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(54) Title: PD-1H AS TARGET IN TREATMENT OF ASTHMA

(57) Abstract: Disclosed is a method for prophylaxis or treatment of asthma in a subject comprising administering to the subject in need thereof a therapeutically effective amount of a PD-1 H agonist.



PD-1H AS TARGET IN TREATMENT OF ASTHMA

TECHNICAL FIELD

The present invention is related to a method for prophylaxis or treatment of asthma in a subject. The instant invention also concerns use of a PD-1H agonist in the preparation of a pharmaceutical compositions for prophylaxis or treatment of asthma in a subject.

BACKGROUND

Asthma is a common, chronic inflammatory disease of the airways and CD4⁺ Th2 cells are shown to play critical role in disease induction, pathogenesis and progression. The hallmarks of Th2 type responses in asthma are eosinophilic airway inflammation with mucus secretion, airway remodeling, and hyper-reactivity. IL-5 and IL-13 are critical for the pathophysiology of asthma. IL-5 has multifaceted roles including direct activation of eosinophils, influencing adhesion and inducing chemotaxis and inflammatory mediator synthesis. IL-13 has a major impact in influencing bronchial hyperreactivity, inflammation, and airway remodeling. Moreover, IL-13 drives epithelial cell maturation and mucus production, synthesis of extracellular matrix proteins and enhanced contractility of airway smooth muscle cells. The mechanisms in the regulation of cytokine production during asthma pathogenesis, however, are yet to be elucidated. Recent therapeutic efforts have focused on blockade of the interaction of these cytokines to their receptors as an approach for asthma treatment.

PD-1H (also called Gi24, VISTA, DD1 α , Dies1) is a cell surface molecule of the B7/CD28 immune modulatory gene family. Alignment of the PD-1H Immunoglobulin V region with CD28 members shows the highest identity with the programmed death one (PD-1) protein. PD-1H is constitutively expressed on the majority of hematopoietic cells including both lymphoid cells (except B cells) and myeloid cells. Several lines of evidence support that PD-1H functions as a coinhibitory receptor on T cells to limit naive T cell activation, whereas PD-1H expressed on antigen-presenting cells (APCs) interacts with an unknown receptor on T cells to suppress T cell responses. A recent study showed that the PD-1H/PD-1H interaction between different cells (homophilic interaction) could promote macrophage-mediated clearance of dead cells and T cell suppression. PD-1H knockout (KO) mice were shown to develop more severe inflammation and autoimmune diseases in several mouse models, indicating a role of PD-1H in the suppression of T cell immunity. On the other hand, agonist monoclonal antibodies (mAb) to mouse PD-1H were shown to suppress T cell-mediated acute hepatitis and prevent acute graft-versus-host disease (GVHD) in semi- and fully allogeneic murine models, leading to full chimerism following treatment. It appears that the effect of PD-1H on the suppression of T cell response could be divided into two stages with an early event in arresting allo-reactive donor T cells from activation and a later event in promoting donor Treg expansion.

While PD-1H could suppress CD4⁺ T cell response to antigens, its role in Th2-like responses, especially under pathogenic condition, is not yet known.

SUMMARY

In the instant invention, we evaluate the role of PD-1H in chicken ovalbumin (OVA)-induced allergic airway inflammation employing PD-1H KO mice and soluble PD-1H fusion protein. Our findings show that PD-1H is a critical regulator of Th2 T cell responses. Furthermore, our studies support the development of an agonist of PD-1H as potential therapeutic agents for the treatment of human asthma.

In one aspect of the invention, a method for prophylaxis or treatment of asthma in a subject is provided. The method comprises administering to the subject in need thereof a therapeutically effective amount of a PD-1H agonist.

In another aspect of the invention, provided is use of a PD-1H agonist in the preparation of a pharmaceutical composition for prophylaxis or treatment of asthma in a subject.

In some embodiments of the invention, the asthma is a Th2-mediated asthma. In some embodiments of the invention, the administration is carried out intravenously. In some embodiments of the invention, the PD-1H agonist is a monoclonal antibody against PD-1H.

Using a PD-1H deficient mouse strain and recombinant PD-1H-Ig fusion protein, we showed that PD-1 deficiency or blocking PD-1H engagement with its ligand by soluble PD-1H led to vastly increased accumulation of infiltrating inflammation in airway with eosinophils as major cell components. BALF contains elevated levels of Th2-type cytokines including IL-5 and IL-13 as well as innate inflammatory cytokine MCP-1 and IL-6, as well as increased Th2-like CD4⁺ T cells and decreased Treg, supporting that PD-1H could function as a negative regulator in the control of airway inflammation in experimental asthma progression.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1. PD-1H KO mice developed severe lung inflammation and mucus secretion in OVA-induced asthma model. (a) Experimental protocol to induce experimental asthma: WT and PD-1H KO mice were immunized by i.p. injection of 20µg OVA mixed with 4mg aluminum hydroxide gel on day 0 and 5, then challenged with 1% OVA (15ml/20min/ day) on 3 consecutive days (12-14) by aerosol instillation. Mice were sacrificed for analyses on day 15. (b, c) BALF was collected and stained for leukocyte counts. (d) H&E staining of lung paraffin sections. (e) PAS staining of lung paraffin sections for mucus-secreting goblet cells. Scale bars in figure 1b, 1d and 1e were 100 µm. *P<0.05 and **P<0.01 (two-tailed Student's t-test). All values are expressed as the means±s.e.m. N=8/group. All experiments were repeated at least 3 times.

Figure 2. PD-1H-Ig promotes airway inflammation in OVA-induced asthma. Allergic asthma was induced by OVA as described in Figure 1a. WT mice were i.v. injected with 20ug PD-1H-Ig plasmid in 2ml PBS through tail on day -1, 4 and 11. Flag-Ig plasmid was used as the control. BALF was collected after the last OVA challenge and stained **(a)** for leukocyte counts **(b)**. Scale bar in figure 2a was 100 μ m. * $P < 0.05$ and *** $P < 0.001$ (two-tailed Student's t-test). All values are expressed as the means $\pm$ s.e.m. N=8/group. All experiments were repeated at least 3 times.

Figure 3. Blockade of PD-1H enhanced innate and Th2- type cytokine production in OVA-induced asthma. **(a)** BALF from WT and PD-1H KO mice was collected, indicate cytokines were assessed by ELISA or CBA (N=8/group). **(b)** Sera from the WT and KO mice was collected and OVA-specific IgE was measured by ELISA (N=8/group). **(c)** BALF from mice treated by the PD-1H-Ig or the flag plasmid was collected, indicate cytokines were assessed by ELISA (N=8/group). **(d)** The percentage of IL-4-producing and IFN- γ -producing CD4⁺ T cells in the WT and KO mice in OVA-induced asthma. Lung lymphocytes isolated after the last OVA challenge were stimulated with PMA and ionomycin for 4 hrs in the presence of GolgiStop with brefeldin A. IL-4 and IFN- γ were measured by intracellular staining (N=8/group). **(e)** The percentage of IL-4-producing and IFN- γ -producing CD4⁺ T cells in the Wild mice treated with PD-1H-Ig or Flag-Ig plasmids in OVA-induced asthma were analyzed (N=8/group). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and **** $P < 0.0001$ (two-tailed Student's t-test). All values are expressed as the means $\pm$ s.e.m. All experiments were repeated at least 3 times.

Figure 4. Decreased Treg during experimental asthma induction in PD-1H KO mice. **(a)** Lung lymphocytes and **(b)** spleens from the WT and KO mice were collected after the last OVA inhalation, percentage of Foxp3⁺CD4⁺ T cells were analyzed by intracellular staining. ** $P < 0.01$ and **** $P < 0.0001$ (two-tailed Student's t-test). All values are expressed as the means $\pm$ s.e.m. N=8/group. All experiments were repeated at least 3 times.

Figure 5. 4C11 monoclonal antibody and its role in suppressing airway inflammation. **(a)** PD-1H⁺ CHO cells were stained with mIgG, mam82 and 4C11 and analyzed by flow cytometry. **(b, c)** Airway inflammation and asthma were induced as described in Figure 1a, but mice were challenged with 1% OVA (25ml/40min/ day) on 3 consecutive days (12-14). Meanwhile, mice were treated by i.p. injection with 200 μ g 4C11 or control mIgG in 200 μ l PBS on day 0, 3, 6, 9 and 12. BALF was collected after the last challenge and stained for leukocyte counts. **(d)** H&E staining of lung paraffin sections. **(e)** PAS staining of lung paraffin sections for mucus-secreting goblet cells. Scale bars in 5b, 5d and 5e were 100 μ m). * $P < 0.05$ and ** $P < 0.01$ (two-tailed Student's t-test). All values are expressed as the means $\pm$ s.e.m. N=8/group. All experiments were repeated at least 3 times.

Figure 6. Mice at groups of 3 were treated with the plasmid encoding murine PD-1HIg fusion protein by hydrodynamic injection as described previously and sera were sampled daily up to 13 days. The

levels of PD-1Hlg fusion protein at ng/ml in sera were determined in 3 individual mouse by specific sandwich ELISA with an anti-murine PD-1H mAb MH5A and an anti-human Ig mAb.

Figure 7. The percentage of CD3⁺CD4⁺T and CD3⁺CD8⁺T cells in lung lymphocytes and spleens of WT and KO mice in OVA-induced asthma were numerated by flow cytometry analysis (N=8/group).

*P<0.05, (two-tailed Student's t-test). All values are expressed as the means±s.e.m. All experiments were repeated at least 3 times.

DETAILED DESCRIPTION

Definitions

As used herein, the terms “treat” or “treatment” refer to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) an undesired physiological change or disorder, such as the progression of cancer. Beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilized (*i.e.*, not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. “Treatment” can also mean prolonging survival as compared to expected survival if not receiving treatment. Those in need of treatment include those already with the condition or disorder as well as those prone to have the condition or disorder or those in which the condition or disorder is to be prevented.

By “subject” or “individual” or “animal” or “patient” or “mammal,” is meant any subject, particularly a mammalian subject, for whom diagnosis, prognosis, or therapy is desired. Mammalian subjects include humans, domestic animals, farm animals, and zoo, sport, or pet animals such as dogs, cats, guinea pigs, rabbits, rats, mice, horses, cattle, cows, and so on. The subject herein is preferably a human.

As used herein, phrases such as “to a patient in need of treatment” or “a subject in need of treatment” includes subjects, such as mammalian subjects, that would benefit from administration of an antibody or composition of the present disclosure used, *e.g.*, for detection, for a diagnostic procedure and/or for treatment.

As used herein, the term “agonist” refers to any agent that increases the level and/or activity of PD-1H. As used herein, the term “agonist” refers to an agent which increases the expression and/or activity of the PD-1H by at least 10% or more, *e.g.* by 10% or more, 50% or more, 100% or more, 200% or more, 500% or more, or 1000% or more. Non-limiting examples of agonists of PD-1H can include PD-1H polypeptides or agonist fragments thereof and nucleic acids encoding a PD-1H polypeptide.

Methods and Therapies

An aspect of the disclosure provides a method for prophylaxis or treatment of asthma in a subject comprising administering to the subject in need thereof a therapeutically effective amount of a PD-1H agonist, or a pharmaceutical composition comprising the PD-1H agonist. Equally, the disclosure provides the PD-1H agonist as described above for use in a method for treating or alleviating symptoms involved with asthma.

In certain embodiments, the PD-1H agonist or the pharmaceutical composition is administered parenterally, e.g. intravenously, intramuscularly, percutaneously or intracutaneously.

In some embodiments, it may be desirable to combine a PD-1H agonist with other agents effective in the treatment of asthma. For example, the treatment of asthma may be implemented with a PD-1H agonist and other anti-asthma therapies, such as β_2 receptor agonists available in the market.

In certain embodiments, the methods of treating asthma prevent the progression of the infection and/or the onset of disease caused by asthma. Thus, in some embodiments, a method for preventing the progression of asthma and/or the onset of disease caused by asthma, comprises administering an effective amount of a PD-1H agonist to a subject in need thereof. In certain embodiments, the methods of treating asthma prevent the onset, progression and/or recurrence of a symptom associated with asthma. Thus, in some embodiments, a method for preventing a symptom associated with asthma in a subject, comprises administering an effective amount of a PD-1H agonist to a subject in need thereof.

Compositions

An aspect of the present invention provides a pharmaceutical composition comprising a therapeutically effective amount of PD-1H agonist and a pharmaceutically acceptable carrier. The pharmaceutical composition is useful for prophylaxis or treatment of asthma in a subject. The PD-1H agonist may be prepared in a suitable pharmaceutically acceptable carrier or excipient.

As used herein, "carrier" includes any and all solvents, dispersion media, vehicles, coatings, diluents, antibacterial and antifungal agents, isotonic and absorption delaying agents, buffers, carrier solutions, suspensions, colloids, and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

The phrase "pharmaceutically acceptable" refers to molecular entities and compositions that do not produce an allergic or similar untoward reaction when administered to a human. The preparation of

an aqueous composition that contains a protein as an active ingredient is well understood in the art. Typically, such compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection can also be prepared.

Examples

Materials and Methods

Mice. All experiments were carried out in accordance with the guidelines of Sun Yat-sen University on animal care and the ethical guidelines for investigation of experimental animals. BALB/c mice were purchased from the Experimental Animal Center of Sun Yat-sen University (Guangzhou, China). PD-1H KO mice were described previously and were backcrossed with BALB/c mice to generate H-2^d/PD-1H KO strain. Mice in 8-10 weeks old were used for all experiments and kept under specific pathogen-free conditions.

Generation of PD-1H mAb. Full-length mouse PD-1H-pcDNA3.1 was stably transfected into Chinese hamster ovary cells (CHO cells) by lipofection and the stably transfected PD-1H⁺ CHO cell was confirmed by flow cytometry using mam82 mAb. PD-1H-Ig fusion protein was produced and purified as described. A PD-1H KO mouse was immunized with mouse PD-1H-Ig and the generation of hybridomas secreting PD-1H mAb were performed as described previously. The specificity of the mAb were validated by ELISA and flow cytometry using PD-1H⁺ CHO cell. A clone of PD-1H mAb, 4C11, was selected for further experiments and produced and purified.

Mouse model of experimental asthma. Mice were sensitized by intraperitoneal (i.p.) injection of 10µg OVA(Sigma-Aldrich) with 4mg aluminum hydroxide (Thermo Fisher) gel on days 0 and 5, then challenged with aerosolized 15 or 25 ml 1% OVA for 20 or 40min on days 12,13, and 14. The aerosol was generated by a nebulizer (NE-U07; Yuyue). Mice were sacrificed for analyses on day 15. For PD-1H-Ig treatment, groups of mice received hydrodynamic injections intravenously (i.v.) of 20 µg PD-1H plasmid in 2ml PBS on days -1, 4 and 11 and the Flag plasmid was the control. The levels of PD-1HIg fusion protein in the sera were detected by a specific sandwich ELISA method (Figure 6). For mAb treatment, groups of mice received i.p. injections of 200 µg anti-mouse PD-1H mAb (4C11), on days 0, 3, 6, 9, and 12, or control mouse immunoglobulin G (mIgG) (Rockland).

Assays of bronchoalveolar lavage fluid (BALF) and sera. Mice were anesthetized with a lethal dose of pentobarbital and lungs were gently lavaged with 0.5 ml PBS for three times (1.5ml of total BALF) via a tracheal cannula. Samples were centrifuged at 2000 rpm for 5 min. Mouse IL-4, IL-5 and IL-13 in BALF were quantified by ELISA Kits (eBioscience) according to the manufacturer's protocols, MCP-1 and IL-6 were measured by CBA Kit (BD Bioscience). The total counts of cells in BALF were determined using a microscope. To identify BALF differential cell counts, BALF cells were spun onto microscope slides by CytoFuge and stained with Diff-Quick (Nanjing Jiancheng Bioengineering

Institute), differential cell counts were performed by counting 400 cells per slides using a high-magnification microscope. Sera from experimental mice were collected and OVA-specific IgE was examined by specific sandwich ELISA.

Histology. After bronchial lavage procedure, lungs were exsanguinated and fixed by intratracheal instillation with 1ml 10% formalin and subsequently removed from mice. After successive dehydration, the lungs were embedded in paraffin and sectioned, subsequently stained with H&E and Periodic Acid-Schiff (PAS).

Flow cytometric analysis. Cells ($0.5-1 \times 10^6$) were first incubated with unlabeled anti-FcR mAb to block nonspecific binding of mAbs to FcR, and then incubated with labeled mAbs. The mAbs against H-2K^d, H-2K^b, mIgG, CD3, CD4, CD8, Siglec-F, IL-4, IFN- γ and Foxp3 were purchased from eBioscience. For intracellular Foxp3 staining, lymphocytes from lungs and spleens were isolated as described and cells were subsequently fixed, permeabilized and stained following the manufacturer's protocol of Cytofix/Cytoperm Plus kit (BD Biosciences). For intracellular IL-4 and IFN- γ staining, isolated lymphocytes were stimulated by 10 ng/ml PMA (Sigma-Aldrich), 1 μ g/ml ionomycin (Sigma-Aldrich) and Golgi plug (BD Biosciences) for 4hrs and stained with Cytofix/Cytoperm Plus kit (BD Biosciences).

Statistical analysis. Data were analyzed with GraphPad Prism 6 (GraphPad Software). An unpaired Student's *t* test was used to determine statistical significance between groups, with $p \leq 0.05$ being considered significant.

Results

PD-1H is required for suppressing airway inflammation in experimental asthma model

WT and PD-1H KO mice were immunized and challenged by OVA to induce asthma (**Figure 1a**). Mice were sacrificed at day 15 and differential cell counts were performed in harvested BALF. The challenge by OVA-induced pulmonary infiltration of inflammatory cells in WT mice and inflammatory cells were dominated by eosinophils, also with increased lymphocytes and macrophages. This prominent eosinophilic response is highly characteristic of allergic asthma in this model. Interestingly, PD-1H KO mice had significantly higher total numbers of inflammatory cells in BALF than WT mice, with significant increases of eosinophils. Infiltrating lymphocytes were in a small degree and macrophages were negligible (**Figure 1b and c**). These findings indicate that PD-1H is required for inhibiting recruitment of inflammatory cells to the lung in this model.

We then compared lung histology of OVA-challenged WT and KO mice. Compared with WT mice, OVA-challenged KO mice showed substantially more inflammatory cell infiltration mainly around bronchi (**Figure 1d**). At the same time, OVA-challenged KO mice showed significantly larger and more PAS⁺ mucin-producing goblet cells lining the bronchial air spaces (**Figure 1e**), accompanied

with profound goblet cell hyperplasia and metaplasia, which indicate primarily bronchial involvement and extensive airway remodeling during inflammation. These results demonstrate substantially more severe histopathologic changes characteristic of inflammatory asthma in OVA-challenged lungs in KO than in WT mice. Taken together, our results indicate that endogenous PD-1H suppressed the development of severe inflammatory cell infiltration and airway remodeling following OVA challenge.

To validate our findings, we also tested the effect of PD-1HIg, a fusion protein of extracellular domain of PD-1H and IgG2a Fc portion. In our preliminary experiments, we found that high pressure injection of PD-1HIg plasmid (hydrodynamic injection) led to high level expression of the PD-1HIg fusion protein (Fig. 6) as detected in mouse sera and this method had more profound effect than the infusion of purified recombinant PD-1HIg fusion protein. In the beginning of OVA-induced asthma model, mice were injected i.v. with PD-1H-Ig or Flag-Ig control plasmids via tail veins in high pressure on day -1, 4 and 11. Mice were sacrificed at day 15 and the BALF was collected. Similar to PD-1HKO mice, PD-1H-Ig-treated mice showed significantly increased eosinophil counts than that of mice treated with Flag-Ig plasmid control (**Figure 2a and b**), indicating PD-1H-Ig may block PD-1H to interact with its counter-receptor to promote airway inflammation in OVA-induced asthma. Our results thus further support a suppressive role of endogenous PD-1H in the development of lung inflammation during the induction of experimental asthma.

Mechanisms of PD-1H mediated suppression of airway inflammation

We first determined the levels of cytokines in the BALF in OVA-challenged WT and PD-1H KO mice. The KO mice challenged with OVA had significantly higher levels of IL-5, and IL-13, IL-6, MCP-1 and TNF α in the BALF than WT mice while the changes of IL-4 and IFN- γ were insignificant (**Figure 3a**). The OVA-specific IgE in sera was measured as well, but no difference was observed (**Figure 3b**). These results are consistent with the cell counts and histopathological findings, showing increased lung inflammation biased to eosinophil recruitment in the KO mice. Similar results were also obtained in the WT mice treated by hydrodynamic inoculation of PD-1H-Ig plasmid with significantly higher levels of IL-5 and IL-13 than the mice treated with the control plasmid (**Figure 3c**). Interestingly, treatment by PD-1H-Ig led to significantly increased IL-4 in BALF (**Figure 3d**). Our results indicate that endogenous PD-1H inhibits production of innate (MCP-1, TNF α , IL-6) and Th2-like cytokines (IL-5, IL-13) during the induction of airway inflammation.

We also determine CD4⁺ T helper cell types in lung lymphocytes upon a brief PMA/ionomycin stimulation in vitro by intracellular staining of cytokines. CD4⁺IL-4⁺ Th2-type cells were significantly higher in KO than that of WT mice in the BALF during the asthma induction. In contrast, the Th1-type cells (CD4⁺IFN- γ ⁺) were significantly less in the KO than that of WT mice (**Figure 3e**). Similar results were also observed in the WT mice treated with PD-1H-Ig vs. control Ig plasmids (**Figure 3f**). These results of intracellular cytokine detection further validate previous findings in

extracellular cytokine measurement in the BALF and support the role of endogenous PD-1H in the suppression of CD4⁺Th2-like responses in the experimental asthma model.

We showed previously that PD-1H promoted Treg expansion in the induction of graft versus host diseases (GVHD) in several mouse models. We next evaluated if there is impaired generation of Treg during the induction of airway inflammatory response in the absence of PD-1H. CD4⁺Foxp3⁺ Treg in the lungs and spleens were evaluated by flow cytometry immediately upon the last OVA challenge of WT and KO mice. The percentage of Treg was significantly lower in both lungs (**Figure 4a**) and spleens (**Figure 4b**) in the KO mice compared with WT mice. Our results suggest a possible contribution of Treg in PD-1H mediated suppression of airway inflammation. Therefore, PD-1H may operate via multiple mechanisms to suppress airway inflammation.

Amelioration of experimental asthma by a new agonistic PD-1H antibody

In the context of the roles of PD-1H in the suppression of airway inflammation, we explore if PD-1H could be targeted to suppress asthma in the experimental model. We reported several agonist mAbs (clones MH5A, mam82) with suppressive function in GVHD in mouse models. In our preliminary studies, these mAbs, however, are less consistent in suppressing airway inflammation in the OVA-induced asthma model (data not shown). We generated additional mAbs by immunization of PD-1H KO mice with PD-1H-Ig. One mAb, designated 4C11, was selected based on its specificity and high affinity for murine PD-1H. The mAb 4C11 reacted strongly with PD-1H⁺ CHO cells in a similar fashion to clone mam82 (**Figure 5a**) and this binding could be blocked by the inclusion of PD-1H-Ig (data not shown).

To test the effect of 4C11, mice were administered with 4C11 or control mIgG on day 0, 3, 6, 9 and 12 during the induction of OVA-induced asthma. Mice treated with control IgG developed a typical accumulation of eosinophils in BALF fluid (**Figure 5b and 5c**). The histopathological examination showed massive inflammatory cell infiltration around the bronchi (**Figure 5d**) and mucus overproduction into the bronchi (**Figure 5e**). In contrast, accumulation of eosinophils in BALF was greatly reduced in the 4C11-treated mice (**Figure 5b and 5c**). Meanwhile, infiltration of inflammatory cells around the bronchi (**Figure 5d**) and overproduction of mucus (**Figure 5e**) also decreased significantly. These results indicate agonistic PD-1H mAb 4C11 can suppress airway inflammation and asthma development and imply that PD-1H could be a potential therapeutic target for asthma treatment.

In OVA-induced experimental asthma model, PD-1H appears to mainly suppress IL-5 and IL-13 production while IL-4 was less altered (**Figure 3**). These findings are consistent to the observation that the majority of accumulated leukocytes in airway are eosinophils but not lymphocytes. IL-5 was originally defined as a T-cell-derived cytokine and now appreciated as a major cytokine to affect many aspects of eosinophils including maturation, differentiation, migration and survival. IL-13 can be produced by various immune and non-immune cells including T cells and eosinophils

and could induce airway eosinophilia, airway hyper-responsiveness, and mucus overproduction. Although IL-4 appears to be less affected by loss of PD-1H, the effect of IL-4 may be replaced by high level of IL-13 because IL-13 and IL-4 share a common α chain receptor subunit as their receptors and have overlapping biological functions. PD-1H is constitutively found on the surface of naïve T lymphocytes whereas its expression on eosinophils is not yet reported. In our experiments, however, inflammatory eosinophils isolated from BALF do not express PD-1H. Because both IL-5 and IL-13 could be produced by T cells, it is thus possible that PD-1H on T cells may directly mediate suppression of cytokine production. On the other hand, IL-13 could be produced by various hematopoietic cells while PD-1H is also broadly expressed on the majority of hematopoietic cells. It is thus tempting to speculate that PD-1H may have a more broad suppressive function beyond T cells for IL-13 production from hematopoietic cells in addition to T cells and eosinophils.

Both naturally occurring thymus-derived CD4⁺CD25⁺Foxp3⁺ Tregs and inducible population of Tregs suppress the development of allergies including allergic asthma through multiple mechanisms and the inhibition of other effector Th1, Th2, and Th17 cells, eosinophils, basophils, mast cells, inflammatory DCs and inflammatory cell migration to tissues. We showed previously that an agonist mAb MH5A for mouse PD-1H selectively promotes Treg cell expansion in murine GVHD models. Our results showed insignificant differences in percentage of CD4⁺ and CD8⁺T cells between WT and KO mice in both lung and spleens during experimental asthma induction (**Figure 7**). However, PD-1H deficiency led to dramatically reduced percentage of CD4⁺ Foxp3⁺ Tregs in lungs compared with WT mice, proportion of Tregs in spleens was reduced as well during the airway inflammation (**Figure 4**). These findings may partially explain more severe asthma pathogenesis in PD-1H KO mice.

The administrations of several PD-1H agonist mAbs could suppress acute hepatitis and GVHD in animal models. By a newly generated mAb 4C11, the treatment could remarkably decrease the number of inflammatory cells in BALF, especially the eosinophils and lymphocytes, as well as pulmonary inflammation and mucus production (**Fig. 5**). Our results thus implicate the possibility to target PD-1H for the treatment of allergic diseases. In our early attempt to treat the experimental asthma model with MH5A and mam82 agonistic mAb, however, the effect was inconsistent among different experiments (data not shown). Currently the reason behind different effect of these PD-1H agonistic mAb is yet to be investigated. It is possible that these mAbs may interact with PD-1H on different functional domains and subsequently trigger signals which are different in quantity or in quality, leading to different biological outcomes. Nevertheless, our results suggest a possible strategy for the treatment of allergic asthma by agonistic mAb against PD-1H.

It should be understood that although the present disclosure has been specifically disclosed by preferred embodiments and optional features, modification, improvement and variation of the disclosures embodied therein herein disclosed may be resorted to by those skilled in the art, and that such modifications, improvements and variations are considered to be within the scope of this

disclosure. The materials, methods, and examples provided here are representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the disclosure.

The disclosure has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the disclosure. This includes the generic description of the disclosure with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein. In addition, where features or aspects of the disclosure are described in terms of Markush groups, those skilled in the art will recognize that the disclosure is also thereby described in terms of any individual member or subgroup of members of the Markush group.

All publications, patent applications, patents, and other references mentioned herein are expressly incorporated by reference in their entirety, to the same extent as if each were incorporated by reference individually. In case of conflict, the present specification, including definitions, will control. The disclosures illustratively described herein may suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the terms “comprising,” “including,” “containing,” etc. shall be read expansively and without limitation. Additionally, the terms and expressions employed herein have been used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the disclosure claimed.

CLAIMS

1. A method for prophylaxis or treatment of asthma in a subject comprising administering to the subject in need thereof a therapeutically effective amount of a PD-1H agonist.
2. The method of claim 1, wherein the asthma is a Th2-mediated asthma.
3. The method of claim 1, wherein the administration is carried out intravenously.
4. The method of claim 1, wherein the PD-1H agonist is a monoclonal antibody against PD-1H.
5. The method of claim 1, wherein the subject is a human being.
6. Use of a PD-1H agonist in the preparation of a pharmaceutical composition for prophylaxis or treatment of asthma in a subject.
7. The use of claim 6, wherein the asthma is a Th2-mediated asthma.
8. The use of claim 6, wherein the PD-1H agonist is a monoclonal antibody against PD-1H.
9. The use of claim 6, wherein the subject is a human being.

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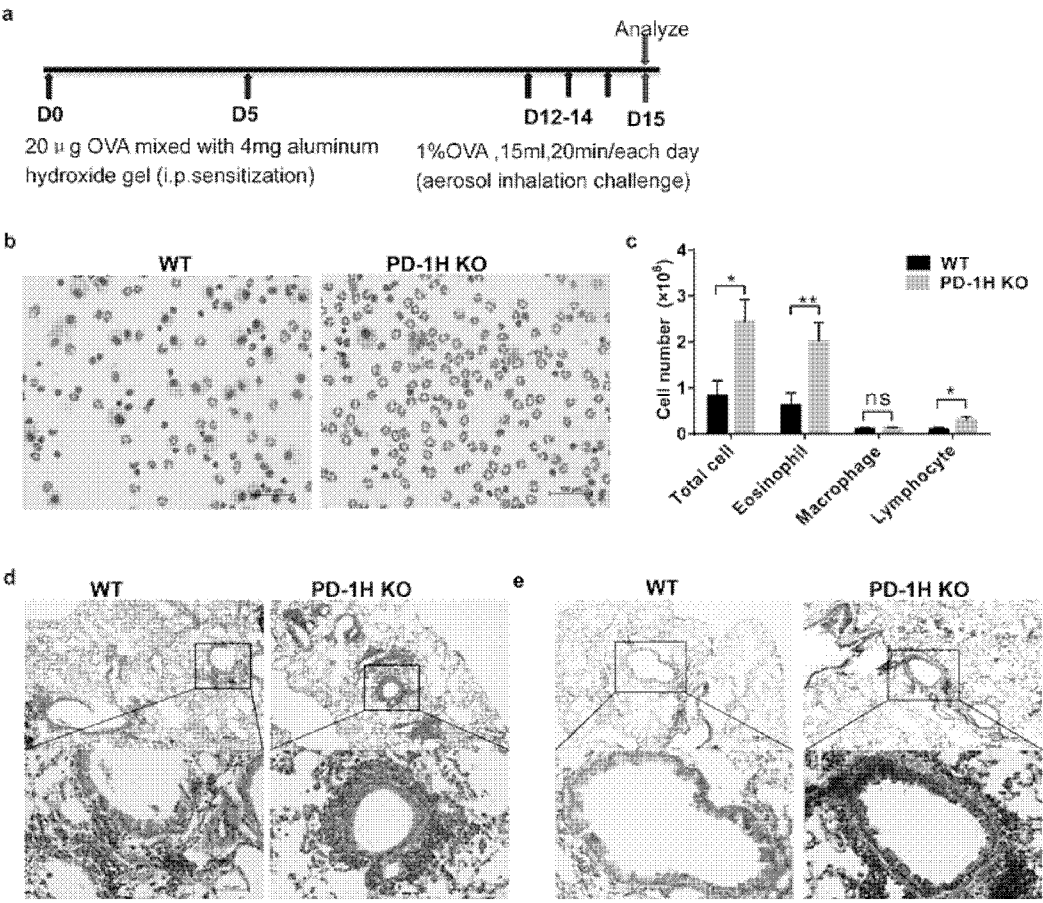


Fig. 1

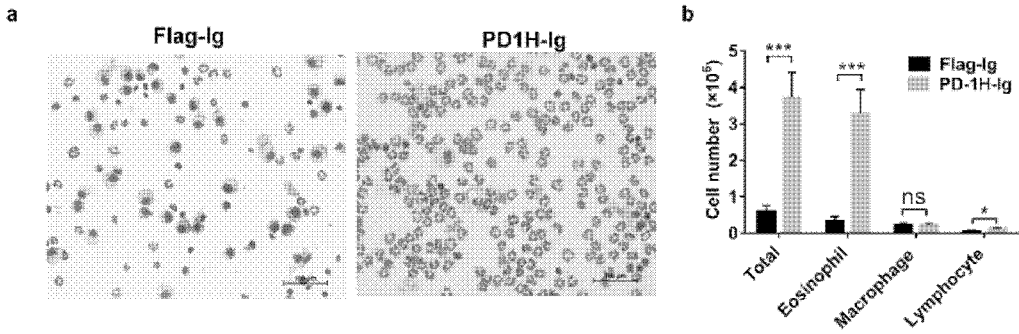


Fig. 2

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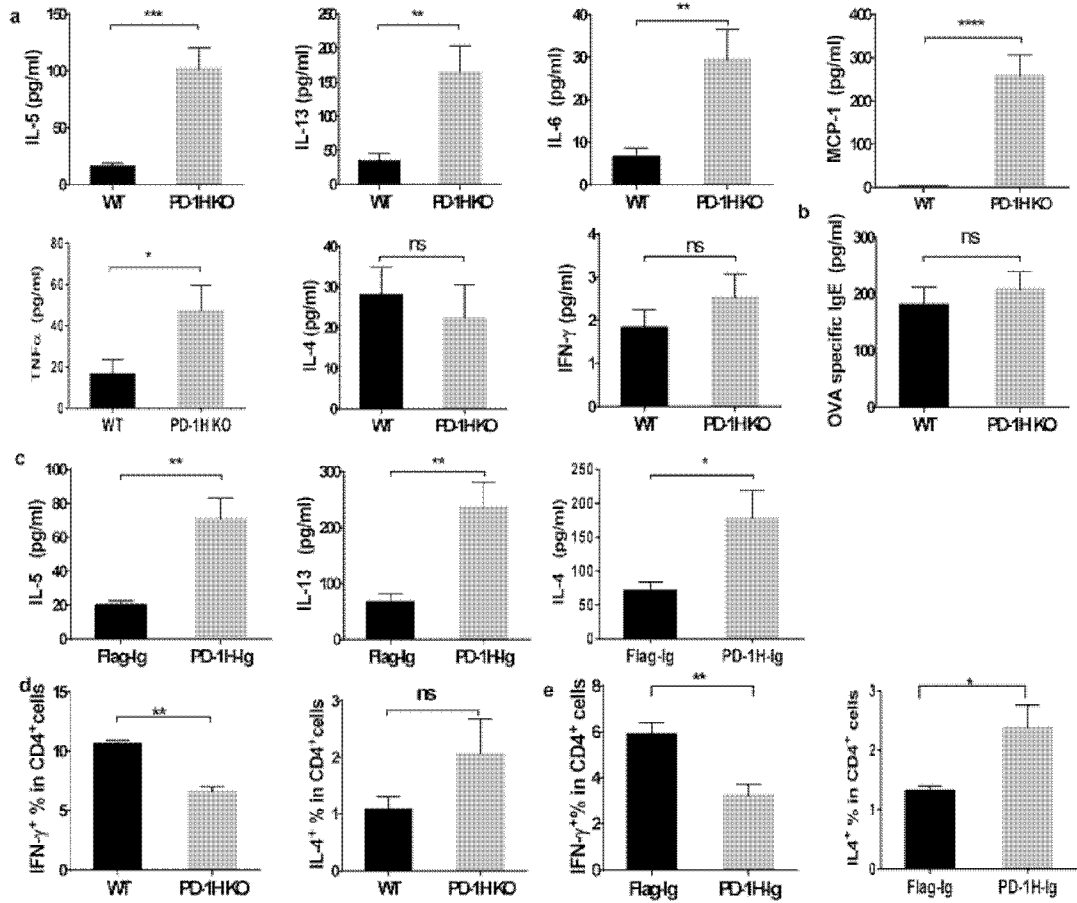


Fig. 3

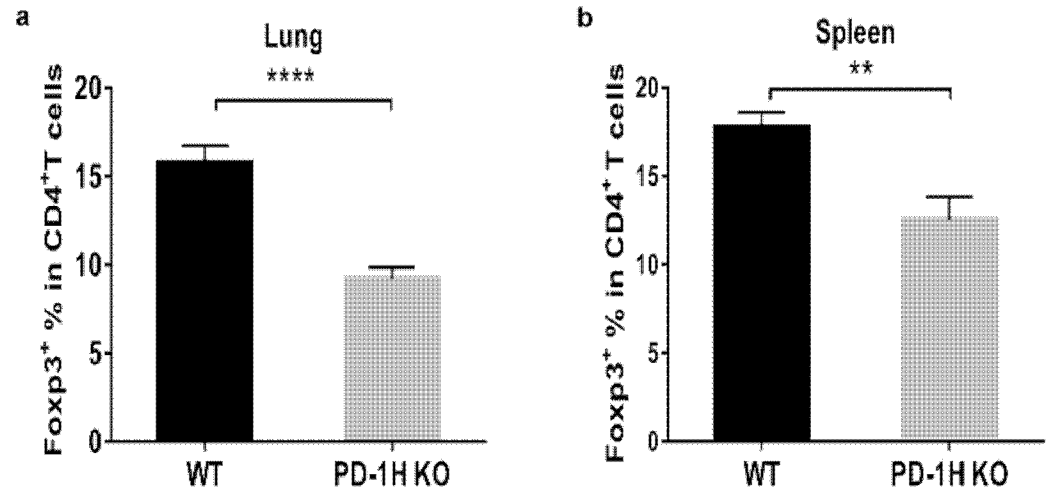


Fig. 4

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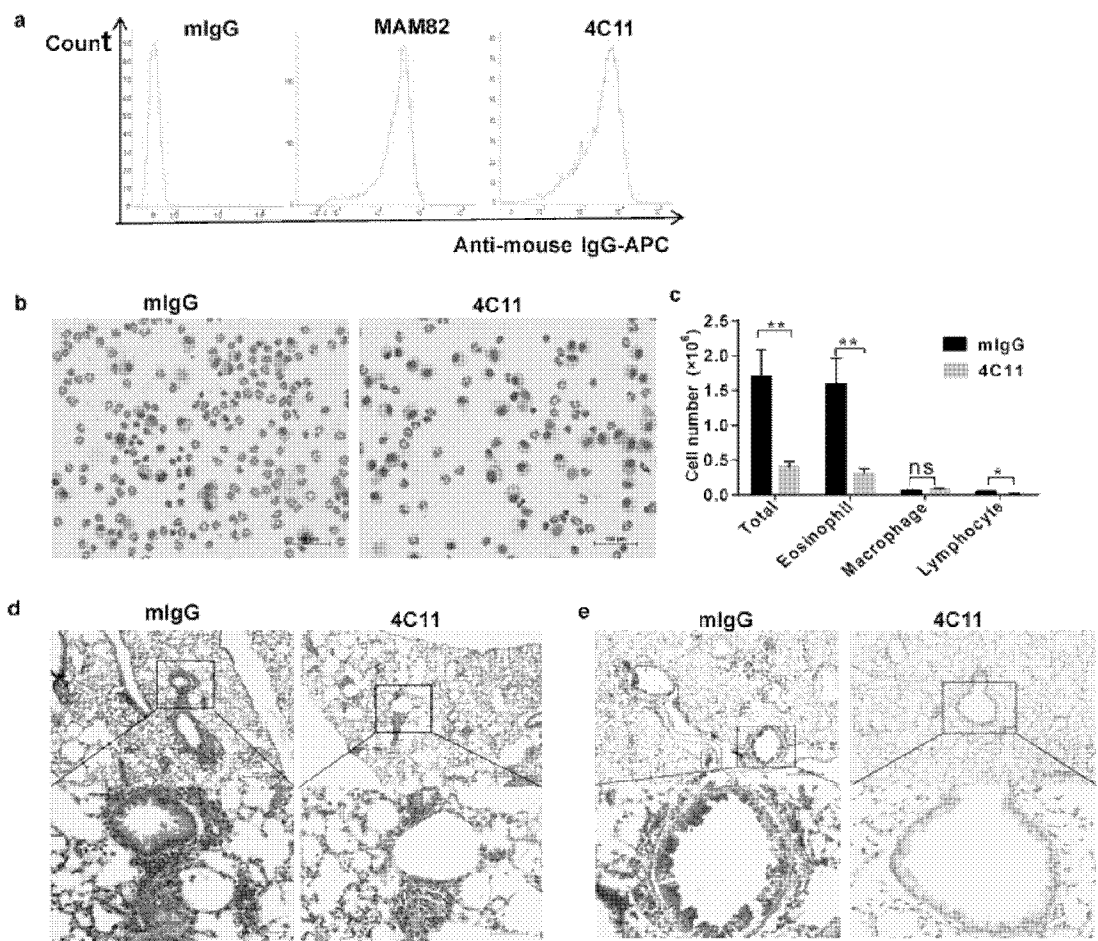


Fig. 5

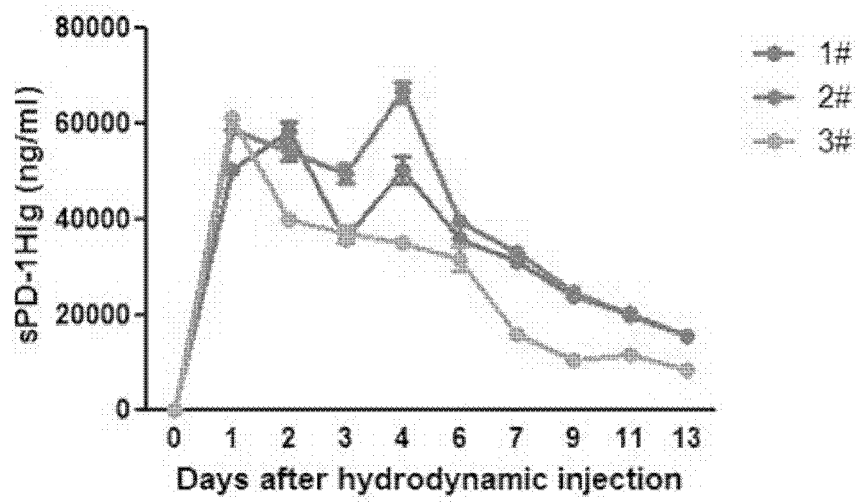


Fig. 6

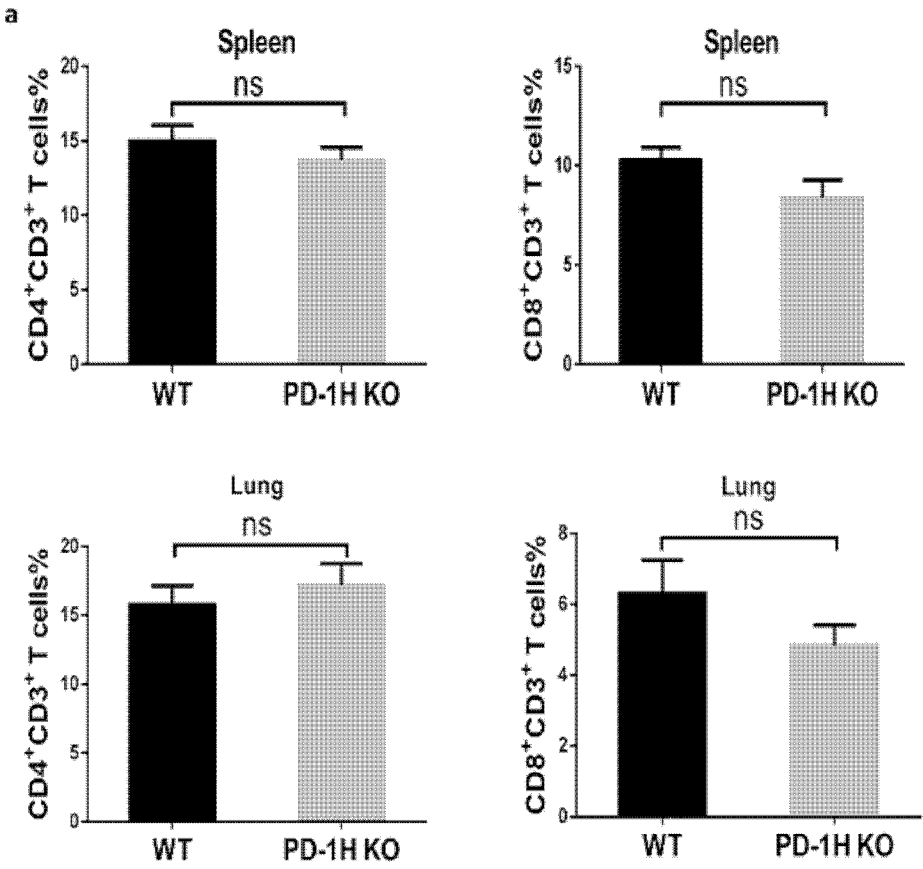


Fig. 7

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2017/081844

A. CLASSIFICATION OF SUBJECT MATTER

A61K 39/395(2006.01)i; A61P 11/06(2006.01)i

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K, A61P

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CNABS,SIPOABS,DWPI,CNXTXT,EPTXT,USTXT,WOTXT,CNKI,WANFANG,EBI,NCBI,GOOGLE,BAIDU,ISI Web of Knowledge,PUBMED: SUN YAT-SEN UNIVERSITY,CHEN,Lieping,LIU, Huafeng, prophylaxis ,treatment , asthma,PD-1 H, agonist, monoclonal antibody,Th2-mediated asthma,Th2,airway inflammation, inflammation,Gi24,VISTA,DD1 α ,Dies1,B7-H5, DD1,agonistic antibody

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CN 104619722 A (TRUSTEES OF DARTMOUTH COLLEGE ET AL.) 13 May 2015 (2015-05-13) claims 107-122, 144-148, 182, 193-198, description, paragraph[0616], the example 35.	1-9
X	CN 103119054 A (TRUSTEES OF DARTMOUTH COLLEGE) 22 May 2013 (2013-05-22) claims 1-52	1-9
A	CA 2913312 A1 (MEDIMMUNE LLC ET AL.) 27 November 2014 (2014-11-27) the whole document	1-9
A	CN 105246507 A (TRUSTEES OF DARTMOUTH COLLEGE ET AL.) 13 January 2016 (2016-01-13) the whole document	1-9
A	WO 2015179799 A1 (THE GENERAL HOSPITAL CORPORATION) 26 November 2015 (2015-11-26) the whole document	1-9



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

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

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

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

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

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

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

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

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

“&” document member of the same patent family

Date of the actual completion of the international search

08 January 2018

Date of mailing of the international search report

17 January 2018

Name and mailing address of the ISA/CN

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Telephone No. (86-10)82246978

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2017/081844

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2017066561 A2 (PRESIDENT AND FELLOWS OF HARVARD COLLEGE) 20 April 2017 (2017-04-20) the whole document	1-9

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2017/081844

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.: **1-5**
because they relate to subject matter not required to be searched by this Authority, namely:

[1] The subject matter of claims 1-5 relates to a method of treating a subject having a disease, and therefore does not warrant an international search according to the criteria set out in Rule 39.1(iv). However, the search has been carried out and based on the use of PD-1H agonist for manufacturing of a medicament.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/CN2017/081844

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
CN	104619722	A	13 May 2015	CA	2877533	A1	27 December 2013
				JP	2015526064	A	10 September 2015
				AU	2013277051	A1	12 February 2015
				TW	201412775	A	01 April 2014
				EP	2864352	A4	25 May 2016
				US	2014220012	A1	07 August 2014
				HK	1214272	A1	22 July 2016
				WO	2013192504	A1	27 December 2013
				EP	2864352	A1	29 April 2015
CN	103119054	A	22 May 2013	KR	20130010906	A	29 January 2013
				AU	2011230537	B2	07 January 2016
				US	9631018	B2	25 April 2017
				NZ	602634	A	26 June 2015
				WO	2011120013	A2	29 September 2011
				MX	2012011089	A	28 June 2013
				EP	2552947	A2	06 February 2013
				WO	2011120013	A3	03 May 2012
				JP	2013527144	A	27 June 2013
				AU	2011230537	A1	29 November 2012
				JP	6034283	B2	30 November 2016
				EP	2552947	A4	13 November 2013
				JP	6175170	B2	02 August 2017
				CN	103119054	B	18 January 2017
				US	2013177557	A1	11 July 2013
				CN	107098958	A	29 August 2017
				EP	3153521	A1	12 April 2017
				JP	2016222702	A	28 December 2016
				WO	2011120013	A9	10 November 2011
				CA	2794483	A1	29 September 2011
				AU	2016202172	A1	28 April 2016
				US	2017334990	A1	23 November 2017
				MX	342017	B	09 September 2016
CA	2913312	A1	27 November 2014	CN	106414489	A	15 February 2017
				AU	2014268298	A1	21 January 2016
				EP	3004158	A2	13 April 2016
				MX	2015016111	A	26 October 2016
				US	2016096891	A1	07 April 2016
				WO	2014190356	A3	23 April 2015
				WO	2014190356	A2	27 November 2014
				SG	11201509618Q	A	30 December 2015
				KR	20160129698	A	09 November 2016
				JP	2016527187	A	08 September 2016
CN	105246507	A	13 January 2016	EP	2892558	A4	20 April 2016
				JP	2015533796	A	26 November 2015
				WO	2014039983	A1	13 March 2014
				US	2017112929	A1	27 April 2017
				CA	2884704	A1	13 March 2014
				US	2014105912	A1	17 April 2014
				US	9381244	B2	05 July 2016
				AU	2013312211	A1	16 April 2015

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/CN2017/081844

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
				EP	2892558	A1	15 July 2015
WO	2015179799	A1	26 November 2015	US	2017184604	A1	29 June 2017
WO	2017066561	A2	20 April 2017	WO	2017066561	A3	08 June 2017