

## Is the H4 receptor a new drug target for allergies and asthma?

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## 1. ABSTRACT

Histamine H4 receptor (H4R) has become a promising target for immuno-inflammatory diseases, such as allergic rhinitis, asthma or dermal allergies. Its distribution pattern in immune cells and the preclinical data obtained from different biological systems using diverse histamine H4 modulators (1) suggest a key role of H4R in immunity and in inflammatory cell trafficking. Recent results with UR-63325, the first H4R antagonist from which clinical data has been reported (2), confirm the feasibility of complete H4R blocking in humans without limiting safety concerns. Also, H4R blockade results in clear pharmacodynamic effects in relevant human cells, e.g. eosinophils. It is believed that allergic rhinitis and asthma are manifestations of one unique syndrome in two parts of the respiratory tract. Dermal allergies are also recognized as related manifestations in a different location. The coexistence of allergic-related diseases in the same patients could permit a single treatment approach e.g., the systemic use of H4R antagonists. Further clinical studies are needed to establish the role of H4R antagonists in the treatment of allergic diseases.

## 2. INTRODUCTION

### 2.1. Allergies and asthma

Allergic inflammatory diseases include a wide range of pathologies, characterized by excessive activation of different immune cells, mainly mast cells and eosinophils, resulting in an inflammatory response. Significant entities include allergic rhinitis asthma, eczema, urticaria and food allergies (1).

Allergic rhinitis is the most common form of non-infectious rhinitis and is associated with an IgE-mediated immune response against allergens. Main symptoms in nasal reactions occurring in allergy are sneezing, nasal obstruction and mucous discharge. Allergic rhinitis does not only affect the nose, but it is often associated with ocular symptoms. Allergic rhinitis is a global health problem that causes major illness and disability worldwide. Also, its economic impact is substantial and often underestimated (3). Asthma is characterized by a chronic inflammation of the airways in susceptible individuals, with recurrent episodes of wheezing, breathlessness, chest tightness and cough, particularly at night and/or in the early

morning. These episodes are usually associated with widespread but variable airflow obstruction that is often reversible either spontaneously or with treatment. The inflammation also causes an associated increase in the existing bronchial hyperresponsiveness to a variety of stimuli (3). Epidemiologic studies have consistently shown that asthma and rhinitis often co-exist in the same patients (3,4). The prevalence of asthma in subjects without rhinitis is usually less than 2%, whereas the prevalence in patients with rhinitis varies from 10% to 40%. Patients with moderate/severe persistent rhinitis may be more likely to suffer from asthma than those with an intermittent and/or a milder form of the disease. There is substantial evidence of the connection of both diseases, and according to the WHO ARIA report (3) it is now believed that the two conditions are manifestations of one syndrome in two parts of the respiratory tract. In asthma and rhinitis, inflammation of the nasal and bronchial mucosa is sustained by a similar inflammatory infiltrate including eosinophils, mast cells, T-lymphocytes, and cells of the monocytic lineage. Also, similar pro-inflammatory mediators (histamine, Cys-leukotrienes), Th2 cytokines and chemokines are found. Allergen-specific IgE is a pre-requisite for the development of allergic inflammation in both allergic rhinitis and asthma; both are commonly associated with raised circulating levels of IgE, and the increased presence of total-serum IgE is a risk factor for asthma even in non-allergic individuals. However, the magnitude of inflammation may not be identical in both diseases. In patients with moderate-severe asthma, eosinophilic inflammation is more pronounced in the bronchi than in the nose, whereas in patients with mild asthma, inflammation appears to be similar in both sites. Moreover, eosinophilic inflammation of the nose exists in asthmatics with or without nasal symptoms, while this is not a feature of other bronchial diseases (3). In patients with allergic diseases, allergen provocation can activate a systemic response that provokes inflammatory cell production by the bone marrow. After the release and differentiation of progenitor cells, eosinophils, basophils and mast cells are typically recruited to tissues in atopic individuals.

Dermal allergies are common skin diseases also associated with other atopic disorders, such as allergic rhinitis and asthma (4,5). Atopic dermatitis (AD) is the most prevalent dermal allergy and has shown to be a major risk factor for the development of asthma, with an increased odds ratio in children with AD in several longitudinal studies compared with children without AD. The main risk factors for progression and persistence of asthma are early onset, IgE sensitization, and severity of AD. Approximately 70% of patients with severe AD develop asthma compared with 20-30% of patients with mild AD and about 8% in the general population (4). The hallmarks of atopic dermatitis are a chronic, relapsing form of skin inflammation, a disturbance of epidermal-barrier function that culminates in dry skin, and IgE-mediated sensitization to food and environmental allergens. The most important symptom is persistent pruritus, which impairs the patient's quality of life. The histological features of acute eczematous patches and plaques are epidermal intercellular oedema (spongiosis) and a prominent perivascular infiltrate

of lymphocytes, monocyte macrophages, dendritic cells, and a few eosinophils in the dermis. Inflammation in atopic dermatitis is biphasic with an acute and a chronic phase. The initial acute phase is associated with an increase in T-helper type 2 (Th2) cells, followed by a later chronic phase characterized by the infiltration of inflammatory dendritic epidermal cells, macrophages, and eosinophils. The interleukin (IL)-12 production by these various immune cells results in the switch from the acute Th2 phase dominated by secretion of IL-4, IL-13, and IL-5 to a Th1-type cytokine milieu associated with increasing presence of interferon (IFN)-gamma-producing Th1 cells (6). The clinical observation that pruritus in patients with AD is often not relieved by antihistamines targeting the histamine H1 receptor (H1R) or histamine H2 receptor (H2R) (7) led to the assumption that histamine is binding to other histamine receptors expressed on the immune cells taking part in AD (8).

The coexistence of tightly related conditions, i.e. allergic rhinitis, asthma and AD supports the search for a single therapeutic approach. It is now clear that this approach cannot be provided by H1 antihistamine drugs, which have repeatedly failed to show efficacy in asthma (9) and produce only partial improvement of symptoms in allergic rhinitis. Also, nasal, inhaled or dermal application of topical corticosteroids is a safe approach but intrinsically limited to provide local release, with no effects on concomitant disease. Poor patient compliance is a limitation inherent to corticosteroid topical administration and potential adverse effects, both local and systemic, remain as a cause of concern. The use of chromones and leukotriene antagonists to modify the underlying allergic mechanisms of both rhinitis and asthma has proven to be better than placebo, but worse than nasal or inhaled corticosteroids, and are useful only in a limited number of patients (10). Thus, further development in the treatment of allergic rhinitis would require approaches allowing an improvement of the efficacy ceiling with the aim of obtaining complete resolution of symptoms through convenient routes of delivery that enhance patient compliance and comfort. Thus, there is room for the development of new drugs that may etiologically address the allergic process as a whole, providing an integrated and systemic approach with efficacy in symptom resolution and prevention on both respiratory and dermal manifestations of allergy.

### 2.2. Histamine H4 receptor

Histamine was first identified in the early 1900s and recognized as a major mediator in many biological functions. Since many decades, the activation of the histamine H1 receptor (H1R) has been considered a major player in allergic processes. Despite their widespread use, H1 antagonists have shown only minor anti-inflammatory activity in allergic processes, and only at high doses (9). In fact, while clinical trials show evidence of significant symptom reductions in patients with allergic rhinitis, residual symptoms at the end of the observation period are not completely resolved, but often close to 50% of those at baseline (11). Such observations leave room for additional roles of histamine that may be mediated by different

**Table 1.** Available information for H4 modulators in clinical trials

Phase	Compound	Reference	Status
	<b>JNJ-39758979</b>		
Phase I Single and Multiple Dose	Single and Multiple Dose Study to Explore the Safety and Pharmacokinetics of JNJ-39758979 In Healthy Male Volunteers of Either Caucasian or Japanese Descent Single oral dose: 50, 100, 300, 600mg or Placebo Multiple dose: Once daily oral dose for 14 days: 300 mg	NCT01081821	Completed No results available
Proof of Concept	A Study To Investigate The Effect of JNJ-39758979 on Histamine Induced Itch in Healthy Male Volunteers Single oral dose: 600 mg Active comparator: Cetirizine	NCT01068223	Completed No results available
Phase II	A Study of the Safety and Efficacy of of JNJ-39758979 in the Treatment of Adults With Asthma 3 x 100 mg capsule-shaped tablet once daily for 12 weeks (total daily dose is 300 mg)	NCT00946569	Completed No results available
	<b>PF-3893787</b>		
Phase I Single Dose	Study title not found Single oral dose: 0.03, 0.3, 3, 12, 48 mg	37	Completed Results available only for GAFS. Doses 12 mg provide full inhibition and maintain effect at 24 h
Phase I Multiple Dose	A Phase I Study To Evaluate The Safety And Tolerability Of Different Doses Of PF-03893787 In Healthy Adult Volunteers Oral solution, 5, 15, 50 mg, QD for 14 days	NTC00992342	Completed No results available
Proof of Concept	A Study To Assess The Effect Of PF-03893787 On Lung Function Following An Allergen Challenge In Asthmatic Subjects Oral, two doses 12 hours apart. No dose specified Active comparator: Montelukast	NTC00856687	Completed No results available
	<b>UR-63325</b>		
Phase I Single Dose	Phase I Single Rising Dose Tolerability and Pharmacokinetics in Healthy Volunteers Single oral dose: 2, 4, 8, 16, 24, 40, 60, 100 mg	2	Completed See main results in text
Phase I Multiple Dose	Phase I Repeated Rising Dose Tolerability and Pharmacokinetics in Healthy Volunteers Repeated (x7 days) oral dose: 5, 10, 15, 25, 50 mg	44	Completed See main results in text
Proof of Concept	Proof of Activity Study of UR-63325 in Allergic Rhinitis Induced by Nasal Challenge Active comparator: Fluticasone propionate nasal spray Repeated QD (x7 days) oral dose	NCT01260753	Ongoing No results available

pathways independent of H1 stimuli. Thus, the possibility of targeting other receptors to complement or even substitute H1R antagonists has been pursued by many research groups. It was just a decade ago when one new receptor came into the play, the histamine 4 receptor (H4R), opening new ways of exploring still unexplained physiological routes and spreading out promising new possibilities of therapeutic intervention (12). Similarly to the other members of its family, this receptor also belongs to the large G-protein coupled receptor (GPCR) family, consisting of seven transmembrane-spanning helices. The H4R is expressed in cells that are key players in regulation of immune disorders: mast cells, eosinophils, basophils, dendritic cells, and several subsets of T cells (1,13,14). Several studies have demonstrated H4R expression also in monocytes, neutrophils, and in peripheral and central nervous system (12-14). This prevalent distribution in hematopoietic cells suggests a prominent role of H4R in immuno-inflammation from allergic origin. This hypothesis has been confirmed with the study of H4R knock-out mice in models of allergic disease, as well as the observations of selective H4R antagonists showing promising activity in down-regulating immune responses in a range of animal disease models of acute inflammation (13,15-19), peritonitis (20), inflammatory bowel disease (21,22), and allergic inflammation, including dermal allergies and associated pruritus (23-29)

### 3. FROM H4 RECEPTOR AFFINITY TO HUMAN ACTIVITY:

In the last few years, potent selective H4R agonists and antagonists have been synthesized and *in vitro*

and *in vivo* studies have been carried out to evaluate their activities (30-32). This review will focus mainly on those histamine H4 antagonists from which clinical data has been reported up to now (see Table 1), and developed for immuno-inflammatory pathologies. Abbott Laboratories is studying its H4 antagonist as a possible treatment for chronic pain which, like itch, may involve a neuronal mechanism, but it is out of this paper scope. Thus, JNJ-39758979, PF-3893787, and UR-63325 have successfully overcome all preclinical steps up to human administration and will be the first candidates with the potential to translate the preclinical data into a therapeutic use. So far, the preclinical data published with those compounds is clearly leading to a better picture of the role of this receptor in the allergic physiopathology and supporting the relevance of the H4R blockade in the therapy for allergic diseases.

#### 3.1. Preclinical evidences: *In vitro* studies

H4R has been implicated in the chemotaxis of several inflammatory cells, such as mast cells, lymphocytes and eosinophils. Mast cell chemotaxis in mice lungs is mediated by histamine, and can be blocked by a selective H4R antagonist (15). Histamine-induced chemotaxis of eosinophils has also been demonstrated *in vitro*. Upon treatment with histamine, eosinophils undergo H4R-mediated shape change and increase the expression of the adhesion molecules CD11b/CD18 and CD54, events that are required for the migration of eosinophils into tissues (24). The expression of the human H4R on monocyte-derived dendritic cells also stimulates the chemotactic

**Table 2.** H4R antagonists: *in vitro*- related findings

Findings	Reference
Release of IL-16 from CD8(+) related with H4R activation. Pre-incubation of lymphocytes with pertussis toxin abolished IL-16 release triggered by activation of the G(i/o)-coupled H(4)	34
H4R inverse agonist block histamine-induced mast cell chemotaxis	25
Upon treatment with histamine, eosinophils undergo H4R-mediated shape change and increase the expression of the adhesion molecules CD11b/CD18 and CD54, required for the migration of eosinophils into tissues	18
High and selective affinity on binding on SK-N-MC cells transfected with human H4R (JNJ7777120; Ki = 4 nM)	15
Functional activity of forskolin-stimulated cAMP on SK-N-MC cells transfected with human (JNJ7777120; pA2= 8.1)	15
Inhibition of histamine-induced human eosinophil chemotaxis (JNJ7777120 , IC50 = 40nM; UR-63325, IC50 =10nM)	15,40
H4R mRNA up-regulation during monocyte derived dendritic cells differentiation. Suppression of histamine induced IL-12p70 production. F-actin polymerization and migration in human MoDC	33
H4R blockade results in reduced cytokine production from CD4+ T cells polarized in vitro	16
Human monocytes express H4R stimulation leads to a Ca(2+) influx and an inhibition of CCL2 production, resulting in a reduction of monocyte recruitment.	19
<i>Ex vivo</i> specific antagonist activity with H4R antagonists (UR-63325; Pfizer compound) on the histamine-induced eosinophil chemotaxis in humans	2,37
Three new H4R gene single nucleotide polymorphisms (SNPs) have been identified in human peripheral cells, associated with atopic dermatitis	24
JNJ7777120 exhibits partial agonist efficacy onto Gi-protein activation (steady-state high-affinity GTPase activity).	35

response *in vitro*, and the activation of the H4R in this system inhibits the production of IL-12p70 (33). Another evidence for the role of the H4R in the immune response is the histamine-mediated regulation of IL-16 release from CD8+ T-cells (34). A summary of the *in vitro* evidences can be seen on Table 2.

JNJ-7777120 has been described as a H4R selective antagonist and it has been widely used as a research tool to characterize the physiological role of the H4R. This compound binds to human H4R with a Ki value of 4 nM (15) and is inactive at a broad range of other enzymes and receptors tested. Functional analysis in H4R transfected cells has demonstrated potent antagonism of JNJ-7777120, with reported pA2 of 8.1 (15). Interestingly, recent works investigating the pharmacological properties of JNJ-7777120 using two distinct downstream signalling readouts, G protein activation and beta-arrestin recruitment have shown that this compound can act as an agonist or partial agonist (35). JNJ-7777120 has also shown antagonistic effects in primary cells, such as in histamine-induced human eosinophil and murine mast cell chemotaxis (15). Moreover, JNJ-7777120 inhibited histamine-induced eosinophil shape change and adhesion molecule upregulation, which proved to be useful *ex vivo* biomarkers for H4R antagonist evaluation in the clinic (2,35). However, this compound did not progress to clinics and JNJ-39758979, another Johnson and Johnson H4R antagonist, is the compound currently being investigated in

clinical trials (see Section 3.3.) although no preclinical data for this compound have been released by the company so far.

Pfizer's PF-3893787 is another H4R antagonist in clinical trials (see Section 3.3.) for which some interesting preclinical data has been released (37,38). PF-3893787's binding affinity (Ki) for human H4R was 1.2 nM. In functional studies it showed a Ki value of 0.7 nM. Activity was established in whole blood and isolated eosinophils by using several end points, such as the shape change, the CD11b expression and the actin polymerization, activity being in the nanomolar range. Pfizer described other compounds, including PF-2988403 and PF-03306138, with H4R binding Ki of 7 and 117 nM, respectively (37), exhibiting a range of functional effects comprising from inverse agonism in humans to full agonism in rats (30). However, these two compounds were abandoned due to serious adverse effects in rats (37).

Palau Pharma has described two H4R antagonists, UR-63325 and UR-64027, whose pharmacology has been also widely characterized (39-42). Both compounds showed similar high affinity for the human H4R, Ki =15 nM and 9 nM, respectively. In mouse, rat, and monkey H4Rs UR-63325's affinity was x0.56, x1.04 and x0.98 fold vs. the human, respectively. Saturation assays with the tritiated compound showed fast association and dissociation properties from the human H4R, with a Kd value of 11 nM. It was very selective vs. the other histamine receptors (>150 fold), as well as in a wide panel of GPCRs, nuclear receptors, enzymes and bioamine transporters ( 240 fold). In eosinophils, UR-63325 behaved as an antagonist, potentially reducing histamine-induced shape change, both in isolated cells (IC50= 10 nM) and in whole-blood (IC50 = 47 nM). Moreover, it reduced histamine-induced eosinophil chemotaxis (39,40).

### 3.2. Preclinical evidences: *In vivo* studies

*In vivo* assays with H4 antagonists have been performed in several pathology models of airway inflammation. There are substantial differences for this receptor along the relevant animal species (mouse, rat, guinea pig, rabbit, dog, pig and human), both in terms of affinity to histamine and of H4R expression (32). Moreover, depending on animal species, experimental settings and readouts, different compounds' behaviour including full agonism, partial agonism, inverse agonism and full antagonism have been described. Thus, caution should be exercised when interpreting the results through the different species studies. A summary of the *in vivo* studies can be seen in Table 3.

No *in vivo* data of Pfizer's PF-3893787 or Johnson and Johnson JNJ-39758979 has been released so far. Thus, many of available *in vivo* information is based on the use of JNJ-7777120 as a pharmacological tool. JNJ-7777120 was tested in a mice asthma model, where ovalbumin was administered intraperitoneally in the sensitization phase and the animal was then challenged with a 5% ovalbumin aerosol. JNJ-7777120 blocked the

**Table 3.** H4R antagonists: *in vivo*- related findings

Findings	Reference
H4R involved in leukotriene B4 production and mast cell-dependent neutrophil recruitment induced by rat zymosan-induced peritonitis	20
JNJ7777120, shows anti-inflammatory properties in mouse histamine-induced mast cell migration <i>in vivo</i> and in mouse zymosan-induced peritonitis model	15
Histamine H4R attenuates experimental ulcerative colitis in rats	21,22
Pruritic responses in mice are induced by intradermally administered selective H4 receptor agonists	23
Histamine H4 receptor antagonists are superior to traditional antihistamines in the attenuation of experimental pruritus	23
Histamine 4 receptor antagonism modulates Th2 cell function, IL13 production, and lung remodelling in a mice model of chronic asthma	17
UR-60427, an H4 receptor-inverse agonist, shows good efficacy in a rat and mice asthma model	39
UR-63325 reduces inflammation in a rat zymosan-induced peritonitis	40
UR-63325 reduces 4-methyl-histamine-induced pruritus in rat	40
UR-63325 reduces Th2 cytokine production in local lymph nodes	40
UR-63325 significantly reduced methacholine-induced Penh in sensitised mice (asthma model), reducing total cells and eosinophils in BAL, serum OVA-IgE, IL-4, IL-5, IL-13 in OVA-restimulated cells from local lymph nodes, IL-4 and IL-13 in lungs and lesion score in lungs	36
UR-63325 reduces the 5-HT- induced airway resistance in the Brown Norway rat asthma model, reverting the reduction of lung compliance, reducing total cells, neutrophil, and eosinophil counts in BAL.	36

inflammatory response to ovalbumin when administered during either the sensitization or the challenge phase (16). This observation demonstrated that the H4R was not only able to suppress the inflammatory response to an allergen, but that this receptor was also involved in an adaptive immune response, possibly by educating helper type 2 (Th2) T-cells. Another *in vivo* anti-inflammatory effect of an H4R antagonist was demonstrated in a murine model of human allergic rhinitis (43). Mice that were sensitized for ovalbumin exhibited a dose-dependent decrease in allergic rhinitis symptoms, such as sneezing and rubbing, after the administration of a selective H4R antagonist. The serum levels of IL-4 and total IgE in these mice decreased, while IFN $\gamma$  levels in nasal lavage fluid increased, thereby contributing to the inhibition of the allergic response.

UR-63325 showed efficacy in several asthma models in rodents in which the pathology was induced by different allergens, such as ovalbumin and house dust mite at several degrees of chronicity (39-42). UR-63325 improved the functional impairment induced by allergen challenge in these models, as well as produced variable effects on the inflammatory response as measured by cellular counts in bronchoalveolar lavage (BAL), cytokine expression, IgE levels and lung histopathology in rats. UR-63325 was evaluated in an ovalbumin (OVA)-induced mouse asthma model where it reduced airway hyperreactivity (Penh) induced by methacholine in conscious and unrestrained animals. Lung inflammation was also reduced as evaluated by total and differential cell count in BAL, and cytokines in BAL and lungs. Moreover,

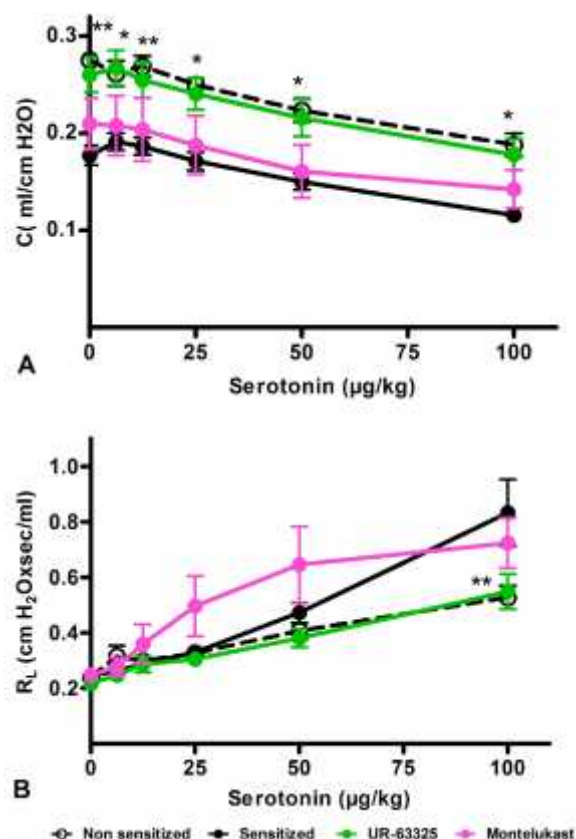
local lymph nodes analysis revealed significant suppression of inflammatory cytokines. To confirm these *in vivo* results in other species, a study in OVA-sensitized rats was performed, and allowed the evaluation of pulmonary function in anesthetized animals. Serotonin-induced increase of pulmonary resistance and decrease in pulmonary compliance were abrogated by the treatment with UR-63325 (30 mg/kg, po). The compound also significantly reduced the number of cell counts in BAL. The positive impact on both functional and cellular parameters was greater with UR-63325 than with the cysteinyl leukotriene inhibitor montelukast (Figure 1).

### 3.3. Clinical evidences

Four companies have reported an H4R antagonist in their clinical pipelines: Johnson and Johnson, Abbott, Pfizer and Palau Pharma. Abbott has reported to be studying their H4R antagonist compound as a possible treatment for chronic pain. Because the focus of the present review is allergy we will not discuss Abbott's compound further.

Palau Pharma was the first group to release human clinical data of an H4R antagonist at the European Academy of Allergy and Clinical Immunology meeting in June 2010. Palau Pharma reported preliminary data on the oral administration of UR-63325 to human healthy volunteers (2). A first-into-man double blind randomized single raising dose trial included 8 sequential cohorts, where healthy volunteers were randomly assigned to UR-63325 (6 subjects) or placebo (2 subjects). Dose escalation was guided by tolerability, pharmacokinetics (PK) and pharmacodynamics (PD), as assessed by a Dose Escalation Safety Committee. Treatment emergent AEs reported were unspecific, generally mild and resolved uneventfully, allowing a full escalation up to the maximum planned dose of 100 mg. PK fitted with *a priori* predictions from animal modelling, showing PK linearity with very low inter-individual variability and half-life suitable for once daily dosing. PD were assessed *ex-vivo* through the shift of the dose-response curve of the change of shape of eosinophils from total blood drawn from the study volunteers at different times, after stimulation with a range of doses of histamine, and assessed by flow cytometry. Dose-dependent and sustained H4R antagonist activity of UR-63325 lasted up to 24 hours, reaching the maximum level of H4R blockade expected according to pre-clinical PK-PD modelling. Because of meeting the PK and PD objectives, no further escalate doses to reach the Minimum Intolerated Dose were required. The study concluded that UR-63325 showed a lineal pharmacokinetic profile suitable for once daily dosing, very good tolerability results that led to full sequential dose escalation as per protocol, and pharmacodynamic results supporting that full H4R blockage was achieved with single doses up to 100 mg.

The conclusions from the first into man study allowed to validate the PK-PD models built with animal data and to enrich the predictive value of the model through integration of human data, in order to estimate a lowest active dose and the dose providing safe systemic exposure at the steady state. The model was used to design a



**Figure 1.** UR-63325 improved airway hyperreactivity and lung inflammation in the Brown Norway rat asthma model, with greater efficacy than montelukast (36). UR-63325 (30 mg/kg p.o.) reverted serotonin-induced lung compliance reduction (A) and serotonin-induced airway resistance increase (B) in sensitized animals. The cyst-leukotriene inhibitor montelukast (30 mg/kg p.o.) was ineffective. \*  $p < 0.05$ , \*\* $p < 0.01$  vs sensitized animals (ANOVA plus Dunnett's test).

Multiple Ascending Dose study (44). Results from this study confirmed the feasibility of complete H4R blocking in humans without limiting safety concerns. A proof of the clinical activity of UR-63325 in respiratory allergy is being investigated in a Proof of Concept study with a nasal allergen challenge in allergic volunteers otherwise healthy. The study is a double-blind fluticasone and placebo controlled cross-over study of UR-63325 where clinical symptoms as well as inflammatory parameters measured from nasal exudate are assessed up to 24 hours after allergic challenge, and is currently ongoing. (ClinicalTrials.gov as consulted on April 8th 2011: Proof of Activity Study of UR-63325 in Allergic Rhinitis Induced by Nasal Challenge; NCT01260753). Results are expected by end 2011.

Three studies have been registered in the database ClinicalTrials.gov with the H4R antagonist compound JNJ-39758979. These include two Phase I studies and a phase II study in asthma. A single and multiple dose study to explore the safety and pharmacokinetics of JNJ-39758979

in healthy male volunteers of either Caucasian or Japanese descent was completed on December 2010. The study assessed the safety and pharmacokinetics of JNJ-39758979 or placebo in a in two parts study, a randomized, double-blind study evaluating the safety, tolerability and pharmacokinetics of single doses of JNJ-39758979 (50, 100, 300, or 600 mg) or placebo in 36 healthy Japanese male volunteers and a randomized, double-blind study evaluating the safety, tolerability, and pharmacokinetics of multiple doses of JNJ-39758979 (300 mg) or placebo once a day for 14 days JNJ-39758979 and placebo in 24 healthy Japanese males and 24 healthy Caucasian males. No results have been communicated yet. (ClinicalTrials.gov as consulted on April 8th 2011: Single and Multiple Dose Study to Explore the Safety and Pharmacokinetics of JNJ-39758979 In Healthy Male Volunteers of Either Caucasian or Japanese Descent, NCT01081821).

The second phase I study was a randomized active and placebo controlled study to assess the effect of JNJ-39758979 on histamine induced itch and hive in 24 healthy male volunteers, which was reported as been completed by June 2010. (ClinicalTrials.gov as consulted on April 8th 2011: A Study to Investigate the Effect of JNJ-39758979 on Histamine Induced Itch in Healthy Male Volunteers, NCT01068223). The study had a double-blind randomized, three-treatment (single doses of either JNJ-39758979 600 mg, cetirizine 10 mg or placebo) cross-over design. A histamine prick test was conducted pre-dose and 2 and 6 hours after dose at each of 3 treatment periods, and itching, wheal and flare were measured, as well as safety assessments. No results have been communicated yet. Finally, a Phase II study where the efficacy of 300 mg of JNJ-39758979 once daily for 12 weeks is compared to placebo in adults with persistent asthma has been communicated. This multi-centre, double-blind randomized parallel-groups, placebo-controlled exploratory study in adults with persistent asthma is assessing patient lung function including FEV1 (forced expiratory volume in one second), FVC (forced vital capacity) and FEF (forced expiratory flow) after 12 weeks of treatment. Also morning/evening peak expiratory flow rates (PEFR), presence of nocturnal awakenings, asthma symptoms score and worsening of asthma, pharmacokinetics, pharmacodynamics and safety will be evaluated. Recruitment has been completed but study is still ongoing. (ClinicalTrials.gov as consulted on April 8th 2011: A Study of the Safety and Efficacy of JNJ-39758979 in the Treatment of Adults With Asthma, NCT01081821)

Two studies have been registered in the database ClinicalTrials.gov with Pfizer's compound, PF-03893787, a repeated dosing Phase I safety study in healthy volunteers (ClinicalTrials.gov as consulted on April 8th 2011: A Phase 1 Study To Evaluate The Safety And Tolerability Of Different Doses Of PF-03893787 In Healthy Adult Volunteers, NCT00992342), and an exploratory study to assess the effect of the compound on lung function following an allergen challenge in asthmatic subjects (ClinicalTrials.gov as consulted on April 8th 2011: A Study To Assess The Effect Of PF-03893787 On Lung Function Following An Allergen Challenge In Asthmatic Subjects,

NCT00856687). The phase I study was a double blind (3rd party open), randomized, placebo-controlled, dose escalation study to investigate the safety, tolerability, and pharmacokinetics of three doses of PF-03893787 (5, 10 and 15 mg) given once daily during 14 days to healthy subjects. The study was completed by March 2010, but no results have been communicated yet. The second study was a randomized, double-blind (3rd party open), double-dummy, placebo and montelukast controlled, 3-way crossover study to determine the effects of two doses of oral PF-03893787 given 12 hours apart on allergen-induced airway responses in mild asthmatic subjects, as compared to two doses given 12 hours apart of montelukast or placebo. The study was reported as completed by January 2010, but no results have been communicated yet. Some additional information on PF-03893787 was released during 2010 at two oral presentations given by Pfizer at the GRC Medicinal Chemistry Symposium (August 2010, New London, EEUU) and the Biomarker World Congress (May 2010, Sandwich, UK). The reports included description of the translational development of the compound between preclinical and clinical phases. The doses required to block H4R pharmacology in volunteers were safe and well tolerated. Moreover, it was said that studies exploring the utility of PF-3893787 in patients would be reported in due course, being the potential indications asthma, pruritus, inflammatory skin diseases and pain, among others. The dose projection presented, taking into account the *in vitro* data (see section 3.1), corrected by receptor occupancy, and/or plasma protein binding, showed a concentration to be used in clinics to observe efficacy of about 10-15 nM. The compound has been reported to be part of the active pipeline of Pfizer for asthma (Pfizer's pipeline, as consulted by April 8<sup>th</sup> 2011 at: [http://www.pfizer.com/research/product\\_pipeline/product\\_pipeline.jsp](http://www.pfizer.com/research/product_pipeline/product_pipeline.jsp)).

### 3.3.1. GAFS: an attractive biomarker:

Histamine (0.004–2 mM) induces a concentration-dependent shape change of human eosinophils, but not of neutrophils or basophils, which is detected as an increase in forward scatter in the gated autofluorescence/forward scatter (GAFS) assay. This effect is mediated through the H4R exclusively and represents therefore a functional good indication of H4R activation (45)

Results from UR-63325's multiple dose clinical trial have very nicely shown that this histamine-induced eosinophil shape change is reduced in a concentration-dependent manner in *ex vivo* blood samples when human subjects are treated with UR-63325 and that the effect also disappears when the compound is cleared from the plasma. In the first into man single dosing study, statistically significant differences to placebo were found at 2 hours following low doses administration and for most post-treatment times at doses from 16 mg onwards. Consistently sustained activity up to 24 hours supporting a good pharmacodynamic profile suitable for once daily dosing was evident from 16 mg onwards. The observed activity was consistent with the predictions made from pre-clinical data (2,44). Preliminary data from the UR-63325 multiple dosing phase I trial further supported the H4R antagonist activity after repeated dosing. Histamine-induced cell shape

changes were significantly different from placebo at most times from doses of 5 mg onwards, in good correlation with product plasma concentrations (2,44). According to the results shared by Pfizer on the Biomarker World Congress (38), the GAFS biomarker has also been used by Pfizer in a first into man clinical trial, with success.

## 4. SUMMARY AND PERSPECTIVE

Future research in the treatment of allergy should search for new therapeutic approaches that achieve complete resolution of symptoms while approaching simultaneously all tissues where an allergic response is evident (mainly respiratory tract and skin), as well as enhancing patient compliance and comfort. Ideally, these should be etiological approaches to new pharmacological targets with appropriate safety profiles, allowing the use of systemic routes of administration that provide an integrated approach to allergic diseases as a whole. There is strong preclinical evidence on H4R as a promising target for the treatment of these diseases. Unlike other histamine receptors, its preferential distribution pattern in immune cells such as mast cells, eosinophils, dendritic cells and T cells, suggest an important role of H4R in the immune response. *In vitro* studies have confirmed an important role of H4R in cellular responses, including chemotaxis, Th2 polarization, and cytokine and chemokine production. *In vivo* studies in different species using several histamine H4 agonists and antagonists have confirmed the potential of H4R modulation in allergy, although species differences regarding compound's behaviour, i.e. agonism/antagonism, have raised some conflicting results. Several H4R antagonists have already initiated clinical trials, both in healthy volunteers and in patients with allergic rhinitis. Recent results from clinical trials with UR-63325, the first H4R antagonist with clinical data reported, have confirmed the possibility of H4R modulation in humans without limiting safety concerns. Also, UR-63325 has demonstrated clear and dose-dependent pharmacodynamic effects in eosinophils, a key cell in asthma and other allergic diseases. In case H4R blockade met expectations in terms of a strong impact in allergic diseases, the possibility of treating patients with related comorbidities would be opened. Results from the ongoing clinical studies are eagerly awaited to establish the potential role of H4R antagonists in the treatment of allergic diseases.

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**Abbreviations:** H4R: Histamine H4 receptor; ARIA: Allergic Rhinitis and its Impact on Asthma; AD: Atopic dermatitis; IL: interleukin; Ki: binding affinity constant; OVA: ovalbumin; BAL: bronchoalveolar lavage; PK: pharmacokinetics; PD: pharmacodynamics; FEV1: forced expiratory volume in one second; FVC: forced vital capacity; FEF: forced expiratory flow; PEF: peak expiratory flow rates; GAFFS: gated autofluorescence/forward scatter; 5-HT: 5-hydroxytryptamine.

#### **H4 receptors in allergy: from basic science to clinics**

**Key Words:** H4 Histamine Receptor, Asthma, Rhinitis, Pruritus, Dermatitis, Immuno-Inflammatory, JNJ-39758979, PF-3893787, UR-63325, Review

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