: Common treatment-emergent all-causality adverse events**

As previously reported,<sup>23</sup> 31 (9%) patients with axitinib and three (1%) patients with sorafenib had elevated haemoglobin, which was managed with phlebotomy in three patients. In patients who had thyroid-stimulating hormone (TSH) concentrations lower than 5 mU/L before treatment, patients treated with axitinib had more elevations of TSH 10 mU/L or more (79 [32%] of 245 patients) than did those treated with sorafenib (25 [11%] of 232 patients). In all, 95 (27%) patients in the axitinib group and 48 (14%) patients in the sorafenib group either started or increased their dose of supplemental thyroid medication. Laboratory abnormalities were greater in the sorafenib arm than in the axitinib arm for anaemia (165 [52%] of 316 vs 113 [35%] of 320 patients) and hypophosphataemia (158 [50%] of 318 vs 43 [13%] of 336 patients).

There were no treatment-related deaths in the axitinib group and two in the sorafenib group (one due to tumour

necrosis causing retroperitoneal bleeding in a patient receiving concomitant dalteparin and one due to gastrointestinal bleed).

Completion rates for analysable symptom deterioration questionnaires during treatment were high (>90%). The time to deterioration FKSI-15 composite endpoint showed a 17% reduction in risk for axitinib compared with sorafenib (figure 4A). Similarly, there was a 16% risk reduction in the time to deterioration FKSI-DRS composite endpoint with axitinib (figure 4B). Thus, when time to symptom worsening is added to the clinical endpoints of death and disease progression, the benefit of axitinib over sorafenib is maintained.

## Discussion

This randomised phase 3 study directly compared two VEGFR tyrosine kinase inhibitors, axitinib and sorafenib, in patients with metastatic renal cell cancer following failure of one previous systemic therapy (panel). Axitinib led to a statistically significant and clinically meaningful increase in the primary efficacy endpoint of PFS compared with sorafenib. These results are notable since an active agent, sorafenib, was used as the comparator rather than placebo. These data also support the hypothesis that biochemically more potent inhibition of the VEGFR, as achieved with axitinib, produces a more robust clinical effect.

The preplanned subgroup analyses showed a significant superiority of axitinib over sorafenib in both previous sunitinib and previous cytokine treatment subgroups. The median PFS of 12·1 months in the subgroup of previous cytokine treatment was similar to previous phase 2 results obtained with axitinib in cytokine-refractory patients<sup>13,15</sup> and to that observed in treatment-naïve patients treated with sunitinib, pazopanib, or bevacizumab plus interferon- $\alpha$ . While cross-trial comparisons are challenging, the axitinib data for the cytokine-refractory subgroup compare favourably with similar patient populations treated with sunitinib (about 8·3 months)<sup>24,25</sup> or pazopanib (7·4 months).<sup>6</sup> For sorafenib, the median PFS of 6·5 months observed in the cytokine-refractory subgroup is consistent with previous phase 3 results.<sup>5</sup> Results in the sunitinib-refractory subgroup also favour axitinib, but with a reduced median PFS in both treatment groups. These data suggest that metastatic renal cell cancer remains sensitive to VEGFR inhibition, even after failure of a previous VEGFR inhibitor, although the clinical benefit of VEGFR inhibition might be reduced with subsequent therapy.

Delay of symptom worsening is a treatment goal in metastatic renal cell cancer with the currently available non-curative therapies. A PFS benefit for a particular drug loses clinical value if symptomatic toxic effects from the drug are significantly worse over the course of more effective treatment. The fact that the PFS advantage with axitinib is maintained in the composite time to deterioration endpoint that included symptom

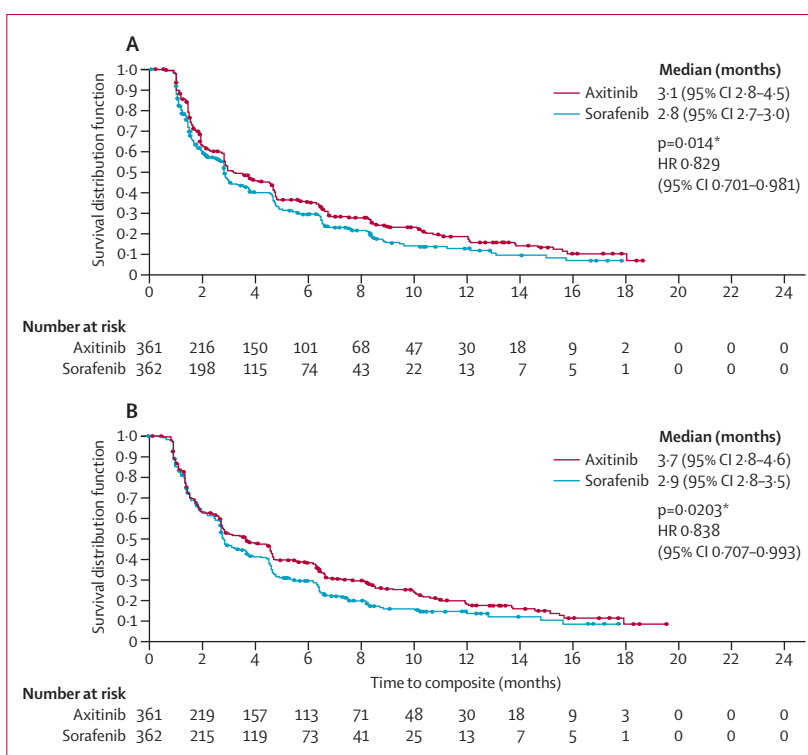


deterioration supports the notion that axitinib is associated with extended disease control and symptom control in this setting.

The tolerability of axitinib was similar to that of sorafenib and other VEGFR inhibitors, with some exceptions.<sup>13,16</sup> Common adverse events seen with axitinib, such as diarrhoea, hypertension, and fatigue, have been noted with other VEGFR inhibitors. Other adverse events often seen with the currently approved VEGFR inhibitors that were less commonly reported with axitinib included palmar-plantar erythrodysesthesia, cutaneous toxicities, and myelosuppression, highlighting one potential advantage of a more specific VEGFR inhibitor. The different toxicity profile of axitinib might be more tolerable for some patients and might allow patients to remain on therapy for extended periods and maintain a clinical benefit.

This study has several important limitations. First, this study was not a blinded trial, although all efficacy endpoints were adjudicated by masked independent radiology review. Nevertheless, bias in assessment of toxic effects might occur with an open-label trial design. Secondly, patients without hypertension and with good tolerability in the axitinib group were allowed to increase their doses, whereas those in the sorafenib group were not. This might have contributed to the efficacy and toxicity profiles of axitinib. The usefulness of axitinib dose increases for the treatment of renal cell carcinoma is not yet defined, but is being prospectively assessed in an ongoing phase 2 trial (ClinicalTrials.gov identifier, NCT00835978). By contrast, dose increases for sorafenib were shown not to be feasible or provide clinical advantages in patients with renal cell carcinoma,<sup>26</sup> and thus the dose and schedule of sorafenib used in this trial represents the standard of care for this drug. Finally, the size of the previous-therapy subgroups, including patients treated with sunitinib, currently the most commonly used first-line drug for renal cancer carcinoma, was small. In the previous-bevacizumab and previous-temsirolimus subgroups, no meaningful conclusions can be made because too few patients were previously treated with these drugs.

Although now an approved option for therapy in patients after failure of treatment with sunitinib or sorafenib, the oral mTOR inhibitor everolimus was an investigational drug when the present trial was begun and thus was not appropriate to include as a comparator in this study. Axitinib was therefore assessed against a standard of care at that time, sorafenib, which has shown activity in patients with treatment-refractory renal cell carcinoma and was commonly used in this setting.<sup>27–29</sup> After AXIS started, data from the phase 3 RECORD-1 trial were published showing the activity of everolimus in refractory patients (median PFS 4.0 months).<sup>30</sup> There are several trial discrepancies that make comparison of the activity of axitinib and everolimus imprecise. Notably, axitinib was tested only



**Figure 4: Kaplan-Meier analysis of composite endpoint of time to deterioration**

FKSI=Functional Assessment of Cancer Therapy Kidney Symptom Index. DRS=Disease-Related Symptoms. Composite endpoint of time to deterioration was defined as time between date of randomisation to first occurrence of death, progression, or deterioration of symptoms, as measured by (A) FKSI-15 and (B) FKSI-DRS. \*p values based on one-sided log-rank test.

### Panel: Research in context

#### Systematic review

We searched PubMed for clinical trials published in 2006–10 in English language with the MeSH terms “renal cell carcinoma”, “vascular endothelial growth factor receptor”, “targeted therapy”, and for specific targeted agents (sorafenib, sunitinib, pazopanib, everolimus, temsirolimus) for renal cell carcinoma. To date, no randomised phase 3 trials comparing the effectiveness of one targeted drug against another have been reported. We did a randomised phase 3 study of axitinib, a potent and selective second-generation inhibitor of vascular endothelial growth factor (VEGF) receptors, against sorafenib, an approved VEGF receptor inhibitor, to compare the efficacy and safety of these agents in 723 patients with advanced renal cell carcinoma who failed initial therapy with sunitinib, bevacizumab, temsirolimus, or cytokines.

#### Interpretation

Our results show that axitinib therapy resulted in a statistically significant and clinically meaningful increase in the primary efficacy endpoint of progression-free survival compared with sorafenib. Axitinib also led to a significantly higher objective response rate. Moreover, time to deterioration (defined as symptom deterioration, progression, or death) also favoured axitinib, supporting the idea that prolonging disease control connotes clinical benefit in this treatment setting. The tolerability of axitinib generally was similar to sorafenib and other similar inhibitors. These data suggest axitinib is a treatment option for second-line therapy of advanced renal cell carcinoma.

in second-line renal cell carcinoma whereas the RECORD-1 trial allowed more than one previous therapy. Although more lines of previous therapy can lead to diminished subsequent response, patients who reach third-line or fourth-line therapy necessarily have more indolent disease, confounding interpretation of PFS. Another interpretation of these data is that everolimus retains clinical activity after two or more VEGF-targeted drugs. Furthermore, by contrast with the AXIS trial, patients who were intolerant of previous tyrosine kinase inhibitors were included in the RECORD-1 trial; these patients had longer PFS than the overall population.<sup>30</sup>

Both axitinib and everolimus should be considered as effective second-line treatment options for renal cell carcinoma. It is not apparent from comparison of PFS of axitinib in this trial and of everolimus in the RECORD-1 trial that switching mechanism of action or maintaining VEGF suppression is a superior strategy in patients with renal cell carcinoma. Drug selection for individual patients is thus influenced by factors such as toxicity profile. The development of validated biomarkers could aid in this regard. Although no biomarkers have been established for everolimus so far, recent data<sup>31</sup> suggest that diastolic blood pressure of 90 mm Hg or higher during study can serve as a predictive biomarker of axitinib efficacy in patients with solid tumours, including renal cell carcinoma. Moreover, characteristics of front-line therapy (eg, response or duration of first-line therapy) or other clinical variables might be important for selecting the best second-line treatment. However, at present, such factors are not well defined and require further study. Additional translational clinical trials are required to understand the mechanisms of response and resistance to targeted therapy in metastatic renal cell cancer to advance patient care.

In conclusion, results from this phase 3 study of axitinib showed a statistically significant and clinically meaningful improvement in median PFS compared with sorafenib in patients with advanced renal cell carcinoma. Although overall survival data are not mature at this time, survival analyses will help to elucidate the utility of axitinib versus sorafenib as second-line therapy for renal cell carcinoma. The safety profile of axitinib was generally similar to sorafenib and manageable. These results establish axitinib as a treatment option for second-line therapy of advanced renal cell carcinoma. An ongoing phase 3 trial is comparing axitinib and sorafenib in patients with metastatic renal cell cancer and no previous systemic first-line therapy or progressive disease after one previous systemic first-line regimen for metastatic disease containing sunitinib, cytokine(s), or both. Additional randomised trials assessing comparative effectiveness of treatments are needed to optimise further the use of targeted therapy in renal cell carcinoma and establish appropriate sequencing of drugs.

#### Contributors

JT was mainly responsible for overall trial design, literature search, data collection, data interpretation, and preparation of the report, with assistance from the study statistician BR. BE was involved with the literature search, study design, data collection, data interpretation, and preparation of the report. TEH, MEG, and HU helped with patient accrual, data collection, data interpretation, and preparation of the report. BIR and MDM were involved with study design, patient accrual, data collection, data interpretation, and preparation of the report. CC, SK, and RJM assisted with study design, data collection, data interpretation, and preparation of the report. VG, CS, and DCa participated in patient accrual, data collection, data interpretation, and preparation of the report. DCE, HYL, SN, PT, and YCO assisted with data collection, data interpretation, and preparation of the report. AK and IR assisted with patient accrual and preparation of the report. All authors approved the final draft of the report.

#### Conflicts of interest

BIR has served as a consultant for and received research funding from Pfizer. DCE has served as a consultant for and received research funding from Pfizer. MEG has served on the speakers' bureau and as an adviser for Bayer and Pfizer. TEH has served as a consultant for and received honoraria and research funding from AVEO, Bayer, Genentech, GlaxoSmithKline, and Pfizer. RJM has served as a consultant for Pfizer. SN has served as a consultant for Pfizer and Roche, and has received honoraria from Novartis and Pfizer and research funding from GlaxoSmithKline. BE has received honoraria from AVEO, Bayer, GlaxoSmithKline, Novartis, and Pfizer. CS has served on an advisory board for Pfizer. JT, CC, BR, and SK are employed by and own stock in Pfizer. AK, DCa, VG, IR, HYL, YCO, HU, MDM, and PT declare that they have no conflicts of interest.

#### Acknowledgments

The study was sponsored by Pfizer Inc. We thank Helen Bhattacharyya, of Pfizer Inc, Global Outcomes Research, for assistance with patient-reported outcomes statistical analysis. Editorial assistance was provided by Larry Rosenberg and Joanna Bloom of UBC Scientific Solutions, and was funded by Pfizer Inc.

#### References

- 1 International Agency for Research on Cancer. The GLOBOCAN project: cancer incidence and mortality worldwide in 2008. <http://globocan.iarc.fr/> (accessed July 6, 2011).
- 2 Motzer RJ, Bander NH, Nanus DM. Renal-cell carcinoma. *N Engl J Med* 1996; **335**: 865–75.
- 3 Rini BI. Metastatic renal cell carcinoma: many treatment options, one patient. *J Clin Oncol* 2009; **27**: 3225–34.
- 4 Motzer RJ, Hutson TE, Tomczak P, et al. Sunitinib versus interferon alfa in metastatic renal-cell carcinoma. *N Engl J Med* 2007; **356**: 115–24.
- 5 Escudier B, Eisen T, Stadler WM, et al. Sorafenib in advanced clear-cell renal-cell carcinoma. *N Engl J Med* 2007; **356**: 125–34.
- 6 Sternberg CN, Davis ID, Mardiak J, et al. Pazopanib in locally advanced or metastatic renal cell carcinoma: results of a randomized phase III trial. *J Clin Oncol* 2010; **28**: 1061–68.
- 7 Escudier B, Pluzanska A, Koralewski P, et al. Bevacizumab plus interferon alfa-2a for treatment of metastatic renal cell carcinoma: a randomised, double-blind phase III trial. *Lancet* 2007; **370**: 2103–11.
- 8 Rini BI, Rixe O, Bukowski RM, et al. AG-013736, a multi-target tyrosine kinase receptor inhibitor, demonstrates anti-tumor activity in a phase 2 study of cytokine-refractory, metastatic renal cell cancer (RCC). *J Clin Oncol* 2005; **23**: Abstr 4509.
- 9 Sonpavde G, Hutson TE, Rini BI. Axitinib for renal cell carcinoma. *Expert Opin Investig Drugs* 2008; **17**: 741–48.
- 10 O'Farrell AM, Abrams TJ, Yuen HA, et al. SU11248 is a novel FLT3 tyrosine kinase inhibitor with potent activity in vitro and in vivo. *Blood* 2003; **101**: 3597–605.
- 11 Abrams TJ, Lee LB, Murray LJ, Pryer NK, Cherrington JM. SU11248 inhibits KIT and platelet-derived growth factor receptor beta in preclinical models of human small cell lung cancer. *Mol Cancer Ther* 2003; **2**: 471–78.

- 12 Flaherty KT. Sorafenib in renal cell carcinoma. *Clin Cancer Res* 2007; **13**: 747s–52s.
- 13 Rixe O, Bukowski RM, Michaelson MD, et al. Axitinib treatment in patients with cytokine-refractory metastatic renal-cell cancer: a phase II study. *Lancet Oncol* 2007; **8**: 975–84.
- 14 Motzer RJ, De la Motte Rouge T, Harzstark AL, et al. Axitinib second-line therapy for metastatic renal cell carcinoma (mRCC): 5-year (yr) overall survival (OS) data from a phase 2 trial. *J Clin Oncol* 2011; **29**: abstr 4547.
- 15 Tomita Y, Uemura H, Fujimoto H, et al. Key predictive factors of axitinib (AG-013736)-induced proteinuria and efficacy: a Japanese phase II study in patients with cytokine-refractory metastatic renal cell cancer (mRCC). *Ann Oncol* 2010; **21** (Suppl 8): Abstr 902P.
- 16 Rini BI, Wilding G, Hudes G, et al. Phase II study of axitinib in sorafenib-refractory metastatic renal cell carcinoma. *J Clin Oncol* 2009; **27**: 4462–68.
- 17 Therasse P, Arbuuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* 2000; **92**: 205–16.
- 18 Cella D, Yount S, Du H, et al. Development and validation of the Functional Assessment of Cancer Therapy-Kidney Symptom Index (FKSI). *J Support Oncol* 2006; **4**: 191–99.
- 19 Cella D, Yount S, Brucker PS, et al. Development and validation of a scale to measure disease-related symptoms of kidney cancer. *Value Health* 2007; **10**: 285–93.
- 20 Escudier B, Eisen T, Stadler WM, et al. Sorafenib for treatment of renal cell carcinoma: final efficacy and safety results of the phase III treatment approaches in renal cancer global evaluation trial. *J Clin Oncol* 2009; **27**: 3312–18.
- 21 Motzer RJ, Bacik J, Schwartz LH, et al. Prognostic factors for survival in previously treated patients with metastatic renal cell carcinoma. *J Clin Oncol* 2004; **22**: 454–63.
- 22 Heng DY, Xie W, Regan MM, et al. Prognostic factors for overall survival in patients with metastatic renal cell carcinoma treated with vascular endothelial growth factor-targeted agents: results from a large, multicenter study. *J Clin Oncol* 2009; **27**: 5794–99.
- 23 Alexandre I, Billefont B, Meric JB, Richard S, Rixe O. Axitinib induces paradoxical erythropoietin synthesis in metastatic renal cell carcinoma. *J Clin Oncol* 2009; **27**: 472–73; author reply 3–4.
- 24 Motzer RJ, Rini BI, Bukowski RM, et al. Sunitinib in patients with metastatic renal cell carcinoma. *JAMA* 2006; **295**: 2516–24.
- 25 Escudier B, Roigas J, Gillessen S, et al. Phase II study of sunitinib administered in a continuous once-daily dosing regimen in patients with cytokine-refractory metastatic renal cell carcinoma. *J Clin Oncol* 2009; **27**: 4068–75.
- 26 Gore ME, Jones RJ, Ravaud A, et al. Efficacy and safety of inpatient dose escalation of sorafenib as first-line treatment for metastatic renal cell carcinoma (mRCC). *J Clin Oncol* 2011; **29**: 4609.
- 27 Ueda T, Imamura Y, Komaru A, et al. Treatment outcomes of sorafenib for first line or cytokinerefractory advanced renal cell carcinoma in Japanese patients. *Int J Urol* 2010; **17**: 811–15.
- 28 Di Lorenzo G, Carteni G, Autorino R, et al. Phase II study of sorafenib in patients with sunitinib-refractory metastatic renal cell cancer. *J Clin Oncol* 2009; **27**: 4469–74.
- 29 Garcia JA, Hutson TE, Elson P, et al. Sorafenib in patients with metastatic renal cell carcinoma refractory to either sunitinib or bevacizumab. *Cancer* 2010; **116**: 5383–90.
- 30 Motzer RJ, Escudier B, Oudard S, et al. Efficacy of everolimus in advanced renal cell carcinoma: a double-blind, randomised, placebo-controlled phase III trial. *Lancet* 2008; **372**: 449–56.
- 31 Rini BI, Schiller JH, Fruehauf JP, et al. Diastolic blood pressure as a biomarker of axitinib efficacy in solid tumors. *Clin Cancer Res* 2011; **17**: 3841–49.



# Simultaneous blockade of programmed death 1 and vascular endothelial growth factor receptor 2 (VEGFR2) induces synergistic anti-tumour effect *in vivo*

Annexure - 5

S. Yasuda,\* M. Sho,\* I. Yamato,\*  
H. Yoshiji,<sup>†</sup> K. Wakatsuki,\*  
S. Nishiwada,\* H. Yagita<sup>‡</sup> and  
Y. Nakajima\*

\*Department of Surgery, <sup>†</sup>Third Department of  
Internal Medicine, Nara Medical University,  
Nara, and <sup>‡</sup>Department of Immunology, Juntendo  
University School of Medicine, Tokyo, Japan

## Summary

Recent basic and clinical studies have shown that the programmed death ligand (PD-L)/PD-1 pathway has a significant role in tumour immunity, and its blockade has a therapeutic potential against several human cancers. We hypothesized that anti-angiogenic treatment might augment the efficacy of PD-1 blockade. To this end, we evaluated combining the blockade of PD-1 and vascular endothelial growth factor receptor 2 (VEGFR2) in a murine cancer model using Colon-26 adenocarcinoma. Interestingly, simultaneous treatment with anti-PD-1 and anti-VEGFR2 monoclonal antibodies (mAbs) inhibited tumour growth synergistically *in vivo* without overt toxicity. Blocking VEGFR2 inhibited tumour neovascularization significantly, as demonstrated by the reduced number of microvessels, while PD-1 blockade had no impact on tumour angiogenesis. PD-1 blockade might promote T cell infiltration into tumours and significantly enhanced local immune activation, as shown by the up-regulation of several proinflammatory cytokine expressions. Importantly, VEGFR2 blockade did not interfere with T cell infiltration and immunological activation induced by PD-1 blockade. In conclusion, simultaneous blockade of PD-1 and VEGFR2 induced a synergistic *in-vivo* anti-tumour effect, possibly through different mechanisms that might not be mutually exclusive. This unique therapeutic strategy may hold significant promise for future clinical application.

**Keywords:** anti-angiogenesis, anti-tumour immunity, immune checkpoint, PD-1, VEGFR2

Accepted for publication 10 January 2013

Correspondence: M. Sho, Department of  
Surgery, Nara Medical University, 840 Shijo-cho,  
Kashihara, Nara 634-8522, Japan.  
E-mail: m-sho@naramed-u.ac.jp

## Introduction

Blocking immune check-points can potentially activate and sustain T cell response against tumours [1]. Cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) (CD152) is well known to serve as a dominant inhibitory receptor on T cells, and plays a key role in immune tolerance and homeostasis [2,3]. CTLA-4 blockade has been long expected as a new cancer immunotherapy. A recent large-scale randomized clinical trial demonstrated usefully that immunotherapy using anti-human CTLA-4 monoclonal antibody had a significant anti-tumour response and improved overall survival in metastatic melanoma [4]. This was the first therapy to extend overall survival in humans, and one of the most successful cancer immunotherapies. However, immune-related adverse events have been also reported, and some patients have died due to severe toxicities related to the study drugs. Therefore, another treatment targeting the

T cell-negative regulatory pathway with less toxicity, as well as substantial efficacy for anti-cancer treatment, would be desirable.

Programmed death 1 (PD-1, CD279) is another potent immune check-point receptor [5–7]. PD-1/programmed death ligand-1 (PD-L1) also functions as a negative regulator of T cell activation and contributes to the prevention of autoimmune diseases. A number of previous studies have shown that the PD-1/PD-L1 pathway has clinical importance in several human malignancies and its blockade has a significant anti-tumour effect in rodent models [8–10]. Furthermore, recent Phase I clinical trials have shown that anti-human PD-1 or PD-L1 antibodies were tolerable for clinical use and might hold great promise as a new anti-cancer treatment for several advanced human malignancies [11,12]. However, the effect of targeting PD-1/PD-L1 alone may be insufficient, especially for advanced or intractable malignant tumours that are resistant to conventional

anti-cancer treatments, including chemotherapy and radiotherapy. Therefore, it is important to investigate the combination treatments for augmenting the potency of PD-1 blockade.

It is known that angiogenesis is a key feature in cancer development and metastasis [13,14]. Among various regulators of angiogenesis, vascular endothelial growth factor (VEGF) and its receptor VEGFR receptor 2 (VEGFR2) are thought to be essential [15]. Basic findings have shown that blocking of the VEGF/VEGFR pathway disrupts tumour microvessels and inhibits tumour growth. Furthermore, it has been also reported that VEGF/VEGFR blockade could normalize abnormal tumour vessels and increase tumour oxygenation, drug supply and immune cells [16–19]. Indeed, anti-VEGF treatment is currently standard therapy for several human malignancies. However, it is also insufficient as a single treatment and usually administered with other cytotoxic anti-cancer drugs.

In this study, we hypothesized that anti-angiogenesis treatment may enhance the anti-tumour effect of targeting the PD-1 pathway without enhancing toxicity by efficiently inducing T cell infiltration into tumours. To this end, we employed an anti-VEGF receptor-2 (VEGFR2, CD309) monoclonal antibody (mAb), designated DC101. VEGFR2 is a major receptor for VEGF and plays a central role in tumour angiogenesis [20]. Furthermore, this mAb has been proved to have a certain anti-tumour effect in murine models [21–24].

## Materials and methods

### Animal and cell line

Female BALB/c mice (5–6 weeks old) were obtained from Clea Japan (Tokyo, Japan). All mice were maintained under specific pathogen-free conditions in the animal facility at Nara Medical University. All experiments were conducted under a protocol approved by our institutional review board. A murine Colon-26 adenocarcinoma was obtained from Riken Cell Bank (Tsukuba, Japan). Cells were grown in RPMI-1640 supplemented with 10% heat-inactivated fetal bovine serum.

### Antibodies

The anti-mouse PD-1 blocking mAb [RB65 mediating protein (RMP)1–14, rat immunoglobulin (Ig)G1] was generated as described previously [25]. The anti-mouse VEGFR2 blocking mAb (DC101, rat IgG1) was kindly provided by ImClone Systems (New York, NY, USA). The anti-mouse CD34 mAb (MEC 14-7, rat IgG2a), anti-CD4 antibody (sc-7219, rabbit polyclonal) and anti-CD8 mAb (EP1150Y, rabbit IgG) were purchased from Abcam (Tokyo, Japan), Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA), and Novus Biologicals (Littleton, CO, USA), respectively.

### Animal experimental protocol

One million Colon-26 cells were inoculated subcutaneously in the flank of syngeneic BALB/c mice with 100 µl of cell suspension with an equal volume of Matrigel (BD Bioscience, San Jose, CA, USA). When the tumour reached 4–5 mm in diameter approximately 3 days after tumour inoculation, treatment was started. In the antibody treatment arm, mice were injected intraperitoneally (i.p.) with 0.25 mg of RMP1–14 and/or 0.8 mg of DC101 every other day for five times. Control mice received control rat IgG. The doses were determined on the basis of our preliminary experiments and previous studies [8,23,24]. Tumour size was determined by electric caliper measurements. Some mice were killed and tumours were removed for further analysis at 11 days after tumour establishment.

### Cell viability analysis

Cell viability was determined using one solution cell proliferation assay kit (MTS assay), according to the instruction manual (Promega Corporation, Madison, WI, USA). Briefly, aliquots of  $3 \times 10^3$  cells per well were cultured in 96-well plates with control IgG, RMP1–14, DC101 or both of RMP1–14 and DC101 for 72 h. Antibody was used at a concentration of 1 or 10 µg/ml. Cell-titre 96 aqueous one solution was added to each well and incubated for an additional 1 h. The absorbance at 490 nm was recorded with a 96-well plate reader. Each experiment was performed in triplicate and repeated at least three times.

### Immunohistochemistry and tumour vessel density measurement

Formalin-fixed or zinc-fixed, paraffin-embedded tissues of primary tumour were cut into 5-µm sections, deparaffinized and rehydrated in a graded series of ethanol. To block endogenous peroxidase, sections were immersed in 0.3% solution of hydrogen peroxide in absolute methanol for 5 min at room temperature and washed three times in fresh phosphate-buffered saline (PBS), each for 5 min duration. Purified rat anti-mouse CD34 mAb, rabbit anti-CD4 antibody or rabbit anti-CD8 mAb diluted with antibody diluent (Dako, Tokyo, Japan) was added and incubated for 1 h at room temperature or overnight at 4°C. Sections were washed three times in PBS, each for 5 min duration, and then Histofine Simple Stain Max PO (Nichirei, Tokyo, Japan) was added and incubated at room temperature for 30 min. After washing three times, the Histofine diaminobenzidine (DAB) substrate kit (Nichirei) was added and incubated at room temperature for 5 min. Sections were rinsed three times in distilled water, counterstained with haematoxylin, dehydrated in ethanol, cleared in Hemo-De and coverslipped. For tumour vessel density measurement,

slides were scanned at low power fields ( $\times 40$ ) to identify areas of highest vascularity. Twenty high-powered ( $\times 200$ ) fields were then selected randomly within these areas, and tumour vessel densities were calculated based on the number of CD34-positive luminal structures. To rule out the possibility that the staining kit reacted with antibodies which had been used for the treatment in mice, we confirmed that there were no positive signals in the samples stained without primary anti-CD34 mAb.

#### Extraction of total RNAs and real-time reverse transcriptase polymerase chain reaction (PCR) analysis

Total RNA was isolated using RNeasy Mini (GE Healthcare, Tokyo, Japan) and the first-strand cDNA was synthesized from 1  $\mu$ g RNA using a high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's protocol. Real-time quantitative PCR analysis was carried out using an ABI Prism 7700 sequence detector system (Applied Biosystems). All primer/probe sets were purchased from Applied Biosystems. PCR was carried out using the TaqMan Universal PCR Master Mix (Applied Biosystems) using 1  $\mu$ l of cDNA in a 20  $\mu$ l final reaction volume. The PCR thermal cycle conditions were as follows: initial step at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. The expression level of the housekeeping gene  $\beta_2$ -microglobulin was measured as an internal reference with a standard curve to determine the integrity of template RNA for all specimens. The ratio of the mRNA level of each gene was calculated as follows: (absolute copy number of each gene)/(absolute copy number of  $\beta_2$ -microglobulin).

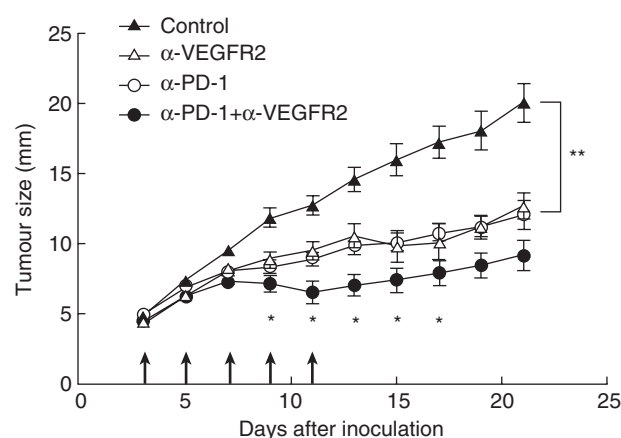
#### Statistical analysis

Results were expressed as mean values  $\pm$  standard error, and Student's *t*-test or Welch's *t*-test were used for evaluating statistical significance. A value less than 0.05 was considered statistically significant.

## Results

#### Simultaneous blockade of PD-1 and VEGFR2 induced synergistic anti-tumour effect

First, we investigated the efficacy of simultaneous blockade with both PD-1 and VEGFR2 *in vivo* using the murine colon cancer model. Tumour cells were inoculated subcutaneously with  $1 \times 10^6$  in the right flank of BALB/c mice and treated with anti-PD-1 mAb (RMP1-14) and/or anti-murine VEGFR2 mAb (DC101). Control rat IgG was used as a control. *In-vivo* treatment either with anti-PD-1 mAb or anti-VEGFR2 mAb induced a substantial anti-tumour effect and inhibited tumour growth significantly compared to control (Fig. 1). There was no significant difference in



**Fig. 1.** Simultaneous blockade of programmed death (PD)-1 and vascular endothelial growth factor receptor 2 (VEGFR2) induced synergistic anti-tumour effect *in vivo*. BALB/c mice were inoculated subcutaneously with Colon-26 cells and were given with control rat immunoglobulin (Ig)G, anti-PD-1 monoclonal antibody (mAb), anti-VEGFR2 mAb or both mAbs five times (arrow). Data are presented as mean  $\pm$  standard error of seven to 10 mice of each group. \**P* < 0.05; \*\**P* < 0.01.

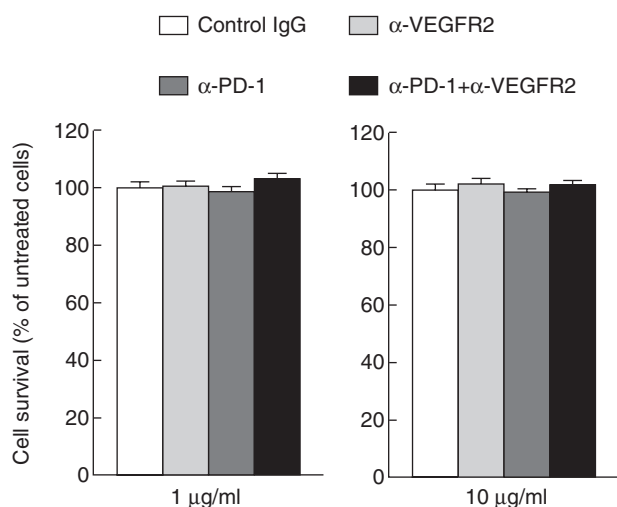
tumour growth between PD-1 and VEGFR2 blockade. Furthermore, dual blockade of both PD-1 and VEGFR2 inhibited tumour growth significantly compared to each mAb treatment (Fig. 1). Thus, the combination therapy of anti-PD-1 and anti-VEGFR2 mAb showed a synergistic anti-tumour effect in tumour growth. There were no overt toxicities in treated mice.

#### Effect of PD-1 and VEGFR2 blockade on cancer cells *in vitro*

To analyse the underlying mechanisms in tumour growth inhibition induced by PD-1 and VEGFR2 blockade, we evaluated the *in-vitro* effect of anti-PD-1 and anti-VEGFR2 mAb on Colon-26. A total of 3000 Colon-26 were co-cultured with anti-PD-1 mAb, anti-VEGFR2 mAb or both mAbs. Control rat IgG was used as a control. The survival rate of Colon-26 was determined by MTS assay. As a result, anti-PD-1 mAb and anti-VEGFR2 mAb did not affect cell survival. Thus, blockade of PD-1 and VEGFR2 does not have any direct effect on cancer cell growth (Fig. 2).

#### VEGFR2 blockade inhibited tumour neovascularization

We then analysed tumour neovascularization by immunohistochemistry with antibody against CD34 (Fig. 3a). Treatment with anti-VEGFR2 mAb or combination therapy inhibited the development of tumour microvessels significantly compared with control (Fig. 3b). Furthermore, anti-PD-1 mAb had no effect on tumour neovascularization



**Fig. 2.** Programmed death (PD)-1 and vascular endothelial growth factor receptor 2 (VEGFR2) blockade did not have any direct effect on cancer cell growth *in vitro*. A total of 3000 Colon-26 cells were co-cultured with anti-PD-1 monoclonal antibody (mAb), anti-VEGFR2 mAb or control rat immunoglobulin (IgG) for 72 h, and cell proliferation was determined by MTS assay.

(Fig. 3b). Thus, PD-1 blockade did not interfere with the anti-cancer treatment targeting tumour angiogenesis.

#### PD-1 blockade enhanced T cell recruitment into tumours

We next evaluated tumour T cell infiltrations by immunohistochemistry and quantitative real-time PCR analysis. Although there were no significant statistical differences, there was a constant tendency of increase in CD4<sup>+</sup> and CD8<sup>+</sup> T cell infiltration in tumour tissues treated with anti-PD-1 mAb alone or combination of anti-PD-1 mAb and anti-VEGFR2 mAb compared to control or anti-VEGFR2 mAb alone (Fig. 4). Even though anti-VEGFR2 mAb disrupted tumour vessels, as shown above, T cell infiltration in tumours treated with anti-VEGFR2 mAb or combination did not decrease. Thus, VEGFR2 blockade did not abrogate recruitment of T lymphocytes into tumours induced by PD-1 blockade. In addition, we examined forkhead box protein 3 (FoxP3) expression in tumours as a marker for regulatory T cells. We found that FoxP3 expression was not reduced by anti-VEGFR2 treatment and elevated by anti-PD-1 treatment (data not shown).

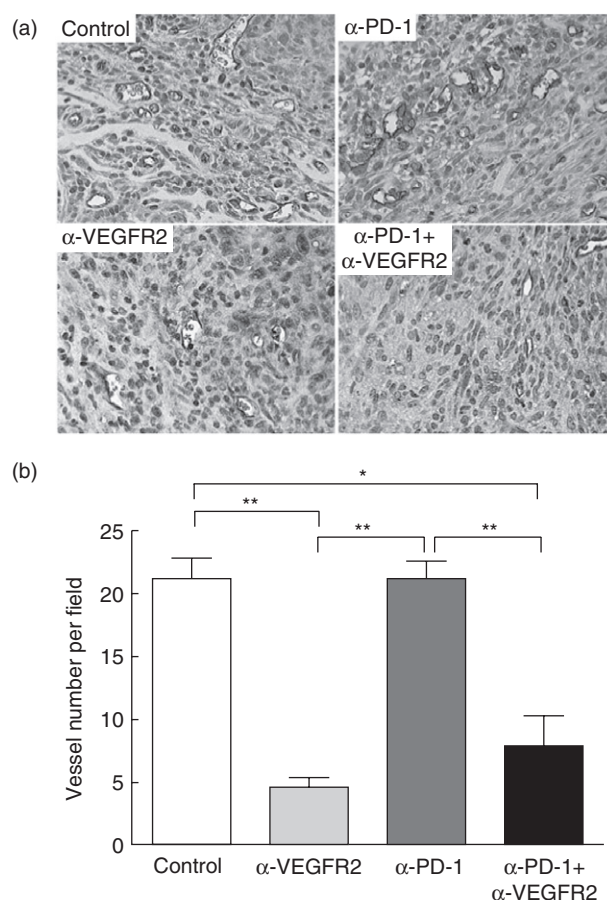
#### PD-1 blockade activated local immunity

To analyse local immune status in tumours, we evaluated the several potent proinflammatory cytokines and mediators. Treatment with anti-PD-1 mAb or combination therapy induced a significant increase in the expression of interferon (IFN)- $\gamma$ , tumour necrosis factor (TNF)- $\alpha$  and

granzyme B in comparison with control (Fig. 5). Thus, PD-1 blockade enhanced T cell recruitment and activated local immune status, thereby resulting in tumour reduction. However, VEGFR2 blockade alone did not induce local immune activation in this model.

#### Discussion

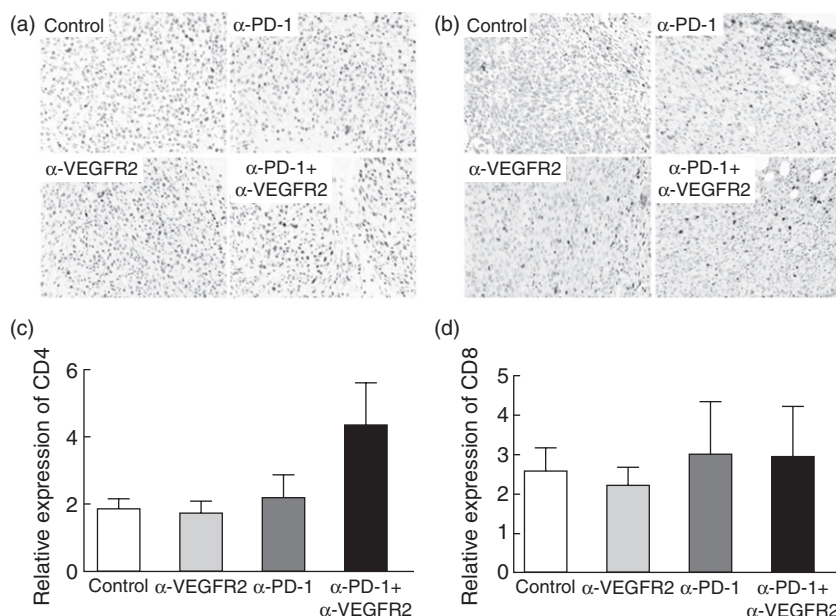
This is the first study to investigate the synergistic anti-tumour effect induced by dual blockade of PD-1 and VEGFR2. Recent multi-centre Phase I clinical trials have shown that PD-1 or PD-L1 mAbs were safe in patients with various types of cancer, and hold promise as new anti-cancer agents. However, in order to enhance anti-tumour efficacy of strategies targeting the PD-1/PD-L pathway, combination therapy may be desirable, especially for refractory tumours such as pancreatic cancer. Because practical



**Fig. 3.** Treatment with anti-vascular endothelial growth factor receptor 2 (VEGFR2) monoclonal antibody (mAb) inhibited tumour neovascularization. (a) Immunohistochemistry analysis by staining with CD34. Representative tumours from mice treated with control rat IgG, anti-programmed death (PD)-1 mAb, anti-VEGFR2 mAb or both mAbs. (b) Tumour microvessels were counted at  $\times 200$  magnification. Data are collected from four to seven mice of each group. \* $P < 0.01$ ; \*\* $P < 0.001$ .



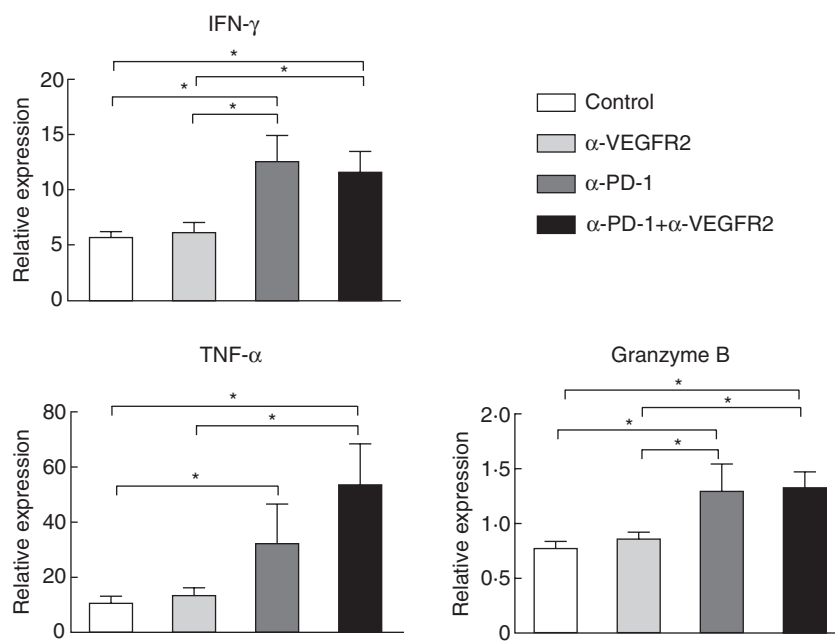
**Fig. 4.** (a) Immunohistochemical staining of CD4<sup>+</sup> and (b) CD8<sup>+</sup> T cells in tumour tissue at day 11. Representative pictures of mice for each treatment are shown. Programmed death (PD)-1 blockade and combination treatment seemed to induce more CD4<sup>+</sup> and CD8<sup>+</sup> T cell infiltration compared to control and vascular endothelial growth factor receptor 2 (VEGFR2) blockade. (c) Quantification of tumour-infiltrating CD4<sup>+</sup> and (d) CD8<sup>+</sup> T cells by real-time polymerase chain reaction (PCR). There was a tendency towards increased T cell infiltration by the treatment of anti-PD-1 monoclonal antibody (mAb) and combination treatment. Anti-VEGFR2 mAb treatment did not interfere with T cell infiltration. Data are collected from four to seven mice of each group.



reagents targeting PD-1 and VEGF pathways are currently available, our proposed strategy may have actual clinical relevance. There are several advantages in dual blockade of completely different pathways. First, the combination therapy may reduce the harmful effect if they have different profiles of toxicity, as they can be used at reduced doses while preserving efficacy. This is important, because both PD-1 and VEGFR blockades are thought to cause unique adverse events. For instance, it is known that PD-1 blockade has a risk of inducing autoimmune reactions and diseases [10,11,26]. The therapeutic dose of anti-PD-1 antibody may cause significant harmful effects. Its reduced, but optimal, dose can be achieved by combination therapy. Although we

have observed no overt toxicity in mice during and after treatment, careful observations will be required in clinical applications. Secondly, when combining two reagents that have different properties, enhanced efficacy may be anticipated because of synergistic interactions. Our data indicate clearly that a synergistic in-vivo anti-tumour effect can be induced successfully by combining PD-1 and VEGFR2 blockade.

Although the underlying mechanisms are not elucidated fully, several interpretations may be drawn from our data. First, as expected, anti-VEGFR2 mAb treatment resulted in a significant decrease of tumour microvessels. Reducing tumour vasculature deprives the tumours of blood supply,



**Fig. 5.** Expression of interferon (IFN)- $\gamma$ , tumour necrosis factor (TNF)- $\alpha$  and granzyme B was up-regulated significantly by anti-programmed death (PD)-1 monoclonal antibody (mAb) or combination mAb treatment compared with control. Treatment of anti-VEGFR2 mAb alone did not increase each cytokine expression. Data are collected from four to seven mice of each group. \* $P < 0.05$ .

thereby leading to the necrosis or apoptosis of tumour cells [19,21]. This was not observed in tumours treated with anti-PD-1 mAb. Secondly, anti-PD-1 mAb treatment enhanced the infiltration of T cells into tumours. Furthermore, significant increases of several proinflammatory cytokines were also confirmed in tumours treated with anti-PD-1 mAb. Thus, PD-1 blockade induced T cell infiltrations, thereby resulting in local immune activation against tumours. Interestingly, although tumour vessels were reduced significantly by VEGFR2 blockade, tumour T cell infiltration was not interfered with the treatment. This paradoxical phenomenon may be explained by the normalization of tumour vessels induced by anti-angiogenesis treatment [16]. The normalized tumour vessels restore blood flow and improve the ability to transport oxygen and anti-cancer drugs as well as immune cells to the tumour [27–30]. Consistent with our data, previous studies investigating the combination of tumour immunotherapy with anti-angiogenic therapy have also shown that anti-angiogenesis treatment does not impede the infiltration of immune-competent cells into tumours [19,21,31]. In addition, as regulatory T cells also express PD-1 selectively, it is possible that PD-1 blockade suppressed regulatory T cells and inhibited tumour growth [32]. However, our data analysing FoxP3 expression suggested that regulatory T cells did not play a significant role in this model. Interestingly, there were no direct effects of PD-1 or VEGFR2 blockade on cancer cell growth, as demonstrated by *in-vitro* studies. Therefore, combining PD-1 and VEGFR2 blockades may exert their anti-tumour efficacy through controlling tumour microenvironments by activating tumour-infiltrating lymphocytes and inhibiting tumour neovascularization. Taken together, anti-angiogenesis strategy may be a good candidate for combination with immune check-point blockade in cancer therapy.

Immunotherapy has long been expected to become a powerful anti-cancer treatment that can be tumour-specific and less toxic [33]. It includes cancer vaccine and adoptive cell therapy. To date, however, there are few definitive evidences for their efficacy in clinical cancers. Besides these conventional immunotherapies, monoclonal antibody-based treatments of targeting T cell negative regulatory pathways, CTLA-4 and PD-1, have been recently introduced and evaluated. A recent large-scale randomized clinical trial demonstrated that immunotherapy using anti-human CTLA-4 monoclonal antibody improved overall survival in metastatic melanoma [4]. To our knowledge, this is the first strong evidence that immunotherapy has worked in actual human cancer. In general, there are many pathways and mechanisms involved in tumour development and progression. Thus, it may be difficult to induce a complete cure by monotherapy or a single anti-cancer method, especially for intractable tumours. Regarding future clinical applications, other combination therapies with blockade of immune check-points should be evaluated in order to

achieve a synergistic anti-tumour effect and less systematic toxicity. In fact, several previous preclinical *in-vivo* studies have shown that the combination of blockade of PD-L1/PD-1 pathway with the simultaneous use of gemcitabine [8], anti-LAG-3 [34] or anti-TIM3 mAb [35] exerted a significant anti-tumour efficacy without overt toxicity. Furthermore, other immune check-points, including B7-H3 [36], LAG3 [34] or TIM3 [35], should also be evaluated in the combination of anti-angiogenesis treatment. In addition, VEGFR1 has become recognized to have unique and diverse activities, including cancer cell survival and migration [37]. Therefore, a combination of PD-1 and VEGFR1 blockades warrants further investigation.

Clearly, further studies will be required to achieve definitive conclusions. First, long-term treatment of combination of PD-1 and VEGFR2 blockade needs to be assessed. In this study, tumour growth became noticeable after withdrawal of antibody treatment. It may be desirable that immunotherapy can induce tumour-specific memory cells that prevent tumour recurrence. Therefore, the sustained beneficial and adverse effects by long-term administration of both mAbs need to be evaluated. Secondly, more fundamental mechanistic studies should be also performed, as some of our data failed to demonstrate statistical significance.

In conclusion, we have shown for the first time that the combination of PD-1 and VEGFR2 had induced a synergistic *in-vivo* anti-tumour effect without overt toxicity. This unique strategy may have clinical relevance and should have the potential to be evaluated in future clinical trials.

## Acknowledgements

This work was supported by the following grants: Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan, no. 19591491, 21591648; Research Grant from the Pancreas Research Foundation of Japan; Research Grant from the Foundation for Promotion of Cancer Research in Japan; Research Grant from Daiwa Securities Health Foundation; Research Grant from The Japanese Society of Gastroenterology; and Research Grant from Nakayama Cancer Research Institute (M. Sho).

## Disclosure

None.

## References

- 1 Vesely MD, Kershaw MH, Schreiber RD, Smyth MJ. Natural innate and adaptive immunity to cancer. *Annu Rev Immunol* 2011; **29**:235–71.
- 2 Schwartz RH. Costimulation of T lymphocytes: the role of CD28, CTLA-4, and B7/BB1 in interleukin-2 production and immunotherapy. *Cell* 1992; **71**:1065–8.

- 3 Lenschow DJ, Walunas TL, Bluestone JA. CD28/B7 system of T cell costimulation. *Annu Rev Immunol* 1996; **14**:233–58.
- 4 Hodi FS, O'Day SJ, McDermott DF *et al.* Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med* 2010; **363**:711–23.
- 5 Greenwald RJ, Freeman GJ, Sharpe AH. The B7 family revisited. *Annu Rev Immunol* 2005; **23**:515–48.
- 6 Nishimura H, Honjo T. PD-1: an inhibitory immunoreceptor involved in peripheral tolerance. *Trends Immunol* 2001; **22**:265–8.
- 7 Ishida Y, Agata Y, Shibahara K, Honjo T. Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. *EMBO J* 1992; **11**:3887–95.
- 8 Nomi T, Sho M, Akahori T *et al.* Clinical significance and therapeutic potential of the programmed death-1 ligand/programmed death-1 pathway in human pancreatic cancer. *Clin Cancer Res* 2007; **13**:2151–7.
- 9 Ohgashi Y, Sho M, Yamada Y *et al.* Clinical significance of programmed death-1 ligand-1 and programmed death-1 ligand-2 expression in human esophageal cancer. *Clin Cancer Res* 2005; **11**:2947–53.
- 10 Berger R, Rotem-Yehudar R, Slama G *et al.* Phase I safety and pharmacokinetic study of CT-011, a humanized antibody interacting with PD-1, in patients with advanced hematologic malignancies. *Clin Cancer Res* 2008; **14**:3044–51.
- 11 Brahmer JR, Tykodi SS, Chow LQ *et al.* Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *N Engl J Med* 2012; **366**:2455–65.
- 12 Topalian SL, Hodi FS, Brahmer JR *et al.* Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med* 2012; **366**:2443–54.
- 13 Folkman J, Shing Y. Angiogenesis. *J Biol Chem* 1992; **267**:10931–4.
- 14 Folkman J. Angiogenesis inhibitors: a new class of drugs. *Cancer Biol Ther* 2003; **2**:S127–33.
- 15 Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell* 1996; **86**:353–64.
- 16 Jain RK. Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. *Science* 2005; **307**:58–62.
- 17 Tong RT, Boucher Y, Kozin SV, Winkler F, Hicklin DJ, Jain RK. Vascular normalization by vascular endothelial growth factor receptor 2 blockade induces a pressure gradient across the vasculature and improves drug penetration in tumors. *Cancer Res* 2004; **64**:3731–6.
- 18 Fukumura D, Jain RK. Tumor microenvironment abnormalities: causes, consequences, and strategies to normalize. *J Cell Biochem* 2007; **101**:937–49.
- 19 Shrinani RK, Yu Z, Theoret MR, Chinnasamy D, Restifo NP, Rosenberg SA. Antiangiogenic agents can increase lymphocyte infiltration into tumor and enhance the effectiveness of adoptive immunotherapy of cancer. *Cancer Res* 2010; **70**:6171–80.
- 20 Shibuya M. Vascular endothelial growth factor receptor-2: its unique signaling and specific ligand, VEGF-E. *Cancer Sci* 2003; **94**:751–6.
- 21 Manning EA, Ullman JG, Leatherman JM *et al.* A vascular endothelial growth factor receptor-2 inhibitor enhances antitumor immunity through an immune-based mechanism. *Clin Cancer Res* 2007; **13**:3951–9.
- 22 Prewett M, Huber J, Li Y *et al.* Antivascular endothelial growth factor receptor (fetal liver kinase 1) monoclonal antibody inhibits tumor angiogenesis and growth of several mouse and human tumors. *Cancer Res* 1999; **59**:5209–18.
- 23 Yoshiji H, Kuriyama S, Yoshii J *et al.* Halting the interaction between vascular endothelial growth factor and its receptors attenuates liver carcinogenesis in mice. *Hepatology* 2004; **39**:1517–24.
- 24 Yoshiji H, Kuriyama S, Hicklin DJ *et al.* The vascular endothelial growth factor receptor KDR/Flk-1 is a major regulator of malignant ascites formation in the mouse hepatocellular carcinoma model. *Hepatology* 2001; **33**:841–7.
- 25 Youngnak P, Kozono Y, Kozono H *et al.* Differential binding properties of B7-H1 and B7-DC to programmed death-1. *Biochem Biophys Res Commun* 2003; **307**:672–7.
- 26 Okazaki T, Honjo T. PD-1 and PD-1 ligands: from discovery to clinical application. *Int Immunol* 2007; **19**:813–24.
- 27 Wei YQ, Huang MJ, Yang L *et al.* Immunogene therapy of tumors with vaccine based on *Xenopus* homologous vascular endothelial growth factor as a model antigen. *Proc Natl Acad Sci USA* 2001; **98**:11545–50.
- 28 Niethammer AG, Xiang R, Becker JC *et al.* A DNA vaccine against VEGF receptor 2 prevents effective angiogenesis and inhibits tumor growth. *Nat Med* 2002; **8**:1369–75.
- 29 Luo Y, Zhou H, Krueger J *et al.* Targeting tumor-associated macrophages as a novel strategy against breast cancer. *J Clin Invest* 2006; **116**:2132–41.
- 30 Dirx AE, oude Egbrink MG, Castermans K *et al.* Anti-angiogenesis therapy can overcome endothelial cell anergy and promote leukocyte–endothelium interactions and infiltration in tumors. *FASEB J* 2006; **20**:621–30.
- 31 Huang X, Wong MK, Yi H *et al.* Combined therapy of local and metastatic 4T1 breast tumor in mice using SU6668, an inhibitor of angiogenic receptor tyrosine kinases, and the immunostimulator B7.2-IgG fusion protein. *Cancer Res* 2002; **62**:5727–35.
- 32 Suzuki H, Onishi H, Wada J *et al.* VEGFR2 is selectively expressed by FOXP3<sup>high</sup> CD4<sup>+</sup> Treg. *Eur J Immunol* 2010; **40**:197–203.
- 33 Rosenberg SA, Restifo NP, Yang JC, Morgan RA, Dudley ME. Adoptive cell transfer: a clinical path to effective cancer immunotherapy. *Nat Rev Cancer* 2008; **8**:299–308.
- 34 Woo SR, Turnis ME, Goldberg MV *et al.* Immune inhibitory molecules LAG-3 and PD-1 synergistically regulate T-cell function to promote tumoral immune escape. *Cancer Res* 2012; **72**:917–27.
- 35 Ngiew SF, von Scheidt B, Akiba H, Yagita H, Teng MW, Smyth MJ. Anti-TIM3 antibody promotes T cell IFN- $\gamma$ -mediated anti-tumor immunity and suppresses established tumors. *Cancer Res* 2011; **71**:3540–51.
- 36 Yamato I, Sho M, Nomi T *et al.* Clinical importance of B7-H3 expression in human pancreatic cancer. *Br J Cancer* 2009; **101**:1709–16.
- 37 Schwartz JD, Rowinsky EK, Yousoufian H, Pytowski B, Wu Y. Vascular endothelial growth factor receptor-1 in human cancer: concise review and rationale for development of IMC-18F1 (human antibody targeting vascular endothelial growth factor receptor-1). *Cancer* 2010; **116**:1027–32.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property  
Organization  
International Bureau(10) International Publication Number  
**WO 2012/135408 A1**(43) International Publication Date  
4 October 2012 (04.10.2012)(51) International Patent Classification:  
A61K 39/395 (2006.01)(21) International Application Number:  
PCT/US2012/031063(22) International Filing Date:  
29 March 2012 (29.03.2012)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
61/470,121 31 March 2011 (31.03.2011) US(71) Applicant (for all designated States except US): **MERCK SHARP & DOHME CORP.** [US/US]; 126 East Lincoln Avenue, Rahway, New Jersey 07065-0907 (US).

(72) Inventors; and

(71) Applicants (for NZ, US only): **SHARMA, Manoj, K.** [IN/US]; 556 Morris Avenue, Summit, New Jersey 07901-1330 (US). **NARASIMHAN, Chakravarthy Nachu** [US/US]; 556 Morris Avenue, Summit, New Jersey 07901-1330 (US). **GERGICH, Kevin James** [US/US]; 351 N. Sumneytown Pike, North Wales, Pennsylvania 19454 (US). **KANG, Soonmo Peter** [US/US]; 126 East Lincoln Avenue, Rahway, New Jersey 07065-0907 (US).(74) Common Representative: **MERCK SHARP & DOHME CORP.**; 126 East Lincoln Avenue, Rahway, New Jersey 07065-0907 (US).

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

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

## Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))

## Published:

- with international search report (Art. 21(3))
- with sequence listing part of description (Rule 5.2(a))

(54) Title: STABLE FORMULATIONS OF ANTIBODIES TO HUMAN PROGRAMMED DEATH RECEPTOR PD-1 AND RELATED TREATMENTS

Stability Data for h409A11 Anti-PD-1 Powder for injection, 50 mg/Vial									
Storage Condition	5°C								
Batch Number	1								
Test	Clinical Acceptance Criteria	Initial	1-month	3-month	6-month	9-month	12-month	18-month	24-month
Description	White to off-white powder	White cake	White cake	White cake	White cake	White cake	White cake	White cake	White cake
Lyophilized Powder									
Reconstitution Time (seconds)	Report Results	39	36	35	42	43	34	28	45
Description									
Reconstituted Solution									
Clarity	Clear to opalescent solution; May contain particulates	Opalescent solution	Opalescent solution	Opalescent solution	Opalescent solution	Opalescent solution	Opalescent solution	Opalescent solution	Opalescent solution
Color	Report results by "Y" ref solution	Colorless**	Colorless**	Colorless**	Colorless**	Colorless**	Colorless**	Colorless**	Colorless**
pH	5.0 - 6.0	5.6	5.5	5.5	5.6	5.6	5.6	5.6	5.6
Assay UV A <sub>280</sub> nm	21.3 - 28.8 mg/mL	24.9	24.8	24.1	24.8	24.4	25.5	24.6	23.6
Biological Potency									
Anti-PD-1 Competitive ELISA (% Relative to control)	50-150% of Reference	95	80	86	116	93	87	76	83
Purity									
HPSEC									
High Molecular Weight Species (%)	≤ 5.00	ND	ND	<QL	<QL	<QL	<QL	<DL	<QL
Late Eluting Peaks (%)	Report Results	ND	ND	ND	ND	ND	ND	ND	ND
Monomer (%)	> 90.0	100.0	100.0	99.8	99.8	99.8	99.8	100.0	99.8
CE-SDS Reducing									
% Impurity	≤ 10.00% species other than heavy and light chains	0.38	0.35	0.39	0.41	0.40	0.44	0.38	0.39

FIG.1A

(57) Abstract: The present invention relates to stable formulations of antibodies against human programmed death receptor PD-1, or antigen binding fragments thereof. The present invention further provides methods for treating various cancers and chronic infections with stable formulations of antibodies against human programmed death receptor PD-1, or antigen binding fragments thereof.



WO 2012/135408 A1



**STABLE FORMULATIONS OF  
ANTIBODIES TO HUMAN PROGRAMMED DEATH RECEPTOR PD-1 AND  
RELATED TREATMENTS**

**CROSS REFERENCE TO RELATED APPLICATIONS**

[0001] This application claims the benefit of U.S. provisional patent application No. 61/470,121, filed March 31, 2011, which is herein incorporated by reference in its entirety.

**FIELD OF THE INVENTION**

[0002] The present invention relates to stable formulations of antibodies against human programmed death receptor PD-1, or antigen binding fragments thereof. The present invention further provides methods for treating various cancers and chronic infections with stable formulations of antibodies against human PD-1, or antigen binding fragments thereof.

**BACKGROUND OF THE INVENTION**

[0003] Programmed Death 1 (PD-1), a member of the CD28 costimulatory gene family, is moderately expressed on naive T, B and NKT cells and up-regulated by T/B cell receptor signaling on lymphocytes, monocytes and myeloid cells (1). PD-1 has two known ligands with distinct expression profiles, PD-L1 (B7-H1) and PD-L2 (B7-DC). PD-L2 expression is relatively restricted and is found on activated dendritic cells, macrophages and monocytes and on vascular endothelial cells (1-3). In contrast, PD-L1 is expressed more broadly including on naive lymphocytes and its expression is induced on activated B and T cells, monocytes and dendritic cells. Furthermore, by mRNA, it is expressed by non-lymphoid tissues including vascular endothelial cells, epithelial cells and muscle cells.

[0004] PD-1 is recognized as an important player in immune regulation and the maintenance of peripheral tolerance. In the mouse, this was shown to require PD-L1 expression on peripheral tissues and ligation of PD-1 on potentially autoreactive T cells to negatively modulate T cell activation involving an ITIM sequence in the PD-1 cytoplasmic domain (1, 4).

[0005] Depending on the specific genetic background, *pdc1<sup>-/-</sup>* mice spontaneously develop lupus-like phenomena or dilated cardiomyopathy (5, 6). Furthermore, antibody-

induced blockade of the PD-1 / PD-L1 pathway was demonstrated to accelerate the onset of autoimmune insulinitis and diabetes in NOD mice (7).

**[0006]** Human cancers arising in various tissues were found to over-express PD-L1 or PD-L2. In large sample sets of *e.g.* ovarian, renal, colorectal, pancreatic, liver cancers and melanoma it was shown that PD-L1 expression correlated with poor prognosis and reduced overall survival irrespective of subsequent treatment (15-26). Similarly, PD-1 expression on tumor infiltrating lymphocytes was found to mark dysfunctional T cells in breast cancer and melanoma (27-28) and to correlate with poor prognosis in renal cancer (29). Using primary patient samples, it was shown that blockade of PD-1 or PD-L1 *in vitro* results in enhancement of human tumor-specific T cell activation and cytokine production (30). Consequently, in several murine syngeneic tumor models, blockade of either PD-1 or PD-L1 significantly inhibited tumor growth or induced complete regression.

**[0007]** A PD-1 blocking mAb (h409A11) was discovered and developed for use to treat human cancer patients and chronic virus-infected patients (described in co-pending application WO2008/156712).

**[0008]** Antigen-specific T cell dysfunction or tolerance is exemplified by the accumulated loss of the potential to produce Interleukin 2 (IL-2), Tumor Necrosis factor (TNF)  $\alpha$ , perforin, interferon (IFN)  $\gamma$  (8) and inability to mount a proliferative response to T cell receptor triggering (1). The PD-1 pathway controls antigen-specific T cell tolerance and was found to be exploited in viral infection and tumor development to control and evade effective T cell immunity.

**[0009]** In chronic infection with LCMV (mouse), HIV, HBV or HCV (human), antigen-specific T cells were found to express aberrantly high levels of PD-1 correlating with their state of anergy or dysfunction (9). Blocking the PD-1 – PD-L1 interaction *in vivo* (LCMV) or *in vitro* (HIV, HCV, HBV) was shown to revive anti-viral T cell activity (10-12). PD-1 blockade in recently Simian Immunodeficiency Virus-infected macaques resulted in strong reduction of viral load and increased survival (13). Similarly, reduction in viral load was confirmed in second study using long-term SIV-infected rhesus macaques (14).

**[0010]** Overall, the PD-1/PD-L1 pathway is a well-validated target for the development of antibody therapeutics for cancer treatment. Anti-PD-1 antibodies are also useful for treating chronic viral infection. Memory CD8<sup>+</sup>T cells generated after an acute viral

infection are highly functional and constitute an important component of protective immunity. In contrast, chronic infections are often characterized by varying degrees of functional impairment (exhaustion) of virus-specific T-cell responses, and this defect is a principal reason for the inability of the host to eliminate the persisting pathogen. Although functional effector T cells are initially generated during the early stages of infection, they gradually lose function during the course of a chronic infection. Barber *et al.* (Barber *et al.*, Nature 439: 682-687 (2006)) showed that mice infected with a laboratory strain of LCMV developed chronic infection resulting in high levels of virus in the blood and other tissues. These mice initially developed a robust T cell response, but eventually succumbed to the infection upon T cell exhaustion. The authors found that the decline in number and function of the effector T cells in chronically infected mice could be reversed by injecting an antibody that blocked the interaction between PD-1 and PD-L1.

**[0011]** PD-1 has also been shown to be highly expressed on T cells from HIV infected individuals and that receptor expression correlates with impaired T cell function and disease progression (Day et al., Nature 443:350-4 (2006); Trautmann L. *et al.*, Nat. Med. 12: 1198-202 (2006)). In both studies, blockade of the PD-1 pathway using antibodies against the ligand PD-L1 significantly increased the expansion of HIV-specific, IFN-gamma producing cells *in vitro*.

**[0012]** Other studies also implicate the importance of the PD-1 pathway in controlling viral infection. PD-1 knockout mice exhibit better control of adenovirus infection than wild-type mice (Iwai et al., Exp. Med. 198:39-50 (2003)). Also, adoptive transfer of HBV- specific T cells into HBV transgenic animals initiated hepatitis (Isogawa M. et al., Immunity 23:53-63 (2005)). The disease state of these animals oscillates as a consequence of antigen recognition in the liver and PD-1 upregulation by liver cells.

**[0013]** Therapeutic antibodies may be used to block cytokine activity. A significant limitation in using antibodies as a therapeutic agent *in vivo* is the immunogenicity of the antibodies. As most monoclonal antibodies are derived from non-human species, repeated use in humans results in the generation of an immune response against the therapeutic antibody. Such an immune response results in a loss of therapeutic efficacy at a minimum, and potentially a fatal anaphylactic response. Accordingly, antibodies of reduced immunogenicity in humans, such as humanized or fully human antibodies, are preferred for treatment of human subjects. Exemplary therapeutic antibodies specific for human PD-1 are

disclosed in commonly-assigned U.S. Patent Application Publication No. US2010/0266617, and in International Patent Publication No. WO2008/156712, the disclosures of which are hereby incorporated by reference in their entireties.

[0014] Antibodies for use in human subjects must be stored prior to use and transported to the point of administration. Reproducibly attaining a desired level of antibody drug in a subject requires that the drug be stored in a formulation that maintains the bioactivity of the drug. The need exists for stable formulations of anti-human PD-1 antibodies for pharmaceutical use, *e.g.*, for treating various cancers and infectious diseases. Preferably, such formulations will exhibit a long shelf-life, be stable when stored and transported, and will be amenable to administration at high concentrations, *e.g.* for use in subcutaneous administration, as well as low concentrations, *e.g.* for intravenous administration.

### SUMMARY OF THE INVENTION

[0015] The present invention relates to stable formulations of antibodies against human programmed death receptor PD-1, or antigen binding fragments thereof. The present invention further provides methods for treating various cancers and chronic infections with stable formulations of antibodies against human programmed death receptor PD-1, or antigen binding fragments thereof.

[0016] In certain embodiments, the invention relates to a lyophilized formulation of an anti-human PD-1 antibody, or antigen binding fragment thereof, comprising: a) said anti-human PD-1 antibody, or antigen binding fragment thereof; b) histidine buffer; c) polysorbate 80; and d) sucrose.

[0017] In certain embodiments, the formulation has a pH between 5.0 and 6.0 when reconstituted.

[0018] In certain embodiments, the lyophilized formulation enables reconstitution of the antibody, or antigen binding fragment thereof, at a concentration of between about 25 mg/mL and 100 mg/mL.

[0019] In certain embodiments, polysorbate 80 is present at a weight ratio of approximately 0.02% (w/v).

[0020] In certain embodiments, sucrose is present at a weight ratio of approximately 7% (w/v).

[0021] In yet additional embodiments, the invention relates to a lyophilized pharmaceutical formulation of an anti-human PD-1 antibody, or antigen binding fragment thereof, made by lyophilizing an aqueous solution comprising: a) 25-100 mg/mL anti-

antibody, or antigen binding fragment thereof; b) about 70 mg/mL sucrose; c) about 0.2 mg/mL polysorbate 80; and d) about 10 mM histidine buffer at pH 5.0-6.0.

**[0022]** In certain embodiments, the anti-human PD-1 antibody, or antigen binding fragment thereof, is present at about 25 mg/mL in the aqueous solution. In certain embodiments, the aqueous solution has a pH of about 5.5.

**[0023]** In yet additional embodiments, the invention relates to a lyophilized pharmaceutical formulation of an anti-human PD-1 antibody, or antigen binding fragment thereof, that when reconstituted comprises: a) 25-100 mg/mL anti-human PD-1 antibody, or antigen binding fragment thereof; b) about 70 mg/mL sucrose; c) about 0.2 mg/mL polysorbate 80; and d) about 10 mM Histidine buffer at about pH 5.0- pH 6.0.

**[0024]** In certain embodiments, the anti-human PD-1 antibody, or antigen binding fragment thereof, is present at about 25 mg/mL in the reconstituted solution. In certain embodiments, the reconstituted solution has a pH of about 5.5.

**[0025]** In yet additional embodiments, the invention relates to a liquid pharmaceutical formulation of an anti-human PD-1 antibody, or antigen binding fragment thereof comprising: a) 25-100 mg/mL anti- antibody, or antigen binding fragment thereof; b) about 70 mg/mL sucrose; c) about 0.2 mg/mL polysorbate 80; and d) about 10 mM histidine buffer at pH 5.0-6.0.

**[0026]** In yet additional embodiments, the invention relates to a pharmaceutical formulation of an anti-human PD-1 antibody, or antigen binding fragment thereof comprising: a) said anti-human PD-1 antibody, or antigen binding fragment thereof; b) histidine buffer; c) polysorbate 80; and d) sucrose. In certain embodiments, the formulation has a pH between 5.0 and 6.0 when reconstituted. In certain embodiments, the polysorbate 80 is present at a weight ratio of approximately 0.02% (w/v). In certain embodiments, the sucrose is present at a weight ratio of approximately 7% (w/v).

**[0027]** In yet additional embodiments, the invention relates to any of the formulations described herein, wherein the antibody, or antigen binding fragment thereof, comprises a light chain comprising three CDR sequences selected from the group consisting of SEQ ID NOs: 9, 10, 11, 15, 16, and 17.

**[0028]** In yet additional embodiments, the invention relates to any of the formulations described herein, wherein the antibody, or antigen binding fragment thereof, comprises a heavy chain comprising three CDR sequences selected from the group consisting of SEQ ID NOs: 12, 13, 14, 18, 19, and 20.

**[0029]** In yet additional embodiments, the invention relates to any of the formulations described herein, wherein the antibody, or antigen binding fragment thereof, comprises: i) a light chain comprising three CDR sequences SEQ ID NOs: 15, 16, and 17; and ii) a heavy chain comprising three CDR sequences SEQ ID NOs: 8, 19, and 20.

**[0030]** In yet additional embodiments, the invention relates to any of the formulations described herein, wherein the antibody, or antigen binding fragment thereof, comprises a light chain variable domain comprising amino acid residues 20 to 130 of SEQ ID NO:32.

**[0031]** In yet additional embodiments, the invention relates to any of the formulations described herein, wherein the antibody, or antigen binding fragment thereof, comprises a heavy chain variable domain comprising SEQ ID NO:31.

**[0032]** In yet additional embodiments, the invention relates to any of the formulations described herein, wherein the antibody, or antigen binding fragment thereof, comprises: i) a light chain comprising amino acid residues 20 to 237 of SEQ ID NO: 36 and ii) a heavy chain comprising amino acid residues 20 to 466 of SEQ ID NO: 31.

**[0033]** In yet additional embodiments, the invention relates to any of the formulations described herein, wherein the antibody is selected from the group consisting of h409A11, h409A16, and h409A17.

**[0034]** In yet additional embodiments, the invention relates to a method of treating chronic infection in a mammalian subject in need thereof comprising: administering an effective amount of any of the formulations described herein.

**[0035]** In yet additional embodiments, the invention relates to a method of treating cancer in a mammalian subject in need thereof, the method comprising administering an effective amount of any of the formulations described herein. In certain embodiments, the effective amount comprises a dose of anti-human PD-1 antibody selected from the group consisting of the 1.0, 3.0, and 10 mg/kg.

#### **BRIEF DESCRIPTION OF THE DRAWINGS**

**[0036]** FIGURES 1A-B show stability data for lyophilized formulations of h409A11 at pH 5.5 stored at 5°C (24 months).

**[0037]** FIGURES 2A-B show stability data for lyophilized formulations of h409A11 at pH 5.5 stored at 25H conditions (25°C, 60% RH, 12 months).

**[0038]** FIGURES 3A-B show stability data for lyophilized formulations of h409A11 at pH 5.5 stored at RH4 conditions (40°C, 75% RH, 6 months).

[0039] FIGURES 4A-B show stability data for lyophilized formulations of h409A11 stored at 5°C (24 months).

[0040] FIGURES 5A-B show stability data for lyophilized formulations of h409A11 at pH 5.5 stored at 25H conditions (25°C, 60% RH, 6 months).

[0041] FIGURES 6A-B show stability data for lyophilized formulations of h409A11 at pH 5.5 stored at RH4 conditions (40°C, 75% RH, 6 months).

[0042] FIGURES 7A-B show stability data for lyophilized formulations of h409A11 stored at 5°C (24 months).

[0043] FIGURES 8A-B show stability data for lyophilized formulations of h409A11 25H conditions (25°C, 60% RH, 6 months).

[0044] FIGURES 9A-B show stability data for lyophilized formulations of h409A11 at RH4 conditions (40°C, 75% RH, 6 months).

#### DETAILED DESCRIPTION

[0045] The present invention provides formulations of anti-PD-1 antibodies and uses thereof for treating various cancers and infectious diseases.

[0046] Anti-PD-1 antibody h409A11 is an exemplary antibody in the stable formulations described herein. Three humanized anti-PD-1 monoclonal antibodies (*i.e.*, h409A11, h409A16, and h509A17) suitable for the present formulations are described in co-pending patent publication WO2008/156712. Additionally, formulations described herein are useful for treating certain cancers as well as chronic infections. Table 2 provides a list of the corresponding CDR sequences for h409A11. Table 6 provides a list of sequences of exemplary anti-PD-1 antibodies.

[0047] In accordance with the present invention there may be employed conventional molecular biology, microbiology, protein expression and purification, antibody, and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. *See, e.g.*, Sambrook *et al.* (2001) *Molecular Cloning: A Laboratory Manual*. 3<sup>rd</sup> ed. Cold Spring Harbor Laboratory Press: Cold Spring Harbor, New York; Ausubel *et al.* eds. (2005) *Current Protocols in Molecular Biology*. John Wiley and Sons, Inc.: Hoboken, NJ; Bonifacino *et al.* eds. (2005) *Current Protocols in Cell Biology*. John Wiley and Sons, Inc.: Hoboken, NJ; Coligan *et al.* eds. (2005) *Current Protocols in Immunology*, John Wiley and Sons, Inc.: Hoboken, NJ; Coico *et al.* eds. (2005) *Current Protocols in Microbiology*, John Wiley and Sons, Inc.: Hoboken, NJ; Coligan *et al.* eds. (2005) *Current Protocols in*

*Protein Science*, John Wiley and Sons, Inc.: Hoboken, NJ; and Enna *et al.* eds. (2005) *Current Protocols in Pharmacology*, John Wiley and Sons, Inc.: Hoboken, NJ.; *Nucleic Acid Hybridization*, Hames & Higgins eds. (1985); *Transcription And Translation*, Hames & Higgins, eds. (1984); *Animal Cell Culture* Freshney, ed. (1986); *Immobilized Cells And Enzymes*, IRL Press (1986); Perbal, *A Practical Guide To Molecular Cloning* (1984); and Harlow and Lane. *Antibodies: A Laboratory Manual* (Cold Spring Harbor Laboratory Press: 1988).

## I. Definitions

[0048] As used herein, the term "antibody" refers to any form of antibody that exhibits the desired biological activity. Thus, it is used in the broadest sense and specifically covers monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, multispecific antibodies (*e.g.*, bispecific antibodies), chimeric antibodies, humanized antibodies, fully human antibodies, etc. so long as they exhibit the desired biological activity.

### Adjuvant

[0049] As used herein, the term "adjuvant" refers to a compound or mixture that enhances the immune response to an antigen. An adjuvant can serve as a tissue depot that slowly releases the antigen and also as a lymphoid system activator that non-specifically enhances the immune response (Hood *et al.*, *Immunology, Second Ed.*, 1984, Benjamin/Cummings: Menlo Park, California, p. 384). Often, a primary challenge with an antigen alone, in the absence of an adjuvant, will fail to elicit a humoral or cellular immune response. Adjuvants include, but are not limited to, complete Freund's adjuvant, incomplete Freund's adjuvant, saponin, mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil or hydrocarbon emulsions, keyhole limpet hemocyanins, and potentially useful human adjuvants such as N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine, N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine, BCG (*bacille Calmette-Guerin*) and *Corynebacterium parvum*. Preferably, the adjuvant is pharmaceutically acceptable.

### Cytokine

[0050] The term "cytokine" is a generic term for proteins released by one cell population which act on another cell as intercellular mediators. Examples of such cytokines



are lymphokines, monokines, chemokines, and traditional polypeptide hormones. Exemplary cytokines include: human IL-2, IFN- $\gamma$ , IL-6, TNF $\alpha$ , IL-17, and IL-5.

### **Cytotoxic Agent**

[0051] The term “cytotoxic agent” as used herein refers to a substance that inhibits or prevents the function of cells and/or causes destruction of cells. The term is intended to include radioactive isotopes (e.g., I<sup>131</sup>, I<sup>125</sup>, Y<sup>90</sup> and Re<sup>186</sup>), chemotherapeutic agents, and toxins such as enzymatically active toxins of bacterial, fungal, plant or animal origin, or fragments thereof.

### **Therapeutic Uses and Methods**

[0052] The PD-1 blocking agents include those which specifically bind to human PD-1, can be used to increase, enhance, stimulate or up-regulate an immune response. Desirable subjects include human patients in need of enhancement of an immune response including patients with cancer and/or a chronic viral infection.

### **Cancer**

[0053] The terms “cancer”, “cancerous”, or “malignant” refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include but are not limited to, carcinoma, lymphoma, leukemia, blastoma, and sarcoma. More particular examples of such cancers include squamous cell carcinoma, myeloma, small-cell lung cancer, non-small cell lung cancer, glioma, hodgkin's lymphoma, non-hodgkin's lymphoma, gastrointestinal (tract) cancer, renal cancer, ovarian cancer, liver cancer, lymphoblastic leukemia, lymphocytic leukemia, colorectal cancer, endometrial cancer, kidney cancer, prostate cancer, thyroid cancer, melanoma, chondrosarcoma, neuroblastoma, pancreatic cancer, glioblastoma multiforme, cervical cancer, brain cancer, stomach cancer, bladder cancer, hepatoma, breast cancer, colon carcinoma, and head and neck cancer.

[0054] PD-1 blocking agents include those used to treat cancer (*i.e.*, to inhibit the growth or survival of tumor cells). Preferred cancers whose growth may be inhibited using anti-PD-1 antibodies such as humanized anti-PD-1 antibody h409A11 and include cancers typically responsive to immunotherapy, but also cancers that have not hitherto been associated with immunotherapy. Non-limiting examples of preferred cancers for treatment include melanoma (e.g., metastatic malignant melanoma), renal cancer (e.g. clear cell carcinoma), prostate cancer (e.g. hormone refractory prostate adenocarcinoma), pancreatic

adenocarcinoma, breast cancer, colon cancer, lung cancer (e.g. non-small cell lung cancer), esophageal cancer, squamous cell carcinoma of the head and neck, liver cancer, ovarian cancer, cervical cancer, thyroid cancer, glioblastoma, glioma, leukemia, lymphoma, and other neoplastic malignancies. Malignancies that demonstrate improved disease-free and overall survival in relation to the presence of tumor-infiltrating lymphocytes in biopsy or surgical material, e.g. melanoma, colorectal, liver, kidney, stomach/esophageal, breast, pancreas, and ovarian cancer are encompassed in the methods and treatments described herein. Such cancer subtypes are known to be susceptible to immune control by T lymphocytes. Additionally, included are refractory or recurrent malignancies whose growth may be inhibited using the antibodies described herein. Particularly preferred cancers include those characterized by elevated expression of PD-1 and/or its ligands PD-L1 and/or PD-L2 in tested tissue samples, including: ovarian, renal, colorectal, pancreatic, breast, liver, gastric, esophageal cancers and melanoma. Additional cancers that can benefit from treatment with anti-PD-1 antibodies such as humanized anti-PD-1 antibody h409A11 include those associated with persistent infection with viruses such as human immunodeficiency viruses, hepatitis viruses class A, B and C, Epstein Barr virus, human papilloma viruses that are known to be causally related to for instance Kaposi's sarcoma, liver cancer, nasopharyngeal cancer, lymphoma, cervical, vulval, anal, penile and oral cancers.

### **Chemotherapeutic Agent**

[0055] A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Anti-PD-1 antibodies can be used with any one or more suitable chemotherapeutic agent. Examples of such chemotherapeutic agents include alkylating agents such as thiotepa and cyclophosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethylenethiophosphoramide and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including the synthetic analogue topotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CBI-TMI); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil

mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; antibiotics such as the enediyne antibiotics (e.g. calicheamicin, especially calicheamicin gammaII and calicheamicin phiII, see, e.g., Agnew, Chem. Intl. Ed. Engl., 33:183-186 (1994); dynemicin, including dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromomophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabycin, caminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiothane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine; razoxane; rhizoxin; sizofuran; spirogermanium; tenuazonic acid; triaziquone; 2, 2',2''-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, e.g. paclitaxel and doxetaxel; chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; vinorelbine; novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; capecitabine; and pharmaceutically acceptable salts, acids or derivatives of any

of the above. Also included are anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens and selective estrogen receptor modulators (SERMs), including, for example, tamoxifen, raloxifene, droloxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and toremifene (Fareston); aromatase inhibitors that inhibit the enzyme aromatase, which regulates estrogen production in the adrenal glands, such as, for example, 4(5)-imidazoles, aminoglutethimide, megestrol acetate, exemestane, formestane, fadrozole, vorozole, letrozole, and anastrozole; and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

### **Growth Inhibitory Agent**

**[0056]** A “growth inhibitory agent” when used herein refers to a compound or composition which inhibits growth of a cell, especially cancer cell over expressing any of the genes identified herein, either in vitro or in vivo. Thus, the growth inhibitory agent is one which significantly reduces the percentage of cells over expressing such genes in S phase. Examples of growth inhibitory agents include agents that block cell cycle progression (at a place other than S phase), such as agents that induce G1 arrest and M-phase arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine) taxanes, and topo II inhibitors such as doxorubicin, epirubicin, daunorubicin, and etoposide. Those agents that arrest G1 also spill over into S-phase arrest, for example, DNA alkylating agents such as dacarbazine, mechlorethamine, and cisplatin. Further information can be found in *The Molecular Basis of Cancer*, Mendelsohn and Israel, eds., Chapter 1, entitled “Cell cycle regulation, oncogens, and antineoplastic drugs” by Murakami et al. (WB Saunders: Philadelphia, 1995).

### **Antibody or Antibody Fragments in Combination with Additional Agents**

**[0057]** Anti-PD-1 antibody or antibody fragments can be used alone or in combination with: other anti-neoplastic agents or immunogenic agents (for example, attenuated cancerous cells, tumor antigens (including recombinant proteins, peptides, and carbohydrate molecules), antigen presenting cells such as dendritic cells pulsed with tumor derived antigen or nucleic acids, immune stimulating cytokines (for example, IL-2, IFN $\alpha$ 2, GM-CSF), and cells transfected with genes encoding immune stimulating cytokines such as but not limited to GM-CSF); standard cancer treatments (for example, chemotherapy, radiotherapy or surgery); or other antibodies (including but not limited to antibodies to VEGF, EGFR, Her2/neu, VEGF receptors, other growth factor receptors, CD20, CD40, CD-40L, CTLA-4, OX-40, 4-1BB, and ICOS).

**Infectious Diseases**

**[0058]** Antagonist anti-PD-1 antibodies or antibody fragments can also be used to prevent or treat infections and infectious disease. These agents can be used alone, or in combination with vaccines, to stimulate the immune response to pathogens, toxins, and self-antigens. The antibodies or antigen-binding fragment thereof can be used to stimulate immune response to viruses infectious to humans, including but not limited to: human immunodeficiency viruses, hepatitis viruses class A, B and C, Epstein Barr virus, human cytomegalovirus, human papilloma viruses, and herpes viruses. Antagonist anti-PD-1 antibodies or antibody fragments can be used to stimulate immune response to infection with bacterial or fungal parasites, and other pathogens. Viral infections with hepatitis B and C and HIV are among those considered to be chronic viral infections.

**[0059]** As used herein, the terms "PD-1 binding fragment," "antigen binding fragment thereof," "binding fragment thereof" or "fragment thereof" encompass a fragment or a derivative of an antibody that still substantially retains its biological activity of binding to antigen (human PD-1) and inhibiting its activity (e.g., blocking the binding of PD-1 to PDL1 and PDL2). Therefore, the term "antibody fragment" or PD-1 binding fragment refers to a portion of a full length antibody, generally the antigen binding or variable region thereof. Examples of antibody fragments include Fab, Fab', F(ab')<sub>2</sub>, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules, e.g., sc-Fv; and multispecific antibodies formed from antibody fragments. Typically, a binding fragment or derivative retains at least 10% of its PD-1 inhibitory activity. Preferably, a binding fragment or derivative retains at least 25%, 50%, 60%, 70%, 80%, 90%, 95%, 99% or 100% (or more) of its PD-1 inhibitory activity, although any binding fragment with sufficient affinity to exert the desired biological effect will be useful. It is also intended that a PD-1 binding fragment can include variants having conservative amino acid substitutions that do not substantially alter its biologic activity.

**[0060]** A "domain antibody" is an immunologically functional immunoglobulin fragment containing only the variable region of a heavy chain or the variable region of a light chain. In some instances, two or more V<sub>H</sub> regions are covalently joined with a peptide linker to create a bivalent domain antibody. The two V<sub>H</sub> regions of a bivalent domain antibody may target the same or different antigens.

**[0061]** A "bivalent antibody" comprises two antigen binding sites. In some instances, the two binding sites have the same antigen specificities. However, bivalent antibodies may

be bispecific. As used herein, the term "bispecific antibody" refers to an antibody, typically a monoclonal antibody, having binding specificities for at least two different antigenic epitopes. In one embodiment, the epitopes are from the same antigen. In another embodiment, the epitopes are from two different antigens. Methods for making bispecific antibodies are known in the art. For example, bispecific antibodies can be produced recombinantly using the co-expression of two immunoglobulin heavy chain/light chain pairs. *See, e.g., Milstein et al. (1983) Nature 305: 537-39.* Alternatively, bispecific antibodies can be prepared using chemical linkage. *See, e.g., Brennan et al. (1985) Science 229:81.* Bispecific antibodies include bispecific antibody fragments. *See, e.g., Holliger et al. (1993) Proc. Natl. Acad. Sci. U.S.A. 90:6444-48, Gruber et al. (1994) J. Immunol. 152:5368.*

**[0062]** As used herein, the term "single-chain Fv" or "scFv" antibody refers to antibody fragments comprising the V<sub>H</sub> and V<sub>L</sub> domains of antibody, wherein these domains are present in a single polypeptide chain. Generally, the Fv polypeptide further comprises a polypeptide linker between the V<sub>H</sub> and V<sub>L</sub> domains which enables the sFv to form the desired structure for antigen binding. For a review of sFv, see Pluckthun (1994) THE PHARMACOLOGY OF MONOCLONAL ANTIBODIES, vol. 113, Rosenberg and Moore eds. Springer-Verlag, New York, pp. 269-315.

**[0063]** The monoclonal antibodies herein also include camelized single domain antibodies. *See, e.g., Muyldermans et al. (2001) Trends Biochem. Sci. 26:230; Reichmann et al. (1999) J. Immunol. Methods 231:25; WO 94/04678; WO 94/25591; U.S. Pat. No. 6,005,079.* Single domain antibodies comprising two V<sub>H</sub> domains with modifications such that single domain antibodies are formed are also included.

**[0064]** As used herein, the term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy chain variable domain (V<sub>H</sub>) connected to a light chain variable domain (V<sub>L</sub>) in the same polypeptide chain (V<sub>H</sub>-V<sub>L</sub> or V<sub>L</sub>-V<sub>H</sub>). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, *e.g., EP 404,097; WO 93/11161; and Holliger et al. (1993) Proc. Natl. Acad. Sci. USA 90: 6444-6448.* For a review of engineered antibody variants generally see Holliger and Hudson (2005) *Nat. Biotechnol.* 23:1126-1136.

**[0065]** As used herein, the term "humanized antibody" refers to forms of antibodies that contain sequences from non-human (*e.g., murine*) antibodies as well as human antibodies. Such antibodies contain minimal sequence derived from non-human

immunoglobulin. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. The humanized forms of rodent antibodies will generally comprise the same CDR sequences of the parental rodent antibodies, although certain amino acid substitutions may be included to increase affinity, increase stability of the humanized antibody, or for other reasons.

**[0066]** The antibodies of the present invention also include antibodies with modified (or blocked) Fc regions to provide altered effector functions. See, e.g., U.S. Pat. No. 5,624,821; WO2003/086310; WO2005/120571; WO2006/0057702; Presta (2006) *Adv. Drug Delivery Rev.* 58:640-656. Such modification can be used to enhance or suppress various reactions of the immune system, with possible beneficial effects in diagnosis and therapy. Alterations of the Fc region include amino acid changes (substitutions, deletions and insertions), glycosylation or deglycosylation, and adding multiple Fc. Changes to the Fc can also alter the half-life of antibodies in therapeutic antibodies, and a longer half-life would result in less frequent dosing, with the concomitant increased convenience and decreased use of material. See Presta (2005) *J. Allergy Clin. Immunol.* 116:731 at 734-35.

**[0067]** The term "fully human antibody" refers to an antibody that comprises human immunoglobulin protein sequences only. A fully human antibody may contain murine carbohydrate chains if produced in a mouse, in a mouse cell, or in a hybridoma derived from a mouse cell. Similarly, "mouse antibody" refers to an antibody which comprises mouse immunoglobulin sequences only. A fully human antibody may be generated in a human being, in a transgenic animal having human immunoglobulin germline sequences, by phage display or other molecular biological methods.

**[0068]** As used herein, the term "hypervariable region" refers to the amino acid residues of an antibody that are responsible for antigen-binding. The hypervariable region comprises amino acid residues from a "complementarity determining region" or "CDR" (e.g. residues 24-34 (CDRL1), 50-56 (CDRL2) and 89-97 (CDRL3) in the light chain variable domain and residues 31-35 (CDRH1), 50-65 (CDRH2) and 95-102 (CDRH3) in the heavy chain variable domain (Kabat *et al.* (1991) *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md.) and/or those residues from a "hypervariable loop" (i.e. residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in

the light chain variable domain and 26-32 (H1), 53-55 (H2) and 96-101 (H3) in the heavy chain variable domain (Chothia and Lesk (1987) *J. Mol. Biol.* 196: 901-917). As used herein, the term "framework" or "FR" residues refers to those variable domain residues other than the hypervariable region residues defined herein as CDR residues. The residue numbering above relates to the Kabat numbering system and does not necessarily correspond in detail to the sequence numbering in the accompanying Sequence Listing.

[0069] “Conservatively modified variants” or “conservative substitution” refers to substitutions of amino acids are known to those of skill in this art and may be made generally without altering the biological activity of the resulting molecule, even in essential regions of the polypeptide. Such exemplary substitutions are preferably made in accordance with those set forth in Table 1 as follows:

**Table 1**  
**Exemplary Conservative Amino Acid Substitutions**

Original residue	Conservative substitution
Ala (A)	Gly; Ser
Arg (R)	Lys, His
Asn (N)	Gln; His
Asp (D)	Glu; Asn
Cys (C)	Ser; Ala
Gln (Q)	Asn
Glu (E)	Asp; Gln
Gly (G)	Ala
His (H)	Asn; Gln
Ile (I)	Leu; Val
Leu (L)	Ile; Val
Lys (K)	Arg; His
Met (M)	Leu; Ile; Tyr
Phe (F)	Tyr; Met; Leu
Pro (P)	Ala
Ser (S)	Thr
Thr (T)	Ser
Trp (W)	Tyr; Phe
Tyr (Y)	Trp; Phe
Val (V)	Ile; Leu



[0070] In addition, those of skill in this art recognize that, in general, single amino acid substitutions in non-essential regions of a polypeptide do not substantially alter biological activity. See, e.g., Watson *et al.* (1987) *Molecular Biology of the Gene*, The Benjamin/Cummings Pub. Co., p. 224 (4th Edition).

[0071] The phrase "consists essentially of," or variations such as "consist essentially of" or "consisting essentially of," as used throughout the specification and claims, indicate the inclusion of any recited elements or group of elements, and the optional inclusion of other elements, of similar or different nature than the recited elements, that do not materially change the basic or novel properties of the specified dosage regimen, method, or composition. As a non-limiting example, a binding compound that consists essentially of a recited amino acid sequence may also include one or more amino acids, including substitutions of one or more amino acid residues, that do not materially affect the properties of the binding compound.

[0072] "Immune condition" or "immune disorder" encompasses, e.g., pathological inflammation, an inflammatory disorder, and an autoimmune disorder or disease. "Immune condition" also refers to infections, persistent infections, and proliferative conditions, such as cancer, tumors, and angiogenesis, including infections, tumors, and cancers that resist eradication by the immune system. "Cancerous condition" includes, e.g., cancer, cancer cells, tumors, angiogenesis, and precancerous conditions such as dysplasia.

[0073] The antibody, or binding composition derived from the antigen-binding site of an antibody, of the contemplated formulation or method binds to its antigen with an affinity that is at least two fold greater, preferably at least ten times greater, more preferably at least 20-times greater, and most preferably at least 100-times greater than the affinity with unrelated antigens. In a preferred embodiment the antibody will have an affinity that is greater than about  $10^9$  liters/mol, as determined, e.g., by Scatchard analysis. Munsen *et al.* (1980) *Analyt. Biochem.* 107:220-239.

#### **Pharmaceutical Composition Definitions**

[0074] The term "bulking agents" comprise agents that provide the structure of the freeze-dried product. Common examples used for bulking agents include mannitol, glycine, lactose and sucrose. In addition to providing a pharmaceutically elegant cake, bulking agents may also impart useful qualities in regard to modifying the collapse temperature, providing freeze-thaw protection, and enhancing the protein stability over long-term storage. These agents can also serve as tonicity modifiers.

**[0075]** The term "buffer" encompasses those agents which maintain the solution pH in an acceptable range prior to lyophilization and may include succinate (sodium or potassium), histidine, phosphate (sodium or potassium), Tris (tris (hydroxymethyl) aminomethane), diethanolamine, citrate (sodium) and the like. The buffer of this invention has a pH in the range from about 5.0 to about 6.0; and preferably has a pH of about 5.5. Examples of buffers that will control the pH in this range include succinate (such as sodium succinate), gluconate, histidine, citrate and other organic acid buffers. In arriving at the exemplary formulation, histidine, acetate and citrate buffers in the pH range of 5.0-6.0 were explored for suitability. Histidine and acetate buffer systems performed better than the citrate system. Histidine buffer is a preferred buffer system, because acetate buffer systems are not compatible with the lyophilization process.

**[0076]** The term "cryoprotectants" generally includes agents which provide stability to the protein against freezing-induced stresses, presumably by being preferentially excluded from the protein surface. They may also offer protection during primary and secondary drying, and long-term product storage. Examples are polymers such as dextran and polyethylene glycol; sugars such as sucrose, glucose, trehalose, and lactose; surfactants such as polysorbates; and amino acids such as glycine, arginine, and serine.

**[0077]** The terms "lyophilization," "lyophilized," and "freeze-dried" refer to a process by which the material to be dried is first frozen and then the ice or frozen solvent is removed by sublimation in a vacuum environment. An excipient may be included in pre-lyophilized formulations to enhance stability of the lyophilized product upon storage.

**[0078]** The term "lyoprotectant" includes agents that provide stability to the protein during the drying or 'dehydration' process (primary and secondary drying cycles), presumably by providing an amorphous glassy matrix and by binding with the protein through hydrogen bonding, replacing the water molecules that are removed during the drying process. This helps to maintain the protein conformation, minimize protein degradation during the lyophilization cycle and improve the long-term product stability. Examples include polyols or sugars such as sucrose and trehalose.

**[0079]** The term "pharmaceutical formulation" refers to preparations which are in such form as to permit the active ingredients to be effective, and which contains no additional components which are toxic to the subjects to which the formulation would be administered.

**[0080]** "Pharmaceutically acceptable" excipients (vehicles, additives) are those which can reasonably be administered to a subject mammal to provide an effective dose of the active ingredient employed.

**[0081]** "Reconstitution time" is the time that is required to rehydrate a lyophilized formulation with a solution to a particle-free clarified solution.

**[0082]** A "stable" formulation is one in which the protein therein essentially retains its physical stability and/or chemical stability and/or biological activity upon storage. Various analytical techniques for measuring protein stability are available in the art and are reviewed in Peptide and Protein Drug Delivery, 247-301, Vincent Lee Ed., Marcel Dekker, Inc., New York, N.Y., Pubs. (1991) and Jones, A. Adv. Drug Delivery Rev. 10:29-90 (1993). Stability can be measured at a selected temperature for a selected time period.

**[0083]** A "stable" lyophilized antibody formulation is a lyophilized antibody formulation with no significant changes observed at a refrigerated temperature (2-8° C) for at least 12 months, preferably 2 years, and more preferably 3 years; or at room temperature (23-27°C) for at least 3 months, preferably 6 months, and more preferably 1 year. Typical acceptable criteria for stability are as follows. No more than 10%, preferably 5%, of antibody monomer is degraded as measured by SEC-HPLC. The rehydrated solution is typically colorless, or clear to slightly opalescent by visual analysis. The concentration, pH and osmolality of the formulation have no more than  $\pm 10\%$  change. Potency is typically within a range of 50-150% of the reference. No more than 10%, preferably 5% of clipping is observed. No more than 10%, preferably 5% of aggregation is formed.

**[0084]** A "stable" pharmaceutical antibody formulation (including a lyophilized formulation, a reconstituted liquid, as well as a liquid formulation that is a "final" formulation (*i.e.*, has not been previously lyophilized)) is a pharmaceutical antibody formulation with no significant changes observed at a refrigerated temperature (2-8°C) for at least 3 months, preferably 6 months, and more preferably 1 year, and even more preferably up through 2 years. Additionally, a "stable" liquid formulation includes one that exhibits desired features at temperatures including at 25°C and 40°C for periods including 1 month, 3 months, 6 months, 12 months, and/or 24 months. Typical acceptable criteria for stability are as follows. Typically, no more than about 10%, preferably about 5%, of antibody monomer is degraded as measured by SEC-HPLC. The pharmaceutical antibody formulation is colorless, or clear to slightly opalescent by visual analysis. The concentration, pH and osmolality of the formulation have no more than  $\pm 10\%$  change. Potency is typically within 50-150 of the reference. Typically, no more than about 10%, preferably about 5% of clipping is observed. Typically, no more than about 10%, preferably about 5% of aggregation is formed.

**[0085]** An antibody "retains its physical stability" in a pharmaceutical formulation if it shows no significant increase of aggregation, precipitation and/or denaturation upon visual

examination of color and/or clarity, or as measured by UV light scattering, size exclusion chromatography (SEC) and dynamic light scattering. The changes of protein conformation can be evaluated by fluorescence spectroscopy, which determines the protein tertiary structure, and by FTIR spectroscopy, which determines the protein secondary structure.

**[0086]** An antibody "retains its chemical stability" in a pharmaceutical formulation, if it shows no significant chemical alteration. Chemical stability can be assessed by detecting and quantifying chemically altered forms of the protein. Degradation processes that often alter the protein chemical structure include hydrolysis or clipping (evaluated by methods such as size exclusion chromatography and SDS-PAGE), oxidation (evaluated by methods such as by peptide mapping in conjunction with mass spectroscopy or MALDI/TOF/MS), deamidation (evaluated by methods such as ion-exchange chromatography, capillary isoelectric focusing, peptide mapping, isoaspartic acid measurement), and isomerization (evaluated by measuring the isoaspartic acid content, peptide mapping, etc.).

**[0087]** An antibody "retains its biological activity" in a pharmaceutical formulation, if the biological activity of the antibody at a given time is within a predetermined range of the biological activity exhibited at the time the pharmaceutical formulation was prepared. The biological activity of an antibody can be determined, for example, by an antigen binding assay.

**[0088]** The term "isotonic" means that the formulation of interest has essentially the same osmotic pressure as human blood. Isotonic formulations will generally have an osmotic pressure from about 270-328 mOsm. Slightly hypotonic pressure is 250-269 and slightly hypertonic pressure is 328-350 mOsm. Osmotic pressure can be measured, for example, using a vapor pressure or ice-freezing type osmometer.

**[0089]** Tonicity Modifiers: Salts (NaCl, KCl, MgCl<sub>2</sub>, CaCl<sub>2</sub>, etc.) are used as tonicity modifiers to control osmotic pressure. In addition, cryoprotectants/lyoprotectants and/or bulking agents such as sucrose, mannitol, glycine etc. can serve as tonicity modifiers.

#### **Analytical Methods**

**[0090]** Analytical methods suitable for evaluating the product stability include size exclusion chromatography (SEC), dynamic light scattering test (DLS), differential scanning calorimetry (DSC), iso-asp quantification, potency, UV at 340 nm, UV spectroscopy, and FTIR. SEC (J. Pharm. Scien., 83:1645-1650, (1994); Pharm. Res., 11:485 (1994); J. Pharm. Bio. Anal., 15:1928 (1997); J. Pharm. Bio. Anal., 14:1133-1140 (1986)) measures percent monomer in the product and gives information of the amount of soluble aggregates. DSC (Pharm. Res., 15:200 (1998); Pharm. Res., 9:109 (1982)) gives information of protein

denaturation temperature and glass transition temperature. DLS (American Lab., November (1991)) measures mean diffusion coefficient, and gives information of the amount of soluble and insoluble aggregates. UV at 340 nm measures scattered light intensity at 340 nm and gives information about the amounts of soluble and insoluble aggregates. UV spectroscopy measures absorbance at 278 nm and gives information of protein concentration. FTIR (Eur. J. Pharm. Biopharm., 45:231 (1998); Pharm. Res., 12:1250 (1995); J. Pharm. Scien., 85:1290 (1996); J. Pharm. Scien., 87:1069 (1998)) measures IR spectrum in the amide one region, and gives information of protein secondary structure.

**[0091]** The iso-asp content in the samples is measured using the Isoquant Isoaspartate Detection System (Promega). The kit uses the enzyme Protein Isoaspartyl Methyltransferase (PIMT) to specifically detect the presence of isoaspartic acid residues in a target protein. PIMT catalyzes the transfer of a methyl group from S-adenosyl-L-methionine to isoaspartic acid at the .alpha.-carboxyl position, generating S-adenosyl-L-homocysteine (SAH) in the process. This is a relatively small molecule, and can usually be isolated and quantitated by reverse phase HPLC using the SAH HPLC standards provided in the kit.

**[0092]** The potency or bioidentity of an antibody can be measured by its ability to bind to its antigen. The specific binding of an antibody to its antigen can be quantitated by any method known to those skilled in the art, for example, an immunoassay, such as ELISA (enzyme-linked immunosorbant assay).

**[0093]** A "reconstituted" formulation is one that has been prepared by dissolving a lyophilized protein formulation in a diluent such that the protein is dispersed in the reconstituted formulation. The reconstituted formulation is suitable for administration, *e.g.* parenteral administration), and may optionally be suitable for subcutaneous administration.

#### **Humanized Anti-PD-1 Antibodies**

**[0094]** DNA constructs encoding the variable regions of the heavy and light chains of the humanized antibodies h409A11, h409A16 and h409A17 are described in WO2008/156712.

**[0095]** The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. The present invention is not to be limited in scope by the culture deposited, since the deposited embodiment is intended as a single illustration of one aspect of the invention and any culture that is functionally equivalent is within the scope of this invention. The deposit of material herein does not constitute an admission that the written description herein contained is inadequate to enable the practice of any aspect of the invention, including the best mode thereof, nor is it to be construed as limiting the scope of

the claims to the specific illustration that it represents. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims.

**[0096]** Sequences are provided for exemplary anti-human PD-1 antibodies; a summary table of the sequences is provided in Table 6. CDRs are provided under separate sequence identifiers, as indicated in Table 2 for h409A11.

**[0097]** Ordinarily, amino acid sequence variants of the humanized anti-PD-1 antibody will have an amino acid sequence having at least 75% amino acid sequence identity with the original humanized antibody amino acid sequences of either the heavy or the light chain more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, and most preferably at least 95, 98, or 99%. Identity or homology with respect to this sequence is defined herein as the percentage of amino acid residues in the candidate sequence that are identical with the humanized anti-PD-1 residues, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. None of N-terminal, C-terminal, or internal extensions, deletions, or insertions into the antibody sequence shall be construed as affecting sequence identity or homology.

**[0098]** The humanized antibody can be selected from any class of immunoglobulins, including IgM, IgG, IgD, IgA, and IgE. Preferably, the antibody is an IgG antibody. Any isotype of IgG can be used, including IgG<sub>1</sub>, IgG<sub>2</sub>, IgG<sub>3</sub>, and IgG<sub>4</sub>. Different constant domains may be appended to the humanized V<sub>L</sub> and V<sub>H</sub> regions provided herein. For example, if a particular intended use of an antibody (or fragment) of the present invention were to call for altered effector functions, a heavy chain constant domain other than IgG1 may be used. Although IgG1 antibodies provide for long half-life and for effector functions, such as complement activation and antibody-dependent cellular cytotoxicity, such activities may not be desirable for all uses of the antibody. In such instances an IgG4 constant domain, for example, may be used.

**[0099]** Likewise, either class of light chain can be used in the compositions and methods herein. Specifically, kappa, lambda, or variants thereof are useful in the present compositions and methods.

**[00100]** CDR and FR residues are determined according to the standard sequence definition of Kabat. Kabat *et al.* (1987) Sequences of Proteins of Immunological Interest, National Institutes of Health, Bethesda Md.

[00101] The signal sequences, or nucleic acid sequences encoding the signal sequences, may be appended to the N-terminus of the respective antibody chains to create a precursor protein for secretion from a host cell. Alternative signal sequences may also be used, and several can be found at “SPdb: a Signal Peptide Database.” Choo *et al.* (2005) *BMC Bioinformatics* 6:249.

[00102]

**TABLE 2**  
**H409A11 CDR Sequences**

<b>Antibody</b>	<b>CDR Sequence</b>	<b>SEQ ID NO:</b>
H409A11	Light chain CDR1 (equivalent to hPD-1.09A light chain CDR1) RASKGVSTSGYSYLH	15
H409A11	Light chain CDR2 (equivalent to hPD-1.09A light chain CDR2) LASYLES	16
H409A11	Light chain CDR3 (equivalent to hPD-1.09A light chain CDR3) QHSRDLPLT	17
H409A11	Heavy chain CDR1 (equivalent to hPD-1.09A heavy chain CDR1) NYMY	18
H409A11	Heavy chain CDR2 (equivalent to hPD-1.09A heavy chain CDR2) GINPSNGGTNFNEKFKN	19
H409A11	Heavy chain CDR3 (equivalent to hPD-1.09A heavy chain CDR3) RDYRFDMGFDY	20

### **Biological Activity of Humanized Anti-PD-1**

[00103] Formulations of the present invention include antibodies and fragments thereof that are biologically active when reconstituted or in liquid form. As used herein, the term “biologically active” refers to an antibody or antibody fragment that is capable of binding the desired the antigenic epitope and directly or indirectly exerting a biologic effect. Typically, these effects result from the failure of PD-1 to bind its ligands. As used herein, the term “specific” refers to the selective binding of the antibody to the target antigen epitope.

Antibodies can be tested for specificity of binding by comparing binding to PD-1 to binding to irrelevant antigen or antigen mixture under a given set of conditions.

### **Lyophilized Pharmaceutical Compositions**

**[00104]** Lyophilized formulations of therapeutic proteins provide several advantages. Lyophilized formulations in general offer better chemical stability than solution formulations, and thus increased half-life. A lyophilized formulation may also be reconstituted at different concentrations depending on clinical factors, such as route of administration or dosing. For example, a lyophilized formulation may be reconstituted at a high concentration (i.e. in a small volume) if necessary for subcutaneous administration, or at a lower concentration if administered intravenously. High concentrations may also be necessary if high dosing is required for a particular subject, particularly if administered subcutaneously where injection volume must be minimized. One such lyophilized antibody formulation is disclosed at U.S. Pat. No. 6,267,958, which is hereby incorporated by reference in its entirety. Lyophilized formulations of another therapeutic protein are disclosed at U.S. Pat. No. 7,247,707, which is hereby incorporated by reference in its entirety.

**[00105]** Typically, the lyophilized formulation is prepared in anticipation of reconstitution at high concentration of drug product (DP, in an exemplary embodiment humanized anti-PD-1 antibody h409A11, or antigen binding fragment thereof), *i.e.* in anticipation of reconstitution in a low volume of water. Subsequent dilution with water or isotonic buffer can then readily be used to dilute the DP to a lower concentration. Typically, excipients are included in a lyophilized formulation of the present invention at levels that will result in a roughly isotonic formulation when reconstituted at high DP concentration, *e.g.* for subcutaneous administration. Reconstitution in a larger volume of water to give a lower DP concentration will necessarily reduce the tonicity of the reconstituted solution, but such reduction may be of little significance in non-subcutaneous, *e.g.* intravenous, administration. If isotonicity is desired at lower DP concentration, the lyophilized powder may be reconstituted in the standard low volume of water and then further diluted with isotonic diluent, such as 0.9% sodium chloride.

**[00106]** In an embodiment of the present invention, humanized anti-PD-1 antibody (or antigen binding fragment thereof) is formulated as a lyophilized powder for reconstituting and utilizing for intravenous administration. Exemplary formulations are described in Tables 3-4, and in Figures 1-9. In certain embodiments, the antibody (or antigen binding fragment thereof) is provided at about 50 mg/vial, and is reconstituted with sterile water for injection prior to use. If desired, the reconstituted antibody may be aseptically diluted with 0.9%



Sodium Chloride Injection USP in a sterile IV container. The target pH of the reconstituted formulation is  $5.5 \pm 0.5$ . In various embodiments, the lyophilized formulation of the present invention enables reconstitution of the anti-PD-1 antibody to high concentrations, such as about 20, 25, 30, 40, 50, 60, 75, 100 or more mg/mL.

**[00107]** The present invention provides in certain embodiments, a lyophilized formulation comprising humanized anti-PD-1 antibody, a histidine buffer at about pH 5.5, or at about pH 5.0, for example at about 5.1, 5.2, 5.3, 5.4, 5.6, 5.7, 5.8, 5.9, or 6.0.

**[00108]** When a range of pH values is recited, such as “a pH between pH 5.5 and 6.0,” the range is intended to be inclusive of the recited values. Unless otherwise indicated, the pH refers to the pH after reconstitution of the lyophilized formulations of the present invention. The pH is typically measured at 25°C using standard glass bulb pH meter. As used herein, a solution comprising “histidine buffer at pH X” refers to a solution at pH X and comprising the histidine buffer, *i.e.* the pH is intended to refer to the pH of the solution.

**[00109]** The formulation in Table 3 reflects the weight of the components in a batch formulation, as lyophilized in vials, and as reconstituted. Lyophilized formulations are by definition essentially dry, and thus the concept of concentration is not useful in describing them. Describing a lyophilized formulation in the terms of the weight of the components in a unit dose vial is more useful, but is problematic because it varies for different doses or vial sizes. In describing the lyophilized formulations of the present invention, it is useful to express the amount of a component as the ratio of the weight of the component compared to the weight of the drug substance (DS) in the same sample (e.g. a vial). This ratio may be expressed as a percentage. Such ratios reflect an intrinsic property of the lyophilized formulations of the present invention, independent of vial size, dosing, and reconstitution protocol.

**[00110]** In other embodiments, the lyophilized formulation of anti-human PD-1 antibody, or antigen binding fragment, is defined in terms of the pre-lyophilization solution used to make the lyophilized formulation, such as the pre-lyophilization solution. In one embodiment the pre-lyophilization solution comprises antibody, or antigen-binding fragment thereof, at a concentration of about 25mg/mL. Such pre-lyophilization solutions may be at pH 4.4 – 5.2 (including about 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1. and 5.2), e.g. preferably about pH 4.8, or about pH 5.5.

**[00111]** In yet other embodiments, the lyophilized formulation of anti-human PD-1 antibody, or antigen binding fragment, is defined in terms of the reconstituted solution

generated from the lyophilized formulation, such as the reconstituted solution disclosed at Table 4.

**[00112]** Reconstituted solutions may comprise antibody, or antigen-binding fragment thereof, at concentrations of about 10, 15, 20, 25, 30, 40, 50, 60, 75, 80, 90 or 100 mg/mL or higher concentrations such as 150mg/mL, 200 mg/mL, 250 mg/mL, or up to about 300 mg/mL. Such reconstituted solutions may be at about pH 5.5, or range from about pH 5.0 to about 6.0

**[00113]** The lyophilized formulations of the present invention are formed by lyophilization (freeze-drying) of a pre-lyophilization solution. Freeze-drying is accomplished by freezing the formulation and subsequently subliming water at a temperature suitable for primary drying. Under this condition, the product temperature is below the eutectic point or the collapse temperature of the formulation. Typically, the shelf temperature for the primary drying will range from about -30 to 25°C (provided the product remains frozen during primary drying) at a suitable pressure, ranging typically from about 50 to 250 mTorr. The formulation, size and type of the container holding the sample (e.g., glass vial) and the volume of liquid will dictate the time required for drying, which can range from a few hours to several days (e.g. 40-60 hrs). A secondary drying stage may be carried out at about 0-40°C, depending primarily on the type and size of container and the type of protein employed. The secondary drying time is dictated by the desired residual moisture level in the product and typically takes at least about 5 hours. Typically, the moisture content of a lyophilized formulation is less than about 5%, and preferably less than about 3%. The pressure may be the same as that employed during the primary drying step. Freeze-drying conditions can be varied depending on the formulation and vial size.

**[00114]** In some instances, it may be desirable to lyophilize the protein formulation in the container in which reconstitution of the protein is to be carried out in order to avoid a transfer step. The container in this instance may, for example, be a 3, 5, 10, 20, 50 or 100 cc vial.

**[00115]** The lyophilized formulations of the present invention are reconstituted prior to administration. The protein may be reconstituted at a concentration of about 10, 15, 20, 25, 30, 40, 50, 60, 75, 80, 90 or 100 mg/mL or higher concentrations such as 150mg/mL, 200 mg/mL, 250 mg/mL, or 300 mg/mL up to about 500 mg/mL. High protein concentrations are particularly useful where subcutaneous delivery of the reconstituted formulation is intended. However, for other routes of administration, such as intravenous administration, lower concentrations of the protein may be desired (e.g. from about 5-50 mg/mL).

**[00116]** Reconstitution generally takes place at a temperature of about 25°C to ensure complete hydration, although other temperatures may be employed as desired. The time required for reconstitution will depend, *e.g.*, on the type of diluent, amount of excipient(s) and protein. Exemplary diluents include sterile water, bacteriostatic water for injection (BWFI), a pH buffered solution (*e.g.* phosphate-buffered saline), sterile saline solution, Ringer's solution or dextrose solution.

**[00117]** The lyophilized formulations of the present invention are expected to be stable for at least about 36 months (based on the stability data from Figures 1-9). In addition, the liquid formulation is expected to exhibit stability for at least 24 months, based on 24 months of stability data from reconstituted h409A11 formulation in polypropylene tubes at 2-8°C.

**[00118]** In line with the results shown in Figures 1-9, stability has been observed through 2 years for a refrigerated reconstituted formulation of h409A11. 2mL samples in polypropylene tubes were stored at 5°C, and 25H and RH4 conditions and tested at initial, 1, 3, 6, 9, 12, 18, and 24 month periods. This reconstituted h409A11 formulation has the same substituents in the same concentration as a liquid h409A11 formulation (*i.e.*, a formulation that was not lyophilized) and the stability is expected to be the same.

#### **Liquid Pharmaceutical Compositions**

**[00119]** A liquid antibody formulation can be made by taking the drug substance (*e.g.*, anti-humanized PD-1) which is in liquid form (*e.g.*, h409A11 in an aqueous pharmaceutical formulation) and buffer exchanging it into the desired buffer as the last step of the purification process. There is no lyophilization step in this embodiment. The drug substance in the final buffer is concentrated to a desired concentration. Excipients such as sucrose and polysorbate 80 are added to the drug substance and it is diluted using the appropriate buffer to final protein concentration. The final formulated drug substance is filtered using 0.22µm filters and filled into a final container (*e.g.* glass vials). Such a liquid formulation is exemplified by a final liquid formulation comprising 10 mM histidine pH 5.5, 7% sucrose, 0.02% polysorbate 80, and 25 mg/mL h409A11.

**[00120]** Various literature references are available to facilitate selection of pharmaceutically acceptable carriers or excipients. *See, e.g., Remington's Pharmaceutical Sciences* and *U.S. Pharmacopeia: National Formulary*, Mack Publishing Company, Easton, PA (1984); Hardman *et al.* (2001) *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, McGraw-Hill, New York, NY; Gennaro (2000) *Remington: The Science and Practice of Pharmacy*, Lippincott, Williams, and Wilkins, New York, NY; Avis *et al.* (eds.) (1993) *Pharmaceutical Dosage Forms: Parenteral Medications*, Marcel Dekker, NY;

Lieberman, *et al.* (eds.) (1990) *Pharmaceutical Dosage Forms: Tablets*, Marcel Dekker, NY; Lieberman *et al.* (eds.) (1990) *Pharmaceutical Dosage Forms: Disperse Systems*, Marcel Dekker, NY; Weiner and Kotkoskie (2000) *Excipient Toxicity and Safety*, Marcel Dekker, Inc., New York, NY.

**[00121]** Toxicity is a consideration in selecting the proper dosing of a therapeutic agent, such as a humanized anti-PD-1 antibody (or antigen binding fragment thereof). Toxicity and therapeutic efficacy of the antibody compositions can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD<sub>50</sub> (the dose lethal to 50% of the population) and the ED<sub>50</sub> (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio of LD<sub>50</sub> to ED<sub>50</sub>. Antibodies exhibiting high therapeutic indices are preferred. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED<sub>50</sub> with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized.

**[00122]** Suitable routes of administration may, for example, include parenteral delivery, including intramuscular, intradermal, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal. Drugs can be administered in a variety of conventional ways, such as intraperitoneal, parenteral, intraarterial or intravenous injection. Modes of administration in which the volume of solution must be limited (*e.g.* subcutaneous administration) require a lyophilized formulation to enable reconstitution at high concentration.

**[00123]** Alternately, one may administer the antibody in a local rather than systemic manner, for example, via injection of the antibody directly into a pathogen-induced lesion characterized by immunopathology, often in a depot or sustained release formulation. Furthermore, one may administer the antibody in a targeted drug delivery system, for example, in a liposome coated with a tissue-specific antibody, targeting, for example, pathogen-induced lesion characterized by immunopathology. The liposomes will be targeted to and taken up selectively by the afflicted tissue.

**[00124]** Selecting an administration regimen for a therapeutic depends on several factors, including the serum or tissue turnover rate of the entity, the level of symptoms, the immunogenicity of the entity, and the accessibility of the target cells in the biological matrix. Preferably, an administration regimen maximizes the amount of therapeutic delivered to the

patient consistent with an acceptable level of side effects. Accordingly, the amount of biologic delivered depends in part on the particular entity and the severity of the condition being treated. Guidance in selecting appropriate doses of antibodies, cytokines, and small molecules are available. See, e.g., Wawrzynczak (1996) *Antibody Therapy*, Bios Scientific Pub. Ltd, Oxfordshire, UK; Kresina (ed.) (1991) *Monoclonal Antibodies, Cytokines and Arthritis*, Marcel Dekker, New York, NY; Bach (ed.) (1993) *Monoclonal Antibodies and Peptide Therapy in Autoimmune Diseases*, Marcel Dekker, New York, NY; Baert *et al.* (2003) *New Engl. J. Med.* 348:601-608; Milgrom *et al.* (1999) *New Engl. J. Med.* 341:1966-1973; Slamon *et al.* (2001) *New Engl. J. Med.* 344:783-792; Beniaminovitz *et al.* (2000) *New Engl. J. Med.* 342:613-619; Ghosh *et al.* (2003) *New Engl. J. Med.* 348:24-32; Lipsky *et al.* (2000) *New Engl. J. Med.* 343:1594-1602; Physicians' Desk Reference 2003 (Physicians' Desk Reference, 57th Ed); Medical Economics Company; ISBN: 1563634457; 57th edition (November 2002).

**[00125]** Determination of the appropriate dose is made by the clinician, e.g., using parameters or factors known or suspected in the art to affect treatment or predicted to affect treatment. The appropriate dosage ("therapeutically effective amount") of the protein will depend, for example, on the condition to be treated, the severity and course of the condition, whether the protein is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the protein, the type of protein used, and the discretion of the attending physician. Generally, the dose begins with an amount somewhat less than the optimum dose and it is increased by small increments thereafter until the desired or optimum effect is achieved relative to any negative side effects. Important diagnostic measures include those of symptoms of, e.g., the inflammation or level of inflammatory cytokines produced. The antibody is suitably administered to the patient at one time or repeatedly. The antibody may be administered alone or in conjunction with other drugs or therapies.

**[00126]** A pharmaceutical antibody formulation can be administered by continuous infusion, or by doses at intervals of, e.g., one day, 1-7 times per week, one week, two weeks, three weeks, monthly, bimonthly, etc. A preferred dose protocol is one involving the maximal dose or dose frequency that avoids significant undesirable side effects. A total weekly dose is generally at least 0.05 µg/kg, 0.2 µg/kg, 0.5 µg/kg, 1 µg/kg, 10 µg/kg, 100 µg/kg, 0.2 mg/kg, 1.0 mg/kg, 2.0 mg/kg, 10 mg/kg, 25 mg/kg, 50 mg/kg body weight or more. See, e.g., Yang *et al.* (2003) *New Engl. J. Med.* 349:427-434; Herold *et al.* (2002) *New Engl. J. Med.* 346:1692-1698; Liu *et al.* (1999) *J. Neurol. Neurosurg. Psych.* 67:451-456; Portielji

*et al.* (20003) *Cancer Immunol. Immunother.* 52:133-144. The desired dose of a small molecule therapeutic, *e.g.*, a peptide mimetic, natural product, or organic chemical, is about the same as for an antibody or polypeptide, on a moles/kg basis.

**[00127]** In certain embodiments, dosing will comprise administering to a subject escalating doses of 1.0, 3.0, and 10 mg/kg of the pharmaceutical formulation, *i.e.*, a formulation comprising h409A11, over the course of treatment. The formulation comprising h409A11 can be a reconstituted liquid formulation, or it can be a liquid formulation not previously lyophilized. Time courses can vary, and can continue as long as desired effects are obtained. In certain embodiments, dose escalation will continue up to a dose of about 10mg/kg. In certain embodiments, the subject will have a histological or cytological diagnosis of melanoma, or other form of solid tumor, and in certain instances, a subject may have non-measurable disease. In certain embodiments, the subject will have been treated with other chemotherapeutics, while in other embodiments, the subject will be treatment naïve.

**[00128]** In yet additional embodiments, the dosing regimen will comprise administering a dose of 1, 3, or 10mg/kg of any of the pharmaceutical formulations described herein (*i.e.*, a formulation comprising h409A11), throughout the course of treatment. For such a constant dosing regimen, the interval between doses will be about 14 days ( $\pm 2$  days). In certain embodiments, the interval between doses will be about 21 days ( $\pm 2$  days).

**[00129]** In certain embodiments, the dosing regimen will comprise administering a dose of from about 0.005mg/kg to about 10mg/kg, with intra-patient dose escalation. In certain embodiments, a dose of 5 mg/kg or 10 mg/kg will be administered at intervals of every 3 weeks, or every 2 weeks. In yet additional embodiments, a dose of 3mg/kg will be administered at three week intervals for melanoma patients or patients with other solid tumors. In these embodiments, patients should have non-resectable disease; however, patients may have had previous surgery.

**[00130]** In certain embodiments, a subject will be administered a 30 minute IV infusion of any of the pharmaceutical formulations described herein. In certain embodiments for the escalating dose, the dosing interval will be about 28 days ( $\pm 1$  day) between the first and second dose. In certain embodiments, the interval between the second and third doses will be about 14 days ( $\pm 2$  days). In certain embodiments, the dosing interval will be about 14 days ( $\pm 2$  days), for doses subsequent to the second dose.

**[00131]** In certain embodiments, the use of cell surface markers and/or cytokine markers, as described in co-pending patent publications WO2012/018538 or

WO2008/156712 will be used in bioassays for monitoring, diagnostic, patient selection, and/or treatment regimens involving blockade of the PD-1 pathway.

**[00132]** Subcutaneous administration may performed by injected using a syringe, or using other injection devices (e.g. the Inject-ease<sup>®</sup> device); injector pens; or needleless devices (e.g. MediJector and BioJector<sup>®</sup>).

**[00133]** The broad scope of this invention is best understood with reference to the following examples, which are not intended to limit the inventions to the specific embodiments. The specific embodiments described herein are offered by way of example only, and the invention is to be limited by the terms of the appended claims, along with the full scope of equivalents to which such claims are entitled.

## EXAMPLES

### EXAMPLE 1

#### Antibody Production

**[00134]** h409A11 is a humanized monoclonal antibody that binds to human PD-1 and blocks the interaction between PD-1 and its ligands PDL1 and PDL2. The antibody is an IgG4/kappa isotype with a stabilizing S228P sequence alteration in the Fc region. Table 2 provides a list of the CDR sequences. The theoretical molecular weights of the heavy and light chains derived from the amino acid sequences, excluding glycosylation, are 49.3kDa and 23.7 kDa, respectively. The parental antibody (hPD-1.09A) was produced by immunizing mice with hPD-1 DNA. The h409A11 antibody was generated by humanization of the parental murine anti-human PD-1 antibody by the Medical Research Council (Cambridge, UK) using CDR grafting technology, (e.g., U.S. Patent No. 5,225,539), as described in co-pending WO2008/156712.

**[00135]** An expression plasmid was constructed for expression of heavy and light chains of h409A11. The nucleotide sequences encoding the heavy and light chains, along with their respective promoters and poly A signal sequence, were confirmed by DNA sequence analysis. The expression vector was subsequently used to transfect a CHO cell line. An antibody-expressing clone was selected for the generation of a Master Seed Bank (MSB), based on growth, productivity, and production stability. This MSB was then used to prepare the antibody and to generate the Master Cell Bank (MCB).

[00136] Cells from the MCB were expanded in shake flasks, culture bags, and a seed bioreactor to generate the inoculum for a production bioreactor to produce the antibody product. Further processing included three chromatography steps (protein A affinity, cation exchange and anion exchange chromatography), two orthogonal viral clearance steps (low pH viral inactivation and viral reduction filtration), ultrafiltration/diafiltration, and a final 0.2µm filtration step.

#### **h409A11 Structure and Features**

[00137] h409A11 is a highly selective humanized monoclonal antibody that blocks the interaction between human PD-1 and its ligands PD-L1 and PD-L2. h409A11 is heterogeneously glycosylated at asparagine 297 within the Fc domain of each heavy chain, yielding molecular weights typically ranging between 148.9 and 149.5 kDa, depending on the attached glycan chains. The amino acid sequences of the heavy and light chains of h409A11 are found in SEQ ID NO:31 and SEQ ID NO:36. The light chain without the leader sequences comprises amino acid residues 20 to 237 of SEQ ID NO: 36 and the heavy chain without the leader sequences comprises amino acid residues 20 to 466 of SEQ ID NO: 31.

#### **Stable humanized PD-1 formulations**

[00138] In certain embodiments, stable humanized PD-1 e.g., h409A11 is an aqueous solution stored under refrigerated conditions (temp. range: typically about 2-8°C, but under certain circumstances, the aqueous formulation may exhibit stability at other temperatures including at about 25°C and about 40°C for periods of up to about 12 months) at a concentration of  $\geq 25$ mg/mL in 10 mM Histidine buffer, pH 5.0-6.0. In certain embodiments, stable humanized PD-1 e.g., h409A11 is an aqueous solution at a concentration of about 25 mg/mL in 10 mM Histidine buffer, pH 5.0-6.0. The stable formulation (*i.e.*, drug substance) is typically a clear to opalescent solution and may contain particulates.

[00139] In certain embodiments, a liquid or frozen solution of h409A11 is formulated in histidine buffer (pH 5.5) containing sucrose and polysorbate 80.

[00140] An additional exemplary formulation includes: h409A11 formulated in histidine buffer (pH 5.5) containing sucrose and polysorbate 80 in lyophilized form.

[00141] In certain embodiments, stable humanized PD-1 formulation is provided as lyophilized powder in vials intended for single-use.

[00142] In certain embodiments, stable humanized PD-1 formulation is reconstituted with water for injection (WFI) and aseptically diluted with appropriate volumes of 0.9% sodium chloride for injection in a sterile IV container to form an admixture solution.

#### **Biological Activity**



[00143] Biological activity of the humanized anti-PD-1 antibody is measured by its ability to compete with PD-L1 (natural ligand of PD-1) in binding to human PD-1, quantified in competitive ELISA relative to a reference material. The stable formulations described herein exhibit biological activity for long periods of time, including up to at least about eighteen months. The stability of several batches of h409A11, under various storage conditions are illustrated in Figures 1-9.

#### **Stable Formulations of Humanized Anti-PD-1 Antibodies**

[00144] Lyophilized formulations of anti-PD-1 antibody are prepared as follows. An exemplary batch formula for h409A11 antibody is provided in Table 3. The final concentration of antibody is 25 mg/mL. This batch formulation may be used to prepare the lyophilized 50 mg/vial units, as discussed with reference to Table 4, *infra*. Polysorbate 80 from a vegetable source is used. Additional hydrochloric acid or sodium hydroxide may be added to adjust the pH to the desired value of approximately 5.5 ( $\pm 0.2$ ). The components are brought to a final volume of 14 L with sterile water for injection (WFI). Correspondingly smaller lots may be prepared by proportional reduction of the amounts listed in Table 3.

[00145] An exemplary liquid antibody formulation is prepared by taking the drug substance (*e.g.*, anti-humanized PD-1 from a batch formula described herein) which is in liquid form (*e.g.*, h409A11 in an aqueous formulation) and buffer exchanging it into the desired buffer as the last step of the purification process. In this instance, there is no previous lyophilization step. The drug substance in the final histidine buffer is concentrated to a desired concentration. Excipients such as sucrose and polysorbate 80 are added to the drug substance and it is diluted using the appropriate buffer to final protein concentration. The final formulated drug substance is filtered using 0.22 $\mu$ m filters and filled into a final container (*e.g.* glass vials). Such a liquid formulation includes final liquid formulation comprising 10 mM histidine pH 5.5, 7% sucrose, 0.02% polysorbate 80, and 25 mg/mL h409A11.

[00146]

**Table 3**

**Batch Formula of Representative 14.0 L Pre-lyophilization Solution for h409A11**

**Powder for Injection, 50 mg/vial**

Component	Compendial Grade	Concentration (mg/mL)	Amount per Batch (g)
h409A11 antibody	N/A	25.0	350.0

L-Histidine	USP	1.55	21.7
Polysorbate 80	NF	0.2	2.8
Sucrose	NF	70	980
Hydrochloric acid <sup>a</sup>	NF	-	-
Sodium Hydroxide <sup>a</sup>	NF	-	pH adjustment
Water for injection <sup>b</sup>	USP	-	14.0 L @ q.s

<sup>a</sup> Hydrochloric acid and sodium hydroxide added if needed to adjust pH to 5.5

<sup>b</sup> Water removed by sublimation and desorption during lyophilization

[00147] The unit composition of an exemplary final lyophilized formulation of humanized anti-PD-1 is provided at Table 4.

**Table 4**

**Unit Composition of Lyophilized Powder Formulation for Injection, 50 mg/vial**

Component	Grade	Amount (mg/vial)	Concentration after Reconstitution (mg/mL) <sup>b</sup>	Function
h409A11	N/A	50	25	Drug Substance/Active Pharmaceutical ingredient
L-Histidine	USP	3.1	1.55	Buffer
Polysorbate 80	NF	0.4	0.2	Surfactant
Sucrose	NF	140	70	Stabilizer/ Tonicity Modifier
Hydrochloric acid <sup>c</sup>	NF	-	-	pH adjustment
Sodium Hydroxide <sup>c</sup>	NF	-	-	pH adjustment
Sterile Water for Injection (sWFI or WFI) <sup>d</sup>	USP	2.0 mL @ q.s.	-	Solvent

<sup>a</sup> An excess fill of 0.4 mL is provided to ensure the recovery of 50 mg h409A11 per vial.

<sup>b</sup> Following reconstitution with 2.3 mL sterile water for injection.

<sup>c</sup> Hydrochloric acid and sodium hydroxide added if needed to adjust pH to 5.5

<sup>d</sup> Water removed by sublimation and desorption during lyophilization

**[00148]** The unit formulation of Table 4 comprises 1/20,000<sup>th</sup> of the batch formulation of Table 3 after lyophilization to remove the water. The 50 mg of DS is added as 2.0 mL of the 25 mg/mL batch formulation of Table 3. Each vial is filled with 2.4 mL and reconstituted with 2.3 mL sWFI, resulting in approximately 2.4 mL of reconstituted solution due to expansion volume of the lyophilized cake.

**[00149]** The drug is packaged in sterile 20 mm neck, 6R DIN, Type 1 glass tubing vials, closed with 20-mm gray butyl rubber stoppers and sealed with aluminum crimp seals. Vials are stored at 2 – 8°C, and refrigerated when shipped.

**[00150]** Compounding involves the following steps. Charge the required amount of water for injection (WFI) into a tared compounding vessel. Charge and dissolve with mixing, sucrose, histidine, and polysorbate 80 from a vegetable source. Measure the pH and adjust if needed to bring the pH to about 5.4 --5.6. Use hydrochloric acid and/or sodium hydroxide to adjust the pH. Equilibrate the drug substance to ambient temperature and charge the drug substance slowly into the compounding vessel. Continue to mix gently to avoid foaming. Measure the pH again and adjust if needed to bring the pH to approximately 5.5. Charge WFI to the final weight of the bulk solution with continued gentle mixing.

**[00151]** Filtration involves the following steps. Connect clarifying filter (0.22 µm) and sterilizing filter (0.22 µm) to the compounding vessel. Collect an aliquot of the bulk solution for bioburden testing after clarifying filtration step. Perform aseptic filtration using a 0.22 µm filter into a sterile container. Remove aliquot of sample after aseptic filtration for bulk sterility testing. Perform filter integrity testing after product filtration.

**[00152]** Filling involves the following steps. Using suitable filling equipment, aseptically fill the product solution into sterilized Type I tubing glass vials to achieve a target fill volume of 2.4 mL. Perform fill weight checks during filling. Partially seat sterilized lyo-shape stoppers into filled vials. Load the filled vials into a suitable freeze-dryer.

**[00153]** Lyophilization, stoppering and capping involve the following steps. Lyophilize the filled vials using an appropriate lyophilization cycle. After lyophilization is complete, backfill the vials with 0.22 µm filtered nitrogen and fully stopper. Unload the stoppered vials from the lyophilizer and seal them.

**[00154]** The resulting vials are inspected for visual defects and stored at 2-8 °C. Finished unit dosage vials are shipped under refrigerated conditions.

## EXAMPLE 2

### Stability Testing of Lyophilized Formulations of Humanized Anti-PD-1 Antibodies

[00155] Figures 1-9 provide data of stability testing of lyophilized formulations of a humanized anti-human PD-1 antibody under various storage conditions. Vials were stored in upright configurations. As discussed in more detail below, formulations of the present invention show stability through at least 24 months for antibodies lyophilized at pH 5.5 (histidine buffer), as well as similar liquid formulations.

[00156] Stability was assessed as follows. Samples were lyophilized in 6R DIN Type I glass vials, and sealed with 20 mm bromobutyl lyo stoppers (Helvoet Rubber & Plastic Technologies BV, Hellevoetsluis, The Netherlands) and flip-off aluminum seals. Vials were placed on stability stations under the following storage conditions: 5°C (5±3°C), 25H (25, 60% relative humidity), or RH4 (40°C, 70% relative humidity). Samples were obtained at an initial time point, and for certain samples at a variety of time points including 1, 2, 3, 6, 9, 12, 18, and 24 months.

[00157] The stability of the samples is illustrated by the various characteristics presented in the tables in FIGS 1-9. The lyophilized samples were visually inspected, reconstituted, and the reconstituted formulation was visually inspected. The pH of the samples after reconstitution was measured, and the protein concentration determined by U.V. absorbance. The samples were analyzed by CE-SDS technique in which protein was denatured with sodium dodecyl sulfate (SDS) under reducing and non-reducing conditions and separated using capillary electrophoresis (CE). The proteins separate based on their apparent molecular weight. Under non-reducing conditions, all species other than the main IgG peak are classified as impurities. Under reducing conditions, the IgG is resolved into the heavy and light chains. All other species are classified as impurities.

[00158] Purity of the sample was further assessed by high performance size exclusion chromatography (HPSEC) in which the percentage of monomer was determined, as well as the percentages of high molecular weight species (possibly aggregates) and late eluting peaks (possibly degradation products).

[00159] Additional sample characterization data are provided in Figures 1-9. High performance ion-exchange chromatography (HP-IEX) was used to assess purity by revealing the presence of acidic or basic variants. Results are presented as a percentage of total observed material. The samples were further characterized for biological function using an enzyme-linked immunosorbent assay (ELISA) for binding to human PD-1. The antibody concentration necessary to achieve half-maximal binding is called EC<sub>50</sub>. Potency of the test

sample was assessed by comparing binding curves of the test samples to a reference material (or control) by the ration of EC<sub>50</sub>'s. Potency was expressed as percent relative potency of reference material (or control). Moisture content of the lyophilized powder was also determined by coulometric titration. Particulate matter count measurements were performed to count particles  $\geq 10 \mu\text{m}$  and  $\geq 25 \mu\text{m}$ . The method used for these measurements was based on USP<788>.

[00160] These results demonstrate high stability formulations of the present invention over at least 24 months at about pH 5.5. The data reveal no trending over time that would reflect instability for samples the tested storage conditions.

### [00161] **EXAMPLE 3: INITIAL CLINICAL RESULTS**

#### **Phase 1 Study of h409A11 (Anti-PD-1 Monoclonal Antibody) in Patients With Advanced Solid Tumors**

[00162]

A phase 1 trial examined safety, PK, PD, and antitumor activity of h409A11. An open-label, dose escalation study was conducted in patients with advanced malignancy refractory to standard chemotherapy. In the initial patient set, patients with advanced solid tumors were treated with a stable h409A11 formulation as described herein. There was no limitation/restriction regarding surgery; however, patients were not currently surgical candidates. Cohorts of 3-6 patients were enrolled (3+3 design) at IV doses of 1, 3, or 10 mg/kg. Following an initial dose and 28-day Cycle 1, patients were allowed to subsequently receive multiple doses given every 2 wks. For phase 1 part A, three patients were treated at 1 mg/kg, three patients were treated at 3mg/kg, and nine patients were treated at 10 mg/kg and all were dosed every 2 weeks. There was no inpatient dose escalation. Radiographic assessment was conducted every 8 wks using RECIST 1.1 guidelines.

Nine patients, 3 at each dose level, completed the dose-limiting toxicity (DLT) period (28 d). Patients had non-small cell lung cancer (NSCLC, n=3), rectal cancer (n=2), melanoma (MEL, n=2), sarcoma (n=1), or carcinoid (n=1). To date, a total of 63 doses were administered (median 7/patient; max 12) without DLT. Drug-related adverse events (AEs) across all doses included Grade 1 fatigue (n=3), nausea (n=2), diarrhea (n=1), dysgeusia (n=1), breast pain (n=1), and pruritus (N=1). One drug-related Grade 2 AE of pruritus was reported. No drug-related AEs  $\geq$  grade 3 were observed. PK data are shown in Table 5. Based on RECIST, 1 patient with MEL on therapy >6 mths had a partial response, and preliminary evidence of tumor size reduction (stable disease) was observed in 3 additional patients with

advanced cancer. These results show that h409A11 was well-tolerated without DLT across 3 tested dose levels. (*i.e.*, 1, 3, and 5 mg/kg). Evidence of antitumor activity was observed.

**[00163] TABLE 5. Mean (CV%) PK Parameter Values of MK-3475 Following Single IV Dose of 1, 3, or 10 mg/kg in Cycle 1**

Dose (mg/kg)	N	C <sub>max</sub> (μg/mL)	AUC <sub>(0-28day)</sub> (μg·day /mL)	t <sub>1/2</sub> <sup>a</sup> (day)
1	4	16.8 (23)	163 (20) <sup>b</sup>	15.1 (41) <sup>b</sup>
3	3	109 (26)	990 (23)	21.7 (11)
10	2	337 (8)	2640 (30)	13.6 (28)
<sup>a</sup> PK sampling up to 28 days following first IV administration, therefore t <sub>1/2</sub> not fully characterized.				
<sup>b</sup> N=3 due to subject discontinuation.				

Table 6 provides a brief description of the sequences in the sequence listing.

#### Sequence Identifiers

SEQ ID NO:	Description
1	hPD-1.08A heavy chain variable region (DNA)
2	hPD-1.08A light chain variable region (DNA)
3	hPD-1.09A heavy chain variable region (DNA)
4	hPD-1.09A light chain variable region (DNA)
5	hPD-1.08A heavy chain variable region (AA)
6	hPD-1.08A light chain variable region (AA)
7	hPD-1.09A heavy chain variable region (AA)
8	hPD-1.09A light chain variable region (AA)
9	hPD-1.08A light chain CDR1 (AA)
10	hPD-1.08A light chain CDR2 (AA)
11	hPD-1.08A light chain CDR3 (AA)
12	hPD-1.08A heavy chain CDR1 (AA)
13	hPD-1.08A heavy chain CDR2 (AA)
14	hPD-1.08A heavy chain CDR3 (AA)
15	hPD-1.09A light chain CDR1 (AA)
16	hPD-1.09A light chain CDR2 (AA)

17	<b>hPD-1.09A light chain CDR3 (AA)</b>
18	<b>hPD-1.09A heavy chain CDR1 (AA)</b>
19	<b>hPD-1.09A heavy chain CDR2 (AA)</b>
20	<b>hPD-1.09A heavy chain CDR3 (AA)</b>
21	<b>109A-H heavy chain variable region (DNA)</b>
22	<b>Codon optimized 109A-H heavy chain variable region (DNA)</b>
23	<b>Codon optimized 409A-H heavy chain full length (DNA)</b>
24	<b>K09A-L-11 light chain variable region (DNA)</b>
25	<b>K09A-L-16 light chain variable region (DNA)</b>
26	<b>K09A-L-17 light chain variable region (DNA)</b>
27	<b>Codon optimized K09A-L-11 light chain variable region (DNA)</b>
28	<b>Codon optimized K09A-L-16 light chain variable region (DNA)</b>
29	<b>Codon optimized K09A-L-17 light chain variable region (DNA)</b>
30	<b>109A-H heavy chain variable region (AA)</b>
31	<b>409A-H heavy chain full length (AA)</b>
32	<b>K09A-L-11 light chain variable region (AA)</b>
33	<b>K09A-L-16 light chain variable region (AA)</b>
34	<b>K09A-L-17 light chain variable region (AA)</b>
35	<b>109A-H heavy chain full length (AA)</b>
36	<b>K09A-L-11 light chain full length (AA)</b>
37	<b>K09A-L-16 light chain full length (AA)</b>
38	<b>K09A-L-17 light chain full length (AA)</b>

[00164] As used herein, including the appended claims, the singular forms of words such as “a,” “an,” and “the,” include their corresponding plural references unless the context clearly dictates otherwise. Unless otherwise indicated, the proteins and subjects referred to herein are human proteins and subject, rather than another species.

[00165] **REFERENCES**

1. Sharpe, A.H, Wherry, E.J., Ahmed R., and Freeman G.J. The function of programmed cell death 1 and its ligands in regulating autoimmunity and infection. *Nature Immunology* (2007); 8:239-245.

2. Greenwald R.J., Freeman G.J., and Sharpe A.H. The B7 family revisited. *Annual Reviews of Immunology* (2005); 23:515-548.
3. Okazaki T and Honjo T. PD-1 and PD-1 ligands: from discovery to clinical application. *International immunology* (2007);19:813-824.
4. Chemnitz *et al.* SHP-1 and SHP-2 associate with immunoreceptor tyrosine-based switch motif of programmed death 1 upon primary human T cell stimulation, but only receptor ligation prevents activation. *J. Immunol.* (2004); 173: 945-954.
5. Nishimura, H., Nose, M., Hiai, H. *et al.* Development of lupus-like autoimmune diseases by disruption of the *PD-1* gene encoding an ITIM motif-carrying immunoreceptor. *Immunity* (1999);11:141-151.
6. Okazaki T *et al.* Autoantibodies against cardiac troponin I are responsible for dilated cardiomyopathy in PD-1 deficient mice. *Nature Medicine* (2003); 9: 1477-1483.
7. Ansari MJ. The programmed death-1 pathway regulates diabetes in nonobese diabetic (NOD) mice. *J Exp. Med.* (2003), Jul 7;198(1):63-9.
8. Riley J and June C. The road to recovery: translating PD-1 biology into clinical benefit. *Trends in Immunology* (2006); 28:48-50.
9. Barber DL. Restoring function in exhausted CD8 T cells during chronic viral infection. *Nature* (2006);439: 682-687.
10. Trautmann L *et al.* Upregulation of PD-1 expression on HIV-specific CD8+ T cells leads to reversible immune dysfunction. *Nature Medicine* (2006) 12: 1198-1202.
11. Petrovas C *et al.* PD-1 is a regulator of virus-specific CD8+ T cell survival in HIV infection. *J Exp. Med.* (2006); 203: 2281-2292.



12. Day CL *et al.* PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression, *Nature*. 2006 Sep 21;443(7109):350-4.
13. Velu V *et al.* 2009. Enhancing SIV-specific immunity in vivo by PD-1 blockade. *Nature* (2009) 458: 206-210.
14. Finnefrock et al. PD-1 blockade in rhesus macaques: impact on chronic infection and prophylactic vaccination. *J. of Immunol.* (2009): 182: 980-987.
15. Dong H *et al.* Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. *Nat Med*. 2002 Aug;8(8):793-800.
16. Yang *et al.* PD-1 interaction contributes to the functional suppression of T-cell responses to human uveal melanoma cells in vitro. *Invest Ophthalmol Vis Sci*. 2008 Jun;49(6 (2008): 49: 2518-2525.
17. Ghebeh *et al.* The B7-H1 (PD-L1) T lymphocyte-inhibitory molecule is expressed in breast cancer patients with infiltrating ductal carcinoma: correlation with important high-risk prognostic factors. *Neoplasia* (2006) 8: 190-198.
18. Hamanishi J *et al.* Programmed cell death 1 ligand 1 and tumor-infiltrating CD8+ T lymphocytes are prognostic factors of human ovarian cancer. *Proceeding of the National Academy of Sciences* (2007): 104: 3360-3365.
19. Thompson RH et al. Significance of B7-H1 overexpression in kidney cancer. *Clinical genitourin Cancer* (2006): 5: 206-211.
20. Nomi, T. Sho, M., Akahori, T., *et al.* Clinical significance and therapeutic potential of the programmed death- 1 ligand/programmed death-1 pathway in human pancreatic cancer. *Clinical Cancer Research* (2007);13:2151-2157.
21. Ohigashi Y et al. Clinical significance of programmed death-1 ligand-1 and programmed death-1 ligand 2 expression in human esophageal cancer. *Clin. Cancer Research* (2005): 11: 2947-2953.

22. Inman *et al.* PD-L1 (B7-H1) expression by urothelial carcinoma of the bladder and BCG-induced granulomata: associations with localized stage progression. *Cancer* (2007): 109: 1499-1505.
23. Shimauchi T *et al.* Augmented expression of programmed death-1 in both neoplastic and nonneoplastic CD4+ T-cells in adult T-cell Leukemia/ Lymphoma. *Int. J. Cancer* (2007): 121:2585-2590.
24. Gao *et al.* Overexpression of PD-L1 significantly associates with tumor aggressiveness and postoperative recurrence in human hepatocellular carcinoma. *Clinical Cancer Research* (2009) 15: 971-979.
25. Nakanishi J. Overexpression of B7-H1 (PD-L1) significantly associates with tumor grade and postoperative prognosis in human urothelial cancers. *Cancer Immunol Immunother.* (2007) 56: 1173- 1182.
26. Hino *et al.* Tumor cell expression of programmed cell death-1 is a prognostic factor for malignant melanoma. *Cancer* (2010): 00: 1-9.

**[00166]** All references cited herein are incorporated by reference to the same extent as if each individual publication, database entry (e.g. Genbank sequences or GeneID entries), patent application, or patent, was specifically and individually indicated to be incorporated by reference. This statement of incorporation by reference is intended by Applicants, pursuant to 37 C.F.R. §1.57(b)(1), to relate to each and every individual publication, database entry (e.g. Genbank sequences or GeneID entries), patent application, or patent, each of which is clearly identified in compliance with 37 C.F.R. §1.57(b)(2), even if such citation is not immediately adjacent to a dedicated statement of incorporation by reference. The inclusion of dedicated statements of incorporation by reference, if any, within the specification does not in any way weaken this general statement of incorporation by reference. Citation of the references herein is not intended as an admission that the reference is pertinent prior art, nor does it constitute any admission as to the contents or date of these publications or documents.

\*\*\*\*\*

<160> 38 Sequences

**WHAT IS CLAIMED IS:**

1. A lyophilized formulation of an anti-human PD-1 antibody, or antigen binding fragment thereof, comprising:
  - a) said anti-human PD-1 antibody, or antigen binding fragment thereof;
  - b) histidine buffer;
  - c) polysorbate 80; and
  - d) sucrose.
2. The lyophilized formulation of Claim 1, wherein the formulation has a pH between 5.0 and 6.0 when reconstituted.
3. The lyophilized formulation of Claim 1 that enables reconstitution of the antibody, or antigen binding fragment thereof, at a concentration of between about 25 mg/mL and 100 mg/mL.
4. The lyophilized formulation of Claim 1, wherein polysorbate 80 is present at a weight ratio of approximately 0.02% (w/v).
5. The lyophilized formulation of Claim 1, wherein sucrose is present at a weight ratio of approximately 7% (w/v).
6. A lyophilized pharmaceutical formulation of an anti-human PD-1 antibody, or antigen binding fragment thereof, made by lyophilizing an aqueous solution comprising:
  - a) 25-100 mg/mL anti- antibody, or antigen binding fragment thereof;
  - b) about 70 mg/mL sucrose;
  - c) about 0.2 mg/mL polysorbate 80; and
  - d) about 10 mM histidine buffer at pH 5.0-6.0.
7. The lyophilized pharmaceutical formulation of Claim 6, wherein the anti-human PD-1 antibody, or antigen binding fragment thereof, is present at about 25 mg/mL in the aqueous solution.

8. The lyophilized pharmaceutical formulation of Claim 6, wherein the aqueous solution has a pH of about 5.5.
9. A lyophilized pharmaceutical formulation of an anti-human PD-1 antibody, or antigen binding fragment thereof, that when reconstituted comprises:
  - a) 25-100 mg/mL anti-human PD-1 antibody, or antigen binding fragment thereof;
  - b) about 70 mg/mL sucrose;
  - c) about 0.2 mg/mL polysorbate 80; and
  - d) about 10 mM Histidine buffer at about pH 5.0- pH 6.0.
10. The lyophilized pharmaceutical formulation of Claim 9, wherein the anti-human PD-1 antibody, or antigen binding fragment thereof, is present at about 25 mg/mL in the reconstituted solution.
11. The lyophilized pharmaceutical formulation of Claim 9, wherein the reconstituted solution has a pH of about 5.5.
12. A pharmaceutical formulation of an anti-human PD-1 antibody, or antigen binding fragment thereof comprising:
  - a) said anti-human PD-1 antibody, or antigen binding fragment thereof;
  - b) histidine buffer;
  - c) polysorbate 80; and
  - d) sucrose.
13. The pharmaceutical formulation of Claim 12, wherein the formulation has a pH between 5.0 and 6.0 when reconstituted.
14. The pharmaceutical formulation of Claim 12, wherein polysorbate 80 is present at a weight ratio of approximately 0.02% (w/v).
15. The pharmaceutical formulation of Claim 12, wherein sucrose is present at a weight ratio of approximately 7% (w/v).

16. A liquid pharmaceutical formulation of an anti-human PD-1 antibody, or antigen binding fragment thereof comprising:
- a) 25-100 mg/mL anti- antibody, or antigen binding fragment thereof;
  - b) about 70 mg/mL sucrose;
  - c) about 0.2 mg/mL polysorbate 80; and
  - d) about 10 mM histidine buffer at pH 5.0-6.0.
17. The formulation of any one of Claims 1, 6, 9, 12, or 16, wherein the antibody, or antigen binding fragment thereof, comprises a light chain comprising three CDR sequences selected from the group consisting of SEQ ID NOs: 9, 10, 11, 15, 16, and 17.
18. The formulation of any one of Claims 1, 6, 9, 12, or 16, wherein the antibody, or antigen binding fragment thereof, comprises a heavy chain comprising three CDR sequences selected from the group consisting of SEQ ID NOs: 12, 13, 14, 18, 19, and 20.
19. The formulation of any one of Claims 1, 6, 9, 12, or 16, wherein the antibody, or antigen binding fragment thereof, comprises:
- i) a light chain comprising three CDR sequences SEQ ID NOs: 15, 16, and 17; and
  - ii) a heavy chain comprising three CDR sequences SEQ ID NOs: 18, 19, and 20.
20. The formulation of any one of Claims 1, 6, 9, 12, or 16, wherein the antibody, or antigen binding fragment thereof, comprises a light chain variable domain comprising amino acid residues 20 to 130 of SEQ ID NO: 32.
21. The formulation of any one of Claims 1, 6, 9, 12, or 16, wherein the antibody, or antigen binding fragment thereof, comprises a heavy chain variable domain comprising amino acid residues 20 to 466 of SEQ ID NO:31.
22. The formulation of any one of Claims 1, 6, 9, 12, or 16, wherein the antibody, or antigen binding fragment thereof, comprises:
- i) a light chain comprising amino acid residues 20 to 237 of SEQ ID NO: 36 and
  - ii) a heavy chain comprising amino acid residues 20 to 466 of SEQ ID NO: 31.

23. The formulation of any one of Claims 1, 6, 9, 12, or 16 wherein the antibody is selected from the group consisting of h409A11, h409A16, and h409A17.
24. A method of treating chronic infection in a mammalian subject in need thereof comprising: administering an effective amount of the formulation of any one of claims 1, 6, 9, 12, or 16.
25. A method of treating cancer in a mammalian subject in need thereof, the method comprising administering an effective amount of the formulation of any one of claims 1, 6, 9, 12, or 16.
26. The method of claim 24 or 25, wherein the effective amount comprises a dose of anti-human PD-1 antibody selected from the group consisting of about 1.0, 3.0, and 10 mg/kg.
27. The method of claim 25 or 26, wherein the subject does not exhibit dose-limiting toxicity (DLT).
28. The formulation of any one of Claims 1, 6, 9, 12, or 16 wherein the antibody exhibits stability over at least about 12 months.
29. The formulation of any one of Claims 1, 6, 9, 12, or 16 wherein the antibody exhibits stability over at least about 24 months.
30. The formulation of any one of Claims 1, 6, 9, 12, or 16 wherein the antibody exhibits stability over at least about 36 months.

1/18

Stability Data for h409A11 Anti-PD-1 Powder for injection, 50 mg/Vial											
Storage Condition											
5°C											
Batch Number											
1											
Stability Test Interval											
Test	Clinical Acceptance Criteria	Initial	1-month	3-month	6-month	9-month	12-month	18-month	24-month		
Description Lyophilized Powder	White to off-white powder	White cake	White cake	White cake	White cake	White cake	White cake	White cake	White cake		
Reconstitution Time (seconds)	Report Results	39	36	35	42	43	34	28	45		
Description Reconstituted Solution											
Clarity	Clear to opalescent solution: May contain particulates	Opalescent solution	Opalescent solution	Opalescent solution	Opalescent solution	Opalescent solution	Opalescent solution	Opalescent solution	Opalescent solution		
Color	Report results by "Y" ref solution	Colorless #	Colorless#	Colorless#	Colorless#	Colorless#	Colorless#	Colorless#	Colorless#		
pH	5.0 – 6.0	5.6	5.5	5.5	5.6	5.6	5.6	5.6	5.6		
Assay UV A280 nm	21.3 – 28.8 mg/mL	24.9	24.8	24.1	24.8	24.4	25.5	24.6	23.6		
Biological Potency Anti-PD-1 Competitive ELISA (% Relative to control)	50–150% of Reference	95	80	86	116	93	87	76	83		
Purity											
HPSEC											
High Molecular Weight Species (%)	≤ 5.00	ND	ND	<QL	<QL	<QL	<QL	<DL	<QL		
Late Eluting Peaks (%)	Report Results	ND	ND	ND	ND	ND	ND	ND	ND		
Monomer (%)	≥ 90.0	100.0	100.0	99.8	99.8	99.8	99.8	100.0	99.8		
CE-SDS Reducing											
% Impurity	≤ 10.00% species other than heavy and light chains	0.38	0.35	0.39	0.41	0.40	0.44	0.38	0.39		

FIG.1A

2/18

Stability Data for h409A11 Anti-PD-1 Powder for injection, 50 mg/Vial										
5°C										
1										
Stability Test Interval										
Test	Clinical Acceptance Criteria	Initial	1-month	3-month	6-month	9-month	12-month	18-month	24-month	
CE-SDS Non-Reducing										
% Impurity	≤ 10.00% species other than main band	0.59	0.57	0.53	0.56	1.12	0.94	0.70	1.22	
HP-IEX										
Acidic variants (%)	Report Results	2.5	2.5	3.3	3.2	3.3	3.4	3.2	3.3	
Acidic 1 (%)	Report Results	3.7	3.7	3.6	3.9	3.9	3.9	3.7	3.8	
Acidic 2 (%)	Report Results	8.2	8.5	7.7	8.0	8.1	8.3	7.8	8.0	
Main (%)	Report Results	50.8	54.0	49.6	47.7	47.1	48.1	45.6	47.3	
Basic 1 (%)	Report Results	11.0	10.6	10.7	11.3	11.7	10.4	11.5	11.0	
Basic 2 (%)	Report Results	8.9	8.4	8.9	8.9	9.3	9.0	9.7	8.6	
Basic Variants (%)	Report Results	14.9	12.4	16.1	17.0	16.5	16.8	18.5	17.9	
Moisture (%)	≤ 5.0%	0.3	0.4	0.3	0.5	0.4	0.4	0.7	0.6	
Particulate Matter (HIAC*)	Complies USP<788>									
≥ 10 µm per container	NMT 6000	51	50	51	50	51	57	43	44	
≥ 25 µm per container	NMT 600	2	3	2	0	4	1	1	0	
Bacterial Endotoxin	≤ 0.25 EU/mg	NT	NT	NT	NT	NT	NT	NT	NT	
Sterility	Meets Sterility Test Requirement	NT	NT	NT	NT	NT	NT	NT	NT	
Container Closure Integrity	No leakage detected	NT	NT	NT	NT	NT	NT	NT	NT	
Quantization limit (QL) = 0.25%, Detection Limit (DL) = 0.10%, NT = not tested, ND = not detected										
# not tested according to "y" ref solution										
* A modified version of USP<788> was used for pre-clinical batch 1										

FIG.1B



3/18

FIG. 2A

Stability Data for h409A11 Anti-PD-1 Powder for injection, 50 mg/Vial						
Storage Condition						
Batch Number						
1						
25H (25°C, 60% RH)						
		Stability Test Interval				
Test	Clinical Acceptance Criteria	Initial	1-month	3-month	6-month	12-month
Description Lyophilized Powder	White to off-white powder	White cake	White cake	White cake	White cake	White cake
Reconstitution Time (seconds)	Report Results	39	39	37	36	32
Description Reconstituted Solution						
Clarity	Clear to opalescent solution: May contain particulates	Opalescent solution	Opalescent solution	Opalescent solution	Opalescent solution	Opalescent solution
Color	Report results by "Y" ref solution	Colorless#	Colorless#	Colorless#	Colorless#	Colorless#
pH	5.0 – 6.0	5.6	5.6	5.5	5.6	5.6
Assay UV A280 nm	21.3 – 28.8 mg/mL	24.9	25.1	23.5	24.2	24.8
Biological Potency Anti-PD-1 Competitive ELISA (% Relative to control)	50–150% of Reference	95	80	81	105	96
Purity						
HPSEC						
High Mol. Wt. Species (%)	≤ 5.00	ND	ND	<QL	<QL	<QL
Late Eluting Peaks (%)	Report Results	ND	ND	ND	ND	ND
Monomer (%)	≥ 90.0	100.0	100.0	99.8	99.8	99.8
CE-SDS Reducing						
% Impurity	≤ 10.00% species other than heavy and light chains	0.38	0.38	0.39	0.41	0.33
CE-SDS Non-Reducing						
% Impurity	≤ 10.00% species other than main band	0.59	0.57	0.71	0.58	0.98

4/18

Stability Data for h409A11 Anti-PD-1 Powder for injection, 50 mg/Vial						
Storage Condition						
25H (25°C, 60% RH)						
Batch Number						
Stability Test Interval						
Test	Clinical Acceptance Criteria	Initial	1-month	3-month	6-month	12-month
HP-IEX						
Acidic variants (%)	Report Results	2.5	2.8	3.1	3.2	3.4
Acidic 1 (%)	Report Results	3.7	3.5	3.7	4.0	4.0
Acidic 2 (%)	Report Results	8.2	8.6	8.3	8.4	9.1
Main (%)	Report Results	50.8	53.4	48.6	46.7	46.6
Basic 1 (%)	Report Results	11.0	10.8	10.8	11.3	10.4
Basic 2 (%)	Report Results	8.9	8.4	9.0	8.9	9.1
Basic Variants (%)	Report Results	14.9	12.6	16.5	17.5	17.4
Moisture (%)	≤ 5.0%	0.3	0.5	0.6	0.8	0.9
Particulate Matter (HIAC*)	Complies USP<788>					
≥ 10 μm per container	NMT 6000	51	31	37	31	59
≥ 25 μm per container	NMT 600	2	1	1	1	0
Bacterial Endotoxin	≤ 0.25 EU/mg	NT	NT	NT	NT	NT
Sterility	Meets Sterility Test Requirement	NT	NT	NT	NT	NT
Container Closure Integrity	No leakage detected	NT	NT	NT	NT	NT
Quantization limit (QL) = 0.25%, Detection Limit (DL) = 0.10%, NT = not tested, ND = not detected						
# not tested according to "Y" ref solution						
* A modified version of USP<788> was used for pre-clinical batch 1						

FIG.2B

5/18

FIG. 3A

Stability Data for h409A11 Anti-PD-1 Powder for injection, 50 mg/Vial									
RH4 (40°C, 75% RH)									
Batch Number 1									
Stability Test Interval									
Test	Clinical Acceptance Criteria	Initial	0.5-month	1-month	2-month	3-month	6-month		
Description Lyophilized Powder	White to off-white powder	White cake	White cake	White cake	White cake	White cake	White cake		
Reconstitution Time (seconds)	Report Results	39	31	33	37	33	39		
Description Reconstituted Solution									
Clarity	Clear to opalescent solution: May contain particulates	Opalescent solution	Opalescent solution	Opalescent solution	Opalescent solution	Opalescent solution	Opalescent solution		
Color	Report results by "Y" ref solution	Colorless#	Colorless#	Colorless#	Colorless#	Colorless#	Colorless#		
pH	5.0 – 6.0	5.6	5.5	5.6	5.6	5.5	5.6		
Assay UV A280 nm	21.3 – 28.8 mg/mL	24.9	25.5	24.2	24.1	23.9	24.3		
Biological Potency Anti-PD-1 Competitive ELISA (% Relative to control)	50–150% of Reference	95	85	83	77	86	97		
Purity									
HPSEC									
High Mol. Wt. Species (%)	≤ 5.00	ND	ND	<DL	<QL	0.27	0.30		
Late Eluting Peaks (%)	Report Results	ND	ND	ND	ND	ND	ND		
Monomer (%)	≥ 90.0	100.0	100.0	99.9	99.8	99.7	99.7		
CE-SDS Reducing									
% Impurity	≤ 10.00% species other than heavy and light chains	0.38	0.38	0.33	0.39	0.42	0.40		
CE-SDS Non-Reducing									
% Impurity	≤ 10.00% species other than main band	0.59	0.58	0.56	0.50	0.60	0.72		

6/18

Stability Data for h409A11 Anti-PD-1 Powder for injection, 50 mg/Vial									
RH4 (40°C, 75% RH)									
1									
Storage Condition		Stability Test Interval							
Batch Number		1							
Test	Clinical Acceptance Criteria	Initial	0.5-month	1-month	2-month	3-month	6-month		
HP-IEX									
Acidic variants (%)	Report Results	2.5	2.7	2.4	2.5	3.0	3.3		
Acidic 1 (%)	Report Results	3.7	3.6	4.0	3.9	3.8	4.0		
Acidic 2 (%)	Report Results	8.2	8.7	9.2	9.4	9.5	10.0		
Main (%)	Report Results	50.8	50.2	52.3	50.4	46.0	43.4		
Basic 1 (%)	Report Results	11.0	11.0	10.8	10.8	10.7	11.8		
Basic 2 (%)	Report Results	8.9	8.8	8.4	8.7	8.7	8.8		
Basic Variants (%)	Report Results	14.9	15.0	12.9	14.4	18.2	18.7		
Moisture (%)	≤ 5.0%	0.3	0.6	0.6	0.7	0.9	1.1		
Particulate Matter (HIAC*)	Complies USP<788>								
≥ 10 μm per container	NMT 6000	51	40	37	65	28	43		
≥ 25 μm per container	NMT 600	2	3	2	2	1	1		
Bacterial Endotoxin	≤ 0.25 EU/mg	NT	NT	NT	NT	NT	NT		
Sterility	Meets Sterility Test Requirement	NT	NT	NT	NT	NT	NT		
Container Closure Integrity	No leakage detected	NT	NT	NT	NT	NT	NT		
Quantization limit (QL) = 0.25%, Detection Limit (DL) = 0.10%, NT = not tested, ND = not detected									
# not tested according to “Y” ref solution									
* A modified version of USP<788> was used for pre-clinical batch 1									

FIG.3B

7/18

Stability Data for h409A11 Anti-PD-1 Powder for injection, 50 mg/Vial											
Storage Condition		5°C									
Batch Number		2									
		Stability Test Interval									
Test	Clinical Acceptance Criteria	Initial	1-month	3-month	6-month	9-month	12-month	18-month	24-month		
Description Lyophilized Powder	White to off-white powder	White cake	White cake	White cake	White cake	White cake	White cake	White cake	White cake		
Reconstitution Time (seconds)	Report Results	49	30	36	27	22	23	27	36		
Description Reconstituted Solution											
Clarity	Clear to opalescent solution: May contain particulates	Opalescent solution	Opalescent solution	Opalescent solution	Opalescent solution	Opalescent solution	Opalescent solution	Opalescent solution	Opalescent solution		
Color	Report results by "Y" ref solution	Colorless #	Colorless #	Colorless #	Colorless #	Colorless #	Colorless #	Colorless #	Colorless #		
pH	5.0 – 6.0	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.5		
Assay UV A280 nm	21.3 – 28.8 mg/mL	25.6	26.4	25.2	26.1	23.2	24.6	24.7	25.5		
Biological Potency Anti-PD-1 Competitive ELISA (% Relative to control)	50–150% of Reference	108	88	86	94	86	82	90	103		
Purity											
HPSEC											
High Molecular Weight Species (%)	≤ 5.00	0.52	0.30	0.31	0.31	0.32	<DL	<QL	0.40		
Late Eluting Peaks (%)	Report Results	ND	ND	ND	ND	ND	ND	ND	ND		
Monomer (%)	≥ 90.0	99.5	99.7	99.7	99.7	99.7	99.9	99.8	99.6		
CE-SDS Reducing											
% Impurity	≤ 10.00% species other than heavy and light chains	0.37	0.36	0.38	0.37	0.38	0.39	0.45	0.64		

FIG.4A

8/18

Stability Data for h409A11 Anti-PD-1 Powder for injection, 50 mg/Vial											
5°C											
2											
Stability Test Interval											
Test	Clinical Acceptance Criteria	Initial	1-month	3-month	6-month	9-month	12-month	18-month	24-month		
CE-SDS Non-Reducing											
% Impurity	≤ 10.00% species other than main band	0.42	0.38	0.77	0.90	0.94	0.73	1.25	1.39		
HP-IEX											
Acidic variants (%)	Report Results	3.1	3.4	3.3	3.4	3.4	3.2	3.3	4.2		
Acidic 1 (%)	Report Results	4.0	3.9	4.0	4.0	4.0	3.8	3.8	3.9		
Acidic 2 (%)	Report Results	8.0	8.1	8.0	8.4	8.3	7.8	8.0	8.1		
Main (%)	Report Results	47.4	47.8	47.4	47.9	47.3	45.8	47.6	46.1		
Basic 1 (%)	Report Results	11.4	11.4	11.6	10.5	12.3	11.6	10.9	11.2		
Basic 2 (%)	Report Results	8.8	8.9	9.2	8.9	9.4	9.6	8.5	8.7		
Basic Variants (%)	Report Results	17.3	16.5	16.4	16.8	15.4	18.2	17.8	17.9		
Moisture (%)	≤ 5.00%	0.8	0.8	0.8	1.1	1.3	1.1	1.1	1.3		
Particulate Matter (HAC*)	Complies USP<788>										
≥ 10 µm per container	NMT 6000	55	6	35	17	23	1	46	20		
≥ 25 µm per container	NMT 600	0	0	1	1	1	0	0	2		
Bacterial Endotoxin	≤ 0.25 EU/mg	NT	NT	NT	NT	NT	NT	NT	NT		
Sterility	Meets Sterility Test Requirement	NT	NT	NT	NT	NT	NT	NT	NT		
Container Closure Integrity	No leakage detected	NT	NT	NT	NT	NT	NT	NT	NT		

NT = not tested, ND = not detected,

# not tested according to "Y" ref solution

\* A modified version of USP&lt;788&gt; was used for pre-clinical batch 2

FIG.4B

9/18

Stability Data for h409A11 Anti-PD-1 Powder for injection, 50 mg/Vial						
Storage Condition						
25H (25°C, 60% RH)						
Batch Number						
B						
		Stability Test Interval				
Test	Clinical Acceptance Criteria	Initial	1-month	3-month	6-month	
Description Lyophilized Powder	White to off-white powder	White powder	White powder	White powder	White powder	
Reconstitution Time (seconds)	Report Results	49	33	31	23	
Description Reconstituted Solution						
Clarity	Clear to opalescent solution: May contain particulates	Opalescent solution	Opalescent solution	Opalescent solution	Opalescent solution	
Color	Report results by "Y" ref solution	Colorless#	Colorless#	Colorless#	Colorless#	
pH	5.0 – 6.0	5.6	5.6	5.6	5.6	
Assay UV A280 nm	21.3 – 28.8 mg/mL	25.6	25.8	24.8	26.4	
Biological Potency Anti-PD-1 Competitive ELISA (%Relative to control)	50–150% of Reference	108	76	78	89	
Purity						
HPSEC						
High Mol. Wt. Species (%)	≤ 5.00	0.52	0.30	0.34	0.33	
Late Eluting Peaks (%)	Report Results	ND	ND	ND	ND	
Monomer (%)	≥ 90.0	99.5	99.7	99.7	99.7	
CE-SDS Reducing						
% Impurity	≤ 10.00% species other than heavy and light chains	0.37	0.31	0.39	0.27	
CE-SDS Non-Reducing						
% Impurity	≤ 10.00% species other than main band	0.42	0.44	1.10	0.96	

FIG.5A

10/18

Stability Data for h409A11 Anti-PD-1 Powder for injection, 50 mg/Vial						
Storage Condition						
25H (25°C, 60% RH)						
Batch Number						
B						
		Stability Test Interval				
Test	Clinical Acceptance Criteria	Initial	1-month	3-month	6-month	
HP-IEX						
Acidic variants (%)	Report Results	3.1	3.3	3.3	3.4	
Acidic 1 (%)	Report Results	4.0	4.0	4.0	4.0	
Acidic 2 (%)	Report Results	8.0	8.2	8.4	8.9	
Main (%)	Report Results	47.4	47.5	47.1	47.2	
Basic 1 (%)	Report Results	11.4	11.4	11.5	10.5	
Basic 2 (%)	Report Results	8.8	9.0	9.1	8.8	
Basic Variants (%)	Report Results	17.3	16.6	16.5	17.2	
Moisture (%)	≤ 5.0%	0.8	0.9	1.1	1.2	
Particulate Matter (HIAC*)	Complies USP<788>					
≥ 10 μm per container	NMT 6000	55	11	34	6	
≥ 25 μm per container	NMT 600	0	0	1	0	
Bacterial Endotoxin	≤ 0.25 EU/mg	NT	NT	NT	NT	
Sterility	Meets Sterility Test Requirement	NT	NT	NT	NT	
Container Closure Integrity	No leakage detected	NT	NT	NT	NT	
NT = Not tested, ND = Not detected						
# not tested according to "Y" ref solution						
* A modified version of USP<788> was used for pre-clinical batch 2						

FIG.5B



11/18

Stability Data for h409A11 Anti-PD-1 Powder for injection, 50 mg/Vial						
Storage Condition						
Batch Number						
2						
RH4 (40°C, 75% RH)						
Stability Test Interval						
Test	Clinical Acceptance Criteria	Initial	1-month	3-month	6-month	
Description Lyophilized Powder	White to off-white powder	White powder	White powder	White powder	White powder	
Reconstitution Time (seconds)	Report Results	49	32	35	20	
Description Reconstituted Solution						
Clarity	Clear to opalescent solution: May contain particulates	Opalescent solution	Opalescent solution	Opalescent solution	Opalescent solution	
Color	Report results by "Y" ref solution	Colorless#	Colorless#	Colorless#	Colorless#	
pH	5.0 – 6.0	5.6	5.6	5.6	5.6	
Assay UV A280 nm	21.3 – 28.8 mg/mL	25.6	24.9	24.9	25.9	
Biological Potency Anti-PD-1 Competitive ELISA (% Relative to control)	50–150% of Reference	108	76	60	66	
Purity						
HPSEC						
High Mol. Wt. Species (%)	≤ 5.00	0.52	0.34	0.37	0.38	
Late Eluting Peaks (%)	Report Results	ND	ND	ND	ND	
Monomer (%)	≥ 90.0	99.5	99.7	99.6	99.6	
CE-SDS Reducing						
% Impurity	≤ 10.00% species other than heavy and light chains	0.37	0.39	0.45	0.34	
CE-SDS Non-Reducing						
% Impurity	≤ 10.00% species other than main band	0.42	0.43	1.16	0.97	

FIG. 6A

12/18

Stability Data for h409A11 Anti-PD-1 Powder for injection, 50 mg/Vial					
Storage Condition		RH4 (40°C, 75% RH)			
Batch Number		3			
		Stability Test Interval			
Test	Clinical Acceptance Criteria	Initial	1-month	3-month	6-month
HP-IEX					
Acidic variants (%)	Report Results	3.1	3.4	3.3	3.5
Acidic 1 (%)	Report Results	4.0	4.0	4.3	4.1
Acidic 2 (%)	Report Results	8.0	8.8	9.4	10.2
Main (%)	Report Results	47.4	46.8	45.9	44.8
Basic 1 (%)	Report Results	11.4	11.7	11.4	10.4
Basic 2 (%)	Report Results	8.8	9.1	8.9	8.9
Basic Variants (%)	Report Results	17.3	16.4	16.8	18.0
Moisture (%)	≤ 5.0%	0.8	1.1	1.3	1.7
Particulate Matter (HIAC*)	Complies USP<788>				
≥ 10 μm per container	NMT 6000	55	11	14	35
≥ 25 μm per container	NMT 600	0	0	0	1
Bacterial Endotoxin	≤ 0.25 EU/mg	NT	NT	NT	NT
Sterility	Meets Sterility Test Requirement	NT	NT	NT	NT
Container Closure Integrity	No leakage detected	NT	NT	NT	NT
NT = not tested, ND = not detected					
# not tested according to "Y" ref solution					
* A modified version of USP<788> was used for pre-clinical batch 2					

FIG.6B

13/18

Stability Data for h409A11 Anti-PD-1 Powder for injection, 50 mg/Vial											
Storage Condition											
5°C											
Batch Number											
3											
		Stability Test Interval									
Test	Clinical Acceptance Criteria	Initial	1-month	3-month	6-month	9-month	12-month	18-month	24-month		
Description Lyophilized Powder	White to off-white powder	White powder	White powder	White powder	White powder	White powder	White powder	Off-white powder	White powder		
Reconstitution Time (seconds)	Report Results	41	35	34	32	32	38	42	32		
Description Reconstituted Solution											
Clarity	Clear to opalescent solution: May contain particulates	Opalescent solution does not contain particulates	Clear solution does not contain particulates	Clear solution does not contain particulates	Opalescent solution does not contain particulates	Opalescent solution does not contain particulates	Opalescent solution does not contain particulates	Opalescent solution does not contain particulates	Opalescent solution does not contain particulates		
Color	Report results by "Y" ref solution	Equivalent to ref solution Y7	Equivalent to ref solution Y7	Equivalent to ref solution Y7	Equivalent to ref solution Y7	Equivalent to ref solution Y7	Equivalent to ref solution Y7	Equivalent to ref solution Y7	Equivalent to ref solution Y7		
pH	5.0 – 6.0	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5		
Assay UV A280 nm	21.3 – 28.8 mg/mL	25.6	25.3	25.5	25.5	25.5	26.3	26.6	25.7		
Biological Potency	50–150% of Reference	90	102	96	102	106	105	71	98		
Purity											
HPSEC											
High Molecular Weight Species (%)	≤ 5.00	0.32	0.27	0.28	0.25	0.31	0.36	0.32	0.36		
Late Eluting Peaks (%)	Report Results	<0.13	<0.13	<0.13	<0.13	<0.13	<0.13	<0.13	<0.13		
Monomer (%)	≥ 90.0	99.7	99.7	99.7	99.8	99.7	99.6	99.7	99.6		
CE-SDS Reducing											
% Impurity	≤ 10.00% species other than heavy and light chains	0.42	0.42	0.46	0.44	0.41	0.56	0.44	0.44		

FIG. 7A

14/18

Stability Data for h409A11 Anti-PD-1 Powder for injection, 50 mg/Vial											
Storage Condition											
5°C											
Batch Number											
3											
Stability Test Interval											
Test	Clinical Acceptance Criteria	Initial	1-month	3-month	6-month	9-month	12-month	18-month	24-month		
CE-SDS Non-Reducing	light chains										
% Impurity											
HP-IEX											
	≤ 10.00% species other than main band	0.45	0.47	0.42	0.55	0.78	0.31	0.60	0.55		
Acidic variants (%)	Report Results	3.97	8.45	8.06	6.79	8.52	9.29	8.71	9.70		
Acidic 1 (%)	Report Results	5.45	4.99	5.05	4.97	4.75	5.07	4.90	4.91		
Acidic 2 (%)	Report Results	7.76	8.26	8.26	8.02	7.91	8.48	8.48	8.59		
Main (%)	Report Results	54.4	48.3	47.3	48.9	46.2	47.0	46.6	44.9		
Basic 1 (%)	Report Results	7.23	7.63	7.82	7.98	7.69	7.77	8.04	8.43		
Basic 2 (%)	Report Results	7.92	8.65	9.23	9.33	9.07	8.87	9.08	9.08		
Basic Variants (%)	Report Results	13.22	13.75	14.29	14.03	15.82	13.51	14.20	14.38		
Moisture (%)	≤ 5.0%	0.8	0.8	0.9	0.8	0.8	1.0	1.0	1.0		
Particulate Matter (HIAC*)	Complies USP<788>	Complies	Complies	Complies	Complies	Complies	Complies	Complies	Complies		
≥ 10 µm per container	NMT 6000	39	24	16	18	18	16	16	47		
≥ 25 µm per container	NMT 600	0	1	0	1	0	0	0	2		
Bacterial Endotoxin	≤ 0.25 EU/mg	<0.05	NT	NT	NT	NT	<0.05	NT	<0.05		
Sterility	Meets Sterility Test Requirement	Meets Requirements	NT	NT	NT	NT	NT	NT	NT		
Container Closure Integrity	No leakage detected	No leakage detected	NT	NT	NT	NT	No leakage detected	NT	No leakage detected		
NT: Not tested											

FIG.7B

15/18

Stability Data for h409A11 Anti-PD-1 Powder for injection, 50 mg/Vial						
Storage Condition						
25H (25°C, 60% RH)						
Batch Number						
3						
Stability Test Interval						
Test	Clinical Acceptance Criteria	Initial	1-month	3-month	6-month	
Description Lyophilized Powder	White to off-white powder	White powder	White powder	White powder	White powder	
Reconstitution Time (seconds)	Report Results	41	35	33	35	
Description Reconstituted Solution						
Clarity	Clear to opalescent solution: May contain particulates	Opalescent solution does not contain particulates	Clear solution does not contain particulates	Clear solution does not contain particulates	Opalescent solution does not contain particulates	
Color	Report results by "Y" ref solution	Equivalent to ref solution Y7	Equivalent to ref solution Y7	Equivalent to ref solution Y7	Equivalent to ref solution Y7	
pH	5.0 – 6.0	5.5	5.5	5.5	5.5	
Assay UV A280 nm	21.3 – 28.8 mg/mL	25.6	25.4	25.8	25.9	
Biological Potency	50–150% of Reference	90	107	89	99	
Purity						
HPSEC						
High Mol. Wt. Species (%)	≤ 5.00	0.32	0.28	0.25	0.32	
Late Eluting Peaks (%)	Report Results	<0.13	<0.13	<0.13	<0.13	
Monomer (%)	≥ 90.0	99.7	99.7	99.7	99.7	
CE-SDS Reducing						
% Impurity	≤ 10.00% species other than heavy and light chains	0.42	0.42	0.43	0.45	
CE-SDS Non-Reducing						
% Impurity	≤ 10.00% species other than main band	0.45	0.71	0.43	0.53	

FIG.8A

16/18

Stability Data for h 409 All Anti-PD-1 Powder for injection, 50 mg/Vial						
Storage Condition						
25H (25°C, 60% RH)						
Batch Number						
3						
Stability Test Interval						
Test	Clinical Acceptance Criteria	Initial	1-month	3-month	6-month	
HP-IEX						
Acidic variants (%)	Report Results	3.97	8.52	8.67	9.79	
Acidic 1 (%)	Report Results	5.45	4.97	5.09	5.04	
Acidic 2 (%)	Report Results	7.76	8.40	8.60	8.49	
Main (%)	Report Results	54.4	48.0	45.9	44.9	
Basic 1 (%)	Report Results	7.23	7.65	7.89	8.09	
Basic 2 (%)	Report Results	7.92	8.60	9.22	9.37	
Basic Variants (%)	Report Results	13.22	13.88	14.67	14.35	
Moisture (%)	≤ 5.0%	0.8	0.9	1.0	1.2	
Particulate Matter (HIAC)	Complies USP<788>	Complies	Complies	Complies	Complies	
≥ 10 μm per container	NMT 6000	39	10	18	21	
≥ 25 μm per container	NMT 600	0	0	0	0	
Bacterial Endotoxin	≤ 0.25 EU/mg	<0.05	NT	NT	NT	
Sterility	Meets Sterility Test Requirement	Meets requirements	NT	NT	NT	
Container Closure Integrity	No leakage detected	No leakage detected	NT	NT	NT	
NT: Not tested						

FIG. 8B

17/18

Stability Data for h409A11 Anti-PD-1 Powder for injection, 50 mg/Vial						
Storage Condition						
RH4 (40°C, 75% RH)						
Batch Number						
3						
Stability Test Interval						
Test	Clinical Acceptance Criteria	Initial	1-month	3-month	6-month	
Description Lyophilized Powder	White to off-white powder	White powder	White powder	White powder	White powder	
Reconstitution Time (seconds)	Report Results	41	35	34	33	
Description Reconstituted Solution						
Clarity	Clear to opalescent solution: May contain particulates	Opalescent solution does not contain particulates	Clear solution does not contain particulates	Clear solution does not contain particulates	Opalescent solution does not contain particulates	
Color	Report results by "Y" ref solution	Equivalent to ref solution Y7	Equivalent to ref solution Y7	Equivalent to ref solution Y7	Equivalent to ref solution Y7	
pH	5.0 – 6.0	5.5	5.5	5.5	5.5	
Assay UV A280 nm	21.3 – 28.8 mg/mL	25.6	25.4	25.5	25.9	
Biological Potency	50–150% of Reference	90	101	105	104	
Purity						
HPSEC						
High Mol. Wt. Species (%)	≤ 5.00	0.32	0.30	0.33	0.40	
Late Eluting Peaks (%)	Report Results	<0.13	<0.13	<0.13	<0.13	
Monomer (%)	≥ 90.0	99.7	99.7	99.7	99.6	
CE-SDS Reducing						
% Impurity	≤ 10.00% species other than heavy and light chains	0.42	0.50	0.48	0.45	
CE-SDS Non-Reducing						
% Impurity	≤ 10.00% species other than main band	0.45	0.52	0.67	0.35	

FIG.9A

18/18

Stability Data for h409A11 Anti-PD-1 Powder for injection, 50 mg/Vial						
Storage Condition						
RH4 (40°C, 75% RH)						
Batch Number						
3						
Stability Test Interval						
Test	Clinical Acceptance Criteria	Initial	1-month	3-month	6-month	
HP-IEX						
Acidic variants (%)	Report Results	3.97	8.16	9.32	9.47	
Acidic 1 (%)	Report Results	5.45	5.11	5.12	5.08	
Acidic 2 (%)	Report Results	7.76	8.88	9.47	10.11	
Main (%)	Report Results	54.4	47.1	43.4	42.2	
Basic 1 (%)	Report Results	7.23	7.75	8.22	8.41	
Basic 2 (%)	Report Results	7.92	8.51	9.11	9.12	
Basic Variants (%)	Report Results	13.22	14.48	15.32	15.66	
Moisture (%)	≤ 5.0%	0.8	1.1	1.3	1.5	
Particulate Matter (HIAC)	Complies USP<788>	Complies	Complies	Complies	Complies	
≥ 10 μm per container	NMT 6000	39	14	19	26	
≥ 25 μm per container	NMT 600	0	0	0	1	
Bacterial Endotoxin	≤ 0.25 EU/mg	<0.05	NT	NT	NT	
Sterility	Meets Sterility Test Requirement	Meets requirements	NT	NT	NT	
Container Closure Integrity	No leakage detected	No leakage detected	NT	NT	NT	
NT: Not tested						

FIG. 9B



## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 12/31063

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> IPC(8) - A61K 39/395 (2012.01) USPC - 424/133.1 According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b> Minimum documentation searched (classification system followed by classification symbols) USPC: 424/133.1  Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched USPC: 424/133.1, 172.1; 536/23.53; 530/387.3; 435/328, 326 (text search)  Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) Electronic data bases: PubWEST (PGPB, USPT, EPAB, JPAB); Google Scholar; GenCore Sequence Search (AA) Search terms: antibody, anti-PD1 antibod\$3, PD-1 (i.e. PD1 or CD279 or PDCD1), formulation, lyophil\$7, histidine buffer, polysorbate 80 or Tween 80, sucrose, stability, high concentration (e.g 50 mg/mL).		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2006/0029599 A1 (KAISHEVA et al.) 9 February 2006 (09.02.2006). Especially [0012], [0013], [0059], [0065].	1-25,28-30
Y	US 2010/0266617 A1 (CARVEN et al.) 21 October 2010 (21.10.2010). Especially para [0017], [0020], [0024], [0063], [0228], SEQ ID NOs: 15-20, 31, 32, 36	1-25,28-30
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/>		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 4 June 2012 (04.06.2012)		Date of mailing of the international search report <b>22 JUN 2012</b>
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201		Authorized officer: Lee W. Young  PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 12/31063

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

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

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
3. ☒ Claims Nos.: 26,27  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

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

**Remark on Protest**

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

# Annexure - 7

## HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use INLYTA safely and effectively. See full prescribing information for INLYTA.

### INLYTA® (axitinib) tablets for oral administration

Initial U.S. Approval: 2012

#### -----INDICATIONS AND USAGE-----

INLYTA is a kinase inhibitor indicated for the treatment of advanced renal cell carcinoma after failure of one prior systemic therapy. (1)

#### -----DOSAGE AND ADMINISTRATION-----

- The starting dose is 5 mg orally twice daily. Dose adjustments can be made based on individual safety and tolerability. (2.1, 2.2)
- Administer INLYTA dose approximately 12 hours apart with or without food. (2.1)
- INLYTA should be swallowed whole with a glass of water. (2.1)
- If a strong CYP3A4/5 inhibitor is required, decrease the INLYTA dose by approximately half. (2.2)
- For patients with moderate hepatic impairment, decrease the starting dose by approximately half. (2.2)

#### -----DOSAGE FORMS AND STRENGTHS-----

1 mg and 5 mg tablets (3)

#### -----CONTRAINDICATIONS-----

None (4)

#### -----WARNINGS AND PRECAUTIONS-----

- Hypertension including hypertensive crisis has been observed. Blood pressure should be well-controlled prior to initiating INLYTA. Monitor for hypertension and treat as needed. For persistent hypertension despite use of anti-hypertensive medications, reduce the INLYTA dose. (5.1)
- Arterial and venous thrombotic events have been observed and can be fatal. Use with caution in patients who are at increased risk for these events. (5.2, 5.3)
- Hemorrhagic events, including fatal events, have been reported. INLYTA has not been studied in patients with evidence of untreated brain metastasis or recent active gastrointestinal bleeding and should not be used in those patients. (5.4)

- Gastrointestinal perforation and fistula, including death, have occurred. Use with caution in patients at risk for gastrointestinal perforation or fistula. (5.5)
- Hypothyroidism requiring thyroid hormone replacement has been reported. Monitor thyroid function before initiation of, and periodically throughout, treatment with INLYTA. (5.6)
- Stop INLYTA at least 24 hours prior to scheduled surgery. (5.7)
- Reversible Posterior Leukoencephalopathy Syndrome (RPLS) has been observed. Permanently discontinue INLYTA if signs or symptoms of RPLS occur. (5.8)
- Monitor for proteinuria before initiation of, and periodically throughout, treatment with INLYTA. For moderate to severe proteinuria, reduce the dose or temporarily interrupt treatment with INLYTA. (5.9)
- Liver enzyme elevation has been observed during treatment with INLYTA. Monitor ALT, AST and bilirubin before initiation of, and periodically throughout, treatment with INLYTA. (5.10)
- The starting dose of INLYTA should be decreased if used in patients with moderate hepatic impairment. INLYTA has not been studied in patients with severe hepatic impairment. (2.2, 5.11)
- INLYTA can cause fetal harm when administered to a pregnant woman based on its mechanism of action. Women of childbearing potential should be advised of the potential hazard to the fetus and to avoid becoming pregnant while receiving INLYTA. (5.12, 8.1)

#### -----ADVERSE REACTIONS-----

The most common ( $\geq 20\%$ ) adverse reactions are diarrhea, hypertension, fatigue, decreased appetite, nausea, dysphonia, palmar-plantar erythrodysesthesia (hand-foot) syndrome, weight decreased, vomiting, asthenia, and constipation. (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact Pfizer, Inc at 1-800-438-1985 or FDA at 1-800-FDA-1088 or [www.fda.gov/medwatch](http://www.fda.gov/medwatch).

#### -----DRUG INTERACTIONS-----

- Avoid strong CYP3A4/5 inhibitors. If unavoidable, reduce the INLYTA dose. (2.2, 7.1)
- Avoid strong CYP3A4/5 inducers. (7.2)

See 17 for PATIENT COUNSELING INFORMATION and FDA-approved patient labeling

## FULL PRESCRIBING INFORMATION: CONTENTS\*

### 1 INDICATIONS AND USAGE

### 2 DOSAGE AND ADMINISTRATION

- 2.1 Recommended Dosing
- 2.2 Dose Modification Guidelines

### 3 DOSAGE FORMS AND STRENGTHS

### 4 CONTRAINDICATIONS

### 5 WARNINGS AND PRECAUTIONS

- 5.1 Hypertension and Hypertensive Crisis
- 5.2 Arterial Thromboembolic Events
- 5.3 Venous Thromboembolic Events
- 5.4 Hemorrhage
- 5.5 Gastrointestinal Perforation and Fistula Formation
- 5.6 Thyroid Dysfunction
- 5.7 Wound Healing Complications
- 5.8 Reversible Posterior Leukoencephalopathy Syndrome
- 5.9 Proteinuria
- 5.10 Elevation of Liver Enzymes
- 5.11 Hepatic Impairment
- 5.12 Pregnancy

### 6 ADVERSE REACTIONS

- 6.1 Clinical Trials Experience

### 7 DRUG INTERACTIONS

- 7.1 CYP3A4/5 Inhibitors
- 7.2 CYP3A4/5 Inducers

### 8 USE IN SPECIFIC POPULATIONS

- 8.1 Pregnancy

- 8.3 Nursing Mothers

- 8.4 Pediatric Use

- 8.5 Geriatric Use

- 8.6 Hepatic Impairment

- 8.7 Renal Impairment

### 10 OVERDOSAGE

### 11 DESCRIPTION

### 12 CLINICAL PHARMACOLOGY

- 12.1 Mechanism of Action
- 12.2 Pharmacodynamics
- 12.3 Pharmacokinetics

### 13 NONCLINICAL TOXICOLOGY

- 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

### 14 CLINICAL STUDIES

### 16 HOW SUPPLIED/STORAGE AND HANDLING

### 17 PATIENT COUNSELING INFORMATION

- 17.1 Hypertension
- 17.2 Arterial/Venous Thromboembolic Events
- 17.3 Hemorrhage
- 17.4 Gastrointestinal Disorders
- 17.5 Abnormal Thyroid Function
- 17.6 Wound Healing Complications
- 17.7 Reversible Posterior Leukoencephalopathy Syndrome
- 17.8 Pregnancy
- 17.9 Concomitant Medications

\*Sections or subsections omitted from the Full Prescribing Information are not listed.

## FULL PRESCRIBING INFORMATION

### 1 INDICATIONS AND USAGE

INLYTA is indicated for the treatment of advanced renal cell carcinoma (RCC) after failure of one prior systemic therapy.

### 2 DOSAGE AND ADMINISTRATION

#### 2.1 Recommended Dosing

The recommended starting oral dose of INLYTA is 5 mg twice daily. Administer INLYTA doses approximately 12 hours apart with or without food [*see Clinical Pharmacology (12.3)*]. INLYTA should be swallowed whole with a glass of water.

If the patient vomits or misses a dose, an additional dose should not be taken. The next prescribed dose should be taken at the usual time.

#### 2.2 Dose Modification Guidelines

Dose increase or reduction is recommended based on individual safety and tolerability.

Over the course of treatment, patients who tolerate INLYTA for at least two consecutive weeks with no adverse reactions >Grade 2 (according to the Common Toxicity Criteria for Adverse Events [CTCAE]), are normotensive, and are not receiving anti-hypertension medication, may have their dose increased. When a dose increase from 5 mg twice daily is recommended, the INLYTA dose may be increased to 7 mg twice daily, and further to 10 mg twice daily using the same criteria.

Over the course of treatment, management of some adverse drug reactions may require temporary interruption or permanent discontinuation and/or dose reduction of INLYTA therapy [*see Warnings and Precautions (5)*]. If dose reduction from 5 mg twice daily is required, the recommended dose is 3 mg twice daily. If additional dose reduction is required, the recommended dose is 2 mg twice daily.

**Strong CYP3A4/5 Inhibitors:** The concomitant use of strong CYP3A4/5 inhibitors should be avoided (e.g., ketoconazole, itraconazole, clarithromycin, atazanavir, indinavir, nefazodone, nelfinavir, ritonavir, saquinavir, telithromycin, and voriconazole). Selection of an alternate concomitant medication with no or minimal CYP3A4/5 inhibition potential is recommended. Although INLYTA dose adjustment has not been studied in patients receiving strong CYP3A4/5 inhibitors, if a strong CYP3A4/5 inhibitor must be co-administered, a dose decrease of INLYTA by approximately half is recommended, as this dose reduction is predicted to adjust the axitinib area under the plasma concentration vs time curve (AUC) to the range observed without inhibitors. The subsequent doses can be increased or decreased based on individual safety and tolerability. If co-administration of the strong inhibitor is discontinued, the INLYTA dose should be returned (after 3 – 5 half-lives of the inhibitor) to that used prior to initiation of the strong CYP3A4/5 inhibitor [*see Drug Interactions (7.1) and Clinical Pharmacology (12.3)*].

**Hepatic Impairment:** No starting dose adjustment is required when administering INLYTA to patients with mild hepatic impairment (Child-Pugh class A). Based on the pharmacokinetic data,

the INLYTA starting dose should be reduced by approximately half in patients with baseline moderate hepatic impairment (Child-Pugh class B). The subsequent doses can be increased or decreased based on individual safety and tolerability. INLYTA has not been studied in patients with severe hepatic impairment (Child-Pugh class C) [*see Warnings and Precautions (5.11), Use in Specific Populations (8.6), and Clinical Pharmacology (12.3)*].

### 3 DOSAGE FORMS AND STRENGTHS

1 mg tablets of INLYTA: red, film-coated, oval tablets, debossed with “Pfizer” on one side and “1 XNB” on the other side.

5 mg tablets of INLYTA: red, film-coated, triangular tablets, debossed with “Pfizer” on one side and “5 XNB” on the other side.

### 4 CONTRAINDICATIONS

None

### 5 WARNINGS AND PRECAUTIONS

#### 5.1 Hypertension and Hypertensive Crisis

In a controlled clinical study with INLYTA for the treatment of patients with RCC, hypertension was reported in 145/359 patients (40%) receiving INLYTA and 103/355 patients (29%) receiving sorafenib. Grade 3/4 hypertension was observed in 56/359 patients (16%) receiving INLYTA and 39/355 patients (11%) receiving sorafenib. Hypertensive crisis was reported in 2/359 patients (<1%) receiving INLYTA and none of the patients receiving sorafenib. The median onset time for hypertension (systolic blood pressure >150 mmHg or diastolic blood pressure >100 mmHg) was within the first month of the start of INLYTA treatment and blood pressure increases have been observed as early as 4 days after starting INLYTA. Hypertension was managed with standard antihypertensive therapy. Discontinuation of INLYTA treatment due to hypertension occurred in 1/359 patients (<1%) receiving INLYTA and none of the patients receiving sorafenib [*see Adverse Reactions (6.1)*].

Blood pressure should be well-controlled prior to initiating INLYTA. Patients should be monitored for hypertension and treated as needed with standard anti-hypertensive therapy. In the case of persistent hypertension despite use of anti-hypertensive medications, reduce the INLYTA dose. Discontinue INLYTA if hypertension is severe and persistent despite anti-hypertensive therapy and dose reduction of INLYTA, and discontinuation should be considered if there is evidence of hypertensive crisis. If INLYTA is interrupted, patients receiving antihypertensive medications should be monitored for hypotension [*see Dosage and Administration (2.2)*].

#### 5.2 Arterial Thromboembolic Events

In clinical trials, arterial thromboembolic events have been reported, including deaths. In a controlled clinical study with INLYTA for the treatment of patients with RCC, Grade 3/4 arterial thromboembolic events were reported in 4/359 patients (1%) receiving INLYTA and 4/355 patients (1%) receiving sorafenib. Fatal cerebrovascular accident was reported in 1/359 patients

(<1%) receiving INLYTA and none of the patients receiving sorafenib [see *Adverse Reactions* (6.1)].

In clinical trials with INLYTA, arterial thromboembolic events (including transient ischemic attack, cerebrovascular accident, myocardial infarction, and retinal artery occlusion) were reported in 17/715 patients (2%), with two deaths secondary to cerebrovascular accident.

Use INLYTA with caution in patients who are at risk for, or who have a history of, these events. INLYTA has not been studied in patients who had an arterial thromboembolic event within the previous 12 months.

### 5.3 Venous Thromboembolic Events

In clinical trials, venous thromboembolic events have been reported, including deaths. In a controlled clinical study with INLYTA for the treatment of patients with RCC, venous thromboembolic events were reported in 11/359 patients (3%) receiving INLYTA and 2/355 patients (1%) receiving sorafenib. Grade 3/4 venous thromboembolic events were reported in 9/359 patients (3%) receiving INLYTA (including pulmonary embolism, deep vein thrombosis, retinal vein occlusion and retinal vein thrombosis) and 2/355 patients (1%) receiving sorafenib. Fatal pulmonary embolism was reported in 1/359 patients (<1%) receiving INLYTA and none of the patients receiving sorafenib. In clinical trials with INLYTA, venous thromboembolic events were reported in 22/715 patients (3%), with two deaths secondary to pulmonary embolism.

Use INLYTA with caution in patients who are at risk for, or who have a history of, these events. INLYTA has not been studied in patients who had a venous thromboembolic event within the previous 6 months.

### 5.4 Hemorrhage

In a controlled clinical study with INLYTA for the treatment of patients with RCC, hemorrhagic events were reported in 58/359 patients (16%) receiving INLYTA and 64/355 patients (18%) receiving sorafenib. Grade 3/4 hemorrhagic events were reported in 5/359 (1%) patients receiving INLYTA (including cerebral hemorrhage, hematuria, hemoptysis, lower gastrointestinal hemorrhage, and melena) and 11/355 (3%) patients receiving sorafenib. Fatal hemorrhage was reported in 1/359 patients (<1%) receiving INLYTA (gastric hemorrhage) and 3/355 patients (1%) receiving sorafenib.

INLYTA has not been studied in patients who have evidence of untreated brain metastasis or recent active gastrointestinal bleeding and should not be used in those patients. If any bleeding requires medical intervention, temporarily interrupt the INLYTA dose.

### 5.5 Gastrointestinal Perforation and Fistula Formation

In a controlled clinical study with INLYTA for the treatment of patients with RCC, gastrointestinal perforation was reported in 1/359 patients (<1%) receiving INLYTA and none of the patients receiving sorafenib. In clinical trials with INLYTA, gastrointestinal perforation was reported in 5/715 patients (1%), including one death. In addition to cases of gastrointestinal perforation, fistulas were reported in 4/715 patients (1%).

Monitor for symptoms of gastrointestinal perforation or fistula periodically throughout treatment with INLYTA.

## 5.6 Thyroid Dysfunction

In a controlled clinical study with INLYTA for the treatment of patients with RCC, hypothyroidism was reported in 69/359 patients (19%) receiving INLYTA and 29/355 patients (8%) receiving sorafenib. Hyperthyroidism was reported in 4/359 patients (1%) receiving INLYTA and 4/355 patients (1%) receiving sorafenib. In patients who had thyroid stimulating hormone (TSH)  $<5$   $\mu\text{U/mL}$  before treatment, elevations of TSH to  $\geq 10$   $\mu\text{U/mL}$  occurred in 79/245 patients (32%) receiving INLYTA and 25/232 patients (11%) receiving sorafenib [*see Adverse Reactions (6.1)*].

Monitor thyroid function before initiation of, and periodically throughout, treatment with INLYTA. Treat hypothyroidism and hyperthyroidism according to standard medical practice to maintain euthyroid state.

## 5.7 Wound Healing Complications

No formal studies of the effect of INLYTA on wound healing have been conducted.

Stop treatment with INLYTA at least 24 hours prior to scheduled surgery. The decision to resume INLYTA therapy after surgery should be based on clinical judgment of adequate wound healing.

## 5.8 Reversible Posterior Leukoencephalopathy Syndrome

In a controlled clinical study with INLYTA for the treatment of patients with RCC, reversible posterior leukoencephalopathy syndrome (RPLS) was reported in 1/359 patients ( $<1\%$ ) receiving INLYTA and none of the patients receiving sorafenib [*see Adverse Reactions (6.1)*]. There were two additional reports of RPLS in other clinical trials with INLYTA.

RPLS is a neurological disorder which can present with headache, seizure, lethargy, confusion, blindness and other visual and neurologic disturbances. Mild to severe hypertension may be present. Magnetic resonance imaging is necessary to confirm the diagnosis of RPLS. Discontinue INLYTA in patients developing RPLS. The safety of reinitiating INLYTA therapy in patients previously experiencing RPLS is not known.

## 5.9 Proteinuria

In a controlled clinical study with INLYTA for the treatment of patients with RCC, proteinuria was reported in 39/359 patients (11%) receiving INLYTA and 26/355 patients (7%) receiving sorafenib. Grade 3 proteinuria was reported in 11/359 patients (3%) receiving INLYTA and 6/355 patients (2%) receiving sorafenib [*see Adverse Reactions (6.1)*].

Monitoring for proteinuria before initiation of, and periodically throughout, treatment with INLYTA is recommended. For patients who develop moderate to severe proteinuria, reduce the dose or temporarily interrupt INLYTA treatment.

## 5.10 Elevation of Liver Enzymes

In a controlled clinical study with INLYTA for the treatment of patients with RCC, alanine aminotransferase (ALT) elevations of all grades occurred in 22% of patients on both arms, with Grade 3/4 events in  $<1\%$  of patients on the INLYTA arm and 2% of patients on the sorafenib arm.

Monitor ALT, aspartate aminotransferase (AST) and bilirubin before initiation of and periodically throughout treatment with INLYTA.

### 5.11 Hepatic Impairment

The systemic exposure to axitinib was higher in subjects with moderate hepatic impairment (Child-Pugh class B) compared to subjects with normal hepatic function. A dose decrease is recommended when administering INLYTA to patients with moderate hepatic impairment (Child-Pugh class B). INLYTA has not been studied in patients with severe hepatic impairment (Child-Pugh class C) [see *Dosage and Administration* (2.2), *Use in Specific Populations* (8.6), and *Clinical Pharmacology* (12.3)].

### 5.12 Pregnancy

INLYTA can cause fetal harm when administered to a pregnant woman based on its mechanism of action. There are no adequate and well-controlled studies in pregnant women using INLYTA. In developmental toxicity studies in mice, axitinib was teratogenic, embryotoxic and fetotoxic at maternal exposures that were lower than human exposures at the recommended clinical dose.

Women of childbearing potential should be advised to avoid becoming pregnant while receiving INLYTA. If this drug is used during pregnancy, or if a patient becomes pregnant while receiving this drug, the patient should be apprised of the potential hazard to the fetus [see *Use in Specific Populations* (8.1)].

## 6 ADVERSE REACTIONS

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in clinical practice.

The safety of INLYTA has been evaluated in 715 patients in monotherapy studies, which included 537 patients with advanced RCC. The data described [see *Adverse Reactions* (6.1)] reflect exposure to INLYTA in 359 patients with advanced RCC who participated in a randomized clinical study versus sorafenib [see *Clinical Studies* (14)].

The following risks, including appropriate action to be taken, are discussed in greater detail in other sections of the label [see *Warnings and Precautions* (5.1-5.10 and 5.12)]: hypertension, arterial thromboembolic events, venous thromboembolic events, hemorrhage, gastrointestinal perforation and fistula formation, thyroid dysfunction, wound healing complications, RPLS, proteinuria, elevation of liver enzymes, and fetal development.

### 6.1 Clinical Trials Experience

The median duration of treatment was 6.4 months (range 0.03 to 22.0) for patients who received INLYTA and 5.0 months (range 0.03 to 20.1) for patients who received sorafenib. Dose modifications or temporary delay of treatment due to an adverse reaction occurred in 199/359 patients (55%) receiving INLYTA and 220/355 patients (62%) receiving sorafenib. Permanent discontinuation due to an adverse reaction occurred in 34/359 patients (9%) receiving INLYTA and 46/355 patients (13%) receiving sorafenib.

The most common ( $\geq 20\%$ ) adverse reactions observed following treatment with INLYTA were diarrhea, hypertension, fatigue, decreased appetite, nausea, dysphonia, palmar-plantar



erythrodysesthesia (hand-foot) syndrome, weight decreased, vomiting, asthenia, and constipation. Table 1 presents adverse reactions reported in  $\geq 10\%$  patients who received INLYTA or sorafenib.

**Table 1. Adverse Reactions Occurring in  $\geq 10\%$  of Patients Who Received INLYTA or Sorafenib**

Adverse Reaction <sup>a</sup>	INLYTA		Sorafenib	
	(N=359)		(N=355)	
	All Grades <sup>b</sup>	Grade 3/4	All Grades <sup>b</sup>	Grade 3/4
	%	%	%	%
Diarrhea	55	11	53	7
Hypertension	40	16	29	11
Fatigue	39	11	32	5
Decreased appetite	34	5	29	4
Nausea	32	3	22	1
Dysphonia	31	0	14	0
Palmar-plantar erythrodysesthesia syndrome	27	5	51	16
Weight decreased	25	2	21	1
Vomiting	24	3	17	1
Asthenia	21	5	14	3
Constipation	20	1	20	1
Hypothyroidism	19	<1	8	0
Cough	15	1	17	1
Mucosal inflammation	15	1	12	1
Arthralgia	15	2	11	1
Stomatitis	15	1	12	<1
Dyspnea	15	3	12	3
Abdominal pain	14	2	11	1
Headache	14	1	11	0
Pain in extremity	13	1	14	1
Rash	13	<1	32	4
Proteinuria	11	3	7	2
Dysgeusia	11	0	8	0
Dry skin	10	0	11	0
Dyspepsia	10	0	2	0
Pruritus	7	0	12	0
Alopecia	4	0	32	0
Erythema	2	0	10	<1

<sup>a</sup> Percentages are treatment-emergent, all-causality events

<sup>b</sup> National Cancer Institute Common Terminology Criteria for Adverse Events, Version 3.0

Selected adverse reactions (all grades) that were reported in  $<10\%$  of patients treated with INLYTA included dizziness (9%), upper abdominal pain (8%), myalgia (7%), dehydration (6%), epistaxis (6%), anemia (4%), hemorrhoids (4%), hematuria (3%), tinnitus (3%), lipase increased (3%), pulmonary embolism (2%), rectal hemorrhage (2%), hemoptysis (2%), deep vein

thrombosis (1%), retinal-vein occlusion/thrombosis (1%), polycythemia (1%), transient ischemic attack (1%), and RPLS (<1%).

Table 2 presents the most common laboratory abnormalities reported in  $\geq 10\%$  patients who received INLYTA or sorafenib.

**Table 2. Laboratory Abnormalities Occurring in  $\geq 10\%$  of Patients Who Received INLYTA or Sorafenib**

Laboratory Abnormality	N	INLYTA		N	Sorafenib	
		All Grades <sup>a</sup>	Grade 3/4		All Grades <sup>a</sup>	Grade 3/4
		%	%		%	%
<b>Hematology</b>						
Hemoglobin decreased	320	35	<1	316	52	4
Lymphocytes (absolute) decreased	317	33	3	309	36	4
Platelets decreased	312	15	<1	310	14	0
White blood cells decreased	320	11	0	315	16	<1
<b>Chemistry</b>						
Creatinine increased	336	55	0	318	41	<1
Bicarbonate decreased	314	44	<1	291	43	0
Hypocalcemia	336	39	1	319	59	2
ALP increased	336	30	1	319	34	1
Hyperglycemia	336	28	2	319	23	2
Lipase increased	338	27	5	319	46	15
Amylase increased	338	25	2	319	33	2
ALT increased	331	22	<1	313	22	2
AST increased	331	20	<1	311	25	1
Hypernatremia	338	17	1	319	13	1
Hypoalbuminemia	337	15	<1	319	18	1
Hyperkalemia	333	15	3	314	10	3
Hypoglycemia	336	11	<1	319	8	<1
Hyponatremia	338	13	4	319	11	2
Hypophosphatemia	336	13	2	318	49	16

<sup>a</sup> National Cancer Institute Common Terminology Criteria for Adverse Events, Version 3.0

ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase

Selected laboratory abnormalities (all grades) that were reported in <10% of patients treated with INLYTA included hemoglobin increased (above the upper limit of normal) (9% for INLYTA versus 1% for sorafenib).

## 7 DRUG INTERACTIONS

*In vitro* data indicate that axitinib is metabolized primarily by CYP3A4/5 and, to a lesser extent, CYP1A2, CYP2C19, and uridine diphosphate-glucuronosyltransferase (UGT) 1A1.

### 7.1 CYP3A4/5 Inhibitors

Co-administration of ketoconazole, a strong inhibitor of CYP3A4/5, increased the plasma exposure of axitinib in healthy volunteers. Co-administration of INLYTA with strong CYP3A4/5

inhibitors should be avoided. Grapefruit or grapefruit juice may also increase axitinib plasma concentrations and should be avoided. Selection of concomitant medication with no or minimal CYP3A4/5 inhibition potential is recommended. If a strong CYP3A4/5 inhibitor must be co-administered, the INLYTA dose should be reduced [*see Dosage and Administration (2.2) and Clinical Pharmacology (12.3)*].

## 7.2 CYP3A4/5 Inducers

Co-administration of rifampin, a strong inducer of CYP3A4/5, reduced the plasma exposure of axitinib in healthy volunteers. Co-administration of INLYTA with strong CYP3A4/5 inducers (e.g., rifampin, dexamethasone, phenytoin, carbamazepine, rifabutin, rifapentin, phenobarbital, and St. John's wort) should be avoided. Selection of concomitant medication with no or minimal CYP3A4/5 induction potential is recommended [*see Dosage and Administration (2.2) and Clinical Pharmacology (12.3)*]. Moderate CYP3A4/5 inducers (e.g., bosentan, efavirenz, etravirine, modafinil, and nafcillin) may also reduce the plasma exposure of axitinib and should be avoided if possible.

## 8 USE IN SPECIFIC POPULATIONS

### 8.1 Pregnancy

Pregnancy Category D [*see Warnings and Precautions (5.12)*].

There are no adequate and well-controlled studies with INLYTA in pregnant women. INLYTA can cause fetal harm when administered to a pregnant woman based on its mechanism of action. Axitinib was teratogenic, embryotoxic and fetotoxic in mice at exposures lower than human exposures at the recommended starting dose. If this drug is used during pregnancy, or if the patient becomes pregnant while receiving this drug, the patient should be apprised of the potential hazard to the fetus.

Oral axitinib administered twice daily to female mice prior to mating and through the first week of pregnancy caused an increase in post-implantation loss at all doses tested ( $\geq 15$  mg/kg/dose, approximately 10 times the systemic exposure (AUC) in patients at the recommended starting dose). In an embryo-fetal developmental toxicity study, pregnant mice received oral doses of 0.15, 0.5 and 1.5 mg/kg/dose axitinib twice daily during the period of organogenesis. Embryo-fetal toxicities observed in the absence of maternal toxicity included malformation (cleft palate) at 1.5 mg/kg/dose (approximately 0.5 times the AUC in patients at the recommended starting dose) and variation in skeletal ossification at  $\geq 0.5$  mg/kg/dose (approximately 0.15 times the AUC in patients at the recommended starting dose).

### 8.3 Nursing Mothers

It is not known whether axitinib is excreted in human milk. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants from INLYTA, a decision should be made whether to discontinue nursing or to discontinue the drug, taking into account the importance of the drug to the mother.

### 8.4 Pediatric Use

The safety and efficacy of INLYTA in pediatric patients have not been studied.

Toxicities in bone and teeth were observed in immature mice and dogs administered oral axitinib twice daily for 1 month or longer. Effects in bone consisted of thickened growth plates in mice and dogs at  $\geq 15$  mg/kg/dose (approximately 6 and 15 times, respectively, the systemic exposure (AUC) in patients at the recommended starting dose). Abnormalities in growing incisor teeth (including dental caries, malocclusions and broken and/or missing teeth) were observed in mice administered oral axitinib twice daily at  $\geq 5$  mg/kg/dose (approximately 1.5 times the AUC in patients at the recommended starting dose). Other toxicities of potential concern to pediatric patients have not been evaluated in juvenile animals.

## 8.5 Geriatric Use

In a controlled clinical study with INLYTA for the treatment of patients with RCC, 123/359 patients (34%) treated with INLYTA were  $\geq 65$  years of age. Although greater sensitivity in some older individuals cannot be ruled out, no overall differences were observed in the safety and effectiveness of INLYTA between patients who were  $\geq 65$  years of age and younger.

No dosage adjustment is required in elderly patients [*see Dosage and Administration (2.2) and Clinical Pharmacology (12.3)*].

## 8.6 Hepatic Impairment

In a dedicated hepatic impairment trial, compared to subjects with normal hepatic function, systemic exposure following a single dose of INLYTA was similar in subjects with baseline mild hepatic impairment (Child-Pugh class A) and higher in subjects with baseline moderate hepatic impairment (Child-Pugh class B).

No starting dose adjustment is required when administering INLYTA to patients with mild hepatic impairment (Child-Pugh class A). A starting dose decrease is recommended when administering INLYTA to patients with moderate hepatic impairment (Child-Pugh class B) [*see Dosage and Administration (2.2), Warnings and Precautions (5.11), and Clinical Pharmacology (12.3)*].

INLYTA has not been studied in subjects with severe hepatic impairment (Child-Pugh class C).

## 8.7 Renal Impairment

No dedicated renal impairment trial for axitinib has been conducted. Based on the population pharmacokinetic analyses, no significant difference in axitinib clearance was observed in patients with pre-existing mild to severe renal impairment ( $15 \text{ mL/min} \leq \text{creatinine clearance [CLcr]} < 89 \text{ mL/min}$ ) [*see Clinical Pharmacology (12.3)*]. No starting dose adjustment is needed for patients with pre-existing mild to severe renal impairment. Caution should be used in patients with end-stage renal disease ( $\text{CLcr} < 15 \text{ mL/min}$ ).

## 10 OVERDOSAGE

There is no specific treatment for INLYTA overdose.

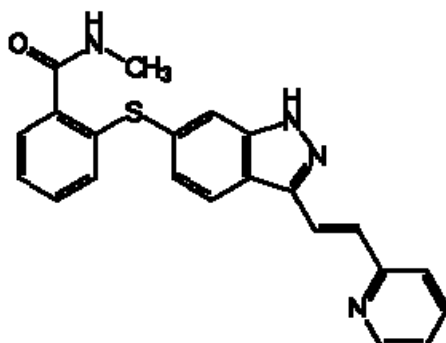
In a controlled clinical study with INLYTA for the treatment of patients with RCC, 1 patient inadvertently received a dose of 20 mg twice daily for 4 days and experienced dizziness (Grade 1).

In a clinical dose finding study with INLYTA, subjects who received starting doses of 10 mg twice daily or 20 mg twice daily experienced adverse reactions which included hypertension, seizures associated with hypertension, and fatal hemoptysis.

In cases of suspected overdose, INLYTA should be withheld and supportive care instituted.

## 11 DESCRIPTION

INLYTA (axitinib) is a kinase inhibitor. Axitinib has the chemical name *N*-methyl-2-[3-((*E*)-2-pyridin-2-yl-vinyl)-1*H*-indazol-6-ylsulfanyl]-benzamide. The molecular formula is C<sub>22</sub>H<sub>18</sub>N<sub>4</sub>OS and the molecular weight is 386.47 Daltons. The chemical structure is:



Axitinib is a white to light-yellow powder with a pKa of 4.8. The solubility of axitinib in aqueous media over the range pH 1.1 to pH 7.8 is in excess of 0.2 µg/mL. The partition coefficient (n-octanol/water) is 3.5.

INLYTA is supplied as red, film-coated tablets containing either 1 mg or 5 mg of axitinib together with microcrystalline cellulose, lactose monohydrate, croscarmellose sodium, magnesium stearate, and Opadry® II red 32K15441 as inactive ingredients. The Opadry II red 32K15441 film coating contains lactose monohydrate, HPMC 2910/Hypromellose 15cP, titanium dioxide, triacetin (glycerol triacetate), and red iron oxide.

## 12 CLINICAL PHARMACOLOGY

### 12.1 Mechanism of Action

Axitinib has been shown to inhibit receptor tyrosine kinases including vascular endothelial growth factor receptors (VEGFR)-1, VEGFR-2, and VEGFR-3 at therapeutic plasma concentrations. These receptors are implicated in pathologic angiogenesis, tumor growth, and cancer progression. VEGF-mediated endothelial cell proliferation and survival were inhibited by axitinib *in vitro* and in mouse models. Axitinib was shown to inhibit tumor growth and phosphorylation of VEGFR-2 in tumor xenograft mouse models.

### 12.2 Pharmacodynamics

The effect of a single oral dose of INLYTA (5 mg) in the absence and presence of 400 mg ketoconazole on the QTc interval was evaluated in a randomized, single-blinded, two-way crossover study in 35 healthy subjects. No large changes in mean QTc interval (i.e., >20 ms) from

placebo were detected up to 3 hours post-dose. However, small increases in mean QTc interval (i.e., <10 ms) cannot be ruled out.

### 12.3 Pharmacokinetics

The population pharmacokinetic analysis pooled data from 17 trials in healthy subjects and patients with cancer. A two-compartment disposition model with first-order absorption and lag-time adequately describes the axitinib concentration-time profile.

**Absorption and Distribution:** Following single oral 5-mg dose administration, the median  $T_{\max}$  ranged from 2.5 to 4.1 hours. Based on the plasma half-life, steady state is expected within 2 to 3 days of dosing. Dosing of axitinib at 5 mg twice daily resulted in approximately 1.4-fold accumulation compared to administration of a single dose. At steady state, axitinib exhibits approximately linear pharmacokinetics within the 1-mg to 20-mg dose range. The mean absolute bioavailability of axitinib after an oral 5 mg dose is 58%.

Compared to overnight fasting, administration of INLYTA with a moderate fat meal resulted in 10% lower AUC and a high fat, high-calorie meal resulted in 19% higher AUC. INLYTA can be administered with or without food [*see Dosage and Administration (2.1)*].

Axitinib is highly bound (>99%) to human plasma proteins with preferential binding to albumin and moderate binding to  $\alpha_1$ -acid glycoprotein. In patients with advanced RCC (n=20), at the 5 mg twice daily dose in the fed state, the geometric mean (CV%)  $C_{\max}$  and  $AUC_{0-24}$  were 27.8 (79%) ng/mL and 265 (77%) ng.h/mL, respectively. The geometric mean (CV%) clearance and apparent volume of distribution were 38 (80%) L/h and 160 (105%) L, respectively.

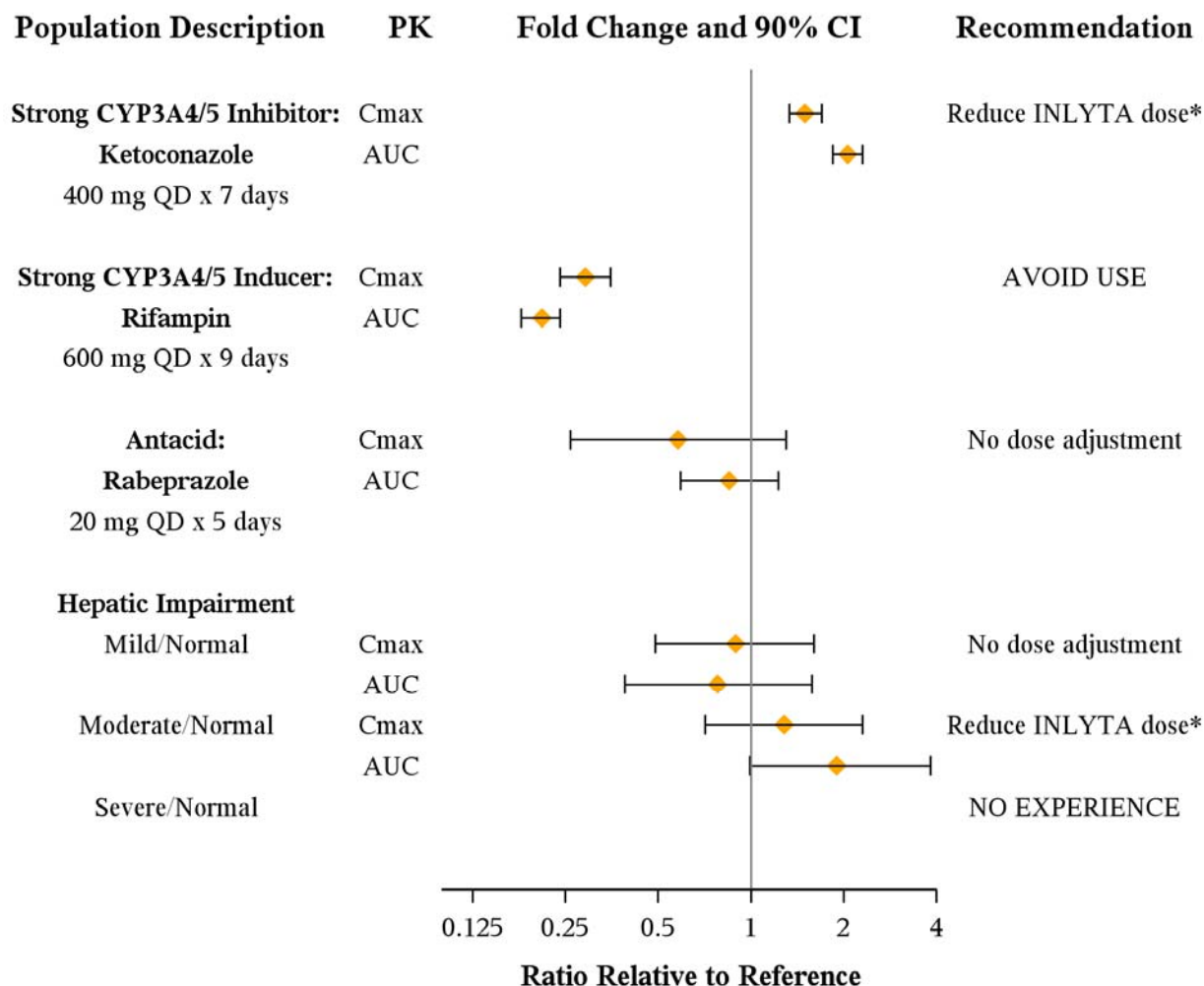
**Metabolism and Elimination:** The plasma half life of INLYTA ranges from 2.5 to 6.1 hours. Axitinib is metabolized primarily in the liver by CYP3A4/5 and to a lesser extent by CYP1A2, CYP2C19, and UGT1A1. Following oral administration of a 5-mg radioactive dose of axitinib, approximately 41% of the radioactivity was recovered in feces and approximately 23% was recovered in urine. Unchanged axitinib, accounting for 12% of the dose, was the major component identified in feces. Unchanged axitinib was not detected in urine; the carboxylic acid and sulfoxide metabolites accounted for the majority of radioactivity in urine. In plasma, the N-glucuronide metabolite represented the predominant radioactive component (50% of circulating radioactivity) and unchanged axitinib and the sulfoxide metabolite each accounted for approximately 20% of the circulating radioactivity.

The sulfoxide and N-glucuronide metabolites show approximately  $\geq 400$ -fold less *in vitro* potency against VEGFR-2 compared to axitinib.

### Drug-Drug Interactions

**Effects of Other Drugs on INLYTA:** Axitinib is metabolized primarily in the liver by CYP3A4/5. Additionally, the aqueous solubility of axitinib is pH dependent, with higher pH resulting in lower solubility. The effects of a strong CYP3A4/5 inhibitor, a strong CYP3A4/5 inducer, and an antacid on the pharmacokinetics of axitinib are presented in Figure 1 [*see Dosage and Administration (2.2) and Drug Interactions (7.1, 7.2)*].

**Figure 1. Impact of Co-administered Drugs and Hepatic Impairment on Axitinib Pharmacokinetics**



AUC: area under the curve; Cmax: maximum concentration. \*See Dosage and Administration (2).

**Effects of INLYTA on Other Drugs:** *In vitro* studies demonstrated that axitinib has the potential to inhibit CYP1A2 and CYP2C8. However, co-administration of axitinib with paclitaxel, a CYP2C8 substrate, did not increase plasma concentrations of paclitaxel in patients.

*In vitro* studies indicated that axitinib does not inhibit CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4/5, or UGT1A1 at therapeutic plasma concentrations. *In vitro* studies in human hepatocytes indicated that axitinib does not induce CYP1A1, CYP1A2, or CYP3A4/5.

Axitinib is an inhibitor of the efflux transporter P-glycoprotein (P-gp) *in vitro*. However, INLYTA is not expected to inhibit P-gp at therapeutic plasma concentrations.

### Pharmacokinetics in Specific Populations

**Pediatric Use:** INLYTA has not been studied in patients <18 years of age.

**Hepatic Impairment:** The effects of hepatic impairment on the pharmacokinetics of axitinib are presented in Figure 1 [see *Dosage and Administration* (2.2), *Warnings and Precautions* (5.11), and *Use in Specific Populations* (8.6)].

**Renal Impairment:** Population pharmacokinetic analysis (based on pre-existing renal function) was carried out in 590 healthy volunteers and patients, including five with severe renal impairment ( $15 \text{ mL/min} \leq \text{CLcr} < 29 \text{ mL/min}$ ), 64 with moderate renal impairment ( $30 \text{ mL/min} \leq \text{CLcr} < 59 \text{ mL/min}$ ), and 139 with mild renal impairment ( $60 \text{ mL/min} \leq \text{CLcr} < 89 \text{ mL/min}$ ). Mild to severe renal impairment did not have meaningful effects on the pharmacokinetics of axitinib. Data from only one patient with end-stage renal disease are available [see *Use in Specific Populations* (8.7)].

**Other Intrinsic Factors:** Population pharmacokinetic analyses indicate that there are no clinically relevant effects of age, gender, race, body weight, body surface area, UGT1A1 genotype, or CYP2C19 genotype on the clearance of axitinib.

## 13 NONCLINICAL TOXICOLOGY

### 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenicity studies have not been conducted with axitinib.

Axitinib was not mutagenic in an *in vitro* bacterial reverse mutation (Ames) assay and was not clastogenic in the *in vitro* human lymphocyte chromosome aberration assay. Axitinib was genotoxic in the *in vivo* mouse bone marrow micronucleus assay.

INLYTA has the potential to impair reproductive function and fertility in humans. In repeat-dose toxicology studies, findings in the male reproductive tract were observed in the testes/epididymis (decreased organ weight, atrophy or degeneration, decreased numbers of germinal cells, hypospermia or abnormal sperm forms, reduced sperm density and count) at  $\geq 15 \text{ mg/kg/dose}$  administered orally twice daily in mice (approximately 7 times the systemic exposure (AUC) in patients at the recommended starting dose) and  $\geq 1.5 \text{ mg/kg/dose}$  administered orally twice daily in dogs (approximately 0.1 times the AUC in patients at the recommended starting dose). Findings in the female reproductive tract in mice and dogs included signs of delayed sexual maturity, reduced or absent corpora lutea, decreased uterine weights and uterine atrophy at  $\geq 5 \text{ mg/kg/dose}$  (approximately 1.5 or 0.3 times the AUC in patients at the recommended starting dose compared to mice and dogs, respectively).

In a fertility study in mice, axitinib did not affect mating or fertility rate when administered orally twice daily to males at any dose tested up to  $50 \text{ mg/kg/dose}$  following at least 70 days of administration (approximately 57 times the AUC in patients at the recommended starting dose). In female mice, reduced fertility and embryonic viability were observed at all doses tested ( $\geq 15 \text{ mg/kg/dose}$  administered orally twice daily) following at least 15 days of treatment with axitinib (approximately 10 times the AUC in patients at the recommended starting dose).

## 14 CLINICAL STUDIES

The safety and efficacy of INLYTA were evaluated in a randomized, open-label, multicenter Phase 3 study. Patients (N=723) with advanced RCC whose disease had progressed on or after



treatment with 1 prior systemic therapy, including sunitinib-, bevacizumab-, temsirolimus-, or cytokine-containing regimens were randomized (1:1) to receive INLYTA (N=361) or sorafenib (N=362). Progression-free survival (PFS) was assessed by a blinded independent central review committee. Other endpoints included objective response rate (ORR) and overall survival (OS).

Of the patients enrolled in this study, 389 patients (54%) had received 1 prior sunitinib-based therapy, 251 patients (35%) had received 1 prior cytokine-based therapy (interleukin-2 or interferon-alfa), 59 patients (8%) had received 1 prior bevacizumab-based therapy, and 24 patients (3%) had received 1 prior temsirolimus-based therapy. The baseline demographic and disease characteristics were similar between the INLYTA and sorafenib groups with regard to age (median 61 years), gender (72% male), race (75% white, 21% Asian), Eastern Cooperative Oncology Group (ECOG) performance status (55% 0, 45% 1), and histology (99% clear cell).

There was a statistically significant advantage for INLYTA over sorafenib for the endpoint of PFS (see Table 3 and Figure 2). There was no statistically significant difference between the arms in OS.

**Table 3. Efficacy Results**

Endpoint/Study Population	INLYTA	Sorafenib	HR (95% CI)	P-value
<b>Overall ITT</b>	N= 361	N = 362		
Median PFS <sup>a,b</sup> in months (95% CI)	6.7 (6.3, 8.6)	4.7 (4.6, 5.6)	0.67 (0.54, 0.81)	<0.0001 <sup>c</sup>
Median OS in months (95% CI)	20.1 (16.7, 23.4)	19.2 (17.5, 22.3)	0.97 (0.80, 1.17)	NS
ORR % (95% CI)	19.4 (15.4, 23.9)	9.4 (6.6, 12.9)	2.06 <sup>d</sup> (1.41, 3.00)	- <sup>e</sup>
<b>PFS by prior treatment</b>				
Sunitinib-refractory subgroup	N=194	N=195		
Median, months (95% CI)	4.8 (4.5, 6.4)	3.4 (2.8, 4.7)	0.74 (0.57, 0.96)	- <sup>e</sup>
Cytokine-refractory subgroup	N=126	N=125		
Median, months (95% CI)	12.1 (10.1, 13.9)	6.5 (6.3, 8.3)	0.46 (0.32, 0.68)	- <sup>e</sup>

CI: Confidence interval; HR: Hazard ratio (INLYTA/sorafenib); ITT: Intent to treat; ORR: Objective response rate; NS: Not significant; OS: Overall survival; PFS: Progression-free survival

<sup>a</sup> Time from randomization to progression or death due to any cause, whichever occurs first.

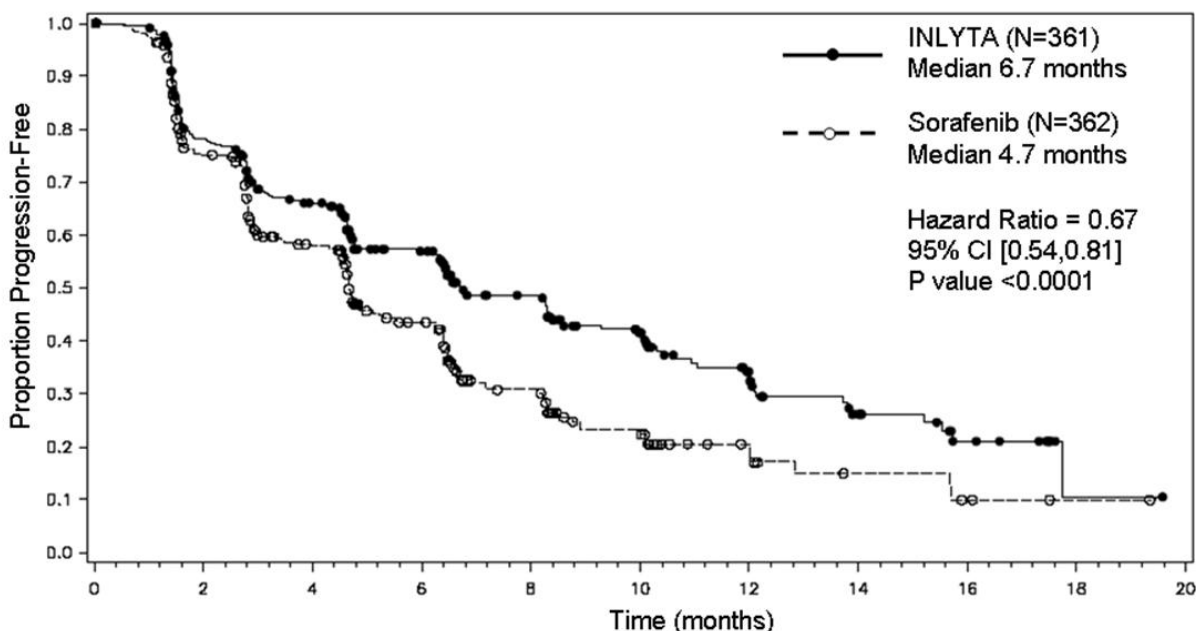
<sup>b</sup> Assessed by independent radiology review according to RECIST.

<sup>c</sup> One-sided p-value from a log-rank test of treatment stratified by ECOG performance status and prior therapy (comparison is considered statistically significant if the one-sided p-value is <0.023).

<sup>d</sup> Risk ratio is used for ORR. A risk ratio >1 indicated a higher likelihood of responding in the axitinib arm; a risk ratio <1 indicated a higher likelihood of responding in the sorafenib arm.

<sup>e</sup> P-value not included since it was not adjusted for multiple testing.

**Figure 2. Kaplan-Meier Curve for Progression Free Survival by Independent Assessment (Intent-to-Treat Population)**



## 16 HOW SUPPLIED/STORAGE AND HANDLING

INLYTA tablets are supplied as follows:

1 mg tablets are red film-coated, oval tablets debossed with “Pfizer” on one side and “1 XNB” on the other; available in bottles of 180: NDC 0069-0145-01.

5 mg tablets are red film-coated, triangular tablets debossed with “Pfizer” on one side and “5 XNB” on the other; available in bottles of 60: NDC 0069-0151-11.

Store at 20°C to 25°C (68°F to 77°F); excursions permitted to 15°C to 30°C (59°F to 86°F) [see USP Controlled Room Temperature].

## 17 PATIENT COUNSELING INFORMATION

*See FDA Approved Patient Labeling*

### 17.1 Hypertension

Advise patients that hypertension may develop during INLYTA treatment and that blood pressure should be monitored regularly during treatment [see *Warnings and Precautions* (5.1)].

### 17.2 Arterial/Venous Thromboembolic Events

Advise patients that arterial and venous thromboembolic events have been observed during INLYTA treatment and to inform their doctor if they experience symptoms suggestive of thromboembolic events [see *Warnings and Precautions* (5.2, 5.3)].

### **17.3 Hemorrhage**

Advise patients that INLYTA may increase the risk of bleeding and to promptly inform their doctor of any bleeding episodes [*see Warnings and Precautions (5.4)*].

### **17.4 Gastrointestinal Disorders**

Advise patients that gastrointestinal disorders such as diarrhea, nausea, vomiting, and constipation may develop during INLYTA treatment and to seek immediate medical attention if they experience persistent or severe abdominal pain because cases of gastrointestinal perforation and fistula have been reported in patients taking INLYTA [*see Warnings and Precautions (5.5) and Adverse Reactions (6.1)*].

### **17.5 Abnormal Thyroid Function**

Advise patients that abnormal thyroid function may develop during INLYTA treatment and to inform their doctor if symptoms of abnormal thyroid function occur [*see Warnings and Precautions (5.6)*].

### **17.6 Wound Healing Complications**

Advise patients to inform their doctor if they have an unhealed wound or if they have surgery scheduled [*see Warnings and Precautions (5.7)*].

### **17.7 Reversible Posterior Leukoencephalopathy Syndrome**

Advise patients to inform their doctor if they have worsening of neurological function consistent with RPLS (headache, seizure, lethargy, confusion, blindness and other visual and neurologic disturbances) [*see Warnings and Precautions (5.8)*].

### **17.8 Pregnancy**

Advise patients that INLYTA may cause birth defects or fetal loss and that they should not become pregnant during treatment with INLYTA. Both male and female patients should be counseled to use effective birth control during treatment with INLYTA. Female patients should also be advised against breast-feeding while receiving INLYTA [*see Warnings and Precautions (5.12) and Use in Specific Populations (8.3)*].

### **17.9 Concomitant Medications**

Advise patients to inform their doctor of all concomitant medications, vitamins, or dietary and herbal supplements.

## **FDA-Approved Patient Labeling**

LAB- 0561-1.0

Issued January 2012

## PATIENT INFORMATION

INLYTA® (in-ly-ta)  
(axitinib)  
Tablets

Read this Patient Information before you start taking INLYTA and each time you get a refill. There may be new information. This information does not take the place of talking with your doctor about your medical condition or your treatment.

### What is INLYTA?

INLYTA is a prescription medicine used to treat advanced kidney cancer (advanced renal cell carcinoma or RCC) when one prior drug treatment for this disease has not worked.

It is not known if INLYTA is safe or effective in children.

### What should I tell my doctor before taking INLYTA?

Before you take INLYTA, tell your doctor if you:

- have high blood pressure
- have thyroid problems
- have liver problems
- have a history of blood clots in your veins or arteries (types of blood vessels), including stroke, heart attack, or change in vision
- have any bleeding problems
- have an unhealed wound
- plan to have surgery. You should stop taking INLYTA at least 24 hours before planned surgery.
- have any other medical conditions

#### For females, tell your doctor if you:

- are pregnant or plan to become pregnant. Taking INLYTA during pregnancy may cause the death of an unborn baby or birth defects. You should not become pregnant while taking INLYTA. Talk to your doctor if you are pregnant or plan to become pregnant.
- are able to become pregnant. You should use effective birth control during your treatment with INLYTA. Talk to your doctor about birth control methods to prevent pregnancy while you are taking INLYTA.
- are breastfeeding or plan to breastfeed. It is not known if INLYTA passes into your breast milk. You and your doctor should decide if you will take INLYTA or breastfeed. You should not do both.

#### For males:

- use effective birth control during your treatment with INLYTA. Talk to your doctor about birth control methods.
- if your female partner becomes pregnant while you are taking INLYTA, tell your doctor right away.

**Tell your doctor about all the medicines you take**, including prescription and non-prescription medicines, vitamins, and herbal supplements. INLYTA and certain other medicines can affect each other causing serious side effects.

Especially tell your doctor if you take:

- dexamethasone
- St. John's Wort (*Hypericum perforatum*)
- Medicine for:
  - asthma
  - tuberculosis
  - seizures
  - bacterial infections
  - fungal infections
  - depression
  - HIV or AIDS

Ask your doctor or pharmacist if you are not sure if your medicine is one listed above. If you are taking any medicines for the conditions listed above, your doctor might need to prescribe a different medicine or your dose of INLYTA may need to be changed. Talk with your doctor before you start taking any new medicine.

Know the medicines you take. Keep a list of them to show your doctor and pharmacist when you get a new medicine.

#### **How should I take INLYTA?**

- Take INLYTA exactly as prescribed by your doctor.
- Your doctor may change your dose if needed.
- INLYTA can be taken with or without food.
- Take INLYTA 2 times a day approximately 12 hours apart.
- Swallow INLYTA tablets whole with a glass of water.
- Your doctor should check your blood pressure regularly during treatment with INLYTA.
- If you vomit or miss a dose of INLYTA, take your next dose at your regular time. Do not take two doses at the same time.
- If you take too much INLYTA, call your doctor or go to the nearest hospital emergency room right away.

#### **What should I avoid while taking INLYTA?**

- Do not drink grapefruit juice or eat grapefruit. Grapefruit may increase the amount of INLYTA in your blood.

#### **What are the possible side effects of INLYTA?**

INLYTA may cause serious side effects, including:

- **High blood pressure (hypertension).** Your doctor should check your blood pressure regularly during treatment with INLYTA. If you develop blood pressure problems, your doctor may prescribe medicine to treat your high blood pressure, lower your dose, or stop your treatment with INLYTA.
- **Thyroid gland problems.** Your doctor should do blood tests to check your thyroid gland function before and during your treatment with INLYTA. Tell your

doctor if you have any of the following symptoms during your treatment with INLYTA:

- tiredness that worsens or that does not go away
- feeling hot or cold
- your voice deepens
- weight gain or weight loss
- hair loss
- muscle cramps and aches
- **Problem with blood clots in your veins or arteries.** Get emergency help and call your doctor if you get any of the following symptoms:
  - chest pain or pressure
  - pain in your arms, back, neck or jaw
  - shortness of breath
  - numbness or weakness on one side of your body
  - trouble talking
  - headache
  - vision changes
- **Bleeding.** INLYTA can cause bleeding which can be serious, and sometimes lead to death. Call your doctor or get medical help if you develop any bleeding, including:
  - unexpected bleeding or bleeding that lasts a long time, such as:
  - unusual bleeding from the gums
  - menstrual bleeding or vaginal bleeding that is heavier than normal
  - bleeding that is severe or you cannot control
  - pink or brown urine
  - red or black stools (looks like tar)
  - bruises that happen without a known cause or get larger
  - cough up blood or blood clots
  - vomit blood or your vomit looks like “coffee grounds”
  - unexpected pain, swelling, or joint pain
  - headaches, feeling dizzy or weak
- **Tear in your stomach or intestinal wall (perforation).** Get medical help right away if you get the following symptoms:
  - severe stomach (abdominal) pain or stomach pain that does not go away
  - vomit blood
  - red or black stools
- **Reversible Posterior Leukoencephalopathy Syndrome (RPLS).** A condition called reversible posterior leukoencephalopathy syndrome (RPLS) can happen while taking INLYTA. Call your doctor right away if you get:
  - headache
  - seizures
  - weakness
  - confusion
  - high blood pressure
  - blindness or change in vision
  - problems thinking
- **Increased protein in your urine.** Your doctor should check your urine for protein before and during your treatment with INLYTA. If you develop protein

in your urine, your doctor may decrease your dose of INLYTA or stop your treatment.

- **Change in liver function.** Your doctor should do blood tests before and during your treatment with INLYTA to check your liver function.

The most common side effects of INLYTA include:

- diarrhea (frequent or loose bowel movements)
- high blood pressure
- tiredness or feeling weak
- decreased appetite
- nausea
- hoarseness
- rash, redness, itching or peeling of your skin on your hands and feet
- decreased weight
- vomiting
- constipation

Tell your doctor if you have any side effect that bothers you or that does not go away.

These are not all the possible side effects of INLYTA. For more information, ask your doctor or pharmacist.

Call your doctor for medical advice about side effects. You may report side effects to FDA at 1-800-FDA-1088.

#### **How should I store INLYTA?**

- Store INLYTA at room temperature between 68°F to 77°F (20°C to 25°C).

**Keep INLYTA and all medicines out of the reach of children.**

#### **General information about INLYTA.**

Medicines are sometimes prescribed for purposes other than those listed in a Patient Information leaflet. Do not use INLYTA for a condition for which it was not prescribed. Do not give INLYTA to other people, even if they have the same symptoms you have. It may harm them.

This Patient Information leaflet summarizes the most important information about INLYTA. If you would like more information, talk with your doctor. You can ask your doctor or pharmacist for information about INLYTA that is written for healthcare professionals.

For more information, go to [www.inlyta.com](http://www.inlyta.com) or call 877-744-5675.

#### **What are the ingredients in INLYTA?**

Active ingredient: axitinib

Inactive ingredients: microcrystalline cellulose, lactose monohydrate, croscarmellose sodium, magnesium stearate, and Opadry® II red 32K15441. The Opadry II red 32K15441 film coating contains: lactose monohydrate, HPMC 2910/Hypromellose 15cp, titanium dioxide, triacetin (glycerol triacetate), and red iron oxide.

This Patient Information has been approved by the U.S. Food and Drug Administration.



LAB-0438-1.0

Issued January 2012